THE CHEMICAL ANALYSIS OF FOODS

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A PRACTICAL TREATISE ON THE
EXAMINATION OF FOODSTUFFS AND
THE DETECTION OF ADULTERANTS

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PREFACE TO THE THIRD EDITION

THE chemistry of food has advanced and is advancing so rapidly that it is almost impossible to keep pace even with the analytical aspects of the subject. The most that can be hoped for in the compass of a small laboratory handbook is a reasonably connected and up-to-date account of well-tried methods, an indication of what tests should be made, and reference to sources of further information.

In the present edition it has been necessary to observe economy of space ; so the type and make-up have been adjusted so that although there is a good deal of new matter, and some excision of old matter, the total volume is not greater than that of former editions.

Methods for the determination of the metallic impurities, usually by means of the newer organic reagents, have been detailed, also methods for other substances of dietetic importance, the estimation of which are now routine to the analyst.

Amongst other new matters are the determination of total solids in milk, available carbon dioxide in baking powders, oxidation and other values in vinegar, soya meal and rancidity tests. Further and better particulars are given in a number of other subjects.

It has been difficult to decide how far to make reference to the large number of war-time regulations or standards for foods. Only those standards which seem likely to be of permanent value have been mentioned. The Labelling of Food Order makes it necessary to evaluate certain vitamins and minerals in appropriate units, so a statement of units of the vitamins has been included, as well as methods for the estimation of those of them which are amenable to chemical assay.

I am indebted to Mr. E. Voelcker and Mr. W. R. Dracass for help in the proof reading.

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PREFACE TO THE FIRST EDITION

THE chemistry of food has developed so much in recent years that it is quite impossible to deal adequately with its analysis in the compass of one small volume. To the specialist no text-book is really adequate or contains just that information which he seeks. Fully conscious of these limitations the writer has endeavoured therefore only to present the elements of the subject and to do that in a manner suitable to the requirements of chemists who have no special experience in this branch of their science. One of the chief difficulties in writing such a book is to decide what not to include.

The methods given are, with only a few exceptions, those which have been well tried and found reliable ; in the few cases no process has yet been found completely satisfactory. It is hoped that sufficient has been included to provide an adequate survey of the foodstuffs considered and to suggest to the intelligent worker lines on which further investigation can be made. The point of view is rather that of the requirements of public health and of the Sale of Food and Drugs Acts than that of the worker in a particular branch of the food industry. Every chemist who undertakes the examination of foodstuffs must have a competent knowledge of ordinary organic and inorganic analysis and be familiar with the usual physical instruments ; no account of such is therefore included.

Microscopy is not given quite the prominence it deserves, not for lack of appreciation of its usefulness but because it is a sufficiently important branch to necessitate a volume to itself ; several such volumes are available, such as Greenish's ' Food and Drugs " and Clayton's " Compendium of Food Microscopy."

The author will be grateful to anyone directing his attention to errors, which are sure to have crept in, so that they may be eliminated.

All temperatures are in degrees Centigrade and refractive indexes at 40°, unless otherwise expressly stated.

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THE CHEMICAL ANALYSIS OF FOODS

CHAPTER I

SUGARS, SYRUPS, TREACLE, HONEY, JAM MARMALADE

CARBOHYDRATES are the leading product of plant life and constitute the largest proportion of an ordinary diet ; they exist in a great variety of forms, which are included in the general formula, $C_x(H_2O)_y$. Until quite recent years only those in which "x" is 6 or a multiple thereof were known, but, as the result of the work of E. Fischer and others, carbohydrates are now known in which there are any number of carbon atoms from 2 upwards, and, as in the case of rhamnose ($C_6H_{12}O_5$), some in which hydrogen and oxygen are not present in the proportion required to form water.

The more important carbohydrates of natural occurrence fall into two distinct classes : those which are sweet and crystallisable, sugars, and those which are not crystallisable, such as the starches. Chemically, five principal groups are recognised : monosaccharoses, di-, tri-, tetra- and polysaccharoses. The monosaccharoses are subdivided into groups distinguished by the name biose, triose, tetrose, pentose, hexose, etc., up to nonose containing 9 carbon atoms ; of these the hexoses are by much the most important. Further, according to whether the particular member has the properties of an aldehyde or a ketone, the name aldose or ketose is applied.

It is proposed here to discuss only those carbohydrates which enter into the composition of foods. The more important members and some of their properties are set out on the next page.

General reactions by which the carbohydrates may be identified or estimated are as follows : When warmed with concentrated sulphuric acid they swell up, blacken, and evolve carbon dioxide, sulphur dioxide, and other gases. When to an aqueous solution in a test tube are added a few drops of an alcoholic solution of α-naphthol and strong sulphuric acid is poured down the side of the tube, a deep violet coloration is produced which is discharged on the addition of excess of alkali

Name.	Formula.	Rotation $[a]^{15}_{D}$.	Melting point. Degrees C.	Melting point of osazone.	Reaction with Fehling's solution.
MONOSACCHAROSES					
Pentoses. Arabinose	$C_5H_{10}O_5$	+ 104°–105°	160°	160°	+
Hexoses. Dextrose	$C_6H_{12}O_6$	+ 52·7°	146°	204·5°	+
Lævulose	,,	− 93·8°	95°	204·5°	+
Galactose	,,	+ 81·5°	163·5°	204·5°	+
Mannose	,,	+ 14·5°	132°	186°	+
DISACCHAROSES.					
Sucrose	$C_{12}H_{22}O_{11}$	+ 66·5°	160°	—	—
Lactose	,,	+ 55·3°	205°	200°	+
Maltose	,,	+ 139·5°	—	190°	+
TRISACCHAROSE.					
Raffinose	$C_{18}H_{32}O_{16}$	+ 104°	118°–119°	—	—
POLYSACCHAROSES.					
Starch	$(C_6H_{10}O_5)n$	+ 200°[1]	—	—	—
Cellulose	,,	+	—	—	—
Inulin	,,	− 38°–40°	178°	—	
Glycogen	,,	+ 191°	—	—	—
Dextrin	,,	+ 198°[1]	—	—	—

(Molisch's reaction). In common with other aldehydes and ketones, many of the sugars react with phenylhydrazine, giving rise to osazones, many of which have a characteristic micro-crystalline appearance and melting point. For the preparation of the osazone about 2 g. of the sugar are dissolved in 10 ml. of water, 4 g. of phenylhydrazine in 10 ml. of dilute acetic acid are added, and the mixture is heated in the water bath for an hour, then set aside to cool. The crystals may then be examined with the microscope, or filtered off, washed, dried and their melting point determined.

Some of the sugars exert a considerable reducing action on copper solutions or silver nitrate, producing a reddish precipitate of cuprous oxide or a silver mirror respectively. The reagent most useful for their detection or quantitative estimation is that of Barreswil or Fehling. This reagent consists of two solutions which must be kept separately ; equal volumes are mixed together as required for use. No. 1 contains 69·28 g. of copper sulphate, $CuSO_45H_2O$, dissolved in water to produce 1 litre, and No. 2 is prepared by dissolving 100 g. of pure sodium hydroxide and

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[1] The specific rotatory power of starch and of dextrin varies according to the variety.

346 g. of sodium potassium tartrate in water and diluting the solution to 1 litre.[1]

A reagent useful for qualitative purposes is that of Barfoed, which is prepared by dissolving 6·5 g. of crystallised copper acetate in 100 ml. of water and adding 2·5 ml. of dilute acetic acid just before use ; it has the peculiarity of being reduced by monosaccharoses and not by disaccharoses, so that it is available for the detection, say, of dextrose or lævulose in the presence of lactose or maltose.

Carbohydrates are generally optically active ; the direction and extent of the rotation depends upon concentration, temperature and other factors, which are considered in connection with the various sugars.

Arabinose. This pentose is widely distributed in plants and occurs to some extent in animal tissues, usually in the form of its anhydrides or pentosans, which yield arabinose (with xyloses) on hydrolysis. It is a crystalline sugar having m.p. 160° and specific rotary power $[\alpha]_D^{15}$ + 104° to + 105°. When distilled with dilute hydrochloric acid it yields furfural which gives characteristic colour reactions with resorcinol or phloroglucinol. Arabinose reduces Fehling's solution and forms a phenylosazone melting at 160°. In admixture with other sugars it may be estimated in the same manner as pentosans (see p. 96), or, if no other reducing sugar is present in the solution, it may be estimated by Fehling's reagent in the manner described on p. 16.

Dextrose. Dextrose, grape sugar or glucose, is a crystalline substance having m.p. 146°, $[\alpha]_D^{15}$ + 52·7 ; it occurs also as crystals having one molecule of water of crystallisation ; it is readily soluble in water and in alcohol, but not in dry ether. It exists in a great variety of animal and vegetable foodstuffs and is produced on a large scale by the hydrolysis of starch : it is also formed by the hydrolysis of disaccharoses such as cane sugar, hence is present to a small extent at least in commercial foodstuffs prepared therefrom. Dextrose readily reduces Fehling's or Barfoed's solutions, forms a characteristic osazone, and is readily fermented by yeast. It is well known that, chemically, dextrose and many other sugars exist in lævo-rotatory and inactive forms, but these varieties do not arise in connection with foods.

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[1] A large number of modifications of Fehling's reagent have been suggested; the above formula is given because it is desirable to keep the amount of alkali to a minimum.

When starch is dissolved in dilute acids, maltose, dextrins and other products are formed which finally yield dextrose ; in this country dilute sulphuric acid is generally employed, whereas in America hydrochloric acid is more usual. Sago, maize, rice or other cereals are boiled in cast-iron or copper vats under pressure with the diluted acid ; after the reaction the solution is neutralised, filtered, decolorised and concentrated in vacuum pans. The corn syrup thus produced contains, besides water, a large proportion of maltose and dextrin, and is generally further purified by concentration to about 85–86 per cent., followed by the addition of a few crystals of dextrose which serve as nuclei for the crystallisation of a large quantity ; the crystals are separated from the mother liquor by centrifuging. The following table shows the composition of good commercial glucose. Methods for the analysis of such substances are given on p. 31.

Water . . .	10 to 20 per cent.
Ash . . .	1 ,, 1·5 ,,
Dextrose . . .	20 ,, 60 ,,
Maltose . . .	10 ,, 40 ,,
Dextrin . . .	5 ,, 20 ,,
Proteins . . .	Trace, up to 0·15 per cent.

Lævulose fructose or fruit sugar, as its name indicates, is commonly present in fruits and their products, and is lævo-rotatory. It exists in considerable quantities in honey and is a colourless substance obtainable as crystals of m.p. 95° ; it readily reduces Fehling's solution and forms with phenylhydrazine a characteristic osazone having m.p. 204°–205°. The specific rotation of lævulose is considerably influenced by temperature, a feature due to alteration in the chemical equilibrium of the isomerides of which it is composed ; at 15° $[\alpha]_D$ is − 93·8°, the temperature change per degree is + 0·6385°, so that the rotation decreases with rise of temperature and at about 87° a solution of invert sugar exhibits no rotation, since the dextro-rotation due to dextrose is exactly neutralised by the lævo-rotation due to the lævulose. It also exhibits to a marked extent the phenomenon of muta-rotation ; when a solution is freshly prepared it has a much higher rotatory power than that of the same solution after standing a few hours ; this is frequently the case with sugar solutions, hence in their polarimetric estimation it is important to eliminate this factor, otherwise serious errors may be introduced. This may be done either by allowing the solution to stand several hours before making the observations or, more conveniently, by adding a few drops of ammonia, which establishes the optical equilibrium at once.

Galactose occurs in a polymerised form in many gums; commercially it is a product of the inversion of lactose. It readily crystallises with one molecule of water—$C_6H_{12}O_6.H_2O$—in the form of a white powder having m.p. 120°, but the anhydrous variety melts at 163·5°. It exhibits muta-rotation and has a considerable temperature coefficient, at equilibrium $[\alpha]^{15}_{D}$ is $+ 81{\cdot}5°$. Fehling's solution is reduced, the taste is sweet, and the osazone melts at 204·5°.

Mannose occurs in nature in its condensation products as mannosans and exists to a small extent in honey. It has m.p. 132°, $[\alpha]^{15}_{D} + 14{\cdot}5$, readily reduces Fehling's solution, and forms an osazone having m.p. 186°.

Sucrose, saccharose or cane sugar, ($C_{12}H_{22}O_{11}$), is well known as the most important member of the sugar group and one which enters largely into the composition of foodstuffs. It forms crystals of m.p. 160°, is strongly dextro-rotatory, having $[\alpha]^{15}_{D} + 66{\cdot}5°$, and does not reduce Fehling's solution. This sugar does not form an osazone. In common with other disaccharoses it is readily inverted by heating with dilute mineral acids, giving rise to equivalent quantities of dextrose and lævulose which together form "invert sugar." The same change may also be brought about by yeast and certain enzymes.

Sucrose is commonly manufactured from the sugar cane, beet or maple; during the purification of the product advantage is often taken of the fact that it forms complex saccharosates with lime or strontia which are not very soluble in water; these are precipitated and filtered out of the crude syrup, and subsequently decomposed by carbon dioxide. Other properties of this important sugar are referred to in connection with its estimation in commercial products.

Lactose, or milk sugar, has the same empirical formula as saccharose but a different structure; hence it differs materially from it in chemical and physical properties. It occurs to the extent of about 5 per cent. in milk (*q.v.*), but has not been demonstrated in plant products. It exists as a white crystalline powder of m.p. 203°–205° with decomposition, has only a mildly sweet taste, and readily forms a monohydrate which is stable at

100° but decomposes at 130°. This point is of importance in connection with the total solids in milk; but although the hydrate $C_{12}H_{22}O_{11}.H_2O$ is not completely dehydrated until the temperature reaches 130°, it seems likely that under the normal conditions of drying in the oven at 100° the total solids include lactose in a partly dehydrated condition. Lactose reduces Fehling's solution, forms an osazone melting at 200°, and is inverted by dilute acids, yielding equal quantities of dextrose and galactose. The rotatory power of this sugar is $+ 55{\cdot}3°$, which is that of the equilibrium or γ form of the α-hydrate and β-anhydride which exist when the solution is first prepared. Fearon (*Analyst*, 1942, **67,** 130) gives a convenient identification test for lactose or maltose; to a dilute neutral solution of the sugar add 3 or 4 drops of a 5 per cent. solution of methylamine hydrochloride, boil for a few seconds then add 5 drops of 20 per cent. sodium hydroxide solution. A yellow colour changing to carmine indicates lactose or maltose; other organic compounds do not so react.

Maltose occurs naturally in plants, leaves and seeds, and in malt. In each case it is probably produced by the decomposition of starch. It consequently occurs in commercial glucose and corn syrups. It is usually prepared by the action of the enzyme diastase on malt, and exists as a slightly sweet sugar forming a hydrate which is decomposed at or below 100°. It reduces Fehling's solution, has $[\alpha]_D^{15} + 139{\cdot}5°$, with a temperature coefficient of $- 0{\cdot}095°$. (The specific rotation of this sugar is considerably dependent on the concentration of the solution; the above figure is for a 10 per cent. solution.) The osazone melts at 190°. Maltose may be hydrolysed by dilute acids at about 80°, and yields thereby two molecules of dextrose; this fact may be utilised for its estimation. A biochemical method is given by Davis (*loc. cit.*, p. 13).

Raffinose (or melitose), $C_{18}H_{32}O_{16}$, is the only important trisaccharose of interest to the food analyst; it occurs in beet sugar, hence in molasses, and in certain cereals. It forms a monohydrate melting at 80°, but in the anhydrous state melts at 118·5°. Its behaviour with Fehling's solution and with phenylhydrazine is exactly like saccharose, the optical rotation $[\alpha]_D^{15}$ is $+ 104°$ without appreciable muta-rotation, and on hydrolysis with strong acids it yields dextrose, lævulose and galactose; weak acids form lævulose and a disaccharosemelibiose. The polarimeter is not reliable for the estimation of raffinose since this sugar is almost invariably associated in practice with relatively large amounts of other sugars. Accurate results are obtainable by fermentation methods based on the fact that top and bottom yeasts hydrolyse raffinose to lævulose and melibiose and to galactose and dextrose respectively. Quite small quantities of raffinose in commercial products can be accurately estimated by this method, the details of which are given on p. 13.

The polysaccharoses will be discussed after the consideration of methods of analysis of commercial sugar products.

Cane Sugar and its Products

The cane sugar of commerce in this country is obtained from the sugar cane or beet, and, as it so readily crystallises, is generally of a high degree of purity; cube sugar and the white granular form usually contain more than 99·5 per cent. of sucrose. It is a remarkable fact that the sweetness of a sample of sugar is not conditioned merely by the amount of sucrose it contains, but is much influenced by the acidity; the presence of 0·2 or more per cent. of organic acids in an 80 per cent. sugar imparts a sweeter taste than that of a pure sugar free from acid. A small quantity of salt has a similar effect. White sugar, in the unrefined condition, whether from beet or cane, usually contains reducing sugars and some gummy substances which are only partly soluble in water; even refined sugar occasionally contains insoluble matter producing a white turbidity; this may be calcium sulphite which has escaped the filter cloth and become oxidised to sulphate. Also fine icing sugars sometimes contain small quantities of starch added to prevent caking. A small quantity of a blue dye is often added to make sugar appear more white. The table on p. 8 shows the usual composition of different kinds of sucrose.

Under the Preservatives, etc., in Food Regulations sugar, including solid glucose and cane syrups, may contain sulphur dioxide not exceeding 70 parts per million. A long-standing practice exists of adding small quantities of tin chloride to certain types of sugar, such as Demerara sugar; it is claimed that the tin acts as a mordant for the natural colour of the sugar. The amount of tin found in these sugars is usually much less than

—	Water.	Sucrose.	Glucose.	Pro-tein.	Ash.	Other non-saccharine matter.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Java white sugar .	0·30	98·60	0·30	0·10	0·20	0·50
Cuban white sugar .	0·40	97·32	1·10	0·14	0·50	0·54
Beet sugar (white) .	0·71	97·70	Trace.	0·55	0·58	0·46
Beet (recrystallised) .	—	99·75	—	—	0·12	0·13
"Demerara" sugar .	1·05	96·85	1·05	0·38	0·45	0·22
Best West Indian cane	0·10	99·50	0·23	—	0·02	0·15
English beet (best) .	0·05	99·90	—	—	0·01	0·04

140 parts per million, but the desirability of the practice is very questionable from the consumer's point of view. Tin, if present, can be determined in the ash by the method given on p. 107.

The exact estimation of sucrose is always carried out by the polarimeter. Methods dependent on specific gravity or refractive index of saccharine solutions, while useful for certain kinds of routine work, are not sufficiently accurate for the analysis of pure sugars. Methods dependent on copper reduction are more tedious and generally less accurate than the polarimeter.

The specific rotatory power, at temperature $t°$, of a substance in solution, using a sodium lamp, is expressed by the formula

$$\frac{100\,a}{l\,g\,d}$$

where a is the observed rotation, l the length of the tube in decimetres, g the number of grams of solute per 100 g. of solution, and d the density. This may be more simply expressed as

$$[\alpha]_D^t = \frac{100\,a}{l\,c}$$

where c is the concentration in grams per 100 ml. of solution, but it has to be borne in mind that the specific rotation depends to a small extent upon the concentration of the solution on which the observation is made. For this reason, when great accuracy is required the so-called "normal weight" of sugar should be taken, this being the quantity of sucrose which in 100 ml. of solution will, in a 200 mm. tube, give a rotation of 100 divisions on an arbitrary scale known as the sugar scale, which will be found on most polarimeters ; thus when the normal weight of sugar is taken, the observed rotation (sometimes termed degrees V.) gives directly the percentage of sucrose. For many purposes it is convenient to use the sugar scale even when not dealing with pure sucrose solutions ; in any case it is a simple matter to convert angular degrees into degrees on the sugar scale by multiplying by the appropriate factor :—

On a Ventzke or Schmidt and Haensch instrument, where the normal weight is 26 g., 1° on the sugar scale = 0·3466 angular degree.

On the Soleil-Dubosq or Laurent saccharimeters, using 16·3 g., 1° sugar scale = 0·2167 angular degree.

The standard weight of sugar which should be taken for different instruments varies slightly with the particular instrument and is usually stated by the maker. For most purposes, however, the following weights may be taken with the common types of polarimeter :—

Ventzke	26·05 g.
Soleil-Dubosq	16·35 g.
Laurent	16·27 g.
Schmidt-Haensch	26·05 g.

For the estimation of sucrose when no other sugars are present, it is sufficient to dissolve the normal weight in water, make the solution up to 100 ml., filter if necessary, and observe the rotation. The amount of the sugar is simply calculated from the formula already given. This process is applicable to any saccharine liquid which contains only one optically active substance ; when two or more are present the methods given in subsequent pages must be employed. The specific rotation of the principal sugars when in solution of about 10–15 per cent. concentration may be taken to be as follows for sodium light at 15·5° C. :—

Arabinose	+ 104·5°
Dextrose	+ 52·7°
Lævulose	− 93·8°
Invert Sugar	— 20·0°
Galactose	+ 81·5°
Mannose	+ 14·5°
Sucrose	+ 66·5°
Lactose (anhydrous)	+ 55·3°
Maltose	+ 139·5°
Raffinose	+ 104·4°

As it is essential for accurate polarimetric readings that the solution to be examined shall be not only clear but colourless, it is frequently necessary to resort to the use of decolorising agents. For this purpose lead acetate and alumina cream are employed; animal charcoal is not recommended. It has been shown that when an excess of basic lead acetate is added, errors may be introduced in strong sugar solutions by the lead precipitate carrying down with it some dextrose or lævulose. It is therefore preferable to add normal lead acetate and to use only a very slight excess (W. A. Davis has shown that no such occlusion occurs in the case of dilute sugar solutions). With a dark-coloured syrup the normal weight should be dissolved in about 50 ml. of water, then a few drops of 10 per cent. lead acetate solution and about 5 ml. of hydrated alumina cream are added, the solution is made up to 100 ml., filtered through a dry paper, and polarised in the usual way.

Alumina cream is prepared by adding a slight excess of ammonia to a saturated solution of alum, then adding more alum solution until the reaction is just acid.

When pure syrups of known composition are being examined, the refractometer affords a rapid and fairly accurate method for the estimation of their sugar content. The ordinary Zeiss-Abbe instrument may be used for this purpose, or a dipping refractometer. The specific gravity is also of use, but does not yield quite such good results. The table on p. 11 shows the specific gravity and refractive index of solutions of cane sugar.

Moisture in sugars may be estimated with fair accuracy by drying in a flat-bottomed dish at 105° for five hours, or until there is no further loss in weight. Fructose must be dried *in vacuo* at 70°—above this temperature it undergoes some decomposition. In the case of lactose it must be remembered that the hydrate is only decomposed at 130°; all other common sugars lose their water of crystallisation at or below 100°.

For the estimation of sucrose in sugars in the presence of invert sugar as in molasses, syrups or similar products, the modified Clerget-Herzfeld method of double polarisation is most convenient; it depends upon the fact that the dextro-rotation of sucrose is changed to a lævo-rotation on hydrolysis by acids or enzymes. Changes of temperature affect the rotatory power of lævulose considerably, so that it is essential to make the readings at a definite temperature; 20° is most convenient.

SPECIFIC GRAVITY & REFRACTIVE INDEX OF SUGAR SOLUTIONS

Specific gravity at 15·5°.	n_D^{20}	Sucrose per cent.	Specific gravity at 15·5°.	n_D^{20}	Sucrose per cent.	Specific gravity at 15·5°.	n_D^{20}	Sucrose per cent.
1·0000	1·3330	0	1·1181	1·3767	27·5	1·2609	1·4307	55·0
1·0019	1·3337	0·5	1·1205	1·3775	28·0	1·2637	1·4318	55·5
1·0039	1·3344	1·0	1·1229	1·3784	28·5	1·2666	1·4329	56·0
1·0060	1·3351	1·5	1·1253	1·3793	29·0	1·2695	1·4340	56·5
1·0078	1·3359	2·0	1·1276	1·3802	29·5	1·2724	1·4351	57·0
1·0099	1·3366	2·5	1·1300	1·3811	30·0	1·2753	1·4362	57·5
1·0118	1·3373	3·0	1·1324	1·3820	30·5	1·2782	1·4373	58·0
1·0138	1·3381	3·5	1·1348	1·3829	31·0	1·2811	1·4385	58·5
1·0158	1·3388	4·0	1·1373	1·3838	31·5	1·2840	1·4396	59·0
1·0178	1·3395	4·5	1·1397	1·3847	32·0	1·2870	1·4408	59·5
1·0198	1·3403	5·0	1·1421	1·3856	32·5	1·2900	1·4418	60·0
1·0218	1·3410	5·5	1·1446	1·3865	33·0	1·2929	1·4431	60·5
1·0238	1·3417	6·0	1·1471	1·3874	33·5	1·2959	1·4441	61·0
1·0259	1·3425	6·5	1·1496	1·3883	34·0	1·2989	1·4454	61·5
1·0279	1·3433	7·0	1·1520	1·3893	34·5	1·3018	1·4464	62·0
1·0299	1·3440	7·5	1·1545	1·3902	35·0	1·3048	1·4475	62·5
1·0320	1·3448	8·0	1·1570	1·3912	35·5	1·3078	1·4486	63·0
1·0341	1·3455	8·5	1·1595	1·3920	36·0	1·3109	1·4497	63·5
1·0361	1·3463	9·0	1·1620	1·3931	36·5	1·3139	1·4509	64·0
1·0382	1·3471	9·5	1·1645	1·3939	37·0	1·3169	1·4521	64·5
1·0403	1·3478	10·0	1·1670	1·3950	37·5	1·3200	1·4532	65·0
1·0424	1·3486	10·5	1·1695	1·3958	38·0	1·3230	1·4544	65·5
1·0445	1·3494	11·0	1·1720	1·3969	38·5	1·3261	1·4555	66·0
1·0466	1·3501	11·5	1·1744	1·3978	39·0	1·3291	1·4567	66·5
1·0487	1·3509	12·0	1·1769	1·3988	39·5	1·3322	1·4579	67·0
1·0508	1·3517	12·5	1·1794	1·3997	40·0	1·3354	1·4591	67·5
1·0529	1·3525	13·0	1·1820	1·4007	40·5	1·3384	1·4603	68·0
1·0551	1·3533	13·5	1·1846	1·4016	41·0	1·3416	1·4615	68·5
1·0572	1·3541	14·0	1·1872	1·4026	41·5	1·3447	1·4627	69·0
1·0593	1·3549	14·5	1·1898	1·4036	42·0	1·3478	1·4639	69·5
1·0615	1·3557	15·0	1·1924	1·4046	42·5	1·3509	1·4651	70·0
1·0637	1·3567	15·5	1·1950	1·4056	43·0	1·3541	1·4663	70·5
1·0659	1·3573	16·0	1·1977	1·4066	43·5	1·3573	1·4676	71·0
1·0681	1·3581	16·5	1·2004	1·4076	44·0	1·3604	1·4688	71·5
1·0703	1·3589	17·0	1·2031	1·4086	44·5	1·3636	1·4700	72·0
1·0725	1·3597	17·5	1·2057	1·4096	45·0	1·3668	1·4713	72·5
1·0747	1·3605	18·0	1·2084	1·4106	45·5	1·3700	1·4725	73·0
1·0769	1·3614	18·5	1·2111	1·4117	46·0	1·3732	1·4737	73·5
1·0791	1·3622	19·0	1·2137	1·4127	46·5	1·3764	1·4749	74·0
1·0813	1·3630	19·5	1·2164	1·4137	47·0	1·3796	1·4762	74·5
1·0835	1·3638	20·0	1·2191	1·4148	47·5	1·3829	1·4774	75·0
1·0857	1·3647	20·5	1·2218	1·4158	48·0	1·3862	1·4787	75·5
1·0879	1·3655	21·0	1·2246	1·4169	48·5	1·3894	1·4799	76·0
1·0902	1·3664	21·5	1·2273	1·4179	49·0	1·3927	1·4812	76·5
1·0925	1·3672	22·0	1.2300	1·4190	49·5	1·3960	1·4825	77·0
1·0949	1·3681	22·5	1·2328	1·4200	50·0	1·3993	1·4838	77·5
1·0972	1·3689	23·0	1·2356	1·4211	50·5	1·4025	1·4850	78·0
1·0995	1·3698	23·5	1·2384	1·4221	51·0	1·4059	1·4863	78·5
1·1018	1·3706	24·0	1·2411	1·4232	51·5	1·4092	1·4876	79·0
1·1041	1·3715	24·5	1·2439	1·4242	52·0	1·4126	1·4888	79·5
1·1064	1·3723	25·0	1·2467	1·4253	52·5	1·4160	1·4901	80·0
1·1087	1·3732	25·5	1·2495	1·4264	53·0	1·4193	1·4914	80·5
1·1110	1·3740	26·0	1·2523	1·4274	53·5	1·4220	1·4927	81·0
1·1133	1·3749	26·5	1·2552	1·4285	54·0	1·4260	1·4941	81·5
1·1157	1·3758	27·0	1·2580	1·4296	54·5	1·4294	1·4954	82·0

Also raffinose and other carbohydrates are inverted by acid and may seriously affect the accuracy of the process unless the inversion is carried out under carefully defined conditions. 26·05 g., or the normal weight of the sugar or syrup, are diluted with about 60 ml. of water, defecated, and made up to 100 ml., and the rotation is observed in a 200-mm. tube, preferably using the sugar scale ; let D be the direct rotation. Now take 50 ml. of the solution (= half the normal weight), add 5 ml. of concentrated hydrochloric acid, dilute to about 90 ml. in a 100-ml. flask, and place the flask with a thermometer in it in a water bath kept at 72°–73° ; note when the temperature of the flask rises to 69° and keep the mixture at this temperature, with occasional shaking, for exactly five minutes, then cool to 20° quickly, adjust the volume to the mark and observe the rotation in a 200-mm. tube at this temperature ; the observed reading is doubled so as to represent the normal weight and may be denoted by I. Then the sucrose percentage, $S = \frac{(D - I)\,100}{142{\cdot}66 - t/2}$, or if t, the temperature, is 20°, $S = 0{\cdot}754\,(D - I)$.

This method may readily be extended to the estimation of another sugar such as invert sugar or lactose in the presence of sucrose. The total weight for sucrose (26·05 g.) is dissolved in water, defecated and diluted to 100 ml. Then if D is the direct rotation and the percentage of sucrose calculated by the Clerget-Herzfeld formula is denoted by S, then the percentage of the other sugar X is given by the equation

$$X = \frac{(D - S)\,66{\cdot}5}{[\alpha]_D \text{ (of the other sugar)}}.$$

In the case of lactose or of invert sugar, the equation becomes

$$X = \frac{(D - S)\,66{\cdot}5}{55{\cdot}3} \text{ and } X = \frac{(D - X)\,66{\cdot}5}{-\,20{\cdot}0}$$

respectively. These formulæ do not apply when a third optically active substance is present, such as glucose in a syrup or treacle which contains cane sugar and invert sugar.

It has been shown that the Clerget constant varies slightly with the concentration, with the rate of heating, and with the time which elapses between inversion and polarisation. A small error is also introduced by the differing volumes of the lead precipitate in the case of molasses or syrups containing much protein matter or amino compounds.

In using this method for the analysis of syrups or jams it must be remembered that hydrochloric acid under the conditions of the experiment will hydrolyse other carbohydrates to a greater or lesser extent. Maltose or dextrins are only slightly affected, as for complete inversion they require about four hours' heating; lactose also is not much hydrolysed under these conditions, so that Clerget's method may be used with reasonable accuracy in the presence of glucose, dextrins, maltose or lactose. The probable limit of accuracy of the Clerget process is about ± 0·5 per cent.

In carrying out the polarimetric estimation of sugars by acid inversion it is usually more convenient to make the initial solution up to 100 ml., after defecation if necessary, then to invert 50 ml. by heating with 5 ml. of hydrochloric acid at the appropriate temperature (p. 12); then, after cooling, make the volume of the inverted solution up to exactly 55 ml. If the direct reading is made in a 200-mm. tube and the invert reading in the 220-mm. tube, no corrections for dilution are required.

Raffinose, which is usually present in beet molasses, may be roughly estimated by Clerget's acid inversion method. Normal weights are used and the readings made on the Ventzke scale; the formulæ giving the percentage of raffinose then are:—

$$S = \frac{0{\cdot}514\,D - I}{0{\cdot}844} \text{ and } R = \frac{D - S}{1{\cdot}85}$$

where D and I are the direct and invert rotations measured at 20° and R and S the percentages of raffinose and sucrose respectively.

A lengthy but more exact method for the estimation of raffinose by yeast is given by Davis (*J. Soc. Chem. Ind.*, 1916, **35,** 201). The sugar solution is defecated by lead acetate and the excess of lead removed by hydrogen sulphide, the latter is boiled off and the solution cooled, neutralised, made just acid with acetic acid, and diluted to 100 ml.; to 95 ml. are added 5 ml. of autolysed solution of invertase from top yeast (for preparation see *J. Soc. Chem. Ind.*, 1910, **29,** 443). The enzyme is allowed to act for eighteen to twenty-four hours, and the rotation is then observed at 20°. To 95 ml. of this hydrolysed solution are now added 5 ml. of autolysed solution from bottom yeast and the mixture kept at room temperature while determining the rotation daily until constant. A change in rotation of 1° Ventzke corresponds with 0·239 g. of melibiose or 0·352 g. of anhydrous raffinose per 100 ml. of the solution, if the readings are taken in the 200-mm. tube.

Estimation of Sugars by Copper Reduction

Two methods are available for the estimation of sugars by Fehling's solution—gravimetric and volumetric ; in either case it has to be borne in mind that proteins and certain other substances reduce alkaline copper tartrate ; hence solutions containing such substances must be purified before the estimation of the sugars.

For quantitative purposes the cuprous oxide precipitated on boiling the sugar solution with Fehling's reagent may be weighed as cuprous oxide, cupric oxide, or as copper ; the last named is perhaps the most convenient and accurate. It is important to adopt uniform conditions of precipitation, even to the point of uniformity of the size and covering of the beaker, otherwise small errors due to surface oxidation will be introduced. The weight of copper oxide found is not strictly proportional to the weight of the particular sugar, so that a simple factor is not accurate for the calculation of the results, although it may sometimes be of use for an approximation.

The recipe for Fehling's solution is given on p. 2. For the gravimetric process 50 ml. of the mixed Fehling solution are introduced into a 400 ml. squat-form beaker covered with a clock glass ; to it is added such volume of water that when the sugar solution is introduced the total volume will be 100 ml. This is heated in a bath of boiling water so adjusted that the height of water outside the beaker is at the level of the solution inside, but the beaker should not rest on the bottom of the bath. After five minutes the measured volume of sugar solution is added, the cover immediately replaced, and the heating continued for exactly twelve minutes. The precipitate is then rapidly filtered off, washed with hot water, ignited and weighed. For the filtration an ordinary Gooch crucible may be used and the cuprous oxide ignited in air to CuO. The writer prefers to use a hard glass tube of the form indicated in Fig. 1 ; when this is used in conjunction with a small funnel and a filter pump, the filtration, washing, drying and ignition are the work of only a few minutes, and the whole estimation may be carried out in about half an hour. The glass tube is lightly plugged with washed asbestos, attached to the Kipp hydrogen generator, and ignited in a current of hydrogen, then cooled and weighed. After filtration the cuprous oxide is washed, first with water, then with 5 ml. of alcohol to facilitate drying, then dried for a few minutes in the oven and ignited in the current of hydrogen at the Kipp for about five minutes, cooled in the hydrogen, and the weight of copper noted. A convenient alternative to weighing the cuprous oxide after filtration is to dissolve it in a known quantity of acid ferric sulphate solution and titrate the iron so reduced with 0·1 N potassium permanganate as recommended by Bertrand (1 ml. = 0·00636 g. Cu.).

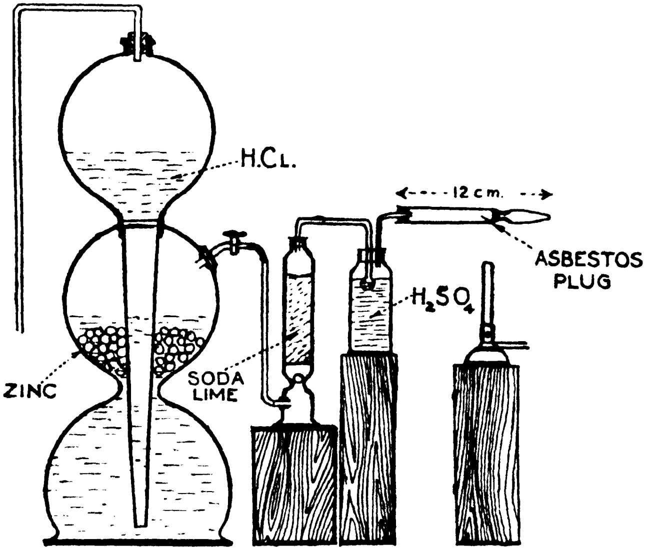

FIG. 1.

When a reducing sugar is to be estimated in the presence of cane sugar, a blank test should be made under the precise conditions given above and the correction applied, as there is usually a small reduction of Fehling by the cane sugar on heating in boiling water. Sugar solutions which have been inverted by mineral acids must be neutralised with sodium carbonate before adding the copper solution.

The amount of sugar equivalent to the quantity of copper or copper oxide reduced may be obtained from the following tables (Elsdon, *Analyst*, 1923, **48,** 436). Such quantity of the sugar solution should be taken that the precipitate of copper oxide weighs between 0·10 and 0·3 g.

For convenience in calculating the sugars in jams and similar substances, the reducing values of carbohydrates are often expressed by " K " which is termed the " cupric reducing power " and is defined as the copper oxide reduced by 100 parts of the substance calculated as dextrose ; thus a syrup which contained 45 per cent. of reducing sugars expressed as dextrose would have a K value of 45.

For many purposes, and particularly when a number of sugar estimations have to be made, a volumetric process based on Fehling's solution is preferable. The elegant method of Lane and Eynon has displaced most of the earlier ones, and has been officially adopted by several sugar associations. This process depends upon the use of a 1 per cent. aqueous solution of methylene blue as internal indicator ; under the appropriate conditions the methylene blue is decolorised by the sugar solution as soon as the Fehling solution has all been reduced. It is important that the sugar solution to be titrated should not contain appreciable quantities of lead or of calcium salts ; these if present may be removed by means of potassium oxalate, excess of which does not interfere with the determination. The quantity of sugar taken should be such that the 10 or 25 ml. of Fehling's reagent, which is made up as stated on p. 2, requires from 15 to 50 ml. of the sugar solution in the titration. The method is as follows : 10 or 25 ml. of Fehling's solution are pipetted into a hard glass flask of about 300 ml. capacity, to it are added from a burette 15 ml. or more of the sugar solution and the mixture is boiled on a wire gauze for 10 to 15 seconds, then further additions of the sugar solution are made, with a few seconds' boiling between each addition until the blue colour is nearly discharged. Then 3 or 4 drops of the methylene blue solution are added, and the addition of the sugar solution to the boiling mixture continued at intervals until the indicator is completely decolorised. It is usually necessary to make two titrations, because the boiling and titration must be completed in not less than two nor more than four minutes. So when the approximate titre has been found this volume, less about 0·5 ml., is run into the 10 or 25 ml. of Fehling's solution and the mixture

QUANTITIES OF COPPER OXIDE PRODUCED UNDER STANDARD CONDITIONS BY VARIOUS CARBOHYDRATES

Quantities expressed in Milligrams in all cases

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{12}H_{22}O_{11}.H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
100	89·9	79·9	—	—	—	59·2	56·2	72·5	—	—
101	90·8	80·7	—	—	—	59·8	56·8	73·2	—	—
102	91·7	81·5	—	—	—	60·4	57·3	74·0	—	—
103	92·6	82·3	—	—	—	61·0	57·9	74·7	—	—
104	93·5	83·1	—	—	—	61·6	58·5	75·4	—	—
105	94·4	83·9	—	—	—	62·2	59·1	76·2	—	—
106	95·3	84·7	—	—	—	62·8	59·6	76·9	—	—
107	96·2	85·5	—	—	—	63·4	60·2	77·6	—	—
108	97·1	86·3	—	—	—	64·0	60·8	78·3	—	—
109	98·0	87·1	—	—	—	64·6	61·3	79·0	44·9	42·6
110	98·9	87·9	—	—	—	65·2	61·9	79·8	45·3	43·0
111	99·8	88·7	—	—	—	65·8	62·5	80·5	45·7	43·4
112	100·7	89·5	—	—	—	66·4	63·0	81·3	46·1	43·7
113	101·6	90·3	—	—	—	67·0	63·6	82·0	46·5	44·1
114	102·5	91·1	—	—	—	67·6	64·2	82·7	46·9	44·5
115	103·4	91·9	—	—	49·8	68·2	64·8	83·5	47·3	44·9
116	104·3	92·7	—	—	50·2	68·8	65·3	84·2	47·7	45·2
117	105·2	93·5	—	—	50·6	69·4	65·9	84·9	48·0	45·6
118	106·1	94·3	—	—	50·9	70·0	66·5	85·7	48·4	46·0
119	107·0	95·1	—	—	51·3	70·6	67·0	86·4	48·8	46·3
120	107·9	95·9	46·5	41·8	51·7	71·2	67·6	87·2	49·2	46·7
121	108·8	96·7	46·9	42·2	52·1	71·8	68·2	87·9	49·6	47·1
122	109·7	97·5	47·3	42·5	52·5	72·4	68·7	88·7	50·0	47·4
123	110·6	98·3	47·7	42·9	52·8	73·0	69·3	89·4	50·4	47·8
124	111·5	99·1	48·1	43·2	53·2	73·6	69·9	90·2	50·8	48·2
125	112·4	99·9	48·5	43·6	53·6	74·2	70·5	91·1	51·2	48·6
126	113·3	100·7	48·8	44·0	54·0	74·8	71·0	91·9	51·5	49·0
127	114·2	101·5	49·2	44·3	54·3	75·4	71·6	92·8	51·9	49·3
128	115·1	102·3	49·6	44·7	54·7	76·0	72·2	93·6	52·3	49·7
129	116·0	103·1	50·0	45·0	55·1	76·6	72·7	94·5	52·7	50·0
130	116·9	103·9	50·4	45·4	55·5	77·2	73·3	95·3	53·1	50·4
131	117·8	104·7	50·8	45·7	55·9	77·8	73·9	95·9	53·5	50·8
132	118·7	105·5	51·2	46·1	56·3	78·4	74·4	96·6	53·9	51·2
133	119·6	106·3	51·5	46·4	56·7	79·0	75·0	97·2	54·2	51·5
134	120·5	107·1	51·9	46·8	57·1	79·6	75·6	97·9	54·6	51·9
135	121·4	107·9	52·3	47·1	57·5	80·2	76·2	98·5	55·0	52·2
136	122·3	108·7	52·7	47·4	57·9	80·8	76·7	99·2	55·4	52·6
137	123·2	109·5	53·1	47·8	58·3	81·4	77·3	99·8	55·8	53·0
138	124·1	110·3	53·5	48·1	58·7	82·0	77·9	100·5	56·2	53·3
139	125·0	111·1	53·8	48·5	59·1	82·6	78·4	101·3	56·6	53·7
140	125·9	111·9	54·2	48·8	59·5	83·2	79·0	102·0	57·0	54·2
141	126·8	112·6	54·6	49·1	59·9	83·8	79·6	102·7	57·4	54·6
142	127·7	113·4	54·9	49·5	60·3	84·4	80·2	103·4	57·8	55·0
143	128·6	114·2	55·3	49·8	60·8	85·0	80·7	104·2	58·2	55·3
144	129·5	115·0	55·7	50·2	61·2	85·6	81·3	104·9	58·6	55·7
145	130·4	115·8	56·0	50·5	61·7	86·2	81·9	105·6	59·0	56·1

QUANTITIES OF COPPER OXIDE—*contd.*

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{12}H_{22}O_{11}H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
146	131·3	116·6	56·4	50·8	62·1	86·9	82·4	106·3	59·4	56·5
147	132·2	117·4	56·8	51·2	62·6	87·5	83·0	107·0	59·8	56·9
148	133·1	118·2	57·2	51·5	63·0	88·1	83·6	107·8	60·2	57·2
149	134·0	119·0	57·6	51·9	63·5	88·7	84·2	108·5	60·6	57·6
150	134·9	119·8	58·0	52·2	63·9	89·3	84·8	109·2	61·0	58·0
151	135·8	120·6	58·3	52·5	64·4	90·0	85·4	109·9	61·4	58·4
152	136·7	121·4	58·7	52·9	64·8	90·6	86·0	110·7	61·8	58·8
153	137·6	122·2	59·1	53·2	65·2	91·2	86·5	111·4	62·2	59·1
154	138·5	123·0	59·5	53·6	65·6	91·8	87·1	112·2	62·7	59·5
155	139·4	123·8	59·9	53·9	66·0	92·4	87·7	113·0	63·1	59·9
156	140·3	124·6	60·3	54·2	66·5	93·0	88·3	113·8	63·5	60·3
157	141·2	125·4	60·7	54·6	66·9	93·6	88·8	114·5	63·9	60·7
158	142·1	126·2	61·1	54·9	67·3	94·2	89·4	115·3	64·3	61·0
159	143·0	127·0	61·5	55·3	67·7	94·8	90·0	116·0	64·7	61·4
160	143·9	127·8	61·8	55·6	68·1	95·4	90·6	116·8	65·1	61·8
161	144·8	128·6	62·2	56·0	68·5	96·0	91·2	117·5	65·5	62·2
162	145·7	129·4	62·6	56·3	68·9	96·6	91·7	118·2	65·9	62·6
163	146·6	130·2	63·0	56·7	69·4	97·2	92·3	119·0	66·3	63·0
164	147·5	131·0	63·4	57·0	69·8	97·8	92·9	119·7	66·7	63·4
165	148·4	131·8	63·8	57·4	70·2	98·4	93·5	120·4	67·1	63·8
166	149·3	132·6	64·2	57·7	70·6	99·0	94·0	121·2	67·6	64·1
167	150·2	133·4	64·5	58·1	71·1	99·6	94·6	121·9	68·0	64·5
168	151·1	134·2	64·9	58·4	71·5	100·2	95·2	122·7	68·4	64·9
169	152·0	135·0	65·3	58·7	71·9	100·8	95·8	123·4	68·8	65·3
170	152·9	135·8	65·7	59·1	72·4	101·4	96·3	124·2	69·2	65·7
171	153·8	136·6	66·1	59·5	72·8	102·0	96·9	124·9	69·6	66·1
172	154·7	137·4	66·5	59·8	73·3	102·6	97·4	125·6	70·0	66·5
173	155·6	138·2	66·9	60·2	73·7	103·2	98·0	126·4	70·4	66·9
174	156·5	139·0	67·3	60·5	74·1	103·8	98·6	127·1	70·8	67·3
175	157·4	139·8	67·6	60·9	74·6	104·4	99·2	127·9	71·2	67·7
176	158·3	140·6	68·0	61·2	75·0	105·0	99·7	128·6	71·7	68·1
177	159·2	141·4	68·4	61·6	75·4	105·6	100·3	129·4	72·1	68·5
178	160·1	142·2	68·8	61·9	75·8	106·2	100·9	130·1	72·5	68·9
179	161·0	143·0	69·2	62·3	76·3	106·8	101·5	130·8	72·9	69·3
180	161·9	143·8	69·6	62·6	76·7	107·4	102·0	131·5	73·4	69·7
181	162·8	144·6	70·0	63·0	77·1	108·0	102·6	132·2	73·8	70·1
182	163·7	145·4	70·4	63·3	77·5	108·6	103·2	123·0	74·2	70·5
183	164·6	146·2	70·8	63·7	77·9	109·2	103·8	133·7	74·6	70·9
184	165·5	147·0	71·2	64·0	78·3	109·8	104·3	134·4	75·0	71·3
185	166·4	147·8	71·6	64·4	78·8	110·4	104·9	135·1	75·4	71·6
186	167·3	148·6	72·0	64·8	79·2	111·0	105·5	135·8	75·8	72·0
187	168·2	149·4	72·4	65·1	79·6	111·6	106·1	136·6	76·2	72·4
188	169·1	150·2	72·8	65·5	80·0	112·2	106·7	137·3	76·6	72·8
189	170·0	151·0	73·2	65·8	80·4	112·8	107·3	138·1	77·0	73·2
190	170·9	151·8	73·6	66·2	80·9	113·5	107·9	138·8	77·5	73·6
191	171·8	152·6	74·0	66·6	81·3	114·1	108·5	139·6	77·9	74·0
192	172·7	153·4	74·4	66·9	81·8	114·7	109·1	140·3	78·3	74·4
193	173·6	154·2	74·8	67·3	82·2	115·4	109·6	141·1	78·7	74·7
194	174·5	155·0	75·2	67·6	82·7	116·0	110·2	141·8	79·1	75·1
195	175·4	155·8	75·6	68·0	83·1	116·6	110·8	142·6	79·5	75·5

QUANTITIES OF COPPER OXIDE—*contd.*

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{12}H_{22}O_{11}.H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
196	176·3	156·6	76·0	68·4	83·6	117·2	111·3	143·3	79·9	75·9
197	177·2	157·4	76·4	68·7	84·0	117·8	111·9	144·1	80·3	76·2
198	178·1	158·2	76·8	69·1	84·5	118·4	112·5	144·8	80·7	76·6
199	179·0	159·0	77·2	69·4	84·9	119·1	113·1	145·5	81·1	77·0
200	179·9	159·8	77·6	69·8	85·3	119·7	113·7	146·3	81·5	77·4
201	180·8	160·6	78·0	70·2	85·7	120·3	114·3	147·0	81·9	77·8
202	181·7	161·4	78·4	70·5	86·1	121·0	114·9	147·7	82·4	78·2
203	182·6	162·2	78·8	70·8	86·6	121·6	115·5	148·4	82·8	78·6
204	183·5	163·0	79·2	71·2	87·0	122·2	116·1	149·2	83·2	79·0
205	184·4	163·8	79·6	71·6	87·4	122·8	116·7	149·9	83·6	79·4
206	185·3	164·6	80·0	71·9	87·8	123·4	117·3	150·6	84·0	79·8
207	186·2	165·4	80·4	72·3	88·2	124·1	117·9	151·4	84·4	80·2
208	187·1	166·2	80·8	72·7	88·7	124·7	118·5	152·1	84·8	80·6
209	188·0	167·0	81·2	73·0	89·1	125·4	119·1	152·9	85·2	81·0
210	188·9	167·8	81·6	73·4	89·5	126·0	119·7	153·6	85·7	81·4
211	189·8	168·6	82·0	73·8	89·9	126·6	120·3	154·4	86·1	81·8
212	190·7	169·4	82·4	74·1	90·4	127·2	120·9	155·1	86·5	82·2
213	191·6	170·2	82·7	74·5	90·8	127·8	121·5	155·8	87·0	82·6
214	192·5	171·0	83·1	74·8	91·3	128·5	122·1	156·6	87·4	83·0
215	193·4	171·8	83·5	75·2	91·7	129·1	122·6	157·3	87·8	83·5
216	194·3	172·6	83·9	75·6	92·2	129·7	123·2	158·1	88·3	83·9
217	195·2	173·4	84·3	75·9	92·6	130·3	123·8	158·8	88·7	84·3
218	196·1	174·2	84·7	76·3	93·1	130·9	124·4	159·6	89·2	84·7
219	197·0	175·0	85·1	76·6	93·5	131·6	125·0	160·3	89·6	85·1
220	197·9	175·8	85·5	77·0	94·0	132·2	125·6	161·0	90·0	85·5
221	198·8	176·6	86·0	77·4	94·4	132·8	126·2	161·8	90·4	85·9
222	199·7	177·4	86·4	77·8	94·9	133·4	126·8	162·5	90·9	86·3
223	200·6	178·2	86·9	78·2	95·4	134·0	127·4	163·2	91·3	86·8
224	201·5	179·0	87·3	78·6	95·8	134·7	127·9	163·9	91·8	87·2
225	202·4	179·8	87·8	79·0	96·3	135·3	128·5	164·7	92·2	87·6
226	203·3	180·6	88·2	79·4	96·8	135·9	129·1	165·4	92·6	88·0
227	204·2	181·4	88·7	79·8	97·2	136·5	129·7	166·2	93·1	88·5
228	205·1	182·2	89·1	80·2	97·7	137·2	130·3	166·9	93·5	88·9
229	206·0	183·0	89·6	80·6	98·1	137·8	130·9	167·6	93·9	89·3
230	206·9	183·8	90·0	81·0	98·6	138·4	131·5	168·3	94·4	89·7
231	207·8	184·6	90·4	81·4	99·1	139·0	132·1	169·1	94·8	90·1
232	208·7	185·4	90·8	81·8	99·5	139·6	132·7	169·8	95·2	90·5
233	209·6	186·2	91·2	82·1	100·0	140·2	133·3	170·5	95·6	90·9
234	210·5	187·0	91·7	82·5	100·4	140·9	133·9	171·3	96·1	91·4
235	211·4	187·7	92·1	82·9	100·9	141·5	134·4	172·0	96·5	91·8
236	212·3	188·5	92·5	83·3	101·3	142·1	135·0	172·8	97·0	92·2
237	213·2	189·3	92·9	83·6	101·7	142·7	135·6	173·5	97·4	92·6
238	214·1	190·1	93·4	84·0	102·1	143·3	136·2	174·3	97·8	93·0
239	215·0	190·9	93·8	84·4	102·6	144·0	136·8	175·0	98·3	93·4
240	215·9	191·7	94·2	84·8	103·0	144·6	137·4	175·7	98·7	93·8
241	216·8	192·5	94·6	85·2	103·4	145·2	138·0	176·5	99·2	94·2
242	217·7	193·3	95·0	85·5	103·8	145·8	138·6	177·2	99·6	94·6
243	218·6	194·1	95·4	85·9	104·3	146·4	139·1	177·9	100·0	95·0
244	219·5	194·9	95·8	86·3	104·7	147·0	139·7	178·6	100·4	95·4
245	220·4	195·7	96·2	86·6	105·1	147·6	140·3	179·3	100·8	95·8

QUANTITIES OF COPPER OXIDE—*contd.*

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{12}H_{22}O_{11}.H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
246	221·3	196·5	96·6	87·0	105·5	148·2	140·8	180·1	101·2	96·2
247	222·2	197·3	97·0	87·3	106·0	148·9	141·4	180·8	101·7	96·6
248	223·1	198·1	97·5	87·7	106·4	149·5	142·0	181·6	102·1	97·0
249	224·0	198·9	97·9	88·1	106·9	150·1	142·6	182·3	102·5	97·4
250	224·9	199·7	98·3	88·5	107·3	150·7	143·2	183·1	102·9	97·8
251	225·8	200·5	98·7	88·9	107·8	151·4	143·8	183·8	103·3	98.2
252	226·7	201·3	99·1	89·3	108·2	152·0	144·4	184·5	103·8	98·6
253	227·6	202·1	99·5	89·6	108·7	152·6	145·0	185·3	104·2	99·0
254	228·5	202·9	99·9	90·0	109·1	153·2	145·6	186·1	104·6	99·4
255	229·4	203·7	100·3	90·4	109·6	153·8	146·2	186·8	105·0	99·7
256	230·3	204·5	100·8	90·7	110·0	154·4	146·8	187·6	105·4	100·1
257	231·2	205·3	101·2	91·1	110·5	155·0	147·4	188·3	105·8	100·5
258	232·1	206·1	101·6	91·5	110·9	155·7	148·0	189·1	106·3	100·9
259	233·0	206·9	102·1	91·9	111·4	156·4	148·6	189·8	106·7	101·3
260	233·9	207·7	102·5	92·3	111·8	157·0	149·2	190·5	107·1	101·7
261	234·8	208·5	102·9	92·7	112·3	157·6	149·8	191·3	107·5	102·1
262	235·7	209·3	103·4	93·0	112·7	158·2	150·3	192·0	108·0	102·5
263	236·6	210·1	103·8	93·4	113·2	158·8	150·9	192·7	108·4	102·9
264	237·5	210·9	104·2	93·8	113·6	159·4	151·5	193·4	108·8	103·3
265	238·4	211·7	104·7	94·1	114·1	160·0	152·1	194·2	109·2	103·8
266	239·3	212·5	105·1	94·5	114·5	160·6	152·6	194·9	109·7	104·2
267	240·2	213·3	105·5	94·8	115·0	161·2	153·2	195·6	110·1	104·6
268	241·1	214·1	105·9	95·2	115·5	161·8	153·8	196·4	110·5	105·0
269	242·0	214·9	106·3	95·6	115·9	162·4	154·4	197·1	111·0	105·4
270	242·9	215·7	106·7	96·0	116·4	163·0	154·9	197·9	111·4	105·8
271	243·8	216·5	107·1	96·4	116·9	163·7	155·5	198·6	111·8	106·2
272	244·7	217·3	107·6	96·7	117·3	164·4	156·1	199·4	112·3	106·6
273	245·6	218·1	108·0	97·1	117·8	165·0	156·6	200·1	112·7	107·0
274	246·5	218·9	108·4	97·4	118·2	165·6	157·2	200·8	113·1	107·4
275	247·4	219·7	108·8	97·8	118·7	166·2	157·8	201·6	113·6	107·9
276	248·3	220·5	109·2	98·2	119·2	166·8	158·4	202·3	114·0	108·3
277	249·2	221·3	109·6	98·5	119·6	167·4	158·9	203·0	114·4	108·7
278	250·1	222·1	110·0	98·9	120·1	168·0	159·5	203·7	114·8	109·1
279	251·0	222·9	110·4	99·3	120·6	168·6	160·1	204·5	115·2	109·5
280	251·9	223·7	110·8	99·7	121·0	169·2	160·7	205·2	115·7	109·9
281	252·8	224·5	111·2	100·1	121·5	169·9	161·3	205·9	116·1	110·3
282	253·7	225·3	111·7	100·5	121·9	170·5	161·9	206·7	116·5	110·7
283	254·6	226·1	112·1	100·8	122·4	171 1	162·5	207·4	117·0	111·2
284	255·5	226·9	112·5	101·2	122·8	171·7	163·1	208·2	117·5	111·6
285	256·3	227·7	112·9	101·6	123·2	172·3	163·7	208·9	117·9	112·0
286	257·2	228·5	113·4	101·9	123·7	173·0	164·3	209·7	118·3	112·5
287	258·1	229·3	113·8	102·3	124·1	173·6	164·9	210·4	118·8	112·9
288	259·0	230·1	114·2	102·7	124·6	174·2	165·5	211·1	119·2	113·3
289	259·9	230·9	114·6	103·1	125·1	174·8	166·1	211·9	119·6	113·7
290	260·8	231·7	115·0	103·5	125·6	175·5	166·7	212·6	120·1	114·1
291	261·7	232·5	115·4	103·9	126·0	176·1	167·4	213·3	120·5	114·5
292	262·6	233·3	115·9	104·3	126·5	176·7	168·0	214·1	121·0	115·0
293	263·5	234·1	116·3	104·7	126·9	177·6	168·7	214·8	121·4	115·4
294	264·4	234·9	116·7	105·1	127·4	178·4	169·3	215·5	121·9	115·8
295	265·3	235·7	117·2	105·5	127·8	179·0	170·0	216·3	122·3	116·3

QUANTITIES OF COPPER OXIDE—*contd.*

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{12}H_{22}O_{11}\cdot H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
296	266·2	236·5	117·6	105·9	128·3	179·8	170·6	217·0	122·8	116·7
297	267·1	237·3	118·1	106·3	128·7	180·4	171·3	217·8	123·2	117·1
298	268·0	238·1	118·5	106·7	129·2	181·0	171·9	218·5	123·7	117·6
299	268·9	238·9	118·9	107·1	129·6	181·7	172·6	219·3	124·1	118·0
300	269·8	239·7	119·4	107·5	130·1	182·3	173·2	220·0	124·6	118·4
301	270·7	240·5	119·8	107·9	130·6	183·0	173·8	220·7	125·0	118·8
302	271·6	241·3	120·2	108·3	131·0	183·6	174·5	221·5	125·5	119·2
303	272·5	242·1	120·6	108·6	131·5	184·3	175·1	222·2	125·9	119·7
304	273·4	242·9	121·1	109·0	132·0	185·0	175·7	222·9	126·4	120·1
305	274·3	243·7	121·5	109·4	132·4	185·6	176·4	223·6	126·8	120·5
306	275·2	244·5	121·9	109·7	132·9	186·2	177·0	224·4	127·3	121·0
307	276·1	245·3	122·2	110·1	133·3	186·9	177·6	225·1	127·7	121·4
308	277·0	246·1	122·8	110·5	133·8	187·6	178·3	225·8	128·2	121·8
309	277·9	246·9	123·2	110·9	134·3	188·2	178·9	226·6	128·6	122·2
310	278·8	247·7	123·7	111·3	134·7	188·9	179·5	227·3	129·1	122·6
311	279·7	248·5	124·1	111·7	135·2	189·6	180·1	228·1	129·6	123·0
312	280·6	249·3	124·5	112·1	135·7	190·2	180·7	228·8	130·0	123·5
313	281·5	250·1	125·0	112·5	136·2	190·8	181·3	229·6	130·5	123·9
314	282·4	250·9	125·5	112·9	136·7	191·4	181·9	230·3	130·9	124·4
315	283·3	251·7	125·9	113·4	137·2	192·2	182·6	231·0	131·4	124·8
316	284·2	252·5	126·4	113·8	137·6	192·8	183·2	231·8	131·9	125·2
317	285·1	253·3	126·8	114·2	138·1	193·4	183·8	232·5	132·3	125·7
318	286·0	254·1	127·3	114·6	138·6	194·1	184·4	233·2	132·8	126·1
319	286·9	254·9	127·7	115·0	139·0	194·8	185·0	234·0	133·2	126·6
320	287·8	255·7	128·2	115·4	139·5	195·4	185·6	234·7	133·7	127·0
321	288·7	256·5	128·6	115·8	140·0	196·0	186·2	235·4	134·2	127·4
322	289·6	257·3	129·1	116·2	140·5	196·7	186·9	236·2	134·6	127·9
323	290·5	258·1	129·5	116·6	141·0	197·4	187·5	236·9	135·1	128·3
324	291·4	258·9	130·0	117·0	141·5	198·0	188·1	287·7	135·5	128·7
325	292·3	259·6	130·4	117·3	142·0	198·7	188·7	238·4	136·0	129·2
326	293·2	260·4	130·9	117·7	142·5	199·3	189·4	239·2	136·4	129·6
327	294·1	261·2	131·3	118·1	143·0	200·0	190·0	239·9	136·9	130·0
328	295·0	262·0	131·8	118·5	143·5	200·7	190·7	240·6	137·3	130·4
329	295·9	262·8	132·2	118·9	144·0	201·4	191·3	241·4	137·8	130·9
330	296·8	263·6	132·6	119·3	144·5	202·0	191·9	242·1	138·2	131·3
331	297·7	264·4	133·1	119·7	145·0	302·7	192·6	242·8	138·7	131·7
332	298·6	265·2	133·5	120·1	145·5	203·4	193·2	243·5	139·1	132·1
333	299·5	266·0	133·9	120·5	146·0	204·0	193·9	244·3	139·6	132·5
334	300·4	266·8	134·4	120·9	146·5	204·7	194·5	245·0	140·0	133·0
335	301·3	267·6	134·8	121·4	147·0	205·4	195·1	245·7	140·4	133·5
336	302·2	268·4	135·3	121·8	147·5	206·0	195·8	246·5	140·9	133·9
337	303·1	269·2	135·7	122·2	148·0	206·7	196·4	247·2	141·4	134·4
338	304·0	270·0	136·2	122·6	148·5	207·4	197·1	248·0	141·9	134·8
339	304·9	270·8	136·7	123·0	149·0	208·0	197·7	248·7	142·3	135·3
340	305·8	271·6	137·1	123·4	149·5	208·7	198·3	249·5	142·8	135·7
341	306·7	272·4	137·6	123·8	150·0	209·4	198·9	250·2	143·2	136·2
342	307·6	273·2	138·0	124·2	150·4	210·0	199·5	250·9	143·7	136·6
343	308·5	274·0	138 5	124·6	150·9	210·7	200·2	351·7	144·2	137·1
344	309·4	274·8	139·0	125·0	151·4	211·3	200·8	252·4	144·7	137·5
345	310·3	275·6	139·4	125·5	151·9	212·0	201·4	253·2	145·1	138·0

QUANTITIES OF COPPER OXIDE—*contd.*

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{12}H_{22}O_{11}.H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
346	311·2	276·4	139·9	125·9	152·3	212·6	202·0	253·9	145·6	138·5
347	312·1	277·2	140·4	126·3	152·8	213·3	202·7	244·7	146·1	138·9
348	313·0	278·0	140·8	126·7	153·2	214·0	203·3	255·4	146·6	139·4
349	313·9	278·8	141·3	127·1	153·7	214·6	203·9	256·1	147·2	139·8
350	314·8	279·6	141·7	127·5	154·2	215·3	204·5	256·9	147·7	140·3
351	315·7	280·4	142·2	127·9	154·6	216·0	205·1	257·6	148·2	140·8
352	316·6	281·2	142·6	128·3	155·1	216·6	205·8	258·3	148·7	141·3
353	317·5	282·0	143·1	128·8	155·6	217·3	206·4	259·1	149·2	141·7
354	318·4	282·8	143·5	129·2	156·1	218·0	207·1	259·8	149·7	142·2
355	319·3	283·6	144·0	129·7	156·5	218·7	207·7	260·5	150·2	142·7
356	320·2	284·4	144·5	130·1	157·0	219·4	208·3	261·3	150·7	143·2
357	321·1	285·2	144·9	130·5	157·5	220·0	209·0	262·0	151·2	143·6
358	322·0	286·0	145·4	131·0	158·0	220·7	209·6	262·8	151·7	144·1
359	322·9	286·8	145·9	131·4	158·5	221·3	210·3	263·5	152·2	144·6
360	323·8	287·6	146·4	131·8	158·9	222·0	210·9	264·3	152·7	145·1
361	324·7	288·4	146·9	132·2	159·4	222·6	211·5	265·0	153·2	145·5
362	325·6	289·2	147·4	132·7	159·9	223·4	212·1	265·7	153·7	146·0
363	326·5	290·0	147·9	133·1	160·4	224·0	212·8	266·5	154·2	146·4
364	327·4	290·8	148·4	133·5	160·9	224·6	213·4	267·2	154·7	146·8
365	328·3	291·6	148·9	134·0	161·3	225·3	214·0	267·9	155·2	147·3
366	329·2	292·4	149·4	134·4	161·8	226·0	214·7	268·6	155·7	147·7
367	330·1	293·2	149·9	134·9	162·3	226·6	215·3	269·4	156·1	148·2
368	331·0	294·0	150·3	135·3	162·8	227·3	216·0	270·1	156·6	148·6
369	331·9	294·8	150·8	135·7	163·3	228·0	216·6	270·8	157·0	149·1
370	332·8	295·6	151·2	136·1	163·8	228·6	217·2	271·6	157·5	149·6
371	333·7	296·4	151·6	136·5	164·2	229·3	217·8	272·3	157·9	150·0
372	334·6	297·2	152·1	136·9	164·7	230·0	218·4	273·0	158·4	150·4
373	335·5	298·0	152·5	137·3	165·2	230·6	219·1	273·7	158·8	150·9
374	336·4	298·8	152·9	137·7	165·7	231·3	219·7	274·5	159·3	151·3
375	337·3	299·6	153·4	138·1	166·1	232·0	220·3	275·2	159·8	151·7
376	338·2	300·4	153·8	138·5	166·6	232·6	220·9	275·9	160·2	152·1
377	339·1	301·2	154·3	138·9	167·1	233·2	221·6	276·7	160·6	152·6
378	340·0	302·0	154·7	139·3	167·6	234·0	222·2	277·5	161·1	153·0
379	340·9	302·8	155·1	139·7	168·0	234·6	222·8	278·2	161·5	153·4
380	341·8	303·6	155·6	140·1	168·5	235·2	223·4	279·0	161·9	153·8
381	342·7	304·4	156·1	140·5	169·0	235·9	224·0	279·7	162·4	154·2
382	343·6	305·2	156·6	141·0	169·4	236·6	224·7	280·4	162·8	154·7
383	344·5	306·0	157·1	141·4	169·9	237·2	225·3	281·2	163·3	155·1
384	345·4	306·8	157·5	141·9	170·4	237·9	225·9	281·9	163·7	155·5
385	346·3	307·6	158·0	142·3	170·9	238·6	226·6	282·7	164·1	156·0
386	347·2	308·4	158·5	142·7	171·4	239·2	227·2	283·4	164·4	156·4
387	348·1	309·2	159·0	143·2	171·9	239·9	227·9	284·2	165·0	156·9
388	349·0	310·0	159·5	143·6	172·5	240·6	228·5	284·9	165·5	157·3
389	349·9	310·8	160·0	144·0	173·0	241·2	229·2	285·6	166·0	157·8
390	350·8	311·6	160·5	144·5	173·5	241·9	229·8	286·4	166·5	158·2
391	351·7	312·4	161·0	144·9	174·0	242·6	230·4	287·1	167·0	158·7
392	252·6	313·2	161·4	145·3	174·5	243·2	231·1	287·8	167·5	159·1
393	353·5	314·0	161·9	145·8	175·0	243·9	231·7	288·5	168·0	159·6
394	354·4	314·8	162·3	146·2	175·5	244·6	232·4	289·3	168·5	160·0
395	355·3	315·6	162·8	146·6	176·0	245·2	233·0	290·0	169·0	160·5

QUANTITIES OF COPPER OXIDE—*contd.*

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{22}H_{22}O_{11}.H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
396	356·2	316·4	163·3	147·1	176·5	245·9	233·6	290·7	169·5	160·9
397	357·1	317·2	163·7	147·5	177·0	246·6	234·3	291·5	170·0	161·4
398	358·0	318·0	164·2	147·9	177·4	247·2	234·9	292·2	170·5	161·8
399	358·9	318·8	164·7	148·3	177·9	247·8	235·6	293·0	170·9	162·3
400	359·8	319·6	165·2	148·7	178·4	248·6	236·2	293·7	171·4	162·8
401	260·7	320·4	165·7	149·1	178·9	249·2	236·8	294·5	171·9	163·2
402	361·6	321·2	166·2	149·6	179·4	249·8	237·4	295·2	172·4	163·7
403	362·5	322·0	166·7	150·0	179·9	250·5	237·9	295·9	172·8	164·1
404	363·4	322·8	167·2	150·5	180·4	251·1	238·5	296·7	173·3	164·5
405	364·3	323·6	167·6	150·9	180·9	251·7	239·1	297·4	173·8	165·0
406	365·2	324·4	168·1	151·3	181·4	252·3	239·6	298·2	174·3	165·4
407	366·1	325·2	168·6	151·8	181·9	252·9	240·2	298·9	174·7	165·9
408	367·0	326·0	169·1	152·2	182·4	253·5	240·8	299·7	175·2	166·3
409	367·9	326·8	169·6	152·7	182·8	254·1	241·4	300·4	175·7	166·8
410	368·8	327·6	170·1	153·1	183·3	254·7	242·0	301·1	176·2	167·2
411	369·7	328·4	170·6	153·5	183·8	255·4	242·6	301·8	176·7	167·7
412	370·6	329·2	171·1	154·0	184·3	256·0	243·2	202·6	177·2	168·2
413	371·5	330·0	171·6	154·4	184·8	256·6	243·8	303·3	177·7	168·7
414	372·4	330·8	172·1	154·9	185·3	257·2	244·4	304·0	178·2	169·2
415	373·3	331·5	172·5	155·3	185·8	257·8	244·9	304·7	178·7	169·6
416	374·2	332·3	173·0	155·7	186·3	258·4	245·5	—	179·2	170·1
417	375·1	333·1	173·5	156·2	186·8	259·0	246·1	—	179·7	170·6
418	376·0	333·9	174·0	156·6	187·3	259·6	246·7	—	180·2	171·1
419	376·9	334·7	174·5	157·1	187·8	260·2	247·3	—	180·7	171·6
420	377·8	335·5	175·0	157·5	188·3	260·9	247·9	—	181·2	172·1
421	378·7	336·3	175·5	157·9	188·8	261·5	248·5	—	181·7	172·6
422	379·6	337·1	176·0	158·4	189·3	262·1	249·1	—	182·2	173·1
423	380·5	337·9	176·5	158·8	189·8	262·8	249·7	—	182·7	173·5
424	381·4	338·7	177·0	159·3	190·3	263·4	250·2	—	183·2	174·0
425	382·3	339·5	177·4	159·7	190·8	264·9	250·8	—	183·7	174·5
426	383·2	340·3	177·9	160·1	191·3	264·5	251·4	—	184·2	175·0
427	384·1	341·1	178·4	160·6	191·8	265·4	252·0	—	184·7	175·4
428	385·0	341·9	178·9	161·0	192·3	266·0	252·6	—	185·2	175·9
429	385·9	342 7	179·4	161·5	192·7	266·6	253·2	—	185·7	176·4
430	386·8	343·5	179·9	161·9	193·2	267·2	253·8	—	186·2	176·9
431	387·7	344·3	180·4	162·4	193·7	267·9	254·4	—	186·7	177·4
432	388·6	345·1	180·9	162·8	194·2	268·6	255·1	—	187·2	177·9
433	389·5	345·9	181·4	163·3	194·7	269·2	255·7	—	187·7	178·3
434	390·4	346·7	181·9	163·7	195·2	269·9	256·3	—	188·2	178·8
435	391·3	347·5	182·5	164·2	195·7	270·5	257·0	—	188·7	179·3
436	392·2	348·3	183·0	164·7	196·3	271·2	257·6	—	189·2	179·8
437	393·1	349·1	183·5	165·1	196·8	271·8	258·2	—	189·7	180·2
438	394·0	349·9	184·0	165·6	197·3	272·4	258·9	—	190·2	180·7
439	394·9	350·7	184·5	166·0	197·8	273·1	259·5	—	190·7	181·2
440	395·8	351·5	185·0	166·5	198·4	273·8	260·1	—	191·2	181·7
441	396·7	253·3	185·5	167·0	198·9	274·4	260·7	—	191·7	182·2
442	397·6	353·1	186·0	167·4	199·5	275·1	261·4	—	192·2	182·7
443	398·5	353·9	186·5	167·9	200·0	275·8	262·0	—	192·7	183·2
444	399·4	354·7	187·0	168·3	200·5	276·4	262·6	—	193·2	183·6
445	400·3	355·5	187·5	168·8	201·1	277·0	263·3	—	193·7	184·1

QUANTITIES OF COPPER OXIDE—*contd.*

Cupric oxide.	Cuprous oxide.	Copper.	Dextrose.	Starch.	Lævulose.	Hydrated lactose. $C_{12}H_{22}O_{11}.H_2O$.	Anhydrous lactose.	Maltose.	Invert sugar.	Cane sugar.
446	401·2	356·3	188·0	169·2	201·6	277·7	263·9	—	194·2	184·6
447	402·1	357·1	188·5	169·7	202·1	278·4	264·5	—	194·7	185·0
448	403·0	357·9	189·0	170·1	202·6	279·0	265·2	—	195·2	185·5
449	403·9	358·7	189·5	170·6	203·1	279·7	265·8	—	195·7	186·0
450	404·8	359·5	190·0	171·0	203·6	280·4	266·4	—	196·3	186·5
451	405·7	360·3	190·5	171·5	204·2	281·0	267·0	—	196·8	187·0
452	406·6	361·1	191·0	171·9	204·7	281·7	267·7	—	197·3	187·5
453	407·5	361·9	191·5	172·4	205·2	282·4	268·3	—	197·9	188·0
454	408·4	362·7	192·0	172·8	205·7	283·0	269·0	—	198·4	188·5
455	409·3	363·5	192·5	173·3	206·2	283·7	269·6	—	198·9	189·0
456	410·2	364·3	193·0	173·7	206·8	284·4	270·2	—	199·5	189·5
457	411·1	365·1	193·5	174·2	207·3	285·1	270·9	—	200·0	190·0
458	412·0	365·9	194·0	174·6	207·8	285·8	271·5	—	200·5	190·5
459	412·9	366·7	194·5	175·1	208·3	286·5	272·2	—	201·1	191·0
460	413·8	367·5	195·0	175·5	208·8	287·2	272·8	—	201·6	191·5

boiled for two minutes, then the methylene blue is added and the titration completed in one further minute by adding the sugar solution, a few drops at a time, at ten-second intervals until the blue colour is discharged. The end point is quite sharp in daylight or with the use of a daylight bulb in artificial light ; the bulb should be quite near the boiling flask and be shaded from the eyes. A burette with a rubber pinch cock is better than one with a glass tap, and it is convenient to have it suspended, but not clamped, above the titration flask. The proportions of the various sugars, equivalent to the 10 or 25 ml. of Fehling's solution, are shown in the tables on pp. 25–29. These give a wide range of sugar concentrations to which the method can be applied. Lower concentrations can be brought up by the addition of small quantities of the appropriate sugar.

Another useful method of the determination of dextrose (and other sugars) is based upon its oxidation by iodine to gluconic acid, as described by Willstätter and Schudel (*Ber.*, 1918, **51,** 781) : and investigated by Hinton and Macara (*Analyst*, 1924, **49,** 2) :

$$C_6H_{12}O_6 + I_2 + 3NaOH \longrightarrow HO.CH_2(CHOH)_4CO_2Na + 2NaI + 2H_2O.$$

The reaction is specific for aldoses and, as ketoses are not affected, may be applied in the presence of sucrose or lævulose.

To a quantity of sugar solution which should contain about 0·05 to 0·08 g. of dextrose is added 20 ml. of 0·1 N iodine solution (*i.e.*, a considerable excess) and 5 ml. of 0·5 N sodium hydroxide solution. The mixture is allowed to stand in a dark place for ten minutes, then acidified with 5 ml. of 2 N sulphuric acid and the excess of iodine titrated, without delay, with 0·1 N sodium thiosulphate solution. A blank test is put on at the same time and the difference between the two titrations is calculated as dextrose ; 1 ml. 0·1 N iodine = 0·00901 g. of dextrose. The method is applicable to the estimation of glucose in honey. Lactose can be similarly estimated in the presence of

MALTOSE TABLE

Ml. of sugar solution required.	For 10 ml. of Fehling's solution.				For 25 ml. of Fehling's solution			
	Hydrated maltose $C_{12}H_{22}O_{11}$ H_2O.		Anhydrous maltose $C_{12}H_{22}O_{11}$		Hydrated maltose $C_{12}H_{22}O_{11}.H_2O$.		Anhydrous maltose $C_{12}H_{22}O_{11}$.	
	Factor.*	Mg. per 100 ml.	Factor.*	Mg. per 100 ml.	Factor.†	Mg. per 100 ml.	Factor.†	Mg. per 100 ml.
15	**81·3**	**542**	**77·2**	**515**	**208·2**	**1388**	**197·8**	**1319**
16	81·2	507	77·1	482	207·8	1298	197·4	1233
17	81·1	477	77·0	453	207·4	1220	197·0	1159
18	81·0	450	77·0	427	207·1	1151	196·7	1093
19	80·9	426	76·9	405	206·8	1088	196·5	1034
20	**80·8**	**404·0**	**76·8**	**383·8**	**206·5**	**1032·3**	**196·2**	**980·7**
21	80·7	384·3	76·7	365·1	206·1	981·6	195·8	932·5
22	80·6	366·4	76·6	348·1	205·8	935·5	195·5	888·7
23	80·5	350·0	76·5	332·5	205·4	893·2	195·1	848·5
24	80·4	335·0	76·4	318·3	205·1	854·5	194·8	811·8
25	**80·4**	**321·5**	**76·4**	**305·4**	**204·8**	**819·0**	**194·5**	**778·1**
26	80·3	308·8	76·3	293·4	204·4	786·3	194·2	747·0
27	80·2	297·0	76·2	282·2	204·1	756·0	193·9	718·2
28	80·1	286·1	76·1	271·8	203·8	727·9	193·6	691·5
29	80·0	276·0	76·0	262·2	203·5	701·7	193·3	666·6
30	**80·0**	**266·6**	**76·0**	**253·3**	**203·2**	**677·3**	**193·0**	**643·4**
31	79·9	257·8	75·9	244·9	202·9	654·3	192·8	621·6
32	79·9	249·7	75·9	237·2	202·6	633·1	192·5	601·4
33	79·8	241·9	75·8	229·8	202·3	613·0	192·2	582·4
34	79·8	234·6	75·8	222·9	202·0	594·3	191·9	564·6
35	**79·7**	**227·6**	**75·7**	**216·2**	**201·8**	**576·5**	**191·7**	**547·7**
36	79·6	221·1	75·6	210·0	201·5	559·7	191·4	531·7
37	79·6	215·0	75·6	204·3	201·2	543·9	191·2	516·7
38	79·5	209·2	75·5	198·7	201·0	528·9	191·0	502·5
39	79·5	203·8	75·5	193·6	200·8	514·7	190·8	489·0
40	**79·4**	**198·5**	**75·4**	**188·6**	**200·5**	**501·3**	**190·5**	**476·2**
41	79·4	193·7	75·4	184·3	200·3	488·5	190·3	464·1
42	79·3	188·8	75·3	179·4	200·1	476·3	190·1	452·5
43	79·3	184·3	75·3	175·1	199·8	464·7	189·8	441·5
44	79·2	180·0	75·2	171·0	199·6	453·6	189·6	430·9
45	**79·2**	**175·9**	**75·2**	**167·1**	**199·4**	**443·0**	**189·4**	**420·9**
46	79·1	172·0	75·1	163·4	199·2	433·1	189·2	411·4
47	79·1	168·3	75·1	159·9	199·0	423·6	189·0	402·4
48	79·1	164·7	75·1	156·5	198·9	414·4	188·9	393·7
49	79·0	161·2	75·0	153·1	198·7	405·5	188·8	385·2
50	**79·0**	**158·0**	**75·0**	**150·1**	**198·6**	**397·2**	**188·7**	**377·3**

* Mg. of maltose corresponding to 10 ml. of Fehling's solution
† ,, ,, ,, ,, 25 ml. ,, ,, ,,

sucrose, 1 ml. 0·1 0·1 N iodine = 0·0180 g. lactose hydrate. Maltose may also be titrated, 1 ml. 0·1 N = 0·017 g. maltose.

Sucrose can be estimated by iodine titration before and after inversion (at 60° C. for ten minutes). The factor for the 0·1 N iodine solution is then 1 ml. = 0·017 g. sucrose.

LACTOSE TABLE

Ml. of sugar solution required.	For 10 ml. of Fehling's solution.				For 25 ml. of Fehling's solution.			
	Hydrated lactose. $C_{12}H_{22}O_{11}, H_2O$,		Anhydrous lactose. $C_{12}H_{22}O_{11}$.		Hydrated lactose. $C_{12}H_{22}O_{11}, H_2O$.		Anhydrous lactose. $C_{12}H_{22}O_{11}$.	
	Factor.*	Mg. per 100 ml.	Factor.*	Mg. per 100 ml.	Factor.†	Mg. per 100 ml.	Factor.†	Mg. per 100 ml.
15	**68·3**	**465**	**64·9**	**432**	**172·5**	**1150**	**163·9**	**1093**
16	68.2	426	64·8	405	172·1	1076	163·5	1022
17	68·2	401	64·8	381	171·7	1010	163·1	960
18	68·1	378	64·7	359	171·4	952	162·8	906
19	68·1	358	64·7	340	171·1	900	162·5	855
20	**68·0**	**340·0**	**64·6**	**323·0**	**170·9**	**854·5**	**162·3**	**811·8**
21	68·0	323·8	64·6	307·6	170·6	812·4	162·0	772·3
22	68·0	309·1	64·6	293·6	170·4	774·5	161·8	735·8
23	67·9	295·4	64·5	280·6	170·2	740·0	161·6	703·0
24	67·9	282·9	64·5	268·8	170·0	708·5	161·5	673·1
25	**67·9**	**271·6**	**64·5**	**258·0**	**169·9**	**679·5**	**161·4**	**645·5**
26	67·9	261·0	64·5	248·0	169·7	652·7	161·2	620·1
27	67·8	251·1	64·4	238·5	169·5	627·9	161·0	596·5
28	67·8	242·1	64·4	230·0	169·3	604·8	160·8	574·6
29	67·8	233·8	64·4	222·2	169·2	583·3	160·7	554·1
30	**67·8**	**226·0**	**64·4**	**214·7**	**169·0**	**563·3**	**160·6**	**535·1**
31	67·8	218·7	64·4	207·8	168·9	544·8	160·5	517·6
32	67·8	211·9	64·4	201·3	168·8	527·4	160·4	501·0
33	67·8	205·6	64·4	195·3	168·6	511·0	160·2	485·5
34	67·9	199·7	64·5	189·7	168·5	495·6	160·1	470·8
35	**67·9**	**194·0**	**64·5**	**184·3**	**168·4**	**481·1**	**160·0**	**457·0**
36	67·9	188·6	64·5	179·2	168·2	467·3	159·8	443·9
37	67·9	183·5	64·5	174·3	168·1	454·3	159·7	431·6
38	67·9	178·7	64·5	169·8	168·0	442·1	159·6	420·0
39	67·9	174·1	64·5	165·4	167·9	430·5	159·5	409·0
40	**67·9**	**169·7**	**64·5**	**161·2**	**167·8**	**419·5**	**159·4**	**398·5**
41	68·0	165·9	64·6	157·6	167·7	409·0	159·3	388·6
42	68·0	161·9	64·6	153·8	167·6	399·1	159·2	379·1
43	68·0	158·1	64·6	150·2	167·6	389·7	159·2	370·2
44	68·0	154·7	64·6	147·0	167·5	380·7	159·1	361·7
45	**68·1**	**151·3**	**64·7**	**143·7**	**167·4**	**372·1**	**159·0**	**353·5**
46	68·1	148·0	64·7	140·6	167·4	363·9	159·0	345·7
47	68·2	145·1	64·8	137·8	167·3	356·0	158·9	338·2
48	68·2	142·1	64·8	135·0	167·2	348·3	158·8	330·9
49	68·2	139·2	64·8	132·2	167·2	341·0	158·8	324·0
50	**68·3**	**136·6**	**64·9**	**129·8**	**167·1**	**334·2**	**158·7**	**317·5**

* Mg. of lactose corresponding to 10 ml. of Fehling's solution.
† ,, ,, ,, ,, 25 ml. ,, ,,

Syrup, Treacle and Honey

By the terms "syrup," "golden syrup," and "treacle," are understood products obtained entirely from beet or cane sugar, and therefore composed of sucrose and invert sugar with only small quantities of other sugars. A common adulterant is glucose or corn syrup, which is sometimes sold under fancy names.

Treacle or molasses varies much in composition, not only according to its quality, but depending to some extent on the variety of sugar, cane or beet, and the district from which it has been obtained ; it exists as an uncrystallisable syrup containing a large percentage of sucrose and invert sugar together with traces or small quantities of proteins, other carbohydrates, such as dextrin or raffinose, and organic acids.

INVERT SUGAR TABLE FOR 10 ml. FEHLING SOLUTION

Ml. of sugar solution required	Solutions containing besides invert sugar :—									
	No sucrose		1 g. sucrose per 100 ml.		5 g. sucrose per 100 ml.		10g. sucrose per 100 ml.		25g. sucrose per 100 ml.	
	Invert sugar factor*	Mg. invert sugar per 100 ml.	Invert sugar factor*	Mg. invert sugar per 100 ml.	Invert sugar factor*	Mg. invert sugar per 100 ml.	Invert sugar factor*	Mg. invert sugar per 100 ml.	Invert sugar factor*	Mg. invert sugar per 100 ml
15	**50·5**	**336**	**49·9**	**333**	**47·6**	**317**	**46·1**	**307**	**43·4**	**289**
16	50·6	316	50·0	312	47·6	297	46·1	288	43·4	271
17	50·7	298	50·1	295	47·6	280	46·1	271	43·4	255
18	50·8	282	50·1	278	47·6	264	46·1	256	43·3	240
19	50·8	267	50·2	264	47·6	250	46·1	243	43·3	227
20	**50·9**	**254·5**	**50·2**	**251·0**	**47·6**	**238·0**	**46·1**	**230·5**	**43·2**	**216**
21	51·0	242·9	50·2	239·0	47·6	226·7	46·1	219·5	43·2	206
22	51·0	231·8	50·3	228·2	47·6	216·4	46·1	209·5	43·1	196
23	51·1	222·2	50·3	218·7	47·6	207·0	46·1	200·4	43·0	187
24	51·2	213·3	50·3	209·8	47·6	198·3	46·1	192·1	42·9	179
25	**51·2**	**204·8**	**50·4**	**201·6**	**47·6**	**190·4**	**46·0**	**184·0**	**42·8**	**171**
26	51·3	197·4	50·4	193·8	47·6	183·1	46·0	176·9	42·8	164
27	51·4	190·4	50·4	186·7	47·6	176·4	46·0	170·4	42·7	158
28	51·4	183·7	50·5	180·2	47·7	170·3	46·0	164·3	42·7	152
29	51·5	177·6	50·5	174·1	47·7	164·5	46·0	158·6	42·6	147
30	**51·5**	**171·7**	**50·5**	**168·3**	**47·7**	**159·0**	**46·0**	**153·3**	**42·5**	**142**
31	51·6	166·3	50·6	163·1	47·7	153·9	45·9	148·1	42·5	137
32	51·6	161·2	50·6	158·1	47·7	149·1	45·9	143·4	42·4	132
33	51·7	156·6	50·6	153·3	47·7	144·5	45·9	139·1	42·3	128
34	51 7	152·2	50·6	148·9	47·7	140·3	45·8	134·9	42·2	124
35	**51·8**	**147·9**	**50·7**	**144·7**	**47·7**	**136·3**	**45·8**	**130·9**	**42·2**	**121**
36	51·8	143·9	50·7	140·7	47·7	132·5	45·8	127·1	42·1	117
37	51·9	140·2	50·7	137·0	47·7	128·9	45·7	123·5	42·0	114
38	51·9	136·6	50·7	133·5	47·7	125·5	45·7	120·3	42·0	111
39	52·0	133·3	50·8	130·2	47·7	122·3	45·7	117·1	41·9	107
40	**52·0**	**130·1**	**50·8**	**127·0**	**47·7**	**119·2**	**45·6**	**114·1**	**41·8**	**104**
41	52·1	127·1	50·8	123·9	47·7	116·3	45·6	111·2	41·8	102
42	52·1	124·2	50·8	121·0	47·7	113·5	45·6	108·5	41·7	99
43	52·2	121·4	50·8	118·2	47·7	110·9	45·5	105·8	41·6	97
44	52·2	118·7	50·9	115·6	47·7	108·4	45·5	103·4	41·5	94
45	**52·3**	**116·1**	**50·9**	**113·1**	**47·7**	**106·0**	**45·4**	**101·0**	**41·4**	**92**
46	52·3	113·7	50·9	110·6	47·7	103·7	45·4	98·7	41·4	90
47	52·4	111·4	50·9	108·2	47·7	101·5	45·3	96·4	41·3	88
48	52·4	109·2	50·9	106·0	47·7	99·4	45·3	94·3	41·2	86
49	52·5	107·1	51·0	104·0	47·7	97·4	45·2	92·3	41·1	84
50	**52·5**	**105 1**	**51·0**	**102·0**	**47·7**	**95·4**	**45·2**	**90·4**	**41·0**	**82**

* Mg. of invert sugar corresponding to 10 ml. of Fehling's solution.

Ling (*J. Inst. Brewing*, 1914, **20**, 185) has shown that it is possible to differentiate between some cane and beet products by the determination of protein (N × 6·25) ; cane sugar and molasses contain less protein than the corresponding beet products, as is shown by the following figures for raw products :—

	Sucrose per cent.	Protein per cent.
Cane sugar . .	85 to 96	0·40 to 0·85
Beet sugar . .	91 ,, 95	0·90 ,, 2·20
Cane molasses .	55 ,, 58	3·00 ,, 4·50
Beet molasses .	48 ,, 49·5	9·50 ,, 10·5

INVERT SUGAR TABLE. 25 ml.

Ml. of sugar solution required	Solutions containing besides invert sugar :—			
	No sucrose.		1 g. sucrose per 100 ml.	
	Invert sugar factor.*	Mg. invert sugar per 100 ml.	Invert sugar factor.*	Mg. invert sugar per 100 ml.
15	123·6	824	122·6	817
16	123·6	772	122·7	767
17	123·6	727	122·7	721
18	123·7	687	122·7	682
19	123·7	651	122·8	646
20	**123·8**	**619·0**	**122·8**	**614·0**
21	123·8	589·5	122·8	584·8
22	123·9	563·2	122·9	558·2
23	123·9	538·7	122·9	534·0
24	124·0	516·7	122·9	512·1
25	**124·0**	**496·0**	**123·0**	**492·0**
26	124·1	477·3	123·0	473·1
27	124·1	459·7	123·0	455·6
28	124·2	443·6	123·1	439·6
29	124·2	428·3	123·1	424·4
30	**124·3**	**414·3**	**123·1**	**410·4**
31	124·3	401·0	123·2	397·4
32	124·4	388·7	123·2	385·0
33	124·4	377·0	123·2	373·4
34	124·5	366·2	123·3	362·6
35	**124·5**	**355·8**	**123·3**	**352·3**
36	124·6	346·1	123·3	342·5
37	124·6	336·8	123·4	333·5
38	124·7	328·1	123·4	324·7
39	124·7	319·7	123·4	316·4
40	**124·8**	**311·9**	**123·4**	**308·6**
41	124·8	304·4	123·5	301·2
42	124·9	297·3	123·5	294·1
43	124·9	290·5	123·5	287·3
44	125·0	284·1	123·6	280·9
45	**125·0**	**277·9**	**123·6**	**274·7**
46	125·1	272·0	123·6	268·7
47	125·1	266·3	123·7	263·1
48	125·2	260·8	123·7	257·7
49	125·2	255·5	123·7	252·5
50	**125·3**	**250·6**	**123·8**	**247·6**

* Mg. of invert sugar corresponding to 25 ml. of Fehling's solution.

DEXTROSE TABLE

Ml. of sugar solution required	For 10 ml. of Fehling's solution.		For 25 ml. of Fehling's solution.	
	Dextrose factor.*	Mg. dextrose per 100 ml.	Dextrose factor.†	Mg. dextrose per 100 ml.
15	49·1	327	120·2	801
16	49·2	307	120·2	751
17	49·3	289	120·2	707
18	49·3	274	120·2	668
19	49·4	260	120·3	633
20	**49·5**	**247·4**	**120·3**	**601·5**
21	49·5	235·8	120·3	572·9
22	49·6	225·5	120·4	547·3
23	49·7	216·1	120·4	523·6
24	49·8	207·4	120·5	501·9
25	**49·8**	**199·3**	**120·5**	**482·0**
26	49·9	191·8	120·6	463·7
27	49·9	184·9	120·6	446·8
28	50·0	178·5	120·7	431·1
29	50·0	172·5	120·7	416·4
30	**50·1**	**167·0**	**120·8**	**402·7**
31	50·2	161·8	120·8	389·7
32	50·2	156·9	120·8	377·6
33	50·3	152·4	120·9	366·3
34	50·3	148·0	120·9	355·6
35	**50·4**	**143·9**	**121·0**	**345·6**
36	50·4	140·0	121·0	336·3
37	50·5	136·4	121·1	327·4
38	50·5	132·9	121·2	318·8
39	50·6	129·6	121·2	310·7
40	**50·6**	**126·5**	**121·2**	**303·1**
41	50·7	123·6	121·3	295·9
42	50·7	120·8	121·4	289·0
43	50·8	118·1	121·4	282·4
44	50·8	115·5	121·5	276·1
45	**50·9**	**113·0**	**121·5**	**270·1**
46	50·9	110·6	121·6	264·3
47	51·0	108·4	121·6	258·8
48	51·0	106·2	121·7	253·5
49	51·0	104·1	121·7	248·4
50	**51·1**	**102·2**	**121·8**	**243·6**

* Mg. of dextrose corresponding to 10 ml. of Fehling's solution.
† ,, ,, ,, ,, 25 ml. ,, ,,

Contrary to the statements in certain published papers, no reliable diagnosis of the origin of a sugar can be made from the ash or its composition ; so much depends upon the particular variety or the country of origin of the cane or beet.

Any considerable quantity of dextrose in syrups or molasses indicates the addition of corn syrup.

LÆVULOSE TABLE

Ml. of sugar solution required	For 10 ml. of Fehling's solution		For 25 ml. of Fehling's solution.	
	Laevulose factor.*	Mg. laevulose per 100 ml.	Laevulose factor.†	Mg. laevulose per 100 ml.
15	**52·2**	**348**	**127·4**	**849**
16	52·3	327	127·4	796
17	52·3	308	127·5	750
18	52·4	291	127·5	708
19	52·5	276	127·6	672
20	**52·5**	**262·5**	**127·6**	**638·0**
21	52·6	250·6	127·7	608·1
22	52·7	239·6	127·7	580·6
23	52·7	229·1	127·8	555·5
24	52·8	220·0	127·8	532·5
25	**52·8**	**211·3**	**127·9**	**511·5**
26	52·9	203·3	127·9	491·9
27	52·9	196·0	128·0	474·0
28	53·0	189·3	128·0	457·2
29	53·1	183·1	128·1	441·6
30	**53·2**	**177·2**	**128·1**	**427·0**
31	53·2	171·7	128·1	413·3
32	53·3	166·5	128·2	400·5
33	53·3	161·6	128·2	388·5
34	53·4	157·0	128·3	377·3
35	**53·4**	**152·6**	**128·3**	**366·7**
36	53·5	148·6	128·4	356·6
37	53·5	144·7	128·4	347·0
38	53·6	140·9	128·5	338·1
39	53·6	137·3	128·5	329·6
40	**53·6**	**134·0**	**128·6**	**321·5**
41	53·7	130·9	128·6	313·7
42	53·7	127·9	128·6	306·2
43	53·8	125·1	128·7	299·2
44	53·8	122·4	128·7	292·5
45	**53·9**	**119·8**	**128·8**	**286·2**
46	53·9	117·2	128·8	280·0
47	53·9	114·7	128·9	274·2
48	54·0	112·4	128·9	268·6
49	54·0	110·2	129·0	263·2
50	**54·0**	**108·0**	**129·0**	**258·0**

* Mg. of laevulose corresponding to 10 ml. of Fehling's solution
† ,, ,, ,, 25 ml. ,, ,,

The following tables show the percentage composition of some of these products :—

GOLDEN SYRUP

	1. Per cent.	2. Per cent.	3. Per cent.	4. Per cent.
Sucrose . . .	31·7	26·1	33·9	34·5
Invert sugar . .	45·3	47·5	39·6	45·6
Ash . . .	1·63	3·5	1·2	2·2
Glucose . . .	None.	—	0·2	
Water and organic matter (not sugar) . . .	21·37	23·0	25·1	17·7
	98·37 1·63	96·5	98·8	

MOLASSES

	Cane.			Beet.	
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Sucrose	36·5	31·7	34·1	48·8	49·5
Reducing sugars .	23·2	21·0	18·0	5·7	6·5
Organic matter (not sugar)	12·3	17·7	15·6	16·8	14·8
Ash	8·0	10·4	9·0	8·5	8·2
Water	20·0	19·2	23·3	20·2	21·0

(PURE) GLUCOSE SYRUP

Dextrose . .	11·7	17·2	38·
Maltose . . .	22·9	16·4	
Dextrin . . .	44·8	47·7	42·0
Water . . .	21·3	19·3	19·0
Total reducing sugar as maltose . .	55·2	61·6	63·2
Ash and protein .	Trace.	Trace.	0·5

The estimations of water and ash in syrups present no difficulty. For the former it is convenient to weigh about 1 g. of the syrup into a tared flat-bottomed dish containing about 5 g. of ignited silver sand, then add and mix with it a few ml. of warm water and dry first on the water bath, then in the oven at 105°. When an appreciable amount of fructose is present in the syrup, drying at 105° introduces a small error, since this sugar begins to decompose at 75° in the presence of water. For accurate results in the presence of fructose, it is necessary to dry the syrup on sand at 70° *in vacuo*. The ash should be determined by ignition over an Argand burner, care being taken that there is no loss due to draughts.

The approximate amount of water may be found by taking the specific gravity of a solution of 20 g. in 100 ml. and dividing this figure minus 1, by 0·00386 ; this gives the total solid matter, and the difference is taken as water per cent.

For the estimation of the sugars present, 26·05 g. are dissolved in water, defecated, diluted to 100 ml., and the rotation observed before and after inversion as described on p. 12.

At this concentration genuine syrups generally show a direct rotation of about + 23° V. in a 200-mm. tube, whereas those adulterated with glucose give a much higher reading, and on inversion the rotation remains positive, or is only slightly negative.

The following examples make this plain :—

Genuine Syrup

Direct rotation (200 mm. tube)	+ 22·4
Invert	− 22·3
Corresponding to :—	
Cane sugar	33·7 per cent.
Invert sugar	37·6 ,,

Adulterated Syrup

Direct rotation	+ 103·2
Invert	+ 76·0
" K " value	31·2
Corresponding to :—	
Cane sugar	20·5 per cent.
Invert sugar	10·1 ,,
Glucose syrup	50·4 ,,

In the case of genuine syrups the proportion of cane sugar is estimated by means of the Clerget-Herzfeld formula, and the invert sugar from the equation given on p. 12, but in one containing glucose the latter formula is no longer applicable. In such case it is necessary also to determine the cupric reducing power (" K " value). The calculation of the proportion of glucose syrup is then based on the assumption, which is approximately true, that glucose syrup is a chemical entity having a mean K value of 42 and rotation + 170° V. at 20° C. The errors so introduced are not large. The necessary equations for the calculation are :—

$$0{\cdot}42\,G + 1{\cdot}0\,I = K \quad . \quad . \quad . \quad . \quad . \quad . \quad (\text{i.})$$

$$1{\cdot}70\,G - 0{\cdot}326\,I = D - S \quad . \quad . \quad . \quad . \quad . \quad (\text{ii.})$$

where G, I and S are the percentages of glucose syrup, invert sugar and sucrose respectively, D is the direct polarisation in Ventzke degrees. S is first calculated from the Clerget formula, then G and I are readily found by solving the two simultaneous equations. Similar results are given by a formula due to Dyer (*Analyst*, 1900, **25,** 95) :—

$$\text{Glucose syrup percentage} = \frac{0{\cdot}31\,K + (D - S)}{1{\cdot}83}.$$

As is shown by the table on p. 30, commercial glucose is a mixture containing, besides water and ash, dextrose, maltose, dextrin, and a small proportion of protein matter. While it is usual and convenient to consider glucose syrup as a distinct substance when estimating its amount in other syrups or in jams, marmalade or honey, it is sometimes desired to examine glucose syrup itself and estimate the various carbohydrates present. In the older text-books a substance named "gallisin" was commonly described as a non-fermentable sugar, and many analyses of glucose syrups state the percentage of this substance. It is now known that "gallisin" consists mainly of isomaltose, and is formed by the action of concentrated hydrochloric acid or emulsin on dextrose. For the analysis of commercial glucose it is therefore necessary only to estimate dextrose, maltose and dextrin, but no really satisfactory method has yet been evolved; that of Wiley is probably the best. Estimate the total organic solids, *i.e.*, total solids less ash, denoted by O, the cupric reducing power K, and the specific rotation S. Then, assuming that $[\alpha]_D$ for glucose, maltose and dextrin are respectively $+ 52{\cdot}7$, $+ 139{\cdot}2$ and $+ 198$, it may be calculated that —

$$\begin{array}{lll}
\text{Maltose} & + \text{ dextrose} + \text{dextrin} = O\ . \quad . & \text{(i.)} \\
\text{Maltose} & = 3{\cdot}195\,S + 4{\cdot}642\,K - 6{\cdot}326\,O & \text{(ii.)} \\
\text{Dextrose} & = K - 0{\cdot}62\ \%\ \text{maltose}\ . \quad . \quad . & \text{(iii.)} \\
\text{Dextrin} & = O - (\text{dextrose and maltose})\ . & \text{(iv.)}
\end{array}$$

If the normal weight of the sample is taken and the polarimeter reading is made in Ventzke degrees, the equation (ii.) becomes

$$\text{Maltose} = 2{\cdot}125\,D + 4{\cdot}642\,K - 6{\cdot}326\,O \quad \text{(v.)}$$

where D is the direct reading.

Glucose is liable to be contaminated with arsenic, hence all samples should be examined for this impurity, which may be estimated directly, without destruction of organic matter, by either the Marsh or Gutzeit process (see p. 67).

Sugars and syrups are permitted to contain small quantities of sulphur dioxide (for quantities see p. 303), so it is usually necessary to estimate the amount. The quickest method is by steam distillation into excess of 0·05 N iodine; this process is reliable and applicable to most foodstuffs provided that sulphides are not present. About 200 ml. of water are placed in a round-bottomed flask, as shown in Fig. 2, and well boiled; then 25 or 50 g. of the material are added through the funnel and followed by 20 ml. of hydrochloric acid. The burner should then be turned up and boiling effected as quickly as possible. The distillate passes down an efficient condenser and is collected, by aid of the adapter illustrated, in water containing a drop or two of 0·05 N iodine and a few drops of starch solution. The iodine solution is then run in from a burette as the distillation proceeds, so that the blue colour just remains. Generally all the sulphur dioxide will be evolved within five minutes, but the end point is taken when the blue colour, due to 0·1 ml. of 0·05 N iodine, persists for a minute. Each 1 ml. of 0·05 N iodine = 0·0016 g. SO_2. In order to confirm that the distillate does contain sulphur dioxide, the solution remaining after titration may be acidified with hydrochloric acid, boiled to a lower bulk, and the sulphuric acid precipitated by addition of barium chloride and weighed as $BaSO_4$; in this case a " blank " on the reagents must be made and the barium sulphate therefrom deducted.

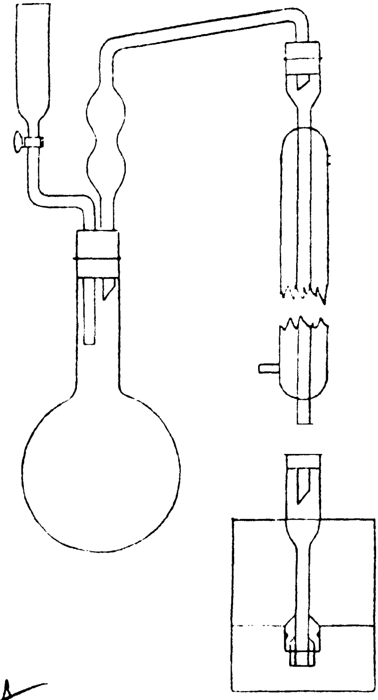

FIG. 2.

Honey

The essential constituents of honey are dextrose, lævulose and sucrose, together with small amounts of mineral matter, proteins, wax, pollen, and sometimes mannitol and dextrins. The composition varies considerably and is largely influenced by the feeding of the bees ; also, as certain enzymes are present which are active in fresh honey, the proportions of different sugars are liable to change on keeping, and a small proportion of sucrose originally present may entirely disappear in the course of months. For a long time it was usual to regard with suspicion any honey which gave a positive optical rotation, as such would be occasioned by the addition either of glucose or cane sugar, but, as will be seen from the following tables of the analyses of known samples, a proportion of cane sugar up to about 12 per cent. is not incompatible with the genuineness of a sample. The presence of more than about 5 per cent. of cane sugar suggests either that the bees have been fed on cane sugar and the honey is not matured; or that it is adulterated. In general, the proportion of invert sugar exceeds 60 per cent., and the rotation before and after inversion is negative.

Honey.	English.	English.	Russian.	Californian.	Coniferous.	Chilian.[1]
Water . per cent.	20·50	19·20	17·32	17·90	16·32	21·50
Ash . ,,	0·20	0·15	0·11	0·11	0·67	2·00
Protein. ,,	0·25	0·14	0·11	Trace.	—	—
Dextrose ,,	36·80	34·20	76·32	37·85	56·30	60·00
Lævulose ,,	32·35	33·10		36·01		
Cane sugar ,,	1·30	Trace.	1·60	—	10·21	16·50
Acidity as formic acid . .	—	—	0·12	—	0·09	—
Direct rotation.	—	—	—	—	+	+

Browne (Bull. No. 110, U.S. Bureau of Chem.) gives the analyses of a number of American honeys which show that in a few cases as much as 12 per cent. of dextrin is present; these samples were strongly dextro-rotatory, as shown in the following table :—

AMERICAN HONEYS

No. of samples.	Polarisation at 20°.	Water.	Invert sugar.	Sucrose.	Ash.	Dextrin.	Undetermined.
		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
34	– 8·5 to – 22·8	15·67 to 19·18	73·16 to 77·57	3·60 to 4·42	0·07 to 0·20	0·34 to 0·82	1·71 to 7·01
22	– 7·00 to –24·00	14·54 to 19·88	71·11 to 76·85	0·03 to 3·12	0·07 to 0·44	1·10 to 4·10	3·27 to 5·69
4	– 4·90 to +11·00	13·56 to 17·47	65·80 to 69·02	2·72 to 4·31	0·51 to 0·79	5·59 to 12·95	1·57 to 4·69

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[1] Of doubtful purity.

A German conference in 1921 adopted a specification for artificial honey with the provision that it must be so labelled. The requirements are: good flavour and keeping properties, ash less than 3 per cent., added starch syrup not to exceed 20 per cent., cane sugar not exceeding 25 per cent., water not more than 22 per cent.; and the honey must give a strong reaction in Fiehe's test.

During the manufacture of invert sugar, from which artificial honey is made, decomposition products are always formed which may be recognised by their reaction with resorcinol or β-naphthol and hydrochloric acid. Fiehe's test for technical invert sugar is as follows: Extract about 1 g. of the honey in a mortar with a little ether, filter, evaporate off the ether and apply a drop of resorcin solution (1 g. in 100 ml. hydrochloric acid); in the presence of technical invert sugar a cherry-red colour, changing to brown-red, is quickly produced. This test is characteristic of artificial honeys and is not given by genuine honeys, even if they have been heated and long stored, although a momentary pink colour sometimes appears if they have been much heated. A useful qualitative test for added starch syrup, which always contains dextrin, is also due to Fiehe. The proteins are precipitated from a 30 per cent. solution of the honey by adding tannin solution and allowing the mixture to stand overnight; then to 2 ml. of the clear filtered solution are added 2 drops of hydrochloric acid and 20 ml. of alcohol (96 per cent.). Genuine honey remains clear, whereas starch dextrin gives a distinct turbidity or a precipitate. The limit of sensitiveness is about 5 per cent., and although absolute reliance should not be placed upon these tests, they are generally trustworthy and afford useful information or confirmation. Another test of utility is based on the fact that natural honey which has not been heated contains diastase and other enzymes; but on account of the natural variation in the diastatic activity of honey, this measurement alone should not be relied upon to detect adulteration. To determine the diastatic activity prepare a series of test tubes with 1 ml. of 0·2 per cent. starch solution, 8 ml. of phosphate buffer solution of *p*H 5·0, and add 5, 4, 3, 2 and 1 ml. of 20 per cent. solution of the honey. Then incubate the tubes at 37° for half an hour, cool and add a drop or two of iodine solution to test for starch. Artificial or heated honey will give the deep blue colour of starch iodide, whereas with genuine honey the starch will have been hydrolysed. (For a full discussion of diastase in honey, see Lampitt, Hughes and Rooke, *Analyst*, 1929, **54**, 381 ; 1930, **55**, 666.) Ley's silver hydroxide test and Lund's tannin reagent are not, in the writer's experience, so reliable as those quoted above.

The total solids of honey may be estimated by drying about 2 g. mixed with sand *in vacuo* at 70° C. ; if dried in air at 100° or in the water oven, the result will be slightly erroneous on account of the presence of lævulose, but the error is not serious for most purposes. The ash is estimated by ignition over an Argand burner ; in general it should not exceed 0·35 per cent., and the presence of more than a mere trace of sulphate suggests adulteration with corn syrup. Protein (N × 6·25) is estimated in the usual way by the Kjeldahl method ; the acidity is calculated as formic acid, litmus being used as indicator, although it is by no means certain that the acid present is formic acid.

The polarimeter affords the most useful information as to the genuineness or otherwise of a sample of honey. The normal weight is dissolved in water, defecated and polarised as usual, and another portion of the solution inverted as described on p. 12. Bi-rotation is liable to occur with honey, and should therefore be guarded against, and since much lævulose is present the temperature should be exactly 20°. Genuine honey, under these conditions, shows a reading of + 5° V. to − 20° V., which is not much altered by inversion. Sometimes a genuine honey will give a direct reading as high as + 8° V. when much cane sugar is present, and this may be considerably reduced or even changed in sign on inversion.

Cane sugar is calculated by the Clerget-Herzfeld formula as usual. When the polarimetric readings fall within the above limits it is not always needful to make further estimations to show that the honey is genuine, but if the readings are outside those limits or for any reason a complete analysis is necessary, the cupric reducing power must be determined and calculated as dextrose. The dextrin should then be estimated by weighing 8 g. of honey into a 100-ml. flask, making up to the mark with 96 per cent. alcohol, then thoroughly shaking and allowing the mixture to stand overnight. The clear solution is decanted, and the precipitate collected on a filter, washed with alcohol, then dissolved in hot water, dried on sand, and weighed. If the weight is quite small, say not more than 0·3 g., it may be taken as dextrin ; if the weight of crude dextrin exceeds this figure it should be dissolved in water and the rotation, before and after inversion, determined, then the weight of sucrose and invert sugar calculated therefrom deducted from that of the crude dextrin.

When the dextrin percentage is quite small the sucrose (s), dextrose (d) and fructose (f) can be calculated from the polarisation and K value, by the formulæ

$$d + 0{\cdot}915f = \mathrm{K}$$

$$f = \frac{0{\cdot}793\,\mathrm{K} + s - \mathrm{D}}{2{\cdot}08}$$

(D = direct polarisation on Ventzke scale at 20°.)
and sucrose by the Clerget formula.

When an appreciable quantity of dextrin is present, the composition may readily be calculated in terms of sucrose, invert sugar and dextrin, as Clerget's formula gives the cane sugar, and the K value divided by 0·915 gives the invert sugar ; or the weight of reduced copper may be calculated directly to invert sugar by means of the tables on p. 17. By this method any added glucose could be included in the calculation as invert sugar, but its presence would be indicated by the abnormal polarisation values and the qualitative test of Fiehe. The iodine titration method referred to on p. 24 is available for the direct estimation of glucose.

A microscopic examination of honey should always be made, genuine honey almost invariably showing some pollen grains and traces of wax besides crystals of sugar. Starch granules should be absent. The honey is diluted with about twice its volume of water and allowed to settle overnight in a conical glass. The sediment, which will include the pollen grains, is removed with a pipette and mounted on microscope slides. Separation with the centrifuge is not so satisfactory.

Jam and Marmalade

Much discussion has taken place on the subject of the proper composition of jam. The following agreed standards were published by the Society of Public Analysts in 1930, and these are generally, though not universally, accepted. Two qualities of jam are fixed : (1) First-Quality Jams, and (2) Second-Quality Jams. The basis of such standards are (a) a minimum percentage of soluble solids, and (*b*) a minimum fruit content for each variety of jam. All jams, whether first or second quality, shall contain at least 68·5 per cent. of total soluble solids by refractometer reading when cold, uncorrected for insoluble solids.

SINGLE-FRUIT JAMS

The minimum percentage of fruit content in finished jams of first quality (or, expressed in other terms, the number of pounds of fruit required to be used in making 100 lb. of finished jam) in each of the following named varieties shall be :—

	Fruit content. Per cent.		Fruit content. Per cent.
Strawberry	42	Red plum	40
Raspberry	38	Damson	38
Black currant	30	Apricot	40 [1]
Red currant	35	Blackberry	38
Green gooseberry	35	Loganberry	38
Red gooseberry	40	Cherry	45
Greengage	40	Peach	40 [1]
Victoria plum	40	Rhubarb	45
Green or golden plum	35		

[1] Apricot and peach jam : The equivalent of four 5-kilo tins of apricot or peach pulp, containing no added water.

MIXED JAMS

The minimum percentage of fruit content in mixed jams and the minimum proportions of each named fruit present shall be as follows :—

	Fruit content. Per cent.
Strawberry and gooseberry	40 (20/20)
Gooseberry and strawberry	40 (30/10)
Raspberry and gooseberry	40 (20/20)
Gooseberry and raspberry	40 (30/10)
Raspberry and red currant	40 (20/20)
Plum and apple	40 (20/20)
Apple and plum	40 (30/10)
Blackberry and apple	40 (20/20)
Apple and blackberry	40 (30/10)
Household, two-fruit jams, or any other jams not mentioned in the schedule	40

Two-fruit jams : The figures in brackets against those denote to the respective proportions of each named fruit to be present. The proportions for two-fruit jams not specially mentioned to be on the same basis.

Single-fruit jams of second quality shall contain not less than 20 per cent. of the named fruit :—

	Minimum fruit content. Per cent.		Minimum fruit content. Per cent.
Strawberry	20	Red plum	20
Rasberry	20	Damson	20
Black currant	20	Apricot	20 [1]
Red currant	20	Blackberry	20
Green gooseberry	20	Loganberry	20
Red gooseberry	20	Cherry	20
Greengage	20	Peach	20
Victoria plum	20	Rhubarb	20
Green or golden plum	20		

[1] Apricot and peach jam : The equivalent of two 5-kilo tins of apricot or peach pulp, containing no added water.

MIXED JAMS

The minimum percentage of fruit content in mixed jams of second quality shall be 20 per cent., and the minimum proportions of each named fruit present shall be as follows :—

	Minimum fruit content total proportion. Per cent.
Strawberry and gooseberry	20 (10/10)
Gooseberry and strawberry	20 (15/5)
Raspberry and gooseberry	20 (10/10)
Gooseberry and raspberry	20 (15/5)
Raspberry and red currant	20 (10/10)
Plum and apple	20 (10/10)
Apple and plum	20 (15/5)
Blackberry and apple	20 (10/10)
Apple and blackberry	20 (15/5)
Household, two-fruit jams, or any other jams not mentioned in the schedule	

Two-fruit jams : The figures in brackets against these denote the respective proportions of each named fruit required to be present. The proportions for two-fruit jams not specially mentioned to be on the same basis.

Any jam not conforming to the standards for first quality shall be described on the label by the name of the fruit or fruits, accompanied by the words " with other fruit juice " in letters of size equal to that of the name of the fruit or fruits ; except that where a jam, *e.g.*, mixed jam, contains no added fruit juice or pectin the words " with other fruit juice " may be omitted.

By the Jam and Marmalade Order, 1942, fresh fruit standard jam must be prepared from fresh fruit only and must be declared to contain no added colouring matter or preservative. Such jam should of course contain vitamin C, ascorbic acid.

The examination of jam may be divided into three parts : the identification and estimation of the fruits or other pulp used, the estimation of the different sugars, and thirdly, the detection or estimation of preservatives, dyes, or saccharin, and artificial thickening substances such as gelatin or agar-agar. The determination of soluble and insoluble solid matter is useful as is also the examination of the pulp with a hand lens and the microscope. For this purpose 50 g. of the jam are weighed out, diluted with warm water and well mixed; then, when all the saccharine matters appear to be in solution, the pulp is filtered off on to a fine linen filter, well washed with hot water, and the filtrate made up when cold to 250 ml. An aliquot part of this is dried in a tared basin. The pulp on the filter is removed to a basin, dried and weighed. In the case of jam containing large stones, such as plum or damson, the weight taken should not include any stones. An approximation to the total soluble solids may be quickly made by determining the refractive index at 20° and referring to the table on p. 11, reading the solids as cane sugar.

The microscopic examination of the pulp must be made on a fresh quantity before drying. Apple pulp may be detected by boiling with water, decanting, and then adding strong iodine solution to the residue; in the presence of apple pulp, little blue-black patches due to amylo-dextrin will be seen. Confirmation must be made by the microscope, as the iodine test is not absolutely reliable. The characteristic appearance of apple pulp is shown in the figure; apricots and pears show a somewhat similar structure. Muttelet (*Analyst*, 1922, **47**, 398) has proposed a quantitative method for the recognition or detection of apple pulp by the isolation and estimation of malic acid. It is a common practice to return the total acidity of jam as malic acid, but in certain fruits there is actually little or none of this acid (see also p. 100).

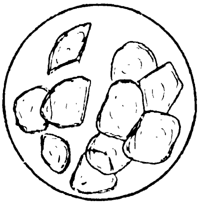


Fig. 3. Apple pulp.

The microscopic appearance of the common fruit pulps is described in Winton's "Structure and Composition of Foods," though it must be remembered that the prolonged boiling which jam undergoes in manufacture materially alters some of the characteristic structures.

The real difficulty in the analysis of jam is the estimation of the fruit content. In addition to determining the insoluble solids as a basis for this, lead numbers and soluble non-sugar solids have been suggested, but in the writer's opinion are not generally so useful, though they may be of service in doubtful cases (cf. *Analyst*, 1934, **59**, 231, 248 ; 1944, **69**, 343). Having determined the insoluble solids, reference to data published by Macara (*Analyst*, 1931, **56**, 35), and by Lampitt and Hughes (*ibid.*, 1928, **53**, 32) will show the range of variation in the natural fruits. The following data have been accumulated for some of the commoner jams and serves for an estimation of fruit content on an average basis. It will be realised that averages are not an adequate basis when a prosecution is contemplated. All possible data, including pectin content and acidity, must be taken into account, and variations due to inequalities in the filling of pots and in sampling considered.

Jam.	Minimum fruit content. Per cent.	Average insoluble solids in fruit. Per cent.	Average pectin in fruit. Per cent.	Average insoluble solids in jam. Per cent.	Average pectin due to fruit in the jam. Per cent.
Strawberry .	42	1·9	0·68	0·80	0·28
Raspberry .	38	5·5	0·71	2·10	0·28
Black currant .	30	5·5	1·50	1·65	0·45
Gooseberry .	35/40	2·25	1·10	0·90	0·43
Greengage .	40	1·5	1·10	0·60	0·44
Victoria plum .	40	1·2	1·00	0·50	0·40
Damson . .	38	1·6	1·10	0·65	0·44
Apricot . .	40	2·0	1·00	0·80	0·40
Blackberry .	38	8·1	0·90	3·10	0·35
Loganberry .	38	7·2	0·65	2·70	0·25
Cherry . .	45	2·1	0·35	0·95	0·16

The average composition found for a number of different kinds of jams and marmalade is shown in the table overleaf ; some of the data is from Hartel and Solling (*Zeit. Unters. Nahr. Genussm.*, 1911, **21**, 168).

An important constituent of many fruits and therefore of jams, is pectin ; on the presence and amount of this substance the solidifying power of the conserve largely depends. Pectin is stated by Tutin (*Biochem. J.*, 1922, **16**, 704) to be the methyl-isopropenyl ester of pectic acid, this latter substance has the formula $C_{17}H_{24}O_{16}$, and forms a calcium salt which is insoluble in acetic acid. It appears from the work of Tutin, Tollens, and others, that the pectin is the same in all the common fruits and

Jam.	In-soluble matter.	Soluble solids.	Acidity (as malic acid).	Invert sugar.	Sucrose.	Sugar free extract.	Ash.	Glucose.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Raspberry .	5·14	70·05	1·08	39·46	26·89	3·70	0·37	—
Apricot .	1·90	80·66	1·87	52·53	21·38	6·75	0·57	—
Strawberry .	2·37	73·45	1·22	36·60	32·82	4·03	0·41	—
Quince .	5·41	51·95	0·68	30·56	15·76	5·63	0·30	—
Gooseberry .	2·74	57·98	1·12	46·90	5·24	5·84	0·27	—
Cherry .	7·42	69·66	1·03	43·00	21·25	5·40	0·45	—
Pumpkin .	1·28	63·98	0·03	3·26	58·03	2·69	0·34	—
Apple .	1·39	52·25	0·42	20·84	29·11	2·29	0·15	—
Blackberry .	3·65	55·70	—	20·65	29·35	1·55	0·50	—
Marmalade .	3·80	70·40	0·25	35·70	19·30	1·95	0·63	12·82
,,	2·70	64·10	—	25·55	34·60	3·50	0·45	—
,,	2·65	66·35	—	14·67	32·65	4·05	0·38	14·60

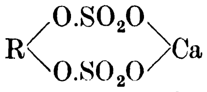
vegetables. In the presence of sugar and acid it forms a gel; the gelatinising power is much influenced by the acidity, quite a small increase in which is said to be equivalent to a considerable quantity of sugar.

Several methods have been published for the estimation of pectin, the more important of which are those of Carré and Haynes (*Biochem. J.*, 1922, **16,** 60), of Wichmann, which has been adopted by the American Association of Official Agricultural Chemists (see p. 95), and that of J. King (*Analyst*, 1925, **50,** 371). King heats 50 g. of the jam on the water bath with hot water and disintegrates the tissue with the aid of a glass rod, then adds hot alcohol little by little, with constant stirring until the volume reaches 300 ml. The mass at the bottom is frequently stirred for about two hours, or until no gelatinous particles are visible; then the whole is filtered on a coarse paper, using the pump if necessary towards the end of the filtration. The filter and its contents are washed back into a beaker with warm alcohol, treated again with 300 ml. of alcohol, and refiltered. The pectin is dissolved from the residue with boiling water and the solution filtered and cooled, a small excess (about 0·02 N) of sodium hydroxide is added, and the solution is allowed to stand for an hour to complete the hydrolysis. After this time acetic acid is added to give a concentration of free acid of about 0·1 N, then 20 ml. of 10 per cent. calcium chloride solution. The mixture is allowed to stand for an hour, then boiled and filtered through a tared paper. The gelatinous precipitate is washed with boiling water, then transferred again to the beaker, boiled with 300 ml. of water, filtered once more through the same paper, dried and weighed as calcium pectate.

King gives the following percentages of calcium pectate for some of the common varieties of jam :—

Plum and apple	0·13
Blackberry and apple	0·25
Plum	0·46
Damson	0·53
Raspberry	0·31
Gooseberry	0·45

Agar-agar is sometimes added to stiffen jellies or jams. This substance is the product of certain marine algæ common in Japan and parts of Australia ; it consists largely of the carbohydrate gelose, and is readily hydrolysed by boiling with even dilute acids, such as are found in fruits ; on this account the estimation of agar-agar is sure to give somewhat low results. It becomes partially hydrolysed during the boiling of the conserve. Parkes (*Analyst*, 1921, **46,** 239) gives a simple method for its detection ; 50 g. of jam and 500 ml. of water at about 50° are well mixed and left to stand in a warm place with occasional stirring until disintegrated, then allowed to settle, and filtered through a folded filter. A little alumina cream may be used to facilitate filtration. The insoluble matter is washed with warm water, the filter and contents are then transferred to a porcelain basin and boiled for a few minutes with about 50 ml. of water, then thrown immediately on to a folded filter. The filtrate, on cooling, will set into a gel if it contains any appreciable quantity of agar ; if only a small amount, such as 0·1 per cent., be present it may be necessary to evaporate the filtrate to a small bulk before it will gel successfully.

King (*loc. cit.*) takes advantage of the presence of the group  $R\begin{matrix}\diagup O.SO_2O \diagdown \\ \diagdown O.SO_2O \diagup\end{matrix}Ca$ which is completely hydrolysed on prolonged boiling with hydrochloric acid, and can be precipitated with barium chloride. One hundred grams of the jam are de-sugared by treatment with hot water and alcohol as described for the estimation of pectin, and filtered. The residue is boiled for some minutes with 200 ml. of water, filtered, again boiled and refiltered, the fluid being kept at a temperature of at least 80° during the filtration to ensure that all the agar passes into the filtrate. The latter is concentrated to, say, 300 ml., and the free sulphate estimated on 100 ml.; care should be taken to add the hydrochloric acid at the last minute only, so as to minimise hydrolysis of the agar. The remaining 200 ml. are boiled for six hours with 100 ml. of hydrochloric acid, concentrated to about 25 ml., diluted, filtered while hot and precipitated with barium chloride. The weight of agar-agar is found from the formula $15\,[1{\cdot}5\,(a - 2b)]$ where a is the weight of $BaSO_4$ from the total sulphate on 200 ml., and b is the weight of $BaSO_4$ from the free sulphate in 100 ml. The solution, after the addition of barium chloride in the final precipitation, should be allowed to stand overnight as the $BaSO_4$ is precipitated very slowly.

Another method for the detection, but not estimation, of agar-agar depends upon the microscopical detection of the diatoms which are almost invariably present in commercial agar. A quantity of the jam is warmed with dilute sulphuric acid, potassium permanganate is added until most of the organic matter is destroyed, and the sediment is then examined under the microscope; diatoms, if present, suggest the addition of agar jelly, but Parkes (*loc. cit.*) draws attention to the fact that kieselguhr or diatomaceous earth is sometimes used in filtering agents for fruit juices; hence the presence of diatoms must be interpreted with care. A full illustrated list of diatoms which are characteristics of agar-agar is given by King (*loc. cit.*).

Dextrin may be detected by alcohol precipitation as described under honey, and its presence is suggestive of added starch syrup, although certain fruits contain dextrins; these may be distinguished by dissolving the crude dextrin in water and adding iodine solution. Erythrodextrin, which is characteristic of corn syrup, gives a red colour.

The sugars present in pure jam should be sucrose with a varying proportion of invert sugar. Glucose is sometimes considered to be an adulterant in the case of jam, although in marmalade it has been held that an amount up to about 15 per cent. is unexceptionable, as it is said that the presence of such quantity renders the marmalade less liable to the growth of moulds. For the estimation of the sugars 65·12 g. are dissolved in about 200 ml. of water, defecated with lead acetate and alumina cream, diluted to 250 ml. and the sugar estimated in the filtrate, both before and after inversion, by Clerget's method.

If the solution is too highly coloured for polarimetric observation after treatment, the gravimetric or volumetric method of copper reduction may be used. In this case the copper weighed is calculated to invert sugar from the tables given, bearing in mind that 100 g. of sucrose yields on inversion 105·25 g. of invert sugar, so that the increase in invert sugar after inversion must be multiplied by the factor 0·95 to give its equivalent in cane sugar.

The presence of glucose is indicated if the rotation after inversion is positive or only slightly negative. In this case it is necessary to determine the cupric reducing power; then the proportions of cane sugar, invert sugar and glucose syrup are given by the formulæ on p. 31. Dry glucose may be deduced with fair accuracy by assuming 20 per cent. of water in glucose syrup. The amount of glucose found by the writer in a number of genuine jams did not exceed 3 per cent. Hartel and Solling (*loc. cit.*, p. 42) found larger quantities of starch syrup in many of the jams they examined.

The preservative—other than sugar—most usually found in jam or marmalade is sulphur dioxide; it is permitted to the extent of 40 parts per million, and may be determined as described on p. 32. Salicylic acid used to be the favourite before the advent of the preservative regulations. In marmalade or pale-coloured jams it may be detected by shaking out with ether and allowing the ether to evaporate, then dissolving the residue in a little water and adding a drop of solution of ferric chloride; a characteristic violet colour is given in the presence of salicylic acid. Under similar conditions benzoic acid, which is sometimes used, gives a buff-coloured precipitate not always easily seen. Some jams, when so treated, give a coloured ether extract which masks the colour of traces of salicylic acid. In such cases the quantitative method of Harry and Mummery (*Analyst*, 1905, **30**, 124) gives good results, and may be applied to foodstuffs in general with but slight modification for particular substances. To 50 g. of the sample in a flask marked at 300 ml. are added a little water, then 15–20 ml. of saturated basic lead acetate solution and a slight excess of sodium hydroxide solution. The alkali throws down excess of lead and dissolves some lead hydroxide and proteins, which are precipitated in a suitable form by the addition of a small excess of dilute hydrochloric acid. The contents of the flask are therefore just acidified, shaken and diluted to 300 ml., then filtered. Two hundred ml. of the clear colourless filtrate are extracted three times with ether, the ether solutions are mixed, distilled off at a low temperature, and the residue dissolved in a few drops of alcohol and made up to say, 100 ml. with water. The salicylic acid is now estimated colorimetrically by adding to 50 ml. (or a lesser volume diluted to 50 ml.) in a Nessler glass 1 ml. of solution of ferric chloride and matching it against a suitable volume of standard 0·01 per cent. salicylic acid solution similarly diluted to 50 ml. For the estimation of benzoic acid, if present, see p. 104.

Boric acid is not often found in jam or marmalade, and in examining the ash for this substance by means of turmeric paper if should be noted that certain fruits contain a minute amount of boric acid or some substance which gives a slight turmeric reaction. If present, boric acid may be estimated by the method described on p. 92.

Saccharin is occasionally found in jam, and may be added to compensate for the lack of sweetness due to an addition of glucose. In the absence of salicylic acid, the simplest method of testing is to extract the acidified and diluted jam with a mixture of equal volumes of petroleum ether and ether, then wash the extract once with water and evaporate off the volatile solvent. The residue should be tasted. If it contains saccharin the characteristic sweet taste will be noticed and confirmation is obtained by fusing gently with a small piece of sodium hydroxide, then dissolving in water and testing for salicylic acid with ferric chloride. The method may be made approximately quantitative, but obviously cannot be applied in the presence of salicylic or benzoic acid. When either of these is present it must be removed before testing—benzoic acid by heating the extracted matter in the oven at 100° to volatilise the benzoic acid, leaving the saccharin unchanged, and salicylic acid, by making the residue from the acid ether extraction alkaline and warming it gently with excess of potassium permanganate ; the salicylic acid is thereby oxidised, excess of permanganate removed by a drop of sulphurous acid, and the residue then fused with sodium hydroxide and tested with ferric chloride as before.

The addition of artificial dyes to jams may be detected by transferring the dye to wool. If the test is carried out as follows it is usually possible to distinguish between added dye and the natural colour of certain fruits. Add a tuft of white wool to about 20 g. of jam diluted with 50 ml. of water, acidify with acetic acid, and boil for five minutes ; remove the wool, wash it under the tap, then boil it with water containing a trace of ammonia, which extracts the colour ; remove the wool, acidify the solution, and boil it with a fresh piece of wool, wash the wool under the tap and dry it between filter paper. The colour may sometimes be identified by the ordinary tests (for details of tests, see Green's "Analysis of Dyestuffs," 1915 ed., p. 59, or Clayton, *J. Soc. Dyers and Color.*, 1937, **53,** 178). For the identification of the prohibited dyes, see Appendix.

The following organic colours are permitted to be added to foodstuffs in America and in Canada :—

	Red Shades.		*Yellow Shades.*
184	Amaranth.	10	Naphthol yellow S.
80	Ponceau 3R.	640	Tartrazine.
773	Erythrosin.	22	Yellow O.B.
		61	Yellow A.B.
	Orange Shades.		
150	Orange I.		
	Green Shades.		*Blue Shades.*
666	Guinea green B.	1180	Indigo carmine di-sulphonic acid.
670	Light green S.F. yellowish.		
—	Fast green F.C.F.		

The numbers refer to the numbers in the Colour Index of the Society of Dyers and Colourists. These dyes may be added to an amount not exceeding 2 grains per pound. Sudan I and butter yellow were formerly included but have been found unsatisfactory and were withdrawn. It is recommended (*Analyst*, 1928, **23,** 217) that dyestuffs for use in food should not contain more than 5 parts of arsenic per million in the undiluted colours. Methods for the determination of arsenic and of lead in food colours are to be found in the *Analyst*, 1930, **55,** 102, and 1935, **60,** 541. A limit of 20 parts per million appears reasonable for lead.

In England a larger variety of dyes appears to be in common use. These are generally mixtures but include eosin, naphthol yellow S, malachite green, ponceau red, rosanilines, tartrazines and rhodamines.

Richardson (*J. Soc. Dyers and Color.*, 1923, **39,** 148) gives a resumé of the toxic action of dyes, pointing out that nitro groups are generally harmful, whereas a sulphonic acid group is harmless even to the extent of neutralising nitro groups.

A review of the dietetic aspects of food colours is given by Cox (*Chem. and Ind.*, 1944, 30).

CHAPTER II

STARCHES, CEREALS, FLOUR, BREAD, CUSTARD AND BLANCMANGE POWDERS

THE starches enter more largely into the composition of human food than any component except water. The chemical constitution of the group is not yet understood, but all have the general formula $(C_6H_{10}O_5)_n$, where n is probably not less than 1,000. Among the more important properties of starch from the analytical point of view may be mentioned that it is readily convertible into sugar by hydrolysis either by an enzyme such as diastase or by heating with an acid ; the estimation of starch usually depends upon this reaction. When heated with water, the grains swell up and burst at about 70°, forming a paste. When iodine solution is added a characteristic blue colour is developed, due to starch iodide, which compound is decomposed on heating, but is re-formed on cooling. Starch has no melting point ; it gradually swells and chars on heating, with the formation of caramel and its derivatives ; it does not reduce Fehling's solution until after hydrolysis, and its optical rotation is variable, depending upon the variety of cereal from which it has been obtained. The specific rotations $[\alpha]_D^{20}$ of some of the commoner forms, according to Ewers, are given on p. 61, but they vary somewhat according to the exact conditions of hydrolysis and purification.

Starch has density of approximately 1·50–1·53 varying a little with the type, it is insoluble in water and in organic solvents, but on grinding with water sufficient is dissolved or passes through a filter to give a blue colour with iodine. It is usually slightly acid in reaction.

The starches or cereals, when ground, are usually identified by their microscopic characteristics. To a small drop of water on a slide is added a minute quantity of the starch on the point of a knife ; then the powder is thoroughly mixed with the water, taking care to break any air bubbles, and covered with a coverslip. Excess of water may be removed by means of a piece of filter paper applied without pressure. The examination should be made first with a low power, and then with a higher one, such as the $\frac{1}{6}$-inch objective. A better idea of the shape of the granules can often be obtained by rolling them by gently moving the coverslip, and the striations and hilum can be brought into relief by modifying the illumination. When there is any doubt as to whether or not particular grains are starch, a little very dilute iodine solution run under the coverslip enables the point to be easily decided.

Some of the commoner starches, and a few of the less common ones which are met sometimes as adulterants of cocoa, ginger, pepper, and other foods, are illustrated here. Details of others may be found in Greenish's "Anatomical Atlas of Vegetable Powders," or in Clayton's "Compendium of Food Microscopy," but in all cases of doubt it is much better to compare the sample with known specimens of the various starches themselves, than to rely upon drawings or illustrations.

Most of the various proposals which have been put forward from time to time for the detection and estimation of foreign starch in mixtures have not been altogether satisfactory. It is best to rely upon the microscope, which will readily show any adulteration by a starch of another group, such, for example, as maize in rice or in wheat, but when two starches of the same group are present it is exceedingly difficult to detect a small quantity with certainty; wheat and barley, for instance, are very similar. Wallis points out that the large grains of wheat starch reach as much as 45 μ, whereas those of barley do not exceed 40 μ. He bases a counting method with lycopodium on this fact (*Pharm. J.*, 1922, **109,** 82).

In mixed cereals fragments of the husk are often to be found by sifting, and examination of them will yield information. An alternative procedure is to boil a quantity of the material with acid and alkali as for a fibre determination, p. 60, and examine the residue on the filter with the microscope. Vogt (*Zeit. Unters. Nahr. Genussm.*, 1921, **42,** 145) gives a method which also may help in these cases, based upon the differential staining of flours by Congo red and the alkalinity of the ash. König and Bartschat (*Zeit. Unters. Nahr. Genussm.*, 1923, **46,** 321) give an empirical method for estimating rye flour in admixture with wheat flour, which is accurate to about 5 per cent., provided that the acidity is not abnormally high. The method is based upon the observation that, of the total protein of wheat, 29·1 per cent., and of rye 51·5 per cent. is soluble in a saturated solution of calcium sulphate. Ten grams of flour are moistened in a 500 ml. flask with the calcium sulphate solution at room temperature, made up to the

500 ml. mark with the solution, shaken in a machine for an hour, the nitrogen then estimated by Kjeldahl's method on 100 ml. of the clear filtered solution, and the result expressed as a percentage of the total nitrogen. The following tables may be used for extrapolating the composition of the mixture :—

Wheat.	*Rye.*		*Wheat*	*Rye.*	
100		29·1	40	60	42·54
90	10	31·34	30	70	44·78
80	22	33·58	20	80	47·02
70	30	35·82	10	90	49·26
60	40	38·06		100	51·50
	50	40·30			

When two starches (which can be readily identified microscopically) are present in admixture their relative proportions can be approximated by comparison with known mixtures, say, of 10, 20, 30 or 50 per cent. It is needful to ensure thorough mixing by trituration, say, of 0·5 g. with 20 ml. of water, then removing 1 drop with a platinum loop and comparing it with an exactly similar quantity of the unknown starch in a like quantity of water. A more elaborate method capable of yielding accurate results depends on mixing the powder with lycopodium and counting the number of granules. For details, see Wallis (*Analyst*, 1916, **41,** 357).

The more important diagnostic characters of the starches are as follows :—

Wheat Starch. Mostly round grains with very faint striation, some are small, but others are of large size, maximum diameter 35–45 μ.[1] The larger grains are oval or lenticular when rolled, and some have a longitudinal furrow, but there is no hilum. With polarised light they exhibit a cross.

Barley Starch. Closely resembles wheat, but the grains are smaller, diameter not greater than 35 μ. There are not so many intermediate sized grains ; the longitudinal furrow is more frequent, and on rolling their form is seen to be lemon shaped. Under polarised light there is a cross.

Rye Starch. This starch also resembles wheat, but the grains are distinctly larger, measuring in some cases as much as 60 μ. There are three- or four-rayed fissures in many of the larger

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[1] 1 μ = 0·001 mm.

granules, and with polarised light the cross is more distinct than with wheat or barley.

Rice Starch. These are closely packed angular grains without hilum, fairly uniform in size, measuring from 6 to 9 μ. Compound grains are numerous, but are broken by slight pressure. The usual cross is seen under the polariser.

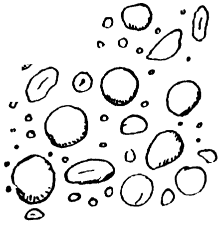

FIG. 4. Wheat starch. × 125.

FIG. 5. Barley starch. × 125.

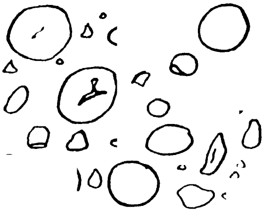

FIG. 6. Rye starch. × 125.

FIG. Rice starch. × 125.

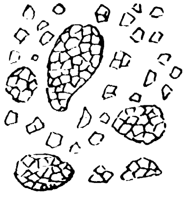

FIG. 8. Oat starch. × 125.

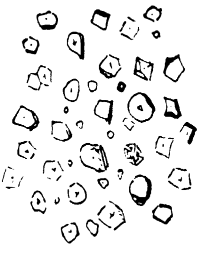

FIG. 9. Maize starch. × 125.

Oat Starch. Is very similar to rice, but the grains are somewhat larger, average 10–11 μ. Compound grains are numerous and are not so readily fractured as are those of rice; this starch is peculiar in not exhibiting the usual cross when examined in polarised light, a feature which provides a means of differentiation from rice.

Maize Starch. The grains are fairly uniform in size and shape, they are polygonal, usually having five to six sides ; the majority measure about 15 μ, though a few reach 20 μ. There is a distinct hilum on most of the grains, sometimes a star-shaped fissure, and a well-defined cross is shown by aid of polarised light.

Potato Starch. Is composed of quite large grains of oval or conchoidal shape with oyster-shell markings, length from 100 μ downwards. A few are rounded or flattened, and are much smaller, 15 μ in length ; a distinct hilum is visible in all grains, usually near one end. Under the polariser there is a cross which is centred at the hilum.

Tous-les-mois, or Queensland Arrowroot. Is the largest of the starches ; the grains are generally similar to those of potato, but are larger, some reaching as much as 135 μ ; the concentric striations are more close than those of potato, and the hilum nearer to the end of the grains.

Curcuma Starch, or East Indian Arrowroot. This is generally similar to potato but smaller ; the grains average about 50 μ in length and have a tapering projection at the larger end. The hilum is not very distinct, but the concentric striations are well marked.

St. Vincent Arrowroot. This also has a similarity to potato starch but is smaller, and of much more uniform size ; its grains have an average length of 45 μ ; neither the striations nor hilum are very distinct.

Manihot Starch, or Brazilian Arrowroot. The grains of this starch are variable in shape, some circular, a few polygonal or muller-shaped ; the average diameter is about 20 μ but some large grains are seen up to 30–33 μ. The hilum is visible on the circular grains as is in the form of a cross ; there are faint concentric striations.

Sago. This starch which is obtained from the stem of the sago palm, *Metroxylon rumphii* and other species, is usually seen in a swollen form as rounded or ovoid granules, some angular and of unequal size, varying from 20–60 μ or more. Some granules are saucer-like in appearance, and have a dark patch in the centre.

Maranta Starch, or Ordinary West Indian Arrowroot. Is closely similar to St. Vincent arrowroot both in size and shape. A distinctive feature is the slit or dark line, frequently transverse, at the hilum. J. R. Stubbs (*Analyst*, 1926, **51,** 400) calls attention to and illustrates sweet potato starch as an adulterant of arrowroot and cornflour. So-called marble arrowroot is not a true arrowroot but is occasionally used as an adulterant (see Jones, *Analyst,* 1934, **59,** 493).

Fig. 10. Potato starch.
× 125.

Fig. 11. Tous-les-mois.
× 125.

Fig. 12. East Indian arrowroot.
× 125.

Fig. 13. St. Vincent arrowroot.
× 125.

Fig. 14. Brazilian arrowroot.
× 125.

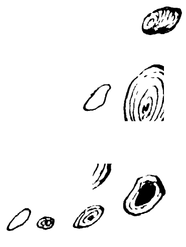

Fig. 15. Sago.
× 125.

Arum Starch, or Portland Arrowroot. Is one of the smaller starches, its grains are usually 7–12 μ in diameter, occasionally as much as 14 μ ; some are circular with a dot-like hilum of small angular or truncated shape, others are angular. There is a general resemblance to manihot starch, but on a smaller scale.

Tapioca Starch. This is prepared from manihot starch by a process of heating the moist mass. In appearance it is similar to sago but smaller. The unswollen grains are roughly circular in shape with concentric rings and usually a hilum ; their size is from 15–25 μ diameter. In the gelatinised tapioca of commerce a few unswollen grains are often visible, but the majority are about three times the above size, and have irregular and some-

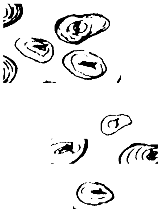

FIG. 16. West Indian arrowroot. × 125.

FIG. 17. Portland arrowroot. × 125.

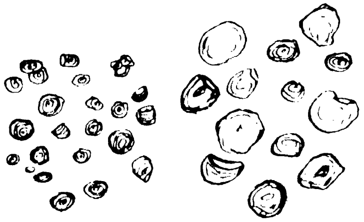

FIG. 18. Tapioca.
Unaltered. Heated.

FIG. 19. Pea starch. × 125.

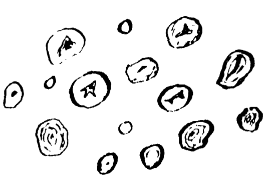

FIG. 20. Bean starch. × 125.

FIG. 21. Dextrin.

times saucer-like shapes with no regular markings. The centre is usually dark.

Pea, Bean and Lentil Starches. These three starches are closely similar in form; they are of irregular bean-shaped or elliptical form, and most of the grains show concentric markings. Bean starch grains are the largest, being up to 57 μ in length; pea starch is intermediate, having grains from 15–47 μ, and lentils are mostly from 20–40 μ with a few as large as 45 μ. The hilum and markings are more distinct in beans than in lentils, and there is with each of these two forms a slit at the hilum. With pea starch the markings and hilum are less pronounced, and there is no slit in most of the grains.

FIG. 22. Banana starch × 125.

FIG. 23. Chestnut starch. × 125.

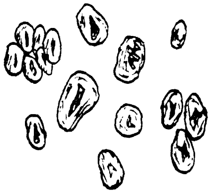

FIG. 24. Acorn starch. × 125.

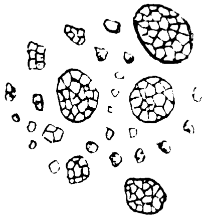

FIG. 25. Darnel starch. × 125.

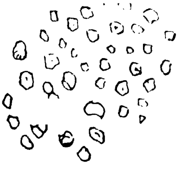

FIG. 26. Buckwheat starch. × 125.

FIG. 27. Millet starch. × 125.

Dextrin. This is seen under the microscope as large swollen grains of irregular size and shape, which give a brown or reddish-brown colour with weak solution of iodine. Oyster-shell markings are visible on some of the particles.

The following starches are of less frequent occurrence but are sometimes met in adulterated foods, or in feeding stuffs :—

Banana Starch. This has a general resemblance to curcuma starch, but is rather smaller, the grains being about 30 μ in length, narrow, and somewhat irregular in shape, with an eccentric hilum and circular striations.

Chestnut Starch. Is very irregular, both in shape and size; amongst the more characteristic types are pear-shaped grains, about 16–18 μ in length, with a hilum near the centre. There are many small grains with no visible markings.

Acorn Starch. Is similar in appearance to bean starch, but the grains are more regular both in their oval shape, large slit in the hilum, and length, which is about 70 μ.

Darnel Starch. Is polygonal and angular like rice, with a hilum in many grains, the average diameter being 4 μ. It usually exhibits numerous compound ovoid lumps which are not easily broken up by pressure on the coverglass.

Buckwheat Starch. Exhibits angular grains of irregular size and shape, usually from 6–12 μ in diameter, and having curved sides. There is no hilum.

Millet Starch. The starch of *Panicum* is of the same general shape as buckwheat and darnel, but there is a hilum on many grains and their size is greater, usually 10–15 μ.

The average composition of the principal cereals is set out in the following table :—

Flour.	Water.	Protein.	Fat.	Starch by difference.	Fibre.	Ash.	
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
Wheat :							
"Strong"	12·40	12·75	1·35	72·80	0·25	0·45	**Dry gluten, 13·1**
"Weak".	13·20	11·90	1·26	72·94	0·26	0·44	**Dry gluten, 9·5**
Barley .	11·05	7·65	1·32	78·47	0·65	0·86	
Oat . .	8·45	15·70	5·68	67·62	0·90	1·65	
Rice . .	11·25	6·88	1·20	79·99	0·38	0·30	
Maize .	12·57	7·13	1·33	78·36	0·87	0·61	
Potato .	75·48	1·95	0·15	20·69	0·75	0·98	**Average of German potatoes (König).**
Rye . .	13·37	11·52	1·84	68·88	2·55	1·94	**German rye meal.**

Wheat Flour. The composition of wheat flour is liable to considerable variation according to the class of wheat, country of origin, and the grade as regulated by the miller. The finest grades contain the least fibre and are quite white, whereas the lowest grades may contain as much as 3 per cent. of fibre and have a brownish tint reminiscent of the standard flour so widely advertised a few years ago. Wheat flour differs from all others in containing a considerable proportion of gluten ; to this its peculiar suitability for bread-making is due. The proportion of gluten varies with different wheats, it being formerly supposed that the "strength" of flour depended upon the quantity of gluten ; it has, however, recently been proved that the strength and water-holding properties of the flour depend on the composition of the gluten, not on its quantity. The two proteins forming the greater part of the gluten are glutenin and gliadin, and, while the latter appears to be identical in strong and weak wheats, the former exists in different varieties. The strength of the flour is largely influenced by the particular glutenin synthesised by the plant (*cf.* Woodman, *J. Agric. Sci.*, 1922, **46,** 231).

The grade of a flour is most readily gauged from the ash content. A straight run flour usually has not more than 0·45 per cent. ash, a 'patent' grade less than 0·4 per cent., whereas the later 'breaks' may show from 0·5 to 0·9 or more per cent. The oil content seldom exceeds 1·5 per cent. unless some germ has been included.

The chemical analysis of flour generally includes the estimation of moisture, oil, ash, fibre, proteins, gluten, acidity, and an examination for so-called improvers and for evidence of bleaching. It may also involve the determination of vitamin B_1 (aneurine), riboflavin and added chalk. The estimation of water and ash may conveniently be done on the same quantity of sample ; after drying in the water oven to constant weight the flour is ashed in platinum over an Argand burner at a low temperature. As cereals are very difficult to burn completely, owing to the salts fusing and enclosing unburned carbon, it is advisable, after well charring, to extract the char with hot water, filter, burn the filter and carbon at a higher temperature, then add the filtrate, evaporate to dryness and ignite gently over the Argand burner. This procedure, although more laborious, often effects a saving of time over attempts to obtain a white ash by direct ignition at higher temperatures.

The oil, or fat, which consists mainly of olein and palmitin, is extracted from the dry flour by petroleum spirit in a Soxhlet thimble.

For the estimation of proteins the well-known Kjeldahl-Gunning process is most suitable. Weigh about 2 g. of the sample into a pear-shaped digestion flask with a long neck, add 20 ml. of sulphuric acid, and about 5 drops of copper sulphate solution. Heat the mixture in the fume cupboard gently, then add 10 g. of potassium sulphate and heat more strongly, until all is dissolved and a clear colourless solution is obtained; the heating must be continued for at least half an hour after this point is reached; the copper serves to accelerate the reaction and oxidation. When this stage is completed, cool the solution and add about 200 ml. of water and a few crystals of potassium permanganate; transfer to a distillation flask, rinse the digestion flask with a further quantity of water, add a fragment of ignited pumice, then an excess of 20 per cent. solution of sodium hydroxide free from carbonate, connect at once to an upright condenser by means of a still-head with a trap, and distil with the condenser end just dipping into 25 ml. of 0·2 N hydrochloric acid (or other suitable quantity to ensure an excess of acid for absorbing the ammonia). Continue distillation until all the ammonia is driven over and absorbed by the standard acid; this point may be recognised by removing the receiver and replacing it by a beaker containing a few ml. of water together with 1 drop of 0·1 N acid and methyl-red solution, or experience soon tells when the distillation may be stopped without actual test. The excess of acid in the receiver is titrated back with 0·2 N sodium hydroxide. The process is of wide application and is suitable for the determination of nitrogen in all classes of foodstuffs. For general purposes N multiplied by 6·25 is taken as the equivalent of protein, but other factors are more accurate for specific proteins such as casein or albumen. A correction should be applied for any ammonia due to reagents.

When nitrates are present, Jodlbauer's modification of the standard process should be employed. To the substance in the digestion flask add 1 g. of salicylic acid dissolved in 20 ml. of sulphuric acid; after standing, cold, for about half an hour with occasional shaking, add 5 g. of sodium thiosulphate, heat gently until frothing ceases, then add 10 g. potassium sulphate, a few drops of copper sulphate solution, and continue the process as described above.

For the estimation of crude fibre the following process has been standardised, and is of general application ; it is necessary to follow the details exactly as the method is empirical, and variations of the strength of the reagents or of the time of boiling affect the results. A weighed quantity of 3–5 g. of the sample is added to 20 ml. of cold 1·25 per cent. sulphuric acid in a large flask ; 180 ml. of the dilute acid are then brought to the boiling point and added to the mixture. The whole is then boiled gently under a reflux condenser for thirty minutes, during which time the flask should be rotated occasionally to remove particles from the sides. After thirty minutes the contents are filtered quickly through a No. 54 filter paper on a Buchner funnel. After filtration the suspended matter on the paper is washed back into the flask with 200 ml. of boiling 1·25 per cent. sodium hydroxide solution, measured cold. The mixture is gently boiled for thirty minutes as before and filtered through a No. 54 paper on an ordinary funnel. The crude fibre on the paper is washed with boiling water, then with 1 per cent. hydrochloric acid, again with water and finally with alcohol and ether. The crude fibre is washed off into a tared platinum basin, dried, weighed and ignited ; the weight of the ash, if any, is deduced from that of the fibre.

The estimation of the acidity of flour is not readily made in aqueous solution ; if the titration is attempted in the presence of the flour, the end-point is very uncertain, and filtration is tedious and inaccurate. A convenient method is to add to 10 g. of flour 100 ml. of neutral 90 per cent. alcohol, and allow the mixture to stand twenty-four hours with occasional shaking, then pour off the alcohol, re-extract for a few minutes with a further 20 ml. of alcohol, pouring the liquid through a filter if necessary ; titrate the alcoholic extract with 0·1 N sodium hydroxide, using phenolphthalein as indicator.

The method usually employed for the estimation of gluten is not altogether satisfactory, and consists in adding to 20 g. of flour about 15 ml. of water so as to make a stiff dough. This is allowed to stand in a beaker of water and is then squeezed in the fingers and gently kneaded under a stream of running water until all the starch is washed away and water squeezed out runs away quite clear ; no loss of gluten need occur in this operation. The residue of moist gluten is squeezed as dry as possible and weighed, or it is better to tear it into tiny pieces and dry in the water oven till the weight is nearly constant, and to return the result as dry gluten. As mentioned previously, the percentage of gluten is not a measure of the strength of the flour, although a weak flour usually contains less than a strong one. Crude gluten obtained in this way contains albumin, globulin, glutenin, gliadin and proteose.

Should it be desired to estimate the starch in flour or any cereal food, two good methods are available—the polarimetric method of Lintner, and the diastase method; the latter is probably the more exact, but the polarimetric estimation is rapid and yields quite satisfactory results. Both processes are available for other foodstuffs with slight modification where necessary.

About 5 g. of the material are triturated with 20 ml. of water, and 40 ml. of hydrochloric acid (sp. gr. 1·15) are added in small portions at a time; then the mixture is washed into a 200-ml. flask with 12 per cent. hydrochloric acid, 10 ml. of phosphotungstic acid solution (5 per cent.) are added to precipitate proteins, and the volume is made up with the precipitate to 200 ml. by the addition of more 12 per cent. hydrochloric acid. The mixture is well shaken, filtered, and the rotation of the filtrate is observed in a 200-mm. tube. The mean specific rotation of starch is taken as + 200°, but for special starches Ewers (*Z. Offent. Chem.*, 1908, **14,** 8) gives the following values at 20°, which strictly are only applicable under the special conditions set out in his paper, using potassium ferrocyanide as the clearing agent :—

Wheat . . .	183·6	Potato . .	186·5
Barley . . .	181·5	Rye . .	181·0
Rice . . .	186·0	Maize . .	184·2
Oats . . .	181·3		

When the reading is made on Ventzke's scale and 5 g. in 100 ml. have been used, the reading, multiplied by 1·912, gives the percentage directly.

O'Sullivan's diastase method may be applied as follows: 3 g. of the finely powdered material are extracted several times with ether in a Soxhlet extractor, afterwards washed on a filter first with dilute (10 per cent.) alcohol, then with strong alcohol; the residue is drained and washed into a flask with about 50 ml. of water, and heated for fifteen minutes in a bath of boiling water with constant stirring, so that all the starch is gelatinised and a homogeneous mixture is obtained. The solution is now cooled to 55°, about 0·03 g. of diastase dissolved in a little water is added, and the mixture kept at 55°–60° for an hour or more (a drop of the solution should give no blue colour with iodine) ; then the temperature is raised to 100°, the solution is filtered and the filtrate and washings are cooled and made up to 250 ml. Two hundred ml. of the filtrate are taken for the acid treatment, 20 ml. of hydrochloric acid are added, and the mixture heated in a boiling water bath for two and a half hours. The solution is cooled, then nearly neutralised with sodium carbonate, diluted to 500 ml., and the dextrose is estimated by the volumetric or gravimetric Fehling method. Dextrose multiplied by 0·90 gives the equivalent of starch.

In order to arrive at the composition of self-raising ingredients or other additions to flour, it is sometimes necessary to estimate the SO_3 in the flour, or in self-raising flour ; this is not conveniently done by an analysis of the ash. The following method is more rapid and accurate ; 10 g. of the sample are heated in a covered beaker on the water bath for one hour with about 25 ml. of hydrochloric acid, 100 ml. of water are then added, the mixture is boiled for a few minutes, and the SO_3 precipitated by the addition of barium chloride. The barium sulphate so obtained is filtered off, washed, ignited and weighed. A small amount of flocculent matter generally appears with the precipitate in the brown-coloured solution, but this all burns away, leaving the barium sulphate quite white. Ordinary flour usually contains from 0·01 to 0·03 per cent. of SO_3 ; the average figure is 0·023 per cent.

This practice of adding so-called improvers to flour, and of bleaching, has much increased in recent years. The substances added usually are potassium or ammonium persulphate, or phosphate of potassium or of calcium. These cannot be detected in the ash, since, quite apart from the decomposition of persulphate, the amount added does not usually exceed about 1 in 5,000. A good general method for detecting and estimating any mineral addition (including alum, which is rarely, or never, met with nowadays) is to shake up 50 g. of the flour with 200 ml. of *dry* chloroform or carbon tetrachloride in a separating funnel and allow the mixture to stand ; the flour floats on the liquid and any salts fall to the bottom and may be tapped off into a basin, washed with chloroform by decantation, and the residue evaporated, weighed and identified by the usual chemical methods. This process is particularly useful for the examination of self-raising flours, as the acidic material and bicarbonate are removed without interaction and may be examined for sulphates or for arsenic separately from the flour ; the method appears to have been used as long ago as 1877 by Himly (*Pharm. Handel*, No. 76). Persulphate may be detected very readily by its reaction with benzidine, with which it gives a characteristic blue colour. Make a paste of about 20 g. of flour with 20 ml. of water, pour over it an alcoholic solution of benzidine. On examination if persulphate is present, deep blue specks will be seen which have been produced by the action of the particles of persulphate in contact with the benzidine.

Another "improver" recently introduced is benzoyl peroxide which is known to be a powerful antiseptic ; it may be detected by the appearance of dark spots when the flour is made into a paste and warmed with a solution of potassium iodide ; this reaction is not, of course, specific for benzoyl peroxide. Nicholls (*Analyst*, 1933, **58,** 4) has shown that benzoyl peroxide can be separated from flour by steam distillation, reduced to benzoic acid and quantitatively determined after oxidation to salicylic acid by hydrogen peroxide.

The natural colour of flour is principally due to a hydrocarbon carotene, $C_{40}H_{56}$; this is readily bleached by chlorine, nitric oxide or ozone, all of which have been employed commercially in order to obtain the desired white flour.[1] Chlorine, if used in moderation, is not very easy to detect after a lapse of time ; there can hardly be any doubt that it is undesirable. For the detection of chlorine about 30 g. of the flour should be extracted with dry petroleum spirit ; after evaporation of the solvent Beilstein's test is applied to the residual fat. A piece of copper wire is heated in the Bunsen flame until black, then dipped into the fat and again brought into the flame ; chlorine produces a green colour at the edge of the flame. Kent-Jones and Herd (*J. Soc. Chem. Ind.*, 1930, **49,** 223T.) describe a more elaborate method for the quantitative determination of chlorine ; it is an operation which requires a laboratory quite free from fumes of hyrochloric acid.

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[1] This subject is discussed in a *Report to the Local Government Board*, 1911, No. 12 : "On the Bleaching of Flour and on the Addition of so-called Improvers to Flour," by J. M. Hamill and G. W. Monier-Williams.

Nitric oxide is absorbed by the flour and may be detected long afterwards as nitrite by the Greiss-Ilosvay reaction. The reagent consists of two solutions, namely: (1) containing 0·1 g. of α-naphthylamine dissolved in 20 ml. glacial acetic acid and 50 ml. of water, then diluted to 150 ml.; and (2) containing 0·5 g. of sulphanilic acid in 150 ml. of dilute acetic acid. A small quantity of water is added to 5 g. of the flour and 2 ml. of each of the above solutions are added; in the presence of nitrites a pink colour develops within a minute. The process is made quantitative by matching the intensity of the colour against standard solutions of sodium nitrite. As unbleached flour may sometimes absorb traces of nitric oxide from the air, the development of the pink colour should not be taken as evidence of bleaching unless the amount of nitric oxide indicated exceeds 1 part per million. Ozone often occurs in conjunction with nitric oxide produced by the electrical method. It is stated that bleaching reduces the iodine value of the fat extracted from flour from about 100 to 80 or 90.

For the determination of aneurine in flour and other substances the vitamin is extracted by suitable methods, oxidised to thiochrome by means of ferricyanide and the blue fluorescence is compared either visually or by a fluorimeter with that of a standard solution of aneurine similarly treated. The details of the method are given by a sub-committee of the Medical Research Council (*Biochem. J.*, 1943, **37**, 433). Briefly it is as follows:—

Cream 2 g. of the flour with 25 ml. of 2 per cent. hydrochloric acid, allow to stand overnight and, after shaking, centrifuge. Transfer 5 ml. of the clear liquor to a 15 ml. centrifuge tube, add 1 drop of 10 per cent. hydrochloric acid and 5 ml. of iso-butyl alcohol. Shake the tube for at least one minute, centrifuge, and note the volume of the aqueous layer.

Into a 25 ml. stoppered bottle (A) place 1·5 ml. of the aqueous layer, add 2 ml. of methyl-alcohol, 1 ml. of 20 per cent. sodium hydroxide solution, then within thirty seconds 0·15 ml. of 5 per cent. solution of potassium ferricyanide, shaking thoroughly after each addition. Then add 0·25 ml. of hydrogen peroxide (20 vol.) and allow to stand for one minute. Add 10 ml. of iso-butyl alcohol, shake vigorously for two minutes and allow to separate. In a second bottle (B) put 1·5 ml of the aqueous layer from the centrifuge tube and follow the same procedure but omitting the ferricyanide. Into a third bottle (C) put 1·5 ml. of dilute standard aneurine solution and follow the same procedure as with bottle A.

To compare the fluorescence of the thiochrome produced, draw off the aqueous layer from each bottle with a pipette, wash the iso-butyl alcohol with 3 ml. of water, remove the water, and add to each 2 ml. of ethyl alcohol. Measure 10 ml. of the clear extract from bottles A and B into separate tubes. Compare the fluorescence in a darkened room in front of the window of the ultra-violet lamp. Add quantities of the standard C to the blank B until the fluorescence observed matches that of A. At the last matching add a volume of iso-butyl alcohol to the sample A equal to that of the standard added to the blank B. To calculate the aneurine content: if x is the volume in ml. of standard iso-butyl alcohol extract (C) required and r_1 and r_2 the volumes in ml. of the extracts before and after the preliminary washing with iso-butyl alcohol, then the aneurine content of the sample

$$\frac{4x}{10} \times \frac{25}{1{\cdot}5 \times 2} \times \frac{r_2}{r_1} = \frac{10\,xr_2}{3r_1}\ \mu g./g.$$

The standard stock solution contains 20 μg of aneurine hydrochloride per ml. of 0·01 N HCl containing 25 per cent. of ethyl alcohol. Dilutions of this may be made on the day of test with 0·01 N HCl so that 1·5 ml. = 4 μg of aneurine hydrochloride. One International unit of vitamin B_1 = 3 μg of aneurine hydrochloride.

Riboflavin (vitamin B_2) can be determined fluorimetrically by the method of Hodson and Norris (*J. Biol. Chem.*, 1939, **131,** 621) or better, by the microbiological method of Barton-Wright and Booth (*Biochem. J.*, 1943, **37,** 25); a useful commentary on the subject is given by Kent-Jones and Meiklejohn, *Analyst*, 1944, **69,** 330). The micro-biological method is also applicable to the estimation of nicotinic acid (Barton-Wright, *Biochem. J.*, 1944, **38,** 314).

Calcium carbonate to the amount of 0·16 per cent. was added to flour during the war. Methods for the determination of this addition are given by Greer *et al.* (*Analyst*, 1942, **67,** 352), and by Green (*ibid.*, 1943, **68,** 111).

Flour, particularly if it contains much of the outer coat of the wheat, is liable to infestation with mites, the growth of which is favoured by a high moisture content. Details of the morphology of meal mites is given in a D.S.I.R. pamphlet by M. E. Solomon.[1]

Self-raising Flour. The manufacture and sale of self-raising flour has increased largely of recent years. It is convenient in use and incidentally enables a poor or weak flour to be strengthened and sold which would otherwise be almost unsaleable.

The usual ingredients are sodium bicarbonate and acid calcium phosphate or sometimes acid sodium pyrophosphate, in the proportion of about 1 per cent. bicarbonate and 1·5 per cent. acid phosphate. A recent order (S. R. & O. 1944, No. 44) requires that self-raising flour shall contain not less than 0·45 per cent. of available carbon dioxide and not more than 0·65 per cent. of total carbon dioxide. Both these quantities are to be determined in a prescribed manner.

The analysis of self-raising flour as to its constituents is most simply carried out by flotation on dry carbon tetrachloride or chloroform. The activating constituents from, say, 100 g., can be isolated and analysed by the ordinary methods. The amount of calcium sulphate permissible in acid phosphate has been the subject of a report to the Local Government Board (No. 13 of 1911); it is recommended that not more than 10 per cent. of $CaSO_4$ should be allowed in the acid phosphate, or that the ratio of SO_3 to P_2O_5 should not exceed 1 : 7. The estimation of SO_3 and P_2O_5 may be made on a weighed portion of the separated constituents, or the SO_3 may be estimated by treatment of the flour itself with hydrochloric acid as already described. In this case it would be necessary to allow for the SO_3 natural to the flour, say 0·023 per cent. For the estimation of available carbon dioxide or the efficiency of self-raising flour, several methods have been proposed, but the presence of such a large bulk of flour renders the direct estimation rather unsatisfactory. The official process requires that the total carbon dioxide shall be determined by weighing the gas evolved when the flour is treated with an excess of sulphuric acid at room temperature and the reaction is completed by boiling for five minutes or by means of reduced pressure. The available carbon dioxide is the difference between the total carbon dioxide and the residual carbon dioxide, determined as follows. To 5 g. of the sample add 100 ml. of water, after standing for 20 minutes at room temperature heat it on the water bath for 20 minutes and finally boil for one minute. The residual carbon dioxide is the weight of gas evolved when the

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[1] Tyroglyphid Mites. H.M.S.O., 1943.

sample so heated is treated with an excess of sulphuric acid as for the determination of total carbon dioxide. The apparatus described on p. 84 for baking powder is convenient.

It sometimes happens that an inferior grade of acid phosphate, which is so contaminated with lead or arsenic that it would not pass muster if sold alone or in baking powder, is used in a self-raising flour. In this connection it is important to note that the Royal Commission on Arsenical Poisoning, in its report in 1903, recommended that no substance used in the manufacture of food or drink should contain more than $\frac{1}{100}$ grain per pound (= 1·4 parts per million) of arsenic as As_2O_3. How far this is to be applied to an ingredient present to the extent of about 1 per cent. is perhaps open to discussion, but in view of the recommendation much importance may attach to the estimation of the arsenic in the acid phosphate which is a substance somewhat liable to arsenical contamination. The arsenic may be estimated either on the self-raising flour itself or on the separated constituents ; in the latter case it is essential to remove all traces of carbon tetrachloride or chloroform before applying the Marsh or the Gutzeit test.

When flour or similar material is to be tested for arsenic it is essential for accurate results that the organic matter be previously destroyed. Three processes are available for this purpose ; two were recommended by the Joint Committee of the Society of Public Analysts and the Society of Chemical Industry, whose report appears in the *Analyst*, 1902, and the third was recommended by a Swedish Commission in 1924.

(1) *The Acid Method.* A convenient quantity, say about 7 g., of the material in a large porcelain crucible is covered with 10–15 ml. of pure nitric acid and heated on a sand bath until nitrous fumes are no longer evolved ; then 1 ml. of sulphuric acid is added (this should be diluted with a little water to avoid too vigorous an action), and the heating is continued until all nitric acid is driven off and a dry char remains. The dry charred residue is extracted three times with water and dilute arsenic-free hydrochloric acid and filtered. The arsenic in the solution, which should be clear and colourless, is estimated either by the Gutzeit or Marsh-Berzelius process.

(2) *The Basic Method.* The finely divided material is intimately mixed with pure lime or magnesia, dried and ignited. The proportion of lime required is about 2 g. for 5 g. of dry matter, or

20 ml. of liquid. The ash is dissolved in hydrochloric acid and the solution tested as before.

The Swedish Commission published results which cast doubts on the efficiency of these methods, and the writer has shown that on certain classes of substances at least, such as fish, low results are obtained (see Cox, *Analyst*, 1925, **50,** 3), and finds that the Swedish method of destruction, followed by the Marsh-Berzelius process, gives the best results. It is now realised that it is impossible to extract all trace of metals such as arsenic from a mass of carbon, so that correct results will only follow the complete oxidation of all carbonaceous matter. The bromate titration method for the final estimation is not suitable for very minute quantities of arsenic. The purity of the reagents is a most important factor in all methods, especially in the Swedish one, in which comparatively large amounts of reagents are used.

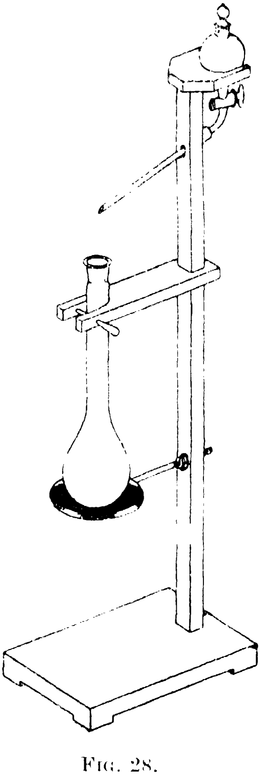

Fig. 28.

(3) *Swedish Commission Method.* A quantity of the substance is introduced into the Kjeldahl digestion flask (see Fig. 28) and thoroughly wetted with 15 ml. of dilute nitric acid. After a few minutes 20 ml. of sulphuric acid are added (less acid may be used with advantage in many cases, but the exact quantities must be noted so as to allow for any "blank" on the materials). The mixture is heated over a small flame and nitric acid is very slowly dropped in by means of the funnel illustrated at the rate of 6 to 8 drops per minute, and the heating continued until all organic matter is oxidised. The atmosphere in the flask should at no time during the oxidation be free from red fumes. When all organic matter is destroyed, the addition of nitric acid is discontinued and the heating continued until no further red fumes are seen to be evolved. After cooling, 20 ml. of saturated ammonium oxalate solution and some fragments of glass are

added, and the liquid is boiled until sulphuric acid fumes appear. After being cooled and diluted the clear colourless solution is ready for the estimation by the Marsh-Berzelius or Gutzeit methods.

The former method is more accurate, but the latter is quick and easy, yielding quite good results, and is suitable for routine purposes. Any doubtful samples should be re-examined by the Marsh test.

It is convenient here to describe the Marsh-Berzelius and the Gutzeit methods. As pure zinc and acids may now be purchased from the usual dealers, and it is only necessary to do " blanks " to ensure their freedom from arsenic, methods of purification will not be described. For a fuller account of the Marsh-Berzelius process, and the recommendations of the committee appointed by the Society of Public Analysts and the Society of Chemical Industry, see *Analyst*, 1902, **27**, 48.

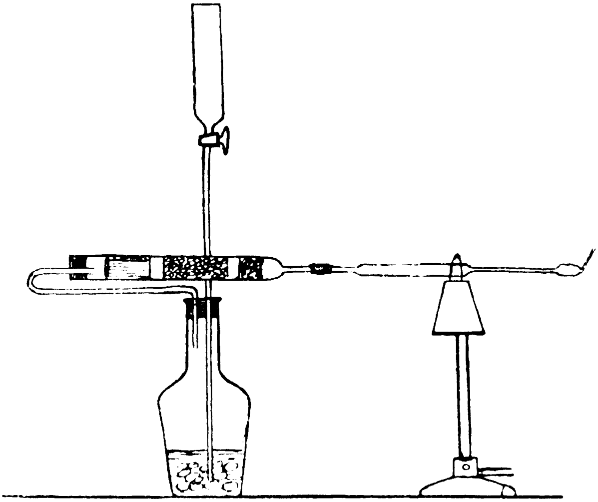

FIG. 29.

The standard bottle is of the shape figured, and of capacity about 250 ml. In this is placed a quantity of arsenic-free granulated zinc which is rendered more sensitive by amalgamation with a little cadmium. For this purpose the zinc, after washing, is covered with a 2 per cent. solution of cadmium sulphate for about ten minutes, then well washed. The flask is fitted with a rubber stopper (the stopper should be boiled with dilute sodium hydrate solution and well washed), through which passes a thistle funnel reaching to the bottom of the flask, and an exit tube connected to a wider tube in which is packed first a plug of cotton wool, then a roll of lead acetate paper, then more cotton wool previously soaked in lead acetate solution and dried, and finally granulated calcium chloride. The calcium chloride should be carefully selected, moistened with concentrated hydrochloric acid, dried, and gently ignited, as some samples absorb hydrogen arsenide so rendering the test inaccurate. After the calcium chloride is placed another plug of wool, then a piece of black rubber tubing connecting to the hard glass tube drawn out in the middle to a thickness of about 2·5 mm. A little water is run into the flask, then 10 ml. of hydrochloric acid; when the air in the flask has been displaced the jet at the end of the tube is ignited and the tube is heated to dull redness by a small flame just before the constriction. In order to secure uniform mirrors it is useful to wrap a short length of platinum wire loosely round the 2 cm. length to be heated so as to distribute the heat uniformly. Further quantities of acid are added as may be necessary to secure steady evolution of gas, and the heating is continued for fifteen minutes; at the end of this time no black or brown stain should be visible in the constricted part of the tube. If this blank test is satisfactory, the solution to be tested is mixed with about 5 ml. of the acid and run into the apparatus. A black or brown mirror of arsenic appears more or less rapidly about 1 cm. beyond the heated area; about twenty minutes are necessary for completion. The mirror so obtained is compared with standard mirrors corresponding to different quantities of As_4O_6. When a test is complete, if the mirror is not to be kept the tube may be cleaned for another experiment by heating the mirror and driving it out at the open end in the current of hydrogen. When this has been done, it is convenient to remove the tube, open the tap of the funnel and plug the rubber connection with a glass rod so that the gas generated forces the liquid back into the funnel whence it may be removed by tipping or by a pipette; this leaves the apparatus full of hydrogen and ready for a further test without the delay of displacing all the air as is necessary when the stopper has been removed. Standard mirrors are prepared containing 0·002–0·01 mg. of As_4O_6. These may be sealed off when filled with hydrogen and kept indefinitely for reference and comparison. It is desirable, especially if the apparatus has not been in use for a few days, not only to run a blank test but also to make a test adding, say, 0·002 mg. of As_4O_6 in order to satisfy oneself that the materials are sensitive, as it sometimes happens that there is a loss of sensitiveness due, possibly, to the zinc or to absorption by the calcium chloride. Some workers prefer to use sulphuric acid instead of hydrochloric acid, in which case standards should be prepared therewith, as the intensity of the mirrors so prepared differs from that of those made from hydrochloric acid. A point to be remembered is that antimony produces a mirror not unlike that of arsenic, but such mirrors are not soluble in a dilute solution of sodium hypochlorite as are those of arsenic.

The electrolytic apparatus in which the hydrogen is generated by an electric current from platinum or lead dipping into dilute sulphuric acid, is often recommended (see *Analyst*, 1923, **48,** 112) but in the writer's opinion, is not so useful for food analysis as the ordinary method because its sensitiveness is apt to become impaired in course of time. When working with liquids which froth badly, the difficulty may often be overcome, with slight loss of sensitiveness, by the addition of two or three drops of amyl alcohol.

The Gutzeit test in a modified form is the official process in the British Pharmacopœia, and, while not quite so sensitive as the Marsh-Berzelius method, is easy of application, rapid, and is particularly useful as a sorting test in routine analysis. The apparatus consists of a wide-mouthed 120 ml. bottle fitted with a rubber cork through which passes a glass tube 200 mm. in length and of diameter 8 mm. externally and 6·5 mm. internally ; the lower end is drawn out to 1 mm. diameter and a hole about 2 mm. diameter blown in the side of the tube just above the constriction. A piece of lead acetate paper is rolled round and inserted in the tube to absorb any hydrogen sulphide. Over the top of the tube is folded a circular piece of mercuric chloride paper, kept in position by a rubber ring. The mercuric chloride papers are prepared from smooth filter paper soaked in saturated mercuric chloride solution and dried ; they must be stored in the dark. It is important to use similar paper for all tests, as the intensity of the yellow stain produced by arsenious hydride varies with different papers.

For the test 10 g. of arsenic-free zinc are put in the bottle together with the solution to be tested, which should be diluted to about 50 ml. and be mixed with 10 ml. of hydrochloric acid containing 1 ml. per cent. of stannous chloride solution. The cork is at once inserted and the bottle placed in a warm place for at least forty minutes. The stain produced on the mercuric chloride cap is matched against those made from solutions containing known amounts of arsenic. The limit of sensitiveness is about 0·003 mg. Many articles of food or chemicals can be treated directly in the Gutzeit bottle by adding to a suitable quantity of the material 50 ml. of water, 10 ml. of brominated hydrochloric acid, and warming until all is dissolved, then discharging the bromine and reducing arsenic compounds to the arsenious condition by adding a few drops of stannous chloride solution. The zinc is then added and the test made as usual. It is essential to remove or oxidise all sulphur compounds ; sulphides or sulphites quite vitiate the test and produce yellow stains with mercuric chloride paper.

Standard solutions of arsenic, when very dilute, do not keep well ; it is best, therefore, to make a stock solution containing 0·1 per cent. of As_4O_6 and from this prepare a solution containing 0·01 mg. per ml., by diluting 1 ml. of the stronger solution to 100 ml.

Self-raising flours and similar mixtures are liable to contain fluorine if an impure acid phosphate has been employed in their manufacture. The Council of the Society of Public Analysts recommended (*Analyst*, 1943, **68,** 233) a maximum limit of 5 parts of fluorine per million. For the determination of fluorine 10 g. of the sample should be ashed and the fluorine determined in the ash by the method described on p. 172. It is necessary that the material to be ashed should contain a small excess of alkali, but this is usually present in self-raising flour.

The examination of other flours may be carried out on the same lines as those for wheat flour, but usually the microscope yields all necessary information. Among the more likely adulterations may be mentioned barley-meal added to oatmeal, and the addition of farina (potato starch) or rice flour to maize. Some self-raising flours contain a small proportion of maize ; in order to identify this under the microscope, slides should be prepared with oil of cloves, in which medium the hilum of maize shows well as a star or dot, but none is apparent in wheat starch. In all doubtful cases the samples should be minutely compared with slides of known starches or starch mixtures. It may be noted that oatmeal contains more protein and fat than any other flour (*cf.* table on p. 57).

Rye flour and (in this country very rarely) wheat flour are occasionally contaminated with ergot. This parasite is the sclerotium of *Claviceps purpurea* and originates in the ovary of *Secale cereale* ; it has ill-defined microscopic characters, hence cannot readily be detected by the microscope, although many staining devices have been proposed from time to time. The most certain test is by the spectroscope, and comparison may be made with a known extract of ergot ; about 50 g. of the flour are extracted with warm alcohol for several hours and the solution filtered, concentrated to about 5 ml., and examined spectroscopically. In the presence of ergot, not only will the solution have a reddish-brown colour, but characteristic absorption bands will be seen in the blue and in the green. The position of these should be noted ; those characteristic of ergot are two bands at 538 and 499 *mμ* respectively, and a faint one at 467 *mμ*. An interesting account of ergot and its alkaloids is given by Barger (*Analyst*, 1937, **62,** 340).

For the approximate estimation of ergot Bernhart (*Z. Unters. Nahr. Genussm.*, 1906, **12,** 321 ; *Analyst*, 1906, **31,** 363) gives a method of treating the flour with hydrochloric acid, carbon tetrachloride and ammoniacal copper solution, and weighing the insoluble residue.

Bread, although such an important item of food, seldom comes to the notice of the analyst ; it is not an easy material to work on, as not only are the starch grains so altered by the heat of the baking process, as to be no longer identifiable with certainty, but " improvers," such as persulphates which are decomposed, or phosphate, which would be present in too small amount for identification in the ash, can no longer be detected. Various mineral adulterants of a gross character have been described in the literature, and, if present, would readily be detected by an observant analyst ; even alum, which is so often described as being commonly added to improve the colour of white bread, is very rarely met nowadays. The following analyses by König show the usual composition of bread, but it must be borne in mind that the possible variations in different classes of bread are great. The usual methods of analysis may be applied.

The following special tests may be applied when occasion

	Fine bread.	Coarse bread.	Whole-meal.
Water . .	33·66	37·27	41·08
Protein . .	6·81	8·44	8·10
Fat . . .	0·54	0·91	0·72
Sugar . .	2·01	3·19	1·47
Starch . .	55·79	47·80	46·09
Fibre. . .	0·31	1·12	1·02
Ash . . .	0·88	1·27	1·52
	100·00	100·00	100·00

requires, and a careful microscopic examination should be made on parts selected from the centre of the loaf, as sometimes starch grains, comparatively little distorted, may be recognised. It is important always to average the sample carefully, as the varying percentage of water may make other figures seem disproportionate. The total ash should not exceed 2·0 per cent.; its estimation is lengthy and troublesome, and may be effected by extracting the char as in the case of flour, or it may be assisted by moistening the charred residue with ammonium nitrate solution and re-heating over an Argand burner; a muffle furnace should on no account be used.

For the detection of alum, 1 ml. of freshly prepared 5 per cent. tincture of logwood is mixed with 5 ml. of 10 per cent. ammonium carbonate solution and about 20 ml. of water; a piece of the bread is soaked in the mixture, then squeezed out and dried in the water oven. In the case of flour about 10 g. are made into a paste with the logwood tincture and ammonium carbonate and similarly dried. In the presence of alum a violet tint remains on drying instead of the buff colour given by pure flour; the colour is influenced by the acidity of the material, so that a comparison should always be made with flour mixed with alum and, if the indication is positive, an estimation of the total alumina present should follow. This may be done on the ash by the ordinary methods of quantitative analysis. An allowance of 0·02 per cent. should be made for the alumina naturally present in bread. When alum is suspected it is desirable also to estimate the silica present; if there be a slight excess of alumina it may be due to fragments of millstone, etc., then the proportion of silica and alumina will be equivalent, whereas if alum has been added there will be alumina in excess over the silica.

Bread almost always contains a small quantity of alcohol; when freshly prepared with yeast about 0·1 per cent. may be separated by distilling a large quantity with water. Stale bread has been shown to contain 0·05–0·9 per cent. The acidity of bread may be estimated by the method already given for flour, and calculated as lactic acid ; in fresh bread the acidity is about 0·1 per cent., but as the bread becomes stale the acidity increases up to about 0·5 per cent.

Cases of poisoning by sapotoxins in bread have been reported in France, due to the presence of corn-cockle in the flour ; these substances may be identified by their hæmolytic action. A simple method of applying the test is given by Stoecklin (*cf.* *Analyst*, 1918, **43,** 142).

For the cause of sourness or mustiness see Wright (*J. Soc. Chem. Ind.*, 1916, 1045), and diseases such as rope are discussed by Watkins (*Ibid.*, 1906, 350). The nutritive value of different varieties of bread is the subject of a report by Dr. Hamill to the Local Government Board in 1911, No. 14.

Rice

Rice is an article of food which has come under the notice of the analyst more frequently in recent years. When ground it may be examined microscopically, and is not likely to be adulterated, though it is to be borne in mind that a rice which is unsaleable in the whole condition may pass muster as satisfactory ground rice. The preparation of rice for sale by "facing" and polishing is described in Hamill's report to the Local Government Board, No. 8 of 1909. Small quantities of blue pigment and of oil are sometimes used to improve the colour and translucency ; talc may be used for "facing" ; the latter is employed in conjunction with glycerine. The practice had become so general in the trade that it was recommended only that a maximum percentage of 0·5 shall be allowed for the extraneous mineral matter, talc, on rice. In more recent years the use of such mineral additions has largely disappeared. In the case of ground rice the only method available is the examination of the ash, which is normally from 0·20–0·30 per cent., and estimation of the ash insoluble in hydrochloric acid. In this connection it may be noted that an unfaced rice generally gives a readily fusible ash; it fuses under ordinary conditions on an Argand burner, yielding a glassy residue, whereas with a faced rice the ash, containing more silica, is not readily fusible, and is flakey. In a ground rice any excess of ash over 0·30 per cent. may be regarded as evidence of facing, and confirmation should be sought by estimation of the ash insoluble in hydrochloric acid. For the determination of the amount of facing on whole grains of rice, the method of Krźiźan (*Z. Unters. Nahr. Genussm.*, 1906, **11**, 641) is recommended and is carried out by shaking the grains with a dilute solution of ammonia and hydrogen peroxide and warming, the talc being completely removed from the surface by the gas evolved. The liquid containing the talc and some of the meal in suspension is poured off, treated with hydrochloric and chromic acids, and boiled; the meal is thus oxidised and destroyed, and the residue may be collected on a filter, ignited and weighed.

The physiological importance of the outer coating of rice grains and the ill effects of the polishing process on the anti-neuritic vitamin B_1 content is clearly shown by Chick and Hume (*Proc. Roy. Soc.*, 1917, B. **90,** 44, 60). Chemically there is an important difference between hulled and milled rice; the former is rich in phospho-protein and lecithin, which is of high nutritive value. Whole rice contains about 0·268 and 0·25 per cent. of phosphorus (P_2O_5) and potassium (K_2O) respectively, whereas polished rice only contains 0·09 and 0·07 per cent. of these substances.

Blancmanges. These products usually consist of a starch basis mixed in many cases with colouring, despite the name, and flavouring essences, vanilla, raspberry, strawberry, and the like. Some well-known brands are made entirely from maize cornflour, others from a mixture with 10 to 20 per cent. of farina or of arrowroot, as these starches impart a gelatinous character to the product. Two vanilla blancmanges were found by the writer to contain:—

	I.	II.
Maize cornflour . . .	50 per cent.	85 per cent.
Potato flour	—	15 ,,
Essence of vanilla . .	Trace.	Trace.
Colouring matter . .	0·01	0·02
Sugar	50	—

Chocolate blancmange is made with varying percentages, from 5–10 of cocoa, about 0·03 per cent. of chocolate colouring, some vanilla essence and, either in the sample or subsequently to be added by the cook, about 50 per cent. of sugar. For the determination of cocoa matter in blancmange powders or similar products the following method due to Moir and Hinks (*Analyst*, 1935, **60,** 439) is available. Moisten 20 g. of the sample in a mortar with alcohol (80 per cent. by vol.) and rub to a smooth paste. Transfer to a flask with the addition of a further 200 ml. of alcohol (80 per cent.), add 2·5 g. of freshly-ignited magnesium oxide and digest for one and a half hours under a reflux air-condenser in a boiling water-bath. The flask should be shaken frequently to prevent the mass from clotting. Filter through a Buchner funnel and re-digest the residue twice with 100-ml. quantities of 80 per cent. alcohol. If the fat is to be determined, collect the alcoholic filtrates in a large measuring flask, make up to the mark with alcohol, and shake vigorously to maintain the fat in an emulsified state throughout the liquid. Remove an aliquot portion for the determination of fat. Evaporate the remainder on the water-bath to about 100 ml., replacing the alcohol by water. Add 2 to 3 drops of dilute hydrochloric acid. Transfer with hot water to a 150-ml. measuring flask, and clarify by adding first 5 ml. of zinc acetate solution, and then, after mixing, 5 ml. of potassium ferrocyanide solution, then make up to 150 ml. with water.

A larger flask and more zinc acetate and potassium ferrocyanide may be used if the amount of fat is large, as it may be in confectionery products.

Filter, evaporate a measured quantity of the filtrate to small bulk (10 to 20 ml.) and extract with chloroform. If the amount of alkaloid is small (2 to 4 mg.), three 50-ml. quantities of chloroform are sufficient. Otherwise five extractions are necessary.

Reserve a portion of the chloroform extract for the qualitative murexide test. Distil the chloroform from the remainder, dissolve the residue in hot water, and determine the nitrogen either by digestion and distillation into N/10 acid, as with cocoa, or by digestion and nesslerisation. In the latter case add 0·1 g. of sucrose and 3 ml. of concentrated sulphuric acid to the residue

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[1] *Zinc Acetate Solution.* 21·9 g. of crystallised zinc acetate, $Zn(C_2H_3O_2)_2.2H_2O$, and 3 ml. of glacial acetic acid, in water, made up to 100 ml.

Potassium Ferrocyanide Solution. 10·6 g. of crystallised potassium ferrocyanide in water made up to 100 ml.

in a small Kjeldahl flask. Heat over a small flame till frothing ceases, add 0·01 g. of selenium,[1] and continue heating till the solution is colourless and then for half an hour longer. Cool, dilute, neutralise with caustic soda and make up to 100 ml. Determine the ammonia- or nitrogen-content of this solution by nesslerisation. Correct the result obtained for the ammonia-content of the digestion reagents. Use the factors of 3·26 and 100/3·15 for conversion of nitrogen into alkaloid, and alkaloid into dry fat-free cocoa-matter respectively.

Among the essences used for flavouring blancmanges and the like may be mentioned amyl acetate, which with ethyl acetate resembles artificial strawberry essence, or, used alone, suggests pears. Essence of lemon is commonly an alcoholic solution of oil of lemon, and essence of vanilla an alcoholic extract of the vanilla bean, but it may be adulterated with coumarin. Ionones also enter into the composition of artificial fruit essences used in flavouring this class of product.

Custard powders are composed usually of maize cornflour with an appropriate yellow dye. It is doubtful whether egg enters into the composition of any of the well-known brands. Should it be necessary to investigate this point, the estimation of organically combined phosphoric acid and protein as discussed under egg substitutes will yield the desired information. The starches present may be identified in the usual way by the microscope. When examined in clove oil, the hilum of wheat flour is invisible, whereas that of maize is seen as a black spot or star (Bevan, *Analyst*, 1900, **25,** 316).

A case heard in 1925 (see *Analyst*, **50,** 19) suggests that an offence against the Sale of Food and Drugs Act is committed if a starch preparation containing no egg is sold as "custard" not qualified by the word "powder." Egg powder in mixtures of this kind can often be detected by flotation, and its amount determined from the organic phosphorus (see p. 88).

Pearl Barley consists, or should consist, of the whole grain of the barley, polished after removal of the husk. Like rice, it is sometimes faced with talc, the purpose of which has been variously stated to be improved appearance or protection from attack by weevils. The latter suggestion is not borne out by experiments by Liverseege and Hawley (*J. Soc. Chem. Ind.*, 1915, 203). Talc,

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[1] The use of selenium as catalyst appears to be essential when applying the Kjeldahl process to small quantities of theobromine.

if present, may be estimated by Kržižan's process (p. 76); foreign starches should be sought for by means of the microscope, and sulphur dioxide, which is not permitted by the regulations, by the method given on p. 32.

Soya Meal. Although soya is not a cereal but a pulse, it is frequently found in admixture with cereals for special purposes, and is often present in sausages or similar mixtures. The composition of soya meal is shown in the following table :—

	Soya beans.	Soya meal.	Soya flour.
Water	10·5	8·1	6·5
Oil	18·0	7·5	20·5
Proteins . . .	35·0	42·1	42·5
Fibre	4·6	5·0	3·5
Ash	4·8	5·5	5·0
Carbohydrate . .	27·1	31·8	22·0
	100·0	100·0	100·0
Lecithin P_2O_5 per cent. .	0·26	0·30	0·28

Soya is peculiar in that it is almost entirely free from starch. The composition varies rather widely depending mainly on the amount of oil which has been extracted. The main constituents are protein in the form of a globulin called glycinin, and a quantity of carbohydrate, which is largely galactan. There is also some lecithin and enzymes, notably urease. The pulse proteins have peculiarly high biological values and, like gelatine, actually improve the biological value of proteins such as those of wheat and other cereals (*cf.* Drummond, *J. Roy. Soc. Arts.*, 1942, **90,** 431).

The detection of soya usually depends upon the microscopic identification of the characteristic palisade cells. These are best seen on the fibrous residue separated by boiling the sample with dilute acid and alkali as in the determination of fibre. If the soya product has not been heated the urease test is useful. This enzyme liberates ammonia from urea. To about 5 g. of the material add 5 ml. of water containing 0·1 g. of urea. Put a piece of red litmus paper in the top of the tube, cork it lightly and incubate at about 40° C. for two or three hours. If urease is present ammonia will be liberated and the litmus paper turns blue. In the absence of egg products or other lecithin the amount of soya can be approximately determined from the organic P_2O_5 as described on p. 88, since soya contains about 2·5 to 3 per cent. lecithin, equivalent to approximately 0·25 per cent. P_2O_5.

The oil extracted from soya meal has a somewhat characteristic taste, and a tendency to rancidity. It shows the following analytical constants :—

Specific gravity	0·922–0·926
Refractive index (40°) . . .	1·467–1·468
Iodine value	130–136
Saponification value . . .	190–194
Reichert value	4–5

CHAPTER III

BAKING POWDER, GOLDEN RAISING POWDERS, EGGS, LIQUID AND DRIED EGGS

BAKING powder consists essentially of an acid or acid salt mixed with an excess of sodium bicarbonate and diluted with starch. The starch used is generally ground rice with or without an admixture of maize; the small pointed grains of rice starch are said to aid the steady evolution of carbon dioxide which takes place on the addition of water. It is usual for the ground rice to be thoroughly dried by passing through a heated chamber before mixing with the aerating substances; this, to some extent, prevents the rapid deterioration which takes place if the powder is damp.

Ground rice contains on the average 13 per cent. of moisture, and a freshly prepared good baking powder, consisting of about 40 per cent. of rice, shows on analysis some 4 to 5 per cent. of water, corresponding to 10 per cent. in the rice. The alkaline constituent is bicarbonate of soda and the composition of the powder should always be such as to leave a small excess of this substance after the reaction. The acidic constituents in general use are cream of tartar, tartaric acid, and acid phosphate of lime or acid sodium pyrophosphate. Alternatively, acid potassium sulphate is employed in a few makes; organic acids such as adipic and other carboxylic acids have also been tried. Alum is used in several brands sold in America, but is not used in this country. Tartaric acid reacts more quickly with bicarbonate than does cream of tartar; in the South of England the better brands of baking powders are made with tartaric acid with a small quantity of cream of tartar; in the North of England and in Scotland, on account of slower methods of baking, a slow-acting powder is generally preferred, such as is made from cream of tartar with a small proportion of tartaric acid. Acid calcium phosphate is inferior to tartaric acid or cream of tartar; powders prepared from it deteriorate more rapidly, evolve their carbon dioxide more slowly and less completely; also they are liable to contain appreciable traces of arsenic and of calcium sulphate. It is a trade practice to market acid phosphate, often under a fancy name such as " phosfacreme " or " cream powder," diluted with 7 or 10 per cent. of starch, with the object of making its acidity equivalent weight for weight to that of cream of tartar, for which it is substituted. The composition of some commercial acid phosphates for baking powder was found to be as follows :—

	1	2.	3.
Moisture	9·70	7·45	5·60
Dry starch . . .	7·00	8·50	10·35
Calcium sulphate . .	7·10	3·05	1·85
Acid calcium phosphate, $CaH_4P_2O_8$ . . .	55·50	60·50	62·60
Tribasic calcium phosphate $Ca_3P_2O_8$ . . .	20·20	20·50	19·30
Insoluble siliceous matter .	0·50	Trace.	0·30
	100·00	100·00	100·00

Hamill (L.G.B. Report, New Series, No. 46, 1911) points out that some samples contain a very large quantity of calcium sulphate ; it is recommended that not more than 10 per cent. be permissible in acid phosphate (see p. 66).

Standards for baking powder generally refer to the amount of " available " carbon dioxide. Since baking powders almost invariably contain an excess of bicarbonate, and sodium bicarbonate decomposes on heating, the question arises as to what is " available " carbon dioxide. When a solution of sodium bicarbonate is boiled decomposition takes place, but the reaction does not proceed to completion in a limited time ; in half an hour the reaction may be represented by the equation :—

$$4NaHCO_3 \longrightarrow 2NaHCO_3 . Na_2CO_3 + H_2O + CO_2,$$

which means that some 25 per cent. of the carbon dioxide present as bicarbonate is evolved, under conditions roughly equivalent to those of the baker's oven. A further quantity of gas is evolved on the addition of a mineral acid.

The problem has been solved in this country by the publication of a Standard for baking powder (S. R. & O. 1944, No. 46) which requires that baking powder shall yield not less than 8 per cent. available carbon dioxide and not more than 1·5 per cent. residual carbon dioxide. Available carbon dioxide is defined as the difference between the total carbon dioxide and the residual carbon dioxide. Directions are given for the determination of total and residual carbon dioxide.

The composition of some well-known brands of English and American baking powders is given in the following table :—

	1.	2	3.	4.	5.	6.	7
Moisture . .	6 50	3·67	4·67	3·40	10·10	7·40	2·97
Tartaric acid .	17·57	6·00	33·65	2·92	—	—	—
Cream of tartar	3·27	38·04	—	40·00	—	—	—
Bicarbonate of soda .	23·68	23·56	40·12	24·02	26·50	22·50	29·08
Acid phosphate	—	—	—	—	45·20	36·30	2·75
Soda alum .	—	—	—	—	—	—	29·40
Dry starch .	48·98	28·73	21·56	29·66	18·20	33·80	35·80
	100·00	100·00	100·00	100·00	100·00	100·00	100·00
Available CO_2	10·99	12·00	19·73	10·64	9·54	8·50	9·84

The complete analysis of baking powder includes the estimation of available and total carbon dioxide, moisture, tartaric acid, potash, phosphoric acid, sulphates, lime, alumina, sodium bicarbonate and starch ; the sample should be examined for arsenic and, if it is phosphatic, for fluorine.[1] In many cases it suffices to estimate the water-freed and total carbon dioxide, test for arsenic, also qualitatively for tartrate or phosphate.

For the purpose of calculating the composition of a baking powder the following equations may be used.

(1) $3\,CaH_4P_2O_8 + 8\,NaHCO_3 \longrightarrow Ca_3P_2O_8 + 4\,Na_2HPO_4 + 8\,CO_2 + 8\,H_2O$

(2) $Na_2H_2P_2O_7 + 2\,NaHCO_3 \longrightarrow Na_4P_2O_7 + 2\,H_2O + 2\,CO_2$

(3) $K_2SO_4 + Al_2(SO_4)_3 + 6NaHCO_3 \longrightarrow Al_2(OH)_6\ \ 6CO_2 + 3Na_2SO_4 + K_2SO_4$

Moisture in baking power or similar products cannot be estimated by drying in the water oven since at this temperature sodium bicarbonate undergoes partial decomposition ; a thin layer of the powder should be exposed for at least twenty-four hours over concentrated sulphuric acid in a vacuum desiccator.

For the determination of carbon dioxide the baking powder is decomposed first with water then with acid, and gas evolved is dried and absorbed either in potassium hydroxide solution or soda lime, and weighed. It is necessary to determine total carbon dioxide, which is the weight of carbon dioxide evolved when

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[1] Suggested limits for fluorine are 200 p.p.m. in acid phosphate, 70 p.p.m. in baking powder, and 50 p.p.m. in golden raising powder (*Analyst*, 1943, **68**, 233).

the baking powder is treated with excess of dilute sulphuric acid at room temperature, and the evolution completed either by boiling for five minutes or by means of reduced pressure. The residual carbon dioxide is determined by treating 2 g. of the powder with 25 ml. of water and evaporating the mixture to dryness on a boiling water bath, subsequently adding a further 25 ml. of water and evaporating again. The residual carbon dioxide is the weight of carbon dioxide evolved when the sample so treated is further treated with excess of dilute sulphuric acid at room temperature, and the evolution is completed either by boiling or by means of reduced pressure.

Available carbon dioxide is the difference between the total and residual carbon dioxide.

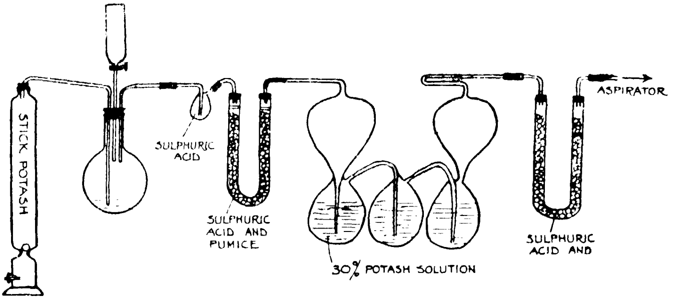

Fig. 30. Determination of CO_2 in baking powder.

A convenient form of apparatus is shown in the accompanying figure. After testing the joints of the apparatus by turning on the aspirator with the air inlet closed, about 15 ml. of water are run in slowly ; then, when the evolution has subsided, CO_2-free air is admitted so as to bubble through the water in the flask, thus removing dissolved CO_2, and washing all the gas into the absorption bulbs. A U tube containing Sofnolite or Carbosorb is a better absorbent than a potash bulb, and has the advantage that the final H_2SO_4 tube can be omitted. With a tartaric acid powder about 2 litres of air in all is sufficient, but with a cream of tartar or phosphatic powder the action is much slower and at least 4 litres should be aspirated. After weighing the water-free CO_2, a little dilute hydrochloric acid may be run in and the acid-freed CO_2 aspirated off and weighed as before.

If desired a small condenser may be interposed between the reaction flask and the drying tubes so that the water may be kept just at the boiling point for half an hour.[1] It will be found that the water-freed CO_2 plus one-quarter of the acid-freed CO_2 is equivalent to the available CO_2, as this corresponds to a 25 per cent. decomposition of the excess of bicarbonate.

For the estimation of potassium 2 g. are charred over an Argand burner and the ash thoroughly extracted with dilute acid ; after the removal of phosphate and lime, if any, the estimation of K_2O is carried out by the perchlorate or platinum chloride method in the usual way. In the calculation of K_2O to cream of tartar an allowance should be made for the K_2O in the ash of rice flour at the rate of about 0·4 per cent. on the rice ; on an average cream of tartar powder an allowance of 0·2 per cent. K_2O is near enough for practical purposes. In a powder prepared from tartaric acid only, the alkalinity of the ash calculated as $NaHCO_3$ should agree with the bicarbonate calculated from the total CO_2, but if the powder has deteriorated through storage some sodium tartrate will be present, which will make the alkalinity of the ash as $NaHCO_3$ higher than the bicarbonate calculated from the carbon dioxide ; on this account it is necessary to estimate the K_2O unless it is definitely known that no cream of tartar enters into the composition of the sample.

Tartaric acid is quickly recognised by the resorcinol test. To 2 ml. of sulphuric acid, add a few mg. of resorcinol and a drop or two of the solution containing tartaric acid. Heat to about 140° ; a bright red colour quickly appears in the presence of tartaric acid.

To estimate the tartaric acid—including cream of tartar—take 5 g of the powder, add 10 ml. of water, followed by 5 ml. of strong acetic acid. Then add 5 ml. of saturated solution of potassium acetate, stir well and add 100 ml. of alcohol. Allow the mixture to stand for a few hours ; filter off the starch and cream of tartar on a Buchner funnel, wash the precipitate with about 150 ml. of alcohol, then transfer it to a beaker, add hot water and titrate the cream of tartar with 0·1 N sodium hydroxide, using phenolphthalein as indicator. Calculate the equivalent of the K_2O found to cream of tartar ; then the difference between the acidity of this and the total cream of tartar gives the equivalent of tartaric acid.

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[1] A small piece of cetyl alcohol may with advantage be introduced ; it prevents frothing.

Another possible acidic ingredient is adipic acid. This acid which has m.p. 153° may be recognised by heating a few mg. with resorcinol and a few drops of sulphuric acid in a test-tube to about 160° for a few moments. An orange colour develops and on cooling and pouring the product into a small excess of sodium hydroxide solution a pink-violet fluorescent colour is produced.

In the case of a phosphatic powder it will be necessary to estimate calcium and phosphoric acid. For this purpose 1 g. is dissolved in hot dilute nitric acid, boiled for at least half an hour to convert pyrophosphate into orthophosphate, and filtered ; the filtrate is divided into two portions ; to one aliquot part is added excess of molybdenum mixture, the solution warmed to 70° and allowed to stand, then the ammonium phospho-molybdate is filtered off and washed *with 2 per cent. solution of potassium nitrate until the wash water is no longer acid. To the precipitate with the paper* is added a small excess of 0·5 N sodium hydroxide solution and the excess titrated back with 0·5 N nitric acid, phenolphthalein being used as indicator (1 ml. N $=$ 0·00307 P_2O_5).[1] Alternatively, the ammonium phospho-molybdate may be dissolved in 2 per cent. ammonia and the phosphoric acid precipitated as magnesium ammonium phosphate by the addition of magnesia mixture ; in this case the precipitate is washed with ammonia, dried, ignited and weighed as $Mg_2P_2O_7$. The lime in the other portion of the solution is estimated by making the cold solution just neutral with ammonia, then adding a considerable excess of ammonium acetate and acetic acid and boiling, then adding ammonium oxalate ; the precipitate of calcium oxalate (which may contain important traces of iron and alumina) is filtered off, washed and titrated with 0·1 N potassium permanganate as usual.

Estimate the SO_3 present by boiling 3 g. with dilute hydrochloric acid to hydrolyse the starch, then precipitate as $BaSO_4$ in the usual way. Calculate the CaO equivalent to the $CaSO_4$ present and deduct it from the total CaO found. The composition of the phosphate can then be calculated from the equations :

$$\left.\begin{aligned} 0{\cdot}607\,x + 0{\cdot}458\,y &= A \\ 0{\cdot}234\,x + 0{\cdot}543\,y &= B \end{aligned}\right\}$$

where x and y are the percentages of $CaH_4P_2O_8$ and $Ca_3P_2O_8$

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[1] For discussion of the sources of error and details of the titration method, *cf.* Richards and Godden, *Analyst*, 1924, **49**, 565.

respectively and A and B are the percentages of P_2O_5 and CaO (after deducting CaO present as $CaSO_4$).

Starch in baking powder is usually taken by difference, but may be determined if desired by dissolving the salts in the powder in a minimum quantity of cold dilute hydrochloric acid, filtering off the crude starchy matter, and weighing it directly. If necessary it can also be determined, either by the polarimetric or by the diastase method (p. 61).

For the estimation of arsenic, to 7 g. are added 40 ml. of water containing a trace of bromine; when the effervescence has subsided, 10 ml. of hydrochloric acid are introduced and the mixture warmed until the starch is dissolved; the excess of bromine is removed by stannous chloride and the B.P. Gutzeit test completed. A standard stain (0·01 mg. As_2O_3) corresponds to $\frac{1}{100}$th grain of arsenious oxide per pound.

In powders not containing phosphate, lead may be determined directly by dissolving the ash from 10 g. of sample in a small excess of nitric acid, then filtering and adding excess of ammonia and 1 ml. of potassium cyanide solution, then 2 drops of solution of sodium sulphide and diluting to 50 ml. The colour is matched against a "blank," containing the same quantity of reagents, by adding the requisite quantity of B.P. standard lead solution. In phosphatic powders the lead should be determined by the general method given on p. 189.

Golden Raising Powder (formerly called Egg Substitute)

These mixtures which were at one time called egg powders or egg substitutes are in almost all cases coloured baking powders quite devoid of eggs or egg products. They may have recently been standardised and are required to yield not less than 6 per cent. of available carbon dioxide with not more than 1·5 per cent. of residual carbon dioxide. The terms available carbon dioxide and residual carbon dioxide, have the same meaning and are determined in the same manner as described under baking powder. The analysis of golden raising powder is carried out exactly as described under baking powder. Some few brands, however, are advertised as containing eggs or dried egg, hence further estimations may be needful to ascertain whether this be true or not and what proportion of egg may be present. An examination with the microscope will generally show whether or not there is any dried egg. Also advantage can be taken of the fact that dried egg is lighter than ground rice or other cereal, so if some of the powder be added to a mixture of petroleum spirit and carbon-tetrachloride of sp. gr. 1·275 the dried egg will float and the baking powder mixture will sink. For the determination of the quality of egg the estimation of organic phosphorus, nitrogen, and ether extract affords the most useful data. Dried egg contains some 7 per cent. of nitrogen, and as baking powder or ground rice may contain from 0·5 up to 0·7 per cent. of nitrogen it would not be possible to detect with certainty from the nitrogen figure, say, 3 per cent. of egg. The ether extract and organic phosphorus afford more information, as will be seen from the following figures (taken from Beach, Needs and Russell, *Analyst*, 1921, **46,** 279).

	Egg substitute.				Egg powder.		Dried egg.[1]		Rice.	Rice + 1 per cent. dried egg.
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
	%	%	%	%	%	%	%	%	%	%
Ether extract .	0·27	0·80	0·30	1·13	0·90	0·34	50·61	39·07	0·35	0·76
Protein .	3·62	5·0	3·44	7·06	5·62	6·02	43·5	46·5	7·00	7·62
Organic P_2O_5 .	0·007	0·017	0·009	0·006	0·013	0·010	1·28	1·27	0·022	0·053

[1] For the fuller composition of dried eggs, see p. 92.

For the estimation of organic phosphorus contained in egg as phosphatides, lecithin and cephalin, the powder is extracted first with dry ether and then with hot alcohol ; the alcoholic solution is evaporated to dryness and the residue heated with 2 ml. each of nitric and sulphuric acids until dissolved, then diluted, and the phosphoric acid precipitated by molybdenum mixture in the usual way. The precipitate may be washed and titrated, or, when the amount is quite small, it should be collected on an asbestos filter, washed with ammonium nitrate solution, transferred to a beaker with a little dilute sulphuric acid ; 0·5 g. of stannous chloride is added ; after half an hour the liquid is filtered and the blue colour matched against a standard solution of phosphoric acid under similar conditions.

Eggs

Eggs. So far as the analyst is usually concerned, the term eggs generally implies those of the hen or duck, though those of certain sea birds are said to enter into the composition of imported dried or liquid eggs. The exact constituents of eggs, both of the white and the yolk, remain not yet fully known; four distinct proteins are recognised in the white, namely, ovomucin or globulin, ovalbumin, ovomucoid and conalbumin, and in the yolk livetin and vitellin. There are also present in the yolk lecithin, egg oil, and an unidentified carbohydrate, besides water and the usual inorganic salts.

The yolk of egg has sp. gr. 1·026, the white 1·055 and the whole egg (not including shell) 1·048. The average composition is:—

	Hen.		Duck.[1]	
	per cent.		per cent.	
Shell . . .	12·0		11·1	
White . . .	55·0		51·0	
Yolk . . .	33·0		37·9	
	White.	Yolk.	White.	Yolk.
	per cent.	per cent.	per cent.	per cent.
Water . . .	87·2	53·1	87·5	45·1
Ash	0·6	2·0	0·7	1·5
Total protein . .	11·5	14·4	10·7	16·3
Fat and lecithin .	0·10	29·3	0·03	36·50
Undetermined . .	0·60	1·20	1·07	0·60

[1] According to Plimmer.

The yolk of the egg is surrounded by the vitelline membrane, which is semi-permeable. The proportion of water in the yolk and white of the egg varies with the age, apart from the question

Number of samples.	Water per cent. in white.			Water per cent. in yolk.		
	max.	min.	average	max.	min.	average
24 fresh eggs .	88·07	85·34	87·13	55·92	49·83	53·82
10 eggs preserved 4 months .	—	—	86·30	—	—	58·81

of decomposition, as water diffuses slowly from the white to the yolk. The figures shown above illustrate this point, which is of interest as it affords help in the discrimination of fresh eggs and those preserved by water-glass. The water content of the yolk increases by about 5 per cent. in four months.

Useful methods for the examination of the shells of eggs suspected of being preserved are given by J. R. Nicholls (*Analyst*, 1931, **56,** 383).

According to Osborne and Campbell (*J. Amer. Chem. Soc.*, 1900, **22,** 422) and Plimmer (*J. Chem. Soc.*, 1908, **98,** 1500) the proportions of the proteins of egg and their nitrogen contents are :—

	Per cent.		Per cent.
Protein of white :—			
Ovomucin	7	Containing N	14·7
Ovalbumin	37	,,	15·5
Conalbumin	34	,,	16·1
Ovomucoid	22	,,	12·4
Protein of yolk :			
Vitellin [1]			5·2
Livetin [2]	—	,,	15·0

Their relative proportions are subject to some variation, but based on these figures the total nitrogen in the protein matter of the white is 14·87 per cent., so that in the estimation of nitrogen by the usual Kjeldahl method the appropriate factor is 6·70 instead of 6·25. In the yolk the mean nitrogen factor is 6·62. For the whole egg the factor 6·68 should be used.

The fatty matter extracted from eggs depends to a small extent upon the solvent used ; there is present in the yolk an oil having the characters given below, and certain phosphoproteins which are extracted by solvents. That the different results obtained by using various solvents are not due to differing amounts of protein being dissolved is shown by the fact that the ratio N : P_2O_5 in the extracts is constant. For the estimation of fat in liquid eggs Gottlieb's process is most convenient and gives good results ; it is applied exactly as described on p. 227 for condensed milk.

The characteristics of egg oil are somewhat influenced by the method of extraction ; the following results are characteristic :—

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[1] Containing 2·29 per cent. of P_2O_5.
[2] Containing 0·23 per cent. of P_2O_5.

Egg Oil

Refractive index at 20° . . .	1·4655–1·4670
Saponification value . . .	188–198
Iodine value	60–70
Unsaponifiable matter . . .	up to 4 per cent.
Phosphoric acid, P_2O_5 . . .	1·4 ,, ,,

Frozen whole Egg is imported on quite a large scale ; as it has been frozen at a temperature below – 6°, the protein has been denatured and, upon thawing, the product does not resume a clear condition like unfrozen egg. The product should contain less than 75 per cent. of water, more than 10 per cent. of egg oil, and the oil should show an acidity not greater than 3 per cent. as oleic acid. The most important chemical criterion of condition, besides the total solids, fat content and acidity, is the absence of indol and skatol. These are tested for by diluting about 200 g. with 500 ml. of water previously acidified with 40 ml. of 5 per cent. acetic acid, then steam distilling until about 300 ml. have passed over. The distillate is shaken out with ether in a separating funnel, the ether is evaporated with about 3 ml. of water until it has nearly all disappeared, when 10 ml. of water are added. This dilute solution is then tested for indol and skatol by means of vanillin. To 5 ml. of solution add 5 drops of 5 per cent. alcoholic solution of vanillin and 2 ml. of sulphuric acid ; an orange colour soluble in chloroform indicates the presence of indol. If skatol is present a deep red or violet colour is given by the same test. Skatol is also recognisable by the violet colour produced on adding a few drops of dimethylaniline and 4 ml. of sulphuric acid.

Dried egg has become an important article of commerce in recent years. It sometimes happens that dried milk or casein, or starch is introduced to aid in the desiccation, which may be done by spraying or by hot rollers. These additions may readily be detected from the analytical results. Boric acid is now not often present in the dried product, although formerly it was present in liquid imported eggs in amounts up to 2 per cent. It is now prohibited by the Preservative Regulations.

The analysis of dried egg can be carried out by the ordinary methods. Water content is important, it may be from 4 to 6 per cent. in freshly prepared samples or rather more in older material ; the determination is made by drying at 103° for six hours.

Nitrogen is determined by the Kjeldahl process, the factor N × 6·68 being used for the calculation of total protein or albumin. The fat is extracted with dry chloroform in a Soxhlet extractor ; the organically combined phosphoric acid may be estimated by heating the chloroform extract with concentrated nitric acid and 2 ml. of sulphuric acid until dissolved ; the solution is then largely diluted with water, molybdenum mixture is added, and the mixture heated to 70°. The yellow precipitate is collected and titrated in the usual way or may be dissolved in ammonia, magnesia mixture added and the phosphoric acid weighed as $Mg_2P_2O_7$.

	Dried eggs.				Liquid eggs.		
	1.	2.	3.	4.	5.	6.	7.
Moisture . .	4·61	6·25	7·17	8·50	69·95	73·02	77·28
Protein . .	40·81	40·44	49·78	45·73	13·81	10·31	10·69
Lecithin and fat	44·30	45·12	31·70	35·45	12·77	13·48	7·46
Ash . .	4·21	3·42	3·92	4·27	2·43	2·59	1·20
Undetermined .	6·07	4·77	7·43	6·05	1·04	0·60	3·37
	100·00	100·00	100·00	100·00	100·00	100·00	100·00
Boric acid .	Absent	Absent	Absent	Absent	1·95	1·89	—
Organic P_2O_5 (per cent.) .	1·30	1·37	1·18	1·25	—	—	—

Nos. 3 and 4 contain added casein ; No. 7 is ostrich egg.

The estimation of boric acid in egg products is rendered less simple by reason of the phosphates present, which must be removed before the boric acid can be titrated in the presence of glycerol in the customary way. The usual method of separation depends upon the fact that calcium borate is soluble in alkaline solution, whilst calcium phosphate is not ; but, as is shown by Monier-Williams (*Analyst*, 1923, **48,** 413), if an excess of alkali is used there is some precipitation of calcium borate with the phosphate and hence a low result. The following procedure avoids this error. Add to about 10 g. of sample 5 ml. of 20 per cent. sodium hydroxide solution, evaporate to dryness and char, extract the charred mass with a small quantity of hot water, filter, then ignite the filter and its contents at a higher temperature, re-extract with water containing a little dilute sulphuric acid. The mixed filtrate contains all the boric and phosphoric acids and some calcium sulphate in solution, and is carefully neutralised with sodium hydroxide, methyl red being used as indicator. Sufficient magnesia mixture is now added to precipitate the whole of the phosphoric acid, but avoiding any large excess ; the mixture is diluted to 100 ml. in a measuring flask, stoppered and vigorously shaken for five to ten minutes and filtered. To 50 ml. of the filtrate are added phenolphthalein and an exess of sodium hydroxide, and the solution boiled to expel ammonia (any precipitate of magnesium salts may be ignored) ; then it is made acid with hydrochloric acid and neutralised with 0·1 N sodium hydroxide, about 25 ml. of neutral glycerol are added, and the boric acid titrated with the 0·1 N sodium hydroxide, 1 ml. of which is equivalent to 0·0062 g. H_3BO_3.

Solubility of Dried Egg. The solubility of dried egg is important as it gives a measure of its quality. A well-prepared fresh sample will show a solubility of upwards of 90 per cent., some being as high as 98 per cent. Accurate methods for the determination of solubility have been studied by J. R. Hawthorne (*J. Soc. Chem. Ind.*, 1944, **63,** 6) ; for practical purposes the following method works quite well.

One g. of the sample is weighed into a dry beaker, 10 ml. of water are added and the mixture is allowed to soak for three hours, then heated on a water-bath at 50° for half an hour. The mixture is transferred to a centrifuge tube without washing the beaker, and spun for at least a minute. The upper layer is poured off without disturbing the sediment, the beaker is then washed with 10 ml. of water at 50°. The water is poured into the centrifuge tube and after mixing is spun as before. The upper layer is poured off and the sediment is washed with a further 10 ml. of water. After this the sediment is transferred to a tared filter or basin, dried and weighed.

CHAPTER IV

FRUITS, VEGETABLES, DRIED FRUITS, TOMATO PUREE, LEMONADE, LIME JUICE, FRUIT SYRUPS, ETC.

THE analysis of fresh fruit and vegetables in terms of water, mineral matter, fibre, carbohydrates and protein is not very frequently required, nor does it present any special difficulties. Much data concerning the average composition of these foods is readily available in the M.R.C. Report on the "Nutritive Value of Fruits, Vegetables and Nuts," by McCance, Widdowson and Shackleton,[1] and in "The Chemical Composition of Foods," by McCance and Widdowson.

The following simple direct method for the estimation of total carbohydrates and soluble sugars, due to Myers and Croll (*J. Biol. Chem.*, 1921, **46,** 537) is convenient, and yields reasonably accurate results. The total available carbohydrates are estimated by boiling about 5 g. of the finely chopped vegetable with 100 ml. of water under a reflux condenser for one and a half hours, then making the total volume up to 200 ml. The mixture is heated, then cooled to 40° ; 5 ml. of 1 per cent. solution of taka diastase and 2 ml. of toluene are added ; the flask is plugged with cotton wool, then incubated at 37° for eighteen hours. The mixture is cooled, diluted to 300 ml., mixed and filtered ; 20 ml. of the filtrate are heated in boiling water for an hour with 1·4 ml. of hydrochloric acid, then cooled, nearly neutralised with concentrated solution of sodium hydroxide, made up to 25 ml., and saturated with picric acid. Of this solution 3 ml. are taken, 1 ml. of 20 per cent. solution of sodium carbonate is added, and the mixture heated in a test tube in boiling water for twenty minutes. At the same time another tube containing 3 ml. of 0·02 per cent. dextrose solution saturated with picric acid, and to which 1 ml. of the carbonate solution has been added, are heated. The contents of the standard tube are made up to 10 ml. after cooling and the colour is matched against that of the sample under examination ; either the sample or the standard being diluted as requisite. A correction is made for any sugar produced by hydrolysis of the taka diastase solution by following the process

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[1] *Medical Research Council Report*, No. 413, 1936, and No. 235, 1942.

through with 200 ml. of water, 5 ml. of the 1 per cent. diastase and 2 ml. of toluene. When this has been done once the blank is known for subsequent experiments.

Important constituents of many fruits, notably apples, pears, plums, and of some vegetables, such as swedes, carrots, turnips, rhubarb and mangels are the pectins. According to Tollens these are substances allied to carbohydrates in structure, they contain from 41 to 45 per cent. of carbon with about 5 to 5·5 per cent. of oxygen. On hydrolysis they yield pentoses and hexoses together with methyl alcohol. Pectose, which is probably combined with cellulose in the plant structure, is the parent substance and in the natural state yields pectin on hydrolysis by the enzyme pectase. Pectin is a water-soluble gelatinising colloid.

Estimation of Pectin. Many methods, differing but slightly, have been proposed for the estimation of pectin, depending upon extraction and hydrolysis by water, then precipitation with alcohol. In such precipitation the product, which is dried and weighed, always contains some impurities. A useful modification of Wichmann's method (*cf. J. Assoc. Off. Agric. Chem.*, 1922, **6,** 34) is as follows: 150 g. of the pulped sample are boiled for an hour with about 750 ml. of water, cooled, made up to 1 litre and filtered; 200 ml. of the filtrate are evaporated to 25 ml. and added to 200 ml. of alcohol. The precipitate which forms is allowed to settle, most of the supernatant liquor is decanted, then the precipitate is filtered off and washed with 80 per cent. alcohol. The residue is taken up with hot water and the volume reduced to 25 ml. by evaporation if necessary; then 25 ml. of 0·2 N sodium hydroxide solution are added. After standing half an hour the mixture is diluted to 100 ml., and boiled for five minutes with the addition of 3 ml. of hydrochloric acid. The pectic acid thus precipitated is filtered off, again dissolved in sodium hydroxide solution (0·2 N) and reprecipitated, filtered off and washed into a platinum dish, dried at 100° and weighed, then ignited; the weight of any ash is deducted from that of the crude pectin.

Ling and others (*J. Soc. Chem. Ind.*, 1925, **44,** 253, T.) report favourably on the method of Carré and Haynes (*Biochem. J.*, 1922, **16,** 60). A suitable quantity of the pulped vegetable or fruit is repeatedly extracted with cold water; the mixed extract is boiled and filtered through paper. An aliquot part of the filtrate is diluted to 300 ml. and to it are added 100 ml. of 0·1 N sodium hydroxide solution; the mixture is allowed to stand overnight, then 50 ml. of N acetic acid are added and five minutes later 50 ml. of 2 N calcium chloride solution. After standing for an hour the liquid is boiled for a few minutes and filtered. The residue is washed with boiling water until free from chlorides, again boiled with water and filtered on a Gooch crucible, washed, dried and weighed as calcium pectate. To this substance Carré and Haynes ascribe the empirical formula $C_{17}H_{22}O_{16}Ca$, but King (*Analyst*, 1925, **50,** 371) gives it the formula $C_{17}H_{24}O_{16}Ca$. King's method has already been given on p. 42.

Besides the pectins there are other substances in fruit which yield five-carbon sugars on hydrolysis; these are the pentosans, which appear to be the nature of anhydrides, having the general formula $(C_5H_8O_4)_n$, and are formed in greater quantity as liquefaction of plant tissue proceeds.

There appears a general relation between the amount of crude fibre and pentosans, which is to be expected as the latter substances are so closely allied to cellulose. The estimation of pentosans is seldom more useful than that of fibre, which is much less tedious; when required it is carried out by distillation with hydrochloric acid with which they yield furfuraldehyde. Two g. of the finely divided material are distilled from a distillation flask fitted with a tap funnel, with 100 ml. of 12 per cent. hydrochloric acid (*i.e.*, sp. gr. 1·06). When about 30 ml. have passed over, a similar volume of the same acid is run into the flask and the distillation repeated. This is continued until all furfural has been distilled off, which may be ascertained by testing a drop of the distillate with aniline acetate paper; no pink colour should appear. It is usually necessary to distil over upwards of 300 ml. To the mixed distillate containing the furfural is added a large excess of solution of phloroglucinol in dilute hydrochloric acid, and the mixture is allowed to stand for twelve hours. The precipitate is filtered off, washed with 150 ml. of cold water, dried in the oven, and weighed. The weight thus obtained, multiplied by 0·544 gives the equivalent of furfuraldehyde; in order to calculate pentosans therefrom, an allowance of 10·4 mg. is deducted (Tollens, *Z. Rubenz. Ind.*, **46,** 480) from the weight of furfural and the difference multiplied by 1·82. A more rapid alternative method is that of Ling and Nanji (*Biochem. J.*, 1921, **15,** 466); the distillate obtained as already described is made up to a definite volume, say 400 ml. Then 25 ml. are carefully neutralised in a 100 ml. flask with 3 N sodium hydroxide solution, using methyl orange as indicator ; it is important to keep the mixture cool at this stage. The solution is acidified with acetic acid, and 10 ml. of 2 per cent. aqueous solution of phenylhydrazine are added, the mixture is diluted nearly to 100 ml. and warmed to 50°–55° for twenty minutes, cooled, made up to the mark and filtered ; 10 ml. of filtrate are added to a like volume of 0·1 N iodine solution, diluted to 100 ml., and titrated back with 0·05 N thiosulphate solution. A similar titration is made on 10 ml. of the phenylhydrazine solution, and the difference calculated as furfuraldehyde in accordance with the equations :

$$PhNH.NH_2 + 2I_2 \longrightarrow 3HI + C_6H_5I + N_2$$
$$C_4H_3O.CHO + PhNH.NH_2 \longrightarrow C_4H_3OCH : N.NHPh + H_2O.$$

Wittmann (*Z. Landw. Versuch. Œsterreich*, 1901, **4**, 131) gives tables of the pentosan content of a large number of fruits and vegetables, including those shown on the next page.

The nature and quantity of the sugar present in fruit depends largely upon the ripeness thereof, and the degree of acidity. Whilst sucrose is a normal constituent of many varieties of fruit, it is often found in the examination of fruit juices or canned fruits to have been nearly all hydrolysed to invert sugar by the vegetable acids naturally occurring.

The composition of the juice of some of the more common fruits is as under, but the variations may be wide. Some of the fruits appear to contain fractional percentages of dextrose in excess of the invert sugar.

In the canning of fruits the proportion of water or syrup to fruit generally approximates either to 2 parts of fruit to 1 of water or syrup, as in the case of pineapple, or equal parts, as in pears or loganberries. When syrup is used, two varieties are recognised in the trade—heavy syrups and light syrups. The former are used on apricots, peaches and plums, and its grades begin with 10 per cent. sugar, increasing with 15 per cent. in each grade. Light syrups begin with 10 per cent. and increase in steps of 10 per cent. in each grade. For example, peaches are packed in California in five grades, known in the trade as "Fancy," "Choice," "Standard," "Second," and "Pie," these being respectively packed in 55, 40, 25, 10 per cent. sugar, and the last-named grade in water. When examining canned fruit syrups it

	Water per cent.	Pentosans per cent.	Sugars as sucrose per cent.
Juniper berries . .	23·86	6·00	16·09
Raspberries . . .	69·54	2·68	9·38
Elderberries. . .	81·87	1·20	6·62
Japanese grapes . .	75·58	1·60	5·51
Blackberries. . .	83·42	1·16	4·00
Strawberries. . .	79·35	0·91	4·55
Cranberries . . .	83·00	0·75	4·34
Bilberries . . .	85·46	0·76	2·39
Gooseberries . .	85·93	0·51	2·20
Currants . . .	82·64	0·41	3·88

Juice of	Sp. gr.	Sucrose. Per cent.	Invert sugar. Per cent.	Acidity (as malic acid). Per cent.	Pectin. Per cent.	As Per c
Apples . .	1·060	0·8	8·3	0·75	0·65	0·2
Pears . .	1·055	Trace	8·0	1·7	—	0·3
Apricots . .	—	4·0	2·6	0·95	—	0·5
Peaches . .	1·054	4·4	3·7	0·70	0·75	0·4
Plums[1] . .	—	3·5	7·5	1·50		0·6
Cherries . .	1·055	0·5	10·5	1·40	0·30	0·4
Pineapple .	1·060	7·5	4·5	0·90	—	0·4
Strawberries .	1·050	1·3	8·5	1·20	1·05	0·4
Gooseberries .	1·040	0·80	5·5	2·50	—	0·4
Loganberries .	1·055	0·9	8·0	1·50	—	0·4
Raspberries .	1·050	1·2	6·5	1·10	—	0·4

is, of course, necessary to take into account the influence of the sugar and water natural to the fruit.

The estimation of the added sugar in canned fruit or, what amounts to the same thing, the determination of the strength of the syrup in which it has been packed is not so easy as might appear at first sight. There is the difficulty of not knowing what was the natural sugar content of the fruit, since this is liable to considerable variation according to the degree of ripeness at the time of packing and the season and variety of the particular fruit ; also there will have been diffusion of sugar into or out of the syrup according to the relative concentrations in the fruit

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[1] Very variable.

juice and syrup respectively. The simplest plan is to estimate the total sugar in the whole tin and deduct therefrom the weight of sugar natural to the fruit present. For this purpose the total weight of fruit and syrup is noted, then the latter is drained off from the fruit so that the weight of fruit and syrup separately can be ascertained. The sugar in the syrup is determined after inversion and reckoned as invert sugar ; then an aliquot part of the fruit is well pulped, boiled with a large volume of water to extract all the sugar, filtered and the total sugar, as invert, estimated by Fehling's solution. This gives a measure of the total sugar content of the tin. Now deduct the amount of sugar, reckoned as invert sugar, contained in the known weight of fruit, which may be supposed to contain the average amount of sugar normal for the particular kind ; calculate the difference to cane sugar, and this is the quantity which must have been present originally in the weight of syrup *plus the weight of the added sugar absorbed into the fruit.*

An example may be given :—

Tinned Strawberries

Total weight of content	834 g.
Weight of fruit	293 g.
Weight of syrup	541 g.
Invert sugar in the fruit	74·2 g.
Invert sugar in the syrup	161·8 g.
Total sugar in the tin	236·0 g.
Allowance for the fruit at 10 per cent.	29·3 g.
Net weight of added sugar as invert	206·7 g.
Equivalent as cane sugar	196·5 g.
Strength of the original syrup	

$$= \frac{196{\cdot}5 \times 100}{541 + (74{\cdot}2 - 29{\cdot}3)} = 33 \text{ per cent.}$$

There is also the possibility of the presence of glucose or starch syrup in tinned fruits. The syrup can be examined by the ordinary methods of sugar analysis explained in connection with jams or honey ; added starch syrup may be detected by Fiehe's reaction (p. 35).

Although it may be convenient to calculate the acidity as malic acid for purposes of comparison, in some fruits tartaric acid predominates and in others citric acid is present in quantity.

The identity and quantity of the acids in fruit is not certain in some cases, and conflicting statements appear in the literature. In apples, bananas, cherries, melons, peaches, plums, quinces and tomatoes, malic acid predominates and the amount of tartaric or citric acid is small. In cranberries and raspberries, citric is the principal acid ; in apricots, gooseberries, pears and peaches, citric and malic acids are about equal in quantity. Grapes, raisins and currants contain tartaric and malic acids. Succinic, formic and other acids have been found in some of the fruits ; it is possible that not only the quantity but also the nature of the particular acids present is variable according to the origin of the fruit.

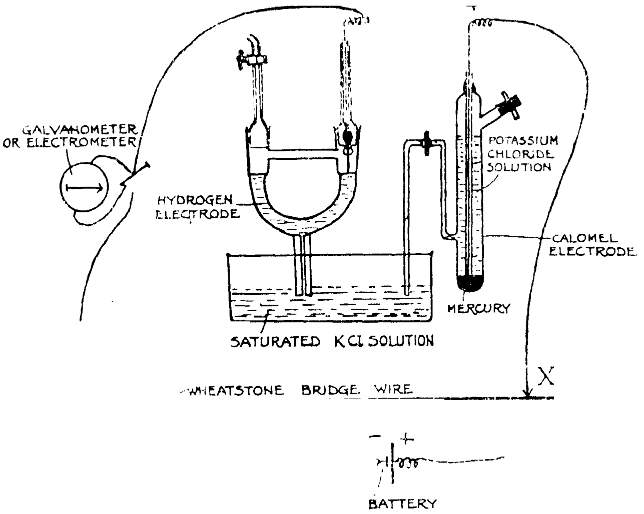

Fig. 31. Determination of pH.

The estimation of the total acidity of fruit juices is often complicated by the pigment present, and even when by largely diluting the liquid a satisfactory end point is obtained with an indicator, the acidity so determined and calculated, say, as malic acid, does not always correspond with the effective acidity or indicate the extent to which metal containers are likely to be attacked. For this purpose the estimation of the hydrogen ion concentration, expressed usually as pH, is of value. The theoretical basis of the pH value is discussed in the usual text-books of physical chemistry, but a simple account of its measurement is given by Monier-Williams (*Analyst*, 1921, **46,** 315). The arrangement of the apparatus is shown in Fig. 31. For the purposes of food analysis, a simple type of hydrogen electrode, such as that illustrated, is quite sufficient and may readily be made in the laboratory. The platinum wire which forms the hydrogen electrode is prepared for use by dipping it into a 2 per cent. solution of palladium chloride, to which a few drops of lead acetate solution have been added, and passing a current for a few minutes in both directions alternately so as to deposit palladium on the platinum; the deposit is washed by electrolysing dilute sulphuric acid for a few minutes and then rinsed in water. To make the measurement, hydrogen which has bubbled through alkaline permanganate solution, then through saturated mercuric chloride solution, is passed through the electrode cell for a few minutes to saturate the electrode. The liquid to be tested is drawn up into the cell by releasing the hydrogen through the plunger or outlet tap until the point of the platinum wire just makes contact therewith. It is desirable that the point of the platinum be not immersed in the liquid, otherwise constant readings on the ridge wire are not readily obtained. The cell, so arranged, is dipped into saturated potassium chloride solution into which also dips a normal calomel electrode, and is connected up to a galvanometer (or capillary electrometer) and metre bridge as illustrated. The E.M.F. is balanced against that of an accumulator (2 volts) [1] by adjusting the sliding contact. Generally the reading may be taken within a minute or two. The *p*H value is found from the simple linear relationship between the E.M.F. against the calomel electrode and the hydrogen ion concentration: E.M.F. = 0·283 + 0·0591 *p*H. The acidity may be titrated electrometrically in the same apparatus by running in 0·1 N sodium hydroxide solution, stirring and determining the E.M.F. at frequent intervals; the neutral point is obtained when the voltage is 0·710 and the *p*H = 7·2, and is best ascertained by plotting the E.M.F. or the length of bridge wire against the volume of alkali added.

The more recent introduction of the glass electrode associated with a thermionic valve has simplified the technique of determining *p*H values. Compact instruments are readily obtainable.

It is often sufficient to determine *p*H values colorimetrically. Provided that the colour of the liquid be not too dark, this may be readily done by means of the B.D.H. comparator or similar

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[1] The exact E.M.F. of the accumulator is determined by balancing it against a standard cadmium cell.

device. A specified quantity of the appropriate indicator is added to the liquid in a test tube and the colour is compared with that of standard buffer tubes to which the same quantity of indicator has been added. In order to eliminate the effect of the colour of the liquid under test the buffer tube is compared by observing it through a similar tube containing the same thickness of the coloured liquid without indicator. An even simpler alternative is to use the coloured glass discs prepared by Lovibond's, which are standardised to give the colour of the appropriate indicator at different *p*H values. The composition of standard buffer solutions and indicators is given in the British Pharmacopœia or in the usual books on volumetric analysis.

For the quantitative separation of the acids in fruits, vegetables or wines, the procedure of Jörgensen is satisfactory (*Zeitsch. Unters. Nahr. Genussm.*, 1907, **13,** 241) for citric, malic, succinic and tartaric acids. Twenty-five ml. of fruit juice or a larger quantity of vegetable extract or wine are made nearly neutral with sodium hydroxide, and 20 ml. of 10 per cent. solution of lead acetate added; the mixture is shaken and then diluted with an equal volume of alcohol. After standing for twenty-four hours the precipitate is filtered off and washed back into the flask with water and again precipitated with an equal volume of alcohol. This treatment is repeated yet a third time. The precipitate is washed into a beaker with hot water and kept warm while a current of hydrogen sulphide is passed through the solution for two hours in order completely to decompose the lead salts. The lead sulphide is removed and washed with water saturated with hydrogen sulphide, and the filtrate concentrated by evaporation, neutralised with potassium hydroxide, litmus paper being used as indicator, and reduced to a volume below 25 ml., then made up to that volume in a cylinder without any filtration. The phosphates, tannin, etc., are precipitated by the addition of 30 ml. of alcohol, washed with 30 per cent. alcohol, and the filtrate acidified with 3 ml. of glacial acetic acid and set aside for twenty-four hours for the separation of acid potassium tartrate, which is filtered off, washed with dilute alcohol, and titrated with 0·1 N sodium hydroxide solution. The filtrate is evaporated to low bulk, acidified with hydrochloric acid, and the succinic acid extracted by shaking out with ether, then dissolved in water and titrated. The residue containing malic and citric acids is carefully neutralised, phenolphthalein being used as indicator, and a large excess of barium chloride solution added; the precipitate, which may contain barium sulphate, phosphate, tannate and some citrate, if any, is collected and washed. The total volume of filtrate and washing should be adjusted to exactly 72 ml., and now contains all the citrate and malate; it is diluted to 100 ml. with alcohol and set aside for the barium citrate, which is practically insoluble in alcohol of approximately 28 per cent. by volume, to crystallise out; the precipitate is dissolved in water and the barium estimated as $BaSO_4$ and calculated to citric acid. The filtrate now contains barium malate and chloride in 28 per cent. alcohol solution; it is diluted with an equal volume of strong alcohol which throws down barium malate; the chloride being soluble in 60 per cent. alcohol. The barium content of the precipitate is again found and the malic acid calculated therefrom. In each case the acid separated should be identified by the usual qualitative tests.

Preservatives in Fruit Products. In connection with the possible presence of preservatives in fruit juices and such products, regard must be had to the possible presence of traces of various substances which may and do occur naturally. It is only the *addition* of preservative which is the subject of regulations. Some fruits such as oranges contain traces of boric acid, so do raisins and other fruits. Benzoic acid and its esters, and possibly minute traces of sulphur compounds, which may give rise to sulphur dioxide may also be found. According to Monier-Williams (*loc. cit.*, p. 104), certain berries may contain as much as 700 parts of benzoic acid per million. Salicylic acid in minute amount has also been reported in strawberries, and formic acid may be found in lemons, grapes and other plant products. So when any substance of this kind is found in a foodstuff prepared from or containing fruit, consideration must be given to the amount of the supposed preservative in relation to the kind and quantity of the vegetable ingredient.

With the possible exception of boric acid, for which the qualitative turmeric reaction is used, it is well to combine the quantitative estimation of the preservative with its qualitative recognition. Sulphur dioxide may be determined by the method given on p. 32 and confirmed by precipitation as $BaSO_4$. Benzoic acid, cinnamic and salicylic acids are separated by the Monier-Williams method and identified by their appropriate reactions after being weighed. A critical study of boric acid in fruits is given by Scott-Dodd and by Brown (*Analyst,* 1927, **52,** 459; 1936, **61,** 671). When dealing with dried fruits by extraction or distillation processes it is essential to mince the sample very finely, and make it into a smooth paste with water, or incomplete extraction will result.

For the estimation of formic acid the following process, based upon that of Fincke, is useful. Apparatus is fitted up consisting of a steam generator, a large flask to contain the sample, a 500 ml. flask, which can be heated and which contains a suspension of about 3 g. of barium carbonate in water, and a condenser. The tube leading from the sample to the barium carbonate should have a spray trap, and preferably a bulb with side holes at the end so as to distribute the incoming vapour in the barium carbonate suspension. About 50 g. of the material are acidified with tartaric acid, placed in the distillation flask, heated, and steam is blown in through the sample and on into the barium carbonate suspension (which is also heated), then condensed and collected in a 1-litre flask. About 1 litre of distillate should be collected, charring must be avoided, and there must be excess of barium carbonate at the end of the distillation. The barium suspension is now filtered, the insoluble carbonate being washed with hot water; the filtrate and washing should measure about 150 ml. or be evaporated to that volume. Add to the filtrate 10 ml. of 50 per cent. sodium acetate solution, 2 ml. of 10 per cent. hydrochloric acid, and 25 ml. of mercuric chloride solution (10 gm. $HgCl_2$ + 15 g. NaCl in 100 ml.). Heat this mixture on a water bath for two hours, collect the precipitate of mercurous chloride on a Gooch crucible, wash, dry and weigh it. The weight × 0·0975 gives the weight of formic acid. A blank test should be made on the reagents.

Perhaps the most frequent preservative in fruit preparations is benzoic acid, or occasionally one of its esters or derivatives. Benzoic acid is most conveniently detected and estimated by the following process, which is due to Monier-Williams.[1]

From 30 to 100 g. of the sample are saturated with common salt, or if the material is solid it is made into a soup with water and an excess of salt. The mixture is then acidified with phosphoric acid and steam-distilled rapidly. About 500 ml. should be collected in a large basin. The condenser is washed

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[1] Reports on Public Health and Medical Subjects, No. 39, 1927 (published by H.M. Stationery Office).

down into the basin with about 25 ml. of 0·1 N sodium hydroxide and the distillate, which must now be alkaline, is evaporated to a volume of about 25 ml. on a water bath. Potassium permanganate is then added until there is a small excess indicated by a permanent pink colour. The mixture is allowed to stand until it is cold, then the excess of permanganate is removed by a few bubbles of sulphur dioxide ; a few drops of sulphuric acid are added to dissolve the precipitate of manganese dioxide, after which the liquid should be clear and colourless. It is transferred to a 100 ml. cylinder, saturated with salt (33 g. per 100 ml.) and well shaken. Then it is extracted four times with 15 ml. of a mixture of equal parts of ether and petroleum spirit. The separated solvent is transferred to a special test tube and evaporated by means of a current of air passing through the test tube which is held in a bath of water at about 30°. These operations are conveniently carried out by the aid of the arrangement shown in Fig. 32 and a special tube with a ground-glass joint, which can be used for the subsequent sublimation as follows :—

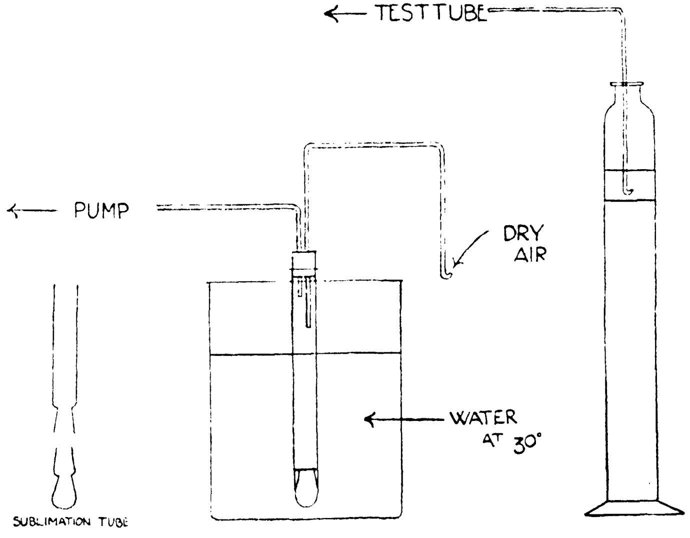

FIG. 32.

The residue at the bottom of the tube consists of benzoic acid in a not quite pure condition. Any deposit on the upper part of the tube is washed down with a few drops of ether, which is then evaporated away. The tube is now removed from the water bath, wiped dry, and enough clean dry sand added to cover the deposit; it is then placed in an air oven, heated at about 160°, until all the benzoic acid has sublimed into the cool upper part. The tube is then disconnected at the ground-glass joint and the benzoic acid in the upper part weighed. The crystals are now available for qualitative tests, and in any cases of doubt as to the identity of this sublimate a mixed melting point determination should be made with known benzoic acid.

Of the many confirmatory tests, other than mixed melting point, which are applicable to the sublimate, Mohler's is perhaps the most delicate. To a few crystals, or to the residue obtained by evaporating 2 or 3 ml. of the ether extract, add 5 drops of sulphuric acid and 0·1 g. of potassium nitrate. Heat the mixture in a glycerine bath at 130° for ten minutes, or in boiling water for twenty minutes. Cool, add 1 ml. of water and a slight excess of ammonia; boil, cool, add carefully 1 drop of ammonium sulphide solution. A reddish-brown ring forms at the junction of the two liquids and spreads on shaking.

The Monier-Williams process described above is not quite specific for benzoic acid. Both salicylic acid and cinnamic acid would be separated by the steam distillation, but destroyed by the permanganate oxidation; this part of the process must therefore be omitted if these acids are being sought. The process given for jam on p. 45 is applicable for salicylic acid in fruit. Saccharin is not appreciably volatilised by steam distillation. *p*-Hydroxy-benzoic acid and its methyl ethyl or propyl ester (often known as Solbrol, Nipagin or Nipasol respectively) are used as preservatives. The para acid is not volatile in steam, though the esters are partly volatile. The acid, or its esters after hydrolysis, may be detected by extraction with ether in acid solution; the separated acid does not respond to Mohler's test, but, like other hydroxy compounds, does give a rose-red colour with Millon's reagent. Millon's reagent is prepared by dissolving one part of mercury in two parts of nitric acid and diluting with two volumes of water. If the supposed hydroxy-acid is separated a mixed melting point affords the most conclusive test. A quantitative method for the estimation of this acid and its esters is given by Edwards, Nanji and Hassan (*Analyst*, 1937, **62**, 178).[1]

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[1] See also Stevenson and Resuggan, *Analyst*, 1938, **63**, 152.

Preserved Fruits

In the examination of preserved fruits should be included a search for either bleaching or preserving agents such as sulphurous acid, and, in such articles as bottled or tinned cherries, for artificial dyes. In one such case within the author's notice cherries had been preserved by sulphurous acid and then brightly pigmented with an aniline red. Dyes may be extracted by the methods already mentioned for jams (p. 46) and the prohibited colours identified by those given in the Appendix. Methods for the determination of preservatives have been given above.

Canned fruits or vegetables in tins are quite commonly contaminated with small amounts of tin, which metal is attacked by the fruit acids. As fruits are sometimes transferred to bottles after being imported in tins, search for tin should not be confined to samples received in tin-plate vessels. The metal, when present, is found associated mainly or entirely with the solid portion of the foodstuff, not in the liquor, as it enters into a loose combination with the proteins of the fruit. The organic matter must be destroyed by combustion before the estimation of the tin contained therein ; dry combustion or ignition is not altogether satisfactory as it sometimes leads to low results, the wet method should therefore be employed. Ten g. of the finely divided sample are digested in a Kjeldahl flask with 30 ml. of sulphuric acid and 10 g. of potassium sulphate until completely oxidised. The dissolved tin is then estimated colorimetrically with dithiol as recommended by De Giacomi (*Analyst*, 1940, **65**, 216) ; the acid solution is diluted to about 60 ml., neutralised with 0·880 ammonia, 2 ml. of hydrochloric acid are added, saturated with hydrogen sulphide and allowed to stand for at least an hour. The mixture is now boiled and filtered ; the filtrate, which should be quite bright, is re-saturated with hydrogen sulphide to ensure that all the tin is precipitated. The filter and precipitate are digested on the water-bath with 10 per cent. sodium hydroxide to dissolve the tin sulphide, washed into a 100 ml. flask just acidified with hydrochloric acid, 0·1 ml. of thioglycollic acid added and the solution made up to the mark. To 5 ml. of the solution in a boiling tube, add 0·5 ml. hydrochloric acid and 0·5 ml. of a reagent made by dissolving 0·2 g. of dithiol and 0·5 of thioglycollic acid in 100 ml. of 1 per cent. sodium hydroxide. The tube is heated in the water-bath for thirty seconds, allowed to stand for a minute and the orange colour compared with that of dilute standard solutions of tin, diluted to the same volume, or if a series of tests is to be made the colour may be measured on a Lovibond tintometer. Strong standard tin solution is conveniently 0·1 per cent. in diluted hydrochloric acid and 1 ml. of this is diluted with 2 drops of thioglycollic acid and made up to 100 ml for the dilute solution (1 ml. = 0·001 mg. of Sn). When larger quantities of tin are present the precipitated sulphide may be ignited and weighed as oxide.

An alternative colorimetric procedure is given by Schryver in a Report to the Local Government Board in 1908, No. 7. Buchanan, in this L.G.B. Report, states that "the presence in the contents of a sample can of tin in quantities approaching 2 grains per pound may be taken to signify that the food has become potentially injurious to health." The limit suggested of 2 grains per pound has been widely adopted as a result of this report.

Tinned or bottled peas, beans and other vegetables formerly were often coloured green by copper sulphate. Under the Regulations made by the Minister of Health (see Appendix) the addition of copper salts to vegetables is entirely prohibited. For the detection or estimation of copper, the sample is ignited and thoroughly charred in a porcelain or silica basin ; the char is boiled with diluted nitric acid and filtered ; the filter paper and any carbon thereon is now completely burned, and the residue dissolved in very dilute nitric acid. The complete removal of carbon is necessary as it retains some of the metal which cannot be extracted from it. The combined filtrates are then made alkaline with dilute ammonia and filtered. The filtrate is boiled until all free ammmonia is driven off, then cooled, transferred to a Nessler cylinder, 1 g. of ammonium acetate is added and 2 drops of potassium ferrocyanide solution added. The pink colour so obtained is matched against a blank containing the reagents and a suitable amount of standard copper solution.[1]

Since the total prohibition of copper in vegetables, the use of zinc salts for the same purpose has been noticed. Zinc appears to act in a similar manner to copper but is less efficacious.

Notwithstanding its volatility, zinc may be determined by ashing the material at a temperature not exceeding about 550° in a silica dish, using a few drops of nitric acid to assist the process

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[1] $CuSO_4 . 5H_2O$ 2·0 g. per litre. 1 ml. = 0·0005 g. Cu.

if necessary. The ash is dissolved in 5 ml. of 5 N hydrochloric acid, diluted and well boiled, then cooled and transferred to a separator with 10 ml. of 5 N ammonium acetate solution. The solution is extracted by shaking vigorously with 5 ml. of diphenylthiocarbazone reagent (prepared by dissolving 0·15 g. of this substance in 100 ml. of chloroform). The chloroform extract is run into a second separator and the aqueous solution extracted with another portion of the reagent. This must be repeated until no further zinc is extracted, which is usually the case with two washings, but is indicated by the colour of the reagent being unchanged. The chloroform extracts are washed by shaking with a mixture of 6 ml. of the ammonium acetate solution, 3 ml. of 5 N hydrochloric acid and 10 ml. of water, and finally with 20 ml. of water. The chloroform extract is now shaken with 5 ml. of 0·5 N hydrochloric acid and the acid solution transferred to a beaker ; then the diphenylthiocarbazone solution is again extracted with dilute acid, and the acid extract added to the beaker. The contents of the beaker are evaporated to dryness, oxidised on a hot plate with a few drops of perchloric acid and hydrogen peroxide until all the organic matter is destroyed. The zinc in the residue so obtained is titrated with dilute standard potassium ferrocyanide solution. This latter solution should be freshly prepared by dissolving 3·24 g. of potassium ferrocyanide in 200 ml. of water and diluting 10 ml. of this solution to 250 ml. One ml. of this dilute solution = 0·2 mg. zinc. To the residue containing the zinc is added 1 ml. of water, 1 ml. of acetic acid and of iso-propyl alcohol, 5 drops of 0·05 per cent. solution of diphenyl benzidine in acetic acid, and 2 drop of potassium ferrocyanide solution. The standard ferrocyanide solution is added until the blue colour just disappears. (*Cf.* Sylvester and Hughes. *Analyst*, 1936, **61,** 734.)

Canned vegetables or fruits are, like meat products, liable to undergo putrefaction, and have sometimes produced poisonous toxins through which illness has been caused. When the condition of the sample is not self-evident, tests for incipient putrefaction may be applied in a manner analogous to those described for meat products.

The possible toxicity of canned fruit and vegetables is not always fully appreciated, as it has been so often popularly supposed that canned meats or sausages are mainly associated with food poisoning. Botulism has frequently been traced to canned vegetables. Thus Savage, in "Canned Foods in Relation to Health," 1923, cites, on p. 74, more than eighty outbreaks of botulism which were due to fruit or vegetables. These have mainly occurred in the United States, where the industry has assumed such large proportions. In this country cases of poisoning other than botulism have been traced to the same class of food. Savage and Bruce White, reporting in 1925 on 100 recent outbreaks of food poisoning in Great Britain, attribute six cases to fresh or preserved fruit. This sufficiently indicates the need for chemical and bacteriological examination of such foodstuffs; feeding experiments on animals are also most desirable in many instances.

Potatoes

Potatoes are well known to contain small quantities of the poisonous alkaloidal glucoside, solanine; in green sprouting potatoes which have been kept in the dark the proportion is liable to increase, and Harris and Cockburn (*Analyst*, 1918, **43**, 133) describe an outbreak of potato poisoning in Glasgow in 1917. According to Bömer and Mattis (*Z. Unters. Nahr. Genussm.*, 1924, **47**, 97) the solanine content of the flesh of potatoes is from 2–10 mg. per 100 g.; when the amounts exceed about 25 mg. per cent. the potatoes are considered injurious. For the estimation of solanine Lampitt *et al.* (*J. Soc. Chem. Ind.*, 1943, **62**, 20), extract about 100 g. of the sample with 150 ml. of alcohol containing 3 ml. of acetic acid, for eighteen hours with occasional shaking. The mixture is filtered on a Buchner funnel and re-extracted with two further quantities of 150 ml. of 64 per cent. alcohol. The combined extracts are evaporated under reduced pressure to 50 ml. Then 5 g. of sodium sulphate are added and the mixture warmed on the water-bath for half an hour. After cooling, 2 ml. of 20 per cent. sulphuric acid are added and the precipitate filtered off, and washed with about 10 ml. of water. The filtrate plus washings are made alkaline with 0·880 ammonia, kept overnight and the precipitate of crude solanine is filtered off and washed with 2 per cent. ammonia. The volume of the filtrate and washings should be noted as 0·2 mg. of solanine is dissolved by each 100 ml. of liquor. The exact solanine plus solanidine content of the precipitate expressed as solanine is determined by hydrolysis and titration of the reducing sugar, preferably after the manner of Hulme and Narain (*Biochem. J.*, 1931, **35,** 1051).

Tomatoes

In America the canning of tomatoes has become an important industry, and tomato *purée* is now sold in enormous quantities. The American product differs considerably from that of parts of Europe ; skins, stalks and any bruised fruit are usually removed in course of manufacture. In order to enhance the keeping property it is usual to concentrate the pulp by about 33 per cent. before canning. The average composition of raw tomato and examples of American and Continental tomato *purée* are as follows :—

	Tomato.	American tomato-*purée.*		Italian tomato-*purée.*	
		1.	2.	3.	4.
Specific gravity	—	1·042	1·050	—	—
Total solids (per cent.)	6·58	9·50	11·73	25·34	21·42
Proteins	0·95	1·62	1·44	3·10	2·75
Ash	0·61	1·27	1·58	3·05	2·75
Total sugar (as invert sugar) (per cent.	3·51	5·21	6·78	9·80	9·50

The predominant acid in tomatoes is citric acid, which may be present in a *purée* to the extent of 1·8 per cent. It is interesting to note that the tomato is, of all fruits, one of the most vigorous in attacking metals, and tomato *purée* is liable to be contaminated with copper ; about 30 p.p.m. may be due to natural causes and a limit of 50 p.p.m. for copper on the dry basis has been recommended (*cf. Analyst*, 1941, **66,** 464). For the determination of copper in tomato products it is convenient to take 5 g. or 10 g. of the *purée* and submit it to the process of wet oxidation with nitric acid and sulphuric acid as described on p. 68. When the oxidation is complete and the excess of nitric acid has been boiled off, the solution is cooled, a small excess of ammonia is added, the precipitated iron is filtered off, and the filtrate diluted to 100 ml. To 10 ml. of this is added 5 ml. of a 0·1 per cent. solution of sodium diethyldithiocarbamate and the mixture is made up to 50 ml. and matched against standard copper solution. The amount of copper should be reckoned on the dry matter and this can be determined either by drying 5 g. of the sample on the water-bath or by the method described in the *Analyst*, 1941, **66,** 319, which is convenient if a large number of samples has to be examined at the same time.

Tomato Catsup contains about 15–20 per cent. of sugar beyond that of the tomato *purée* from which it is made.

The analyses, shown below, are given by Stüber (*Z. Unters. Nahr. Genussm.*, 1906, **11,** 578) for the whole fruit and juice of German tomatoes. Further data are given by Cockburn and Herd (*Analyst*, 1938, **63,** 482).

	Whole fruit.		Juice.	
	1.	2.	1.	2.
	Per cent.	Per cent.	Per cent.	Per cent.
Water . . .	94·52	95·13	96·06	96·19
Nitrogen . . .	0·12	0·16	0·10	0·09
= Proteins . .	0·73	0·99	0·63	0·61
Ash . . .	0·50	0·63	0·50	0·63
Alkalinity of ash (ml. N per 100 gm.) .	4·62	5·50	5·20	6·00
Water-insoluble matter	2·17	1·49	—	—
Sugars (as invert sugar)	2·51	3·19	2·36	1·96
Non-sugars . .	3·00	1·66	0·61	0·46
Acidity as citric acid	0·41	0·48	0·60	0·69
Phosphoric acid P_2O_5	0·04	0·06	0·03	0·04
Specific gravity .	—	—	1·019	1·019

A sample of strawberry *purée* examined had the following composition.

Total solids	6·80
Ash	0·70
Sugars	4·35

In testing strawberry *purée* for salicylic acid a very slight positive reaction is obtained with the pure product, as a minute quantity of this acid occurs in the form of its esters in the strawberries.

Lemonade, Lime Juice and Fruit Syrups

Lemon juice, which forms the basis of a number of beverages, is the expressed juice of *Citrus limonum*, similarly, lime juice is that of *C. limetta* and of certain other varieties of citrons. The composition of the juice is, of course, determined partly by the degree of ripeness of the fruit, and it is important in this connection to note that the colour is not a reliable indication of ripeness or otherwise on account of the widespread practice of fumigating. Citrus fruits are often exposed in rooms fumigated by a paraffin stove; such stoves evolve a small quantity of ethylene which rapidly develops the bright yellow colour associated with ripeness, even when the fruit was originally quite green and unripe.

The characteristic constituent of lemon or lime juice is citric acid; phosphoric acid, which is largely used as a substitute or adulterant, is almost absent from the pure juice. The B.P. 1914 required that lemon juice should have a sp. gr. of 1·030–1·040, that the total acidity should be not less than 7 nor more than 9 g. of citric acid per 100 ml., and that the ash should not exceed 3 per cent.

Lührig (*Z. Unters. Nahr. Genussm.*, 1906, **11**, 441) gives detailed analyses of a number of authentic samples of lemon juice of which the following is a summary:—

PRESERVED JUICE CONTAINING 10 PER CENT. OF ALCOHOL

	Minimum.	Maximum.	Average.
Specific gravity . . .	1·0234	1·0286	1·0260
Total solids (per cent.) . .	8·55	9·75	9·16
Acidity as citric acid (per cent.)	6·21	7·30	6·83
Ash (per cent.) . . .	0·29	0·35	0·33
Alkalinity of ash (ml. N) .	3·93	4·95	4·49
Alcohol (by volume per cent.)	9·59	10·38	9·85
Nitrogen (per cent.) . .	0·05	0·06	0·053
Total sugar as invert (per cent.)	0·83	2·24	1·42
Phosphoric acid P_2O_5 (per cent.)	0·02	0·02	0·02

UNDILUTED LEMON JUICE

	Minimum.	Maximum.	Average.
Total solids (per cent.) . .	9·49	10·82	10·18
Acidity (citric acid per cent.) .	6·90	8·11	7·59
Ash (per cent.) . . .	0·35	0·39	0·36
Alkalinity of ash (ml. N) .	4·37	5·50	4·99
Nitrogen (per cent.) . .	0·05	0·07	0·06
Invert sugar (per cent.) . .	0·92	2·49	1·57
Phosphoric acid P_2O_5 (per cent.)	0·021	0·026	0·023

Lime juice (*Citrus limetta*) differs only slightly from that of the lemon, and has the following composition :—

Specific gravity	1·035
Total solids	9·22 per cent.
Citric acid	7·20
Ash	0·42
Sugar	1·00

Artificial or adulterated lemon juices are very common ; some contain tartaric acid, and some phosphoric acid, with or without the addition of some citric acid. In Germany the use of phosphoric acid is prohibited, and Wolff (*Pharm. Weekblad.*, 1922, **59,** 622) states that this substance may give rise to stomach-ache, sleeplessness and other disturbances. An artificial lemon juice examined by Matthes and Müller (*Z. Unters, Nahr. Genussm.*, 1906, **11,** 20) gave the following figures, which are characteristic of product :—

Specific gravity	1·0425
Alcohol per cent.	5·43
Total solids	13·47
Acidity as citric acid	8·19
Ash (per cent.)	0·21
Alkalinity of ash (ml. N)	0·49
Phosphoric acid P_2O_5	0·05
Rotation (after distillation)	100 mm. + 4·50°

This sample consisted of an artificial mixture made up from starch syrup, citric acid and sugar, with a trace of phosphoric acid to stimulate real lemon juice. More obvious substitutes were the following " lemon essences," both of which contained tartaric acid :—

	I.	II.
Total solids	18·54	9·74
Alcohol	19·91	27·84
Ash	0·08	0·12
Acidity as tartaric acid	15·57	9·38
Sugar	None	None
Aniline dye	Absent	Present

The following simple test for citric acid is useful ; to the solution add 1 ml. of solution of mercuric sulphate (5 g. HgO dissolved in 20 per cent. H_2SO_4), boil, add 1 ml. of saturated solution of potassium permanganate; citric acid is oxidised to acetone dicarboxylic acid and produces a white precipitate.

A small amount of formic acid is occasionally added as a preservative, but the more usual ones are sulphites or benzoic acid. Lead is not infrequently found as an impurity in citric acid and its preparations, and search should always be made for it.

The estimation of the amount of real citric acid, free and combined, is simple when there is no other organic acid present, but when there is tartaric, malic, or other acid present the problem is more difficult; the method of Scopes should then be applied. For the determination of the citric acid free and combined, the total acidity is first titrated and calculated as citric acid; then the solution is evaporated to dryness on the water bath and gently ignited. The ash is dissolved and titrated with 0·1 N hydrochloric acid, preferably by adding excess and titrating back with alkali. From this is calculated the total organic acid as citric acid based on the fact that the sodium salt of the acid leaves sodium carbonate on ignition. The difference between total citric acid and free acid is, of course, the combined citric acid.

Tartaric acid is sometimes added as a diluent or adulterant. It may be determined as acid potassium tartrate in the usual way; to 25 ml. of the liquid add a small excess of potassium carbonate, evaporate to about 10 ml., then add 2 ml. of acetic acid and 50 ml. of alcohol and allow the mixture to stand overnight; then filter off the precipitate, wash with alcohol and titrate it with 0·1 N NaOH. The identity of the precipitate of cream of tartar should always be confirmed by qualitative tests such as the silver mirror or resorcinol test.

For the estimation of citric acid in the presence of other organic acids the following method is satisfactory (Gowing-Scopes, *Analyst*, 1913, **38**, 12). To 10 ml., or such volume as will contain about 0·03 g. of citric acid, add 10 ml. of a reagent containing 51 g. of mercuric nitrate, 51 g. of manganese nitrate and 68 ml. of nitric acid in 250 ml., and dilute to 200 ml. with water. The mixture is boiled under a reflux condenser for three hours; then the precipitate is filtered off through a tared Gooch crucible, washed with water, and dried to constant weight at 100°. The precipitate should be white or cream coloured, and not yellow. The weight of the precipitate × 0·1667 gives the weight of citric acid. Malic, lactic or salicylic acids introduce error if present, but tartaric, oxalic, succinic, benzoic, phosphoric or sulphuric acids do not interfere. An alternative process is that of D. S. Pratt (abstract, *Analyst*, 1912, **37,** 199).

Free mineral acid may be detected by the ordinary reactions for chlorides or sulphates since only traces of these substances occur naturally in the lemon. If present they may be estimated either as chloride, sulphate or nitrate, or calculated from the alkalinity of the ash of the neutralised juice. There can be no free mineral acid if the ash of the sample itself is alkaline, since free acid would decompose the small quantity of alkali carbonate which would be left on ignition of the citrate. The estimation of free acid from the ash is made exactly as described under vinegar on p. 196.

The most probable adulterants of lemon or lime juice are phosphoric or other mineral acid, tartaric acid, dyes and preservatives, specially salicylic or benzoic acids. Methods for the detection of these have already been given.

Orange Juice

Orange juice has constituents similar to those of lemon juice, but is much less acid. The analyses of three samples of fresh juice by Farnsteiner and Stüber (*Z. Unters. Nahr. Genussm.*, 1904, **8,** 603) are :—

Grams per 100 ml.	Unknown oranges.	Valencia oranges.	Messina oranges.
Specific gravity . . .	1·043	1·046	1·045
Total solids . . .	10·73	10·92	10·85
Citric acid . . .	1·19	1·79	1·47
Total sugar as invert sugar.	8·26	7·65	7·86
Ash	0·41	0·52	0·50
Alkalinity of ash (ml. N) .	5·40	7·20	6·40
Phosphoric acid P_2O_5. .	—	0·03	0·04

Its examination is carried out exactly as that of lemon juice.

The analytical data of some of the common fruit juices which form the basis of fruit drinks will be found in the tables on p. 98 ; the following further data are taken from König :—

Grams per 100 ml.	Total solids.	Protein.	Acidity as malic acid.	Ash.	P_2O_5.
Apple .	15·16	0·12	0·32	0·44	0·019
Pear . .	15·85	0·13	0·33	0·36	0·016
Cherry .	19·35	0·42	0·75	0·45	0·021
Strawberry .	9·00	—	1·04	0·66	0·026
Raspberry .	4·27	—	1·84	0·50	0·032
Bilberry .	10·60	—	1·13	0·29	—
Gooseberry .	10·19	0·06	1·65	0·27	—
Currant .	14·02	0·28	2·92	0·59	0·036

Ascorbic Acid Content of Fruit and Vegetables

Much importance now attaches to the ascorbic acid content of fruits and vegetables, so that it is frequently necessary to determine this. Fortunately a method discovered by Tillmans and developed by others is readily available. It depends upon the fact that a blue dye 2 : 6-dichlorophenolindophenol is decolourised by ascorbic acid, but not by any other substances which are known to occur in vegetables. Details and possible sources of error are given by L. J. Harris and M. Olliver (*Biochem. J.*, 1942, **36,** 155). For the determination a small weighed quantity of the fruit or vegetable is ground up with sand and sufficient 20 per cent. trichloroacetic acid to give a final concentration of about 5 per cent. This extract is filtered, diluted if necessary, and transferred to a burette. A measured volume of standardised buffered solution of the indophenol is titrated with the trichloroacetic acid extract until the red colour which this indicator assumes in acid solution is just discharged. The strength of the dichlorophenolindophenol solution may conveniently be 0·08 g. in 100 ml. of phosphate buffer solution of *p*H 7·2 ; it should be standardised against known ascorbic acid. Miss Olliver (*J. Soc. Chem. Ind.*, 1936, **55,** 153T) reports quantities of ascorbic acid in some common fruits or vegetables as shown on p. 118.

Apple juice contains on the average about 0·024 mg. per g., almonds and walnuts the surprisingly large quantity of about 0·2 and 16 mg. per g. respectively (Pyke, *Nature*, 1942, **150,** 268).

Sulphur dioxide is liable to interfere in the titration of ascorbic acid with the indophenol dye if it is present in large quantities. In fruit juices containing 10 per cent. of sugar it is combined with the hexoses and up to 1,000 p.p.m. does not interfere. Where there is insufficient sugar to eliminate the sulphur dioxide its effect is removed by adding 5 ml. of acetone to the liquid before titration ; the reaction with the dye is somewhat slower in the presence of acetone. Tin in small quantities does not interfere.

Ascorbic acid content of raw fruits and vegetables

Fruit :—	No. of different batches tested	Mg. ascorbic acid per gm. Range of values	Mean value
Black currant ...	2	1·722—2·200	1·961
Strawberry	8	0·517—0·775	0·661
Orange juice	53	0·196—0·769	0·448
Gooseberry	6	0·276—0·470	0·366
Loganberry	3	0·204—0·484	0·358
Raspberry	1	0·305	0·305
Apple (cortex) . .	10	0·029—0·139	0·083
Greengage	9	<0·010—0·069	<0·047
Pear	3	0·031—0·053	0·044
Plum	10	<0·010—0·046	<0·046
Melon	3	0·016—0·034	0·023
Vegetable :—			
Sprout	10	0·960—1·460	1·262
Asparagus—Tips	3	0·450—1·654	0·979
Green edible	3	0·237—0·717	0·443
Spinach	4	0·321—1·181	0·804
Potato (new)	2	0·291—0·406	0·348
Broad bean	2	0·277	0·277
Turnip....	4	0·173—0·357	0·261
Pea	6	0·197—0·312	0·248
Runner bean	1	0·181—0·222	0·198
Stringless bean	4	0·070—0·135	0·107
Onion	2	0·050—0·097	0·074
Carrot	10	<0·010—0·080	<0·040

Sometimes a small proportion of the ascorbic acid in foods becomes reversibly oxidised during ageing and forms dehydroascorbic acid. If this is suspected first estimate the ascorbic acid as already described, then through another proportion of the solution pass a stream of hydrogen sulphide for ten minutes. Stopper the flask and allow it to stand overnight in a refrigerator ; then remove the hydrogen sulphide by bubbling nitrogen through the mixture and titrate as before. The difference between the two titrations gives a measure of the dehydroascorbic acid. For a discussion of the possible sources of error, see Harris and Olliver (*loc. cit.*). One International Unit of vitamin C = 50 μg of ascorbic acid.

Lemon Cheese

The manufacture of lemon cheese has much increased in recent years; this has led to the modification of the time-honoured recipes given in cookery books. Instead of a simple mixture of lemon, eggs, butter, and sugar, one finds oil of lemon, margarine instead of butter, little or no egg in some preparations, and glucose. How far these changes are permissible is discussed by Elsdon and others (*cf. Analyst*, 1925, **50,** 230, 396). Sometimes preservatives are present, and gum tragacanth; both these are certainly undesirable, but starch to the extent of 10 or 15 per cent. seems unexceptionable, and serves the purpose of preventing the separation of fat, and an addition of glucose prevents the crystallisation of the sugar. Tartaric acid is sometimes substituted for citric acid. The analyses of two samples of somewhat unsatisfactory composition were as under:—

	1.	2.
	Per cent.	Per cent.
Water	22·7	41·2
Ash	0·83	0·32
Proteins	0·98	1·65
Total sugar	55·9	42·3
Acidity (tartaric acid)	0·58	0·79
Starch	27	16
Boric acid	—	Trace.
Salicylic acid	0·02	—
Fat	4·9	3·4

The sugar present in these samples was largely invert sugar, but some glucose had been added; the fat was margarine.

Lemon cheese is sometimes difficult to analyse; it is easy to devise a method for the determination of any constituent but mechanical difficulties are apt to rise. For the estimation of fat the Gottlieb process (see p. 227) works fairly well; direct extraction with ether, or extraction after destruction with hydrochloric acid, is not satisfactory.

CHAPTER V

TEA, COFFEE, COFFEE ESSENCES, CHICORY COCOA, CHOCOLATE, COCOA-BUTTER

THE tea leaves employed as a beverage are those of various species of *Thea*, the commonest being *T. sinensis*, *T. bohea*, *T. viridis* and *T. assamica*. The appearance of these leaves and their microscopic characteristics are described in the usual text-books of botany and histology. Whilst one sometimes meets an undue proportion of stalks, the admixture of foreign leaves such as hawthorn, elder or beech is very uncommon. In this country all tea imported is examined by the Customs authorities before distribution; on this account, adulterated tea is rarely met with and the gross frauds described in the early literature are now quite apocryphal, but a case recently reported (*Z. Unters. Nahr. Genussm.*, 1922, **44**, 89), in which tea was seriously contaminated with lead through having been stored in foil which had become brittle, points to the necessity for the analyst to keep a sharp look-out for unlikely substances. Tea from plants sprayed with Bordeaux mixtures has been found to contain appreciable quantities of copper. There is still the possibility of the admixture of exhausted tea leaves with the genuine article; this sophistication may be recognised by consideration of the ash, extract, and other data.

Black or green tea may be the product of the same plant and merely represent differences in the mode of preparation; when the leaves are baked and packed immediately after picking, they remain more or less green, but if they are allowed to undergo the usual fermentation before the drying process black leaves result.

It is unfortunate that the chemistry of tea, like that of wines, has not yet reached the art of the taster, and tea is valued more accurately by its flavour, expressed in unscientific but well-known terms, than by the most elaborate chemical analysis.

The chief constituents of tea so far as flavour is concerned appear to be caffeine, tannin, and a volatile oil present to the extent of only about 0·5 per cent., the latter being mainly the product of fermentation after picking. It has been shown that in good teas there is a constant caffeine-tannin ratio of 1 : 3, and upon this the flavour largely depends. Other estimations which should be made when a full examination is desired include moisture, total, soluble and insoluble ash, nitrogen and extract; a microscopic examination of the leaves and dust should always be added. The proportions of the different constituents vary very much, as is to be expected from the wide differences in quality, kind and price of the article. The following table shows what may be expected from genuine teas; some of the figures are the writer's, and others are collected from various sources.

	India.	China.	Ceylon.	Japan.
	Per cent.	Per cent.	Per cent.	Per cent.
Moisture . . .	5·7–8·5	6·0–9·1	4·5–9·5	3·93–4·00
Total ash . . .	4·9–6·0	5·0–6·5	5·0–6·5	5·62–6·23
Ash soluble in water .	3·0–4·1	3·0–4·0	3·1–4·2	3·49–3·54
Ash insoluble in acid .	0·1–0·35	0·1–0·35	0·1–0·35	0·27–0·46
Extractive . . .	43–50	38–47	40–48	42·00–43·20
Caffeine . . .	1·95–3·45	2·15–3·50	1·95–3·60	2·22–2·81
Tannin . . .	13·3–15·0	7·3–10·9	10·1–14·0	14·29–15·08
Total nitrogen . .	5·8	5·0–6·1	5·2–6·2	—

The crude fibre and total ether extract in teas, which may be estimated in the usual way, generally amount to 14–18 and 10–11 per cent. respectively. Under Canadian law tea is regarded as adulterated which contains foreign leaves, or in which the hot water extract is less than 30 per cent.; the total ash must not exceed 8 per cent.; and the water-soluble ash must not be less than 2·75 per cent.

The estimation of the water extract of tea is of much significance, but, unfortunately, the results recorded in some of the literature are not always of value because of the lack of uniformity in the methods employed. The process of boiling with repeated quantities of distilled water until all soluble matter is extracted is tedious, as so many boilings are required; the procedure of Tatlock and Thomson (*Analyst*, 1910, **35**, 103), which gives constant results, is more convenient, although the figures are somewhat lower than those of complete exhaustion. The quantity of water used is such that no material is deposited or goes out of solution on cooling. One g. of the powdered tea is boiled for an hour under a reflux condenser with 400 ml. of water; then the insoluble matter is collected on a filter, washed with 80 ml. of hot water, dried and weighed. The difference between 100 and the sum of the moisture and insoluble matter gives the extract per cent. The degree of fineness of the tea does not make any important difference to the result. A coffee mill is convenient for the coarse powdering of the leaves.

These authors show that the extreme limits of genuine tea when extracted by the above method are from 38·4–49·8 per cent., but it is important to note that certain other authors have given figures widely different from these, which, however, are not reliable criteria under precisely stated conditions. Tatlock and Thomson's figures for the water extracts of teas from India, Ceylon and China are :—

	Limit of variation Per cent.	Average. Per cent.
Indian tea	43·47–49·75	46·43
Ceylon tea	41·32–48·25	44·10
China tea	38·43–46·94	43·09

For the tasting or analysis of tea, infusions of different strength are commonly used. The professional tea taster pours 100 ml. of water which has just boiled on to 3 g. of the leaves and allows it to infuse for five minutes, then pours off the liquor. For the estimation of tannin or caffeine, three and five minute extractions with 2·5 per cent. of tea are frequently made as this strength corresponds approximately with the liquor usually consumed, but such figures, although useful for comparative purposes, do not bear any definite relation to the total quantities of the constituents concerned ; the amounts so extracted vary from 40 to 80 per cent. of the total.

The estimation of moisture, ash, soluble and insoluble ash, follows the usual course. From one to one and a half hours in the oven at 100° is sufficient for the drying ; prolonged heating leads to an increase in weight. The ash of tea is usually of a green colour, due to the presence of manganese ; its leading constituents are potash (K_2O), 27–36 per cent., and phosphoric acid (P_2O_5), 14–18 per cent. ; the average composition, according to Bell, is :—

Silica (including sand)	6·2	per cent.
Chlorine	1·1	,,
Potash, K_2O	33·2	,,
Soda, Na_2O	0·8	,,
Iron oxide, FeO	1·5	,,
Alumina, Al_2O_3	3·5	,,

Manganese oxide, Mn_3O_4	.	1·7 per cent.
Calcium oxide, CaO	. .	8·8 ,,
Magnesium oxide, MgO .	.	4·3 ,,
Phosphoric oxide, P_2O_5 .	.	15·0 ,,
Sulphuric acid, SO_3	. .	6·2 ,,
Carbonic acid, CO_2	. .	11·5 ,,

The tannin of tea has been the subject of much study. Its true nature is still in doubt; it seems likely that different teas contain different tannins, and in particular that the tannin in green tea differs from that in black tea. Recently a number of so-called tannin-less teas have appeared on the market, but analysis of these generally shows them to contain quite normal quantities of tannin – they would hardly be tea without it.

For the estimation of tannin the following process (*cf.* H. L. Smith, *Analyst*, 1913, **38,** 312) is perhaps the most satisfactory, and depends upon the complete precipitation of tannin by means of saturated cinchonine sulphate solution; the cinchonine tannate so obtained contains 55 per cent. of tannin and therefore 4·3 per cent. of nitrogen. Ten g. of the tea are boiled under a reflux condenser for half an hour with 800 ml. of water, filtered on a Büchner funnel while hot, and washed with 200 ml. of boiling water, and the volume adjusted to 1,000 ml. when cold. Fifty ml. of the cold decoction, which will be cloudy, are diluted with water, and extracted with three successive quantities of 30 ml. of chloroform to remove the caffeine, which would otherwise be precipitated with the tannin; the aqueous solution is then evaporated to about 20 ml. and 50 ml. saturated solution of cinchonine sulphate are added while the liquid is still hot and clear. After standing for some hours, the precipitate is filtered off through asbestos in a Gooch crucible which has been previously washed with half-saturated cinchonine sulphate solution and dried at 100°; the precipitate also is washed with the same solution and well drained by suction; then it should be dried over sulphuric acid *in vacuo* overnight, and finally in the oven at 100°; if the precipitate, while still quite wet, is put directly into the oven it fuses and becomes difficult to dry to constant weight. The dry precipitate is assumed to contain 55 per cent. of tannin.

Tatlock and Thomson (*loc. cit.*) precipitate tannin as quinine tannate; the filtrate from the estimation of the insoluble matter is cooled to 15°; then 1 g. of basic quinine sulphate dissolved in 25 ml. of water and 2·5 ml. N sulphuric acid are added. After standing, the precipitate is filtered off, but is not washed with water; it is dried in a basin at 100° to constant weight. The little quinine tannate which remains in the filtrate is compensated for by the few milligrams of soluble matter remaining in the liquid retained by the precipitate. The weight of quinine tannate × 0·75 gives the equivalent of tannin in the tea. (This factor is not applicable to other tannins.)

An important much-used method is Proctor's modification of the Löwenthal process of oxidation by potassium permanganate. For this method are required: (1) Standard potassium permanganate solution containing about 1 g. per litre, standardised against 0·1 N oxalic acid; (2) 2 per cent. solution of gelatin; (3) solution of 5 g. of indigo-carmine in 1 litre of water containing 50 ml. of sulphuric acid. Three titrations are made, working on a 1 per cent. extract of the tea: (*a*) dilute 4 ml. of extract to about 500 ml. with water, add 20 ml. of the indigo solution and run into the potassium permanganate solution until the indigo is bleached and a permanent pink colour remains which is visible at the margin of the yellow solution; (*b*) a similar titration is made on 40 ml. of the indigo solution diluted to 500 ml.; (*c*) 8 ml. of the tea extract are added to 25 ml. of the gelatin solution and an excess of ordinary salt is added, together with 10 ml. of dilute sulphuric acid. The mixture is diluted, shaken with a little kieselguhr, filtered, and the precipitate washed with water. To the filtrate is added 40 ml. of indigo solution; then it is titrated with the permanganate solution. For calculation of the result it is clear that $2a-b$ represents the volume of permanganate required to oxidise the tannin and other oxidisable matter in 0·08 g. of the tea, and that c represents the permanganate required to oxidise the non-tannin matters in 0·08 g. of tea and 40 ml. of the indigo solution. Hence, $2a-c$ represents the volume required to oxidise the tannin in 0·08 g. of the tea. It is important for accuracy that the amount of permanganate used to oxidise the tannin and other matters in the tea, *i.e.*, $a-b$, should not exceed one-third of b; if it does so, a less quantity of tea extract should be taken. The results are returned in terms of oxalic acid which has an equivalent of 63, and as it happens that the actual reduction equivalent of tea tannin is about 62·3 the results so expressed are almost identical with those obtained by calculation as tannins.

The well-known hide powder method is also available for this estimation, but it is not so convenient as the foregoing, and is, therefore, not described here.

So much importance has been attached to the caffeine in tea that many methods have been put forward for its estimation. Of these the A.O.A.C. method is accurate, and somewhat simpler and more convenient than the well-known Stahlschmidt method. The procedure is as follows :—

To 5 g. of material in a 500 ml. flask add 10 g. of heavy magnesium oxide and 200 ml. of water. Boil gently under a reflux condenser for two hours ; then cool, dilute to 500 ml., and filter through a dry paper. Of the filtrate 300 ml. are boiled in a large flask with 10-20 ml. of 10 per cent. sulphuric acid until the volume is reduced to about 100 ml. The liquid is now filtered into a separating funnel, the flask and filter being washed with 1 per cent. sulphuric acid, and shaken out with six successive portions of chloroform, 25, 20, 15, 10, 10, 10 ml. portions being used. The combined extracts are treated with 5 ml. of 1 per cent. potassium hydroxide solution and the latter washed with a further 10 ml. of chloroform. The total chloroform extract is evaporated in a tared flask and the residue dried in the water oven and weighed as caffeine. The residue is then transferred to a Kjeldahl flask with small portions of sulphuric acid, and the nitrogen determined in the usual way, the factor used being N × 3·464 = caffeine.

Stahlschmidt's method (*J. Amer. Off. Agric. Chem.*, **2,** 332) consists in boiling 3·125 g. of the powdered sample with 225 ml. of water under a reflux condenser for two hours ; 2 g. of basic lead acetate are added, and the boiling continued for ten minutes. The liquid is then cooled, transferred to a 250 ml. flask, made up to the mark, and filtered. Of the filtrate, 200 ml. in a 250 ml. flask are saturated with hydrogen sulphide to remove the excess of lead, then the mixture is made up to the mark and filtered. Two hundred ml. of the filtrate are evaporated to about 40 ml., then extracted in a separator with six portions of chloroform, 25, 20, 15, 10, 10, 10 ml. respectively being used. The combined chloroform extracts are washed with potassium hydroxide solution, evaporated, weighed, and the nitrogen estimated as already described in the A.O.A.C. method.

In Switzerland a maximum limit for the percentage of stalks in tea has been fixed at 22 ; this standard is possibly rather too stringent, as it may exclude quite good qualities of Indian, Ceylon or Java teas. The estimation is made according to Besson's method by boiling 5 g. of the tea for fifteen minutes with 500 ml. of water, then picking out the stalks with forceps. The leaves and stalks are dried separately in the water oven and weighed. For the examination of the tea leaves they should be soaked in hot water, then pressed out between folds of blotting paper and examined with a hand lens; the characteristic serrated margin and venation are readily seen. For microscopical examination they should, after thorough extraction in hot water, be soaked in 50 per cent. chloral hydrate solution for several hours, then bleached with hypochlorite and examined; the peculiar idioblasts and long hairs are characteristic. The identification of foreign leaves is not always easy, but if necessary may be done by careful comparison with similar leaves of known origin.

Coffee

The coffee berry, as it appears on the market, is the endosperm, with fragments of seed coats adhering, of the seed of *Coffea arabica* and other species which are indigenous to India, Arabia, Brazil, and other tropical countries. The raw berries do not lend themselves to sophistication other than gross forms which would be obvious to the experienced eye, but when roasted and ground they are liable to adulteration of various kinds; the commonest forms are roasted chicory, acorns, wheat, peas or barley. The roasting of coffee consists in heating to a temperature of about 200°–250° C., by which process most of the moisture present is driven off, the sugars are partially caramellised and the fat, proteins, alkaloids and other constituents are more or less changed, giving rise to traces of heavy oils and certain aromatic compounds which confer the characteristic aroma.

The more important organic constituents of coffee are caffeine, sugars, cellulose and protein, together with small quantities of fat, pentoses and essential oils. The usual range of figures on raw and roasted coffee is given on p. 127.

The amount of caffeine present varies from 1 to 2·5 per cent.; the latter figure is not often reached except in African coffees. It has been shown that there is loss of caffeine on roasting amounting to about 5 per cent. of the total present, but as there is also loss of water the amount found in the roasted coffee varies but little from that in the original bean. The sugars present

	Raw coffee.				Roasted coffee.	
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Moisture	8·25	10·95	11·70	9·10	0·32	2·03
Ash	3·97	3·50	4·45	4·00	4·40	4·55
Ash, soluble in water .	2·80	2·70	3·01	2·90	2·98	2·90
Water-soluble matter .	25·50	27·80	33·50	31·80	22·75	28·50
Caffeine	1·15	1·25	1·29	1·58	1·25	1·02
Nitrogen . . .	3·10	—	2·55	2·70	3·30	2·79
Ether extract . . .	11·45	12·30	13·65	12·15	8·00	11·75

amount to about 4 per cent. as dextrose in the raw berries and 1·5–2 per cent. after roasting. According to Hehner and Skertchly (*Analyst*, 1899, **24,** 178) the pentosans and crude fibre after roasting are 2·50 and 7·36 per cent. respectively. Tannin is present to the extent of about 4·5 per cent. in raw coffee, but this disappears on roasting, probably forming caffeic acid and quinic acid.

The non-volatile oil extracted from coffee has, according to Tatlock and Thomson (*J. Soc. Chem. Ind.*, 1910, **29,** 138), the following characteristics, which are the same in both raw and roasted coffee :—

Iodine value . . .	99
Saponification value . .	179·5
Unsaponifiable matter (per cent.)	5·2
Specific gravity . . .	

Some importance attaches to the ash of coffee ; this is usually less than 4·6 per cent., of which about two-thirds is water-soluble, and that insoluble in acid is only a trace. Chicory, dandelion, or other roots, which are sometimes found, all contain a substantially higher proportion of silica and insoluble ash. The common roots also contain less oil than does the coffee berry, and more crude fibre and pentosans. These latter may be estimated by the methods given for vegetables on p. 26.

A determination which should always be made is that of the extract ; there are two well-known methods ; one is to make a 10 per cent. extract by boiling 10 g. of the finely powdered sample with water under a reflux condenser, then filter and make the filtrate when cold up to 100 ml. and take the specific gravity. The specific gravity of a 10 per cent. extract of roasted coffee varies from 1·008 to 1·010, and that of similar extract of chicory is 1·028. The percentages of coffee and chicory indicated by an intermediate figure are not altogether reliable. The second and better method is that of E. W. T. Jones, which is to boil 5 g. of the dried sample with 200 ml. of water for fifteen minutes, then pour off the liquor and boil again with 50 ml. of water, mix the two extracts, cool, filter, and make the volume up to 250 ml., evaporate 50 ml. on the water bath, dry, and weigh the residue. This is the most satisfactory way of estimating chicory, since coffee has an average extract under these conditions of 24·0 per cent. and that of chicory averages 70. Simple proportion therefore indicates the percentage of chicory in a mixture. The calculation may be made from the formula $x = \frac{E - 24}{0{\cdot}46}$ where x and E are the percentages of chicory and extract respectively, or from the following table :—

Extract.	Chicory per cent.	Extract.	Chicory per cent.
24·0	0·0	48·0	52·0
26·0	4·0	50·0	56·5
28·0	8·0	52·0	61·0
30·0	13·0	54·0	65·0
32·0	17·5	56·0	69·5
34·0	22·0	58·0	74·0
36·0	26·0	60·0	78·0
38·0	30·0	62·0	82·5
40·0	35·0	64·0	87·0
42·0	39·0	66·0	91·0
44·0	43·0	68·0	95·5
46·0	48·0	70·0	100·0

The extract from coffee or a coffee mixture is somewhat influenced by the roasting and by the fineness with which it is ground. The above figures are therefore only approximate ; some pure coffee gives an extract as high as 26 or 27 per cent., so it is essential that chicory or other suspected adulterant be picked out and identified. It would be quite unsafe to deduce adulteration solely from the extract.

Some data on common adulterants of coffee are given in tabular form below.

Tests for Chicory. The only really reliable way of detecting

	Moisture	Ash.	Soluble ash.	Nitrogen.	Ether extract.	Water extract.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Chicory (roasted).	2·5	4·9	3·1	1·4	2·1	72·5
Chicory (roasted).	3·5	5·5	3·0	1·5	3·3	71·0
Acorn coffee .	12·8	2·0	0·9	1·0	4·0	—
Barley coffee .	3·5	3·4	—	1·5	3·3	68·7
Roasted wheat .	—	1·8	—	—	2·8	55·0
Dandelion root .	10·0	2·0	—	—	—	50·0

chicory in a coffee mixture is to isolate the fragments and identify them under the microscope. If a few grams of the coffee are boiled with water for five minutes and the liquor poured off, on examining the residue on a white tile the chicory may be picked out on the point of a knife by its whitish appearance and softness, whereas the coffee particles remain quite hard even after boiling. The pieces so picked out should always be identified by the microscope ; the spiral vessels, sieve tubes with transverse plates, and the large pitted vessels of chicory are quite characteristic. A rough test may be made by floating a layer of the coffee on the surface of water in a tall glass cylinder. On pushing the surface layer down with a glass rod some of the particles sink, and those of chicory leave behind them a trail of colour due to the large amount of caramel they contain ; coffee, unless much roasted, does not colour the water appreciably. The high extract of chicory which serves as a basis for its estimation has already been noted.

Many processes have been proposed for the estimation of caffeine in coffee. They are usually only slight modifications of the same principle : *i.e.*, extraction with water, followed by precipitation of proteins, etc., then extraction with chloroform. The methods of Allen or Vautier are perhaps the best. The former author extracts 12 g. with 500 ml. of boiling water for six hours under a reflux condenser, then filters the liquid, making the volume up to 600 ml. To this solution 4 g. of lead acetate are added, and the mixture is boiled for ten minutes, cooled, filtered, and the volume again made up to 600 ml. A further addition of lead acetate and re-boiling is necessary if the precipitate does not settle well and leave a clear supernatant liquor. Five hundred ml. of the clear filtrate are evaporated to about

50 ml. and 5 ml. of sodium phosphate solution are added to remove excess of lead, then the liquid is filtered, evaporated to about 40 ml. and extracted five times with chloroform, 20 ml. being used for the first and 10 ml. for each subsequent extraction. The chloroform extracts are mixed, evaporated in a tared flask and the residue is weighed as caffeine. Vautier's method (*Ann. Chem. Anal.*, 1918, **23,** 207) consists of extracting 5 g. of the coffee, moistened with 5 ml. of solution of ammonia, in a Soxhlet extractor for five hours with ether. The ammoniacal ether extract is evaporated and the residue dissolved in hot water and filtered. The insoluble portion of the residue should be well washed with boiling water. The water extracts are evaporated on the water bath, and the residue, which consists of crude caffeine, is sublimed into a tared beaker, dried at 100° and weighed.

Both these methods are satisfactory for ordinary coffee, but in the case of certain coffee substitutes and so-called caffeine-less coffee (which contain only a small fraction of the usual percentage of caffeine), the impurities associated with the caffeine as separated introduce a material error. In such substances therefore the real caffeine in the residue should be determined by estimating the nitrogen by Kjeldahl's method: $N \times 3{\cdot}464 = \text{caffeine}$. When the caffeine percentage falls below 1·0 it is probable that some has been extracted or that there is admixture with extracted coffee.

As tannin is present only in raw coffee, there is seldom occasion to estimate it, but if required, the Löwenthal process is satisfactory, with slight modification.

Analysis of coffee extracts or essences with or without chicory may be made in a manner similar to that of coffee. As sugar is often an ingredient it is necessary to determine the cupric reducing power as dextrose, and to allow for that naturally present in the coffee or chicory; these amounts may be taken as 0·7 and 20 per cent. respectively in the roasted product.

In the determination of the sugar the protein and other substances which would exert a copper reducing power must be removed; about 5 g. of the essence are diluted to 50 ml. with water, 10 ml. of copper sulphate solution are added, then 5 ml. of 0·5 N sodium hydroxide, and the volume made up to 100 ml. and filtered. Of the filtrate 50 ml. are inverted by hydrochloric acid, then neutralised and diluted to 100 ml. The cupric reducing power is determined with Fehling's solution in the usual way.

It is difficult or impossible to apply Clerget's process for the sugar estimation on account of the dark colour due to caramel, which is not sufficiently removed by the ordinary clarifying agents for accurate polarimetric observations.

To arrive at the probable composition of an unknown coffee essence is difficult, indeed, only possible on the assumption that the ingredients are coffee, chicory and caramel. It is necessary to determine the caffeine, sugars as dextrose, total soluble matter and ash. Tatlock and Thomson (*J. Soc. Chem. Ind.*, 1910, **29,** 138) observed that whatever the degree of extraction of coffee the ratio of caffeine and ash to total extract is constant. The coffee equivalent to the caffeine found is first calculated on the average basis of 1·25 per cent.; then the ash due to the coffee is taken as 4·2 per cent. of the coffee and the excess of ash calculated as due to chicory which contains on the average 5·2 per cent. of ash. The following are examples of coffee essences examined in this manner. There is usually a considerable proportion of caramel present, as is shown by the figures :—

	1	2	3	4	5.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water	34·90	35·25	55·57	33·79	36·66
Sugars (as dextrose) . .	45·60	60·00	37·00	41·20	40·35
Organic matters not sugars	17·35	2·50	5·70	22·56	21·42
Ash	1·85	2·10	1·50	2·19	1·34
Caffeine	0·30	0·15	0·23	0·26	0·23
Coffee (dry) . . .	25	12	20	22	20
Chicory (dry) . . .	16	32	13	25	10

Edwards and Nanji (*Analyst*, 1937, **62,** 841) show that the ratio of extractives to ash in coffee is 6 : 1 and of ash to caffeine 3 : 1 ; these ratios are not generally influenced by the degree of roasting. The results are, of course, only rough approximations as they assume the absence of ash in the caramel or added sugar. The same authors apply similar principles to the analysis of cups of coffee (*Analyst*, 1938, **63,** 323). The Ministry of Food in a recent Order[1] indicate that liquid coffee essences should contain not less than 0·5 per cent. of caffeine and that coffee and chicory essences should contain at least 0·25 per cent. These figures correspond to 4 and 2 lb. of roasted coffee per gallon.

Preservatives should always be sought in coffee essences.

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[1] S.R. & O., 1945, No. 389.

Benzoic acid is commonly used, as it is permitted up to 450 p.p.m. by the Regulations, and is allowed in the U.S.A. It may be determined by the distillation method given on p. 104. For qualitative purposes benzoic acid or salicylic acid can often be detected by shaking out with ether in acid solution and testing the residue after evaporation of the ether.

Cocoa and Chocolate

The characters and cultivation of the cocoa tree, *Theobroma cacao* (N.O. Sterculiaceæ) and the processes employed in making cocoa and chocolate are described in some detail by Whymper in " Cocoa and Chocolate." The operations of most interest from the analytical point of view are the roasting, cleaning, de-fatting, and sometimes the treatment with alkali. The roasting develops the aroma of the product probably by the formation of a substance known as cocoa-red, which appears to be an **oxidation** product of the tannin present in the seed ; it also darkens the colour.

The cleaning process consists in the separation of the nib from the shell ; the proportion of shell in the whole-roasted bean varies from 7 to 18 per cent. in different varieties, averaging 12 per cent. The separated shell finds some use as a cattle food in small quantities—large quantities are said to cause scouring—and as so-called cocoa teas, but is to a considerable extent a waste product ; hence, in inferior grades of cocoa or chocolate, a proportion of finely ground shell is included, and there is some temptation to the manufacturer to utilise as much as possible, especially now that it is more easily ground to a very fine powder. Consequently, one of the most important estimations the analyst has to make on cocoa is that of shell.

Cocoa butter, or *oleum theobromatis*, is the most important food constituent of the cocoa nib, of which it constitutes some 45–50 per cent., but, as it is neither readily digestible nor soluble, and, further, has a higher commercial value than cocoa powder, it is usual to remove about half of it by hot pressing before marketing the finished cocoa. In some inferior brands of chocolate some of the cocoa butter may even be replaced by illipé fat or hydrogenated oil. Some " cocoa essences " or " soluble cocoas " are treated with alkali, usually carbonate or phosphate, which saponifies some of the fat and incidentally softens the cellulose tissue, so rendering the cocoa more readily emulsified in the cup. Some analyses of raw cocoa nibs and shells are given below :—

	Cocoa nibs.			Cocoa shell.	
	1.	2.	3.	1.	2.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water	1·85	3·75	4·80	9·90	12·24
Fat	54·62	45·98	50·55	3·75	3·10
Cold water extract	14·20	15·25	12·03	—	—
Total ash	4·15	4·02	2·98	9·50	7·42
Water-soluble ash	2·32	1·95	1·60	5·35	4·91
Alkalinity of ash as K_2CO_3	2·35	2·04	1·74	4·80	4·80
Nitrogen	2·40	2·35	2·38	2·05	1·97
Fibre	2·36	2·52	2·08	13·84	14·24
Theobromine	1·40	0·98	0·88	0·98	0·92
Starch	5·85	4·68	4·93	—	—

Besides cocoa butter, shell and cocoa-red, which have already been mentioned, an important constituent of cocoa or chocolate from the analyst's point of view is the alkaloid theobromine, 3 : 7-dimethylxanthine ($C_5H_2(CH_3)_2N_4O_2$), which is closely allied to caffeine, tri-methylxanthine. It is present in the nibs to the extent of about 1·3 per cent., together with a fractional percentage of caffeine. The proteins, of which about 14 per cent. are present in the nib or about 18 per cent. in manufactured cocoa, consist of amino compounds, soluble albumins and insoluble compounds.

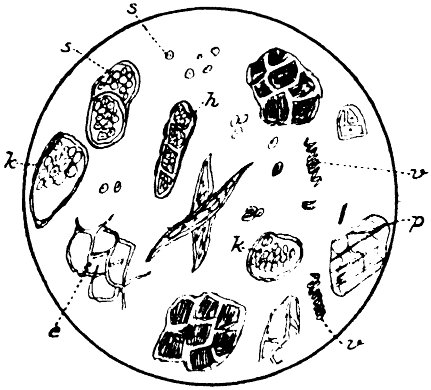

Fig 33. Cocoa with shell. *s*, starch ; *h*, multicellular hairs ; *k*, kernel cells ; *v*, spiral vessels ; *p*, epidermis ; *e*, inner pericarp.

Starch exists in the bean to the amount of some 4 or 5 per cent. or 7–8 per cent. in the manufactured product. This is important, as some soluble cocoas and chocolates contain much added starch, which must be identified microscopically and estimated chemically. Cocoa starch grains are of small size, comparable with rice, but they are round, not angular, and frequently have a hilum. Details of the microscopic structure of cocoa nibs or shell may be found in the usual text-books; characteristic features of the nib are the multicellular hairs and the cellular tissue enclosing oil and starch grains; the presence of shell may be recognised by pericarp fibres and spiral vessels which are not present in the nib (see Fig. 33), and the presence of mucilage which stains with Ruthenium red.

The ash of roasted cocoa nibs varies between 3·0 and 4·5 per cent., of which 50 per cent. should be water-soluble. In cocoa powder it is somewhat higher owing to the removal of some of the fat, usually being between 5 and 7 per cent., of which 55–65 per cent. is soluble in water. The alkalinity of the ash affords useful information as to any treatment with alkali which may have taken place. The normal figure is from 2–3·5 per cent. reckoned as K_2CO_3; if there is substantially more, treatment with potash is indicated. In connection with alkali treatment it may be noted that serious arsenical contamination was at one time found with certain cocoas and traced to the use of impure potash; in view of this an examination of cocoa or chocolate is not complete without a search for arsenic.

Examination of a sample of cocoa should include estimation of moisture, soluble and insoluble ash, alkalinity of ash, fat, fibre, starch, sugar, if any, nitrogen, arsenic, and a microscopic examination. In special cases estimation of theobromine and caffeine may be required. Pentosans are, of course, present in cocoa husk and in the nib, the respective percentages being 9·3 and 3·3; their estimation has been proposed as a method of determining shell, but the process is neither so accurate nor so convenient as those described in the following pages. Albahary has shown that cocoa also contains oxalic acid combined with lime and alkalies to the extent of about 0·45 per cent., and gives a method for its estimation (see *J. Soc. Chem. Ind.*, 1909, **28**,738).

The following table shows the results of analyses of a number of well-known brands of prepared cocoa.

Estimation of the fat in cocoa is most accurately obtained by direct extraction, but requires a lengthy period of time. An approximate result, accurate to about 0·5 per cent., is obtainable more rapidly by digesting a weighed quantity with hot hydro-

	1.	2.	3.	4.	5.	6.	7. Block cocoa.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Moisture	3·28	3·96	2·75	3·52	5·82	6·35	—
Fat	28·93	32·22	28·21	25·28	20·91	22·73	49·47
Cold-water extract	18·26	18·40	20·00	19·40	21·30	17·80	14·1
Ash	9·18	8·14	8·39	8·30	5·65	5·80	4·21
Water-soluble ash	5·72	5·57	5·54	5·61	2·81	4·15	2·58
Alkalinity, K_2CO_3	4·36	3·72	4·82	4·67	1·84	2·01	2·01
Nitrogen	3·44	3·30	3·35	3·67	3·43	3·52	2·43
Fibre	4·08	4·46	4·40	3·60	5·20	4·01	3·61
Shell (calculated)	1·8	7·1	4·3	—	9·1	—	7·5
Added starch	—	—	—	—	—	—	10·3
Added sugar	—	—	—	—	—	—	—
Theobromine	1·25	—	1·31	—	—	—	—

chloric acid, then extracting with ether as in the Werner-Schmidt method for milk-fat, but figures so obtained are usually a little low when compared with those of direct extraction. It is convenient to extract about 3 g. so as to obtain 2 g. of fat-free dry residue on which the crude fibre estimation may be made. The solvent used should be petroleum ether, as this dissolves but little theobromine, and the extraction should be continued for twelve hours in a Soxhlet ; then the residue is dried, powdered again, and re-extracted for a further three hours. The fat, if not quite clean when melted, should be dissolved in warm petroleum ether, filtered, and the solution evaporated, dried and weighed. The fat may be examined by the methods given on p. 141. Bywaters *et al.* give a method particularly useful for detecting illipé fat (*Analyst*, 1927, **52**, 324).

Numerous methods have been published for the estimation of theobromine, many of which are reviewed and criticised by Wadsworth (*Analyst*, 1921, **46**, 33), who recommends the following process. Ten g. of the material are placed in a small porcelain dish and mixed with 2–3 g. of freshly calcined magnesia ; the mixture is triturated with 14 ml. of water until all the particles are wetted ; then the basin is placed on a water bath for half an hour in order to dry it partially ; the mass should be mixed at intervals to prevent any part from becoming perfectly dry. At the end of the half-hour the mass is well triturated and transferred to a flask of about 250 ml. capacity ; 150 ml. of tetrachlorethane are added, and the whole is boiled under a reflux condenser for half an hour. It is filtered, while almost boiling, into a second flask. The residue is transferred, together with the filter, back to the first flask and boiled again with 120 ml. of tetrachlorethane ; after twenty minutes this process is repeated, and the residue is washed twice more as before. The united washings are distilled through an air condenser until reduced to 3–5 ml. To the cold residue are added 60–70 ml. of ether (sp. gr. 0·720) and the whole well mixed and allowed to stand overnight. The precipitate is collected on a tared filter, washed with ether, dried at 100° and weighed. To this weight is added 0·004 g., which represents the amount of theobromine dissolved in the 70 ml. of ether used for the precipitation and washings. The alkaloid so obtained should be clean and white.

Considerable importance attaches to the estimation of shell in cocoa ; methods and their limitations are discussed critically by Baker and Hulton (*Analyst*, 1918, **43**, 197). No single process affords really reliable information, especially now that it is customary to grind cocoas much more finely than formerly. When the proportion of shell is considerable, the error in its estimation is not great or important, but with small quantities serious error may easily be introduced. Most reliance is placed on the estimation of crude fibre, and for confirmation on Macara's levigation process. In both of these methods the estimation is carried out on the fat-free dry residue ; the removal of all traces of fat is especially important in the levigation process. The fibre is estimated in the usual way, using 1·25 per cent. sulphuric acid and sodium hydroxide solutions (p. 60).

The percentage of dry fat-free shell, S, in the sample is then given by the equation

$$S = \frac{(K - 5{\cdot}7)\{100 - (F + W)\}}{11{\cdot}1},$$

which is based upon an average fibre content of 5·7 and 16·8 per cent. in the fat-free dry nib and shell respectively. K is the observed percentage of fibre in the fat-free dry sample and F and W the percentages of fat and water respectively. $S \times \frac{100}{88}$ will give the percentage of average shell.

Macara's levigation process is carried out as follows (Baker and Hulton, *loc. cit.*) ; Ten g. of the finely ground material are extracted with ether in a Soxhlet extractor for twenty hours, dried in the oven, and then well ground in a mortar, stirred into a thin paste with water and washed into a 500 ml. measuring cylinder. The volume in the cylinder is made up to 400 ml., the whole inverted a few times to ensure admixture, and a wash bottle fitting inserted in the neck for the purpose of blowing off the supernatant liquor. The lead-in tube should be turned up at the lower end in order to avoid any disturbance of the sediment. The contents of the cylinder are allowed to stand for fifteen minutes and the liquid blown off. The volume is again made up to 400 ml., and the procedure is repeated after ten minutes and again after two periods of five minutes each. Should the residue in the cylinder show the presence of much starch, it should be finely ground in a mortar and again submitted to levigation. The residual sediment is transferred to a platinum basin, the water removed by evaporation, and the residue dried in a water oven and weighed. The residue is ignited, and the weight of the ash is deducted. Bolton and Revis find an average of 3 per cent. of residue in the fat-free dry nib and 30 per cent. in the fat-free dry shell ; hence the proportion of shell in the sample is given by the expression

$$S = \left(\frac{100\,M}{100 - (F + W)} - 3\right) \cdot \frac{100 - (F + W)}{27}.$$

where M, F and W are the percentages of ash-free levigation sediment, fat and water in the sample.

It has also been proposed to calculate shell from a nitrogen estimation by the Kjeldahl method, but as there is such wide variation in the amount of nitrogen present in the nib and shell, calculations based upon averages of 4·9 and 2·6 per cent. respectively in the dry fat-free state are very approximate.

Some importance attaches to the ash of cocoa, particularly as the estimation of soluble and insoluble ash with alkalinity of the former affords information as to treatment with potassium carbonate or other alkali. Five g. are ignited over an Argand burner until all carbonaceous matter is burned off, leaving usually a light grey ash ; after weighing, this is boiled with water for a few minutes ; the water-insoluble ash is filtered off, ignited with the filter paper and weighed. The filtrate is cooled and titrated with 0·2 N hydrochloric acid, methyl-orange being used as indicator, and the alkalinity is calculated as K_2CO_3. Of the 5–7 per cent. of ash commonly found in cocoa, the soluble ash does not usually exceed 55–60 per cent. in the case of untreated cocoa, but if treatment with alkali has taken place the soluble ash rises to 60 or 70 per cent. of the total, and the alkalinity as K_2CO_3 rises from 2–3·5 per cent. to 4–6 per cent. The total ash in such cases may also be high.

The presence of added starch in cocoa or in chocolate will have been indicated by a microscopical examination; when quantitative information is required the diastase method is employed. Polarimetric methods are not altogether satisfactory on account of the difficulty in obtaining a clear and colourless solution. The fat is removed from 8 g. of the sample by ether extraction, and 5 g. of the dry residue are extracted by shaking in a flask with 100 ml. of dilute (10–15 per cent.) alcohol, then filtered and washed with 20 ml. of industrial alcohol. The extracted residue is now washed into a flask with 50 ml. of ammonia-free water and heated in boiling water for twenty minutes, then cooled; 0·1 g. of diastase mixed with a little water is added, and the mixture is kept at 50°–55° for three hours, then cooled, made up to 250 ml. and filtered. Of the filtrate 200 ml. are mixed with 20 ml. of hydrochloric acid and heated in boiling water for three hours; the solution is nearly neutralised with sodium hydroxide and made up to 250 ml. after cooling; then the dextrose is estimated on an aliquot part by Fehling's solution in the usual way. An ordinary cocoa to which no farinaceous matter has been added contains about 7 per cent. of starch, so that 50 ml. is a convenient volume of the dextrose solution to take for the copper reduction; to convert the dextrose found into starch the factor is 0·90.

Cane sugar is sometimes present in prepared cocoas and usually in chocolate; it may be estimated by copper reduction after inversion, preferably with citric acid. Two g. of the fat-free dry material are shaken with water to dissolve all the sugar; the solution is filtered and the undissolved matter well washed with cold water, the filtrate and washings being made up to about 90 ml. To this is added 1 g. of citric acid, and the liquid is heated in boiling water for an hour, then cooled and neutralised; a few drops of copper sulphate solution are added, to precipitate any proteins. The mixture is made up to 100 ml., filtered, and the sugar is estimated on the filtrate by Fehling's solution in the usual way. In milk chocolates lactose will be present as well as cane sugar, so that the methods applicable to condensed milk may be employed. The lactose may be estimated as above described for cane sugar, but omitting the inversion with citric acid. It is convenient to divide the filtrate containing the sugars into two parts. The protein from one part is removed by zinc sulphate and potassium ferrocyanide solutions as on p. 77, and the lactose estimated in the filtrate by Fehling's method as usual. The other part is heated with 1 per cent. of citric acid to invert the cane sugar ; then the protein is removed by zinc and ferrocyanide solutions, and the total sugar estimated by copper reduction. By subtracting the copper reduced by the lactose from the total the cane sugar is readily calculated. Some commercial samples of chocolate have given the following results :—

	Ordinary chocolate.		Nut chocolate.	Milk chocolate.	
	Per cent. 1.	Per cent. 2.	Per cent. 3.	Per cent. 4.	Per cent. 5.
Moisture	1·56	0·92	1·06	1·66	1·50
Fat	28·94	39·80	36·58	35·48	33·59
Lactose	—	—	6·86	9·28	7·94
Sucrose	41·12	42·74	45·22	35·43	37·25
Nitrogen	0·61	0·84	1·12	1·11	1·18
Ash	1·38	2·54	1·53	1·77	1·96
Starch	24·3	4·3	—	—	—
On the fat :					
Reichert-value	—	—	4·8	5·6	4·3
Polenske value	—	—	1·3	1·1	1·2
Kirschner value	—	—	4·2	5·3	4·0

A number of analyses of chocolate are given by Booth Cribb and Richards (*Analyst*, 1909, **34**, 134).

In calculating the make-up of a milk chocolate many difficulties arise owing to the wide range of possible composition of the ingredients. Full cream or skimmed milk may have been used, either whole or condensed ; the cocoa part may have been derived from the nib only, with its entire fat content, or from partly de-fatted nib, and there is the possibility of other fats having been incorporated. It is therefore possible to indicate only the general lines of procedure ; each case presents its own difficulties, but these may usually be solved by patience and common sense. The amount of non-fatty milk solids may be calculated from the lactose, and the milk fat from the Kirschner value of the separated fat. The protein corresponding to the amount of milk present deducted from the total protein affords information as to the proportion of fat-free cocoa present ; then the added starch can be calculated and the added sugar estimated directly.

A useful method for determining the amount of cocoa matter in chocolate is that of Moir and Hinks (*Analyst*, 1935, **60,** 439) who find that the total alkaloid content of dry fat-free cocoa matter is 3·22 per cent. on the average. The method is as follows :—

Grind 2 g. of prepared cocoa or 2 or more g. of cocoa-nib or chocolate to a smooth paste with a little alcohol (80 per cent. v/v), and transfer to a 200-ml. flask with more alcohol of the same strength, sufficient to produce a total volume of about 100 ml. Add 1 g. of freshly-ignited magnesium oxide and digest in a boiling water-bath for one and a half hours under a reflux air-condenser, with occasional shaking. Filter while hot through a small Büchner funnel, return the residue to the flask, and re-digest for half an hour with 50 ml. of the alcohol. Filter and repeat the digestion with a further 50 ml. of the alcohol (80 per cent.). Evaporate the combined extracts on the water-bath, adding hot water from time to time to replace the alcohol lost. When all the alcohol is removed, finally evaporate to about 100 ml., add 2 or 3 drops of hydrochloric acid (10 per cent. HCl) and transfer the liquid to a 150-ml. measuring flask. Cool, add 5 ml. of zinc acetate solution and mix, and then add 5 ml. of potassium ferrocyanide[1] solution. Make up to the mark with water and mix thoroughly by shaking. Allow the flask to stand for a few minutes, and filter through a dry filter paper.

Evaporate a measured quantity (about 120 ml.) of the filtrate to about 10 ml., transfer to a separator and extract by vigorous shaking with five successive 30-ml. quantities of chloroform. Run off the chloroform after each extraction into a second separator, and wash the combined extracts with 3 to 5 ml. of water. Repeat the above process of extraction with five successive quantities of chloroform, wash the second chloroform extracts with the same wash-water, combine the whole of the extracts, and remove the chloroform by distillation. Dissolve the residue in a little hot water, transfer to a Kjeldahl flask, add 0·2 g. of sucrose and 10 ml. of concentrated sulphuric acid. Heat over a small flame till frothing ceases ; add 0·02 g. of selenium and

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[1] Of the strengths given on p. 77.

digest till colourless. Heat for one hour longer, and determine the ammonia in the usual way by distillation into N/10 acid.

Use the factor 3·26 for conversion of nitrogen into alkaloid.

Cocoa Butter

This may be examined by the methods described in the chapters dealing with fats, but as the analysis of chocolate generally includes some examination of the fat for the purpose of detecting foreign fat or estimating the amount of milk fat, the usual data for cocoa butter are given here.

	1.	2.	Average.
Specific gravity at 15·5°[1]	0·967	0·971	0·968
Melting point[1] °C.	32·6	32·9	33·1
Refractive index at 40°	1·4567	1·4550	1·4560
Titer °C.	48·7	49·5	49·2
Free acid as oleic	1·06	1·51	Up to 2 per cent.
Saponification value	194·1	195·7	195·2
Iodine value	34·3	35·8	36·0
Unsaponifiable matter %	0·27	0·38	0·3
Reichert value	1·1	1·0	1·0
Polenske value	0·7	0·44	0·5
Kirschner value	0·2	0·3	0·2

A useful special test, due to Björklund is available for the detection of beef fat; 3 g. of the fat are dissolved in 10 ml. of ether, slightly warming if necessary; a plug of cotton wool is placed in the mouth of the test tube, which is set aside in a cool place. Pure cocoa butter usually separates only below 10°, but when it does, it is in tufts, leaving a clear solution, whereas beef fat, if present, separates in flocculent masses and the supernatant liquor remains turbid. Cocoa butter crystals are all dissolved on warming to 18°, whereas beef crystals are not. A microscopic examination of the crystals should be made (see under "Lard," p. 283).

The A.O.A.C. in 1922 adopted two tests for cacao butter: (1) The critical temperature of solution in acetic acid and (2) an

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[1] When cocoa butter is melted it does not, on cooling, regain its normal specific gravity and melting point for some time; seventy-two hours should elapse between the time of melting and of observing these constants.

acetone and carbon tetrachloride test. These are carried out as follows (R. Doolittle, *J. Assoc. Off. Agric. Chem.*, 1923, **6**, 278; *Analyst*, 1923, **48**, 224):—

The Critical Temperature Test. The apparatus is a 6 × ¾-inch test tube fitted with a cork carrying a thermometer reading to 0·1° and extending so far into the tube that the bulb will be covered by 10 c.c. of the liquid. The test tube is fitted into a larger tube, 4 × 1 inch, containing glycerin, and is held firmly in place by a cork having a groove cut in its side to equalise the pressure when heat is applied. For the determination there are required glacial acetic acid free from water and 0·1 N potassium hydroxide solution. Filter a portion of the sample to be examined through a dry filter paper in an oven where a temperature of about 110° is maintained, to remove traces of water. Allow the filtered sample to cool until barely warm, and weigh 5 g. of the sample and 5 g. of the acetic acid reagent into the test tube. Insert the cork holding the thermometer, and place the test tube in the glycerin bath. Heat and shake the apparatus frequently until a clear solution of the fat and acetic acid is obtained. Allow the solution to cool with constant shaking without removing it from the bath. Note the temperature at which the first sign of turbidity appears. Make a similar test with the same acetic acid on a sample of pure cacao butter. Free-fatty acids lower the turbidity temperature; a correction must therefore be made for the acid value of the sample.

Correction Factor. If the strength of the acetic acid is such that the turbidity temperature for a pure cacao butter is approximately 90°, one unit of acid value will cause a reduction of 1·4° in the critical temperature of solution. If the turbidity temperature is approximately 100°, one unit of acid value will cause a reduction of 1·2°; for intermediate temperatures, the reduction is proportional.

Determine the acid value (*i.e.*, milligrams of potassium hydroxide required to neutralise the free fatty acid in 1 g. of the fat) of both the sample and the pure cacao butter; multiply the acid value by the correction factor, and add the result to the observed turbidity temperature. The figure so obtained is the true critical temperature of solution. If this is lower by more than 2° than that of the pure sample, adulteration with coconut, palm kernel, cotton-seed oils or stearines, maize oil, arachis oil, or other vegetable oils is indicated.

For the *acetone and carbon tetrachloride test* the reagent required is a mixture of equal volumes of these two substances.

Dissolve 5 g. of the warm fat, which has been previously filtered through dry filter paper in an oven at about 110° to remove traces of moisture, in 5 ml. of the acetone carbon tetrachloride reagent in a test tube. Allow the solution to stand in iced water for twenty or thirty minutes. Put on a test on a sample of pure cacao butter at the same time for comparison. If hydrogenated oil, tallow, oleostearine or paraffin wax is present, a white flocculent precipitate will soon appear. If the water is cold enough, cacao butter may solidify. If a precipitate is formed, remove the sample from the iced water and allow it to remain at the room temperature for a time. Solidified cacao butter will rapidly melt and go into solution, but if the precipitate is due to any of the above-mentioned possible adulterants, a much longer time will be required for complete solution to take place.

For the detection of cacao butter substitutes, see Knapp, Moss and Melley (*Analyst*, 1927, **52**, 452), and Bywaters *et al.* (*ibid.*, p. 324).

CHAPTER VI

MUSTARD, PEPPER, CAYENNE, GINGER, SPICES AND CURRY POWDER

MUSTARD as it appears on the market almost always consists of a mixture—the seeds of two species of *Sinapis*, *S. nigra* and *S.* (or *Brassica*) *alba*, which are brown and white mustard respectively. Brown mustard is distinguished by the pungent oil it yields when crushed with water. Under similar conditions white mustard does not yield any volatile oil, though it has an acrid and bitter taste. The active ingredients in each variety are thiocyanates, allyl-iso-thiocyanate in *S. nigra* and acrinyl-iso-thiocyanate in *S. alba*. These are produced by enzymic hydrolysis of the glucosides, sinigrin and sinalbin respectively. There is also present a considerable percentage of fixed oil and of protein.

Experience shows that if the seeds are simply crushed and mixed they soon become discoloured and mouldy ; this decomposition is inhibited by mixing with a moderate quantity of starch and by the manufacturing process to which the seeds are subjected. The quality of mustard depends partly upon the proportions of the two varieties which have been mixed, and upon the efficient removal of the outer husks by grinding and sifting ; it is also largely dependent on the season, storage and selection of the seed. The amounts of mustard oil vary in the case of brown mustard from, say, 0·3–1·9 per cent., but this alone is not sufficient to account for variations in quality and aroma. In white mustard the volatile oil does not exceed about 0·05–0·1 per cent. ; for medicinal purposes, which, of course, depend upon the volatile oil, it is therefore useless. In order to comply with the Food and Drugs Act, mustard, containing added starch, must be labelled as mustard mixture or mustard condiment. And under the Food Standards (Mustard) Order,[1] 1944, mustard, compound mustard or mustard condiment must yield not less than 0·35 per cent. of allyl-iso-thiocyanate ; the proportion of amylaceous flour or spices must not exceed 20 per cent.

The following are analyses of brown and white mustard :—

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[1] S.R. & O., 1944, No. 275.

	Brown flour.	White flour.	Brown husk.	White husk.
	Per cent.	Per cent.	Per cent.	Per cent.
Moisture . . .	4·90	5·16	10·30	7·25
Fixed oil . . .	39·81	38·06	14·52	22·81
*Starch (by diastase) .	2·82	5·15	0·70	1·20
Nitrogen . . .	4·42	4·76	2·95	4·69
Volatile oil. . .	1·63	0·07	—	—
Total sulphur . .	1·49	1·50	0·85	1·20
Ash	4·11	4·12	4·60	4·25
Phosphoric acid (P_2O_5)	1·80	1·47	0·75	1·30
Pentosans . . .	3·03	3·33	9·50	7·40
Crude fibre. . .	3·70	3·40	15·70	13·20

* Apparent starch.

Examination of some well-known mixed mustards yielded the following figures :—

	1. "Genuine."	2. "D.S.F."	3. "Fine."	4. —	5. "A1."
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Moisture .	5·01	4·91	4·77	5·30	4·96
Fixed oil .	38·82	30·29	32·24	23·98	38·10
Starch . .	3·87	10·29	3·31	14·25	3·50
Nitrogen .	5·05	4·82	5·15	4·10	5·30
Volatile oil .	0·91	0·80	0·57	0·58	0·86
Total sulphur.	1·30	1·14	1·57	1·39	1·50
Ash[1] . .	4·21	3·93	4·08	3·72	4·48
Phosphoric acid	2·02	1·71	1·82	1·51	1·75
Pentosans .	2·77	1·23	3·59	3·50	1·10
Crude fibre .	3·15	1·45	4·00	4·20	1·55
	Probable Composition				
Brown mustard	80·00	75·00	40·00	20·00	60·00
White mustard	20·00	14·00	60·00	65·00	40·00
Added flour .	—	11·00	—	15·00	—

[1] For the composition of the ash, see p. 152.

It will be seen from the above tables that the proportions of fixed oil, nitrogen, ash, sulphur, phosphoric acid and fibre are much the same in the two varieties. A figure which affords a basis for the calculation of the proportions of the two seeds is the sulphur *in the proteins*. Consideration of the reaction

$$\underset{\substack{\text{Potassium myronate}\\ \text{(sinigrin)}}}{KC_{10}H_{18}NS_2O_{10}} \longrightarrow C_6H_{12}O_6 + \underset{\substack{\text{allyl-iso-}\\ \text{thiocyanate}}}{C_3H_5NCS} + KHSO_4$$

shows that the weight of volatile oil multiplied by 0·64 gives the amount of sulphur combined therewith. This, subtracted from the total sulphur, gives the sulphur combined with the other nitrogenous constituents, since the total sulphur in the ash is only some 2–3 per cent. of the ash, which is almost negligible. When so calculated, white mustard flour contains 5·0 per cent. of sulphur in nitrogen combination and the brown flour only 1·60 per cent.; from these data the proportion of the two in any given mixture may be calculated.

An example will make this plain. Taking the data from No. 1 in the above table :—

Total nitrogen	5·05
Nitrogen combined as mustard oil (0·91 × 0·14)	0·13
Difference	4·92
× 6·25 = Proteins	31·49
Total sulphur	1·30
Sulphur combined as mustard oil (0·91 × 0·64) .	0·58
Difference = sulphur combined with proteins .	0·72
∴ Percentage of sulphur in the proteins .	2·3
∴ Percentage of white mustard = $\frac{(2\cdot3 - 1\cdot6)\ 100}{(5\cdot0 - 1\cdot6)}$	= 20
∴ Percentage of brown mustard by difference	= 80

It is clear that the results determined in this way cannot claim to be more than approximate; there is no method yet available whereby the exact proportions of the two varieties can be ascertained. An alternative method of calculation can be based on the percentage of allyl-iso-thiocyanate, estimated as described on the next page, since brown mustard contains about 1·7 per cent. and white mustard only a mere trace, say 0·07 per cent.

True starch is not present in mustard seed, but small quantities not exceeding 2·5 per cent. (U.S.A. limit) may arise from the mucilage and traces of foreign seeds. It must be estimated by diastase and not hydrochloric acid inversion, since the latter gives a much higher figure, which is not true starch. Added starch should always be identified microscopically ; indeed, almost all the likely adulterants of mustard (except aniline dyes) are to be detected in this manner ; such substances include turmeric, pepper, capsicum, or occasionally mineral additions.

Turmeric may be identified chemically by the characteristic red colour produced when it is extracted with alcohol, soaked up on a piece of filter paper and dried in the oven with boric acid. The colour so produced turns green when touched with sodium hydroxide solution. It may be detected microchemically by the red colour produced when concentrated sulphuric acid is run on to the slide containing it. Ordinary or cayenne pepper may also be detected by extracting with alcohol and evaporating the extract, the taste and smell of which will reveal the presence of either, but the microscope is more reliable. The amount of starch present in natural mustard is so small that it does not respond to the iodine reaction unless added starch containing substance is present. Aniline dyes, if present, may be extracted by means of alcohol, then absorbed on white wool or silk, and identified by the usual systematic tests.

In the analysis of mustard, a few special methods are necessary. Moisture is best estimated *in vacuo* over sulphuric acid and fixed oil by direct extraction with petroleum ether ; in the presence of added starch the amounts of both ash and fixed oil will probably be low. For the estimation of total sulphur, about 1 g. is weighed out and added in small quantities to 10 ml. of fuming nitric acid. When solution is complete, water is added, and the mixture is boiled, filtered if necessary, and precipitated by the addition of barium chloride ; then the barium sulphate is filtered off, ignited and weighed. Several methods have been proposed for the estimation of the volatile mustard oil, of which the following is perhaps the best (*cf.* Raquet, *Ann. Chim. Anal.*, 1912, **17**, 174). It is based upon the reaction of allyl-thiocyanate with silver nitrate in ammoniacal solution. Five g. of the mustard are kept for about six hours in a closed flask with 100 ml. of water and 20 ml. of alcohol in order to allow the complete formation of the allyl-iso-thiocyanate ; then the mixture is distilled and the distillate collected in a 100 ml. flask containing 10 ml. of dilute ammonia solution. When 50 ml. of distillate have been collected, 20 ml. of 0·1 N silver nitrate solution are added, and a further 15 ml. are distilled over. The flask should now be closed by a stopper carrying a long glass tube to act as an air condenser; then it is immersed in a bath of water at 85° for an hour. After cooling, the liquid is made up to the 100 ml. mark and filtered; 50 ml. of the filtrate are withdrawn, acidified with nitric acid, a few drops of ferric ammonium sulphate solution are added, and the excess of silver is titrated with 0·1 N sodium thiocyanate solution (1 ml. of 0·1 N silver nitrate = 0·00495 g. of allyl-iso-thiocyanate). Mustard oil was official in the B.P., 1914, and may be assayed by the above process.

Starch should be estimated by diastase; 5 g. (or less if microscopic examination indicates a large addition of other flour) are thoroughly extracted in a Soxhlet extractor, first with petroleum spirit, then with alcohol (95 per cent.), and dried. The residue is washed into a 100-ml. flask with water, which should be free from ammonia, then heated in a water bath for about half an hour, cooled to 50°, and 0·1 g. of disastase dissolved in a little water is added. After standing for three hours in a warm place 5 ml. of 0·1 N sodium hydroxide are added, and the mixture is diluted to 100 ml. The sugar so formed is estimated gravimetrically by Fehling's solution. For purposes of calculation it may be taken that wheat flour, which is most usually found in mustard mixtures, contains 72 per cent. of starch. The estimation of crude fibre follows the ordinary process detailed on p. 60, and yields more information with less expenditure of time and materials than that of pentosans.

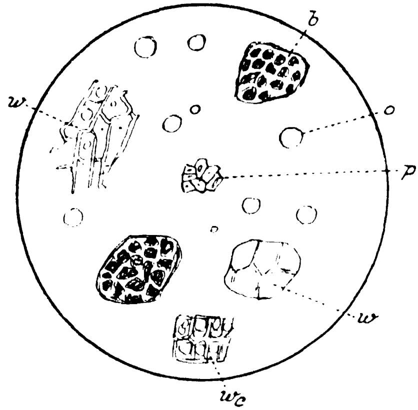

FIG. 34. Mustard. *o*, oil globules; *b*, black mustard; *w*, white mustard; *p*, palisade cells; w_c, cells from cotyledon.

For the microscopic examination it is convenient to extract a small quantity in a test tube with ether, then with alcohol, so as to remove the fixed oil; then on mounting in water or weak glycerin the structures are more easily made out. Most characteristic are the fragments of the sclerenchymatous layer which are seen as small strongly thickened polygonal cells, black or dark brown in the case of black mustard and yellow in the case of white mustard. There are also large polygonal thin-walled epidermal cells filled with mucilage and cotyledonous cells filled with minute aleurone grains. Only a few, if any, starch grains can be seen, and these are small and round, not angular like rice. In the unextracted mustard the round oil globules are numerous (see Fig. 34).

If a small quantity of concentrated sulphuric acid be carefully run under the coverslip, particles of turmeric, if present, will be at once shown up by their characteristic red colour. Dilute iodine solution brings any added starch into clear relief.

Pepper

Both black and white pepper consist of the fruit, more or less ripe, of *Piper nigrum*, the difference in the two grades depending only on the removal of the outer portions of the pericarp in the case of the white variety. Cases have been recorded of black pepper coated with talc, plaster of Paris, or kaolin, being passed off as white pepper; such sophistication would readily be detected by the increased ash content, as naturally the ash of white pepper is much lower than that of black. Apart from the question of gross adulteration, much interest centres round the amount of shell; this depends mainly upon the mode of preparation for the market. For the preparation of ordinary black pepper, peppers of different origin are usually mixed in the grinding stage. If white pepper is desired the seeds are soaked in water, with or without the addition of lime, in order to soften the pericarp, which is then removed by rolling, and the resulting white berries are dried in the sun; the amount of shell thus removed is about 15 per cent. Bleaching with chloride of lime is sometimes resorted to, or the separated hulls may be so bleached, then ground and sold or mixed with genuine white pepper. The detection of such sophistications depends largely upon the estimation of crude fibre, the amounts of which present in the different products will be seen from the tables.

Long pepper, which is not usually retailed in this country, but is used largely in the manufacture of pickles, is the fruit of *Piper officinarum* or *P. longum*, and has the form of spikes about 2–6 cm. in length. It has been reported to be used for the adulteration of black or white pepper, but its presence would readily be detected microscopically.

The active constituent of pepper is the alkaloid, piperine ($C_{17}H_{19}O_3N$). which is present to the extent of 7–8 per cent., and piperidine ($C_5H_{11}N$), amounting to 0·3–0·8 per cent., although the presence of the latter has been disputed. Important constituents also include a volatile oil—about 1–2 per cent.—and a resin. Starch accounts for about 35 per cent. of the berry. Pepper including much husk is slightly more pungent than that prepared only from the kernel ; thus black pepper is more potent than white, and contains rather more piperine (although Gladhill, *Amer. Journ. Pharm.*, 1904, **76**, 71, states that there is no piperine in the husk). It will be found that in general the piperine content of a pepper is 0·8–1·0 per cent. less than the total ether extract.

Although the analytical data on pepper varies somewhat according to the country of origin, the figures below are a fair average.

	Black pepper.	White pepper.	Husks.
	Per cent.	Per cent.	Per cent.
Moisture . . .	7·5–10·0	7·5–11·5	7·0–10·0
Ash[1]	3·0–5·2	0·95–2·5	7·0–15·0
Water-soluble ash .	1·5–3·0	0·25–1·0	2·0–4·0
Ether extract . .	7·5–9·7	6·5–7·5	3·5–7·0
Piperine . . .	6·7–9·0	5·7–6·7	2·3–6·3
Alcohol extract .	8·5–11·0	7·5–9·0	5·0–7·5
Crude fibre . .	8·5–16·0	0·5–6·5	20·0–31·0
Starch . . .	28·0–40·0	45·0–61·0	5·0–13·0
Total nitrogen. .	2·0–2·5	1·80–2·3	2·5–3·0

[1] For the composition of the ash, see p. 152.

The Australian Board of Trade Regulations (1917) fix limits for black and white pepper as :—

	Black.	White.
	Per cent.	Per cent.
Ash not more than	7·0	3·5
Alcohol extract not less than .	8·0	7·0
Ether extract not less than .	6·0	6·0

The following data are given by König as the mean of six analyses of long pepper :—

	Long Pepper. Per cent.
Moisture . .	10·69
Ash . . .	7·11
Water-soluble ash .	3·83
Ether extract, total	8·72
Ether extract, volatile	1·56
Piperine . .	4·47
Alcohol extract .	8·60
Crude fibre . .	5·47
Starch . . .	42·88

The proportion of piperine in long pepper is much lower than in the ordinary variety.

Other adulterants, besides starches, which have been found in pepper, include "pepperette," or ground olive stones, almond, date, walnut, or coconut shells, some of the common analytical data on which may be given here :—

	Olive stones.	Date stones.	Walnut shells.	Coconut shells.	Almond shells.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Moisture . . .	5·30	9·20	8·91	7·36	9·65
Ash . . .	3·50	1·95	1·29	0·54	3·25
Water-soluble ash .	1·10	0·90	—	—	—
Alcohol extract .	2·46	15·2	1·43	1·12	5·16
Ether extract . .	15·30	7·3	1·06	0·25	1·15
Nitrogen . .	1·15	1·10	0·45	0·04	0·35
Crude fibre . .	63·0	64·5	51·74	56·2	48·8

The presence of such substances will be at once recognised by the high fibre figure, but this may be partially masked by the addition of starch ; the low ether extract and piperine content would, however, betray such a mixture, as also would a microscopic examination. Spent ginger (*q.v.*, p. 160) and magnesium carbonate have also been reported on recent occasions. Small proportions, such as 0·2 per cent., of turmeric are sometimes added for colouring purposes and would hardly be regarded as an adulterant. The quantity present may be estimated by comparison of the colour of the alcohol extract with that of dilutions of an alcoholic tincture of *freshly ground* turmeric.

According to Mehring (*J. Agric. Res.*, 1924, **29,** 569), the composition of the ash of mustard and pepper is given below :—

	Black pepper.	Whit pepp	Mustard.	Paprika or Cayenne pepper.
	Per cent.	Per cen	Per cent	Per cent.
K_2O . .	27·56	6·1	18·90	54·37
Na_2O . .	3·89	0·7	0·37	3·98
CaO . .	13·73	32·0	15·57	5·15
MgO . .	7·55	10·5	10·51	6·02
Fe_2O_3 . .	0·58	2·0	1·09	1·97
Al_2O_3 .	—	—		0·09
Mn_3O_4 . .	0·20	0·5		—
CuO . .	—	—		0·10
P_2O_5 . .	9·42	29·5	38·22	16·43
SO_3 . .	8·48	3·1	5·76	5·70
Cl . .	9.13	—	0·17	3·51
CO_2 . .	12·90	14·8	2·62	—
SiO_2 . .	6·56	0·3	6·79	2·68

The special methods of analysis to be applied to pepper include alcohol and ether extract, which may be estimated approximately by shaking 5 g. of the fine powder with 100 ml. alcohol (95 per cent.) or ether at frequent intervals during twenty-four hours, then filtering through a dry paper and evaporating 20 ml. of the clear filtrate in a tared flask. Complete exhaustion in a Soxhlet extractor with these solvents yields more exact figures, but is not usually necessary.

Nitrogen, starch and crude fibre are estimated in the ordinary way, using Kjeldahl's method for nitrogen, the diastase method (p. 61) for starch, and 1·25 per cent. acid and alkali for fibre (p. 60) ; it is convenient to extract 6 g. in a Soxhlet extractor first with ether, then with alcohol, to remove oil and resin, and so obtain a residue on aliquot parts of which the estimations of fibre and starch may be made. Nitrogen should be determined without any previous ether extraction, as the extract so obtained contains some nitrogen.

For the estimation of the alkaloid, piperine, 10 g. of the finely-ground sample are extracted for at least six hours in a hot jacketed Soxhlet extractor with 95 per cent. alcohol; then the extract is evaporated on a water bath at a temperature not exceeding 60° to a syrup; 10 ml. of dilute aqueous solution of potassium hydroxide are added to dissolve the resinous substances which are extracted by alcohol. The solution is poured off through a small pleated filter which is washed with a minimum quantity of water; the residue is dissolved in alcohol, evaporated at 60° as before, dried in a vacuum over sulphuric acid and weighed. An approximate estimation may be made by evaporating and drying the alcohol extract, then subtracting 0·8 per cent., which represents the average quantity of resin dissolved by the alcohol.

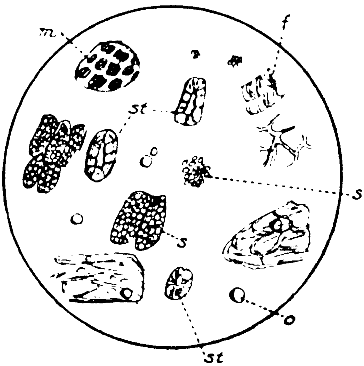

FIG. 35. Black pepper. *m*, mesocarp; *f*, fibro-vascular bundles; *s*, starch; *st*, stone cells; *o*, oil globules.

The microscopic examination of every sample of pepper is essential; of the starches, that of rice is somewhat similar to that of pepper, but the latter is smaller and more rounded. It is most convenient to mount the powdered sample in dilute glycerin, and in cases of doubt it is useful to sift out the coarser particles and examine them separately. For details of the structure of pepper or the many possible adulterants, such as ginger, capsicum, mustard husk, or ground fruit stones, reference must be made to the usual text-books on the microscopy of food and drugs, also Wallis (*Analyst*, 1915, **40,** 190). Characteristic features (see Fig. 35) of genuine pepper are the polygonal cells (perisperm) packed with minute rounded starch grains, fibro-vascular bundles and sclerenchymatous cells, which latter are of more or less regular pentagonal shape, differing from those of ground olive or other stones. For the better detection of poivrette or other ground stones the phloroglucinol reaction is useful. Digest about 0·1 g. of the sample for a few minutes with 5 ml. of warm alcoholic solution of phloroglucinol, pour off the solution, rinse with water, transfer part of the residue to a micro-slide, cover it with hydrochloric acid, and warm till the latter fumes strongly; cover and examine under a low power; pepperette and like substances give an intense red colour. In all cases of doubt, comparison with genuine pepper or known mixture should be made. Capsicum or cayenne can be detected by the method given for mustard on p. 147.

Cayenne Pepper

This is the dried ripe fruit of various species of capsicum, which are indigenous to tropical parts of Africa, India and America ; in commerce the seeds are ground with the pericarp. It is also known as chillies, or pod pepper, as it occurs in thin red pods which contain about eighteen seeds. It should be noted that the B.P. capsicum is the dried ripe fruit of *Capsicum minimum*, which is not necessarily the cayenne pepper of commerce. The characteristic pungent taste is due to a substance, capsicin ($CH_3OC_{17}H_{24}.NO_2H$), although it is only present to the extent of some 0·07 per cent. Capsicum is also notable for its high content of vitamin C (ascorbic acid), which is present to the extent of about 0·2 per cent. Other substances present include fixed oil and resin, 5–7 per cent., and some fatty acids. The usual analytical constants are :—

	Per cent.
Moisture	7·5–10·0
Ash	5·1–6·4
Water-soluble ash	2·6–3·5
Ether extract [1]	15·5–19·2
Alcohol extract	24·0–30·0
Crude fibre	17·5–24·5
Nitrogen	1·8–2·2
Starch	0·5–1·5

The standard prescribed by the U.S. Department of Agriculture is not less than 15 per cent. of non-volatile ether extract, not more than 6·5 per cent. of ash, not more than 0·5 per cent. of ash insoluble in hydrochloric acid, not more than 1·5 per cent. of starch by the diastase method, and not more than 28 per cent. of crude fibre.

Tolman and Mitchell (*U.S. Dept. of Agric. Chem. Bureau Bull.*, No. 163, 1913) give many analyses of different varieties of cayenne and pimento :—

---

[1] The ether extract on the seeds only is much higher and may be as much as 30 per cent.

CAYENNE PEPPER OR CHILLIES

	African chillies.		Japanese chillies.	
	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.
Total ash	4·86	6·16	5·06	5·82
Ash insoluble in HCl . .	0·37	0·85	0·27	0·53
Non-volatile ether extract .	16·07	19·68	17·67	22·89
Crude fibre	25·39	29·46	22·86	26·08

The figure which gives reliable information as to the presence of exhausted capsicum is the ether extract. This solvent dissolves out the whole of the pungent substances, leaving an almost tasteless residue. A partially exhausted cayenne may contain non-volatile ether-soluble substances from practically nothing up to 12 per cent., whereas genuine cayenne yields from 15–20 per cent. As usual with this class of substance, much information is obtainable by careful microscopic examination; for this purpose about 0·5 g. should be taken up with ether and allowed to stand for an hour, then the ether poured off and the residue mounted in 50 per cent. chloral hydrate solution, another portion being examined in weak iodine solution. The details of structure vary with the species of capsicum, but the epidermal cells are characteristic; they have very thick walls, with a few pits and, except in the Japanese variety, well-marked striations. Since cayenne contains only a few minute starch grains, the iodine-water preparation will at once indicate the presence of any farinaceous adulterant, ginger or other starchy substance. Mustard and rice husks and turmeric, besides mineral additions, have been occasionally reported. For illustrations of powdered cayenne see Greenish's "The Microscopic Examination of Food and Drugs"; detailed accounts of the different species are given by Wallis (*Pharm. J.*, 4, **15**, 3).

Capsicum annuum, a species grown largely in Central Europe, is known as paprika. It closely resembles *C. minimum* in general characters, but is less pungent; under the microscope the epidermal cells are much larger, being as much as 100μ long in some cases. The non-volatile ether extract is somewhat lower

HUNGARIAN PAPRIKA (*a variety of Capsicum annuum L.*)

	Shells, seeds, placentæ and stems.		Shells, seeds, and placentæ.		Seeds and placentæ.		Shells.		Stems.	
	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.
Loss at 70° C. *in vacuo* .	3·29	3·76	3·11	4·16	3·73	4·23	3·44	4·00	4·66	5·78
Total ash . . .	5·08	6·03	4·66	5·56	3·38	3·80	6·29	7·11	9·84	11·35
Ash insoluble in HCl .	0·24	0·33	0·20	0·31	0·21	0·30	0·22	0·37	0·66	1·20
Non-volatile ether extract.	12·21	16·43	13·94	17·35	25·97	27·56	5·14	6·90	2·11	3·05
Crude fibre . . .	20·69	22·76	20·47	23·18	20·89	21·60	22·20	24·07	29·34	32·86

SPANISH PIMENTO (*also a variety of Capsicum annuum L.*)

	Shells, seeds, placentæ and stems.		Shells, seeds, and placentæ.		Shells.		Seeds and placentæ.		Stems.	
	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.	Min. per cent.	Max. per cent.
Loss at 70° C. *in vacuo* .	4·31	5·98	4·52	5·09	4·74	5·02	3·59	4·12	4·86	5·98
Total ash . . .	6·98	7·86	6·60	7·35	7·55	8·46	4·43	5·02	13·00	15·77
Ash insoluble in HCl .	0·29	0·48	0·24	0·40	0·26	0·37	0·22	0·31	0·58	1·07
Non-volatile ether extract.	11·30	12·58	11·58	13·34	5·44	6·81	21·82	24·58	1·22	2·05
Crude fibre . . .	19·53	20·59	18·76	20·34	17·26	18·70	19·90	24·08	27·66	30·98

than that of ordinary cayenne, as is shown by the following analyses of the whole pods :—

	Per cent.		Per cent.
Moisture	7·05	..	7·60
Ash [1]	6·10	..	6·45
Water-soluble ash	4·75	..	4·95
Ether extract	9·48	..	11·43
Alcohol extract	14·75	..	15·80
Crude fibre	14·50	..	15·00
Nitrogen	2·40	..	2·85
Starch	—	..	—

[1] The composition of the ash has been given on p. 152.

Hensler and Hassler (*Z. Unters. Nahr. Genussm.*, 1914, **27**, *201*) show that a direct estimation of the iodine value of paprika affords definite evidence of the presence or otherwise of extracted material. Hübl's method is used, and the solution is allowed to act for six hours, under which conditions genuine paprika gives iodine values from 31·8 to 34·8, whereas paprika from which the active principles have been extracted yields values from 19·4 to 20·7.

Allspice

Allspice, or pimento, is another pepper-like spice, but is much less pungent than any species of capsicum ; it is the dried ripe berry of *Pimenta officinalis*, which is largely grown in Jamaica. Prominent differences in the analyses from those of cayenne are that the ash does not exceed 5 per cent., that there is some 5 per cent. of non-volatile oil suggesting cloves, and that there is much starch which resembles that of nutmeg. Under the microscope the general appearance is suggestive of a coarse black pepper, but the stone cells and starch grains are much larger ; details of the microscopic structure are given by Clayton ("Compendium of Food Microscopy," p. 210). The usual composition of pimento is :—

	Per cent.
Moisture	9·69
Ash	4·75
Water-soluble ash	2·62
Ether extract	10·4
Alcohol extract	12·68
Crude fibre	20·9
Nitrogen	0·80
Starch	3·04

As in the case of other spices, the starch should be estimated by the diastase method (p. 61), not by hydrochloric acid inversion.

Ginger

Ginger, according to the B.P., is the scraped and dried rhizome of *Zingiber officinale*. In commerce numerous varieties occur, including Japanese ginger, which differs materially from other kinds and is derived from *Z. mioga*. In the English market Jamaica, Bengal, Cochin and African gingers are commonest, the last named being sold with its epidermis, whereas the others are sold scraped. These varieties may be identified by the size and shape of the "fingers" when whole (for detailed description see the U.S.P.). *In the powder form* they may sometimes be identified from the analytical data by comparison with known standards. The detailed analyses of the different commercial varieties of ginger according to Reich (*Z. Unters. Nahr. Genussm.*, 1907, **14**, 549) are as follows :—

	Water.	Total ash.	Water-soluble ash.	Sand.	Ether extract.		Alcohol extract (after ether extraction).
					Volatile	Non-volatile	
	Per cent	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent
		Cochin Ginger.					
Maximum .	12·59	4·98	2·96	0·20	1·65	3·77	2·11
Minimum .	10·40	3·33	1·64	0·05	1·05	3·02	1·49
Average .	11·64	4·18	2·33	0·15	1·38	3·40	1·86
		Japan Ginger					
Maximum .	13·80	6·44	3·04	0·52	1·89	5·16	4·46
Minimum .	9·20	3·24	1·52	0·14	0·90	3·72	2·69
Average .	11·68	4·65	2·04	0·30	1·38	4·48	3·45
		Bengal Ginger					
Maximum .	13·85	9·33	4·51	3·79	2·15	4·94	2·98
Minimum .	11·69	5·48	2·56	1·21	1·17	2·84	1·12
Average .	12·51	7·06	3·45	2·05	1·60	3·97	1·88
		African Ginger					
Maximum .	13·65	6·22	2·56	2·47	3·08	8·08	2·42
Minimum .	11·16	3·29	1·63	0·08	2·10	5·66	1·39
Average .	12·74	4·37	1·97	0·84	2·54	6·50	1·70

Ginger rhizomes are often whitened by immersion in milk of lime, which has the merit of preserving them to some extent from the ravages of insects or fungi ; bleaching by sulphurous acid or chloride of lime has been reported sometimes, as has also coating with plaster of Paris.

Among the important constituents of ginger may be mentioned a volatile oil having the following characters : sp. gr., 0·875–0·885 ; optical rotation in 100 mm. tube, – 25° to – 45°, distilling from 150° to 300°. To this oil is due the characteristic pungent aroma and taste ; it contains, *inter alia*, cineol, phellandrene, zingiberene and camphene. There are also present a fixed oleo-resin, "gingerol," which varies much in amount, starch, and certain resins and a ketone zingerone. For the chemistry of gingerol see Nomura (*J. Chem. Soc.*, 1917, **111,** 769).

The following standards are put forward for ginger (B.P., 1932) : total ash not more than 6 per cent. ; water-soluble ash not less than 1·7 per cent. ; alcohol extract not less than 4·5 per cent. ; cold water extract not less than 10 per cent. The B.P. Codex (1934) adds 1–3 per cent. volatile oil. The U.S.P. (XII) requires non-volatile ether extract not less than 4·5 per cent., and cold water extract not less than 12 per cent. Commercial ginger does not always conform to pharmacopœial requirements.

The maxima and minima of a large number of analyses of ginger are as under :—

	Genuine.	Exhausted.	
		1.	2.
	Per cent.	Per cent.	Per cent.
Moisture	8·4–12·5	11·5	12·7
Total ash	3·4–7·6	3·80	5·75
Water-soluble ash	1·0–3·7	0·55	1·02
Volatile oil	1·6–2·9	0·55	1·05
Fixed oil and resin	3·0–7·5	1·2	1·4
Alcohol extract	4·5–8·1	3·1	2·5
Crude fibre	1·7–6·5	—	—
Nitrogen	1·0–1·5	1·05	1·10
Cold water extract	7·0–14·0	5·3	4·5
Starch	48·5–53·0	—	—
Gingerol	0·9–2·5	—	—

The most frequent sophistication is admixture with exhausted or spent ginger, which is a by-product of the ginger beer factory; this is detectable by means of the alcohol and cold water extracts particularly, which are seriously lowered by such treatment. Much reliance should not be attached to an increased ash figure as evidence of adulteration, as the natural variation is so wide, and some kinds, such as Bengal ginger, frequently fall outside the pharmacopœial limits. The water-soluble ash in exhausted ginger is always low, being about 25 per cent. of the total ash instead of the usual 40–50 per cent.

Added starches will be detected by the microscope; ginger starch may be likened to sacks tied at the mouth; the grains are from 15 to 35 μ in length, have very faint oyster-shell striations and a hilum near the smaller end. Other characteristic features of ground ginger are the large, thin-walled, pale yellow sclerenchymatous fibres, wide spiral vessels and the cortical parenchyma containing oil globules (see Fig. 36). Possible adulterants include, various starches, exhausted ginger, turmeric, galanga or capsicum. A special test for the last-named substance depends upon the destruction of gingerol by heating with alcoholic potash solution, which treatment does not affect capsicin. Twenty ml. of the aqueous extract are acidified with dilute sulphuric acid and extracted with an equal volume of ether; the ether extract is evaporated at a low temperature, and the residue is heated under a reflux air condenser for half an hour with 5 ml. of 0·5 N alcoholic solution of potash, then allowed to evaporate nearly to dryness; 10 ml. of water are added and 5 ml. of ether; after shaking the ether is separated, evaporated, and the residue taken up in 2 ml. of water and tasted; any pungency is due to capsicum; pure ginger so treated gives only a slight camphor-like taste.

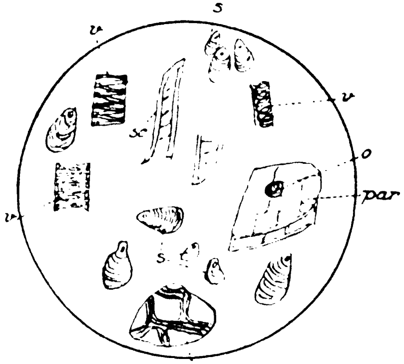

Fig. 36. Ginger. *v*, vessels; *par*, parenchyma; *o*, oil globule; *s*, starch; *sc*, sclerenchymatous fibres; *c*, cork.

For the estimation of the alcohol extract and water extracts of ginger 5 g. of the fine powder should be shaken frequently in a closed flask with 100 ml. of alcohol (96 per cent.) and water respectively for at least twelve hours ; then, after filtration, 20 ml. of the extract are evaporated in a tared flask or basin. More correct results are obtained by exhaustion in a Soxhlet extractor. The fixed oil or resin, which includes gingerol and allied substances, may be estimated similarly by extracting the powder with dry ether ; the residue should be dried to constant weight at a temperature not exceeding 65°. If it is desired to separate the gingerol, the procedure of Garnett and Grier may be followed (*Y. B. of Pharm.*, 1909, 344). The ether extract is well boiled under a reflux condenser with petroleum ether, which is poured or filtered off, and extracted three times in a separator with 35 per cent. alcohol ; the latter is washed once with petroleum ether and then evaporated until all alcohol is removed ; the residue is extracted with ether, which is in turn evaporated, and the resulting crude gingerol weighed. It should be pointed out that *pure* gingerol has not yet been obtained (see Lapworth, Pearson and Royale, *J. Chem. Soc.*, 1917, **111**, 777).

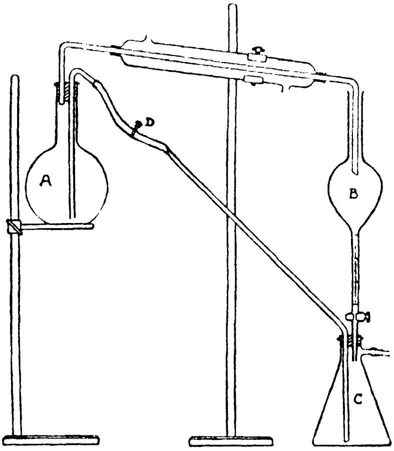

FIG. 37

Estimation of the volatile oil in ginger, or indeed, in any spice or condiment, is not quite a simple matter unless large quantities are available. A useful method, however, has been developed by Sage and Fleck (*Analyst*, 1934, **59,** 614) and has the advantage that the apparatus involved (shown in Fig. 37) is only ordinary laboratory equipment.

Description of Apparatus. The boiler A is an ordinary 1-litre flat-bottomed flask, and is connected with a Liebig condenser, having a narrow-bore inner tube bent as shown. The receiver B is a standard separating funnel receiver (V.4), as specified by the Standardisation of Tar-Products Tests Committee, and C is a 250-ml. Büchner flask. The flask C is connected with A by means of a glass tube with rubber connection, and Hofmann screw-clip at D. The liquid in A is boiled, the distillate is collected in B, and after 100 ml. or so has come over, the lower aqueous layer is separated and pumped back into A by opening the clip at D, and applying a small hand-bellows at C. This cycle is repeated several times until no more oil passes over. The size of the distillation flask may be varied according to the bulk of the material to be examined, and for oils which sink in water the separation is accomplished by placing a measured volume of toluene in the separator, which enables the oily mixture to float, allowance being made for this when making the final measurement.

Solubility of the oil in the distillate cannot be allowed for with any precision, but the repeated distillation of the separated water leaves the bulk of the oil for measurement. Sage and Fleck give the following examples of results obtained by this method :—

DRUGS AND SPICES

Sample.	Oil Per cent. v/w.	n_D^{20} of oil.
Coriander fruit . . .	0·34	1·4632
Ground ginger	1·04	1·4966
Nutmeg	5·12	1·4809
Cinnamon	0·48	—
Cloves	15·40	—
Caraway	4·33	1·4898
Juniper berries . . .	0·19	1·4876
Cardamoms (entire) . . .	4·72	1·4650
Cardamom seeds . . .	3·28	1·4616
Anise fruits (Pimpinella) . .	2·34	1·5544
Fennel fruits	1·64	1·5472
Cummin fruits	3·14	1·5034

Particulars of some other spices or condiments may be summarised as under.

Cloves

These are the flower-buds of *Eugenia caryophyllata* ; they are described in detail in the B.P., by which authority a limit of ash of 10 per cent. is prescribed. Of the ash at least 55–60 per cent. should be water-soluble, and the acid insoluble ash should be not more than 0·75. The principal constituents include 15–20 per cent. of volatile oil (the stalks contain only 5–7 per cent. of volatile oil, and it is not identical with clove oil), a phytosterol, and gallotannic acid to the extent of some 13 per cent. The average composition of cloves, which include the stems, is given in the table on p. 166. A likely adulterant is exhausted cloves, which may be detected by the low ether extract and alcohol extract or by the more tedious estimation of the volatile oil. Tannin may be estimated if desired on the ether-extracted residue by the Löwenthal method (p. 124). In the case of powdered cloves a microscopic examination is essential. There is no starch in the genuine powder; the stone cells, sclerenchymatous fibres, crystals of calcium oxalate and spiral vessels are characteristic. For illustrations see Clayton's "Compendium of Food Microscopy," p. 204.

Caraway

Caraway fruit or "seed" is the dried ripe fruit of *Carum carvi*, which is largely cultivated in Holland. The fruit, which is described in the B.P., contains 3–7 per cent. of volatile oil and about 18 per cent. of fixed oil. The ash limit prescribed by the B.P. is 9 per cent. including not more than 1·5 per cent. of acid insoluble ash; usually the total ash does not exceed 6 per cent., of which 35 per cent. is water-soluble. Dyer and Gilbard (*Analyst*, 1896, **21,** 207) point out that caraway is sometimes adulterated with "drawn" or exhausted seed; this sophistication can be recognised by the low fixed ether extract, which is reduced from about 20 per cent. to 16 or less; there is also almost complete absence of volatile oil in the exhausted seed. The analytical constants of the genuine seeds are given on p. 166.

Nutmeg and Mace

Both these are products of *Myristica fragrans*, a tree indigenous to the West Indies; nutmeg is the kernel of the seed and mace the dried arillus of the fruit. There are various other species of myristica, the seeds of which are sometimes used, but which are not genuine nutmeg or mace, notably Bombay mace or nutmeg and Macassar mace; a description of these varieties is given by Holmes (*Pharm. J.*, 1908, **81,** 652), but spurious nutmegs will be at once detected on comparing them with the genuine article. Damaged nuts "repaired" by means of lime or other extraneous matter have been described; such will be detected by the high ash content and low yield of volatile oil and fat, which in genuine nutmeg amounts to 10–15 per cent. and 40 per cent. respectively. In mace the volatile oil is somewhat less, being from 4 to 10 per cent.; a large amount of amylodextrin is also present. Essential oil of nutmeg is the *oleum myristicæ* of the B.P., which has the following specification:—

Specific gravity	0·880 to 0·925
Optical rotation, 100 mm. tube . .	+ 10° to + 30°
Refractive index at 20° . . .	1·474 to 1·488
Fixed residue not more than . .	3 per cent.

The essential oil from mace has similar characteristics. Microscopic examination of powdered nutmeg shows the presence of small round starch grains with a hilum, numerous crystals of fatty acids, and characteristic endosperm cells. For illustrations see Clayton's "Compendium of Food Microscopy," p. 202.

Mace shows no starch, but much amylodextrin, which stains red with iodine solution; it has much parenchymatous tissue and elongated thick-walled epidermal cells.

For the detection of spurious mace in powder form, the fixed ether extract, which should be dried till no more volatile oil is driven off, and the alcohol extract yield much information. Both these figures are much lower in genuine mace than in Macassar or Bombay mace.

	Genuine mace.	Macassar mace.	Bombay mace.
	Per cent.	Per cent.	Per cent.
Ether extract . .	24—33	50—52	55—60
Alcohol extract .	21—25	35—40	40—45

Griebel (*Z. Unters. Nahr. Genussm.*, 1909, **18**, 202; abstract, *Analyst*, 1909, **34**, 441) and Schindler-Zwickau (*Z. Öffentl. Chem.*, 1902, **8**, 288; abstract, *Analyst*, 1902, **27**, 327) give colour reactions for the detection of Macassar and Bombay maces respectively, but these should not be relied on except as affording confirmation of the deductions from the extracts and other figures. Griebel's test is performed by shaking about 0·1 g. of the mace with 10 ml. of petroleum spirit, then filtering into a test tube and adding sulphuric acid so that it forms a layer underneath; under these conditions Macassar mace gives a red ring at the junction, whereas genuine mace gives only a yellowish brown. It is desirable always to carry out the test on genuine mace for comparison. Schindler proceeds as follows: 5 g. of the powder are wetted with 8 ml. of alcohol and packed in a percolating tube placed over a small flask, and 8 ml. of alcohol are added and allowed to run through; 8 ml. more are run through into another flask and the process repeated, say, six times, separate receivers being used, and to each is added a drop of solution of lead acetate. The first flask shows a yellowish-red precipitate, the second a slight precipitate, and third a very little, and the fourth none, whereas with Bombay mace a coloured precipitate will be formed even up to twenty-five percolations.

The usual analytical constants of these spices are shown below :—

	Cloves.	Caraway.	Nutmeg.	Mace.
	Per cent.	Per cent.	Per cent.	Per cent.
Moisture	5·0–11·0	11·5–15·5	4·0–8·0	3·5–7·0
Ash	4·5–7·0	5·5–6·7	2·5–4·5	1·9–2·5
Water-soluble ash . .	2·7–4·2	2·0–2·2	1·0–2·0	0·9–1·7
Volatile oil . .	14·5–20·0	2·7–8·2	10·0–15·0	4·0–10·0
Fixed oil and resin . .	6·2–10·1	18·5–20·5	34·0–40·0	24·0–33·0
Alcohol extract . .	13·5–15·5	—	10·0–16·5	21·5–25·0
Crude fibre . . .	7·0–9·0	17·5–22·3	—	4·7–7·3
Nitrogen . . .	0·9–1·2	5·9–6·4	1·1–1·4	0·85–1·15
Cold water extract . .	—	—	—	—
Starch	2·0–2·5	—	7·5–12·0	—

Curry Powder

This preparation formerly consisted of the ground-up leaves of an Indian plant, *Murraya Kœnigii*, admixed with various other ingredients, which may include turmeric, fenugreek, cinnamon, cassia, cardamoms, mace, cumin, dill, pimento, coriander, pepper, chillies, ginger, and salt. In many powders at the present time there is no murraya. The active principles of this leaf are a volatile oil, a fixed oil sometimes called simabolee oil, and a glucoside, kœnigin. A sample of curry powder recently examined gave the following results :—

	Per cent.
Moisture	11·17
Total ash	11·42
Water-soluble ash	8·20
Silica	0·64
Ether extract	13·08
Water extract	20·05

Sometimes curry powders are unduly diluted with salt, ground rice or occasionally bean flour. They not infrequently contain undesirable quantities of lead, so all samples should be tested ; in view of the presence of much iron and phosphate the method given on p. 210 should be used. A limit of 20 p.p.m. is commonly adopted.

Mint

Mint, that is the leaves of *Mentha viridis*, comes to the notice of the analyst mainly in the form of dried leaves or in mint sauce. The dried leaves commonly contain from 10 to 12 per cent. of total ash, including about 1 per cent. of ash insoluble in acid. The volatile oil content, which may be determined by the method of Sage and Fleck (p. 162) is approximately 1 per cent. ; this quantity is about the same as that present in the fresh leaf. Within the last few years a number of cases have been reported in which mint has been adulterated with ailanthus leaves, and such mint has found its way into mint sauce. This and other adulteration with foreign leaves is best detected by the aid of a lens or the microscope. The oil glands of mint which are quite a characteristic are not to be found on ailanthus leaves. The latter may be identified by their characteristic calcium oxalate crystals which are distributed along the veins of the leaf and by the curved unicellular hairs. Illustrations of ailanthus leaves appear in *The Analyst*, 1934, **59,** 33.

Onions

Onions, dried onions and onion extracts are now familiar articles of commerce. The composition of fresh and dried onions is given by Sherratt (*Analyst*, 1943, **68,** 200). The essential oil constituent is allyl-propyl-disulphide with traces of other volatile sulphur compounds, which together amount to about 100 parts per million reckoned as volatile sulphur. Vitamin C is present to the extent of 0·05 to 0·1 mgm. per gram. Asafœtida has been found as an adulterant in onion extracts ; it may be detected by the intense blue fluorescence which it gives in neutral or alkaline solution under ultra-violet light. Onion extracts do not fluoresce.

CHAPTER VII

WINES, BRITISH WINES AND CORDIALS, BRANDY, WHISKY, RUM, GIN, BEER, CIDER, AND VINEGAR

THE chemistry of wine and spirits has been much more studied in the wine-producing countries, notably France and Germany, than in England. The detailed examination of different wines does not commonly afford to the analyst so much information as is obtainable from the palate of an experienced taster. In this chapter, therefore, it is not proposed to discuss in detail the large number of estimations which have been recommended by various authorities for the examination of wine, but only to indicate broadly the commoner processes directed at the detection of adulteration and to give references to the literature where further information may be obtained.

It is difficult even to define wine. Formerly it meant simply the fermented juice of the grape, but many kinds are now fortified by the addition of more alcohol, which may or may not have been derived from the grape. Some wines are " still," and others contain much carbon dioxide ; some are red and some are white, depending upon the colour of the grape used ; and there are the wide variations associated with the different classes of wine, such as port, sherry, claret, burgundy, hock, champagne, etc., indicating in many cases the country or district of origin. These may be modified by a geographical prefix such as " Australian burgundy."

By virtue of the Anglo-Portuguese Treaty Acts the term Port is restricted to the produce of Portugal shipped with a certificate from the Portuguese Authority. Sherry is the wine which takes its name from Xeres and is shipped only from certain parts of Spain.

Analytical data on the common types of wines are shown in the table on p. 169, information for which has been collected from various sources ; the results are expressed in grams per 100 ml.

The limits found in the above table must be interpreted with caution, as so much depends on the place of origin of the particular kind of wine, and on the age, vintage, and other circumstances.

COMPOSITION OF THE COMMONER WINES

	Port.	Sherry.	Claret.	Burgundy.	Hock.	Champagne.
Specific gravity	0·995–1·050	0·992–1·015	0·990–1·020	0·995–1·010	0·990–1·008	1·040–1·055
Alcohol, grams per 100 ml. . .	13·5–20·0	13·5–20·5	7·5–12·5	7·5–12·5	7·5–12·5	10·0–14·0
Total solid matter	3·3–8·4	2·0–5·0	2·0–3·5	2·0–3·5	1·5–2·5	9·5–18·0
Free volatile acid (as acetic) . .	0·05–0·10	0·15–0·23	0·09–0·15	0·2–0·35	0·05–0·15	0·03–0·20
Fixed acid (as tartaric) . . .	0·35–0·55	0·25–0·50	0·30–0·50	0·3–0·60	0·25–0·45	0·30–0·45
Ash	0·25–0·35	0·35–0·55	0·2–0·3	0·2–0·40	0·10–0·25	0·25–0·45
Sugars	2·5–6·5	2·0–5·0	0·0–0·7	0·03–0·55	0·0–0·35	8·5–16·0
Phosphoric acid (P_2O_5) . . .	0·03–0·05	0·03–0·05	0·03–0·04	0·02–0·03	0·02–0·03	0·03–0·05
Glycerin	0·3–1·3	0·4–1·0	0·3–1·0	0·3–1·0	0·25–1·3	0·3–1·0

Analyses of large numbers of wines from different localities are given by König.

As the composition of wines varies so much, no very definite conclusion can be drawn from the consideration of any particular figure, but the following generalisations are widely accepted on the Continent. The specific gravity should not be below 0·985 ; if the alcohol content is more than 14·5 g. per 100 ml. (in the case of port or sherry this figure is often exceeded), added alcohol is indicated ; the glycerol content, which is not often more than 1 per cent., should be one-fifteenth to one-fifth of the alcohol, and the total acid should not exceed 1·5 per cent. The total solid matter or extract varies within wide limits, being largely determined by the sugar present ; " dry " wines contain about 0·1 per cent. of sugar, others contain up to 6 per cent., and champagne even up to 16 per cent. According to the Paris Municipal Laboratory, if the sum of the sugar and twice the alcohol exceeds 32·5, the wine may be considered to have received an addition of sugar or alcohol. Importance also attaches to the ratio alcohol : extract ; this for red wines should not exceed 4·5, or for white wines 6·5.

A complete examination of wine should include the estimation of specific gravity, alcohol, extract, acidity, ash, sugar, glycerol, sulphate, and a search for sulphites, or other preservative. Saccharin may also be present, and sometimes it is necessary to search for methyl alcohol or for added colouring matters.

For the estimation of alcohol the general method of distillation, followed by determination of specific gravity, is employed. Protein matters should be precipitated by the addition of lead acetate or tannin, and volatile acid must be neutralised by alkali. Excess of the latter is to be avoided, lest any ammonia be distilled over. For wines it is convenient to take 100 ml. measured accurately at 15·5° and wash it into a distillation flask with 50 ml. of water, add a slight excess of sodium hydroxide solution, then 2·5 g. of lead acetate and then distil until about 90 ml. have passed over ; this is adjusted to 100 ml. at 15·5° by the addition of water, and its specific gravity is taken by means of a pyknometer. Reference to the Alcohol Tables issued by the Commissioners of Customs and Excise gives the percentage of alcohol corresponding to the observed specific gravity. It is convenient to return the results as grams per 100 ml.

Strong spirits or alcoholic mixtures containing more than 50 per cent. of alcohol should be diluted with water before distillation ; for example, 50 ml. may be diluted to 150 ml. and 100 ml. distilled.

When the material under examination contains essential oils or other volatile ingredients the method of Thorpe and Holmes is applied (*J. Chem. Soc.*, 1903, **83,** 314). The sample or distillate from it is diluted so that the alcohol content does not exceed about 25 per cent., then shaken in a separator with sufficient salt to saturate the liquid. It is then vigorously shaken with 50 ml. or more of petroleum spirit, the aqueous layer is run out and the alcohol distilled off ; the essential oils or fatty matter will all be in the petroleum layer.

Many methods have been recommended for the detection and estimation of methyl alcohol in ethyl alcohol, the presence of the former being an indication of adulteration with denatured spirit ; perhaps the best method is that of Denigès, which is as follows : The alcoholic solution is first purified, where necessary, by distillation and diluted to 10 per cent. by volume. To 5 ml. of this prepared liquid, contained in a wide test tube, are added 2·5 ml. of 2 per cent. solution of potassium permanganate and 0·2 ml. of sulphuric acid. When the reaction has proceeded for three minutes, 0·5 ml. of oxalic acid solution (9·6 per cent.) is added. On shaking, the liquid becomes clear and nearly colourless ; 1 ml. of strong sulphuric acid is now run in and well mixed with the solution, which is finally treated with 5 ml. of Schiff's reagent. A violet colour is developed in the course of a few minutes unless only traces of methyl alcohol are present, when twenty or thirty minutes may be required ; it is essential that the details be strictly adhered to, and the Schiff's reagent must not be too strongly acid. Schiff's reagent may be prepared by dissolving 0·2 g. of basic fuchsin in 120 ml. of hot water, then decolorising the solution, after cooling, by adding 2 g. anhydrous sodium sulphite dissolved in 20 ml. of water, and finally 2 ml. of hydrochloric acid. The solution should be kept in a stoppered amber-coloured bottle. Denigès method can be applied quantitatively by comparing the colour developed by the sample with that of 10 per cent. ethyl alcohol containing small additions of methyl alcohol and treated by the same process.

Fluoride is sometimes present in wines as in other foodstuffs. Its detection is based on the fact that a solution of thorium nitrate and alizarin sulphonic acid give a violet colour which is discharged by fluoride. The solutions required are (*a*) thorium nitrate 0·25 g. per litre ; (*b*) 0·01 per cent. alizarin S ; (*c*) standard sodium fluoride 0·0221 g. per litre ; 1 ml. = 10 μg. of fluorine.

The method has been studied in detail by the Analytical Methods Committee of the S.P.A. (see *Analyst*, 1944, **69,** 243). About 10 g. of the sample are mixed with 1 g. of fluorine-free lime and a little water. The mixture is evaporated to dryness and ignited at dull red heat. The ash is transferred to a Claissen flask with about 5 ml. of water and rinsed in with 15 ml. of 60 per cent. perchloric acid ; then add 0·2 g. of silver sulphate, to precipitate all the chloride, and a few fragments of pyrex glass. The Claissen flask is fitted with a thermometer and steam inlet tube, both of which extend almost to the bottom of the flask. The side tube is stoppered and connected to an efficient condenser.

The flask is heated over a burner, using an asbestos board with a hole about 1 inch diameter to prevent local overheating, till the temperature rises to 120°–125°, then connected to the steam supply and the burner and steam regulated so that the temperature of distillation is maintained at 137°–140°. Distil 150 ml. in twenty-five to thirty-five minutes.

Titrate 50 ml. of the distillate with 0·05 *N* NaOH in a Nessler tube, using methyl orange as indicator and comparing the colour with that of distilled water containing the same amount of methyl orange. Transfer the remaining 100 ml. of distillate to a Nessler cylinder and add an amount of 0·05 N hydrochloric acid to make the total acidity equal to 5·0 ml. of 0·05 N acid. Prepare a control cylinder containing 5·0 ml. of 0·05 N hydrochloric acid and distilled water and add to both test and control cylinders 2 ml. of 0·01 per cent. alizarin S solution. From a burette add to the test cylinder solution of thorium nitrate until a slight pink colour persists as compared with the yellow of the control cylinder. Add an exactly similar volume of the thorium nitrate solution to the control cylinder; it then becomes more pink than the test solution. Then add slowly standard solution of sodium fluoride until the tints of test and control solutions exactly match. The volume of standard fluoride solution added corresponds to the amount present in the test portion of the distillate. Calculate the amount of fluorine present in the 150 ml. of distillate and subtract the apparatus blank, which should be determined, using the same amount of all reagents, including the lime.

In considering the results of a fluoride test it should be borne in mind that very many natural products contain small quantities of fluoride; some instances are mentioned by the Committee (*loc. cit.*).

The increasing industrial use of iso-propyl alcohol renders it frequently necessary to test for it in alcoholic distillates. The best method is that of Adams and Nicholls (*Analyst*, 1929, **54,** 2) which depends upon the oxidation of iso-propyl alcohol to acetone, which condensed with *o*-nitrobenzaldehyde forms indigo.

The solutions to be tested are diluted to a strength of approximately 10 per cent. of alcohol. To 10 ml. of each solution in test tubes 5 ml. of saturated bromine water are added. The tubes are lightly corked and allowed to stand for about three to six hours in the cold, or even overnight, in a cool dark cupboard. After the period of standing, 1 ml. of a 1 per cent. solution of *o*-nitrobenzaldehyde in 50 per cent. ethyl alcohol is added, and the solutions are mixed by gentle shaking. Finally, 2 ml. of a 30 per cent. solution of sodium hydroxide are added, and the solutions shaken once more. The tubes are then allowed to stand for about fifteen minutes in diffused daylight, and the colours developed are compared with those produced in standard iso-propyl alcohol solutions similarly treated. A convenient standard solution is one containing 2·5 ml. of pure iso-propyl alcohol in 100 ml. of 10 per cent. ethyl alcohol. The standard colours are those produced by using 1·0, 1·5, 2·0 ml., etc., of this solution, made up to 10 ml. with 10 per cent. ethyl alcohol.

Rigidly defined methods for the determination of extract are given by Continental workers; perhaps the best is to evaporate 50 ml. of a dry wine or 25 ml. of a sweet wine in a flat-bottomed dish of 3-inches diameter, then dry the residue in a steam oven at 100° for two hours. If the extract exceeds 5 per cent., indirect estimation is preferable; determine the specific gravity of the wine (S) and that of the distillate (S′) when diluted to the original volume; then the extract (grams per 100 ml.) $= \frac{S - S'}{0{\cdot}00386}$; the results so derived are more accurate than those obtained by direct estimation, on account of the sugars and glycerol present.

The ash is obtained by ignition of the extract; its alkalinity and solubility in water may also be determined.

The estimation of total acidity of wine is rendered less easy by reason of carbon dioxide and of colouring matters, which obscure the indicator ; the simplest expedient is to use phenolphthalein spotted on a white tile as an outside indicator. For the separate estimation of volatile acid, which is reckoned as acetic, to 25 ml. add about 1 g. of tannin, then water, distil the liquid until the volume is reduced to one half, then introduce more water and continue the distillation until at least 200 ml. have been collected ; titrate the distillate as usual. Fixed acid is calculated by difference, but it is to be noted that the volatile acid as acetic should be calculated to its equivalent as tartaric acid before being subtracted from the total acidity, which is usually reckoned as tartaric except in France, where convention requires that acidity should be expressed as H_2SO_4 per 1,000 ml.

The sugars present may include sucrose as well as dextrose and lævulose, and possibly others, so that it is necessary to determine the cupric reducing power before and after inversion, the former being returned as invert sugar and the latter—after allowing for reducing sugar—as sucrose. One hundred ml. of the sample are carefully neutralised with sodium hydroxide, then evaporated to half that volume to remove alcohol ; the solution is cooled, cleared with zinc and ferrocyanide (p. 77), made up to 100 ml. and then filtered. The copper reducing power is determined volumetrically on the filtrate, and similarly on another 25 ml. of the sample after inversion (p. 12).

Glycerol in wine may be estimated approximately by the German official process, which involves direct weighing of the glycerol. If the wine contains sugar not exceeding 2 per cent., 100 ml. are evaporated on a water bath to 10 ml. ; then for each gram of extract is added 1 g. of fine sand and 2 ml. of concentrated (40 per cent.) milk of lime. The mixture is evaporated nearly to dryness ; then 5 ml. of alcohol are added, and the mass worked up into a paste in the basin with the aid of a spatula, more alcohol being added if requisite ; then the paste is warmed nearly to the boiling point and is washed into a 100 ml. flask with several small quantities of warm alcohol, cooled to 15° and made up to the mark with alcohol. After shaking and filtration, 90 ml. of the clear liquid are evaporated very gently almost to dryness, and the residue taken up by a small quantity of absolute alcohol, transferred to a stoppered cylinder and rinsed in with more alcohol until the volume is 15 ml. Now three separate portions of 7·5 ml. of ether are added with shaking between each addition. The clear liquid is poured into a tared basin, rinsed in with a little alcohol-ether mixture (2 : 3) and very gently evaporated, and finally is dried in the oven for an hour and weighed.

In the case of sweet wines containing more than 2 per cent. of sugar, 50 ml. are warmed in a flask on the water bath with 2 g. of sand and an excess of milk of lime—excess is indicated by the colour changing from dark to pale—the mixture is cooled, 100 ml. of alcohol are added and the precipitate is filtered off and washed with alcohol ; the filtrate is treated exactly as an unsweetened wine as above.

Sulphites are commonly used in the sulphuring of casks, and may sometimes be added to wine ; for the estimation the following direct process gives reasonably accurate results with white wines : Add 50 ml. of the wine to 25 ml. of N sodium hydroxide solution and, after allowing the mixture to stand for fifteen minutes, 10 ml. of dilute sulphuric acid (1 + 3) and a few drops of starch solution. Then titrate with 0·05 N iodine solution ; each ml. of 0·05 N iodine is equivalent to 0·0016 g. SO_2. For coloured liquids recourse must usually be had to the distillation process described on p. 33. Benzoic or salicylic acid can be detected and estimated by methods already given (pp. 104, 106).

Saccharin is detected by extracting a large volume, say 250 ml., of the acidified wine from which the alcohol has been removed by evaporation and replaced by the addition of water, with three portions of 30 ml. of ether ; then, after removal of the ether, the wine is evaporated to half its bulk, and again extracted with ether. The mixed ether extracts are distilled off at a low temperature and finally evaporated spontaneously in an open basin. The residue is dissolved in about 30 ml. of water and again evaporated to dryness, then dissolved in about 5 ml. of 0·2 N sodium hydroxide solution and slight excess of potassium permanganate solution is added. The pink colour should persist after a few minutes' warming ; then the mixture is acidified and just decolorised by the addition of sulphurous acid. The liquid is filtered and extracted three times with ether ; the ether is allowed to evaporate spontaneously and the residue taken up with water and tasted. A characteristic sweet taste is indicative of saccharin ; it should be confirmed by the qualitative reaction given below. Benzoic acid may be detected by extraction with ether from an acidified alcohol-free solution, or by the method given on p. 104. Dulcin is detected by extracting the alcohol-free liquid with ether after making it alkaline. The ether is evaporated and the sweet-tasting dulcin remains. Dulcin and saccharin may be identified by heating a few mgm. with an equal quantity of resorcinol and a few drops of H_2SO_4 to 180° for about two minutes. Then cool, add water and make alkaline with caustic soda. A red colour with green fluorescence indicates saccharin. To part of the solution add a few drops of bromine water. In the presence of dulcin a violet colour appears on standing. A quantitative method for dulcin is given by Longwell and Bass (*Analyst*, 1942, **67,** 15).

British Wines and Cordials

These have been the subject of a report to the Ministry of Health, No. 24, 1924, which gives details of the composition of 357 samples. A small number of fermented British wines consist of the juice of various fruits with addition of sugar, but most of them are made by fermenting an infusion of raisins, currants, rhubarb or grape, and most appropriate flavouring agents, such as ginger or orange peel. Most are coloured by elderberry or vegetable colours, and a few with aniline dyes. They contain from 10 to 27 per cent. of proof spirit and usually added sugar. The following tables show analyses selected from the report mentioned.

Unfermented wines and cordials consist of a basis of syrup with or without natural fruit juice, supplemented sometimes by saccharin or glucose, and flavoured with fruit essences or artificial substances ; they usually contain added citric acid, and sometimes aniline dyes or added phosphoric acid. Non-alcoholic wines show about 25 per cent. of total sugars and cordials 45–50 per cent.

The analysis presents no special difficulty, the amount of fruit juice if any can be inferred approximately from the amounts of potash and phosphoric acid in the ash. Samples should always be examined for preservatives, and for lead, copper and tin. In connection with the copper the presence of traces of this metal must be interpreted with care, as it has been found to occur

ALCOHOLIC WINES.

	Specific gravity.	Alcohol.	Extract.	Reducing sugars calculated as invert sugar.	Sucrose.	Ash.	Phosphoric anhydride (P_2O_5).
		Grams per 100 c.c.					
Black currant .	1·0655	9·88	21·23	14·70	1·89	0·56	0·02
Cherry brandy .	1·0950	16·25	31·18	29·38	*Nil.*	0·16	0·02
Elderberry . .	1·0771	9·74	24·22	19·64	0·16	0·64	0·02
Ginger . . .	1·0565	10·39	19·05	15·77	0·04	0·30	0·07
Ginger . . .	1·0868	4·95	24·88	22·12	0·10	0·28	0·01
Ginger brandy .	1·0965	10·33	29·54	3·18	25·94	0·10	Trace.
Orange . . .	1·0620	10·32	20·47	17·59	0·18	0·27	0·03
Orange . . .	1·0535	10·18	18·19	14·59	0·30	0·19	0·01
Orange quinine .	1·0570	10·25	19·13	16·36	*Nil.*	0·26	0·11
[1] Port, British .	1·0356	12·64	14·38	11·34	0·60	0·24	0·03
Raisin wine .	1·0810	9·88	25·30	21·75	0·49	0·59	0·07
Raspberry wine .	1·0626	10·69	20·75	16·30	1·37	0·37	0·02
Red currant . .	1·0619	10·54	20·52	17·60	0·66	0·29	0·01
Sherry, British .	1·0043	12·81	6·31	3·75	0·11	0·30	0·03
NON-ALCOHOLIC WINES							
Black currant .	1·0847	1·22	22·62	16·99	4·32	0·07	Trace.
Cherry . . .	1·0656	1·01	17·51	11·83	4·21	0·04	,,
Elderberry . .	1·0921	0·14	24·04	20·92	1·39	0·07	,,
Ginger . .	1·0889	0·32	23·28	22·67	*Nil.*	0·09	,,
Orange . .	1·0922	0·39	24·17	22·25	0·16	0·04	,,
Raisin . . .	1·0911	0·29	23·85	11·61	9·09	0·06	,,
Raspberry . .	1·0637	0·53	16·78	5·23	10·62	0·10	0·01
Sherry . . .	1·1086	0·74	28·67	6·56	21·43	0·13	0·03
NON-ALCOHOLIC CORDIALS							
Black currant .	1·1303	0·69	34·39	24·51	9·22	0·04	0·01
Cherry brandy .	1·1513	0·53	39·88	31·14	8·22	0·03	Trace.
Ginger . .	1·0918	0·05	23·91	23·06	0·29	0·06	*Nil.*
Ginger brandy .	1·1964	0·26	51·84	31·42	17·89	0·03	Trace.
Lemon . . .	1·2176	0·11	57·50	56·93	0·21	0·04	*Nil.*
Lemon (syrup of) .	1·3071	0·32	82·29	79·22	1·65	0·08	,,
Lemon squash .	1·1427	0·16	37·41	34·00	0·45	0·21	Trace.
Lime juice . .	1·1427	0·07	37·38	33·94	0·48	0·20	,,
Orange . .	1·2052	0·58	54·38	37·41	16·38	0·03	*Nil.*
Peppermint .	1·1812	0·58	47·91	35·48	9·65	0·03	Trace.
Raisin . . .	1·1243	0·96	32·93	32·33	0·83	0·11	,,
Raspberry . .	1·1962	0·11	51·71	32·13	16·98	0·03	,,
Raspberry vinegar .	1·2475	0·05	65·64	42·04	22·62	0·15	,,

naturally in minute quantities in a number of cereals, fruits and fruit juices.

Spirits

Spirits differ essentially from other alcoholic liquors in being produced by distillation ; they contain much less extractive

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[1] The description " port " is not permissible for these wines.

matter and no glycerin, but a high percentage of alcohol together with certain esters which, though small in amount, are of much importance, as they confer the characteristic flavour of the particular spirit.

Brandy is distilled from wine and subsequently matured by age. That sold in England comes largely from the Cognac district of France, but so many other brandies are now on the market that care is needed in interpreting the results of the chemical analysis. Much importance attaches to the taste or characteristic "bouquet" which is produced by the appropriate conditions of manufacture and ageing. Chemically it is due to the presence of small but significant amounts of higher alcohols, acids and esters, among the more important of which are acetic, butyric, œnanthic, and valerianic esters, furfuraldehyde, and ethyl pelargonate. It is usual to express the "impurities" as milligrams per 100 ml. of absolute alcohol.

The table on p. 179 shows the usual composition of brandy. Of these numbers 1 to 6 are French brandies, probably genuine, and numbers 7, 8 and 9 are Greek, Spanish and Australian spirits respectively ; in these the alcohol content is much higher. Much has been made in the past of a standard of 80 for the minimum content of esters and of 300 for the total impurities, which include volatile acids, esters, aldehydes, furfural and higher alcohols, but how far these standards are really justified is debatable (*cf.* Hehner, *Analyst*, 1905, **30**, 36), and they should be applied with care and supported by tasting by an experienced palate. It appears to be the practice of certain manufacturers to import "silent spirit" (a term applied to almost pure alcohol obtained by distillation in a patent still), and add to it artificial ethers to produce so-called brandy ; obviously such a liquid could easily be made to pass the chemical standards suggested. The matter is further complicated by the wide variation in brandies of known purity, the "impurities" varying from 25 to 1,200 and ethers from 18 to 450.

Whisky was formerly spirit distilled from malted barley only, but of late years the definition has much widened and includes spirit distilled from a variety of fermented cereals. Like brandy, therefore, the standards by which it must be judged are somewhat elastic. Much depends upon the type of still employed ; the product of a simple pot-still generally has a higher content of impurities than that of a patent still in which there is considerable

Constituents of Brandy

	1.	2.	3.	4.	5.	6.	7. Greek.	8. Spanish.	9. Australian.
Specific gravity	0·9566	0·9481	0·9477	0·9427	0·9414	0·9471	0·8896	0·8539	0·9180
Proof spirit (per cent.) . . .	73·05	76·78	78·71	82·78	83·84	75·70	122·8	146·6	103·5
Total solids (per cent.) . . .	1·904	0·646	1·024	0·757	0·608	0·132	0·01	0·08	0·704
Ash (per cent.)	0·012	0·012	0·006	0·005	0·004	0·004	0·001	0·004	0·006
Total acidity as acetic (per cent.) .	0·046	0·039	0·072	0·054	0·041	0·004	0·022	0·034	0·027
= Milligrammes per 100 ml. alcohol.	110	89	160	115	86	9	31	41	46
Total esters (ethyl acetate) . .	580	463	564	502	335	112	—	—	—
Volatile esters	122	57	88	101	123	44	71	48	33
Furfural	6	2	3	4	5	0	1·4	1·2	0
Aldehyde	69	41	71	76	29	24	31	6	8
Higher alcohols	146	315	782	675	611	374	464	314	316

rectification. There is some overlapping, however, so that it is difficult to draw conclusions from the analytical figures alone. The effect of storage is important. Certain tannins and colouring matters are derived therefrom and definite changes take place in the liquid (*cf.* Crampton and Tolman, *J. Amer. Chem. Soc.*, 1908, **30,** 98), among which it may be noted that the alcohol content decreases slightly—about 1 per cent. per annum—and the furfural and esters increase during the first three or four years, afterwards remaining nearly constant.

Much information as to the different varieties of whisky is given in the Minutes of the Royal Commission on Whisky and other Potable Spirits, 1908–9, which defines whisky as the spirit obtained by distillation from a mash of cereal grains saccharified by the diastase of malt; Scotch whisky being distilled in Scotland and Irish whisky in Ireland. The following are analyses of known genuine whiskies :—

	1	2.	3.	4
Specific gravity . .	0·9425	0·9425	0·9463	0·9400
Proof spirit (per cent.) .	80·53	80·53	76·34	82·60
Total solids (per cent.) .	0·122	0·135	0·070	0·103
Ash (per cent.) . .	0·012	0·015	0·003	0·010
Total acidity as acetic (per cent.)	0·021	0·010	0·013	0·025
= mgms. per 100 ml. alcohol	46·0	22·0	31·7	53·0
Esters	50·0	56·5	75·9	35·5
Furfural	4·8	5·5	0·7	1·7
Aldehydes	65·2	52·1	126·4	87·0
Higher alcohols . .	404·3	439·1	326·4	360·5

For a large number of analyses of whiskies, see Schidrowitz (*J. Soc. Chem. Ind.*, 1902, **21,** 815; 1905, **24,** 585).

The standards by which whisky may be judged are similar to those of brandy, but care must be taken not to place too much reliance on figures alone; if the total impurities are much less than 300 there is a probability that some silent spirit has been added. The esters should always be lower than the higher alcohols; the reverse is often the case with rums.

In common with brandy, gin and rum, whisky must contain not less than 65 per cent. proof spirit in order to comply with the Food and Drugs Act, s. 4. Proof spirit is alcohol of such strength that it has density of twelve-thirteenths that of water (at 51° F.); it is now known to be equivalent to 57·1 per cent. by volume of anhydrous alcohol. Percentages of proof spirit are given in the usual alcohol tables.

Rum is a spirit distilled from the fermented products—often waste products—of the sugar cane. Formerly it was almost exclusively derived from Jamaica and the Indies, but imitation rum is now imported from countries in which the sugar cane is not cultivated. Some kinds are prepared from beet molasses, others from silent spirit. Rum differs essentially from whisky or brandy in the preponderance of esters over higher alcohols; ethyl acetate and butyrate predominate. Partly on this account it is particularly easy to produce fictitious rums by the addition of these esters to suitably diluted and coloured silent spirit. The proportion of higher alcohols is quite low in rum; usually it is less than 250 per 100,000, and sometimes less than 100.

Gin is a more artificial product than the other spirits, and is usually prepared by the addition of various flavouring essences to a highly rectified spirit; the essences added commonly include oils from cardamom and coriander seeds and juniper berries. In view of the process of manufacture it is clear that there can be no standard applied or conclusions drawn from the proportions of esters, higher alcohols, or other impurities as in the case of whisky, brandy or rum. The oils added from the seeds and essences used are mainly of the terpene class, and should not be included as higher alcohols. The following are analyses of rum and gin:—

	Rum.			Gin.	
	1	2.	3.	4.	5.
Specific gravity .	0·9436	0·9375	0·9403	0·9531	0·9504
Proof spirit (per cent.)	79·60	85·96	82·50	69·95	72·04
Total solids (per cent.)	0·385	0·420	0·295	0·095	0·013
Ash (per cent.) . .	0·041	0·039	0·025	0·008	0·009
Total acidity as acetic (per cent.) . .	0·102	0·095	0·082	0·015	0·020
= Milligrammes per 100 ml. alcohol .	224	193	174	—	—
Esters . . .	241·3	275·0	190·3	—	—
Furfural . . .	10·5	9·6	6·8	—	—
Aldehydes . .	27·8	35·2	24·5	—	—
Higher alcohols . .	174·5	190·3	160·1	—	—

Methods for the Examination of Spirits

The estimation of alcohol in spirits in which the total solid matter is small may be made approximately by simply determining the specific gravity, but it is more accurate to make allowance for the "obscuration," which is the amount by which the true alcoholic strength differs from the apparent strength indicated by the specific gravity. This may be done by determining the specific gravity at 15·5°, and evaporating a known volume to a low bulk so that all the alcohol is removed, then making the liquid up to its original volume and again taking the specific gravity. The figure so obtained, less 1·0000, is subtracted from the original specific gravity and the percentage of alcohol corresponding to the corrected figure is observed from the alcohol tables.

For the estimation of the higher alcohols there are two useful methods, of which the writer prefers the second.

(1) *The Allen-Marquardt Process* (*Analyst*, 1891, **16,** 102, and *J. Soc. Chem. Ind.*, 1902, **21,** 815). The method is as follows (care must be given to all the details): To 200 ml. of the spirit are added 4 ml. N sodium hydroxide solution and the mixture is boiled under a reflux condenser for an hour. The liquid is transferred to a flask fitted for steam distillation, and distilled over a flame until the volume is reduced to about 20 ml. Then steam is introduced and a further 110 ml. are distilled over so that about 10 ml. remain in the flask and the distillate measures 300 ml. This is divided into two portions and strong brine solution is added to one portion until the specific gravity of the mixture is 1·10. This is then shaken out in a separator with 40 ml., 30 ml., 20 ml., and lastly 10 ml. of carbon tetrachloride. The mixed carbon tetrachloride solution is shaken out with 50 ml. of brine to remove any ethyl alcohol, and then with 50 ml. of saturated sodium sulphate solution to remove chlorides; then it is filtered through a dry paper and the higher alcohols oxidised to their corresponding acids in the following manner. Five grams of potassium dichromate, 2 ml. of sulphuric acid and 10 ml. of water are mixed in a flask fitted to a reflux condenser, the carbon tetrachloride is added and the mixture boiled for eight hours. The condenser is then turned to the position for ordinary distillation, 30 ml. of water are added, and the liquid is distilled to a volume of 20 ml., when steam is admitted and the heating continued until 300 ml. have passed over and only 5–10 ml. remain

in the flask. The distillate is now neutralised with 0·1 N barium hydroxide solution, methyl orange being used as indicator ; when neutral, phenolphthalein is added, and the titration is continued to the neutral point of this indicator. One ml. of 0·1 N barium hydroxide = 0·0088 g. of higher alcohols expressed as amyl alcohol. The original distillate having been divided into two portions, it is convenient to make the estimation in duplicate. It is essential that pure carbon tetrachloride be used, so it is advisable to boil a stock of it with chromic acid and subsequently distil it from barium carbonate for purification. All corks used should be carefully selected and covered with tinfoil (rubber is unsuitable), and for the eight hours' oxidation glass joints are preferable. The same corks should not be used for the original distillation and subsequent oxidation. The methyl orange acidity of the distillate should not exceed 2 ml. 0·1 N.

(2) *The Colorimetric Method* (Royal Commission on Whisky and Potable Spirits : Report, 1909, Appendix).—This method depends upon the colour produced by furfural and sulphuric acid in contact with higher alcohols, but not with ethyl alcohol. It is much more expeditious than the Allen-Marquardt process. A standard solution of higher alcohols is prepared by dissolving 1 g. of a mixture of propyl alcohol 1 part, isobutyl alcohol 2 parts, amyl alcohol 3 parts, and capryl alcohol 1 part in 100 ml. of pure 50 per cent. ethyl alcohol. This solution is further diluted 1 : 10 in 50 per cent. alcohol for use, then each ml. = 0·001 g. of higher alcohols. The spirit to be examined is diluted to contain 50 per cent. of alcohol ; then 200 ml. are distilled until 190 ml. have passed over ; the distillate is made up to 200 ml. ; 10 ml. are mixed in a small flask with 0·5 ml. of 1 per cent. furfural solution and 10 ml. of sulphuric acid. The acid should be run in to form a layer on the bottom of the flask ; then the mixture is shaken for thirty seconds while cooled in iced water. In similar flasks mixtures are made containing 0·5 ml., 1·0 ml., 2·0 ml., and upwards of the standard higher alcohol solution in 10 ml. of 50 per cent. alcohol. In each case the mixture is made by shaking gently for thirty seconds in iced water. The reddish-violet colours produced are matched after standing for an hour at ordinary temperature and the higher alcohols calculated in milligrams per 100 ml. of absolute alcohol.

Esters are estimated by hydrolysis with alkali and returned as ethyl acetate. One hundred ml. of the sample are distilled into a flask which has been well steamed out, until there remains about 10 ml.; steam is then passed in and distillation continued till the distillate measures 150 ml. and the residue 5 ml. The distillate and the residue are titrated separately until exactly neutral to phenolphthalein and the total acidity calculated in terms of acetic acid. If desired, the volatile acid may be returned separately as acetic and the non-volatile acid as tartaric acid. To the neutralised distillate are added 10 ml. of 0·1 N sodium hydroxide solution and the mixture is boiled under a reflux condenser for half an hour, then cooled and titrated back with 0·1 N acid. The alkali used is calculated in terms of ethyl acetate, although other esters are probably present in the spirit.

The *total acidity* may also be estimated by the direct titration of 50 ml. with 0·1 N alkali; barium hydroxide is better than soda for this purpose.

For the estimation of *furfural*, Hewitt's method (*J. Soc. Chem. Ind.*, 1902, **21,** 98) is perhaps the best, although Schidrowitz (*ibid.*, 816) criticises it and points out that there is the possibility of the formation of some furfural during distillation. Fifty ml. of the sample are distilled to a low bulk; to the residue is added pure alcohol, and the distillation repeated; this operation is repeated several times, then the mixed distillate is diluted to contain 50 per cent. of alcohol and the volume noted. To 10 ml. of the distillate is added 1 ml. of a mixture of equal volumes of aniline and glacial acetic acid, and the colour compared after twenty minutes with that of 10 ml. of standard 50 per cent. alcohol to which has been added 10 mgm. per litre of furfural. The comparison should be made in a colorimeter, and the depths of liquids which show an equivalent colour are noted and the proportion of furfural is calculated therefrom. If a colorimeter is not available, the process of "nesslerising" may be applied.

For the determination of *aldehydes* advantage is taken of their property of restoring the colour of magenta which has been decolorised with sulphurous acid—Schiff's reagent, the formula of which is given on p. 171. A standard aldehyde solution is required and is prepared by dissolving 1·386 g. of pure dry aldehyde-ammonia in 50 ml. of pure 95 per cent. alcohol, then adding 22·7 ml. N sulphuric acid solution and making the volume up to 100·8 ml. with alcohol (the 0·8 ml. is to compensate for the volume of ammonium sulphate). The liquid is allowed to stand for twenty-four hours and filtered; each 1 ml. = 0·01 g. aldehyde.

To carry out the estimation 20 ml. of the spirit (which must be decolorised with lead acetate and de-leaded with sodium sulphate if it is highly coloured) are treated with 5 ml. of the Schiff reagent and the colour compared after twenty minutes with that of 20 ml. of alcohol of similar strength to which known volumes of aldehyde solution have been added. The method is approximate, not absolute, as the colour is to some extent influenced by the particular aldehydes present and in a small degree by the furfural, so is not quite proportional to the amount of aldehyde present.

The estimation of sulphates, total solids, ash, and, if necessary, of tannins, may be carried out by the ordinary methods.

Beer

Beer, as is well known, consists essentially of a fermented liquor prepared from malt and other cereals, flavoured with hops. Gentian or quassia or other non-deleterious bitter may be used as a flavouring agent, but such are not employed to any great extent in England. Although the products of the fermentation are very numerous, and it is possible to make detailed analyses showing, in addition to alcohol, acidity, specific gravity and extract, such minor constituents as albuminoids, sugar, dextrin, glycerin, tannin, and the composition of the ash, detailed analyses are not often required and do not yield much valuable information except, perhaps, to a brewer. The methods used are substantially those already described for these estimations in wines; for the significance of such figures reference may be made to the well-known manuals of brewing chemistry.

The estimations commonly required on a beer include that of specific gravity, alcohol, original gravity, acidity, total solids, and an examination for preservatives, lead and arsenic. Analyses of some well-known types of beer are given below :—

	India pale ale.	Mild ale.	Bock.	Lager beer.	Irish stout.
Specific gravity . .	1012·5	1010·1	1020·5	1014·5	1016·0
Alcohol, per cent. by weight . . .	4·30	3·15	4·50	3·20	4·30
Total solids % . .	4·95	3·55	6·80	5·38	5·70
Acidity as acetic % .	0·15	0·08	0·12	0·17	0·17
Ash % . . .	0·26	0·20	0·29	0·20	0·23
Original gravity. .	1053·0	1042·1	1063·5	1046·5	1057·5

The estimation of alcohol in beer is made during the course of the determination of the original gravity, *i.e.*, the indicated specific gravity of the wort before attenuation. The investigations of Thorpe, Brown, and others, have made it possible to calculate with accuracy the quantity of saccharine matter destroyed during the fermentation, thus arriving at the loss of density, and hence the original gravity of the unfermented wort.

If the beer contains yeast and much carbon dioxide, it is filtered through paper, the funnel being kept covered to obviate loss of alcohol, or, if there is no appreciable turbidity, the carbon dioxide may be removed by gently pouring the beer from one beaker to another a few times. One hundred ml. of the beer measured at 15·5° are transferred to a distillation flask and rinsed in with about 40 ml. of water, then distilled into a 100 ml. flask until 80–90 ml. have passed over. The distillate is made up to 100 ml. at 15·5°; the residue is cooled and diluted also to 100 ml. at 15·5°. The specific gravity of the distillate and of the residue is taken with an accurate pyknometer. The specific gravity of the distillate may be used for determining the alcohol by reference to the usual tables; the difference between this specific gravity and 1,000·0 gives the so-called spirit indication. Reference to the table on p. 187 shows the degrees of gravity lost. (This table is reproduced from that adopted in the Finance Act of 1914.)

Allowance must be made for the acid present, if it exceeds 0·1 per cent.; for this purpose 10 ml. of the beer are titrated with 0·1 N sodium hydroxide, litmus being used as an outside indicator. The result is calculated as acetic acid and the allowance reckoned for the acid in excess of 0·10 from the following table:—

Correction for Excess Acid.

Acetic acid	Corresponding degress of spirit indication.									
	0·00	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
0·0	—	0·02	0·04	0·06	0·07	0·08	0·09	0·11	0·12	0·13
0·1	0·14	0·15	0·17	0·18	0·19	0·21	0·22	0·23	0·24	0·26
0·2	0·27	0·28	0·29	0·31	0·32	0·33	0·34	0·35	0·36	0·37
0·3	0·39	0·40	0·42	0·43	0·44	0·46	0·47	0·48	0·49	0·51
0·4	0·52	0·53	0·55	0·56	0·57	0·59	0·60	0·61	0·62	0·64
0·5	0·65	0·66	0·67	0·69	0·70	0·71	0·72	0·73	0·75	0·76
0·6	0·77	0·78	0·80	0·81	0·82	0·84	0·85	0·86	0·87	0·89
0·7	0·90	0·91	0·93	0·94	0·95	0·97	0·98	0·99	1·00	1·02
0·8	1·03	1·04	1·06	1·07	1·08	1·09	1·10	1·11	1·13	1·14
0·9	1·15	1·16	1·18	1·19	1·21	1·22	1·23	1·25	1·26	1·28
1·0	1·29	1·31	1·33	1·35	1·36	1·37	1·38	1·40	1·41	1·42

FINANCE ACT, 1914 (SESS. 2).

TABLE OF GRAVITY LOST FOR DETERMINING THE ORIGINAL GRAVITY OF WORTS OF BEER.

Degrees of Spirit Indication.	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
0	0·00	0·42	0·85	1·27	1·70	2·12	2·55	2·97	3·40	3·82
1	4·25	4·67	5·10	5·52	5·95	6·37	6·80	7·22	7·65	8·07
2	8·50	8·94	9·38	9·82	10·26	10·70	11·14	11·58	12·02	12·46
3	12·90	13·34	13·78	14·22	14·66	15·10	15·54	15·98	16·42	16·86
4	17·30	17·75	18·21	18·66	19·12	19·57	20·03	20·48	20·94	21·39
5	21·85	22·30	22·76	23·21	23·67	24·12	24·58	25·03	25·49	25·94
6	26·40	26·86	27·32	27·78	28·24	28·70	29·16	29·62	30·08	30·54
7	31·00	31·46	31·93	32·39	32·86	33·32	33·79	34·25	34·72	35·18
8	35·65	36·11	36·58	37·04	37·51	37·97	38·41	38·90	39·37	39·83
9	40·30	40·77	41·24	41·71	42·18	42·65	43·12	43·59	44·06	44·53
10	45·00	45·48	45·97	46·45	46·94	47·42	47·91	48·39	48·88	49·36
11	49·85	50·35	50·85	51·35	51·85	52·35	52·85	53·35	53·85	54·35
12	54·85	55·36	55·87	56·38	56·89	57·40	57·91	58·42	58·93	59·44
13	59·95	60·46	60·97	61·48	61·99	62·51	63·01	63·52	64·03	64·54
14	65·10	65·62	66·14	66·66	67·18	67·70	68·22	68·74	69·26	69·78
15	70·30	70·83	71·36	71·89	72·42	72·95	73·48	74·01	74·54	75·07
16	75·60	—	—	—	—	—	—	—	—	—

The gravity lost equivalent to the corrected spirit indication is now added to the specific gravity of the extract, *i.e.*, residue in the flask after being made up to the original volume ; the sum gives the original gravity. An example will make this plain :—

Specific gravity of distillate	0·9935
Specific gravity of extract . . .	1·0204
Acidity as acetic acid (per cent.) . .	0·31
Specific gravity of water	1,000·0
Specific gravity of distillate . . .	993·5
Spirit indication	6·5
Allowance for 0·21 excess acid . . .	0·28
Corrected spirit indication . . .	6·78
Equivalent to gravity lost . . .	30·0
Add specific gravity of extract . .	1,020·4
Original gravity	1,050·4

Traces of arsenic have sometimes been found in beer. The Royal Commission Report on this subject recommended that $\frac{1}{100}$th grain per gallon should be the maximum permitted. For its detection and estimation the Marsh-Berzelius method is much the most satisfactory ; it may be applied without preliminary destruction. Troublesome frothing may sometimes be stopped by adding 1 drop of amyl alcohol.

Preservatives which may be present in beer include salicylic acid, benzoic acid and its esters, sulphur dioxide, and rarely, fluorides. These may be detected or estimated by the processes already described (see pp. 33, 44, 105 and 172). A simple method for sulphites is also given by Baker and Day (*Analyst*, 1912, **37,** 439), who show that sulphites in beer may be estimated by direct titration of the distillate with 0·1 N iodine solution ; the results tend to be slightly low.

Small traces of lead are liable to be present in beer if it has been in contact with pewter or composition pipes, a limit of 0·5 p.p.m. is sometimes adopted. The whole subject of lead in food has been reviewed by Monier-Williams, in a Report to the Minister of Health,[1] who also surveys methods for its determination.

The best general method is a modification of that of Allport and Skrimshire using dithizone. Five to 20 g. of sample, according

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[1] *Lead in Food.* H.M. Stationery Office. Report on Public Health Subjects, 1938, No. 88.

to its nature, are subjected to wet oxidation using 5 ml. of sulphuric acid and as little nitric acid as possible. When oxidation is complete the mixture is cooled, 20 ml. of water are added and the solution again boiled to fuming point. Cool, add 20 ml. water and 2 g. of citric acid ; filter if necessary, and wash the insoluble matter with hot 1 : 1 hydrochloric acid. Concentrate to about 50 ml. and add a small excess of 0·880 ammonia. Cool, add 1 ml. of 10 per cent. potassium cyanide. Shake the mixture in a small separator with 10, 10 and 5 ml of 0·1 per cent. dithizone in chloroform. The chloroform extracts are washed with a little water and evaporated in a test tube on the water bath. The residue is dissolved in 0·7 ml. sulphuric acid, and oxidised with nitric acid, diluted with water and re-heated to fuming.

To the sulphate solution so obtained is added 5 ml. of absolute alcohol and 10 ml. of water and it is set aside overnight. The lead sulphate (visible or not) is filtered off on an acid-washed No. 44 filter paper, dissolved in 10 ml. of 10 per cent. ammonium acetate ; 1·5 ml. 0·880 ammonia and 1 ml. of potassium cyanide solution are added and the lead is estimated colorimetrically as sulphide as usual. Substances containing much iron and phosphate are more conveniently examined by the method given on p. 210.

Cider

Although there is in this country no legal definition of cider or perry, such exists in France, where " no drink is to be sold under the name of cider unless it is derived exclusively from the fermentation of the juice of fresh apples, or a mixture of fresh apples and pears extracted with or without the addition of water. . . . The term cider or perry is reserved for cider or perry containing at least 2·5 per cent. of alcohol, 12 g. per litre of extract (sugar being deducted), and 1·2 g. of mineral matter per litre."

The A.O.A.C. suggests a standard of :—

Alcohol (maximum)	8·0 per cent.
Extract (minimum)	1·8 ,,
Ash (minimum)	0·2 ,,

Such standards, although affording a rough guide by which to judge the authenticity of a cider, must be accepted with reserve, for it would be easy for an unscrupulous person to prepare a fictitious liquid which would pass such standards. For a discussion of the composition of cider, see Barker and Russell (*Analyst*, 1909, **34**, 125), from whose paper the following analyses of genuine cider are taken :—

	1.	2.	3.	4.	5.	6.
Specific gravity .	1·000	1·022	1·0125	1·009	1·007	1·0075
Acid (as malic acid), per cent. . .	0·48	0·59	0·32	0·33	0·34	0·45
Tannin, per cent. .	0·26	0·26	0·21	0·25	0·18	0·27
Alcohol, per cent. .	5·83	3·55	4·20	4·76	5·07	4·82
Solids, per cent. .	2·41	6·88	5·74	3·92	3·88	3·61
Ash, per cent. .	0·32	0·34	0·31	0·31	0·32	0·35
Alkalinity as K_2CO_3, per cent. . .	0·116	0·127	0·095	0·113	0·108	0·026
P_2O_5, per cent. .	0·015	0·023	0·019	0·017	0·019	0·013

These figures represent blends such as are common on the market. From other data it appears that the acidity varies from 0·22–0·59 per cent., and the tannins from 0·04–0·37 per cent. Some light may be thrown on the question of whether or not a cider has been watered by the calculation of the original solids, which may be done approximately by adding the sum of 2·1 × the alcohol and 1·5 × the malic acid to the total solids found. The original solids thus calculated should not be below 12 per cent. in genuine cider. As it may happen that an artificial product containing no apple juice may appear as cider, the following test is given by Barker and Russell for its detection, based on the colour reaction of the tannins extracted by ethyl acetate, with lime water. One hundred ml. of the sample are evaporated to 10 ml. and shaken in a test tube for five minutes with 10 ml. of ethyl acetate ; when the mixture has separated the upper layer is drawn off and poured carefully on to the surface of a few millilitres of lime water in a test tube. At the junction of the liquids a clear band of yellow colour appears in the presence of apple or pear juice. The colour soon disappears, but the reaction is sufficiently sensitive to detect as little as 1 part of apple juice in 1,000.

Many methods have been proposed for the quantitative differentiation of small quantities of tartaric, malic and citric acids (*cf*., pp. 102, 115), but it is doubtful whether the results afford information comparable with the labour involved.

Search should be made for preservatives, particularly benzoic and salicylic acids or sulphites ; in this connection it is important to note that apples usually contain small amounts of boric acid, so that this is also present in cider ; also that cider casks are not infrequently treated with sulphur dioxide, hence traces of sulphites may be present in the liquid from this cause. The quantity found will generally indicate whether boric or sulphurous acid has been added intentionally or not. The estimation of boric acid in cider and similar products is complicated by the presence of phosphates in comparatively large amounts ; these must be removed by calcium chloride, which may be effected in the following way. To 100 ml. of the liquid add 2 ml. N sodium hydroxide more than is required to neutralise the free acid present, evaporate to dryness and ignite over an Argand burner to a white ash. Dissolve the ash in a slight excess of dilute hydrochloric acid, making the volume up to about 80 ml., and warm for half an hour on the water bath to expel carbon dioxide ; then cool, and add 1 ml. of 5 per cent. calcium chloride solution and 0·5 ml. of phenolphthalein solution ; titrate slowly with N sodium hydroxide until there is a faint permanent pink colour, not more ; make up to 100 ml. and filter off 50 ml. for the final titration. Make the 50 ml. quantity just neutral to methyl orange, add 20 ml. of neutralised glycerol and titrate with 0·1 N sodium hydroxide in the usual way ; each ml. 0·1 N = 0·0062 g. boric acid.

Vinegar

Although everybody knows what vinegar is in general terms, we are still without a definition acceptable to all parties concerned. In 1911 the Local Government Board suggested one but it was not universally accepted, and controversy still exists. In 1934 the following definitions were suggested for vinegar as the result of deliberations between the Society of Public Analysts and the Malt Vinegar Brewers' Federation :—

(1) Vinegar is a product of the alcoholic and acetous fermentation of a saccharine solution without any intermediate distillation, except in the case of spirit vinegar as defined in (4).

(2) Malt vinegar should be derived, without intermediate distillation, wholly from malted barley, with or without the addition of entire cereal grain malted or otherwise, the starch of which has been saccharified by the diastase of malt.

(3) When vinegar is demanded, a purchaser should be supplied with malt vinegar, unless due notification is given to the purchaser of the article supplied.

(4) The name "Vinegar" may be applied to other products which comply with the definition of vinegar (No. (1) above), provided a prefix is used to denote the origin of the product; thus, "Distilled Vinegar," "Spirit Vinegar."

Distilled vinegar is the product of the distillation of vinegar as defined in No. (1) above, and its source should be denoted; such as, distilled malt vinegar.

Spirit vinegar is the product of the acetous fermentation of a distilled alcoholic fluid.

(5) "Imitation" or "Artificial" vinegar should in every case be sold specifically marked "Imitation" or "Artificial" vinegar. It is any substitute for vinegar containing acetic acid which is not wholly the product of alcoholic and subsequent acetous fermentation, and shall not contain any acid other than acetic acid.

(6) All vinegars and imitation or artificial vinegars shall contain not less than 4 per cent. w/v of acetic acid. They shall not contain any acid other than acetic acid or those acids produced by normal fermentative processes.

(7) Caramel may be used as a colouring matter in all vinegar and in "Imitation" or "Artificial" vinegars.

Of recent years the practice has grown up describing artificial vinegar as non-brewed vinegar. In 1937 the London Sessions Appeal Committee affirmed that the "substance sold as vinegar or table vinegar without qualification or explanation implied that it was produced by fermentation."

The table below shows the composition of some well-known

	No. 24 malt vinegar.	No. 20 malt vinegar.	No. 18 malt vinegar.	No. 16 malt vinegar.	Artificial or wood vinegar.		
	1.	2.	3.	4.	5.	6.	7.
Specific gravity .	1·0198	1·017	1·013	1·015	1·007	1·009	1·012
Acetic acid (per cent.)	6·13	5·03	4·53	4·11	4·03	4·51	4·64
Total solids . .	2·48	2·45	1·35	2·53	0·32	0·36	0·41
Ash . . .	0·44	0·45	0·30	0·47	0·02	0·03	0·05
Total nitrogen .	0·08	0·08	0·75	0·09	0·03	0·04	0·02
SO_3 . . .	0·11	0·10	0·06	0·08	0·01	0·01	0·01
Phosphoric acid (P_2O_5) . .	0·06	0·09	0·075	0·085	0·02	0·03	0·03
Sodium chloride .	0·24	0·21	0·17	0·15	0·01	0·02	0·02
Oxidation value .	—	1,250	950	950	3	5	6
Iodine value . .	—	1,050	1,020	800	40	150	76
Ester value	—	40	35	35	1	2	2

brands recently analysed. As will be seen from the figures, wood vinegar is little more than dilute acetic acid coloured with caramel, and to which a small amount of brewed vinegar may have been added to impart flavour.

There is, in general, no great difficulty in recognising a genuine malt vinegar or an ordinary artificial vinegar. The total solids, ash, nitrogen, and phosphoric acid figures are widely different. When, however, the question arises as to whether a vinegar has been prepared wholly from malted barley, or if other cereal has been used, or whether a weak malt vinegar has been fortified by the addition of strong acetic acid, there is often much difficulty. The figures to which most importance attaches are those for nitrogen and phosphoric acid. The amounts of these two substances in a brewed vinegar are determined by the variety of the grain used ; in the product of malted or unmalted barley they are generally high, in no case less than 0·05 per cent. of each. In the product of the fermentation of sugar obtained from other sources the phosphoric acid figure may be lower than this ; the nitrogen percentage is likely to be low in proportion but may be higher by reason of the use of yeast products or other nitrogenous substances. Thus, when rice has been used in conjunction with malt the nitrogen figure is generally decidedly high in relation to the phosphoric acid ; it must, however, be remembered that phosphates in some form or other may be added to the vinegar, and that it is not unusual to add small quantities of salt in brewing vinegar.

An interesting development of recent date is the finding that malt vinegar contains significant proportions of aneurine (vitamin B_1), riboflavin (vitamin B_2), nicotinic acid, pantothenic acid, and pyridoxin. There would, of course, be no trace of these in an artificial vinegar.

Spirit vinegars, like many artificial vinegars, usually contain small traces of nitrogen and phosphoric acid which provided food for acetifying bacteria ; in addition, however, they usually show a fractional percentage of alcohol. The presence of alcohol is therefore an indication, though not necessarily proof, of spirit vinegar.

In connection with the use of strong (80 per cent.) acetic acid to fortify a weak vinegar, it may be remarked that the presence of a minute quantity of mercury is strongly indicative of such addition, but the converse does not hold. Much acetic acid is now prepared synthetically from acetylene with the use of a mercury catalyst ; traces of this metal may therefore be found in the strong acid. In view of the minute quantity present, ordinary chemical tests will be quite useless for its detection, and the process described below (p. 196) should be used ; even this is useless unless a considerable volume of the vinegar is available.

A useful method for differentiating different types of vinegar has been developed by Edwards and Nanji (*Analyst*, 1938, **63,** 410), who show that malt vinegar has a high oxidation value, iodine value and ester value, whereas artificial vinegars have very low figures, and spirit vinegars though higher than artificial vinegars are much lower than malt vinegars. These values are determined under standardised conditions as shown below.

The oxidation value is the number of ml. of 0·01 N permanganate required by 100 ml. of the vinegar ; the iodine value is the number of ml. of 0·01 N iodine absorbed by 100 ml. of the vinegar and the ester value is the number of ml. of 0·01 N potassium hydroxide required to saponify the esters from 100 ml. of the vinegar, all determined under the standardised conditions.

The complete analysis of a vinegar should therefore include specific gravity, acetic acid, total solids, ash, nitrogen, phosphoric acid, determination of oxidation value, iodine value and ester value, tests for arsenic and, if necessary, for caramel, ferrocyanide, lead, copper, and mercury.

The acetic acid is usually estimated by direct titration with the use of phenolphthalein as indicator, and nitrogen by the Kjeldahl process, which may be applied to 50 ml., most of the water being boiled off before the potassium sulphate and sulphuric acid are added. Phosphoric acid is estimated on the ash, which must therefore not be ignited too strongly ; the ash is dissolved by boiling in the smallest possible quantity of dilute nitric acid and a large excess of ammonium molybdate solution added, the mixture is heated to 70°, by warming on the water bath, then allowed to cool. The yellow precipitate is filtered, washed until free from acid with 2 per cent. solution of potassium nitrate, dissolved with the filter paper in excess of 0·5 N sodium hydroxide and titrated back with 0·5 N nitric acid, phenolphthalein being used as indicator (1 ml. of 0·5 N = 0·00154 P_2O_5).

Oxidation Value and Iodine Value. These values are determined by distilling 60 ml. of the vinegar with 15 ml. of water in a 400 ml. flask, with a little pumice, until 60 ml. have been collected. To

25 ml. of distillate add 10 ml. of dilute sulphuric acid (1 : 3) and 10 ml. of 0·1 N $KMnO_4$ solution ; stand the mixture for thirty minutes at 18° add potassium iodide and titrate back with 0·1 N sodium thiosulphate. Calculate the amount of 0·01 N permanganate required by 100 ml. of the vinegar. Make another 25 ml. of the distillate just neutral to litmus, add 10 ml. of N potassium hydroxide and 10 ml. 0·1 N iodine solution, stand for fifteen minutes at 18°, make the mixture acid with dilute sulphuric acid for two hours and titrate the excess alkali with 0·1 N acid. Calculate the ester value as ml. of 0·01 N per 100 ml. of vinegar.

Ester Value. Distil 100 ml. of sample in a 400 ml. flask and collect 30 ml. of distillate ; neutralise exactly with N potassium hydroxide, then add 10 ml. of 0·1 N potassium hydroxide, heat for two hours and titrate the excess alkali with 0·1 N acid. Calculate the ester value as ml. of 0·01 N per 100 ml. of vinegar.

The following approximate values will be found :—

	Oxidation value.	Iodine value.	Ester value.
Malt vinegar . . .	500–1.500	600–1.200	25–60
Spirit vinegar (5 per cent. acid)	80–250	5–30	6–20
Artificial vinegar . .	0–15	2–200	0–5

Caramel may be detected by Fiehe's reaction, but the test is not infallible, as a positive result is given by any liquor, such as cider vinegar, which contains furfural. One hundred ml. of the vinegar are extracted with 50 ml. of ether ; the separated ether is allowed to evaporate spontaneously ; to the residue are then added 3 drops of 1 per cent. solution of resorcinol in hydrochloric acid ; in the presence of caramel a rose colour is produced.

Sulphuric Acid. It is not sufficient to identify sulphates in the total solids or ash, as these may be due to the water used in the brewery, but if their amount exceeds, say, 0·03 per cent. as H_2SO_4, it raises some suspicion. This quantity or more might, however, be due to sulphur treatment of the casks or to a hard water. The best method of detecting or estimating added sulphuric acid is still that of Hehner (*Analyst*, 1877, **1**, 105), which is based on the fact that sulphuric acid will decompose a corresponding quantity of acetate which is always present in the vinegar solids. If the ash is alkaline, there is probably no sulphuric acid, although an amount less than that required to neutralise all the acetate might be present. If the ash is neutral or acid, it is presumptive of added mineral acid. Fifty ml. of the vinegar are evaporated with 25 ml. of 0·1 N sodium hydroxide; the residue is charred at a low temperature, mixed with 25 ml. 0·1 N sulphuric acid, boiled, filtered and washed. The filtrate is titrated with 0·1 N sodium hydroxide with the use of litmus as indicator; the volume of alkali used corresponds to the free mineral acid present, if any. For an alternative method *cf.* Richardson and Bowen (*J. Soc. Chem. Ind.*, 1906, **25,** 836).

Ferrocyanides may occasionally be found in a vinegar, and are an indication that the vinegar has been fined with potassium or sodium ferrocyanide, which is an objectionable process. They are easily detected by the blue colour formed on the addition of a trace of ferric chloride. Conversely, iron may be detected and estimated by adding ferrocyanide.

Copper is conveniently determined on the ash by dissolving it in dilute nitric acid, then adding excess of ammonia, filtering, and adding 5 ml. of 0·1 per cent. solution of sodium diethyldithiocarbamate. The brown colour so obtained is matched against standard copper solution.

Lead can also be estimated on the ash by dissolving it in dilute hydrochloric acid adding 10 ml. of ammonium citrate solution (40 per cent.) then 1 ml. of potassium cyanide solution and 2 drops of sodium sulphide solution. The colour should be matched against an equal quantity of ammonium citrate solution to which standard lead solution is added. Alternatively, the process given on p. 210 is applicable.

Arsenic may be estimated directly by the B.P. modification of the Gutzeit test, or by destruction of organic matter followed by a Marsh test (see p. 69).

Mercury. For the detection of minute traces of this metal recourse must be had to electrolysis. To 200 ml. of the vinegar are added 10 ml. of nitric acid, and the mixture is electrolysed for an hour with the use of a platinum anode and a small gold cathode about 1 sq. cm. in area, to which a piece of gold wire is attached. This latter may conveniently be attached to the copper leads on a glass rod covered with a short piece of black rubber tubing. The current should be 3 ampères and voltage about 6. After an hour the gold cathode is examined; if there is any considerable quantity of mercury, it will be obvious by the silvering effect, but if none is apparent, the gold foil is washed in alcohol and ether and allowed to dry for a few moments; then it is folded and placed in a glass tube drawn out and sealed at one end, the sealed end of which contains a minute fragment of iodine. The open end is closed by means of a cork or otherwise; the tube is gently heated from the wide end towards the narrow part, so that any mercury present sublimes into the constricted part of the tube. Here it meets the vapour of iodine, and on cooling a ring of red mercuric iodide, somewhat resembling a Marsh arsenic mirror, will be formed in the narrow part. Some little practice is requisite to obtain the best results, but with care 0·02 mgm. can be detected. The spectroscope affords an even more sensitive test.

In addition to malt vinegar, spirit vinegar and artificial or wood vinegar, some less common varieties appear on the market. Examples of such are wine vinegar, cider vinegar and vinegar essence.

Wine vinegar is mainly the product of Continental countries, where it is prepared by the acetic fermentation of grape juice or of inferior white or red wines. Such vinegars have a characteristic aroma by which they may easily be recognised; they usually contain a small proportion of free alcohol, traces of reducing sugars, and from 0·1–0·4 per cent. of acid potassium tartrate.

Cider vinegar is not common in England, though small amounts are produced in Monmouthshire and Herefordshire, but it is fairly usual in America. It contains a considerable quantity of malic acid, is lævo-rotatory, and yields a strongly alkaline ash rich in potassium. Leach and Lythgoe (*J. Amer. Chem. Soc.*, 1904, **26,** 375) give the following analyses of twenty-two samples of genuine cider vinegar :—

CIDER VINEGAR.

	Minimum.	Maximum.	Average.
	Per cent.	Per cent.	Per cent.
Acetic acid	3·92	5·82	4·84
Total solids	1·84	3·20	2·49
Ash	0·20	0·42	0·34
Alkalinity of ash, ml. N	2·22	3·61	2·97
Total P_2O_5	0·04	0·26	0·13
Reducing sugars as dextrose after inversion	0·15	0·53	0·25
Malic acid	0·08	0·16	0·11

The test for malic acid is made with lead acetate and with calcium chloride. To a few ml. of the vinegar add 1 ml. of solution of lead acetate ; if it does not give a precipitate settling in a few minutes and leaving a clear supernatant liquor, the sample is not cider vinegar. To 5 ml. of the vinegar add 1 ml. of 10 per cent. calcium chloride solution, then make alkaline with ammonia, filter off the precipitate, add to the filtrate 3 volumes of alcohol, and heat just to boiling. In the presence of malic acid a flocculent precipitate settles.

For the estimation of malic acid 100 ml. are treated with 10 ml. of calcium chloride solution, made alkaline with ammonia and filtered after standing for an hour. The filtrate is evaporated to 25 ml., and 75 ml. of alcohol are added. The solution is heated to boiling and filtered, the calcium malate precipitate is washed with 75 per cent. alcohol, dried and ignited, and the ash dissolved in excess of 0·1 N hydrochloric acid, boiled and titrated back with 0·1 N sodium hydroxide. The number of 0·1 N acid used × 0·0067 gives the percentage of malic acid.

The term " vinegar essence " covers a variable range of acetic products, in commerce it usually means a product containing 12 per cent. of acetic acid, coloured and perhaps flavoured. Spirit vinegar is a popular form of vinegar prepared by fermentation from molasses ; the alcohol so formed is distilled off, traces of food for the acetic bacteria are added and the alcohol is acetified in the same way as malt vinegar. As a result of this method of manufacture traces of alcohol are usually to be found in the product. Some spirit vinegars are prepared abroad by the

	Wine vinegar.[1]			Vinegar essence.	Spirit vinegar (strong).	Spirit vinegar.
	Maximum.	Minimum.	Average.			
Specific gravity .	1·0213	1·0129	1·0175	1·0167	1·020	1·007
Total solids (per cent.)	3·19	1·38	1·93	0·16	0·18	0·32
Sugar . ,,	0·56	0·22	0·46	—	0·09	0·03
Acetic acid ,,	7·38	4·44	6·55	11·90	12·1	4·22
Ash . ,,	0·69	0·16	0·32	0·06	0·02	0·04
Alcohol ,,	—	—	—	—	0·15	trace
Oxidation value .	—	—	300[2]	120	165	106
Iodine value . .	—	—	28[2]	25	22	13
Ester value . .	—	—	80[2]	5	3	1

[1] Analyses by the Paris Municipal Laboratory, quoted by Mitchell.
[2] One sample only.

fractionation of ordinary vinegars, and therefore contain but little besides acetic acid, water and traces of higher alcohols. The acetic strength varies from 4 to 12 or more per cent. Typical analyses of these vinegars are shown on the opposite page.

Spiced vinegars such as tarragon, garlic, elder and others are prepared by steeping the leaves or spices in malt vinegar ; the products show oxidation, iodine and ester values approximating those of malt vinegar, so these values afford an indication of the origin of the vinegar used in the manufacture.

For further particulars of these and other less common kinds of vinegar, such as date vinegar, raisin vinegar, etc., see C. Ainsworth Mitchell, " Vinegar : its Manufacture and Examination," 2nd ed. (Chas. Griffin, London, 1925).

CHAPTER VIII

FLESH FOODS, MEAT, SAUSAGES, POTTED MEATS, MEAT EXTRACT, GELATIN, ISINGLASS

THE questions which bring raw meat, apart from sausages or canned foods, to the notice of the analyst, are generally those respecting incipient putrefaction, preservatives, and occasionally the presence of parasites. Smell is not a safe criterion of soundness in meat, as it is well known that slight taint may often be removed by washing the meat with solution of bisulphite, permanganate, hydrogen peroxide or other such substance. If the decomposition is deep-seated or has proceeded far, it becomes obvious, but the chemist is not usually required in such cases; the following criteria may therefore be applied in doubtful cases.

Fresh meat, with the exception of certain special parts, such as the spleen, is normally slightly acid to litmus; alkalinity is usually an unfavourable sign; pickled bacon or ham has, however, an alkaline reaction. Sound meat is firm to the touch, and its surface tends to dry and not to absorb moisture. The strong reducing property of putrefying meat affords a chemical means for its detection, even in quite early stages. Nitrates are reduced to nitrites within two or three hours, and methylene blue is decolorised in a similar period. The most convenient test in the writer's experience is that of dissolved oxygen absorption. Two 5 g. quantities of the minced lean meat are mashed with tap water at 22°–23° and transferred to 250 ml. stoppered bottles, which are completely filled with water at this temperature (all air bubbles being carefully excluded) and incubated at 22° for two and four hours respectively. At the end of these periods there is added to each one 1 ml. of sulphuric acid, then excess of 0·1 N potassium permanganate; after five minutes the colour is discharged by 1 ml. of solution of oxalic acid, then is added 1 ml. of 33 per cent. solution of manganous chloride and 1 ml. of a mixture containing 33 per cent. of sodium hydroxide and 10 per cent. of potassium iodide. The precipitate will be practically white if all oxygen has been absorbed, or brown if there is oxygen still present in solution. If there is any putrefaction, the dissolved oxygen will all have disappeared within two hours, and if it is in the incipient stage, all will have disappeared within four hours.

In connection with the detection of artificial preservation of meat, there are several possibilities to be considered. Some are discussed in the Report of the Departmental Committee on the Use of Preservatives and Colouring Matters in Food, 1924. Treatment with formalin, as in the Linley process, is considered specially reprehensible ; other methods include the use of sulphur dioxide, either alone or mixed with eucalyptus and other substances, or of borax and boric acid ; salicylic or benzoic acids or their esters may be present in packed foods, but are not often met with in raw or cooked meat. Potassium nitrate and salt are not regarded as preservatives in this connection. When nitrate has been used in pickling meat both nitrate and nitrite can be detected in an extract of the meat ; the nitrite arises from bacterial reduction of the nitrate, or is sometimes itself an ingredient in the pickle. However the nitrite originates there is little doubt that its quantity in the product should not be large ; figures such as 250 p.p.m. have been suggested as a maximum. Nitrates are detectable by the familiar phenol-sulphonic acid reaction which may be applied to a filtered aqueous extract of the meat. To the residue obtained by evaporating, say, 10 ml. of cold water extract, are added 2 ml. of phenol-sulphonic acid solution in sulphuric acid ; after warming, the mixture is diluted with water, and excess of ammonia is added ; in the presence of nitrates the yellow colour of ammonium picrate appears. The method does not give quantitative results in the presence of any considerable amounts of sodium chloride. Nitrites are determined by means of the Griess-Ilosvay reagents as on p. 64.

Sulphur dioxide in flesh products may be determined by the iodimetric method described on p. 32, which is quite appropriate for sausages and such products. When, however, there is a possibility of the presence of other volatile sulphur compounds, such as sulphide or mustard oil, the method devised by Monier-Williams[1] is preferable. It depends upon the oxidation of sulphur dioxide to sulphuric acid by pure hydrogen peroxide and eliminates errors due to hydrogen sulphide, or other sulphur compounds or volatile acids. The apparatus is as shown in Fig. 38.

The flask D and tube E each contain 10 ml. of neutralised hydrogen peroxide solution, and F contains 5 ml. of hydrogen peroxide and barium chloride acidified with hydrochloric acid.

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[1] Ministry of Health Report, No. 43, 1927.

About 500 ml. of water are put into the flask A with 20 ml. of hydrochloric acid. The solution is boiled for a short time and a current of pure carbon dioxide from a cylinder passed through the whole apparatus to remove the air. Then about 100 g. of the sample are introduced into the flask A: the mixture is boiled for one hour in a slow current of carbon dioxide. At the end of this time the water supplied to the condenser is turned off so that it may become hot and any sulphur dioxide retained in it may be driven over into the receiver D, which is conveniently kept cold by standing it in a basin of water. The flask D and tube E are removed and their contents titrated with 0·1 N sodium hydroxide using bromphenol blue as indicator. The tube F serves to check

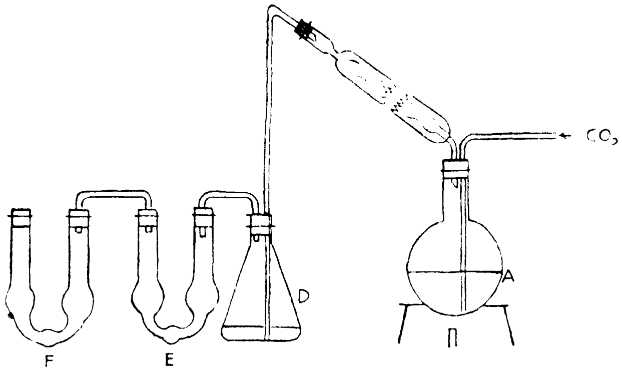

FIG. 38.

that the absorption of the sulphur dioxide in the first vessels has been complete.

Boric acid may be recognised by igniting a portion of the meat with alkali and treating the ash with dilute hydrochloric acid, then drying with a piece of turmeric paper in the well-known manner; if present, boric acid may be estimated in the ash, following the procedure outlined on p. 191. A point of some importance to be considered in connection with boric acid is whether the meat has been pickled in a bath containing borax or whether it has been subsequently packed in, or sprinkled with, this substance. It has been shown that when a piece of meat or a ham is packed in a powder containing boric acid, this substance rapidly permeates the entire mass and may be detected even near the bone after about a week; hence some significance may attach to the amount of boric acid found and its distribution. A small quantity near the surface and a smaller quantity near the centre may indicate packing or sprinkling with boric acid rather than its use in the pickling fluid. It will always be found that there is much more boric acid in the lean portions than in the fat ; indeed, it is probable that the acid is mainly dissolved in the water of the lean issue. Fluoborates will give the qualitative reactions of borates and of fluorides.

Benzoic acid and its esters are detected or determined by the method given on p. 104.

The detection of added formaldehyde in meat or fish products is not always simple. Difficulty arises because traces of formaldehyde may occur naturally in certain smoked meats and as a result of the oxidation of volatile amines in fish products. On this account care should be taken in interpreting the results of the various tests specially if only very faint reactions are obtained. Tests may be made on aqueous extracts of the product ; distillation should not be employed unless it is really necessary because of the possibility of producing the substance sought, by oxidation of trimethylamine oxide. About 10 g. of the minced meat are heated in a water bath for five minutes with 10 ml. of water containing 2 ml. of 1 per cent. phenyl-hydrazine hydrochloride solution ; after cooling, the mixture is filtered and to the filtrate is added 1 ml. of 5 per cent. solution of potassium ferricyanide solution and 4 ml. of hydrochloric acid. In the presence of formaldehyde a brilliant magenta colour is produced (Schryver's test). This colour may be compared in intensity with that of known dilutions of formalin. This test will detect about 1 part of formaldehyde per million ; it is nearly but not quite specific as certain methylamines may give a colour. These latter substances, however, do not give the phloroglucinol reaction which is obtained by adding to an aqueous extraction a mixture of 1 per cent. phloroglucinol and an equal volume of 25 per cent. sodium hydroxide solution ; a rose-red colour appears in the presence of formaldehyde.

Meat of all kinds is liable to contain various animal parasites or their cysts, and even though there is careful inspection at the slaughter-houses, some kinds, specially ham and other pig meats, are occasionally met with infected in this way. Examination of the lean meat, particularly near the bone, with the aid of a lens, usually reveals the parasites, and microscopical examination suffices for their identification. The commonest are different species of *Cysticercus*, which cause " measles " in pork ; *Trichina*, which give rise to trichinosis ; and *Tænia*, or tape worms. For detailed descriptions of these and other types, see Mitchell's " Flesh Foods " (C. Griffin & Co., London.) When the parasites cannot be detected in a section of the flesh, they can sometimes be isolated by chemical means ; these are specially useful for sausages and similar meat preparations. Fragments of the lean meat are treated with 100 ml. of 0·5 per cent. hydrochloric acid and 5 ml. of glycerin-pepsin, and the mixture is kept at 45°–50° for three to six hours, which suffices to dissolve the tissue and leaves the cysticerci or other parasites on the bottom like rice grains. They can then be examined microscopically. In the case of ham it is sometimes convenient to cut small pieces of the muscle tissue, wet with acetic acid, and press between the two stout microscope slides until the tissue is thin enough, and then examine them under a low power. If the question arises whether the parasites or their cysts are alive or dead, it may be answered by application of solution of hæmatoxylin or of carmine ; the living organisms do not really stain, but the tissue when dead quite readily retains the colour. Another parasite which is found in liver, and occasionally in other parts, is the liver fluke, which is a member of the group *Distomidæ* of the class *Trematodes*. Flukes are so large that they can be readily detected by the unaided eye ; a full-grown fluke may be as much as 1½ inches in length, but the ova and sporocysts are of microscopic dimensions.

Sausages and Potted Meat

In addition to the examination for preservatives, putrefaction or parasites as already mentioned, it is often necessary to estimate starch or breadstuffs in sausages or potted meats, and occasionally in imported sausages to examine for the presence of horseflesh. The common types of meat are free from either carbohydrates or cellulose ; hence these, if present, may be all attributed to fillers such as bread or biscuits. A method for the analysis of sausages or meat paste is given by Stubbs and More (*Analyst*, 1919, **44**, 125), which works well if not complicated by the presence of soya meal. Determine water, fat, protein (*i.e.*, nitrogen × 6·25) and ash. The percentage of non-fatty solids less the sum of protein and ash gives the carbohydrates which multiplied by 2 gives the approximate weight of bread or filler containing 40 per cent. of water. Deduct from the total nitrogen, 1 per cent. of the weight of the filler to allow for the nitrogen in the filler. Multiply the balance of nitrogen by 100/3·4 for beef or mutton or by 100/3·6 for pork to obtain the percentage of de-fatted meat; add the fat to obtain the total meat content. To check the result note that 100 minus filler and meat gives the added water, then the total water is 40 per cent. of the filler plus 75 per cent. of the de-fatted meat plus added water. If this figure differs by more than 2 per cent. from the total water found, it is probable that some soya meal has been used. Methods for the detection of soya meal by the urease reaction and the microscope are given in p. 79. No completely satisfactory method exists for determining soya quantitatively in meat products, though some useful data is given by Illing and Whittle (*Food*, 1944, 32), who rely upon the fact that dry oil-free soya contains 11·4 per cent. of sucrose (expressed as dextrose), 6·8 per cent. of salt-free ash and 39 p.p.m. of manganese. Low fat soya such as is used in sausages has a protein content equal to three times its weight of lean meat. For the direct estimation of starch, Mayrhofer's method is still the simplest and most convenient (*Z. Unters. Nahr. Genussm.*, 1896, 331). Sixty to 80 g. of the sausage, or less of a potted meat, are heated on the water bath with 8 per cent. solution of potassium hydroxide in strong alcohol (96 per cent. by volume) until it is obvious that all meat substance has dissolved; then the mixture is diluted with warm alcohol and filtered on a Buchner funnel, and the residue washed free from alkali. The crude starch is suspended in 5 per cent. acetic acid, and made up to a definite volume; an aliquot part is taken and the starch precipitated in a flocculent form by the addition of alcohol. The precipitate is collected on a tared filter, washed, dried and weighed. When using acetic acid in the manner described, it is not necessary to ignite and deduct the weight of the ash, as it will be negligible. In view of the nature of the material in sausages and meat pastes and the uncertainty as to the amount of water in the original filler, refinements such as the polarimetric or inversion methods for the estimation of the separated starch are superfluous, and direct weighing on a considerable quantity of sample is preferable.

Many reactions have been proposed for the detection of horseflesh in sausages and meat products generally. These mostly depend on the recognition or estimation of glycogen, which is a carbohydrate present in the liver of most animals, but only to a small extent in other parts of their flesh, except in the case of the horse. Further investigation by various chemists has shown that it is not safe to conclude the presence of horseflesh unless the glycogen content is upwards of 2 per cent. on the dry matter, and as this amount is not always present in raw horse-meat itself, the test is of limited value. For a summary of results of tests on different meats, see Mitchell (*loc. cit.*, p. 211). Another test of limited value, but useful for confirmation, is that of Ehrlich, who shows that when horse-meat is treated with formalin, it develops an intense and characteristic smell, suggestive of roast goose, within forty-eight hours ; this smell is not given by any other meat. The only specific test is a biological one, which, unfortunately, is not always readily performed by the chemist, owing to the difficulty of obtaining a supply of the appropriate serum. The serum is obtained by injecting fresh filtered horse serum into a rabbit ; after some days blood is withdrawn from the rabbit and a dilute solution of its serum in physiological salt solution is prepared. This has the property of precipitating an extract from meat containing horseflesh, but not extracts from other flesh (Fiehe, *Z. Unters. Nahr. Genussm.*, 1907, **13**, 744).

Detection and Estimation of Glycogen. This carbohydrate forms *a characteristic wine-red colour with solution of iodine.* The reaction may be applied by boiling 50 g. of the meat with 200 ml. of water for an hour, then cooling and adding enough dilute nitric acid to reduce the colour, and then filtering : to the filtrate is added a layer of iodine solution, which gives a red ring at the point of contact in the presence of glycogen. It has been suggested that the test so carried out will reveal the presence of 5 per cent. of horseflesh, but this is improbable. For the estimation of glycogen, Piettre's method is to boil 25 g. of the minced meat with 80 ml. of 10 per cent. alcoholic potassium hydroxide solution until all the tissue is dissolved ; then the insoluble residue is filtered off and washed with acidified alcohol until free from alkali ; it is then dissolved by heat in water made just alkaline. An equal volume of strong alcohol is added to precipitate the starch, which is filtered off and washed with 50 per cent. alcohol ; the filtrate is evaporated to low bulk, and the glycogen is precipitated by adding strong alcohol, collected, dried and weighed. Obviously the process is not susceptible of great accuracy. The following amounts of glycogen are reported by Bujard :—

	Water.	Glycogen.	Glycogen on dried substance.
	Per cent.	Per cent.	Per cent.
Horseflesh	61·83–72·90	0·174–1·366	0·64–4·62
Beef	73·62	0·206	0·74
Beef	75·55	0·018	0·07
Veal	76·12	0·346	1·44
Veal	74·47	0·066	0·25
Pork	54·0–66·3	Traces	Traces
Horse sausages :			
Red sausage	70·04	0·504	1·68
Liver sausage	67·00	1·762	5·34
Salami sausage	33·00	0·034	0·05

Corroboration of the presence of horseflesh may sometimes be obtained by examination of the fat, which may be extracted by solvents in the ordinary way. Horse-fat crystallises from ether in tufts resembling beef-stearin, also it has a higher iodine value (80–95) than other fats such as those of pork, veal, beef and mutton, but as the iodine values of these are so variable and are sometimes rather high, deductions from the iodine value are uncertain, specially in cases of admixture.

Fish and Fish Pastes

For the purposes of the analyst, fish and fish products are conveniently divided into four groups : (i) Fatty fish ; (ii) non-fatty fish ; (iii) salted fish ; and (iv) crustaceans. The percentages of fat and nitrogen in these groups are fairly characteristic and serve as a basis for the calculation of the proportion of fish in the fish pastes made from them. Unfortunately, there are no known methods for determining the identity of fish in a cooked paste, though careful microscopic examination, together with determination of the chemical composition, often affords useful indications. The microscopic appearance of any scales or bits of shell or bristles may help. The following table shows the average composition of the commoner types of fish, of which salmon is much the most important from the present aspect. The data are collected from various sources ; all the figures refer to the edible parts of the fish.

	Water Per cent.	Fat Per Cent	Protein Per Cent.	Ash Per Cent.	Salt Per Cent	Nitrogen on fat-free basis Per Cent.
FATTY FISH						
Herring ..	72·5	7·1	19·5	1·5	0·3	3·4
Mackerel ..	73·4	7·1	18·7	1·2	—	3·2
Salmon* ..	66·6	10·5	21·5	2·0	0·6	3·9
Sprat ..	66·8	14·5	17·1	1·8	—	3·2
Sardine ..	58·5	8·0	20·5	9·5	—	3·6
NON-FATTY FISH						
Cod	82·6	0·4	16·5	1·2	0·2	2·6
Haddock ..	81·7	0·3	17·2	1·2	0·2	2·7
Hake	83·1	0·7	15·4	1·0	—	2·5
Whiting ..	80·4	0·2	17·7	1·1	—	2·8
SALTED FISH						
Anchovy ..	62·5	8·3	20·9	—	[7 to 20]	3·6
Bloater ..	64·0	14·0	19·0	2·0	0·9	3·6
Kipper ..	61·0	8·5	20·5	6·0	4·9	3·6
CRUSTACEANS						
Crab	73·6	0·2	22·4	2·7	0·3	3·6
Prawn ..	71·2	1·3	22·8	5·2	0·3	3·6
Lobster ..	71·5	1·0	20·7	3·4	—	3·3
Shrimp ..	70·0	0·9	22·0	6·8	1·5	3·6

* As canned.

In all cases protein means nitrogen multiplied by the conventional factor, 6·25. Usually the analysis of fish in these terms adds up to something rather less than 100 per cent., and the presence of small quantities of glycogen or other carbohydrate has been reported in many cases. Starch is never found as a natural constituent, so that, apart from any minute quantity due to condiments, its presence in fish paste always indicates bread or other filler. The amount of salt in anchovies, kippers and bloaters is apt to vary widely, and sometimes anchovy or bloater paste will contain quite large additions of salt. The following tables set out the analytical results obtained with a number of fish pastes :—

	Salmon and shrimp. 16 Samples			Bloater. 9 Samples		
	Max. Per Cent.	Min. Per Cent.	Average Per Cent	Max Per Cent	Min Per Cent.	Average Per Cent.
Water ..	68·04	57·58	63·8	59·26	45·00	55·6
Fat ..	11·73	7·56	9·3	16·86	7·80	12·0
Protein ..	20·21	16·46	18·2	23·06	19·31	20·1
Ash	6·22	2·96	4·0	9·31	4·29	6·7
Salt ..	4·50	1·60	2·0	7·15	2·50	4·8
Starch ..	8·68	None	4·2	12·30	None	4·0

	Anchovy. 9 Samples			Sardine and tomato.	Salmon and anchovy.
	Max. Per Cent.	Min Per Cent.	Average Per Cent.	Per Cent	Per Cent.
Water	65·60	38·12	55·7	60·39	63·02
Fat	38·13	0·90	12·1	9·60	9·12
Protein	22·98	17·60	20·6	18·95	14·04
Ash	16·70	4·01	7·1	3·35	5·32
Salt	14·20	3·32	5·9	Sugar 7·73	—
Starch	12·7	Absent	2·5	None	8·50

	Lobster. Per cent.	Crab. Per cent.	Prawn. Per cent.	Shrimp. Per cent.
Water	68·77	66·70	66·20	64·72
Fat	9·83	5·19	8·56	11·50
Protein	15·53	18·79	21·60	18·37
Ash	3·10	2·56	3·98	2·99
Salt	1·02	0·60	1·70	1·82
Starch	3·05	6·76	None	2·50

The calculation of the approximate composition of the paste is not difficult when the identity of the fish used is known ; without such knowledge one can only proceed on the basis of probability fortified by the name on the label. The method is an extension of that adopted by Stubbs and More for the determination of the proportion of meat in sausages.

The starch percentage, multiplied by 2, gives moist bread containing 40 per cent. of water and 1 per cent. of nitrogen. The nitrogen found, less 1 per cent. of the bread, multiplied by 100 and divided by the nitrogen per cent. in the fat-free fish used, gives the fat-free fish, to which must be added the fat found if its amount be not in excess of that natural to the percentage of fish indicated. When there is much fat, it is desirable to examine it in detail to ascertain its nature and origin. If there is no indication of the addition of butter or other foreign-fat, any excess usually indicates the use of a fatty-fish basis such as salmon, which is common in crab, lobster and shrimp pastes. The proportions of salt and water natural to the fish are calculated from the fish basis, and any excess is noted.

As salmon is the commonest basis in mixed pastes, it is convenient and approximately correct to take its composition as the starting-point in attempting to calculate the ingredients of a mixture.

Sometimes canned fish or crustaceans are found to contain small crystals, liable to be mistaken for glass, of struvite, which is magnesium ammonium phosphate. Notes on the form of these crystals are given by Purcell and Hickey (*Analyst*, 1922, **47,** 16); no physiological significance attaches to them.

Canned fish such as sardines may contain excessive quantities of lead and limits of 10 or even 5 p.p.m. have been suggested for this impurity. As the ash from fish will contain much iron and phosphate the best method is that of Hamence (*Analyst*, 1932, **57,** 622). A quantity (14 g.) of the sample is ashed in a porcelain basin; if any carbon remains the ash may be moistened with nitric acid and re-heated. It is then boiled with a few ml. of nitric acid in a little water, then cooled and transferred to a separator. There are added 5 ml. of saturated ammonium thiocyanate solution, then 15 ml. of amyl alcohol and 15 ml. of ether. On vigorous shaking the iron is removed as thiocyanate which passes into the mixed solvent. Sometimes a second extraction is necessary to remove all the iron. The iron-free residue is divided into two parts representing 12 and 2 g. respectively. To these are added 5 ml. (more if necessary) of 20 per cent. citric acid solution to prevent precipitation of phosphate and then an excess of ammonia and 1 ml. of potassium cyanide solution. Two drops of sodium sulphide are added and the amount of lead determined colorimetrically in the usual way.

Meat and Fish Pastes or Potted Meats in jars or tins are widely sold and frequently come under suspicion as the cause of cases or outbreaks of food poisoning. Putrefaction, if not obvious, can be detected by the oxygen absorption test (p. 200), and the reaction to litmus should be noted, as an alkaline reaction is unfavourable. Tin, preservatives, and artificial colouring matters should be searched for and arsenic estimated. The commonest colouring matter in such preparations is Armenian bole, a variety of iron oxide; this is sometimes heavily contaminated with arsenic. If any trace of arsenic is found it may probably be due to the use of a mineral pigment, but the following quantities of arsenic have been found in fresh fish

	As_4O_6. Mgm. per 100 g.		As_4O_6. Mgm. per 100 g.
Whiting	0·04	Haddock	0·06
Plaice	0·14—0·30	Brill	0·03
Sole	0·03	Mackerel	0·05
Hake	0·03	Halibut	0·03
Cod	0·05	Turbot	0·05
John Dory	0·01	Herring	0·03

(*Analyst*, 1925, **50, 3**), and even larger quantities in shell fish, so that the possibility of traces of arsenic being due to such fish must be borne in mind.

Cases of suspected food poisoning generally call for bacteriological examination ;[1] and this should, if possible, be supplemented by physiological tests on animals. The first step is to macerate a small quantity of the paste with sterile water, then plate out the suspension on gelatin and agar plates for incubation aerobically and anaerobically at 22° and at 37°, and further quantities on special McConkey agar for the detection of salmonella bacteria. Suspect white colonies are picked out and inoculated into peptone water, litmus bile-salt peptone tubes and neutral litmus-milk tubes for the identification of these food poisoning organisms in the manner described in the usual text-books of bacteriology. For the separation and identification of these and other forms of bacteria reference must be made to such text-books. A most valuable procedure is the feeding of rats on portions of the suspected food ; captive rats are generally very susceptible to food poisons, and if they fall ill or die, the cultivation of the microbe from the corpse is the surest means of its identification. It may happen that no live pathogenic bacteria are present in the food, yet it may be intensely toxic by reason of the presence of poisonous toxins produced by them before sterilisation.

When the ill-effects of the food are due to bacteria or their toxins it is generally quite useless to examine other samples from the same batch of material ; many cases are on record where only one pot out of a batch of several thousands has been found to be infected or poisonous.

Meat Extracts

The composition of commercial meat extracts has undergone some little change in recent years by reason of the increased utilisation of those parts of the animal which were formerly discarded. The general character of the leading brands seems to be similar so far as analysis discloses : they are prepared by extracting the minced defatted meat with hot water, subsequently evaporating the water and skimming off any fat. The

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[1] See particularly Memo 188/Med. Pamphlet on Food Poisoning issued by the Ministry of Health, 1935.

concentration is usually carried out in vacuum pans. Additions which the analyst may have to trace include that of gelatin and of yeast extract; the latter is sharply differentiated from meat extract by the absence of creatine or creatinine. For commercial purposes a partial analysis showing water, organic matter soluble in 80 per cent. alcohol, organic matter insoluble in 80 per cent. alcohol, inorganic matter, and creatine or creatinine, is often required, and suffices to show the quality of the extract and the presence, if any, of added yeast extract.

Traces of copper sometimes amounting to 50 or even 100 p.p.m. are occasionally present in extracts. This metal should therefore be searched for and estimated.

The quantitative separation of creatine from creatinine is of doubtful value because the creatine of meat loses water during evaporation and forms the anhydride creatinine, which latter tends to recombine with water during storage. The partial analysis of some recent samples of genuine meat extract is as under :—

	1.	2.	3.	4.
	Per cent.	Per cent.	Per cent.	Per cent.
Water	16·51	18·34	19·60	15·78
Organic matter soluble in 80 per cent. alcohol . .	40·95	37·63	43·46	47·52
Organic matter insoluble .	18·23	20·86	12·15	12·83
Mineral matter . . .	24·31	23·17	24·79	22·87
including NaCl . .	3·43	3·76	5·56	4·32
and P_2O_5 . . .	6·58	7·14	7·32	7·10
Creatine and creatinine .	9·5	9·5	11·0	11·0

A sample of yeast extract, somewhat highly salted, examined in the same way, showed :—

	Per cent.
Water . . .	12·45
Organic matter soluble	34·70
Organic matter insoluble	12·20
Mineral matter . .	40·68
Salt . . .	38·51
Creatine and creatinine	*Nil*
Total nitrogen . .	7·33

A more detailed analysis of recent samples shows :—

	1.	2.	3.	4.	5.	6.	7.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water	20·8	17·9	28·1	34·6	23·3	42·9	17·3
Organic matter	58·2	58·5	51·6	47·2	48·2	41·2	62·7
Mineral matter	21·0	23·6	20·3	18·2	28·5	15·9	20·0
Salt	4·6	4·8	10·4	11·1	18·6	10·9	3·9
Total nitrogen	8·9	8·9	6·2	7·1	5·8	5·5	9·6
Meat fibrin	1·3	1·9	5·3	6·0	8·6	0·6	—
Albumoses and peptones	0·5	1·0	0·4	22·4	13·3.	17·5	0·7
Gelatin	0·2	0·3	0·5	0·5	0·4	8·7	trace
Non-nitrogenous extractives		traces only			10·0	7·0	trace
Creatine and creatinine	8·5	8·1	5·5	6·1	6·3	1·4	4·7

On a whole beef extract it will be found that the total creatine and creatinine does not fall much below 10 per cent. when calculated on the dry matter; if the amount present is materially lower than this, it is probable that some yeast extract is present. It is, however, necessary to bear in mind that extracts made wholly or largely from other parts or organs of the body may contain much less than this.

There exists no official standard for meat extract in this country, but a definition has been suggested by the A.O.A.C.: "Meat extract is the product obtained by extracting fresh meat with boiling water and concentrating the liquid by evaporation after removal of the fat. It contains at least 75 per cent. of total solid matter, of which not more than 27 per cent. is ash, and not over 12 per cent. sodium chloride. The fat should not exceed 0·6 per cent. and the nitrogen be not less than 8 per cent. The nitrogenous compounds contain not less than 40 per cent. of meat bases and not less than 10 per cent. of creatine and creatinine."

The methods involved in the partial analysis given above are simple. For the estimation of water, 20 ml. of a solution of 10 g. in 100 ml. are evaporated in a tared platinum basin containing some ignited sand and dried at 100° to constant weight. The ash is determined on another 20 ml. portion: it is ignited at as low a temperature as possible to avoid loss of sodium chloride; the salt and phosphoric acid may be determined on the ash in the ordinary way. For the alcohol extract, to 10 ml. of the 10 per cent. solution are added 50 ml. of alcohol of sp. gr. 0·823, and the mixture is allowed to stand overnight at 16°–18°; then the alcohol extract is poured off into a tared dish and gently evaporated; the residue is re-extracted twice with 80 per cent. (by vol.) alcohol, being shaken and allowed to settle before pouring off the extracts. The total soluble matter is dried in the oven at 100° and weighed, then gently ignited; the loss in weight gives the organic matter soluble in 80 per cent. alcohol.

Creatine and creatinine are estimated by a modification of Folin's method. To 10 ml. of 10 per cent. solution are added 30 ml. of N hydrochloric acid and 60 ml. of water; the mixture is heated in a 100 ml. flask in an autoclave at 117°–120° for twenty minutes, then cooled and the volume adjusted to 100 ml. In this way all the creatine is converted into creatinine. Fifty mg. of creatine ($H_2N.C(NH)\ N\ CH_3.CH_2.CO_2H,H_2O$) in hydrochloric acid solution are treated in exactly the same manner and diluted in 100 ml. Then 10 ml. of the treated meat extract and a similar volume of the standard creatine solution are taken for comparison; to each are added 30 ml. of saturated picric acid solution and 15 ml. of 10 per cent. solution of sodium hydroxide. It is essential that pure picric acid be used. After standing for *five* minutes the solutions are diluted to 500 ml., and the colours are matched in any convenient colorimeter; the proportion of creatinine in the extract is then calculated. It is essential to adhere to the time strictly lest error arises from any sugars present. Instead of converting the creatine as above, a standard solution of potassium dichromate may be used; a column 8 mm. high of solution containing 24·56 g. per litre corresponds in colour with that of 10 mgm. of creatine in 500 ml. of liquid, but it is imperative that all comparisons be made with the 8 mm. column; the colour relation is not valid for other depths.

Molisch's reaction is a useful test for application to a meat extract, since it readily shows the presence of carbohydrates. These occur naturally only in extracts prepared from livers or horseflesh; otherwise a positive result indicates added starch or sugars. The test is applied as follows: 1 ml. of the 10 per cent. solution is placed in a stoppered 25 ml. cylinder, and to it are added gently down the side 9 ml. of sulphuric acid, followed by 6–10 drops to 20 per cent. alcoholic solution of α-naphthol. The tube is stoppered and shaken, then allowed to stand; an intense reddish-purple colour rapidly develops in the presence of carbohydrates. When a positive reaction is obtained, starch should be specially searched for unless the low total nitrogen, low creatinine and low meat-base nitrogen indicate the presence of a quantity of liver extract.

When a more elaborate analysis of meat extract is required, the following additional estimations may be made : total nitrogen and nitrogen as—(1) readily coagulated albumin and fibrine ; (2) soluble albumin ; (3) albumoses and peptones ; (4) gelatin ; (5) meat-base nitrogen. The last-named is the total nitrogen less the sum of (1), (2), (3) and (4) ; it therefore includes creatine and creatinine unless they are expressly excluded.

For the estimation of the nitrogen in these various forms it is convenient to work on a 10 per cent. solution ; then the total nitrogen is determined on 10 ml. or 15 ml., and (1) the readily coagulated albumin and fibrine by diluting 25 ml. to 100 ml. and allowing it to settle overnight, then filtering and determining the nitrogen on the washed insoluble matter. The soluble albumin (2) in the filtrate is precipitated by boiling for five minutes with 5 per cent. of acetic acid, then collecting the coagulated albumin and making the usual Kjeldahl determination on the coagulum. Albumoses and peptones (3) are estimated with the gelatin by acidifying 50 ml. of the original solution with an equal volume of 10 per cent. sulphuric acid and precipitating with an excess of phosphotungstic acid, and filtering after allowing the precipitate to settle. The nitrogen in the precipitate is determined as before. To estimate the gelatin (4), 25 ml. are diluted with 250 ml. of alcohol (96 per cent.) ; after standing overnight the supernatant liquor is poured off, and the insoluble gelatin is washed with cold 10 per cent. alcohol at a temperature not higher than 5° until the washings are free from colour ; the gelatin nitrogen is deducted from that found as albumoses, peptones and gelatin together. An alternative method for albumoses and peptones plus gelatin is to acidify 50 ml. of the solution with dilute hydrochloric acid (5 ml.) and add a considerable excess of bromine water ; the precipitate which contains these three substances is most conveniently separated by centrifuging and is washed with bromine water in the same way. The remaining nitrogen, after deducting that present as fibrine and readily coagulated albumin, soluble albumin, albumoses, peptones and gelatin, is reckoned as meat-base nitrogen ; this figure includes creatine and creatinine, or these may be deducted and the fact stated.

Considerable difference of opinion exists as to the proper factors by which to multiply the nitrogen determined in the above fractions. As the true factors are not known some have advocated the arbitrary factor of 6·25 for all, on the ground of general convenience, although it does not pretend to accuracy. Others prefer different factors for each class, based on the best available data ; these have the merit of some attempt at accuracy, but there is the difficulty that it introduces a want of uniformity. It seems the best plan at present to determine nitrogen and leave it as nitrogen ; if any factor is used it should be stated. For a discussion of this subject, see *Analyst*, 1915, **40,** 310.

Beef Cubes

A development of the meat extract industry has been the manufacture of beef cubes, of which there are many brands. The table shows the composition of twelve makes of meat cubes—not soup cubes—examined recently. It is appropriate in order to arrive at the amount of meat extract in them to assume that meat extract contains 6 per cent. of creatine and creatinine, but the true amount of these two substances in a meat extract may be much less or more than this figure, so that considerable caution is needed in applying an arbitrary factor.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water . .	13·0	13·6	12·7	14·7	6·5	10·0	13·7	10·8	13·4	10·0	11·8	8·1
Organic matter .	48·7	57·9	50·7	54·2	34·4	60·6	55·5	51·9	50·5	62·6	51·5	66·2
Mineral matter .	38·3	28·5	36·6	31·1	59·1	29·4	30·8	37·3	36·1	27·4	36·7	25·7
Salt . . .	31·5	23·0	29·5	27·2	50·3	22·7	25·2	34·7	30·1	20·8	30·0	18·8
Fat . . .	2·4	4·4	0·9	—	—	—	—	—	—	—	—	—
Carbohydrates .	19·3	8·4	29·1	29·0	9·5	12·0	12·3	40·4	29·7	11·5	25·0	33·2
Total nitrogen .	4·2	8·0	3·3	3·9	3·9	7·8	6·8	1·7	3·2	8·2	4·2	5·0
= Protein. .	26·4	50·2	20·8	24·2	24·4	48·6	42·5	10·9	19·8	51·4	25·9	31·1
Gelatin . .	Traces	+	Traces	+	–	–	+	–	–	+	–	+
Starch . .	+	–	+	–	+	+	–	+	+	–	+	–
Creatine and creatinine .	1·6	2·0	1·5	0·9	0·8	1·6	1·9	0·7	1·6	1·8	1·6	1·8

The commonest binding material is gelatin, though a number of preparations on the market are bound with starch.

The amount of gelatin required in a beef cube for binding purposes depends upon the nature and quantity of vegetable flavouring ; usually it is between 7 per cent. and 15 per cent. As there is practically no gelatin in meat extract it may be assumed that the whole amount present has been added as binder. The salt content varies from 20 per cent. to 30 per cent. or even more ; most of this is a direct addition. Carbohydrate is always found.

Gelatin

Although the value of gelatin as a food is indirect, it enters into the make-up of many articles, and hence it has to be examined from the point of view of purity; it differs from glue only in the matter of purity. It is usually prepared from selected parts of the connective tissue, skin, hoofs, etc., of animals. Chittenden gives the following analyses showing the ultimate composition of gelatin :—

	1.	2.
Carbon	50·12	50·00
Hydrogen	6·68	6·52
Nitrogen	17·84	17·88
Sulphur	0·32	0·23
Oxygen	25·10	25·35
Ash	0·32	0·36
	100·38	100·34

When the total nitrogen is determined the appropriate factor is N × 5·55 = gelatin. Tannin solution and platinum salts precipitate gelatin, but iron, aluminium, lead, copper and gold salts do not. It is soluble in acetic acid but not in alcohol or in ether. A full examination of gelatin should include the estimation of ash, fat, sulphur dioxide or other preservative, acidity, arsenic, lead, copper, zinc and iron. There are no legal standards for gelatin in this country except for sulphur dioxide, which is limited to 1,000 p.p.m.; the following limits have been propounded by the Society of Public Analysts. Arsenic (As_2O_3) 2, copper 30, lead 10, zinc 100 p.p.m. (*Analyst*, 1944, **69,** 106). The U.S.A. have fixed the following limits in that country: arsenic, 1·4; sulphur dioxide, 350; lead, nil; zinc, 100; copper, 30 p.p.m. According to Cattelain (*J. Pharm. Chim.*, 1924, **49,** 444) the acidity as hydrochloric acid should not exceed 1·5 per cent., and the iron as Fe_2O_3 should not constitute more than 2 per cent. of the total ash. Trotman and Sutton (*Analyst*, 1924, **49,** 271) give a critical account of methods for the estimation of some of the impurities mentioned above.

The ash of gelatin is estimated by direct incineration, preferably in a large platinum basin. The determination of fat is more important from a photographic point of view than as connected with foodstuffs; direct extraction in a Soxhlet does not yield satisfactory results, because the amount of fat is small and it is difficult to divide the gelatin into sufficiently fine particles for complete extraction. A better method is to destroy about 25 g. of the sample by digestion with hydrochloric acid and water (1 : 1), then cool and extract three times with ether in a separating funnel; the mixed ether extracts are washed free from acid, then evaporated in a tared flask and the residual fat dried and weighed. In order to remove any trace of non-fatty matter, the crude fat may be dissolved in dry petroleum spirit and poured into another flask and the weight of non-fatty residue subtracted from that of the fat originally weighed.

The acidity of gelatin is titrated directly with the use of phenolphthalein as indicator; it is calculated as hydrochloric acid.

For the estimation of arsenic, complete destruction of the organic matter is necessary; this may be effected by the wet combustion method (p. 68) or by ignition with excess of pure calcium oxide. The solution thus obtained is tested by the Gutzeit or Marsh-Berzelius method. Copper, lead and zinc can be determined on the ash by the method given on pp. 108 and 210.

β-naphthol is sometimes to be found as a preservative in gelatin. To detect it stand some of the gelatin with dilute hydrochloric acid 1 : 4 overnight, then warm the mixture for half an hour, cool and extract it twice with ether; evaporate off the ether, and test the residue for β-naphthol. Dissolve it in alcohol, then to 1 ml. of the solution add 1 ml. of water and 5 ml. of glacial acetic acid. Run 5 ml. of sulphuric acid in as an under layer: in the presence of β-naphthol a yellow-green colour with strong fluorescence is produced at the junction.

Sulphur dioxide is separated from gelatin by steam distillation as described on p. 32. Trotman and Sutton (*loc. cit.*) point out that discrepancies may occur through want of uniformity in determining (*a*) free sulphur dioxide, and (*b*) total sulphur dioxide after treatment with acid.

Isinglass

Isinglass is the dried swimming bladder of fish. It contains from 66 to 88 per cent. of gelatin, with 13–20 per cent. of moisture, and up to 1·5 per cent. of fat. The ash should not exceed 1 per cent. Moisture and ash are estimated in the usual way and fat either by direct extraction in a Soxhlet extractor or, better, by destroying the glutin with hydrochloric acid and shaking out with ether as described under gelatin. The total soluble matter should be determined by soaking 10 g. with 500 ml. of water, then boiling until the frothing subsides to a great extent ; the clear liquor is run off into a litre flask, the residue boiled again with a further quantity of water, and the solution poured off into the litre flask. This process is repeated three or four times ; then the solution is cooled and made up to the mark and filtered, if necessary. Fifty ml. of the clear liquor are evaporated and the residue weighed. The difference between the sum of moisture, fat, soluble and insoluble matter is taken as collagen ; this includes the gelatin. which may be estimated by evaporating 500 ml. of the above solution to about 50 ml. and precipitating the gelatin therein by the addition, while still hot, of 250 ml. of alcohol ; the precipitate is allowed to stand overnight and is then filtered off, washed with alcohol, dried and weighed. Starch is occasionally present in artificial isinglass ; it is readily detectable by iodine. Impurities are determined as described under gelatin.

CHAPTER IX

MILK, CREAM, CONDENSED MILK, DRIED MILK, INFANT FOODS, CASEIN, MALTED MILK

FEW articles of diet have been so much studied as milk, so that a vast amount of data is available concerning normal cow's milk and that of other mammals. The following constituents besides water have been described: fat, which consists of the mixed glycerides of stearic, oleic, lauric, palmitic and lower fatty acids, principally butyric, capric and caproic acids; proteins, which include casein, lactalbumin, globulin and possibly small amounts of others; carbohydrates, including mainly lactose; the organic acids are lactic, citric and acetic acids, the latter being in very small quantity. Other organic substances present include urea, lecithin, creatinine, hypoxanthine, alcohol, lactochrome and a variety of enzymes, reductases and oxydases. The mineral constituents include hydrochloric, phosphoric and sulphuric acids combined with sodium, potassium, calcium, magnesium and iron. The following physical properties are those of average cow's milk:—

Specific gravity (at 15·5°) . .	1·032
Refractive index (at 20°) . .	1·348
Freezing point	0·554°
Specific heat	0·96
Electrical conductivity: K_{15} . .	0·0041 [1]
pH value	6·6

The average composition of the milk of the cow and some other mammals, according to Richmond, is given on p. 221.

The difference in the milk of various mammals is not confined merely to the quantities as indicated in the table, but there are important differences in the proteins actually present and in the enzymes.

A few days before and after parturition a liquid—colostrum—is secreted which differs materially from normal milk, but which gradually changes in composition towards the normal, which is reached in about four days. Colostrum contains a large quantity

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[1] Coste and Shelbourne, *Analyst*, 1919, **44**, 159.

	Cow.	Goat.	Woman.	Sheep.	Mare.	Ass.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water .	87·25	86·04	88·20	81·31	89·80	90·12
Fat . .	3·75	4·63	3·30	6·86	1·17	1·26
Lactose .	4·75	4·22	6·80	5·23	6·89	6·50
Casein .	3·00	3·49	1·00	4·62	1·84	1·32
Albumin .	0·40	0·86	0·50	1·00		0·34
Ash . .	0·75	0·76	0·20	0·98	0·30	0·46

of albumin, and under the microscope exhibits characteristic large compound granular cells.

Cow's milk is acid to phenolphthalein, alkaline to methyl-orange, but amphoteric to litmus by reason of the phosphates present. Human milk is alkaline to litmus. On keeping, in air, milk gradually becomes more acid by the activity of the micro-organisms present, and when the acidity amounts to about 1 per cent. lactic acid curdling takes place in the cold ; at higher temperatures this change takes place with a much lower concentration of acid. Milk which has been collected under strictly aseptic conditions and kept in a cool place will remain sweet for three weeks or even more, without either preservative or pasteurisation.

Apart from pathological conditions producing abnormal milk, there are a number of factors which affect its composition ; these must be taken into account in considering the results of analyses. Large variations, especially in the fat content, are given by the different breeds of cows, as is shown in the table of results due to Vieth and others on p. 222.

It has also been noted that the average diameter of the fat globules varies in the different breeds.

The period of lactation has a fairly regular effect on the milk ; the total solids, fat and solids-not-fat fall rapidly in the second month, and then steadily rise until the ninth or tenth month, after which there is a general diminution as the lactation ceases. A point of importance in this connection is that with cows far advanced in lactation the proportion of volatile fatty acids in the fat diminishes, and may, in extreme cases, so affect the Reichert and other values of the butter prepared therefrom as to give rise to suspicion if the facts are unknown. The time of year also has its influence ; milk tends to be poorest in summer

Breed.	Average total solids. Per cent.	Fat. Per cent.	Solids-not-fat. Per cent.
Dairy Shorthorn	12·90	4·03	8·87
Jersey	14·89	5·66	9·23
Kerry	13·70	4·72	8·98
Red Polled	13·22	4·34	8·88
Sussex	14·18	4·87	9·31
Welsh	14·15	4·91	9·24
Ayrshire	13·46	4·24	9·22
Frisian	11·50	3·00	8·50
Devon	13·77	5·07	8·70

and richest in autumn, as will be seen from the table below, which shows the mean composition of milk delivered from a number of farms supplying a condensery. While this may in part be due to the changes in diet as the cows go into stalls or out to grass, diet alone is not a sufficient explanation; if there is an adequate diet in respect of the quantity of fat and protein, the milk is not largely influenced by the food, with the important exception that the flavour of the fat may be influenced by the fats of the food, as some characteristics of the fat from the food pass unchanged into the milk. Thus the fat from cows fed largely on cotton cake may give a cotton-seed oil reaction with Halphen's reagent.

Although there is evidence that the secretion of milk is a continuous process and no one constituent is secreted before another, it does appear that some separation or rising of cream may take place in the udder, for it is a well-known fact that the first runnings or " fore milk " are low in fat, whereas the last runnings or " strippings " are very high. In view of this fact, if a cow be imperfectly milked or if, say, half the milk be drawn off and a calf allowed to take the remainder, the milk so gathered may, and probably will, be seriously deficient in fat. The milk in the four quarters of the udder often shows quite important differences, but no regularity in this has been established; sometimes it is one quarter which is poorest and sometimes another.

It is common experience that evening milk is richer in fat than that drawn in the morning from the same animal. This is a regular phenomenon traceable to the interval between

MEAN MONTHLY VARIATIONS

	Fat. Per cent.	Solids-not-fat. Per cent.
January	3·63	8·84
February	3·65	8·84
March	3·56	8·80
April	3·56	8·80
May	3·60	8·82
June	3·54	8·80
July	3·62	8·75
August	3·68	8·74
September	3·75	8·75
October	3·84	8·85
November	3·88	8·90
December	3·78	8·91

the milkings. If the period were twelve hours in each case, the milk would be of similar composition, but in general the period from evening to morning is fourteen or fifteen hours and that from morning to evening only nine to ten hours. The percentage of fat is approximately in inverse proportion to the interval between the milkings, so nearly so that Collins (*Proc. Durham Phil. Soc.*, 1911, 1) worked out a formula $E - M = \frac{e - m}{4} - 0{\cdot}2$, where E and M are fat percentages in the evening and morning milk, and *e* and *m* are the times in hours between the evening and morning, and morning and evening milkings respectively. It has also to be recognised that the milk of individual cows may fluctuate much in quality and quantity from day to day, but these variations are largely eliminated in practice if the milk is from a mixed herd. Excitement, sexual or otherwise, worry by dogs or insects, distasteful food or physical discomfort will cause an adverse change in the quality or quantity of milk.

Milk Standards. Acting in exercise of the power conferred by Section 23 of the Sale of Food and Drugs Act, 1938, the Minister of Agriculture, in the Sale of Milk Regulations, 1939, prescribed that where a sample of milk (not being sold as skimmed, separated or condensed milk) contains less than 3 per cent. of milk fat it shall be presumed, until the contrary is proved, that the milk is not genuine by reason of the abstraction therefrom of milk fat, or the addition thereto of water. Also, it is prescribed that where a sample contains less than 8·5 per cent. of milk solids other than milk fat it shall be presumed, until the contrary is proved, that the milk is not genuine by reason of the abstraction therefrom of milk solids other than fat or the addition thereto of water. For skimmed or separated milk the Regulations prescribe a standard of not less than 8·7 per cent. of milk solids other than milk fat ; below this the skimmed or separated milk is presumed not to be genuine until the contrary is proved.

Section 24 of the Act (1938) prohibits the addition of colouring matter, water, dried or condensed milk, or any fluid reconstituted therefrom, to milk or skimmed milk. The addition of preservatives is prohibited by the Preservatives Regulations.

The Milk (Special Designations) Regulations, 1936 to 1941, defines three classes of milk which may be sold under licence. These are : (1) " Tuberculin Tested " ; (2) " Accredited " ; and (3) " Pasteurised." Tuberculin tested milk is the product of cows which have been subjected to the prescribed test ; it must satisfy the methylene blue reduction test (p. 237), and contain no coliform bacillus in 0·01 ml. If it is also pasteurised it must not contain more than 30,000 bacteria per ml. Accredited milk must also satisfy the methylene blue reduction test and contain no coliform bacillus in 0·01 ml. Pasteurised milk is milk which has been retained at a temperature of not less than 162° F. for at least fifteen seconds and immediately cooled to 55° F.[1] It must not contain more than 100,000 bacterial per ml.

Analysis of Milk. The preliminary examination of milk includes the determination of specific gravity, total solids, and fat ; other determinations frequently required are those of casein and albumin, lactose, acidity, citric acid, ash, tests for cleanliness, dyestuffs, preservatives, pasteurisation, and a bacteriological examination. Less common estimations which are sometimes desirable are those of alcohol, ammonia, aldehydes, lecithins, urea, volatile acids (acetic), fixed acidity, and an analysis of the ash.

The specific gravity should be taken at 15·5° by a pyknometer or Sprengel tube fitted with glass end-caps. A lactometer may be used when a number of samples have to be

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[1] The " holder " process is alternative, and requires not less than 145° nor more than 150° for thirty minutes.

examined ; the reading must be corrected for temperature. The results so obtained are not so accurate as those with the pyknometer. The temperature correction, between 50° F. and 70° F., is approximately − 0·11 for each 1° F. below 60° and + 0·11 for each degree above 60° F. Thus, if the specific gravity observed at 65° F. is 32·5° (*i.e.*, 1·0325), the true specific gravity at 60° F. will be 32·5° + (5 × 0·11) = 33·1° or 1·0331. If the temperature is much above or below 60° F., it is better to bring it to 60° F. rather than apply corrections, since these are only accurate over a small range.

A curious change in specific gravity, named, after its discoverer, Recknagel's phenomenon, may be remarked. The specific gravity of milk taken an hour or so after milking (when all air bubbles have disappeared) is lower than that observed subsequently. During the first twelve hours after milking there may be a rise of as much as 0·0013 in the observed specific gravity. Conflicting statements as to the reason for this change have been put forward.

For the determination of total solids,[1] weigh about 5 g. into a round flat-bottomed metal dish about 7 cm. diameter and provided with a closely fitting lid which is weighed with the dish. Then place the dish uncovered on a boiling water-bath for 30 minutes. Wipe the bottom of the dish and transfer it with the lid to a well-ventilated oven at 98–100° as recorded by a thermometer above the dish. After three hours in the oven cover the dish and transfer it to a desiccator using a separate desiccator for each dish. Cool for 30 minutes and weigh the dish with its lid. Return the dish and lid to the oven, heat for one hour with the dish uncovered. Then remove it to the desiccator, cool and weigh as before. Repeat the heating if necessary until the loss in weight between successive weighings does not exceed 0·5 mg. The total solids are approximately determinable by calculation or by means of a slide rule from the specific gravity and fat content, thus : T = 0·25G + 1·2F + 0·14, where G is the specific gravity in lactometer degrees. Richmond has devised a slide rule, obtainable from the usual dealers, by which this and most other milk calculations can be rapidly made ; for an exposition of this, see *Analyst*, 1920, **46**, 218.

It is convenient to determine the ash after weighing the total solids. It is essential that it be not heated to redness, or there

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[1] *Cf.* Report, *Analyst*, 1945, **70**, 105.

will be loss of chloride ; if the solids are well spread over the bottom of the dish the ash can be burned white over an Argand burner in quite a short time. According to Fleischmann the ash of normal milk is composed of :—

K_2O	24·5	per cent.
Na_2O	11·0	,,
CaO	22·5	,,
MgO	2·6	,,
Fe_2O_3	0·3	,,
P_2O_5	26·5	
SO_3	1·0	
Cl	15·6	

From the total of this falls to be deducted the oxygen equivalent to the chlorine.

It will be noted that there is no carbonate present ; more than a minute trace of such found in the ash suggests a preservative such as sodium benzoate or carbonate, which would obviously cause the percentage of ash to be high.

There are several methods available for a rapid and approximate estimation of fat which are suitable for the routine examination of samples. Such are the Gerber process, the sinacid and sal methods (*cf.* Golding, *Analyst*, 1911, **36,** 203), which avoid the use of sulphuric acid, and the Mojonnier method. Of these it will be sufficient to describe briefly the Gerber process. The others are dealt with at length in the catalogues of the various makers of the apparatus. Gerber's process is capable of a limit of accuracy of about ± 0·05 per cent. in careful hands and using butyrometers which have been accurately standardised. For a discussion of the sources of error, see Day and Grimes (*Analyst*, 1918, **43,** 123). When fresh sets of butyrometers are brought into use it is essential to check their calibration ; for this purpose it is simple to determine the fat gravimetrically in a sample of milk, then determine it in the new butyrometer tubes, and reject any which, after a duplicate test, fall outside the limits of ± 0·1 per cent. For a more elaborate method of testing the bottles, see Smith (*Analyst*, 1923, **48,** 477) and Day and Grimes (*loc. cit.*). For the estimation of the fat 10 ml. of 90 per cent. sulphuric acid (sp. gr. 1·820–1·825) are pipetted into the butyrometer tube, followed by 11 ml. of the milk, then by 1 ml. of amyl alcohol (sp. gr. 0·815). The tubes are now closed with the rubber corks, the contents are well mixed, then they are centrifuged at

1,000 revolutions per minute for five minutes. The tubes are now placed in water at 65° C. for a few minutes, and the percentage of fat is read off. Some tubes are made to operate on half the above quantities.

McQuerney and Troy (Abstract, *Analyst,* 1921, **46,** 50) show that modification of this process is needed in order to obtain accurate results on skimmed milk. When a large number of samples has to be dealt with it is convenient to have automatic measuring devices for delivering the acid and alcohol, such as those supplied by the makers of the Gerber apparatus.

For the more exact determination of fat, several good methods are available. Of these the most important are the Gottlieb method, the modified Werner-Schmidt method, and, because of its historic value as the official process adopted by the Society of Public Analysts in 1886, the Adams' method. The best method is the Gottlieb, but it requires careful adherence to the prescribed conditions. Ten ml. of the milk are pipetted into a tall narrow cylinder (the variety shown in the figure is convenient). 1·5 ml. of solution of ammonia (sp. gr. 0·880) are added, the mixture is shaken, then 10 ml. of alcohol (96 per cent.) are added, and the mixture is again shaken. Then 25 ml. of ordinary methylated ether are added, the tube is corked and shaken vigorously for a full minute, this is followed by 25 ml. of petroleum spirit, and the whole shaken for another minute. When the separation is complete the ethereal solution is run off by means of the side tap into a tared flask, and the residue is extracted again with the use of 12·5 ml. of ether, then 12·5 ml. of petroleum spirit as before. A third extraction is desirable ; this may conveniently be made with the mixed solvent recovered from previous operations. The mixed solvent is distilled off and the fat is weighed, then it is dissolved out by dry petroleum ether, and the weight of any residue left in the flask is deducted. This correction should not be neglected in any of the three methods.

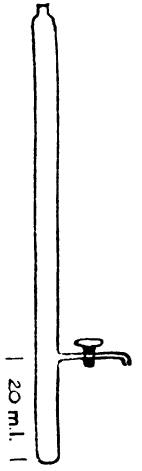

FIG. 39.

For the Werner-Schmidt method 10 ml. of milk are weighed or measured into a tube of capacity 50 ml., and 10 ml. of hydrochloric acid are added. The mixture is heated in boiling water until all casein is apparently dissolved—at this stage the mixture is of a violet or brown colour. Then the tube is cooled, 10 ml. of alcohol are added, the mixture shaken, then 15 ml. of ether and 15 ml. of petroleum spirit are poured in, the tube is corked and well shaken. When the liquids have separated the upper layer is blown off into a tared flask by means of a wash-bottle fitting in which the syphon tube is slightly turned up at the lower end to avoid disturbing the lower layer. The extraction is repeated three times and the mixed ether extracts are evaporated; the fat is weighed and re-extracted to allow for the weight of the trace of non-fatty residue which invariably appears with the fat. Important points are that the preliminary heating should not be carried too far or caramel will be formed which will be partly dissolved by the wet ether, and that the ether be allowed to separate completely so that it may be free from a watery turbidity. This method has the advantage of being readily applicable to curdled or sour milk.

In Adams' method (*cf. Analyst*, 1885, **10,** 46; 1886, **11,** 71) 5 ml. of the milk are spread in a film by means of a pipette on one side of a strip of special fat-free thick filter paper, 22 × 2½ inches; this is suspended by means of a pin, and when the milk is nearly dry the coil is rolled up and tied with cotton, then it is transferred to the water oven to dry; after this it is thoroughly extracted with dry ether or petroleum-spirit in a Soxhlet tube for at least four hours. The fat, after being weighed, should be dissolved out and the weight of any residue deducted. Adams' method is not readily applicable to badly soured milk or to homogenised milk; samples which are curdled may often be rendered sufficiently homogeneous by the addition of a few drops of ammonia (0·880).

It not infrequently happens that sour or altered milk has to be examined and its composition when fresh deducted. In such cases the method worked out by Thorpe (*J. Chem. Soc.*, 1905, **87,** 206) and criticised by Richmond and Miller (*Analyst*, 1906, **31,** 317) is adopted. The fat and solids-not-fat are determined in one operation known as the maceration method.

Before proceeding with the analysis of a sample of sour milk, the contents of the bottle are transferred to a suitable vessel and thoroughly mixed with a wire whisk. Portions of about 10 g. of the sample are weighed into flat-bottomed platinum basins which have been tared with a short glass rod having a flattened end. The weighed quantities are neutralised with 0·1 N strontia solution, phenolphthalein being used as indicator. The milk is evaporated on the water bath with constant stirring towards the end, until it attains the consistency of dry cheese. About 20 ml. of dry ether are poured over the solids, which are then carefully triturated with the glass rod. The ethereal solution of the fat is passed through a dried and tared filter and the maceration of the milk is continued with at least eight successive quantities of ether. At the conclusion of this process the solids should be in a fine state of division like precipitated chalk. Before becoming quite dry they are transferred as far as possible to the filter paper, washed free from fat and dried in the oven in a weighing bottle to constant weight, as is also the platinum basin with the remaining solids. From the total weight of non-fatty solids is deducted 0·00428 g. for each ml. of 0·1 N strontium hydroxide added. The weight of fat is obtained by evaporating the ethereal solution and drying in the usual way.

The following corrections, which are of course additive, must be applied to the non-fatty solids. The alcohol correction is obtained by distilling 50–100 g. of the milk, then neutralising the distillate, using litmus paper as indicator, and re-distilling it. The final distillate is made up to the original volume and its specific gravity is determined by means of a delicate pyknometer. The corresponding percentage by weight of alcohol multiplied by 90/46 gives the percentage of lactose which has disappeared in the production of the alcohol. Next is determined the volatile acid correction. Ten g. of the milk are neutralised to the extent of one-half of the total acidity with the use of 0·1 N sodium hydroxide and phenolphthalein. The mixture is evaporated to dryness on the water bath with frequent stirring, and after treatment with 20 ml. of boiling water so as thoroughly to break up and detach the solids from the basin, a further addition of alkali is made until complete neutrality is reached. The difference between the original acidity of the milk and that of the evaporated portion is regarded as the acetic acid; 60 parts of this acid correspond to a loss of 62 parts of lactose, so an addition must be made of 62/60 times the acetic acid found.

The other corrections may be made—for butyric acid and for ammonia; but these are very small, and may be omitted when the sum of the other two corrections is less than 0·2 per cent., as they are then not likely to exceed 0·05 per cent. For the estimation of butyric acid the volatile acids are separated from the quantity of milk which has been taken for the determination of the alcohol. A portion of the mixed aqueous acids is neutralised with barium hydroxide, evaporated and dried to constant weight. From the percentage of barium contained in the mixed salts the proportions of the two acids, acetic and butyric, are calculated. Each 88 parts of butyric acid corresponds to a loss of 92 parts of lactose ; the loss due to acetic acid has already been calculated.

To estimate the small quantity of ammonia formed, 2 g. of the milk are made up to a volume of 100 ml. with ammonia-free water and filtered through a washed paper. The ammonia in 10 ml. of the filtrate diluted to 50 ml. is estimated by Nessler's reagent in the usual way, a solution of ammonium chloride equivalent to 0·01 mg. per ml. being used as standard.

Methods for the determination of other constituents of normal milk include the following.

Acidity. This is estimated by direct titration of 20 ml. with 0·1 N sodium hydroxide, phenolphthalein being used as indicator. Several modes of expression are common, of which the most convenient (though not really accurate) is as lactic acid per cent. Another well-known method is as degrees of acidity, by which is understood the number of ml. of N sodium hydroxide required to neutralise 1 litre of the milk. A somewhat better end-point is obtainable by using 0·1 N strontia instead of soda.

Proteins. Although it is most convenient to estimate the nitrogen on, say, 10 g., and calculate the proteins therefrom by means of the factor 6·38, this method gives slightly high results on account of certain nitrogenous extractive matters present. The error so introduced is about 0·25 per cent., or less ; for ordinary purposes it may be neglected.

Almen's method is the most exact for the direct estimation of proteins. About 5 g. are diluted to 20 ml. with water, a few drops of magnesium sulphate solution are added, then an excess of a tannin solution. This is prepared by dissolving 4 g. of tannin in 190 ml. of 50 per cent. alcohol and adding 8 ml. of 25 per cent. acetic acid. After standing for some few hours the precipitate is filtered off and washed with ice-cold water. The nitrogen in the precipitate and filter is then determined by the Kjeldahl method ; an allowance is made for any nitrogen in the filter paper. The appropriate factor is 6·38, which is exact for casein and albumin.

If it be desired to estimate casein, albumin and globulin separately, the casein may be precipitated by saturating 10 ml.

of the milk with a paste of magnesium sulphate and adding 100 ml. of saturated solution of the salt ; the precipitate, after standing, is filtered off, washed with magnesium sulphate solution, and the nitrogen is determined on the precipitate. The albumin is precipitated by adding acetic acid and boiling the filtrate from the casein ; the precipitate is washed with acetic acid and alcohol, and the nitrogen estimated therein as usual. The difference between the total protein and the sum of casein and albumin may be taken as globulin.

Lactose may be estimated either gravimetrically, volumetrically or by the polarimeter. To 20–30 g. of the milk add about 50 ml. of water, then 5 ml. of zinc acetate solution (p. 77) and 5 ml. of potassium ferrocyanide, mixing gently after each addition ; dilute to 250 ml. and filter. The lactose in the filtrate is conveniently determined by titration with Fehling solution, using the Lane and Eynon process as described on p. 16. Calculate the lactose as anhydrous lactose.

For the polarimetric estimation the proteins must be removed, for which purpose mercuric nitrate is suitable, although Richmond (*Analyst*, 1910, **35,** 516) shows that this does not completely precipitate all proteins, and that phosphotungstic acid should also be added ; the error so introduced is negligible except in dried milk. To 100 ml. of milk are added 3 ml. of mercuric nitrate (mercury dissolved in twice its weight of concentrated nitric acid), the mixture is shaken, filtered and polarised in a 200 mm. tube at 20° ; then the reading in angular degrees $\times \dfrac{0{\cdot}95 \times (100 - 1{\cdot}057 \times \text{fat}\%)}{100 \times 105 \times \text{sp. gr.}}$ gives the percentage by weight of anhydrous milk sugar. For a discussion of the errors involved by the volume of fat and proteins, see Richmond and Boseley (*Analyst*, 1897, **22,** 98).

For the determination of citric acid in milk the pentabromacetone method is recommended by Lampitt and Rooke (*Analyst*, 1936, **61,** 654). To 150 g. of the milk heated to 50°–60° in a 250 ml. flask add 25 ml. of 2 per cent. potassium oxalate solution, then after shaking, 20 ml. of diluted sulphuric acid (1 : 1). After cooling add 10 ml. of phosphotungstic acid solution (15 per cent.), dilute to 250 ml. and allow to stand for the serum to separate. To 50 ml. of the serum add 5 ml. of 37·5 per cent. potassium bromide solution. Then add potassium permanganate solution (5 per cent.) from a burette until a slight brown precipitate persists after standing for an hour. Sufficient ferrous sulphate solution[1] is added to produce a pale yellow solution with a white precipitate ; then the mixture is set aside in an ice chest overnight. The precipitate is collected on a sintered glass crucible, washed with 10, 10, and 5 ml. of cold water and dried in a vacuum dessicator to constant weight, W. The weight of anhydrous citric acid is $0{\cdot}424\left(W + \frac{0{\cdot}005V}{100}\right)$ where V is the volume of the filtrate.

Adulteration of Milk. In addition to the more obvious forms of adulteration by adding water or removing fat, it is often necessary to consider the possible presence of preservatives, dyes, thickening substances, and condensed milk, and whether the milk has been heated or pasteurised, though this is not in the nature of adulteration. Tests may also be required for the amount of dirt and bacteriological contamination. In connection with the application of the official minimum standards of 3·0 per cent. of fat and 8·5 per cent. of non-fatty milk solids, it has to be borne in mind that they raise only a presumptive proof of the addition of water or abstraction of fat, which may be rebutted by evidence either internal or external. There is no doubt that sometimes the milk from an individual cow may fall below these minimum figures, but that of a mixed herd of cows such as is sold in most dairies seldom falls materially below these limits if unsophisticated. Information of value is given sometimes by a more detailed examination, for, when milk is watered, obviously the proportion of non-fatty solids is reduced, but the ratio of the various constituents is unaltered. When cows yield abnormal milk it is almost always the lactose which is deficient ; the protein and ash remain normal. Vieth, as the result of the examination of some thousands of samples, draws attention to the ratio of anhydrous lactose, proteins and ash, which is 13 : 9 : 2, and these figures are confirmed by the large experience of Richmond. A material divergence from this ratio would therefore suggest abnormality rather than adulteration. Vieth has also shown that the ash is almost invariably 8 per cent. of the solids-not-fat. A higher proportion of albumin than 0·6 per cent. in cows' milk is suggestive of the presence of colostrum or of disease in the animal.

Nitrates do not occur in genuine milk even if the cow has been

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[1] Twenty g. $FeSO_4 . 7H_2O$ in 100 ml. of 1 per cent. sulphuric acid.

dosed therewith, but most natural waters contain small quantities of nitrate; so the detection of nitrate affords confirmatory evidence of the addition of water. To detect nitrate add 6 drops of mercury reagent to 5 ml. of the milk, shake, filter through a filter paper well washed with distilled water, into a test tube containing 2 ml. of diphenyl-benzidine reagent,[1] so that the filtrate runs down the side of the tube and forms a layer on the diphenyl-benzidine solution. A distinct blue colour develops in the presence of nitrate. A genuine milk should be tested as a "blank" to avoid errors due to traces of nitrate from the laboratory or apparatus.

In general, the presence of added water in milk should not be certified merely on the basis of a deficiency in non-fatty solids; confirmation should always be obtained by other tests. The nitrate test described above is useful, but the best method yet available is the determination of freezing point by the Hortvet method. It has been established that the freezing point of genuine milk varies within only narrow limits, and if the Hortvet technique be closely adhered to, the usual limits are from − 0·530° to − 0·550°. Few instances of milk from individual cows falling outside this range have been recorded, but isolated cases showing a depression of 0·525° may be found. The value of the method lies in the fact that circumstances which affect the non-fatty solids of milk quite markedly, do not alter the osmotic pressure of the milk, and hence its freezing point.

It is important that the milk to which the freezing-point test is to be applied should be fresh and free from preservative. The acidity should not exceed 0·18 per cent. reckoned as lactic acid. When the acidity exceeds 0·18 per cent. but does not exceed 0·70 per cent. a correction may be applied.[2] (Δ = freezing point depression, Hortvet) : Δ = apparent Δ − 0·34 (lactic acid per cent. − 0·18). The standard Hortvet apparatus and technique should be closely followed; the standardisation of the thermometer scale may be done once for all, but the zero error must be determined every time the instrument is used. For a critical survey of the technique, see Elsdon and Stubbs, *Analyst*, 1934, **59,** 585, and other papers by the same authors.

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[1] The reagents are (1) 20 g. mercuric chloride, 5 g. ammonium chloride, and 20 ml. hydrochloric acid made up to 100 ml. with water. (2) Diphenyl-benzidine 0·085 g., water 50 ml. and sulphuric acid 450 ml.

[2] Report of sub-committee on the Freezing Point (Hortvet) test of Milk. Department of Health for Scotland. H.M. Stationery Office, 1945.

The Hortvet Apparatus and Method. The essential parts of Hortvet's cryoscope are as follows: A cylindrical Dewar flask of capacity 1 litre fitted with a cork through which passes a thin-walled metal tube 250 × 33 mm. external measurements. At one side of the cork is a metal inlet tube which passes into a perforated loop near the bottom of the flask and serves for the blowing in of air. On the other side is an outlet T piece which serves for the escape of the ether vapour. At the back of the cork is an ordinary thermometer of which the bulb reaches nearly to the bottom of the Dewar flask. Inside the thin-walled tube is the freezing tube, which should be 240 × 29 mm. in size. In the centre of the rubber stopper of this tube is the Hortvet thermometer; at one side is a small looped stirrer and at the other a metal tube with a small hole to carry a fragment of ice which serves as a starter for the freezing. At one side of the apparatus is a Folin drying tube containing sulphuric acid and at the other a drainage tube for carrying any vapours away from the operator. A small telescope is provided for reading the thermometer more accurately, and it is convenient to have a mechanical stirrer and blower. It is also convenient to connect the outlet to a pipe leading out of the window so as to remove all inflammable vapour from the laboratory.

The Hortvet thermometer has a solid stem 580–600 mm. in length, and a bulb 6·5–7 mm. × 55–65 mm. Its total scale is from + 1° to − 2°, each degree being 99–100 mm. in length and divided into tenths and hundredths. This thermometer should be standardised with water and against pure sucrose solutions, using the same procedure as when determining the freezing point of milks. Solutions of pure sucrose 7 g. in water made up to 100 ml. at 20°, and of 10 g. made up to 100 ml. have freezing point − 0·422° and − 0·621° respectively. The depression Δ is the difference between the observed freezing point of water and that of the sucrose solution. The zero point of the thermometer is likely to vary from day to day, or even a little during the same day. If the mean of two or more determinations of Δ obtained for the sucrose solutions differs materially from the figures given another thermometer should be sought; if the differences are but small a correction may be applied.

The procedure in making a freezing-point determination is as follows: Pour 400 ml. of cold ether[1] through the T piece into the Dewar flask—note the depth in the flask—close the inlet with a cork and start the blower so that air bubbles through the drying tube and through the ether at such a rate that the thermometer in the ether falls to 0° in about ten minutes, and continue till the temperature is about – 3°. Then gauge, by means of a glass tube through the T piece, the depth of the ether and add enough to restore the volume to 400 ml. Now pour into the freezing tube enough boiled cold water to cover the bulb of the Hortvet thermometer; this should be about 35 ml. Also pour a few ml. of alcohol into the space between the two test tubes to serve as conducting medium. Start the stirrer at a steady motion of about 30 strokes per minute and continue the blowing until the temperature in the cooling bath is – 2·5°. Continue the slow passage of air through the ether until the temperature on the Hortvet thermometer indicates a super-cooling of 1°–1·2°. At about this temperature the water begins to freeze and the temperature rises. Stop the stirrer and move it up and down three or four times as the mercury in the thermometer becomes stationary; it should remain stationary for about a minute. The thermometer is gently tapped and the temperature carefully read with a small telescope, taking precautions to avoid parallax, to the nearest 0·001°. Pour out the water, rinse the tube with the milk and put in 35 ml. of the cooled milk. Bring the level of the ether up to 400 ml. again and maintain the cooling bath 2·5° below the freezing point of the milk by blowing air through it as before. When the milk has reached about 1° super-cooling as shown on the Hortvet thermometer, with a penknife place a tiny fragment of ice in the hole of the starter rod and insert it quickly into the super-cooled milk. A rapid rise of the thermometer should occur; stop the stirrer and manipulate it three or four times as before, tap the thermometer and observe the maximum reading, which is the freezing point. The depression is given by the difference between the zero with water and the observed temperature. At least two determinations should be made, and these should not differ by more than 0·002° or 0·003° with the same worker and the same thermometer.

[1] The writer prefers to use petroleum spirit, 40°–60°, which is previously cooled in a refrigerator. The temperature does not fall so quickly as with ether, but there is no risk of peroxide formation, and this solvent is drier than ether usually is.

If an appeal-to-the-cow sample is available in respect of a suspect milk the added water present may be calculated with accuracy by means of the formula $\frac{T - T^1}{T} \times (100 - \text{t.s.})$, where T and T^1 are the depressions for the genuine and adulterated sample respectively and t.s. the total solids in the adulterated sample. When an appeal-to-the-cow is not available the simple formula $\frac{T - T^1}{T} \times 100$ suffices, and T may be taken as 0·53°.

Dirt in Milk. The determination of dirt in milk has been thoroughly studied by a committee of the Society of Public Analysts (for details, see *Analyst,* 1937, **62,** 287). This committee considered that a limit of 2 parts of moist dirt per 100,000 is a proper maximum, though legal action is not recommended in respect of an isolated sample showing 3 parts. The method for the determination of dirt consists, briefly, of adding formalin to the milk and allowing it to stand in a covered sedimentation vessel connected to a standardised centrifuge tube, also filled with the milk, for seventy-two hours. Then the lower end of the vessel is stoppered by a glass rod with a rubber cork, the centrifuge tube is removed and spun at 2,000 r.p.m. for five minutes. After pouring off the milk the separated dirt is washed by filling the tube with water and centrifuging again for three minutes, then washing it similarly with N ammonia solution and N hydrochloric acid. The volume of moist dirt is read off in the calibrated tube. For details of the calibration and size of the tubes, see the report above quoted. A microscopical examination of the dirt can be made before the ammonia wash, and the part removed returned to the tube before the final washes and measurement.

Two tests have been adopted for official purposes, the methylene blue test and the phosphatase test. The former is prescribed for accredited milk and the latter indicates whether or not the pasteurisation has been efficiently performed. The official techniques are described in detail in Memo 139/Foods/1937 and addendum 1943. The descriptions given below are abbreviated.

A rapid test much used as in indicator of bacterial condition is by means of resazurin, a blue oxidation-reduction dye which is reduced to pink by bacteria of low reducing power and even to a colourless leuco compound by more active bacteria. Ten ml. of the milk are introduced into a sterile test tube, 1·0 ml. of 0·0005 per cent. resazurin solution is added and the closed tube incubated at 37° for exactly one hour. Complete or rapid reduction indicates severe infection and possibly mastitis ; reduction to pink or a lilac colour is indicative of unsatisfactory bacterial condition (*cf.* J. G. Davis, *Analyst*, 1939, **64,** 607).

The Methylene Blue Test. The decolorisation of methylene blue is brought about by the action of bacteria and reducing substances present in raw milk. As these substances are associated with the fat it is important that the fat should be evenly distributed throughout the test ; also strict precautions against accidental contamination are essential. The stoppers of the bottles and test tubes should be flamed as usual in bacteriological tests.

Pour 10 ml. of the mixed milk into a sterile test tube, add 1 ml. of the standard methylene blue solution, and as controls, pour into other tubes 10 ml. of a good mixed milk + 1 ml. of tap water and 10 ml. of mixed milk with 1 ml. of the methylene blue solution. Close the tubes with sterile rubber stoppers and mix the contents by inversion. Place the test sample in a water bath at 37°–38° at once and heat the two controls in boiling water for three minutes, then cool them and put them in the water bath with the test sample. Inspect the tubes at half-hourly intervals and mix their contents by inversion. The milk is regarded as decolorised when it is completely decolorised up to within 5 mm. of the surface. The two controls serve to show when decolorisation is beginning and when it is complete. Tuberculin tested or accredited milk must not decolorise the methylene blue within four and a half hours if sampled between May 1st and October 31st, or within five and a half hours if sampled between November 1st and April 30th. The methylene blue solution is prepared by dissolving one standard tablet in 200 ml. of sterile glass-distilled water and diluting to 800 ml. ; it should be freshly prepared.

Pasteurised Milk. The Phosphastase Test. Pasteurisation is defined officially as on p. 224. Of the many methods which have been described for determining whether milk has or has not been pasteurised, the phosphatase test of Kay and Graham is probably the best. This test, which is described fully in *J. Dairy Research*, 1935, **VI,** 191, depends upon whether or not the enzyme phosphatase which occurs in raw milk has been destroyed. The milk is added to an excess of a phenylphosphoric ester then, after incubation, test is made for free phenol. The buffer substrate [1] and Folin and Ciocalteu's reagent are obtainable from the usual dealers, and the test is applied as follows :—

To 10 ml. of substrate in each of two test tubes (20 ml.) marked at 10 ml. add 0·5 ml. of the milk, then 3 drops of chloroform, mix, stopper the tubes and incubate them at 37° for twenty-four hours. At the end of that time, cool, add to each 4·5 ml. of F. and C. reagent. Mix, stand for three minutes and filter. To 10 ml. of filtrate add 2 ml. of sodium carbonate solution (14 per cent. w/v), mix, place the tubes in boiling water for exactly two minutes. Cool and read the colour in Lovibond tintometer, using a 13 mm. cell. A milk giving a blue colour reading of 2·3 units or less is said to give a negative test, and has been sufficiently heat treated ; milk showing 2·4 to 6 blue units has been insufficiently heated, and milk showing over 6 units has been grossly underheated. After putting on the tests the milk samples should be kept in the refrigerator, then when the test is completed, controls are put down for those samples which show a positive test. This is done by adding 4·5 ml. of reagent to 10 ml. of substrate, then 0·5 ml. of milk, allowing to stand for three minutes and filtering. Then add sodium carbonate solution and heat in boiling water exactly as before. Cool and read the colour ; it should not exceed 1·5 blue units. (If this colour is exceeded it usually means accidental contamination or faulty technique.)

Preservatives. The commonest are boric acid and formaldehyde, but benzoic acid, fluorides, salicylates, β-naphthol, sodium carbonate and glycerine may be present. For the detection of boric acid or borax, make about 5 ml. of the milk just alkaline with lime water, evaporate and ignite the residue, acidify the ash with hydrochloric acid, and dip into the liquid a piece of turmeric paper, then dry it on the water bath. In the presence of boric acid the paper turns pink or red according to the amount present,

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[1] Buffer-substrate is made by dissolving 1·09 g. of disodium phenyl phosphate and 11·54 g. of sodium diethyl-barbiturate in 1 litre of chloroform water. Folin and Ciocalteu's reagent is prepared by dissolving 100 g. of sodium tungstate ($Na_2WO_2 . 2H_2O$) and 25 g. of sodium molybdate in 700 ml. of water, add 50 ml. of phosphoric acid (85 per cent.) and 100 ml. of hydrochloric acid. Reflux the mixture gently for ten hours, using glass jointed apparatus ; cool, add 15 g. of lithium sulphate, 50 ml. of water and 4 to 6 drops of bromine. Boil off the excess of bromine, cool and make up to 1 litre with water. For use take 1 volume of the F. and C. reagent and add 2 volumes of 5 per cent. sodium hexameta-phosphate solution. The reagents are conveniently obtainable in tablet form.

and on touching the coloured paper with a drop of sodium hydroxide solution the colour changes to green ; this part of the test should not be neglected. For the estimation of boric acid Richardson and Walton's process is quick and accurate. To 25 ml. of the milk are added 2·5 ml. of 5 per cent. solution of copper sulphate, the mixture is well stirred, then heated to the boiling point and filtered ; the precipitate is washed four or five times with small quantities of boiling water. To the cold filtrate is added 1 ml. of 1 per cent. phenolphthalein solution and 0·1 N sodium hydroxide until a blue shade appears ; then there is added 15 ml. of neutralised glycerine and the liquid is titrated with 0·1 N alkali until the characteristic blue shade again appears ; under these conditions 1 ml. 0·1 N = 0·0071 g. H_3BO_3.

An elaboration of Thomson's method in use at the Government Laboratory (*cf. Analyst*, 1923, **48,** 416), though exact, is tedious.

Of the many simple tests available for the detection of formaldehyde that of Hehner remains the best. Add to 5 ml. of the milk in a test tube 1 drop of dilute ferric chloride solution and 10 ml. of water, then pour down the side of the tube 5 ml. of sulphuric acid ; a violet-blue coloration appears at the junction of the two liquids in the presence of formaldehyde. If a few drops of ferric chloride solution be added to the sulphuric acid, this test is readily combined with Gerber's estimation of fat ; a violet ring appears at the junction of the acid and milk in the butyrometer when formalin is present. Schryver's test described on p. 203 may be used for confirmation. Hehner's test fails if a large amount of formalin is present.

The delicate colorimetric method of Shrewsbury and Knapp serves either for quantitative or qualitative purposes ; it must be remembered that small quantities of formalin rapidly disappear from milk, so that the amount found is usually less than that added. To 5 ml. of the milk are added 10 ml. of hydrochloric acid containing 0·1 ml. per cent. of nitric acid (the reagent should be freshly prepared) and the mixture is heated for ten minutes in a water bath at 50°. The purple colour produced is approximately proportional to the amount of formaldehyde actually present, hence comparison may be made with milk to which known volumes of formalin have been added.

Years ago a preservative appeared on the market which did not readily give the ordinary formalin test ; it consisted of formalin mixed with sodium nitrite ; milk should therefore be tested for nitrites, which may readily be done by means of the Griess-Ilosvay reagents (p. 64).

Benzoic acid may be detected and estimated in milk or in cream by the distillation process as described on p. 104, or more simply detected by the method devised by Hinks. Twenty-five ml. of the milk or 10–20 g. of cream are heated with an equal volume of hydrochloric acid as in the Werner-Schmidt method of estimating fat, until the curd is completely dissolved. The cooled mixture is shaken with an equal volume of mixed ether and petroleum ether (1 : 2), the ethereal solution is separated, and 1 drop of (0·880) ammonia is added; in the presence of benzoic acid there is at once produced a turbidity or even a crystalline precipitate which is quite characteristic. In order to confirm this in cases of doubt, 5 ml. of water are added, the mixture is shaken, and separated; the aqueous solution is heated on the water bath for a few minutes to expel excess of ammonia, and is then tested for benzoic acid with ferric chloride, which yields a buff-coloured precipitate. This should be carefully distinguished from ferric hydroxide which might be caused by an excess of ammonia.

Salicylic acid may be similarly detected, and for the estimation the amount in the residue may be determined colorimetrically with ferric chloride.

Hydrogen peroxide is, like formalin, a preservative which tends rapidly to disappear from milk. It may be readily detected by means of benzidine. Add to 10 ml. of the milk 0·5 ml. of 5 per cent. solution of benzidine acetate and a few drops of acetic acid; a deep blue colour indicates peroxide. This test is not successful if the milk has been first pasteurised and the enzymes thereby destroyed; if this is suspected add some fresh milk and again apply the test. Peroxide may be estimated by precipitating the casein from 50 ml. of milk with dilute sulphuric acid (0·5 ml.) and adding excess of potassium iodide to the clear whey; the liberated iodine is titrated with 0·01 N sodium thiosulphate after three hours standing in a cool dark place.

Sodium carbonate, if present, will be found in the ash, which in genuine milk contains only at most a mere trace of carbonate.

β-naphthol as a preservative is rare; it may, however, be separated by extraction with ether in acid solution, then extracting the acid ether with alkali and re-extracting this with ether after acidification exactly as described for benzoic acid. The residue may be identified as β-naphthol by the reaction given on p. 218, or by the diazo test, in the ordinary way.

Fluorides also are rarely found: they may be detected in the ash. Fluoborates give the reactions both of fluorides and borates.

Glycerine is an unusual but possible adulterant of milk. If present in quantity it will be apparent in the total solids, which will give off fumes in the oven, and of course the sum of ash, protein, fat, lactose and water will be less than 100. It may be estimated by evaporating nearly to dryness at a temperature of about 60° and extracting the residue with ether, then shaking with a small volume of water and evaporating the aqueous solution of glycerine in a vacuum desiccator over sulphuric acid at ordinary temperature; a boiling water bath should not be used. The identity of the glycerine should be confirmed by the usual reactions.

Starch has rarely been found in milk, added, doubtless, for thickening purposes; it may readily be detected by the iodine test. Dried milk powder has occasionally been found. When present, there is usually a small residue undissolved which may be detected by aid of the centrifuge, but there is no infallible test for it.

Gelatin used to be fairly common as an adulterant of cream, but rarely of milk. For its detection 50 ml. of sample are curdled by the addition of 3 ml. of mercuric nitrate solution as described in the polarimetric estimation of lactose; the filtrate is concentrated to about 20 ml., then cooled and 10 ml. of saturated picric acid solution are added; this produces a precipitate with gelatin which appears at once in the presence of a large quantity, but only after some hours if the quantity present is very small.

Artificial colouring matters are prohibited in milk. It should be remembered that the eating of certain plants by the cow affects the colour of milk to a small extent, *e.g.*, saffron and rhubarb. Also, added colouring matters may be reduced or otherwise destroyed by the action of bacteria, so that any tests for added dyes must be made while the milk is fresh. If the milk gives a pink colour on the addition of acid an azo-dye is indicated. Annatto is detected by making the milk alkaline with sodium carbonate and soaking a piece of cotton wool therein; after some hours the wool is stained brown in the presence of annatto and turns pink on the addition of a drop of stannous chloride. Annatto may also be detected in the separated fat extracted by the Werner-Schmidt process by shaking it with dilute soda solution and filtering, then washing the filter paper under the tap, drying it in the water oven and touching the paper with a drop of stannous *chloride solution ; a characteristic pink colour is given in the presence of annatto. To detect caramel, coagulate 10 ml. by* warming with a drop of acetic acid, separate the curd and cover it in a basin with hydrochloric acid. A blue colour slowly develops ; comparison should be made with known samples.

Bacteriological Tests. In connection with bacteriological standards prescribed for graded milks (p. 224) the following technique has been laid down.[1] Morning milk is to be kept until 6 p.m., and evening milk until 10 a.m. if unbottled, or may be kept for two hours if bottled, before testing. Thereafter if it is not convenient to begin the test at once the samples may be kept at 32°–40° F., but not for more than eighteen hours. The medium for plates contains : yeastrel, 3 g. ; peptone (B.D.H. or equivalent), 5 g. ; agar, 15 g. ; milk, 10 ml. ; water, 1,000 ml., and has pH 7·2. The dilutions required are $\frac{1}{10}$, $\frac{1}{100}$ and $\frac{1}{1000}$ ml., made from (*a*) 90 ml. water (or $\frac{1}{4}$ strength Ringer[2] solution) + 10 ml. milk ; (*b*) 90 ml. of water + 10 ml. of (*a*) ; (*c*) 90 ml. of water + 10 ml. of (*b*).

Three straight-sided, not bulbed, pipettes are required for each sample, one for each dilution. In making the dilutions the sample and each dilution bottle should be shaken twenty-five times with an up-and-down motion of about 1 foot. To the required quantity (1 ml.) of the diluted milk in a sterile tube are added 10 ml. of melted agar cooled to 45°, the mixture is poured into a standard No. 611 petri dish and incubated for forty-eight hours at 37°.

If among the dilutions there are plates containing from 30 to 500 colonies, these should all be counted and the number, multiplied by the dilution, reported as the final count. No plate containing less than 30 colonies is to be counted unless it was made with a $\frac{1}{10}$ dilution.

B. Coli Tests. Three tubes each containing 5 ml. of McConkey broth and a Durham's fermentation tube are inoculated with

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[1] For details see Memo. 139, Foods, January, 1937, issued by the Ministry of Health.

[2] Ringer's solution contains in 1 litre NaCl 4·25 g., Kcl 0·11 g., $CaCl_2$ 0.12 g., $NaHCO_3$ 0·05 g.

1 ml. of $\frac{1}{100}$ dilution of the sample, and incubated at 37°. The milk is satisfactory if two of the three tubes are free from acid and gas at the end of forty-eight hours. McConkey broth is prepared by dissolving 10 g. of lactose, 5 g. each of sodium chloride and sodium taurocholate, and 20 g. of peptone in 1 litre of water, by heating. The liquid is filtered, adjusted to P_H 7·4, and 10 ml. of 1 per cent. solution of neutral red added.

Cream

Although cream has been the subject of various laws and regulations, it has not been defined ; hence there is a wide variation in the composition of the cream sold to the public. It is well understood that it consists of the layer which rises to the top when milk is allowed to stand, but the amount of fat which is, of course, the leading constituent, varies from, say, 9 per cent., which is not unknown for the whole milk of a Jersey cow, to 65 per cent., or even more. It is important to note that in genuine cream, or cream from genuine cows' milk, the ratio of solids-not-fat to water is exactly the same as that in the milk from which it was prepared. It may be a trifle higher in the cream on account of evaporation during the rising period in the cream pan, but it should not be lower. From this it follows that the ratio, ash : protein : lactose should be, and is, the same in cream, milk and separated milk. Cream from pasteurised milk, Devonshire or Cornish clotted creams, have a higher ratio of solids-not-fat to water by reason of evaporation during heating. The ratio is variable, but in Devonshire and Cornish cream is about 1 : 5.

To decide, therefore, whether a cream has been watered or has been prepared from watered milk, the solids-not-fat as estimated may be compared with that calculated from the formula : S.N.F. $= \frac{W}{10}$; if the solids-not-fat are substantially lower than that calculated, it indicates watering. The lactose, protein, and ash should be $\frac{13}{24}$ths, $\frac{9}{24}$ths and $\frac{2}{24}$ths respectively of the solids-not-fat ; any divergence is suggestive of an adulterant and should therefore be the subject of further investigation.

Richmond gives the following table for the composition of cream showing the relation between the total solids, fat, and ash :—

Total solids. Per cent.	Solids-not-fat. Per cent.	Fat. Per cent.	Ash. Per cent.
32·50	6·83	25·67	0·57
37·59	6·14	31·45	0·52
50·92	5·02	45·90	0·42
55·05	4·65	50·40	0·38
55·18	4·77	50·41	0·39
55·97	4·47	51·50	0·38
56·37	4·40	51·97	0·38
57·99	4·17	53·82	0·41
68·18	3·30	64·88	0·28

Although the best dairy cream usually contains upwards of 50 per cent. of fat, there is now a large sale of cream containing about 23 to 25 per cent. of fat under such names as coffee cream or half-price cream. Also tinned cream is widely sold with a content of about 25 per cent. of fat. It is said that it is not practicable to tin cream containing higher percentages of fat because of separation of the fat during the sterilisation process.

The addition of preservative, colouring matter or thickeners to cream is forbidden.

Starch, gelatin or other thickening substances or preservatives may be detected by the tests mentioned for milk. Cane sugar alone, or in conjunction with lime, should be specially looked for, as it is sometimes added under the name of " viscogen."

It occasionally happens that cream is enriched by the addition of a foreign fat such as soya bean oil. For the investigation in such cases, a quantity of the fat must be separated, say by the Werner-Schmidt method ; it can then be examined by the methods given under butter fat.

Artificial Cream

The Artificial Cream Act, 1929, defines artificial cream as an article of food resembling cream and containing no ingredient which is not derived from milk except water. Such a substance is more appropriately described as reconstituted cream. Unfortunately no really satisfactory method exists whereby a well-emulsified reconstituted cream can be definitely differentiated from genuine cream. Determination of the ash, casein and fat may disclose an abnormality in their ratios if the reconstitution has been in incorrect proportions. The method proposed by F. W. Richardson (*Analyst*, 1933, **58,** 686) works well sometimes, but not if the cream is turning sour, or with reconstituted cream which has been thoroughly emulsified. About 5 ml. of the cream are well shaken in a centrifuge tube with an equal volume of acetone, then centrifuged—about 100 spins ; natural cream does not separate but reconstituted cream usually separates into two or more layers. A specimen of fresh cream should be tested side by side with the suspect sample. Artificial cream made from fat other than milk fat may be recognised by the Reichert value and other properties of the fat separated by the Gottlieb or Werner-Schmidt methods.

Condensed and Dried Milk

Condensed milk is cows' milk, skimmed or full cream, from which a large proportion of water has been evaporated and to which cane sugar may have been added. There are three varieties of unsweetened condensed milk, known to the trade as evaporated milk, bulk condensed milk, and concentrated milk, referring to the degree of concentration. In this country, however, the composition—hence concentration—is limited by the Condensed Milk Regulations, 1923, the labelling schedule of which requires the tins to be labelled in accordance with one of the four schemes given below :—

1.

CONDENSED FULL-CREAM MILK.
UNSWEETENED

This tin contains the equivalent of (*a*) pints of milk.

2.

CONDENSED FULL-CREAM MILK
SWEETENED.

This tin contains the equivalent of (*a*) pints of milk with sugar added.

3.

CONDENSED MACHINE - SKIMMED MILK (OR CONDENSED SKIMMED MILK), UNSWEETENED.

Unfit for babies. This tin contains the equivalent of (*a*) pints of skimmed milk.

4.

CONDENSED MACHINE - SKIMMED MILK (OR CONDENSED SKIMMED MILK), SWEETENED.

Unfit for babies. This tin contains the equivalent of (*a*) pints of skimmed milk with sugar added.

The insertion (*a*) is the appropriate number of pints with fractions expressed as eights, quarters, or half.

For the purposes of the Regulations milk means milk containing not less than 12·4 per cent. of milk solids (including not less than 3·6 per cent. of milk fat) and skimmed milk—means milk which contains not less than 9 per cent. of milk solids other than milk fat.

All condensed milk shall contain not less than :—

	Milk fat per cent.	Milk solids per cent. (including fat).
1. Full cream (unsweetened) .	9·0	31·0
2. Full cream (sweetened). .	9·0	31·0
3. Skimmed (unsweetened) .	—	20·0
4. Skimmed (sweetened) . .	—	26·0

Unsweetened condensed milk is always sterilised during manufacture, after canning, and though usually not absolutely sterile, depends for its keeping quality on the sterilisation ; hence it soon goes bad after the tin has been opened. Sweetened condensed milk is not sterilised. It contains upwards of 40 per cent. of sucrose which acts as a preservative. It has been stated that glucose is sometimes substituted for cane sugar, but this is quite unlikely, as glucose tends to the growth of yeast and moulds, and to the development of acidity, which is disastrous to the manufacturer. The presence of more than 45 per cent. of added sugar tends to lumpiness or grittiness, and less than 40 per cent. does not suffice to keep the milk. Since acidity of the milk is one of the principal difficulties the manufacturer has to overcome, as any excess leads to curdling or attack on the tin, it is a fairly common practice to add a small quantity of ammonia or sodium bicarbonate to the milk before condensing. Unless a considerable quantity, such as 0·08 per cent. has been added, which is unlikely, it will be difficult or even impossible to detect this with certainty in the finished product. In order to retard the separation of fat in unsweetened milk, it is usual to homogenise it by forcing it under pressure through a narrow orifice and causing the jet so formed to impinge on to a steel plate ; in spite of this treatment, however, there is a tendency, both in sweetened and unsweetened milk, for the fat to rise and for crystals of " milk salts " to settle on the bottom. For this reason it is essential carefully to mix by stirring all samples of condensed milk before removal from the tin. For the extreme variations possible in tins of milk which have been standing for a long time, see Bäcke (*Analyst*, 1911, **36,** 138). Obviously the ratio of the components of condensed milk, apart from the addition of sugar and the subtraction of water, will correspond to that of fresh milk, but as it has been concentrated 2·5 : 1 or 3 : 1 the lesser constituents become more important, and still more so in dried milk. Thus the acidity of fresh milk is only 0·15 per cent. lactic acid or thereabouts, but in condensed milk it may amount to 0·45 per cent. Citric acid or citrate, too, may be present in a like amount ; hence the sum of ash, casein and albumin, fat and lactose does not quite represent the total milk solids, and will not quite agree with the difference between the total solids determined and added sugar ; the difference is usually about 0·5 per cent., which represents the acidity and citrate present.

A point arises in connection with milk sugar ; this undoubtedly exists in condensed milk in the hydrated form, and as such often crystallises out, giving the milk a rough texture. Lactose hydrate loses its water only at 130°, but on evaporating a solution thereof at 100° the anhydrous form remains. The question therefore arises whether or not total milk solids should include the water of hydration of lactose : in view of the facts, in the writer's opinion it should not, but it is interesting to note that the difference which amounts in ordinary condensed milk to 0·4 to 0·5 per cent., just corresponds to the citric acid and other constituents not usually determined ; hence, by calculating the lactose as hydrated, a close approximation to the true total milk solids is obtainable without the rather tedious estimation of citric acid.

The following table shows the results of some analyses of well-known brands of condensed milk made since the introduction of the 1923 Regulations :

	Sweetened milk.				Unsweetened milk.		
	Full cream.		Skimmed.		Full cream.		Skimmed.
	1.	2.	3.	4.	5.	6.	7.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water . .	25·05	25·14	26·62	27·03	66·18	67·47	76·62
Milk solids .	33·39	32·94	27·21	26·38	33·82	32·53	23·38
Fat . .	10·64	9·60	0·22	0·29	9·28	9·10	0·75
Lactose[1]. .	12·25	13·07	14·93	14·25	13·33	12·74	12·50
Protein . .	8·57	8·41	9·61	9·53	9·16	8·75	8·35
Sucrose . .	41·56	41·92	46·17	46·59	—	—	—
Ash . .	1·93	1·86	2·45	2·31	2·05	1·94	1·78
Acidity as lactic acid, % .	0·32	0·28	0·35	0·30	0·35	0·40	0·41
Specific gravity	1·305	—	—	—	1·089	1·085	—

[1] Hydrated lactose.

Methods of Analysis. The analysis of condensed milk presents certain difficulties, and on account of the concentration care is needed in order to obtain really accurate results. For a full discussion of these reference may be made to the Reports of a committee of the Society of Public Analysts in the *Analyst*, 1927, **52,** 402 ; 1930, **55,** 111 ; 1932, **57,** 630.

The total solids should, in the case of sweetened milks, be dried on ignited sand, and even for unsweetened milk this is preferable. For the estimation of fat Gottlieb's method is undoubtedly the best. Weigh the unopened tin, then shake, open the tin, thoroughly mix the contents and pour into a wide-mouth stoppered bottle ; wash, dry and weigh the empty tin so that the equivalent of standard milk may subsequently be calculated. It is convenient to do all the weighing-up first : 3 to 3·5 g. into a Gottlieb tube for the fat, then 40 g. for the estimation of sucrose polarimetrically, and, if the lactose is also to be estimated volumetrically, 10 to 12 g. for this purpose. For the total solids, ash and nitrogen determinations, separate portions of about 2, 5 and 3 g. respectively may be weighed out. For the total solids a platinum basin is tared with about 20 g. of ignited sand and a glass rod, the weighed portion of the sample is put in a cleared place on one side of the dish, about 5 ml. of water are added and the diluted milk is mixed thoroughly with the sand. The water is driven off on the steam bath, then the solids are stirred and dried in the oven at 100° (not 95°) for four hours, weighed and re-weighed after a further hour. The nitrogen portion is diluted with a little water in a Kjeldahl digestion flask, treated with sulphuric acid, potassium sulphate and a trace of copper sulphate in the usual way. N × 6·38 = casein.

For the fat estimation the quantity weighed should not exceed 3·5 g. in the case of full cream milks. To this quantity in the Gottlieb tube is added 10 ml. of water and 1·5 ml. of 0·880 ammonia, and the whole carefully mixed. Then add 10 ml. of alcohol and mix again ; the subsequent procedure is the same as described for fresh milk on p. 227.

Many methods are available for the estimation of the sugars. Lactose can be determined volumetrically, gravimetrically, or by the polarimeter ; or the sucrose can be determined and lactose taken by difference. The writer prefers to determine the lactose separately by the volumetric process, partly because any abnormality is thereby more easily detected. Sucrose can then be taken by difference, or if desired, be determined by means of the polarimeter.

For the estimation of lactose weigh 10 to 12 g. into a 250 ml. flask, dilute with 200 ml. of hot water and allow the diluted liquid to stand for at least half an hour to ensure complete solution. Cool, add 4 ml. of zinc acetate solution, mix gently, add 4 ml. of potassium ferrocyanide solution ; both reagents are of the strength given on p. 77. Dilute the mixture to 250 ml., filter through a dry paper and titrate the filtrate against 25 ml. of Fehling solution by the Lane and Eynon method. The result may be calculated as hydrated lactose ; it should be remembered that the volume of the precipitate which should be deducted from the total 250 ml. is : $\frac{W}{100}(1 \cdot 08\ F + 1 \cdot 55\ P)$. W is the weight taken, F and P are the percentages of fat and protein respectively. This volume correction corresponds to about 0·1 per cent. of lactose.

For the determination of sucrose, weigh about 40 g. (W) of the well-mixed sample into a 200 ml. flask, add 100 ml. of warm water, mix, cool ; add 5 ml. of dilute ammonia solution (1 : 10) and allow the mixture to stand for fifteen minutes. Neutralise with acetic acid and add 12·5 ml. of the zinc acetate solution, mix and add 12·5 ml. of the potassium ferrocyanide solution. Dilute the mixture to 200 ml. at 20°, mix and filter through a dry paper. Determine the rotation (D) of the filtrate at 20°. Then pipette 40 ml. of filtrate into a 50 ml. flask, add 6 ml. of 6·34 N hydrochloric acid. Immerse the flask in water at 60° for twelve minutes with occasional shaking. Cool, dilute to 50 ml. at 20°, allow the solution to stand for an hour and observe the rotation (I) at 20°. The percentage of sucrose is :—

$$\frac{D - \frac{5}{4} I}{Q} \times \frac{V - v}{V} \times \frac{V}{l \times W}.$$

Q is the inversion division factor which is 0·8825 for the sodium lamp, or 1·0392 for the mercury green line. V is the volume before filtration, *i.e.*, 200 ml., v is the volume of the precipitate calculated as shown under lactose ; l is the length in decimeters of the polarimeter tube.

For methods applicable to condensed milks in which hydrolysis or other change in the sugars has taken place, reference should be made to the Reports of the Committee already mentioned.

As already indicated, the difference between 100 and the sum of the constituents is usually about 0·5 to 0·6 per cent., which is largely due to citric and lactic acids and their salts. To determine the citric acid, about 30 g. of the milk are diluted with water and treated by the process quoted on p. 231. Acidity, as lactic acid, may be titrated on a weighed quantity, phenolphthalein being used as indicator ; the amount of water added to dilute the milk should be such as to bring the liquid to the strength of fresh milk, that is, the amount of water should be about twice the weight of milk taken ; if a larger volume of water be used the end-point is affected. A milk which is just pink with phenolphthalein becomes quite strongly coloured on the addition of an equal volume of water.

Unsweetened condensed milk is specially liable to be contaminated with tin by action on the container. If this shows any signs of etching, estimation of dissolved tin may be made in the manner described on p. 107.

Calculation of the Equivalent Pints. The Condensed Milk Regulations require that the equivalent in pints of whole milk or skimmed milk shall be declared on the label. This involves the knowledge of the specific gravities of standard whole milk having 3·6 per cent. of fat and 12·4 per cent. of total solids, and of skimmed milk having 9 per cent. of non-fatty solids ; these would be 1·032 and 1·0355 respectively. The calculation may be simplified to the following on the basis of milk fat and total milk solids :—

$$\text{(1) Equivalent pints on T.M.S.} = \frac{\text{T.M.S. per cent.} \times \text{nett weight}}{7255}.$$

$$\text{(2) on fat} = \frac{\text{Fat per cent.} \times \text{nett weight}}{2106}.$$

As the Regulations prescribe not less than 12·4 per cent. of total milk solids with 3·6 per cent. of fat, it is necessary to calculate on each basis and take the lower figure. In the case of skimmed milk only one formula is required, namely :—

$$\text{(3) Equivalent pints} = \frac{\text{S.N.F. per cent.} \times \text{nett weight}}{5284}.$$

These may be further simplified to :—

(1) $\log \text{E.P.}^1 = \log \text{T.M.S.} + \log \text{N.W.} + 0{\cdot}44039.$
(2) $\log \text{E.P.} = \log \text{F.} + \log \text{N.W.} + 0{\cdot}97757.$
(3) $\log \text{E.P.} = \log \text{S.N.F.} + \log \text{N.W.} + 0{\cdot}57807.$

The tables on pp. 252, 253 and 254, covering the usual range for condensed milk tins, will enable the equivalent in ounces to be read off at once when the composition is known.

Dried Milk

Dried milk has, like condensed milk, been the subject of Regulations. The Public Health (Dried Milk) Regulations 1923 and 1927, prescribe for the labelling and description of dried milk and milk powder. The Regulations contemplate four classes of dried milk : (1) dried full-cream milk ; (2) dried three-quarter cream milk ; (3) dried half-cream milk ; and (4) dried quarter-cream milk. They apply to dried milk to which no other substance has been added and to the dried milk contained in any powder or solid of which not less than 70 per cent. consists of dried milk. It is laid down that such milk must contain the following percentages of milk fat :—

Dried full-cream milk . . .	26	per cent.
Dried three-quarter-cream milk .	20	,,
Dried half-cream milk . . .	14	,,
Dried quarter-cream milk . .	8	,,

The labels on the tins must state the equivalent of full cream, three-quarter-cream, half-cream, quarter-cream, or skimmed milk contained in the tin, and for the purpose of the Rules these terms are to mean milk containing not less than the following percentages of milk fat and milk solids :—

—	Milk fat.	Milk solids (including fat).
Milk	3·6	12·4
Three-quarter-cream milk . .	2·7	11·6
Half-cream milk . . .	1·8	10·8
Quarter-cream milk . . .	0·9	9·9

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[1] In ounces.

EQUIVALENT OF STANDARD WHOLE MILK IN OUNCES.

Total milk solids per cent.	Net weight grams.																Total milk solids per cent.
	350	355	360	365	370	375	380	385	390	395	400	405	410	415	420	425	
31·0	29·9	30·3	30·7	31·1	31·5	32·0	32·4	32·9	33·3	33·7	34·1	34·6	35·0	35·4	35·8	36·3	31·0
31·1	30·0	30·4	30·8	31·2	31·6	32·1	32·5	33·0	33·4	33·8	34·3	34·7	35·1	35·5	36·0	36·4	31·1
31·2	30·1	30·5	30·9	31·3	31·7	32·2	32·6	33·1	33·5	33·9	34·4	34·8	35·2	35·6	36·1	36·5	31·2
31·3	30·2	30·6	31·0	31·4	31·8	32·3	32·7	33·2	33·6	34·0	34·5	34·9	35·3	35·7	36·2	36·6	31·3
31·4	30 3	30·7	31·1	31·5	31·9	32·4	32·8	33·3	33·7	34·1	34·6	35·0	35·4	35·8	36·3	36·7	31·4
31·5	30·4	30·8	31·2	31·6	32·0	32·5	32·9	33·4	33·8	34·3	34·7	35·1	35·6	36·0	36·4	36·8	31·5
31·6	30·5	30·9	31·3	31·7	32·1	32·6	33·0	33·5	33·9	34·4	34·8	35·2	35·7	36·1	36·5	36·9	31·6
31·7	30·5	31·0	31·4	31·8	32·2	32·7	33·1	33·6	34·0	34·5	34·9	35·3	35·8	36·2	36·6	37·0	31·7
31·8	30·6	31·1	31·5	31·9	32·3	32·8	33·2	33·7	34·1	34·6	35·0	35·5	35·9	36·3	36·7	37·1	31·8
31·9	30·7	31·2	31·6	32·0	32·4	32·9	33·3	33·8	34·3	34·7	35·1	35·6	36·0	36·4	36·9	37·3	31·9
32·0	30·8	31·3	31·7	32·1	32·5	33·0	33·5	33·9	34·4	34·8	35·2	35·7	36·1	36·6	37·0	37·4	32·0
32·1	30·9	31·4	31·8	32·2	32·6	33·1	33·6	34·0	34·5	34·9	35·3	35·8	36·2	36·7	37·1	37·5	32·1
32·2	31·0	31·5	31·9	32·3	32·7	33·2	33·7	34·1	34·6	35·0	35·5	35·9	36·3	36·8	37·2	37·6	32·2
32·3	31·1	31·6	32·0	32·4	32·8	33·3	33·8	34·2	34·7	35·1	35·6	36·0	36·5	36·9	37·3	37·7	32·3
32·4	31·2	31·7	32·1	32·5	32·9	33·4	33·9	34·3	34·8	35·2	35·7	36·1	36·6	37·0	37·4	37·9	32·4
32·5	31·3	31·8	32·2	32·6	33·0	33·5	34·0	34·4	34·9	35·3	35·8	36·2	36·7	37·1	37·5	38·0	32·5
32·6	31·4	31·9	32·3	32·7	33·1	33·6	34·1	34·5	35·0	35·4	35·9	36·3	36·8	37·2	37·6	38·1	32·6
32·7	31·5	32·0	32·4	32·8	33·2	33·7	34·2	34·7	35·1	35·6	36·0	36·4	36·9	37·4	37·8	38·2	32·7
32·8	31·6	32·1	32·5	32·9	33·3	33·8	34·3	34·8	35·2	35·7	36·1	36·6	37·0	37·5	37·9	38·3	32·8
32·9	31·7	32·3	32·6	33 0	33·4	33·9	34·4	34·9	35·3	35·8	36·2	36·7	37·1	37·6	38·0	38·4	32·9
33·0	31·8	32·3	32·7	33·1	33·5	34·0	34·5	35·0	35·4	35·9	36·4	36·8	37·2	37·7	38·1	38·6	33·0

EQUIVALENT OF STANDARD WHOLE MILK IN OUNCES.

Fat per cent.	Net weight Grams.																Fat per cent.
	350	355	360	365	370	375	380	385	390	395	400	405	410	415	420	425	
9·00	29·8	30·3	30·7	31·1	31·5	31·9	32·3	32·8	33·2	33·7	34·1	34·5	34·9	35·4	35·8	36·2	9·00
9·10	30·2	30·6	31·0	31·4	31·8	32·3	32·7	33·2	33·6	34·0	34·5	34·9	35·3	35·8	36·2	36·6	9·10
9·20	30·5	30·9	31·4	31·7	32·2	32·7	33·1	33·6	34·0	34·4	34·9	35·3	35·7	36·2	36·6	37·0	9·20
9·30	30·8	31·3	31·7	32·1	32·6	33·1	33·4	33·9	34·3	34·8	35·3	35·7	36·2	36·6	37·0	37·4	9·30
9·40	31·2	31·6	32·1	32·5	33·0	33·4	33·8	34·3	34·7	35·2	35·6	36·1	36·6	37·0	37·4	37·8	9·40
9·50	31·5	31·9	32·4	32·9	33·3	33·8	34·2	34·6	35·1	35·6	36·0	36·5	37·0	37·3	37·8	38·2	9·50
9·60	31·8	32·3	32·7	33·2	33·7	34·1	34·6	35·0	35·4	35·9	36·4	36·8	37·3	37·7	38·2	38·7	9·60
9·70	32·2	32·7	33·1	33·5	34·0	34·5	34·9	35·4	35·8	36·4	36·7	37·2	37·7	38·1	38·6	39·1	9·70
9·80	32·5	33·0	33·4	33·9	34·4	34·8	35·3	35·8	36·2	36·7	37·1	37·6	38·1	38·5	39·0	39·5	9·80
9·90	32·8	33·3	33·7	34·2	34·7	35·1	35·6	36·1	36·6	37·1	37·5	37·9	38·4	38·9	39·4	39·9	9·90
10·00	33·2	33·6	34·1	34·6	35·0	35·5	36·0	36·5	37·0	37·4	37·9	38·3	38·8	39·3	39·8	40·3	10·00
10·10	33·5	33·9	34·4	34·9	35·4	35·8	36·3	36·9	37·3	37·8	38·3	38·7	39·2	39·7	40·2	40·7	10·10
10·20	33·8	34·3	34·7	35·3	35·8	36·2	36·7	37·2	37·7	38·2	38·7	39·1	39·6	40·1	40·6	41·1	10·20
10·30	34·2	34·6	35·1	35·6	36·1	36·5	37·0	37·5	38·0	38·5	39·0	39·5	40·0	40·5	41·0	41·5	10·30
10·40	34·5	35·0	35·5	35·9	36·5	36·9	37·4	37·9	38·4	38·9	39·4	39·9	40·4	40·9	41·4	41·9	10·40
10·50	34·8	35·3	35·8	36·3	36·8	37·3	37·8	38·3	38·8	39·3	39·8	40·3	40·8	41·3	41·8	42·3	10·50

EQUIVALENT OF STANDARD SKIMMED MILK IN OUNCES.

Solids-not-fat %.	Net weight grams.																Solids-not-fat %.
	350	355	360	365	370	375	380	385	390	395	400	405	410	415	420	425	
26·0	34·4	34·9	35·4	35·9	36·4	36·8	37·3	37·8	38·3	38·8	39·3	39·8	40·3	40·8	41·3	41·8	26·0
26·1	34·5	35·0	35·5	36·0	36·5	37·0	37·5	38·0	38·5	39·0	39·5	40·0	40·4	40·9	41·4	41·9	26·1
26·2	34·7	35·2	35·7	36·1	36·6	37·1	37·6	38·1	38·6	39·1	39·6	40·1	40·6	41·1	41·6	42·1	26·2
26·3	34·8	35·3	35·8	36·3	36·8	37·3	37·8	38·3	38·8	39·3	39·8	40·3	40·8	41·3	41·8	42·3	26·3
26·4	34·9	35·4	35·9	36·4	36·9	37·4	37·9	38·4	38·9	39·4	39·9	40·4	40·9	41·4	41·9	42·4	26·4
26·5	35·1	35·6	36·0	36·5	37·1	37·6	38·1	38·6	39·1	39·6	40·1	40·6	41·0	41·6	42·1	42·6	26·5
26·6	35·2	35·7	36·1	36·6	37·2	37·7	38·2	38·7	39·2	39·7	40·2	40·7	41·2	41·7	42·2	42·7	26·6
26·7	35·3	35·8	36·3	36·8	37·3	37·8	38·3	38·8	39·4	39·9	40·4	40·9	41·4	41·9	42·4	42·9	26·7
26·8	35·4	35·9	36·5	37·0	37·5	38·0	38·5	39·0	39·5	40·0	40·5	41·0	41·5	42·0	42·5	43·0	26·8
26·9	35·6	36·1	36·6	37·1	37·7	38·1	38·6	39·1	39·7	40·1	40·7	41·2	41·7	42·2	42·7	43·2	26·9
27·0	35·7	36·2	36·7	37·2	37·8	38·3	38·8	39·3	39·8	40·3	40·8	41·3	41·8	42·4	42·9	43·4	27·0
27·1	35·8	36·4	36·9	37·4	37·9	38·4	38·9	39·4	40·0	40·4	41·0	41·5	42·0	42·5	43·0	43·5	27·1
27·2	35·9	36·5	37·0	37·5	38·0	38·5	39·1	39·6	40·1	40·6	41·1	41·6	42·2	42·7	43·2	43·7	27·2
27·3	36·1	36·6	37·1	37·6	38·2	38·7	39·2	39·7	40·2	40·7	41·3	41·8	42·3	42·8	43·3	43·9	27·3
27·4	36·2	36·8	37·3	37·8	38·3	38·8	39·3	39·8	40·4	40·9	41·4	42·0	42·5	43·0	43·5	44·0	27·4
27·5	36·3	36·9	37·4	37·9	38·4	39·0	39·5	40·0	40·5	41·1	41·6	42·1	42·6	43·1	43·6	44·2	27·5
27·6	36·5	37·0	37·6	38·1	38·6	39·1	39·6	40·2	40·7	41·2	71·7	42·3	42·8	43·3	43·8	44·4	27·6
27·7	36·6	37·1	37·7	38·2	38·7	39·2	39·7	40·3	40·8	41·4	41·9	42·4	42·9	43·4	44·0	44·5	27·7
27·8	36·7	37·3	37·8	38·3	38·8	39·4	39·9	40·4	41·0	41·5	42·0	42·6	43·1	43·6	44·1	44·7	27·8
27·9	36·9	37·4	38·0	38·5	39·0	39·5	40·0	40·6	41·1	41·7	42·2	42·7	43·2	43·8	44·3	44·8	27·9
28·0	37·0	37·5	38·1	38·6	39·1	39·7	40·2	40·7	41·3	41·8	42·3	42·9	43·4	43·9	44·4	45·0	28·0

Skimmed milk means milk containing not less than 9 per cent. of milk solids other than milk fat.

The analysis of dried milk is, of course, similar to that of fresh milk, but since this substance is about eight times as concentrated as fresh milk small errors are much magnified and become important. Thus the citric and lactic acids which would be ignored in the ordinary methods of analysis of fresh milk may amount to over 1 per cent. For a detailed study of methods, see the Report of the Milk Products Sub-Committee (*Analyst*, 1936, **61,** 105).

The water content of freshly-dried milk is variable, depending on the type of plant used ; generally the spray process yields a drier milk than does the film process. The moisture in spray milk is often less than 1 per cent., in film-dried milk it may amount to 3 per cent. ; but in either case, as the powder is hygroscopic, this amount often increases during packing or storage and by the time it is retailed, may reach 6 to 7 per cent. It may be estimated in an ordinary oven at 101° to 102° by about two hours' drying, but the weighings must be done in stoppered bottles. As is shown by Jephcott (*Analyst*, 1923, **48,** 529), direct extraction always gives seriously low results for fat ; the Werner-Schmidt process is undoubtedly the best. One g. of the power is weighed into a boiling tube and dissolved in 10 ml. of warm water with the addition of 2 or 3 drops of ammonia. When all is dissolved an equal volume of hydrochloric acid is added, and the mixture is heated in boiling water until all the casein is dissolved, just as in the case of ordinary milk. The cooled mixture is extracted four times with ether in the usual way.

For the estimation of lactose, a solution may be prepared with the aid of a few drops of ammonia, and the estimation made as with fresh milk. All the other estimations are made precisely as for milk.

A point of some commercial importance is the " solubility " of dried milk ; this is, of course, not true solubility, as natural milk is an emulsion rather than a solution, but it is an important feature as determining how far the powder will yield on mixing a liquid similar to natural milk. Acidity of the original milk and high temperatures during desiccation are factors which tend to low solubility. For the determination of solubility Lampitt and Hughes recommend that 5 g. of the milk powder should be shaken for three minutes with 38 ml. of water, or 45 ml. in case of skim milk powder, at 20°. The mixture is then centrifuged and the fat layer poured off. Five ml. of the solution are evaporated to dryness and weighed. The rest of the liquid is decanted and any fat carefully removed from the side of the tube, and the weight of residue plus the associated liquid is noted. Then the residue is dried and the weight of soluble solids in the volume of solution, indicated by the loss in weight due to water, deducted from the total weight. The difference is the insoluble residue on the weight taken.

Infant Foods.

There are three distinct classes of infant foods on the market: pure milk preparations; those prepared from cows' milk with various additions or alterations; and those prepared from farinaceous materials rendered soluble by the action of heat or enzymes, and which are to be used with milk. Doubtless all are, to some extent, modelled on human milk, but they usually differ widely therefrom in composition in respect of the ratio of fat and carbohydrate. The following table shows the composition of some of the best known English brands :—

1. MILK PRODUCTS.

—	*a*	*b*
	Per cent.	Per cent.
Water	4·01	12·80
Fat	19·20	1·05
Casein	24·40	76·50
Lactose	35·50	Trace.
Cane sugar	10·58	—
Ash	5·74	8·70

2. MILK PRODUCTS WITH ADDITIONS.

—	*c*	*d*	*e*
	Per cent.	Per cent.	Per cent.
Water	5·60	4·02	3·80
Fat	13·10	5·31	8·50
Casein	10·20	11·85	14·10
Lactose	32·90	Trace.	Trace.
Cane sugar	33·55	36·10	30·50
Ash	3·80	2·01	3·15
Maltose and dextrin	—	21·50	42·40
Starch	Absent	Present	Present

3. COOKED CEREAL PRODUCTS.

—	*f*	*g*	*h*
	Per cent.	Per cent.	Per cent.
Water	6·10	9·03	4·35
Fat	1·32	0·62	1·20
Protein	12·50	11·75	10·54
Lactose	? 1·50	—	—
Other carbohydrates, mainly starch, maltose and dextrin	77·58	77·29	80·12
Ash	1·00	1·31	3·79

Of the above samples, *a* is dried full-cream milk with an addition of cane sugar ; *b* is mainly casein precipitated from skimmed milk ; *c* is dried milk plus added lactose and cane sugar ; *d* and *e* are milk casein with fat, cane sugar and maltose ; *f*, *g* and *h* are baked flours.

It is impracticable to describe methods of analysis which are applicable to all infant foods. The determinations of water, fat, protein, ash, salts and fibre follow the well-known lines. A qualitative and microscopical examination will usually indicate what substances are present. If the constituents are those of dried milk plus sugar, lactose or sucrose, the methods applicable to condensed milks may be used, though it may be convenient to determine the sucrose volumetrically by inverting the solution containing the lactose and sucrose after precipitation with zinc and ferrocyanide. The inversion is conveniently carried out by heating 50 ml. with 5 ml. of hydrochloric acid at 70° for five minutes, then cooling and titrating the inverted sugar by the Lane and Eynon method. If starch is present, it may be determined approximately by the method of Thorne and Jeffers. Five g. of the sample are triturated in a mortar with 15 ml. of water and 15 ml. of hydrochloric acid added in small quantities. The mass is allowed to stand for half an hour, then diluted ; 10 ml. of 4 per cent. phosphotungstic acid solution and 20 ml. of hydrochloric acid are added and the volume made up to 200 ml. The mixture is allowed to stand for a further hour then filtered and the rotation (R) is observed in a 200 mm. tube at 20°. The percentage of starch is $\frac{40\ R}{11{\cdot}6}$. This figure is based on the average specific rotation of starch which is taken as + 200°. If other sugars are present the rotation is corrected by adding 0·028 S and deducting 0·07 (D + L), where S, D and L are the percentages of sucrose, dextrose and lactose respectively.

When hydrolysed starch products such as maltose and malto-dextrins are present the analysis becomes complicated. The maltose and dextrin can be calculated from the total soluble matter, cupric reducing power and optical activity in the manner indicated on p. 32. For the determination of lactose in such mixtures advantage may be taken of the fact established by Baker and Hulton that ordinary brewers' yeast ferments dextrose, maltose and sucrose, but not lactose. So if a neutral inverted solution of the material is incubated with washed brewers' yeast for about seventy-two hours at 25°–27° all the sugars except the lactose will have been decomposed, and the lactose may be titrated after clarifying the liquid in the usual way. Some other useful methods are quoted by Davis, *J. Soc. Chem. Ind.*, 1916, **35,** 201.

Casein

Reference has already been made to casein as the principal nitrogenous constituent of milk. Much casein is now prepared, both for food purposes and for various industrial processes. The usual method is by curdling separated milk, either by acid or by rennet, then washing and drying the precipitated casein. The details of the process vary considerably, so that there are produced two distinct qualities of casein, one for food purposes and one for industrial use. The following analyses show the difference between the two grades :—

	Industrial.		Edible.	
	1.	2	3.	4.
	Per cent.	Per cent.	Per cent.	Per cent.
Water . . .	10·16	8·40	2·45	3·70
Ash . . .	0·65	4·73	0·36	0·54
Fat . . .	3·95	2·60	0·40	0·62
Nitrogen . .	13·25	12·90	14·99	14·81

An edible casein should contain less than 1 per cent. of ash, and be almost free from fat. Lactic or sulphuric acid are commonly used for the precipitation, but should be completely removed by washing. To test for these, 10 g. of the sample are extracted with 100 ml. of water, then filtered ; the filtrate should not be acid in reaction, and evaporation should leave no residue, indicating the absence of lactose. For the determination of fat the Werner-Schmidt process should be used ; direct extraction with ether gives quite erroneous results. When estimating the ash, it is expedient to extract the charred residue with water, and ignite the carbonaceous matter separately, then return the extract to the basin, evaporate and ignite.

Casein has the property of being soluble in alkalies or alkaline salts, such as sodium phosphate, borate or carbonate. This property is utilised in the manufacture of certain well-known foodstuffs, such as Plasmon, Sanatogen and Nutrose, which contain casein with alkaline salts and phosphates.

Malted Milk

Malted Milk. This product is made by combining whole milk with a mash of ground barley malt and meal with or without the addition of salt and bicarbonate of soda. Sometimes it is prepared from liquid milk and malt extract, sometimes from the mixture of dried milk and malt powders. Additions of skim milk, lactose, sucrose, wheat or casein are sometimes present. Malted milk should contain at least 7·5 per cent. of fat, and the moisture should not exceed 3·5 per cent. The following are recent analyses :—

	1	2	3	4	5	6
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Moisture . . .	3·48	3·25	3·41	3·64	3·29	5·83
Fat . . .	9·31	7·91	7·42	7·50	8·43	8·17
Ash . . .	5·28	3·49	3·43	4·39	4·04	3·30
Proteins . . .	20·07	14·63	10·23	19·95	15·06	15·06
Lactose . . .	12·72	10·95	11·89	16·93	24·71	12·29
Other reducing sugars as maltose . .	37·88	45·45	45·00	46·82	34·75	37·16
Sucrose . . .	9·52	—	—	—	—	—
Non-reducing sugars .	—	14·3	18·60	0·80	9·7	19·8

Of these No. 1 contains added sucrose and skim milk powder, No. 2 contains wheat products, Nos. 4 and 5 contain added lactose. The methods of analysis follow the same lines as dried milk or infant foods and a microscopical examination should not be overlooked.

There are on the market several milk products containing added vitamins. Of these aneurine and ascorbic acid can be determined by appropriate modification of the methods given on pp. 117 and 64. Methods for the determination of vitamin A, which is fat-soluble, are given on p. 297. The estimation of vitamin D is only possible under certain favourable circumstances. The spectrophotometric method of Nield, Russell and Zimmerlie (*J. Biol. Chem.*, 1940, **136,** 73) works satisfactorily if the pink colour produced by the acetyl chloride-antimony chloride reagent is not overborne by the persistent blue colour due to preponderance of vitamin A.

CHAPTER X

BUTTER, MARGARINE AND CHEESE

Butter, like milk, is an article subject to wide variations in composition ; it is affected by seasonal or climatic changes and by any circumstances affecting the food or health of the cow. From all points of view the fat is the most important constituent, and at times the problem of deciding whether a particular sample is genuinely abnormal, or whether it is adulterated, presents great difficulty to the analyst. The non-fatty constituents of butter include water, which, by Section 32 of the Food and Drugs Act, must not exceed 16 per cent. in butter or margarine.[1] The solids-not-fat or curd in butter consist of casein with some lactose and small quantities of mineral matter. There may also be present added salt, colouring matter or preservatives. The composition of some typical butters is as follows :—

	1	2.	3.	4.	5.	6
	Fresh.	Fresh.	Salt.	Salt.	Salt.	Salt.
	Per cent.	Per cent.	Per cent.	Per cent	Per cent.	Per cent.
Water	12·15	14·26	15·03	11·74	14·15	14·00
Fat	86·82	84·94	79·97	82·22	83·28	81·79
Casein	0·73	0·53	0·47	0·73	0·61	0·76
Lactose	0·20	0·18	0·18	0·22	0·30	0·31
Ash *	0·10	0·09	2·35	5·09	1·06	3·14
(* Including salt)	0·03	0·05	2·30	5·03	0·61	2·90

An essential preliminary to the analysis of butter is the thorough mixing of the sample. For this purpose it is convenient to place the stoppered jar containing the sample in the warm incubator at 40° until the butter is thoroughly softened ; then stir it well with a spatula while cooling.

For the estimation of water, curd and ash, a porcelain basin is tared with a short glass rod and about 10 g. of the sample are weighed into it ; this is then heated over an Argand burner turned very low, and the fat stirred until all water is driven off —this point is easily recognised, as there is no further crackling

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[1] In milk-blended butter, which has now almost disappeared from the market, the limit is 24 per cent.

or bubbling, and the curd just begins to darken in colour. The loss in weight after cooling represents the water. The fat is now just melted, then dissolved in dry petroleum ether, allowing the curd and salt to settle, and the solution carefully decanted ; the residue is washed three times more with petroleum ether, dried and weighed as curd plus ash. As it is not usually necessary to separate the casein and lactose in the curd, it suffices now to ignite the residue at a low temperature over an Argand burner and weigh the resulting ash. For the estimation of salt, dissolve the residue or ash in water, filter and titrate with 0·1 N silver nitrate solution, using potassium chromate as indicator. The small amount of phosphate present does not interfere with the estimation of the salt in this way. The amount of ash which is not salt seldom exceeds 0·1 per cent. When salt only has to be estimated it is more convenient to wash the salt out directly from about 10 g. of the sample with hot water. Fat is usually taken by difference, but may easily be estimated directly if desired. Lactose and casein can be determined in the curd by means of Fehling solution and Kjeldahl's method respectively. Sodium carbonate may occasionally be found in the ash and would suggest either treatment of the butter with bicarbonate or the presence of an alkali benzoate or salicylate as preservative.

Boric acid being now prohibited is seldom found in butter, but it is still necessary to test for it. For the qualitative detection of boric acid, the most convenient method is to melt about 5 g. in a small beaker and withdraw from the bottom by means of a capillary tube a few drops of the aqueous layer ; this is acidified with 1 drop of dilute hydrochloric acid and dried on a piece of turmeric paper in the usual way. For the quantitative estimation Richmond's process is quick and accurate : 25 g. of the butter are melted in a beaker in the water oven, then 25 ml. of hot water are added and the mixture is well stirred. The aqueous portion is allowed to settle, then is again stirred into the fat and again allowed to settle ; 20 ml. are withdrawn by pipette, 1 ml. of 0·5 per cent. phenolphthalein solution is added and the liquid brought just to the boil and neutralised with 0·2 N. sodium hydroxide ; 10 ml. of neutral glycerol are then added and the boric acid is titrated in the usual way. The weight of boric acid $\times \dfrac{100 + \text{water per cent.}}{20}$ gives the percentage in the butter ; generally the factor 0·07 the number of ml. of 0·2 N soda is sufficiently accurate, as it assumes an average of 13 per cent. of water in butter.

Any of the preservatives mentioned under "Milk" may be present in butter, but the only likely ones other than boric acid are benzoic acid and, more rarely, fluorides. The latter may be detected in the aqueous layer by the alizarin S reagent mentioned on p. 171. Benzoic acid is estimated as on p. 104.

In recent years the flavour of butter has often been improved by the addition of small quantities of diacetyl. This substance with its precursor acetyl-methyl-carbinol may occur naturally in the butter, so that the finding of diacetyl does not in itself prove its addition. The amount present is about 0·1–2 parts per million. Diacetyl may be detected by steam distilling a large sample of the butter—about 500 g.—just acidified with acetic acid. The condenser dips into a mixture of 4 ml. each of 20 per cent. sodium acetate and 20 per cent. hydroxylamine hydrochloride solution, and 2 ml. of 5 per cent. nickel chloride solution. The presence of diacetyl is shown by a red colour or precipitate of nickel glyoxime. This test can be made quantitative by attention to the details as given by Barnicoat (*Analyst*, 1935, **60**, 653).

The real problem in butter or margarine analysis is the examination of the fat. The composition of butter fat is subject to a certain amount of variation due to the food of the cows and to other factors, but the most important constituent from the analytical point of view is butyric acid. The fat consists mainly of glycerides of the higher fatty acids, including variable percentages of butyric, caproic, caprylic, capric, lauric, myristic, stearic, palmitic and oleic acids, together with small quantities of other less well-known acids.

The fact that there is no such thing as "average" butter leads to the breakdown of many elaborate formulæ for estimating butter in mixtures. The table overleaf shows the usual range of constants for genuine butter fat.

The Reichert value is subject to slight seasonal variations ; it tends to be high in the spring of the year and to be lowest in October and November, just when the fat content of milk tends to a maximum.

The determination of the specific gravity at 37·8° (= 100° F.) as compared with water at the same temperature is perhaps a relic of the past, but has the merit that at this temperature the difference between the specific gravity of butter fat and other

	Normal maximum.	Normal minimum.	Average.	Abnormal.
Specific gravity at $\frac{37 \cdot 8^\circ \text{ C.}}{37 \cdot 8^\circ}$	0·913	0·910	0·9118	0·909–0·914
Refractive index at 40°	1·4561	1·4524	1·4548.	1·4517–1·4566
= Zeiss refractometer number	45·5	40·0	43·5	39–46·0
Saponification value	232	222	228·5	218–233
Iodine value	40·0	26·0	—	22–52
Reichert value	32·8	24·5	27–28	20·5–24·5
Polenske value	3·7	1·5	2·3–2·8	1·4– 4·0
Kirschner value	27	21	24	19·0–20·0
Baryta value	– 0·7	– 23·8	– 9·6	—
Mean molecular weight of fatty acids	267	258	260–261	—

fats is nearly at a maximum. At 100° C., owing to differing coefficients of expansion, the sensitiveness is much diminished.

A few years ago the refractive index of butter fat gave a valuable indication of its purity or otherwise ; coconut and palm kernel oils give a low value, and beef fat or lard give high values, but of recent years mixtures are incorporated into margarine which have the same refractive index as butter, so that the value of the test is much diminished. The temperature of the observation should always be 40° and, although a large amount of data is given in terms of butyrometer numbers on the Zeiss scale, it is preferable to use the refractive index, which is a definite physical constant, rather than a reading on an arbitrary scale. Readings at temperatures above or below 40° may be converted to 40° by adding or subtracting 0·00038 for each degree above or below 40° ; the refractive index decreases with increasing temperature. The factor cannot be applied with accuracy over any considerable range of temperature.

The iodine value does not afford very much information on a butter, on account of the large variations due to feeding, but if it is required it may readily be estimated by Wijs' method (p. 279). Similarly the importance of the saponification value is much diminished by the fact that most edible fats have values above 190, so that a small addition of foreign fat would not have any marked effect on the value obtained for the butter.

The Reichert—Polenske—Kirschner Processes. These processes, which are by far the most valuable for the elucidation of butter and margarine problems, are the outcome of the classic work of Hehner and Angell, who, in 1872, first devised a method which aimed at the estimation of butyric acid. Without tracing the many modifications the process has undergone since that time, it may be given in the form standardised by the Analytical Methods Committee of the Society of Public Analysts (*Analyst,* 1936, **61,** 404). For accurate results it is essential to adhere to the prescribed conditions in detail, especially when butter-margarine mixtures are being examined. The standard apparatus is of the dimensions shown in the accompanying figure.

Five g. of the clear filtered butter fat are weighed into a 310 ml. Polenske flask and 20 g. of glycerin are added, together with 2 ml. of 50 per cent. sodium hydroxide solution. It is convenient to have a special short cylinder graduated to deliver 20 g. of glycerin and an automatic syphon arrangement to deliver 2 ml. of the 50 per cent. soda. The flask is now heated over a flame, with continuous mixing, until the soap so formed suddenly clears ; this point is quite definite with butter fat ; with margarine it is sometimes not quite so easy to see. When the soap has cooled slightly, but not set hard, 93 ml. of boiling distilled water, previously well boiled, are added from a 100 ml. cylinder. Then 0·1 g. of powdered pumice (passing a No. 50 sieve but retained on No. 90 sieve) and 50 ml. of dilute sulphuric acid are added. The dilute acid is prepared by diluting 25 ml. of concentrated sulphuric acid to 1 litre and adjusting the strength so that 40 ml. neutralise the 2 ml. of the sodium hydroxide solution.

The flask is at once connected to the standard distilling apparatus and heated over a small flame until the insoluble fatty acids are completely melted ; then the flame is increased so that distillation commences. The condenser water should be from 18°–20° and the rate of distillation such that 110 ml. are collected in nineteen to twenty minutes. The distillate is further cooled in water at 15° for ten minutes ; after mixing, the liquid is filtered through a dry 9 cm. No. 4 Whatman filter paper, and 100 ml. are collected and titrated with 0·1 N barium hydroxide (sodium hydroxide may be used if the Kirschner value is not subsequently to be determined), phenolphthalein being used as indicator. The titrated liquid is reserved for the Kirschner estimation. The number of ml. of 0·1 N alkali (less the " blank " estimated in the same way but without any fat) increased by $\frac{1}{10}$th is the Reichert value. A small cylinder is placed under the condenser when the distillation is completed ; the condenser, cylinder, and 110 ml. flask are washed out on to the filter paper already used with small quantities (3 × 15 ml.) of water, and the washings rejected; the insoluble acids are then dissolved by

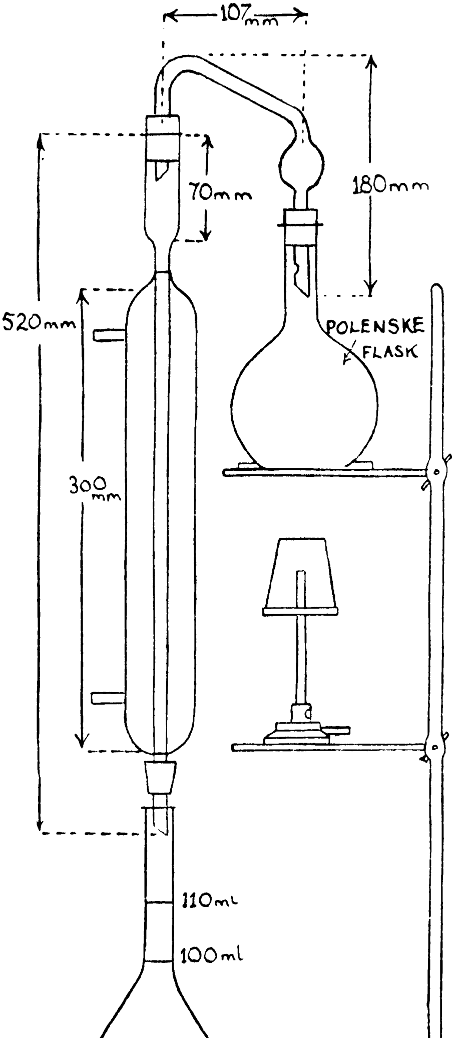

Fig. 40. Standard Reichert-Polenske Kirschner Apparatus.

three similar washings of the condenser, cylinder, flask, and the filter with 15 ml. of neutralised alcohol. The alcoholic solution of the water-insoluble acids is titrated with 0·1 N alkali, using phenolphthalein as indicator; the number of ml. alkali used, less the blank, is the Polenske value.

To the neutralised solution from the Reichert value is added 0·5 g. of silver sulphate, and the mixture is allowed to stand with occasional shaking for at least an hour, and then filtered. To 100 ml. of the filtrate are added 35 ml. of water, 10 ml. of the dilute sulphuric acid, and a small coiled length (30 cm.) of aluminium wire; the liquid is then distilled in the standard apparatus so that 110 ml. are collected in twenty minutes. One hundred ml. of the mixed distillate are titrated with 0·1 N barium hydroxide solution, and after deduction of any "blank," the Kirschner value is calculated from the equation

$$K = \frac{121\,x\,(100 + y)}{10{,}000}$$

where x is the titration (less blank) and y the number of ml. of 0·1 N baryta added to neutralise the original 100 ml. of Reichert distillate.

The Barium method of Avé Lallemant, as modified by Bolton and Revis, is carried out as follows: Saponify 5 g. of the fat with a measured volume of 0·5 N alcoholic sodium hydroxide solution by boiling under a reflux condenser for thirty minutes, then titrate back with 0·2 N hydrochloric acid and so estimate the saponification value; this value × 1·367 gives the barium oxide value (a) for 1 g. of fat. Remove all the alcohol by heating on a water bath and blowing in air, then dissolve the soap in a little hot water, dilute to 250 ml. at 38°, pipette out 100 ml. of this solution into a 250 ml. flask, add about 75 ml. of hot water and exactly 50 ml. of 2·5 per cent. barium chloride solution. Heat the mixture on a water bath for a few minutes to make the barium soap coalesce, then cool, dilute to 250 ml., filter off 200 ml., heat to boiling, add hydrochloric acid and 10 ml. of dilute sulphuric acid. Filter off, ignite and weigh the barium sulphate, add to this one-quarter of its weight and convert to BaO by multiplying by 0·6571. Treat 50 ml. of the barium chloride solution in a similar manner to determine its equivalent of BaO, subtract from this the BaO equivalent already found and so obtain the barium oxide equivalent of the acids forming insoluble barium salts. This, calculated on 1 g. of fat, is the insoluble barium oxide value (b); $a - b$ is the soluble barium oxide value (c). Calculate $b - (200 + c)$.

The order of practical value of these tests is undoubtedly that in which they are given above. In the routine examination of butter the estimation of the Reichert and Polenske values generally suffices. In the case of margarine, when the question of the presence or amount of butter fat arises, the Kirschner value is of next importance, and the Avé Lallemant process is of special merit in the examination of borderline samples of butter.

Certain practical points may be noticed. Genuine butter fat usually melts clear ; margarine fat very seldom does so. During the distillation it is useful to watch the appearance of the insoluble acids (Polenske). In the presence of much coconut oil these are generally in the form of oily drops, whereas, if there is much palm-kernel oil they take the form of white flakes. A genuine butter usually gives not less than 24·5 for the Reichert value. Siberian, South Russian butters, and those from badly fed cows often fall below this figure, occasionally even down to 20, but a sample giving Reichert value 24 or less should always be the subject of further investigation. The majority of genuine butters give a value of 26 or more, so that, if the value found be 23, there may be quite an appreciable addition of other fat or margarine. Consideration of the Polenske value is often a help at this stage. Coconut and palm-kernel oils are almost the only common fats giving a considerable Reichert or Polenske value (the figures are given in the table on p. 284, and these are perhaps the commonest ingredients of a margarine mixture. There exists in genuine butter an approximate proportionality between the Reichert and Polenske and Kirschner values ; thus the average results are :—

Reichert.	Polenske.	Kirschner.
32	3·5	26·4
31	3·2	25·8
30	3·0	25·0
29	2·9	24·8
28	2·6	24·0
27	2·4	23·3
26	2·0	21·8
25	1·8	20·9
24	1·7	20·5
23	1·6	20·0

These values do not often fluctuate more than, say, 0·5 in the Polenske, and 0·6 in the Kirschner processes, hence a low Reichert value with a high Polenske value is suspicious and suggests the presence of margarine, whereas a low Reichert value with a correspondingly low Polenske value may be simply due to the origin of the butter. It has been pointed out (see, for example, Cranfield, *Analyst*, 1915, **40,** 439; 1916, **41,** 240, 336) that the diet affects these figures and that certain substances may lead to high Polenske values. Since the Kirschner value is approximately a measure of butyric acid, it is often a great help in deciding whether a low Reichert value is due to abnormality or to adulteration. As an example, an unknown butter gave a Reichert value 23, and Polenske value 3·6; there is the possibility that it is abnormal, or that it is a mixture of 80 per cent. of butter having Reichert 26 and Polenske 2 with 20 per cent. of palm-kernel oil which has a Reichert value of 6, and Polenske 10. The Kirschner value was found to be 17·6, which is too low for a genuine butter; the normal Kirschner corresponding to Reichert 23 is 20. Thus the Reichert and Polenske figures cast suspicion on the sample, and the Kirschner value showed definitely that it was adulterated. If the Reichert—Polenske—Kirschner values are in the correct ratio, it is not safe to assume adulteration just because they are somewhat low. Positive proof of the adulterant should be sought by the phytosterol acetate test, the determination of iso-oleic acid or the various colour reactions of particular oils.

The Kirschner value is also a most useful indicator of butter in margarine. Bolton, Richmond and Revis (*Analyst*, 1912, **37,** 183) have shown that the proportion of butter fat in a margarine composed of fats other than coconut or palm-kernel is in agreement with the expression

$$\mathrm{K} = 0{\cdot}244\ \mathrm{B} + 0{\cdot}28, \text{ or } \mathrm{B} = \frac{\mathrm{K} - 0{\cdot}28}{0{\cdot}244}.$$

When palm-kernel or coconut oil is present, the proportion of butter fat in margarine may be deduced from the Reichert, Polenske and Kirschner values by the equation

$$\text{Butter fat per cent.} = \frac{\mathrm{K} - 0{\cdot}1\ \mathrm{P} - 0{\cdot}24}{0{\cdot}244}.$$

The presence of other fats does not usually interfere with the approximate accuracy of this expression.

The above expressions are not strictly applicable when the amount of butter fat is much over 10 per cent., but as this is the maximum permissible in margarine it is unusual to find samples containing more than this quantity. Elsdon and Smith (*Analyst*, 1925, **50,** 53) prefer the equation

$$\text{Butter fat per cent.} = \frac{\text{K} - 0{\cdot}2 - 0{\cdot}1\ \text{P}}{0{\cdot}235}$$

and give a series of corrections according to the exact value of P. The most satisfactory results are obtained if the analyst works out his own data, using known mixtures ; in this way the personal factor may be eliminated. In the case of unknown mixtures the percentage of butter fat cannot be returned with certainty nearer than say 1 per cent. When the presence of butter is claimed in margarine the amount must be stated within 2 per cent. (Section 32 of the Food and Drugs Act).

The method of Avé Lallemant is also useful in the examination of butter which gives an abnormally low Reichert value, although it is rather laborious and does not possess any great advantage over the more expeditious Kirschner determination. A genuine butter, even with a low Reichert value, almost always gives a negative value for $b - (200 + c)$, whereas the addition of any other fat tends to make this positive. Thus a butter giving Reichert value 23·5 gave $b - (200 + c) = -1{\cdot}2$, and the same butter mixed with 5 per cent. of margarine gave + 1·5.

Another method of note specially devised for the estimation of butter and coconut fat in margarine is that of Blichfeldt as modified by Gilmour (*Analyst*, 1920, **45,** 2). It affords a useful supplementary test in doubtful cases, but suffers from the disadvantage of requiring a specially designed apparatus which is rather prone to fracture. The apparatus there illustrated may be obtained from F. E. Becker & Co., London.

Among other tests which may be applied to margarine and doubtful samples of butter are the determination of unsaponifiable matter, Halphen's reaction for cotton-seed oil, Baudouin's test for sesamé oil (the addition of this latter to the extent of 10 per cent. is compulsory in certain Continental countries). These are described under " Lard " (p. 278). Starch may also be found in margarine as it has been added by Order to standardised war-time margarine to the extent of 0·025 per cent.

Hydrogenated oils add to the difficulties of the analyst in dealing with margarine ; if present in quantity they may sometimes be recognised by the palate. Since nickel is the usual catalyst in the hydrogenation process, its presence in the fat confirms the diagnosis of hardened oil, but the use of the catalyst in tube form, rather than in fine powder as when the process was first applied, has rendered it less likely that any nickel remains in the fat. To test for it, 50 g. or more are ignited in a clean silica basin and the ash dissolved in a few drops of dilute hydrochloric acid ; then a drop of nitric acid is added and the solution evaporated nearly to dryness, then excess of ammonia is added to the residue and 2 ml. of a 0·2 per cent. ammoniacal solution of α-benzildioxime. Nickel, if present, gives a rose-red colour or precipitate within an hour (Atack, *Analyst*, 1913, **38,** 316).

A more certain detection of hydrogenated fat is based upon the determination of solid unsaturated fatty acids, for which the method of Cocks, Christian and Harding is the best. This method is described on p. 281. Solid unsaturated acids do not occur in butter, but constitute from 25–50 per cent. of the acids in hardened oils.

Microscopic examination of butter and margarine sometimes yields valuable information., Butter which has not been melted is devoid of crystalline structure, whereas margarine fat or renovated butter often shows crystalline forms. A fresh piece of the fat is pressed out under the cover slip on a micro slide and examined with a 1-inch objective, a Nicol's prism and analyser being used. If extraneous light is carefully excluded, with pure butter all is dark, but with margarine or renovated butter bright portions and indefinite crystalline forms may usually be seen. Care should be taken to ascertain that the crystals are not those of salt or boric acid, by examining the slide without the polarising apparatus.

The phytosterol acetate test, although somewhat tedious, is of great value as a definite indication of vegetable oil when admixed with animal fats. The latter contain cholesterol but no phytosterol, and even though a butter may give a slight Halphen reaction, due to the cow having been fed on cotton cake, such butter never gives any phytosterol. The test also detects hydrogenated vegetable fats in animal fats. Of the clear fat 15 g. are boiled under a reflux condenser with 10 ml. of potassium hydroxide solution (1,000 g. in 1,400 ml. of water) and 20 ml. of alcohol until all the fat is dissolved and then for a further half hour. Larger quantities of the fat and alkali are preferable if available. The mixture is cooled, 60 ml. of water and 108 ml. of alcohol are added, and 20 ml. of 1 per cent. alcoholic solution of digitonin. After standing over night the sterol digitonin compound is filtered off on a small Buchner funnel, washed with a few ml. of alcohol, then acetylated by heating in a boiling water bath with 3 ml. of acetic anhydride. The acetic anhydride is allowed to evaporate and the sterol acetates dissolved in a minimum amount of hot absolute alcohol and allowed to crystallise. The crystals are separated by withdrawing the alcohol through a glass tube plugged with a piece of cotton wool, redissolved in more hot alcohol and recrystallised. This recrystallisation process is repeated at least three times, and in doubtful cases five times ; it may be carried out on quite small quantities of the acetate. Then the melting point of the pure acetate is determined, correcting the observed temperature for the emergent stem of the thermometer. Phytosterol acetate has a melting point of 127° or higher, and cholesterol acetate 113°-115°. If, therefore, the melting point observed is above 116° the acetate should be recrystallised again, and if it is higher, or is still above 116°, the presence of phytosterol, hence of vegetable oil, is certain. In the converse way animal fat in vegetable fat may be detected by the melting point being substantially lower than that of pure phytosterol acetate, 127°.

Another test, which, although quite empirical, is sometimes of value in the detection of adulteration in butter, is that proposed by Valenta in 1884, as modified by Fryer and Weston (*Analyst*, 1918, **43,** 3).

Vitamin A in Butter or Margarine

As a wartime measure margarine has been fortified with a vitamin concentrate containing A and D so that it has a value approximately equivalent to that of summer butter (which is about 50 International Units of vitamin A per g.). The determination of vitamin D in margarine is not at present practicable by chemical methods. Vitamin A cannot be satisfactorily determined by the direct Ultra-Violet absorption of the unsaponifiable matter (see p. 297) because the irrelevant absorption is apt to be greater than that due to the vitamin, so the most trustworthy method of test is the measurement of the absorption at 620 mμ of the colour produced on adding the antimony trichloride reagent to a solution of the unsaponifiable matter. Edisbury (*Analyst*, 1940, **65,** 484) recommends the following procedure.

From 8 to 10 g. of margarine are boiled for ten to fifteen minutes with 30 ml. of alcohol containing 4 ml. of 60 per cent. potassium hydroxide solution. The mixture is diluted with 60 to 80 ml. of water and extracted three or four times with 50 ml. of freshly distilled ether. The combined extracts are washed with 50 ml.

of water, 50 ml. of dilute potassium hydroxide or carbonate solution, and three times with 50 ml. of water, all at 30° C. After removal of the ether a few drops of alcohol are added and the wet residue is blown until dry two or three times at 100° C. under nitrogen or carbon dioxide. The dried unsaponifiable matter is immediately made up to 2 ml. with alcohol-free dry chloroform and the $E^{1\%}_{1\,cm.}$ 616–620 $m\mu$ value is determined as if the solution contained 400–500 per cent. (w/v) of margarine, the intensities being matched as before at as near an optical density (log I_0/I) of 2·0 as possible.

For a quick, though less accurate, test, the ethereal extractions may be reduced to two, washing confined to one portion of 50 ml. of water, and the ether dried with sodium sulphate. Such a test will detect gross irregularities and can be quickly carried out.

Cheese

The making of good cheese is still more of an art than a science. Much is known about the different conditions which are essential for the production of the different varieties of cheese, but the chemical changes are largely obscure. The general action of the rennet added to milk is to split up the casein into two parts, one of which forms an insoluble calcium compound which is the curd and which carries with it most of the fat in the milk. The exact composition and physical condition of the curd is influenced by the amount of rennet added, the acidity of the milk, and the temperature. For most varieties of cheese mixed evening and morning milk is used, and has an acidity of about 0·20 per cent. This is often controlled by a " starter " which ensures the presence of the right kind of micro-organisms in the cheese ; the initial temperature is commonly about 84°–88° F.

According to Richmond the distribution of the various milk constituents when made into curd and whey is :—

	Milk.	Curd.	Whey.
	Per cent.	Per cent.	Per cent.
Water . . .	87·30	6·50	80·80
Fat . . .	3·75	3·45	0·30
Lactose . . .	4·70	0·30	4·40
Casein . . .	3·00	2·60	0·40
Albumin. . .	0·40	Trace	0·40
Ash . . .	0·75	0·15	0·60

The ratio of the constituents, apart from water, of a whole milk cheese must therefore be approximately that shown for the curd. From the analyst's point of view cheeses may be classified as :—

(1) Soft cheese made from—
    (*a*) Milk and cream.
    (*b*) Whole milk.
(2) Hard cheeses made from—
    (*a*) Whole milk.
    (*b*) Partly skimmed milk.
(3) Pasteurised cheeses, usually wrapped in tin-foil.

In addition there are a few cheeses made from goat's milk or sheep's milk, of which an example is Roquefort cheese. Pasteurised or process cheese is a variety originally imported from America ; it is prepared by heating a mild whole-milk cheese such as Cheddar with water to a temperature of 175° F. or thereabouts to pasteurise it, then pouring into moulds and wrapping in tinfoil. Small amounts of Rochelle salt, or tartrate citrate or phosphate mixtures are added. A few cheeses are also

1 (*a*). SOFT CHEESES MADE FROM CREAM

	Water.	Fat.	Protein.	Acidity. lactic.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Lactic . .	25·4	65·30	6·95	0·75	0·40
Lactic . .	30·3	60·52	5·34	0·56	0·35
Gervais . .	42·32	49·18	7·75	0·27	0·49
Pommel. .	44·9	45·5	7·2	1·16	0·50

1 (*b*). SOFT CHEESES MADE FROM WHOLE MILK

	Water.	Fat.	Protein.	Acidity.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Camembert .	45·24	30·31	19·75	—	4·70
Camembert .	49·31	22·50	20·10	0·44	4·40
Brie . .	50·04	27·50	18·32	—	4·12
Bondon. .	59·5	24·4	9·4	—	0·7
Bondon. .	55·2	20·8	15·4	1·65	6·98
Neufchâtel .	37·90	41·3	23·6	—	3·4
Caerphilly .	57·5	18·4	19·5	—	3·2

2 (*a*). Hard Cheeses made from Whole Milk

	Water.	Fat.	Protein.	Acidity.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Stilton	23·57	30·13	32·55	1·24	3·51
Stilton	28·60	30·70	35·60	1·08	4·02
Gorgonzola	31·85	34·34	27·88	1·35	4·58
Gorgonzola	27·80	35·46	28·00	1·07	4·02
Wensleydale	28·3	38·3	27·43	—	3·7
Gruyère	33·16	30·69	30·67	0·27	4·71
Cheddar	30·50	30·70	30·20	0·85	4·10
Cheddar	28·54	31·20	30·70	1·25	4·30
Cheshire	37·11	30·68	26·93	0·86	4·42
Roquefort	36·85	30·61	25·25	1·90	5·39

2 (*b*). Cheeses made from Partly Skimmed Milk

	Water.	Fat.	Protein.	Acidity.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Dutch	41·30	22·78	28·25	0·57	7·10
Dutch	45·24	17·20	27·35	0·84	5·60
Gloucester	25·25	25·80	30·05	—	4·80
Parmesan	32·5	17·1	43·6	—	6·2

3. Processed Cheeses

	Water.	Fat.	Protein.	Acidity.	Ash.	Stabiliser.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
1.	35·92	28·65	26·91	0·80	5·43	Citrate present.
2.	39·61	30·52	23·35	1·10	4·16	Citrate and Tartrate.
3.	42·64	26·32	25·66	1·05	5·06	Tartrate.

made with lactic acid without the addition of rennet. The accompanying tables show the composition of cheese. Some figures are due to Richmond, some are the author's analyses.

Perhaps the most important item in the analysis of cheese is the proportion of fat calculated on the dry cheese ; if this is materially less than 50 per cent. it is indicative that the cheese has been made from partly skimmed milk or has been "filled." Another criterion of genuineness is the ratio between the fat and protein; this should not be less than 1 : 1, and is often as high as 1·25 : 1. Richmond gives a formula for calculating from the composition of the cheese the probable composition of the milk from which it was made:—

$$\text{Fat in milk} = \frac{100\ F}{35{\cdot}4\ P + F} \times 0{\cdot}25$$

where F and P are the percentages of fat and protein respectively in the cheese. Owing to the natural variations in the ratio of fat and protein in cheese it is not wise to certify a cheese to be made from skim milk unless the calculation indicates less than 2·75 per cent. of fat, or to condemn a cream cheese unless the indicated fat percentage is less than 10. For a study of the water-fat ratios in about 2,000 samples of English cheese, see Nicholls (*Analyst*, 1941, **66**, 265).

The demand for red or yellow cheese is rather remarkable, as it must obviously be dyed to effect the desired colour, since milk and its curd are practically white. Annatto is the usual pigment; it may be detected if necessary by the method given on p. 242.

As a preliminary to the analysis of cheese, careful sampling is necessary; a soft cheese may be pounded in a mortar or a hard cheese grated on a nutmeg grater. The rapidity with which a freshly cut sample of cheese loses water is surprising; a loss of 2 per cent. may occur within an hour. Direct estimation of water by drying in the oven on sand is fairly satisfactory, but a long time is necessary to dry the sample completely. The better method is to extract the fat from a weighed quantity in a Soxhlet thimble with a dry petroleum ether, then dry the residue; the total loss in weight less that of the fat extracted gives the water. For the exact estimation of fat the Werner-Schmidt method is most convenient. A weighed quantity is digested with dilute hydrochloric acid, then shaken out with ether as described under "Milk." The ash of the cheese should be examined for boric acid and the soluble alkalinity estimated. The proteins in cheese are determined by the usual Kjeldahl method, using the factor 6·38. It will be found that the sum of water, fat, ash and protein differs from 100 by 3 or 4 per cent.; this is due to the presence of albumoses, peptones, amines and other products of ripening. The proteins of cheese can be separated approximately by treating the fat-free sample as described for meat extracts, but the results so obtained do not yield much information in the present state of our knowledge. Richmond has proposed to estimate the total products of ripening directly by grinding 10 g. of the cheese with nine successive quantities of 25 ml. of water and pouring the extracts through a filter ; the volume is made up to 250 ml., the total solids are determined on 50 ml., then ignited. The total solids so obtained less the ash give the products of ripening, and the difference between 100 and the sum of fat, water and ash plus products of ripening may be taken as unaltered casein.

Acidity, reckoned as lactic acid, is estimated by direct titration with the use of phenolphthalein ; it is not very accurate on account of the difficult end point.

To detect citrate or tartrate it is best to de-fat a portion of the cheese by mixing it with sand and extracting in a Soxhlet extractor ; then emulsify the de-fatted material with water, add just enough acetic acid to precipitate the casein and filter off the clear whey. Citrate, tartrate, and sometimes added phosphate can be detected in the liquor by their ordinary reactions.

Tin should be determined in process cheese by the method given on p. 107. Even the small wrapped portion should not show more than 2 grains per lb.

As cheese is sometimes prepared partly from margarine or other fat it is often necessary to examine the fat in the cheese. For this purpose sufficient fat may usually be separated by warming about 30 g. of the grated cheese in a funnel plugged with cotton wool, keeping it in the water-oven for the fat to run out. If necessary the fat can be separated by the Werner-Schmidt method, but such fat usually gives a rather low Reichert value. The dry fat is examined as butter fat ; usually the determination of the Reichert and Polenske values is sufficient. In interpreting the results of these tests in doubtful cases it is desirable to bear in mind the possible (unknown) alterations which may be produced in the fat during the ripening process, and which may cause a slight departure from normal butter figures.

CHAPTER XI

LARD, SUET, SALAD AND OTHER OILS, DETERMINATION OF THE FAT-SOLUBLE VITAMINS

THE definition and composition of lard have changed somewhat in recent years by reason of the inclusion of the fat of all parts of the pig, instead of only that from the omentum, as formerly. The U.S. Department of Agriculture gives the following definitions : Lard is the rendered fresh fat from hogs in good health at the time of slaughter ; it must be clear, free from rancidity, and contain not more than 1 per cent. of substances other than fatty acids and fat. Leaf lard is similarly defined as lard rendered at a moderately high temperature from the internal fat of the abdomen of the hog, excluding that adherent to the intestines, and having an iodine number not greater than sixty. Neutral lard is lard rendered at low temperatures.

Lard consists of glycerides of the following fatty acids : oleic, linolenic, lauric, myristic, palmitic and stearic ; there is also usually present a minute amount of tissue substance, a trace of moisture and unsaponifiable matter. In genuine lard the melting point affords an indication of the origin of the fat. Leaf lard usually has a melting point of 44°–45°, lard from the kidneys 39°–42°, that from the back and other parts 34°–39° ; these figures are, of course, somewhat variable.

The usual constants of lard are as follows :—

Specific gravity at 40°	0·902–0·907
Melting point	34°–45°
Refractive index at 40°	1·4583–1·4610
= Zeiss butyrometer No.	48·5–52
Free fatty acids, as oleic, per cent.	Up to 1·0 per cent.
Saponification value	192–200
Iodine value	51–65
Unsaponifiable matter, per cent.	0·2–0·7

The melting point and specific gravity are not of much value, as most of the likely adulterants of lard give similar figures. The refractive index and iodine value are perhaps the most important constants to determine, and when supplemented by the qualitative tests described below usually suffice for the identification of pure or adulterated samples. The range of iodine values for the finer qualities of lard is smaller than that quoted above ; usually such fat gives iodine value between 55 and 63, but the tendency of recent years has been for the iodine value to be nearer 65 in commercial samples. The free acidity should not exceed 0·6 per cent. calculated as oleic acid ; there should be no more than a trace of water. A useful survey of the constants of lard is given by Sutton *et al* (*Analyst*, 1940, **65,** 623).

The determination of the iodine value is made by Wijs' method. The solutions required are 0·1 N sodium thiosulphate accurately standardised, pure carbon tetrachloride, and Wijs' iodine solution. This is prepared by dissolving 9 g. of iodine and 8 g. of iodine trichloride each in about 500 ml. of glacial acetic acid, mixing the two solutions and making up to 1 litre. It is necessary to use the purest possible acetic acid. Into a bottle of about 250 ml. capacity, cleaned with chromic acid and dried, and having a well-fitting stopper, is weighed 0·3–0·4 g. of the fat (with a drying or semi-drying oil, a much smaller quantity should be taken—about 0·2 g.). Ten ml. of carbon tetrachloride are added to dissolve the fat, then by pipette 25 ml. of the Wijs' solution. In pipetting this solution a pipette with a narrow outlet should be used, always the same one, and it should be allowed to drain for exactly the same time on each occasion—one minute is convenient. The stopper, moistened with potassium iodide solution, is inserted at once. A blank is put on at the same time with similar quantities of solvent and reagent. The bottles should be placed in a cool dark place until absorption is complete, *i.e.*, in the case of non-drying oils like lard, half an hour (less would probably be sufficient, but it is safe to give more) ; drying oils such as linseed or tung oil require a longer period, say three hours at least.

To the blank and the sample are then added 20 ml. of 10 per cent. solution of potassium iodide and about 100 ml. of water ; the excess of iodine is titrated with 0·1 N thiosulphate solution, starch solution being added as indicator towards the end of the titration. It is essential that there should be at least 100 per cent. excess of the reagent during the absorption, so that the difference between the blank titration and that of the test should be less than half the blank. The iodine value is the percentage of iodine absorbed by the fat.

The acid value, or free fatty acids, and saponification value are conveniently estimated on the same weight of sample ; but if the acid value only is to be determined, 10 g. may be taken and the titration made with 0·1 N alkali. For the combined estimation of acid value and saponification value, 2–2·5 g. of the fat are weighed into a small flask and 10 ml. of neutral alcohol are added, the mixture is boiled on the water bath under a reflux condenser for a few minutes, then titrated with standard (0·5 N) alcoholic potassium hydroxide solution, phenolphthalein being used as indicator. The acid value is the number of milligrams of KOH required to neutralise the free acid in 1 g. of the fat ; or the result may be expressed as oleic acid per cent. by multiplying the acid value by 0·502. After reading the burette for the acid value, a further quantity of alcoholic potash is added, usually 30 ml. altogether, and a like quantity is run into another flask for a blank. The two flasks are boiled under reflux condensers on the water bath with occasional shaking for one hour, then each is titrated with standard acid 0·5 N. There must, of course, be an excess of alkali in the flask containing the fat. From the difference between the two titrations is calculated the milligrams of KOH absorbed by 1 g. of the fat ; this is the saponification value and includes the alkali used to neutralise the free fatty acids.

The extraction of the *unsaponifiable matter* from the fat may conveniently be combined with the determination of acid value and saponification value as above. After the titration the soap solution is made alkaline again with about 1 ml. of the alcoholic potash solution, transferred to a separator and washed in with 50 ml., less the volume of standard acid used, of water. The solution while just warm is extracted three times with 50 ml. quantities of ether. The first portion is used to wash out the saponification flask. The mixture in the separator is vigorously shaken each time, allowed to stand, then the lower layer is run off into another separator and the ether poured into another containing 20 ml. of water. The mixed ether extracts are washed gently with the 20 ml. of water, and again with two further 20 ml. quantities, shaking vigorously. The ether extract is washed twice with 20 ml. of aqueous 0·2 N potassium hydroxide solution and twice or more with 20 ml. of water, until the wash water is no longer alkaline to phenolphthalein. The ether extract is poured into a tared flask, evaporated, and dried in the oven (preferably at temperature not exceeding 80°) to constant weight. As a control the unsaponifiable matter is dissolved in neutral alcohol and titrated with 0·1 N alkali ; not more than 0·1 ml. should be required to neutralise the free fatty acid present.

The most reliable test for vegetable fats in lard is the separation of the sterol acetates from the unsaponifiable matter as described on p. 271 ; the finding of any phytosterol would be conclusive evidence of the presence of vegetable fat. Modern lard substitutes and cooking fats consist usually of hydrogenated oils ; there may be of animal or vegetable origin, and their detection in a lard mixture is sometimes difficult. The finding of any significant proportion of iso-oleic acid or other solid unsaturated acid is, however, a definite indication of the presence of such hardened oil.

Determination of Solid Unsaturated Fatty Acids. The method described by Cocks, Christian and Harding (*Analyst*, 1931, **56,** 368) is convenient and accurate. About 5 g. of the fat are saponified with alcoholic potash, and the fatty acid liberated by the addition of dilute sulphuric acid. The fatty acids are washed by boiling with water and dried by passing through dry filter paper. Then 3·5 g. of dry, freshly prepared fatty acids are dissolved in 50 ml. of alcohol and heated to boiling point ; to the solutions are added 50 ml. of boiling alcoholic solution of lead acetate (3·45 g. lead acetate for lard or other fat containing upwards of 25 per cent. of total solid acids, 1 g. for oils containing less than 25 per cent.). The mixture is brought to the boil and allowed to stand overnight at a temperature of 15°–20°. Next morning the lead salts are collected on a Buchner funnel and, after changing the receiver, washed with 100 ml. of petroleum spirit. The petroleum spirit is removed by distillation and the residue dissolved in 20 ml. of boiling alcohol and 1 drop of glacial acetic acid added from a pipette. The solution is allowed to crystallise at 15°–20° for three hours. The lead salts so precipitated are filtered off and washed with 20 ml. of cold alcohol and added to the main bulk of lead salts. These are suspended in a little water, and decomposed by addition of a small excess of dilute nitric acid in the presence of ether. The ethereal solution of the acids is washed with water to remove mineral acid, then evaporated in a tared flask and the total solid fatty acids weighed. A portion of this is taken for determination of the iodine value. From the iodine value the proportion of unsaturated acids is calculated on the basis of an iodine value of 90 (unless rape oil is known to be present, the erucic acid of which has iodine value 75·5). Natural oils other than rape oil will contain less than 3 per cent. of solid unsaturated acids, except tallow, which may show 5 or 6 per cent. Rape oil contains about 50 per cent. Hardened fats usually show from 20 to 50 per cent. of solid unsaturated acids.

Among the more important qualitative tests which should be applied to lard may be mentioned the following :—

Halphen's test for cotton-seed oil depends upon the formation of a bright red colour on heating the oil, diluted with amyl alcohol, with a solution of sulphur in carbon disulphide. To 5 ml. of the oil are added 5 ml. of amyl alcohol and 3 ml. of 1 per cent. solution of sulphur in carbon disulphide ; the test tube is corked and the cork secured by a piece of calico tied over the top. Then the mixture is heated in boiling water for half an hour ; in the presence of cotton-seed oil from 2 per cent. and upwards a rose colour is produced, the intensity of which is approximately proportional to the amount of this oil. As the chromogenic constituent varies in different specimens of the oil, it is necessary to use the same oil always in making colour comparisons and to interpret them quantitatively with caution. The colour is less with oil which has been strongly heated, and is no longer produced by oil which has been exposed to a temperature of 250°. Hydrogenation wholly or partly destroys the chromogenic bodies according to the stage to which the process has been carried. The indications of Halphen's test must be interpreted with special care in the case of butter or lard, since the feeding of the cows or pigs on cotton cake may cause a slight indication. Certain uncommon oils (kapok and baobab) are said to give the rose colour with Halphen's reagent, but may be disregarded from the point of view of likely adulterants of butter or lard.

Becchi also proposed a useful test for cotton-seed oil which may be applied in the following modified form : A solution is prepared by dissolving 1 g. of silver nitrate in about 5 ml. of water and adding to it 200 ml. of alcohol, 40 ml. of ether, and 0·1 ml. of nitric acid. To 10 ml. of the oil are added 10 ml. of amyl alcohol containing 10 per cent. of rape oil and 1 ml. of the above reagent ; the mixture is divided into two parts, one of which is heated in boiling water for fifteen minutes, then compared with the other half. Cotton-seed oil is indicated by the development of a reddish-brown colour.

Baudouin has provided a good reaction for sesame oil. To 5 ml. of the oil or melted fat are added an equal volume of concentrated hydrochloric acid and 2 drops of a 1 per cent. alcoholic solution of furfural; the mixture is vigorously shaken for a minute, then allowed to separate. In the presence of sesamé oil a characteristic reddish colour is produced in the lower layer. Many oils give a dirty brown tint with this test, so that in any case of doubt a comparison should be made with a known oil and the same reagents. Also it should not be overlooked that certain dyes which might be present in butter give a pink colour with hydrochloric acid alone.

Holdé described a simple and useful test for paraffin in fats such as lard; it is available also for the detection of any mineral oil in vegetable oils. To 10 drops of the oil in a test tube are added 5 ml. of 0·5 N alcoholic potash solution and the mixture is well boiled for at least three minutes to ensure complete saponification. To the soap solution is added water, 0·5 ml. at a time until 15 ml. altogether have been added, the mixture being well shaken and observed after each addition. In the presence of mineral oil a characteristic turbidity is produced, but when a positive result is obtained it is essential to repeat the test to avoid possibility of error due to incomplete saponification; also the observation must be close, as in the presence of very small amounts of paraffin only a faint turbidity appears, which often appears on the further addition of water.

The detection of beef or mutton fat in lard is one of the difficult problems which may arise in the examination of fats. Formerly much stress was laid upon the form of the crystals deposited from ether, but experience shows that the observations must be interpreted with caution, and a positive result ought to be supported by other evidence, such as an abnormal iodine value. Two ml. of the melted fat are dissolved in 10 ml. of ether in a cylinder closed with a plug of cotton-wool and the solution set aside in a cool place until a deposit of white crystals appears. The ether is poured off and a few of the crystals are rapidly transferred to a microscope slide with a drop of neutral oil and examined, first under a low power and then under a high power, 200–250 diameters. The characteristic appearance of lard crystals is in large tufts, each crystal having chisel-shaped ends, or as oblong plates with oblique ends, whereas beef or mutton fat gives fan-like groups with pointed ends. It has been shown by Hehner and Mitchell that the form of the crystals depends on the proportion of stearic acid and that some genuine lards give crystals resembling those of beef fat. Repeated crystallisation of genuine lard from ether causes the crystals to assume the beef-like form ; after five or six crystallisations quite misleading results will be obtained. The important matter is to note the ends ; lard often forms beef-like tufts, but when examined under the high power the ends of the crystals are always unique, not pointed. In all cases of suspicion confirmation must be obtained from other data or Bömer's test. Hydrogenated fats often yield tufts of crystals closely resembling those of beef or mutton. Bömer has given a useful confirmatory test for beef fat, depending on the melting point of the distearin, which in lard is 68·5° and in beef is 63·3°, although these values are not obtained on the mixed glycerides. Fifty ml. of the fat are dissolved in an equal volume of acetone, and the solution is cooled to 15° and allowed to stand for an hour, then the crystals are rapidly filtered off and dried in a desiccator over sulphuric acid ; they are now re-dissolved and crystallised from ether. The melting point of the crystals is taken, then they are saponified, and the melting point of the liberated fatty acids is observed. The melting point of the glycerides should be more than 62° and of the fatty acids not less than 56°, whereas the corresponding figures for beef are much lower. The presence of hydrogenated fats may vitiate this test, but otherwise it is reliable when the amount of beef present is not less than 10 per cent., and is favourably reported on by Prescher (*Z. Unters. Nahr. Genussm.*, 1915, **29**, 433), who investigated many samples by this and other methods.

The following table shows the usual constants of some of the more likely adulterants of lard :—

	Lard.	Beef fat.	Cotton stearine.	Coconut oil.	Palm-kernel oil.
Saponification value .	192–200	194–200	194–199	245–265	245–255
Iodine value . .	51–66	35–44	88–92	8–10	10–15
Refractive index 40° .	1·458–1·461	1·455–1·459	1·475	1·440	1·440
Melting point of fatty acids . .	37°–47°	40°–44°	28°–37°	18°–25°	22°–28°
Reichert value .	Less than 1·0	Less than 1·0	Less than 1·0	6·7–8·4	5–6·5
Polenske value. .	Trace	Trace	—	16	10

In the examination of lard or other edible fats the question of rancidity may arise ; this may be due to many factors, including moisture, moulds and exposure to light and air. Theoretical explanations of its causes and chemical methods for its detection are not altogether satisfactory. It has been mentioned that the free acidity as oleic acid should not exceed 0·6 per cent., but acidity alone is not a true measure of rancidity. The best tests are those of Kreis and of Lea. Kreis's test as modified by Pyke (*Analyst*, 1935, **60,** 515) is applied by dissolving 0·5–1 g. of the fat in sufficient 1 per cent. phloroglucinol in acetone to give a 10 per cent. solution. One drop of sulphuric for each ml. of solution is added and the mixture placed in water at 10° for fifteen minutes. The colour is then read in a 1 cm. cell in a Lovibond tintometer. The lower limit is about 2 red units, corresponding to 0·014 per cent. of epihydrin aldehyde. Rancid fats give stronger red colours.

C. H. Lea (*Proc. Roy. Soc.*, 1931, B, **108,** 188) has shown the importance of peroxide oxygen as a measure of the oxidative rancidity of fats ; this may occur without the production of the epihydrin aldehyde to which the Kreis colour is due. For the test 1 g. of the fat is weighed into a stout test tube (27 × 2·7 cm.). One g. of powdered potassium iodide and 20 ml. of a mixture 2 volumes of glacial acetic acid with 1 volume of chloroform are added. A one-hole rubber stopper is inserted into the test tube and nitrogen or carbon dioxide passed into air space above the liquid for two minutes to displace all air. The inlet tube is withdrawn and the hole closed with the finger while the inclined tube is heated over a small flame until the liquid boils. When the liquid boils the tube is put into a boiling water bath, so that the chloroform boils briskly and the vapour begins to escape from the hole. Then the hole is plugged with a glass rod, the test tube quickly cooled under the tap and 25 ml. of water added, the tube well shaken and the liberated iodine titrated with freshly prepared 0·002 N sodium thiosulphate solution, using starch as indicator. A blank test should be made and its result should not exceed 0·2 ml. of the thiosulphate solution. The results may be expressed as ml. of 0·002 N per g. or calculated as millimols of peroxide oxygen per kilo. The determination may be carried out in subdued daylight without the use of inert gas.

Lard Substitutes

Lard substitutes, sold as such or under fancy names, are now quite common, as also are cooking fats. These mixtures may contain hydrogenated vegetable or whale oils together with perhaps coconut oil, stearin or tallow. They may be examined by the methods applicable to lard, with the addition of the Reichert-Polenske values. Lard substitutes do not usually contain water or any appreciable free fatty acid, but some cooking fats approximate more to butter or margarine in comparison, and include substantial proportions of water emulsified with the aid of lecithin or an alkyl cellulose.

Suet

Suet may be defined as the fatty tissue situated about the loins or kidneys of oxen or sheep ; it consists of an accumulation of microscopic particles of fat, each contained in a membranous bag. There is present about 1 per cent. of cellular tissue with some 4 per cent. of water and 95 per cent. of fat. The fat, when run out by heating, forms tallow. It is difficult or impossible to differentiate chemically between beef and mutton fats, though the superior taste and smell of beef may be recognised. The usual constants are given in the table, but these fats are not often adulterated.

Specific gravity at 15·5°	0·940–0·950
Melting point	47°–54°
Saponification value	193–200
Iodine value	35–44
Refractive index 40°	1·447–1·455
Reichert value	0·5–1·0

The microscopical appearance of the crystals obtained from an ether solution of these fats has been mentioned on p. 283.

Suet can be shredded or powdered because of the membranous substance present, whilst tallow cannot be so divided without the addition of starchy matter. This fact has led to the introduction in recent years of prepared suets. The usual method is by forcing the fat through fine holes so that it comes out in thin fragments, and in order to prevent the pieces from caking together again or becoming rancid rapidly by exposure to the air, rice flour is mixed with the product so that each fragment is coated with the starch. This addition enhances the keeping property of the flaked or shredded suet, and incidentally removes some of the moisture. The proportion of rice flour usually found necessary is from 11–17 per cent., as is shown by the following analyses of well-known brands. In 1931 (*Analyst*, **56,** 778) the Society of Public Analysts recommended that shredded suet should contain not less than 83 per cent. of fat, and that the nature of any admixture should be declared. This limit is adopted in S. R. & O. 1944, No. 45.

	1.	2.	3.	4.	5.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Beef fat .	84·86	85·40	78·85	83·75	83·35
Dry rice flour	12·70	12·30	19·50	14·20	14·10
Water .	2·44	2·30	1·65	2·05	2·55
	100·00	100·00	100·00	100·00	100·00

It is most important before analysing these mixtures to ensure uniformity of sample by emulsifying the whole pot or packet. The examination of the fat may be made by the methods already described ; water may be estimated by heating the mixture over a fine flame with constant stirring as in the case of butter, and the starch by extraction of all the fat with petroleum ether. It is convenient to filter a weighed quantity on a tared filter in the oven until most of the fat has run through, then to extract the filter and residue with petroleum spirit in a Soxhlet thimble.

Salad Oil, Olive Oil

The term " salad oil " is no longer synonymous with olive oil, so that, whilst the best qualities of salad oil are usually pure olive oil, a number of other vegetable oils of edible qualities are legitimately sold as salad oil. Mixtures containing cotton-seed, earthnut or sesamé oil are common. Attempts have been made also to market mineral oils, " paraffinum liquidum," or mixtures containing unsaponifiable fat ; such oils are quite without food value and may have physiological properties as has liquid paraffin, so that their sale, unless covered by a declaration of the composition, would certainly be an offence under the Food and Drugs Act.

The analytical examination of salad oil resolves itself into the

OILS COMMONLY OCCURRING IN SALAD OIL

	Specific gravity at 15·5°.	Unsaponifiable matter, per cent.	Saponification value.	Free fatty acids as oleic, per cent.	Iodine value.	Refractive index 40°.	Special tests.
Olive oil . .	0·915–0·918	0·5–1·0	190–195	0·3–1·0	79– 88	1·4605–1·4635	—
Arachis oil .	0·916–0·920	0·4–1·0	188–196	0·4–1·0	85– 99	1·4625–1·4645	Bellier's test (p. 294).
Rape oil . .	0·914–0·916	0·4–1·0	170–176	0·3–2·0	95–105	1·4653–1·4660	Tortelli and Fortini's (p. 290).
Sesamé oil .	0·921–0·924	0·7–1·2	188–193	0·4–2·0	103–114	1·4650–1·4665	Baudouin's test (p. 282).
Almond oil .	0·915–0·920	0·4–1·0	188–196	0·4–1·0	95–100	1·4624–1·4650	Bieber's nitric acid test (p. 295).
Peach-kernel oil	0·918–0·920	0·5–1·0	189–192	0·4–1·0	93–105	1·4640–1·4650	„ „
Apricot-kernel oil	0·918–0·921	0·5–1·0	188–192	0·4–1·0	98–110	1·4640–1·4650	„ „
Sunflower oil .	0·924–0·926	0·5–1·0	190–194	0·5–2·0	125–140	1·4660–1·4680	—
Poppyseed oil .	0·924–0·926	0·5–1·5	192–196	0·5–2·0	130–140	1·4680–1·4690	—
Cotton-seed oil .	0·922–0·925	0·4–1·0	192–196	0·5–1·5	105–115	1·4640–1·4655	Halphen's test (p. 282).

determination of the usual constants and the unsaponifiable matter supplemented by special tests for particular oils. The table on p. 288 shows the constants for those oils which are likely to be met with in this connection.

It will be seen from the table that the constants for several of these oils are so similar that it is not always possible to identify an unknown one of them by this means, or even to be certain of the purity of a given sample. It is essential, therefore, to supplement the examination by special tests. Halphen's reaction will reveal cotton-seed soil, Baudouin's test sesamé oil, and Bellier's test as modified by Evers (*Analyst*, 1912, **37**, 487) will give a quantitative indication of arachis oil when present in vegetable oil mixtures; this test is not applicable quantitatively in the presence of animal fats.

Five g. of the oil are saponified under a reflux condenser with 25 ml. alcoholic potash (80 g. potassium hydroxide dissolved in 80 ml. of water and diluted to 1 litre with 90 per cent. alcohol). To the hot solution is added 7·5 ml. of acetic acid (1 volume of glacial acetic acid to 2 volumes of water) and 100 ml. of 70 per cent. alcohol, containing 1 per cent. of hydrochloric acid by volume. The solution is cooled to 12°–14° for an hour, then filtered and washed with acidified 70 per cent. alcohol, the washing being continued until the filtrate gives no turbidity with water. The total volume of wash liquid should be measured. The precipitate is dissolved according to its bulk in 25–70 ml. of hot 90 per cent. alcohol and cooled to a fixed temperature between 15° and 20°. The solution is allowed to stand at this temperature for three hours, filtered and the crystals washed with a measured volume of 90 per cent. alcohol and finally with 50 ml. of 70 per cent. alcohol. Then the crystals are washed into a tared flask with ether, dried and weighed. If the melting point is lower than 71° the product should be re-crystallised from 90 per cent. alcohol. To the weight of arachidic and lignoceric acid so obtained is added a correction for the solubility in 90 per cent. alcohol plus a correction for the total volume of 70 per cent. alcohol used. The corrected weight of acids is multiplied by the factor 17, 20 or 22 according as the melting point is 71°, 72° or 73° to convert it to percentage of arachis oil. The corrections to be added for each 100 ml. are (1) for solubility in 90 per cent. alcohol:—

Weight of Mixed Acids.	Temperature of Washing Liquor.		
	15°	17·5°	20°
0·05 gm.	+ 0·031	0·040	0·046
0·10 ,,	0·036	0·045	0·052
0·20 ,,	0·048	0·056	0·062
0·40 ,,	0·061	0·071	0·078
0·60 ,,	0·066	0·080	0·088
0·80 ,,	0·069	0·083	0·092
1·00 ,,	0·071	0·084	0·091

and (2) for the 70 per cent. alcohol :—

Weight of Acids corrected for 90 per cent. Alcohol.	Melting Point of Crystals.		
	71°	72°	73
Less than 0·02 gm.	0·006	0·005	0·0
0·02–0·05	0·007	0·006	0·0
0·05–0·08	0·009	0·007	0·0
0·08–0·10	0·011	0·007	0·0
Over 0·10	0·013	0·008	0·0

For the detection of rape oil, Tortelli and Fortini (*Chem. Zeit.*, 1910, **24**, 689), proceed as follows : Twenty g. of the oil are saponified with 6 g. of potassium hydroxide in 50 ml. of alcohol and the solution neutralised to phenolphthalein by titration with 10 per cent. acetic acid, then slowly poured into 300 ml. of boiling water containing 20 g. of lead acetate. The solution is cooled while being rotated so as to cause the soap to stick to the sides of the beaker, then the water is poured off and the soap washed three times with 200 ml. of warm water (60°–70°) and dried with filter paper. The dried soap is heated and shaken with 80 ml. of ether under a reflux condenser for half an hour, then the flask is cooled, corked and kept at exactly 15° for an hour. The solution is now filtered into a separator, the funnel being kept covered as closely as possible to avoid loss by evaporation ; the filter and residue are re-treated with 40 ml. of ether, cooled again for an hour and filtered into the separator. The mixed ether solution of the lead soap is decomposed by 150 ml. of 10 per cent. hydrochloric acid ; then the ether is washed with two 100 ml. quantities of water and evaporated at a low temperature (spontaneous evaporation is best) in a dry flask. The liquid acids are dissolved in 40 ml. of strong alcohol and a slight excess of saturated aqueous solution of sodium carbonate is added, *i.e.*, until sodium carbonate just begins to separate. The alcohol is now distilled off and the residual sodium soap is dried in a vacuum desiccator for forty-eight hours, then heated with 50 ml. of absolute alcohol and quickly filtered ; the residue is re-treated with absolute alcohol until all the soap is dissolved. The alcoholic soap solution is evaporated and the residue dried over sulphuric acid. A quantity of 0·5 g. of the dry soap is dissolved in a large test tube by heating with 20 ml. of absolute alcohol ; then the solution is cooled slowly while it is stirred with a thermometer, and the temperature at which it becomes turbid is noted. The turbidity temperatures are as under :—

Oil.	Turbidity temperature.
Olive oil	24°–20°
Rape oil	50°–45°
Olive + 50 per cent. rape oil	40°–35°
+ 30 ,, ,,	35°–30°
+ 20 ,, ,,	35°–30°
+ 10 ,, ,,	34°–30°
Sesamé oil	20°–18°
Arachis oil	22°–18°
Cotton-seed oil	16°–14°

The form of the deposit also may afford some information ; it is flocculent and gelatinous in the case of olive oil, whilst in the case of rape oil it consists of minute crystalline needles which float in the liquid.

The following tests should be applied to the oils named, but it should be remembered that other kinds of oil may appear on the market from time to time and find sale as salad oil :—

Olive Oil

The B.P. prescribes that this oil is that expressed from the ripe fruit of *Olea europæa.* It should have specific gravity 0·915–0·918, saponification value 190–195, iodine value 79–88, acid value not more than 2·0, and refractive index at 40°, 1·4605–1·4635. It should give negative results with Halphen's test and with the following (Bellier's) test for arachis oil: 1 ml. of the oil is saponified under a reflux condenser for five minutes with 1·5 ml. of N alcoholic potassium hydroxide solution, then there is added 1·5 ml. of acetic acid and 50 ml. of 70 per cent. alcohol. The solution is warmed until it is clear, then set aside with a thermometer in it and cooled to 15·5°. If at this temperature it remains clear arachis oil is absent.

Olive oil usually deposits a stearine when cooled below 10°. If arachis oil is suspected or is indicated by the B.P. test, Evers' modified Bellier test should be applied. Lard oil is another possible adulterant; it may be detected by examining microscopically the crystals deposited from an ether solution cooled to 0°. Tea-seed oil is sometimes added to olive oil; its presence may often be detected by the nitric acid test, but this is not always reliable. To 10 ml. of the oil are added 10 ml. of a mixture of equal parts by weight of nitric and sulphuric acids; the mixture is shaken and heated in boiling water for a quarter of an hour, then allowed to cool. Pure olive oil gives a yellowish mass, whereas oil containing 20 per cent. or more of tea-seed oil gives a cherry-red colour. The physical constants of tea-seed oil are closely similar to those of olive oil, except that the refractive index is a trifle higher—1·4635–1·4645 at 40°—so that its detection is a matter of great difficulty. Bolton and Williams (*Analyst*, 1930, **55,** 5) have used the iodine value of the unsaponifiable portion as a test for the tea-seed oil, but this too is not infallible as a means of detecting admixture.

Arachis Oil

This oil, which is also known as earthnut oil, groundnut oil, or peanut oil, is expressed from the seeds of *Arachis hypogæa*. The B.P. describes it as having specific gravity 0·916–0·920, saponification value 188–196, iodine value 85–99, acid value not more than 4, refractive index at 40°, 1·4625–1·4645. When Bellier's test is applied in the manner described under olive oil, the temperature at which the solution becomes turbid should not be lower than 39°. It should give negative results with Halphen's and Baudouin's reactions. Evers' test (p. 289) is available for its quantitative estimation in admixture with other vegetable oils.

Rape Oil

This oil is not included in the B.P. It is expressed from the seeds of various species of *Brassica* and is known in commerce as colza oil. Its constants are given in the table on p. 288, from which it will be seen that the saponification value is lower than that of the other vegetable oils ; as it may be adulterated with mineral oil, it is necessary to determine the unsaponifiable matter. Rape oil is of unusually high viscosity for a vegetable oil ; for this reason it is used as a standard in Redwood's viscometer, and this determination may be of service in the detection of adulterants, as a low value would indicate the presence of some other oil. Tortelli and Fortini's method, by which it may be estimated, has been given on p. 290. The hexabromide test is useful for the detection of fish oil. To 0·5 ml. of the oil dissolved in 10 ml. of dry ether are added 10 ml. of a mixture of 28 volumes of glacial acetic acid, 1 volume of bromine, and 4 volumes of nitrobenzene. The mixture is vigorously shaken. If only rape oil is present, the mixture remains clear, but in the presence of fish oil or a drying oil such as linseed, a precipitate will soon appear. If this test be applied to the oil directly without dilution with ether, drying oils or fish oils give an immediate precipitate, rape oil and soya-bean oil give a turbidity, whereas other non-drying vegetable oils give no precipitate or only the slightest turbidity. Animal fats may give a turbidity when tested in this way.

Sesamé Oil

The B.P. describes this as the oil expressed from the seeds of *Sesamum indicum*. According to this authority it has specific gravity 0·921–0·924, saponification value 188–193, iodine value 103–114, acid value not more than 4, refractive index at 40°, 1·4650–1·4665. It should respond to Baudouin's reaction (p. 282), but not to Halphen's test nor to the test described for arachis oil in olive oil. It may be noted that the chromogenic substances which produce Baudouin's reaction are destroyed by heating the oil to 200° or more for half an hour. In Germany and Denmark the law requires the presence of at least 10 per cent. of sesamé oil in margarine fat.

Almond Oil, Peach-kernel Oil, and Apricot-kernel Oil

These three oils are so closely similar that their distinction rests almost entirely upon the colour reactions, which must be interpreted with caution. The B.P. describes almond oil as that expressed from the bitter or sweet almond and gives the following specification: pale yellow, nearly inodorous, taste bland and nutty; specific gravity 0·915–0·920, saponification value 188–196, iodine value 95–100, acid value not more than 4, refractive index at 40°, 1·4624–1·4650; it remains clear after exposure for three hours at – 10° and does not congeal until the temperature has been reduced to about – 18°. When 1 ml. of a freshly prepared mixture of equal parts by weight of sulphuric acid, fuming nitric acid and water, kept cool while cautiously mixed, is vigorously shaken with 5 ml. of the oil for one minute a whitish mixture with not more than the slightest tinge of red or brown is produced; after some hours a whitish solid sometimes tinged with green separates, the lower acid layer remaining colourless (absence of peach oil or apricot oil).[1]

For the detection of arachis oil in almond oil Evers' modification of Bellier's test is more sensitive than that given in the B.P. (*Analyst*, 1937, **62,** 96). One ml. of the oil is saponified with 5 ml. of 1·5 N alcoholic potassium hydroxide solution by heating on a water bath for five minutes, avoiding loss of alcohol; 50 ml. of 70 per cent. alcohol are added, followed by 0·8 ml. of hydrochloric acid (specific gravity 1·16). After heating to dissolve any precipitate that may be formed the solution is cooled in water, stirring continuously with a thermometer, so that the temperature falls at the rate of about 1° C. per minute. If a turbidity appears before the temperature reaches 9° C., the usual confirmatory test for arachis oil must be applied; if the liquid remains clear at this temperature, arachis oil may be regarded as absent.

It is essential that the stirring should be continuous, since local cooling will cause the premature formation of a turbidity. For this reason the cooling water should not rise above the level of the liquid in the flask.

The table shows the results obtained with various oils. The turbidity temperature of arachis oil itself is remarkably constant. Thirteen samples of various origins and grades examined by Evers

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[1] Bieber's test.

all gave turbidity temperatures of 39°–40° C. The test thus forms a guide to the purity of arachis oil.

Oil.	Clouding-point. ° C.
Arachis (thirteen samples)	39·0–40·0
Rape	22·5
Sesamé	15
Cottonseed	13
Olive-kernel	10
Maize	7·5
Almond	- 1 to + 1
Apricot-kernel	4 to − 8

In the absence of oils other than olive and arachis the turbidity temperature is a quantitative measure of the arachis oil present

Peach-kernel oil is often sold as a substitute for almond oil, sometimes under the name of *Oleum amygdalæ persicæ* or kernel oil. *Oleum persicæ* is not true almond oil ; *Amygdalus persicæ* is the peach and not an almond. It is not officinal ; its chemical and physical characters have been given in the table on p. 288. Treated with nitric and sulphuric acids as described under almond oil it gives a pink colour after standing for half an hour. Shaken with an equal volume of concentrated nitric acid, almond oil gives a pale yellowish-brown colour, whereas peach-kernel oil gives a darker orange colour.

Apricot-kernel oil is hardly distinguishable from the peach-kernel oil. It is obtained from *Prunus amygdalus armenica*, and shows only a faint pink with the B.P. nitric acid test and an orange yellow when shaken with nitric acid of specific gravity 1·42, as described above. The iodine value of apricot-kernel oil is rather higher than that of peach-kernel or almond oil.

Ground almonds are prepared from the sweet almond, often with some small proportion of the bitter almond. The product normally contains :—

Fixed oil	55–60 per cent.
Ash	3–4 ,,
Moisture	4–6 ,,

If any bitter almond is present there will also be a small proportion of hydrocyanic acid. Processes exist for the de-bittering of bitter almonds, and it is possible that some ground almonds contain such a product. The principal analytical problem presented by ground almonds is the detection of foreign nuts.

Two methods are available; first, a microscopic examination of the de-fatted material, and second, tests on the extracted oil. Almonds are said to contain no starch; nevertheless, on microscopical examination one or two small starch grains may be found in most fields. Barcelona nuts, cob nuts and peanuts contain larger amounts of starch, so that if they are present in any substantial quantity the starch is easily detected. Chestnuts contain much starch. Examination of the ash is of interest, for although the total amount of the ash of the different nuts is not of much diagnostic value, cob nuts and others contain traces of manganese, and Brazil nuts contain barium, whereas almonds contain no barium, and only a minute quantity of manganese. For the examination of the oil, it is better to separate some by pressure rather than by solvent. If a solvent is used, particular care should be taken to remove all of it before applying the Bieber test and Evers' test, as quoted above.

Sunflower Oil

This oil comes from several parts of the world, as the plant, *Helianthus annuus*, from the seeds of which it is obtained, grows in almost any temperate or sub-tropical climate. There are no special tests available for its identification other than the constants given on p. 288. The usual tests for adulterants may be applied; but such are not very likely to be found.

Poppyseed Oil

This is obtained from the seeds of the opium poppy, *Papaver somniferum*. There are no special tests for this oil, but reference to the table on p. 288 shows that it has a higher iodine value and refractive index than the other oils of this class. It may occur as an adulterant of olive oil. Utz has pointed out that it sometimes contains small amounts of sesamé oil, not added as an adulterant, but due to careless methods of manufacture; such an oil gives a slight reaction with Baudouin's test.

Cotton-seed Oil

The source of this well-known oil is the seed of various species of *Gossypium*. The crude oil has a dark colour and characteristic smell, but that met with as salad oil or similar preparation is usually more or less refined, though its taste and smell are always recognisable. Apart from the usual constants, which are not widely different from those of other oils of its class, it is easily identified by Halphen's reaction, p. 282. The only other oils which behave similarly are kapok and baobab, and these are not very common ; they do not give Becchi's reaction, p. 282. As with sesamé oil, the chromogenic substances are wholly or partly destroyed by exposure to a high temperature or by hydrogenation. If a few drops of 70 per cent. sulphuric acid are added to 1 ml. of the oil diluted with 10 ml. of carbon disulphide, a reddish-brown colour is produced. When shaken with an equal volume of nitric acid and allowed to stand for some hours, a coffee-brown colour appears.

The titre of cotton-seed fatty acids is unusually high, generally upwards of 32°.

Determinations of the Fat-Soluble Vitamins

It is frequently necessary to estimate the content of certain vitamins in edible fats or in the fatty portion extracted from foods. Of these vitamins A and E are readily determined by physical or chemical methods. No general method, other than biological, is yet available for vitamin D. For the determination of vitamin A two processes are available, the Carr-Price blue value which is very useful as a preliminary test, and the spectrophotometric method which, although it involves expensive instruments, is much preferable as it is more specific and more accurate. The blue value being a quicker test, is useful in finding the approximate quantity of vitamin A present, so that the appropriate dilution can be made for the spectroscopic method. The International Unit of vitamin A is defined as the activity of 0·6 μg of β-carotene. When the expected concentration of vitamin in the oil is 10,000 I.U.'s or more, the blue value or the absorption spectrum can be determined directly, but with most samples, other than such concentrates, determination should be made on the unsaponifiable matter to eliminate interfering substances. As some vitamin may be lost during the ordinary process of determining unsaponifiable matter, it is desirable to use a special technique avoiding oxidation and other losses.

One g. of the fat is saponified with 10 ml. of 0·5 N alcoholic potassium hydroxide solution, which should be freshly prepared, for ten minutes ; then 20 ml. of water are added and the cool mixture is extracted with two successive portions of 25 ml. of peroxide-free ether. The mixed ether extract is washed twice with 10 ml. of water, then with 10 ml. of 0·5 N KOH, and once with water by gentle rotation without shaking, then twice with water, shaking as usual. The ether solution is evaporated, preferably using a current of nitrogen or carbon dioxide. The unsaponifiable matter is dissolved in 10 ml. of pure chloroform or purified cyclohexane. It is then necessary to make an appropriate dilution ; if the amount of vitamin expected is known the following concentrations, expressed on the weight of the original fat, are convenient :—

I.U.'s per g.	For blue value.	For spectrophotometer (1 cm. cell)
500	10 per cent.	3 per cent.
1,000	6 ,,	1·5 ,,
5,000	1·2 ,,	0·3 ,,
10,000	0·6 ,,	0·15 ,,

The appropriate dilution is made with chloroform or cyclohexane for the blue value, or pure ethyl alcohol may be used for the spectrophotometric method.

To observe the blue value pipette 0·2 ml. of the diluted solution into a 1 cm. tintometer cell, add 2 ml. of antimony chloride reagent,[1] and at once match the colour against Lovibond blue glasses ; the observation should be made of the maximum intensity, which is usually reached within about thirty seconds, and for maximum accuracy the value should lie between 4 and 6 blue units. The blue value is then calculated to 1 g. of the sample and this figure multiplied by 1·28 gives the I.U.'s.

For the spectrophotometric test, a 1 cm. cell with quartz ends is filled with the appropriately diluted solution and the extinction coefficient E is determined in the usual way, according to the particular type of spectrophotometer, at wave length 325 mμ (formerly 328 mμ was much used). The E value observed is calculated to $E_{1\,\text{cm.}}^{1\%}$ and this figure multiplied by 1,600 gives International Units per gram. Longer or shorter cells may be used if necessary to bring the observed E within the optimum range of measurement which with the Hilger instrument is about 1·0. Fats having a very low vitamin A potency can be treated as described under margarine, p. 272.

Vitamin E is also to be found in the fatty portion of some foods. The unit is 1·0 mg. of standard α-tocopheryl acetate ; this is

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[1] Antimony chloride reagent is a 22 per cent. w/v solution of antimony trichloride in pure dry chloroform ; it should be stored in the dark.

equivalent to 0·91 mg. of α-tocopherol. For the determination the method of Emmerie and Engel (*Rec. Trav. Chim.*, 1938, **57,** 1351) is used. This depends upon the oxidation of the tocopherol in alcoholic solution by ferric chloride and the subsequent measurement of the resultant ferrous iron with $\alpha\alpha'$-dipyridyl. The unsaponifiable matter from the fat is extracted as described under vitamin A, dissolved in pure alcohol and diluted to such volume that 1 ml. contains about 0·1 mg. of tocopherol. The unsaponifiable matter may be more easily dissolved by adding 2 ml. of benzene, then diluting with alcohol. A standard solution of α-tocopherol acetate is prepared by hydrolysing 0·1 g. with alcoholic potassium hydroxide, neutralising and diluting with alcohol to 100 ml.; to portions of this from 0·1 to 1·0 ml. are added 1 ml. of 0·2 per cent. alcoholic solution of ferric chloride and 1·0 ml. of alcoholic solution of $\alpha\alpha'$-dipyridyl and the mixtures diluted to 25 ml. with alcohol. The reagents should be freshly prepared. After standing for about ten minutes the red colour is measured, preferable with a photometer, and a graph prepared showing the colour corresponding to the different quantities of tocopherol. To an aliquot part of the solution of the unsaponifiable matter are added the same quantities of the reagents, the solution is made up to 25 ml. and the red colour is measured. The amount of vitamin E is then read off from the standard curve.

International Units of Vitamins

It may be convenient to tabulate the International Units in which vitamins are expressed and the units in which they are to be expressed for purposes of the Labelling of Food Order.

Vitamin A .	1 I.U. = 0·6 μg of β-carotene.
Vitamin B$_1$.	1 I.U. = 3 μg of aneurine hydrochloride. Vitamin B$_1$ is to be declared as milligrams of aneurine hydrochloride.
Vitamin B$_2$.	No. I.U., but is declared as milligrams of riboflavin or of nicotinic acid respectively.
Vitamin C .	1 I.U. = 50 μg of ascorbic acid; to be declared as milligrams of ascorbic acid.
Vitamin D .	1 I.U. = 1 μg of standard irradiated ergosterol.
Vitamin D$_2$.	1 I.U. is generally agreed to be equivalent to 0·025 μg of calciferol; to be declared as I.U.'s of vitamin D.

Vitamin E . 1 I.U. = 1 mgm. α-tocopheryl acetate.

$1\ \mu g = 1\gamma = 10^{-6}$ g.

The following table, which includes the figures laid down by the Third Session of the Technical Commission on Nutrition, 1937, of the League of Nations Health Organisation, shows the normal daily requirement of the vitamins and minerals scheduled in the Labelling of Food Order :—

Vitamin A	3,000 I.U.	Vitamin D	500 I.U.
Vitamin B_1	300 I.U.	Calcium	0·75 gram.
Vitamin B_2	1·8 mg.	Iodine	0·1 mg.
Nicotinic acid	12·0 mg.	Iron	10·0 mg.
Vitamin C	30·0 mg.	Phosphorus	0·75 gram.

Determination of Iodine

The estimation of vitamins A, B, C and E has already been outlined. Calcium, iron and phosphorus may be determined in the ash of most foods by well-known methods, but the determination of iodine calls for special precautions as it is usually present only in quite small amounts. The following method is satisfactory for most products, with such slight modifications as are needed for particular substances. Five to ten grams of the oil or non-fatty food are mixed with excess of alcoholic potassium hydroxide solution and heated on the water-bath to complete the saponification of any fat which is present, and fix the iodine which may be in organic combination. The mass is then evaporated to dryness on the water-bath in a nickel or platinum basin, covered with a lid and gently ignited, preferably in a muffle furnace, gradually raising the temperature not higher than 600°, until the whole is thoroughly charred. This may take two or three hours. To the charred mass when cool add 100 ml. of water, boil gently for ten minutes and filter. Re-extract the residue with a further 100 ml. of water, mix the extracts and evaporate to about 50 ml., add dilute sulphuric acid until the liquid is just acid to methyl orange, then add 0·2 ml. of bromine and boil off the excess of bromine. Cool to room temperature, transfer to a stoppered bottle and add a crystal of potassium iodide. The liberated iodine if in sufficient quantity may be titrated with 0·02N sodium thiosulphate (1 ml. 0·02N = 0·424 mgm. iodine), or if too small for titration may be shaken out with 10 ml. of carbon tetrachloride and matched colorimetrically with known quantities of iodine. It is desirable to make a blank test on the reagents used.

APPENDIX

In the Public Health (Preservatives, etc., in Food) Regulations, 1925,[1] made by the Minister of Health, it is laid down that (Part II.) :—

(1) No person shall manufacture for sale or sell any article of food which contains any added preservative[2] or any added colouring matter being one of those specified in Part II. of the First Schedule to these Regulations :—

Provided that—

(i.) any article of food specified in Part I. of the said Schedule may contain preservative of the nature and in the proportion therein specified ;

(ii.) where an article of food specified in Part I. of the said Schedule is used in the preparation of any other article of food, the latter article may contain any preservative necessarily introduced by the use of the former article, but the total proportion of any one preservative contained in any article of food specified in that Part of the Schedule shall not exceed the proportion therein specified.

No person shall sell cream which contains any thickening substance.

" Preservative " means any substance which is capable of inhibiting, retarding or arresting the process of fermentation, acidification, or other decomposition of food or of masking any of the evidences of putrefaction ; but does not include common salt (sodium chloride), saltpetre (sodium or potassium nitrate), sugars, lactic acid, acetic acid or vinegar, glycerine, alcohol or potable spirits, herbs, hop extract, spices and

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[1] Amended 1926 and 1927.

[2] Amended S. R. & O. 1944, No. 1358, permits the addition of Boric acid 0·25 per cent. to margarine and in bacon.

essential oils used for flavouring purposes or any substance added to food by the process of curing known as smoking ;

" Thickening substance " means sucrate of lime, gelatine, starch paste or any other substance, which when added to cream is capable of increasing its viscosity, but does not include cane or beet sugar;

" Sulphur dioxide " includes sulphites, and " benzoic acid " includes benzoates.

Percentages shall be calculated by weight.

Sulphite shall be calculated as sulphur dioxide (SO_2) and benzoates as benzoic acid (C_6H_5COOH).

THE FIRST SCHEDULE

PART I

Articles of Food which may contain Preservative, and Nature and Proportion of Preservative in each Case.

The articles of food specified in the first column of the following table may contain the preservative specified in the second column in proportions not exceeding the number of parts (estimated by weight) per million specified in the third column :—

Food.	Preservative.	Parts per Million.
1. Sausages and sausage meat containing raw meat, cereals and condiments.	Sulphur dioxide .	450
2. Fruit and fruit pulp (not dried) for conversion into jam or crystallised glacé or cured fruit :		
(a) Cherries	Do.	3,000
(b) Strawberries and raspberries .	Do.	2,000
(c) Other fruit	Do.	1,500
3. Dried fruit :		
(a) Apricots, peaches, nectarines, apples and pears.	Sulphur dioxide	2,000
(b) Raisins and sultanas . .	Do.	750

Food.	Preservative.	Parts per Million.
4. Unfermented grape juice and non-alcoholic wine made from such grape juice if labelled in accordance with the rules contained in the Second Schedule to these Regulations.	Benzoic acid	2,000
5. Other non-alcoholic wines, cordials and fruit juices, sweetened or unsweetened.	Either sulphur dioxide . or benzoic acid .	 350 600
6. Jam (including marmalade and fruit jelly prepared in the way in which jam is prepared, but not including marmalade made from citrous fruits).	Sulphur dioxide .	40
7. Crystallised glacé or cured fruit, including candied peel.	Do.	100
(*a*) Fruit and fruit pulp not otherwise specified in this Schedule.	Do.	350
8. Sugar (including solid glucose and cane syrups).	Do.	70
(*a*) Cornflour (maize starch) and other prepared starches.	Do.	100
9. Corn syrup (liquid glucose) . . .	Do.	450
10. Gelatine	Do.	1,000
11. Beer	Do.	70
12. Cider	Do.	200
13. Alcoholic wines	Do.	450
14. Sweetened mineral waters . . .	Either sulphur dioxide . or benzoic acid .	 70 120
15. Brewed ginger beer	Benzoic acid .	120
16. Coffee extract	Do.	450
17. Pickles and sauces made from fruit or vegetables.	Do.	250

PART II

Colouring Matters which may not be Added to Articles of Food.

1. *Metallic Colouring Matters.*

Compounds of any of the following metals : antimony, arsenic, cadmium, chromium, copper, mercury, lead, zinc.

2. *Vegetable Colouring Matter.*

Gamboge.

3. *Coal Tar Colours.*

Number in colour index of Society of Dyers and Colourists, 1924.	Name.	Synonyms.
7	Picric acid . .	Carbazotic acid.
8	Victoria yellow .	Saffron substitute, Dinitrocresol.
9	Manchester yellow .	Naphthol yellow, Martius yellow.
12	Aurantia . .	Imperial yellow.
724	Aurine. . .	Rosolic acid, Yellow coralline.

THE SECOND SCHEDULE.

Labelling of Articles of Food containing Preservatives and of Preservatives.

1. The articles of food containing preservative to which the rules as to labelling set out in this Schedule apply are :—Sausages, sausage meat, coffee extract, pickles and sauces, and (where the proportion of benzoic acid exceeds 600 parts per million) grape juice and wine.

2. Any of the said articles of food which contain preservative shall bear a label on which is printed the following declaration, or such other declaration substantially to the like effect as may be allowed by the Minister :—

(*a*) contains(s)
preservative

The declaration shall be completed by inserting at (*a*) the word 'this' or 'these' followed by the name of the food as used in paragraph 1 of this Schedule.

3. (1) The declaration on the label of an article sold as a preservative shall be in the following form :—

This preservative contains
(*a*) per cent. of sulphur
dioxide.

(2) Where the preservative contains benzoic acid the words " benzoic acid " shall be substituted for the words " sulphur dioxide."

(3) The declaration shall be completed by inserting at (*a*) in words and figures, excluding fractions, the true percentage of the sulphur dioxide or benzoic acid present in the article.

No instructions are given as to the methods of the separation or identification of the five colours on the prohibited list. The following well-known procedure is the best general method for the detection of dyes in foodstuffs ; it serves well for most dyes, but as Nicholls has shown, does not work well with the prohibited colours. About 100 ml. of liquid or watery mixture of the foodstuff are acidified with 1 ml. of hydrochloric acid and boiled for five minutes with a strip of thin woollen fabric (previously de-fatted). The coloured wool is rinsed in cold water, then the colour is stripped from the fibre by boiling it in 2 per cent. ammonia for a few minutes. The wool is removed and replaced by a new piece ; the solution is now acidified and again boiled to transfer the dye to the wool fabric. In the case of fruit juices or jams it is advisable to purify the colour again by a further transference to another piece of wool. The dyed wool is subjected to the necessary tests for identifying the dye.

Suitable tests are given by A. G. Green (" Analysis of Dyestuffs," C. Griffin, London), and more recent dyes are included in the tables elaborated by E. Clayton (*J. Soc. Dyers and Colorists*, 1937, **53,** 178).

A comprehensive scheme for the detection of the prohibited colours has been worked out by J. R. Nicholls (*Analyst*, 1927, **52,** 585), by whose courtesy the essential parts are reproduced on pp. 308 and 309.

Make an ammoniacal extract of the foodstuff by any suitable means, filtering if necessary and using as large a quantity as is

Extract from methylated ether is coloured yellow by picric acid; yellow to orange by Victoria yellow, Manchester yellow and gamboge; red by aurine.

(A) To 1 ml. of solution add 1 ml. alcohol, 1 ml. meth. ether and 1 ml. 30 per cent. sodium hydroxide solution in that order, and shake.

Ethereal solution is colourless and soda solution is pink if aurine is present. Confirm by tests 11 and 21.

Ethereal solution is yellow and soda solution colourless if others are present.

(B) To 1 ml. of solution add 2 ml. of 30 per cent. sodium hydroxide solution, mix and leave for one minute. Add 1 ml. methylated ether and 1 ml. alcohol and shake.

Extract from petroleum spirit and methylated ether is orange-red if aurantia is present. Apply tests 10 and 19.

Ethereal solution is colourless and soda solution is (*a*) yellow if picric acid is present, (*b*) pink if aurine is present. A yellow colour in the soda layer may not be seen if both are present. In that event apply the confirmatory tests for picric acid, viz., tests 3, 9, 11, and the formation of picrates with naphthalene, quinine, etc.

Ethereal solution is yellow and soda solution colourless if others are present.

(C) To original solution add acid and extract with petroleum spirit.

Ether coloured yellow indicates gamboge. Confirm by tests 21 and 13, 14, 15 (if necessary concentrating the solution).

Victoria yellow and Manchester yellow give colourless petroleum spirit, but on removing this and adding petroleum spirit which has been shaken with ammonia a yellow turbidity is obtained. (Gamboge also gives this.)

Victoria yellow. Apply test 12.

Manchester yellow. Apply test 17.

convenient. To the extract add 1 drop of methyl orange solution, neutralise and make acid to the extent of 0·01 N to 0·02 N. Extract once or twice with ether, then extract the ether with successive quantities of 5 ml. to 10 ml. of 0·01 N sodium hydroxide solution until no more colour is removed. Add an equal quantity of petroleum spirit to the methylated ether and again extract with dilute sodium hydroxide solution.

Absence of colour in alkaline layers indicates absence of prohibited dyes.

The confirmatory tests are shown in the following table :—

Tests on Dilute Soda Solutions obtained in Scheme for Detection of Prohibited Dyes.	Picric Acid.	Victoria Yellow.	Naphthol Yellow.	Aurantia.	Aurine.	Gamboge.
	Trinitrophenol.	Dinitrocresol (*o*- and -*p*).	Dinitronaphthol.	Hexanitrodiphenylamine.	Trihydroxytriphenylmethane.	
(1) Melting point of ethereal extract from acid soln. (from literature).	121°–122·5° C.	85°–86° C., or 80·5° C	138° C	238° C., with decomp.	Not melted at 220° C.	75°–80° C.
(2) Colour of weakly alkaline solution :						
Strong in colour	Yellow.	Orange yellow.	Orange yellow.	Orange red.	Red	Orange red.
Weak in colour	Yellow.	Yellow.	Yellow.	Orange yellow.	Red.	Yellow.
(3) Taste of soln. diluted till almost colourless . .	Bitter.	—	—	—	—	—
(4) Strong soda added to alkaline soln. . . .	Deepens considerably.	No change.	No change.	Reddens slightly.	No change.	Slightly turbid.
(5) Excess of salt added to alkaline soln. . . .	No change.	No change.	No change	No change.	No change.	Soda salt thrown out as reddish yellow ppt.
(6) Dilute acid added to alkaline soln. :						
Strong .	No change.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt	Yellow ppt., which on boiling collects as brown drops.
Weak .	No change.	Colourless.	Colourless.	Colourless.	Yellow soln.	Colourless.
(7) To 1 vol. add 10 vols. concentrated sulphuric acid	Colourless.	Colourless.	Faint yellow.	Faint yellow.	Yellow.	Brownish.
(8) Alk. soln. boiled with ammonium sulphide . .	Deep reddish-brown.	Faint reddish-brown	Faint reddish-brown	Deep brown.	No change.	No change.
(9) Alk. soln. boiled with KCN.	Deep reddish-brown.	Deepens to orange-brown.	Deepens.	Dull brown.	Slowly discoloured.	No change.
(10) Acid soln. boiled with $SnCl_2$	Decolorised.	Decolorised.	Decolorised.	Yellowish-brown to deep reddish-brown	No change.	No change.
(11) Boiled with zinc dust and ammonia . . .	Deepens to reddish-brown, then gradually decolorised.	Decolorised	Decolorised.	Decolorised.	Decolorised.	Colour slightly fades to yellow.
Re-oxidised with sodium persulphate . . .	No change.	No change.	No change.	No change.	Colour partially returns.	No change.
(12) Boiled with ferrous sulphate and ammonia for one minute : filtered.						
Filtrate	Deep red.	Yellow.	Orange.	Brownish.	Practically decolorised.	Yellowish.
Filtrate acidified	Yellow.	Rose pink to deep red.	Orange.	Brownish-red.	Faint yellow.	Yellowish.

(13) Soln. treated with lead acetate	No change.	Yellow to yellow orange when warmed with acetic.	No change.	No change.	No change.	Reddish-yellow ppt.
(14) ,, ,, ,, zinc acetate	No change.	Ditto.	No change.	No change.	No change.	Yellow ppt.
(15) ,, ,, ,, barium chloride	No change.	No change.	No change.	No change.	No change.	Reddish ppt.
(16) To 1 vol. of the alk. soln. add ½ vol. of concentrated sulphuric acid and a little solid permanganate. Boil for one minute and then decolorise the soln. by the addition, drop by drop, of sodium sulphite soln. Add a few crystals of resorcinol and boil gently until the water has evaporated and fuming starts. Pour into water and extract once with ether. Wash the separated ether with water, discarding the latter, and then shake the ether with a little dilute ammonia soln.	—	—	Strong green fluorescence.	—	—	—
(17) To 1 vol. alk. soln. add 1 vol. alcohol, 1 vol. ether and 1 vol. 30 per cent. soda and shake :						
Upper layer	Yellow.	Yellow.	Yellow.	Yellow.	Colourless.	Yellow.
Lower layer	Colourless.	Colourless.	Colourless.	Colourless.	Pink.	Colourless.
(18) To 1 vol. alk. soln. add 2 vols. 30 per cent. soda, mix and stand one minute. Add 1 vol. alcohol, 1 vol. ether and shake :						
Upper layer	Colourless.	Yellow.	Yellow.	Colourless.	Colourless.	Yellow.
Lower layer	Yellow.	Colourless.	Colourless.	Red to pink.	Pink.	Colourless.
(19) Make acid and extract with petroleum spirit. Petroleum spirit layer.	Colourless.	Colourless.	Colourless.	Colourless (ppt. not soluble).	Colourless.	Yellow (faintly yellow suspension).
(20) Add few drops alcoholic $FeCl_3$ soln. to petroleum spirit layer (No. 19), shake and allow to stand.	No change.	No change.	No change.	No change.	No change.	Greenish-brown to greenish-black.
(21) To 1 ml. soln. add dilute acid till just acid, then dilute soda till just alkaline. Add 5 ml. alcohol and 2 drops saturated bromine water. Then add N/10 soda, drop by drop.	No change.	No change.	No change.	No change.	Colour becomes yellow, and on adding N/10 soda turns green, then blue, then violet-red.	Colourless, then yellow when alkaline.

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