

THE CHEMICAL ANALYSIS OF FOODS

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

By

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PREFACE TO THE FOURTH EDITION

It is always difficult to keep any text book on food chemistry up to date and specially so when the book is limited in size and one must exercise rigid economy in space. It has therefore been necessary to make room for new information by deleting matter or methods which are passing out of date. Space has been found in this way, and by adjustments of type, for many new methods and further detail of others which seemed to need some expansion. Effort has been made to maintain the plan of giving only well-tried methods, with references to sources of further information.

New matter includes the expansion of methods for determination of various trace elements and preservatives ; also of methods for the chemical or biochemical estimation of certain of the vitamins. There is new data in relation to baking powders, vinegars, soya meal, onions, meat extracts, sausages, tomatoes, ketchups, egg products, and other foodstuffs.

Again there is the problem of knowing how many of the numerous Orders or Standards issued by the Ministry of Food should be included. Only those have been mentioned which seem likely to be of permanent value or to set standards for the future.

I have to thank Mr. J. H. Halliday for help in reading the proofs.

H. E. Cox.

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LONDON, E.C. 3.

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 textbook 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.

H. E. Cox.

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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

Name.	Formula.	Rotation [α] _D ¹⁵ .	Melting point. Degrees C.	Melting point of osa- zone.	Re- action with Feh- ling's solu- tion.
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	$C_{18}H_{34}O_{16}$	+ 104°	118°-119°	—	—
POLYSACCHAROSES.					
Starch	$(C_6H_{10}O_5)_n$	+ 200° ¹	—	—	—
Cellulose	"	+	—	—	—
Inulin	"	- 38°-40°	178°	—	—
Glycogen	"	+ 191°	—	—	—
Dextrin	"	+ 198° ¹	—	—	—

produced which is discharged on the addition of excess of alkali (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

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

mixed together as required for use. No. 1 contains 69.28 g. of copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, dissolved in water to produce 1 litre, and No. 2 is prepared by dissolving 100 g. of pure sodium hydroxide and 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 rotatory power $[\alpha]_D^{15} + 104^\circ$ to $+ 105^\circ$. 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. 102), 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. 15.

Dextrose. Dextrose, grape sugar or glucose, is a crystalline substance having m.p. 146° , $[\alpha]_D^{15} + 52.7^\circ$; 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

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.

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.

When starch is dissolved in dilute acids, maltose, dextrans 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. 32.

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 $+0.6385^{\circ}$, 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— $C_6H_{12}O_6 \cdot H_2O$ —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 $[\alpha]_D^{15}$ is $+81.5^{\circ}$. 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° , $[\alpha]_D^{15} + 14.5^{\circ}$, 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 $[\alpha]_D^{15} + 66.5^{\circ}$, 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 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 $C_{12}H_{22}O_{11} \cdot H_2O$ 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 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 $+55.3^{\circ}$, 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 $[\alpha]_D^{15} + 139.5^{\circ}$, with a temperature coefficient of -0.095° . (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. 14).

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 $+104^{\circ}$ without appreciable muta-rotation, and on hydrolysis with strong acids it yields dextrose, lævulose and galactose ; weak acids form lævulose and a disaccharose—melibiose. 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. 14.

The polysaccharoses will be discussed after the consideration of methods of analysis of commercial sugar products. The relative sweetness of the sugars more common in foods has been given as follows :—

Sucrose	100
Lævulose	173
Dextrose	74
Invert sugar	130
Lactose	16
Maltose	32
Galactose	32

so a mixture containing invert sugar is sweeter than the equivalent of sucrose—a fact which some traders have utilised.

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 following table shows the usual composition of different kinds of sucrose.

—	Water.	Sucrose.	Glucose.	Protein.	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

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 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. 113.

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

$$[\alpha]_D^t = \frac{100 a}{l g d}$$

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°
Dextrin	+	198–200°

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. 12 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. 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