ISSN 0254-4725

Manuals of food quality control

8. Food analysis: quality, adulteration and tests of identity

FAO
FOOD AND
NUTRITION
PAPER

14/8

Food
and
Agriculture
Organization
of
the
United
Nations

Manuals of food quality control

8. Food analysis: quality, adulteration and tests of identity

prepared with the support of the
Swedish International Development Authority (SIDA)

FAO
FOOD AND
NUTRITION
PAPER

14/8

Food
and
Agriculture
Organization
of
the
United
Nations

Rome, 1986

Reprinted 1997

M-82
ISBN 92-5-102412-X

FOREWORD

The control of food safety and quality is an integral part of national programmes for development. National food control systems are designed to protect the health and welfare of the consumer, to promote the development of trade in food and food products, and to protect the interests of the fair and honest food producer, processor or marketer against dishonest and unfair competition. Emphasis is placed on the prevention of chemical and biological hazards which result from contamination, adulteration or simple mishandling of foods. Also important are the maintenance of general food quality and the control of the use of food additives and food processing procedures.

In order to establish a workable food control system, a national government must:

1. Enact food control legislation.
2. Promulgate regulations to enforce that legislation.
3. Create an agency to conduct the enforcement.
4. Establish food inspection and analysis staff within the agency or agencies concerned.
5. Provide physical facilities including a food control laboratory.

To assist the national governments of developing countries in this process, FAO, with the support of the Swedish International Development Authority (SIDA) has published the series Manuals of Food Quality Control. These are incorporated as part of the FAO Food and Nutrition Paper Series No. 14, and include:

No. 14/1 The Food Control Laboratory

No. 14/2 Additives, Contaminants, and Techniques (out of print)

No. 14/3 Commodities (out of print)

No. 14/4 Microbiological Analysis

No. 14/5 Food Inspection

No. 14/6 Food for Export

No. 14/7 Food Analysis: General Techniques, Additives, Contaminants, and Composition

No. 14/8 Food Analysis: Quality, Adulteration, and Tests of Identity

In addition, FAO, WHO and UNEP jointly have published many guidelines and other documents designed to further assist developing countries in forming adequate food control systems. These publications include:

Methods of Sampling and Analysis of Contaminants in Food
A Report of the Second Joint FAO/WHO Expert Consultation,
Rome - 1978

Guidelines for Establishing or Strengthening National Food Contamination Monitoring Programmes - FAO Food Control Series No. 5 - 1979

Guidelines for the Study of Dietary Intakes of Chemical Contaminants - WHO Offset Publication No. 87 - 1985

Guide to Codex Recommendations concerning Pesticide Residues, Part 2 - Maximum Limits for Pesticide Residues, Second Preliminary Issue - Rome - 1985

Recommended Practices for the Prevention of Mycotoxins in Food, Feed and their Products - FAO Food and Nutrition Paper No. 10, Rome - 1979

Food Standards, Codes of Practice and Methods of Analysis Recommended by the Codex Alimentarius Commission - Joint FAO/WHO Food Standards Programme (several titles)

Food Additive Evaluations and Specifications of Purity and Identity - Reports and Monographs of the Joint FAO/WHO Expert Committee on Food Additives (several titles)

The above publications, and others, are available to persons and organizations. FAO is also interested in receiving comments regarding this volume and suggestions for future improvement. Please send to:

The Chief
Food Quality and Standards Service
Food Policy and Nutrition Division
Food Agriculture Organization of the
United Nations
Via delle Terme di Caracalla
00100 Rome, Italy

FAO wishes to acknowledge the generous support of the Swedish International Development Authority (SIDA), in the preparation of this volume, and the efforts of Mr. J. Weatherwax and Mr. P.G. Martin who were responsible for the preparation of the text.

SPECIAL NOTE

The methods and analytical procedures described in this Manual are designed to be carried out by properly trained personnel in a suitably equipped laboratory. In common with many laboratory procedures, the methods quoted frequently involve hazardous materials.

For the correct and safe execution of these methods it is essential that laboratory personnel follow standard safety procedures for the handling of hazardous materials.

While the greatest care has been exercised in the preparation of this information, FAO expressly disclaims any liability to users of these procedures for consequential damages of any kind arising out of or connected with their use.

The methods are also not to be regarded as official because of their inclusion in this Manual. They are simply methods which have been found by experience to be usable in the average laboratory.

CONTENTS

1. SCOPE OF THIS MANUAL OF FOOD ANALYSIS

This Manual covers nine food groups which include most of the foods consumed throughout the world. Individual foods within a food group (such as 'Dried Milk' within 'Milk and Dairy Products') are discussed regarding their composition and/or routine analysis, as well as other appropriate features. Compositional information may include published analytical data as well as international standards such as those of the Codex Alimentarius, and some individual country legal standards. All these are to serve only as a guide in the event that local standards are not available. The routine analytical information is also to serve as a guide and often includes alternative procedures as well as background literature sources.

The analytical methods given for each food are generally those with specific application to the food. Some are proximate analyses while others include tests for contamination or adulteration. Note that all general analytical methods for contaminants and additives are found in the Manual, "Food Analysis: Techniques, Additives, Contaminants, Composition." . Also note that for all analytical procedures, the following precautions apply:

1. Use only distilled water or the equivalent. (Deionized water is often suitable).

2. Use the best grade of reagent chemicals available and purify if necessary.

3. Follow the method instructions exactly as many of the procedures are empirical.

4. Use all laboratory safety procedures and equipment.

The reference section at the end of each food group gives both the references listed in the text, as well as some general references which may provide background information.

The first edition of this Manual was written in 1977 by Mr. Peter G. Martin presently of Lyne, Martin and Radford, Public Analysts, Reading, Berkshire, England. The present revised edition has been prepared with Mr. Martin's support and assistance by Mr. John R. Weatherwax, retired Laboratory Director for the United States Food and Drug Administration, Los Angeles, California, USA.

2. MILK AND DAIRY PRODUCTS

2.1 WHOLE MILK

COMPOSITION

The composition of bovine milk varies widely depending on a large number of factors including breed, season, stage of lactation, milking interval, health of the cow, and level and type of feed. Quantities of milk large enough (say, over 10,000 litres) and from sources divergent enough to obliterate these variations will tend towards a typical composition given by various authorities as follows:

	Kichmond(1)	Davis(1)	Pearson(2)	Webb et al(3)
Fat	3.75	3.67	3.61	3.5 - 3.7
Protein	3.20	3.42	3.29	3.5
Lactose, hydrated	4.70	4.78	4.65	4.9
Ash	0.75	0.73	0.75	0.7

Webb et al (3) have collated the compositional data for milks from 15 countries and many breeds and the averages of the analytical results fell within the following ranges:

Total solids:	11.52 - 14.56%
Fat:	3.75 - 5.52%
Protein:	2.87 - 3.76%
Lactose hydrate:	4.34 - 4.98%
Ash:	0.66 - 0.72%

The fat content of milk is especially variable. The milk fat of certain breeds such as Jersey and Guernsey rises to 8% or higher, while that from Friesians may be close to 3%. Normal milk from individual cows or small herds may have a composition outside the range quoted above.

The mineral and citric acid composition of milk is typically as follows:

	Ca	Mg	P	Na	K	Cl	S	Citric Acid
mg/100 ml whole milk:	123	12	95	58	141	110	30	160

About 79-80% of the nitrogen in milk is present as casein, the rest being derived from albumin, globulin, proteoses and non-protein nitrogen. In normal milk, the ash is 8% of the solids-not-fat (SNF) and the albumin is not normally above 0.6% of the whole milk.

The composition of the ash of milk lies within the following ranges:

	K_2O	CaO	Na_2O	MgO	Fe_2O_3	P_2O_5	Cl	SO_3
% of ash:	23-30	20-27	6-12	2.3-3.1	0.05-0.4	21-20	13.6-16.4	0-4

ROUTINE ANALYSIS

The chemical tests that must be carried out immediately on receipt of the milk at the laboratory are the determinations of fat and solids-not-fat. If these are below the expected values, the freezing point may be determined. The freezing-point test is invalidated by the presence of formaldehyde, often used as a preservative for samples, but not by the mercuric chloride/salt mixture recommended by Harding and Royal (4). If this is used, the samples must be clearly labelled "Poison". The test is affected by the acidity and corrections have to be made if this is higher than 0.18% expressed as lactic acid. If the acidity exceeds 0.30% as lactic acid, the freezing-point test is not valid. Nitrates do not normally occur in milk and therefore detection of these is confirmation of the addition of water.

Routine tests are designed to check that the composition of milk is normal and that it has not been subject to adulteration. The bacteriological quality can be checked by the methylene blue test or a similar dye reduction test, but more extensive bacteriological tests may also be required. Examination for antibiotics, dirt, added alkali and added preservative is important. The phosphatase test is used to check if the milk has been adequately pasteurised, and the turbidity test to check if it has been sterilized. Kempinski (5) gives a method similar to the turbidity test for the detection of UHT (ultra heat treated) milk.

Analysis of the ash can be useful in detecting abnormality. Anhydrous lactose, proteins, and ash occur in the proportion 13:9:2 (Vieth's ratio) in the milk from healthy cows and therefore their determination is useful in checking that the milk is not abnormal. The addition of water to the milk does not alter this ratio.

Instrumental methods of milk testing are reviewed by Harding (6) and Bergmann (7). Corradini, C. (8) and Haave, I.J.J. (9) discuss the formation of a gel in UHT milk that has been stored a long time. The gel forms by the slow enzymic coagulation of the casein. For the analysis of sour milk suspected of being adulterated, see Hanson (10) and Davis and Macdonald (1).

DETECTION OF ADULTERATION

The natural acidity of milk immediately it comes from the cow is about 0.13-0.14% expressed as lactic acid, although mainly derived from phosphate, casein and to a less extent citrate and CO_2. The lactic level is about 2 mg% (0.002%) in very fresh milk. As the milk ages, the milk bacteria proliferate and produce lactic acid. Once the total acidity reaches about 0.18%, incipient souring can be detected by smell and taste. If storage conditions and hygiene are poor, there may have been an attempt to mask this process by the addition of alkali, which will tend to produce low acidity, high pH, high sodium and high lactate, although not necessarily outside the natural range. The freezing-point depression will also be greater than normal.

The older methods to detect neutralization of milk such as those of Tillmans and Luckenback (11)(12)(13), Woidrich and Schmid (14) and Hankinson and Anderson (15) depend on determining the buffering capacity of the milk by one or more acid-alkali titrations after addition of uranyl nitrate or ferric hydroxide. Adequate methods for the determination of lactate are available, and comparison of total acidity with lactate should usually be sufficient to detect neutralizers. Iwaida et al (16) have used Davidson's method for lactic acid (17) for this purpose. Davidson's procedure, modified by Lawrence (18),

depends upon oxidation of the lactic acid to acetaldehyde and forming a colour with p-hydroxydiphenyl, in the presence of concentrated sulphuric acid. It can be used with milk powders and condensed milks as well as fresh milk. The AOAC procedure has been developed from Hillig's method (19), involving extraction of the lactic acid with ether and development of a colour with ferric chloride after the removal of interferences. Steffen (20) has described an enzymic method.

Roy and Basak(21) investigated the subject of neutralisers in milk in detail. pH of ash of milk, titratable acidity of the soluble ash and direct titrations of the filtrate (or centrifugate) after coagulation of the proteins can detect added carbonate or bicarbonate (or hydroxides) of alkali metals. But in all these parameters those from unadmixed milk have to be subtracted or taken into consideration. As these vary in pure milk within a range, a generally agreed mean value has to be determined. Small additions cannot be detected as these will be concealed within the range of natural variation. TLC and ion-exchange methods devised in the study can be used for detection. Recovery of added sodium carbonate in the TLC and ion-exchange is poor but the titrimetric determination yields a recovery of the added sodium carbonate of 90 $\pm$ 5%.

Addition of salts of any sort to milk will lower the freezing-point. Samples with a correct freezing-point but low solids-not-fat may be genuine but poor quality possibly from diseased animals, or watered and neutralized. Further analysis will be required. Theoretically, addition of 0.84% sodium bicarbonate depresses the freezing-point 0.1850°C but in practice this is probably less, partly due to ionization of lactic acid and partly to loss of CO_2 on neutralization.

OFF FLAVOURS

Milk freshly drawn from a healthy udder has a "cowy" flavour distinguishable from various taints of bacterial origin due either to mastitis or subsequent bacterial growth or contamination. Although souring is the commonest off-flavour derived from bacterial action, this may also cause bitterness, sweet curdling and odours specific to a particular microorganism.

Feeds and weeds, such as some of the Brassicaceae may cause a taint in the milk. These tend to pass off on standing or with aeration.

The fishy flavour that sometimes occurs in the milk from cows on wheat pasture has been shown to be due to trimethylamine (Mehta (22)). Ingested land cress, Coronopus didymus, is one of the weeds more recently reported to cause an off-flavour in milk (see Walker and Gray (23)).

The most important enzymically-induced taint is that of rancidity. Milk kept at low temperatures in the presence of copper and sometimes even in its absence may develop an oxidized flavour. Irradiation and direct sunlight can induce a taint variously described as "flat", "burnt" or "emery". Milk that has been heated over about 80°C has a cooked or scorched taste and smell.

The cause of a complaint in relation to a sample of milk may be one of the above, or may be the accidental contamination of the milk with kerosene, soap, chlorine disinfectants or other chemicals. Occasionally, microorganisms can produce off-flavours which might be thought to have chemical origins. For example, sterilized milk may have a carbolic flavour, and atypical strains of Streptococcus lactis can produce caramel or malt flavours. The flavour of vinegar or some fruits may be due to the action of bacteria or yeasts. Milk easily picks up flavours from the surrounding atmosphere due to the large surface area offered by the fat globules.

There is an article on off-flavours in milk by Kratzer (24) showing that in over 18,000 samples the commonest cause of taint was the feed.

MASTITIS

The term "abnormal milk" in its restricted sense refers to milk from an udder showing mastitis or other disease but may be used to refer to any milk of unusual composition.

Milk from cows with mastitis has an altered composition. The solids-not-fat, fat, lactose, casein, calcium and potassium levels fall and the pH, sodium, soluble protein and chloride levels rise. Dzhorov et al(25) have shown that serum albumin and immunoglobulins increase while beta lactoglobulins and alpha lactalbumin decrease compared with normal milk.

It is important to remember that the presence of mastitis does not alter the freezing-point of the milk, which must show the same osmotic pressure as the cow's blood. The main contribution to the freezing-point depression is from lactose and chlorides. As the lactose level falls due to mastitis, the proportion of chloride, bicarbonate and particularly sodium increases to compensate. Zagaevskii (26) describes the use of a reagent claimed capable of detecting down to 1-3% of mastitis milk in bulk supplies.

Tests designed to detect mastitis in individual cows, or individual quarters of the udder are beyond the scope of this Manual. The reader is referred to Schalm et al (27). The food inspector may have been able to obtain information about the prevalence of disease in the animals from which a milk sample was derived. Thus the occasions when it is necessary to carry out laboratory tests for mastitis in relation to statutory milk samples are relatively few. However, the analyst must be aware of the significance of any information he receives from the inspector or derives from analysis

The California Mastitis Test and its various modifications (The Milk Quality Test, the Michigan Mastitis Test, the Brabant Mastitis Test and the Wisconsin Mastitis Test), the modified Whiteside test and the catalase test are those most commonly used outside the laboratory. Destruction of excess hydrogen peroxide with catalase will give anomalous results in the catalase test for mastitis. The total cell count is commonly used in the USA; the California Mastitis test and its modifications are basically measuring the leucocyte count which is likely to be far in excess of the number of bacteria present, at any rate for all cases except very severe mastitis and is therefore a more readily measured parameter. The centrifuged deposit test and the cell count are alternative ways of measuring essentially the same thing. The resazurin test is positive when the number of bacteria present is higher than normal. Schultze et al (28) report a comparative study on these tests. A chloride content above about 0.13 - 0.14% suggests mastitis, but it is not sufficient by itself as it may indicate the presence of colostrum or milk from late in the lactation period. Newstead and Ormsby (29) give details of a test for colostrum that relies on the detection of high levels of immunoglobulin.

Chemical tests to detect mastitis include the measurement of the pH (6.4 - 6.6 for normal fresh milk), the rennet test, the Koestler ratio and the casein number (Rowland and Zeid-el-dine (30)). Very bad samples of mastitis milk will fail to clot with rennet and will reduce resazurin rapidly. The Koestler ratio,

$$\frac{100 \times \text{chloride}}{\text{lactose}}$$

is about 2.3 in normal milk and above 3 in mastitis milk. The determination of this ratio is usually adequate for the detection of mastitis milk but the casein number may also be determined if desired. This is,

$$\frac{\text{casein N\%} \times 100}{\text{Total N\%}}$$

and is 70-80 for normal milk, falling as low as 70-74 in mastitis milk.

Until confidence is gained, it is safer to determine the nitrogen in the filtrate and the total nitrogen to ensure the three figures agree before concluding from the results that mastitis is present. It must be remembered that these remarks apply to milk that is entirely or substantially derived from diseased quarters of the udder.

ANTIBIOTICS

Various dye reduction tests such as those using methylene blue, resazurin, triphenyl tetrazolium chloride, and brilliant black have been used to assess the bacteriological quality of milk. Milk of poor quality, containing large numbers of bacteria, has a relatively low and decreasing reduction potential and hence reduces these dyes more quickly. In using these dye reduction tests to detect antibiotics, the milk is pasteurised to destroy most of the organisms present and then inoculated with an organism which will be able to cause dye reduction within a certain time under the conditions of test. The presence of an inhibitor is shown by failure to do so. The inhibitor may be an antibiotic, detergent disinfectant, pesticide residue or preservative. Any antibiotics other than penicillin are likely to be difficult to identify. Circumstantial evidence from the inspector may assist in deciding what further tests should be carried out. It is usual to add penicillinase to another portion of the sample. A normal dye reduction in the treated portion indicates that the inhibitor is penicillin.

If the inhibitor is not penicillin and in the absence of evidence as to the likely cause, it is best to carry out some of the simpler tests first. For example, test for peroxide, quaternary ammonium compounds, formaldehyde, free chlorine and phenolic disinfectants. Iodophors will increase the iodine content of the milk. It is useful to check the pH also. If these tests prove negative, the milk may be examined for pesticide residues. Wheeler(31) has reviewed the use of iodophors.

The following may prove useful if the analysis has to be taken further. Hamann et al (32) give a TLC-GLC method for isoxazolyl penicillins and for chloramphenicol. Rybinska (33) used a plate assay method and Micrococcus flavus test organism to detect down to 0.05 IU/ml of bacitracin in milk. Kumar et al (34) found that if the phosphatase test was carried out using Folin-Ciocalteu reagent to detect the phenol produced, some insecticides, such as Propoxur, Carbaryl, Tetramethylthiuram disulphide (Thiram), Phosphamidon and Dichlorvos interfered in the test. Thus a discrepancy between the phosphatase test done in this way, and using p-nitrophenyl phosphate or phenolphthalein might indicate the presence of these compounds. Reinbold (35) uses TLC to detect tylosin in biological materials including milk. Aggarwal (36) found that Thiram affected the methylene blue test (for milk quality, not for antibiotics) even at the 0.05 microgram/ml level, and that the test was much more sensitive to this residue than the others tested. Standard Methods for the Examination of Dairy Products(168) contains a chapter on the detection of inhibitors. Kramer et al (37) describes methods for specific antibiotics.

For a general review of the determination of antibiotic residues in milk, see Feagan (38). Cox and McNamara (39) and Richard and Kerherve (40) describe detection of disinfectants on a paper disc and an acid titration method for antibiotics. The publication of Kramer et al (37) gives methods for penicillin, bacitracin, the tetracyclines, streptomycin, neomycin, polymixin, erythromycin and novobiocin.

SUGARS

The only naturally occurring sugar in milk is lactose, although glucose and galactose may be present due to bacterial or fermentative breakdown of lactose. Lactose may be determined colorimetrically as in Nickerson et al (41), and Fernandez and Ramirez (42). Colorimetric and polarimetric methods are compared by Wilson et al (43). The Bertrand method has been modified to a colorimetric finish by Rukina and Rastegaeva (44). GLC (Jaynes and Asan (45)), liquid

chromatography (Hobbs and Lawrence (46)) and an enzymic method (Bahl (47)) have been used.

For the qualitative analysis of sugars in milk, the TLC method on the filtrate obtained after protein precipitation and clarification by potassium ferryocyanide/zinc acetate, or TLC on cellulose may be used (Vahdehita and Lopez (48)). Dougall and Morgan (49) use TLC to detect adulteration with sucrose or reconstituted sweetened dried skim milk. Madrid Vicente (50) describes a rapid colour test for glucose in milk; the test of Mittal and Roy (51) appears to be more sensitive. Their paper includes a rapid test for added ammonia or urea sulphate. A method by PC is given by Gupta and Mathew (52). Dhar et al (53) point out that if sucrose has been added to milk, it may have been partially hydrolysed by the acidity present naturally. It is therefore more reliable to calculate the milk solids other than milk fat by multiplying the protein (N x 6.38) by 24/9 rather than subtracting the fat and sucrose from the total solids. The factor 24/9 is derived from Vieth's ratio.

NON-BOVINE MILK

The milk of goats or other animals may be analyzed in the same way as cows' milk. For details of the composition of milk from different animals, see Webb (3). Goats' milk is somewhat more variable in composition than cows' milk. The reader is referred to the paper by James (54) for details of some recent goat milk analyses. Also see Jaouen (55) and Rao and Bector (56).

Pruthi et al (57) discuss the Recknagel phenomenon in buffalo milk. In this phenomenon the observed specific gravity of milk increases slowly during the first 12 hours after milking. This is possibly due to changes in the casein or in the physical condition of the fat. The observed gravity may increase by as much as 0.0013. Davide and Domingo (58) have produced a series of papers on Carabao milk. The polyacrylamide gel electrophoresis of buffalo milk is described by Mincione et al (59). See also the book by Swaisgood (60) on the gel electrophoresis of milk proteins. For recent papers on ewes milk see Al-Shabibi et al (61), Williams et al (62) and Wagner (63). For camels' milk and its products see Rao, Gupta and Dastur (64) and Vaghela (65).

Milk from some animals may cause an allergic reaction in sensitive individuals and therefore misdescription can represent a health hazard. Milk from a particular species may command a higher price, so that passing off cheaper milk would be economically attractive.

Monacelli and Cantagalli (66) and Carini and Busca (67) use an agar immunodiffusion test to detect cows' milk in ewes' milk and ewes' milk cheese. Foissy (68) finds electrophoresis best for this purpose, and also applied it to the presence of cows' milk in goats' milk (see also Aschaffenburg and Dance (69), Gombocz et al (169) and Mitchell (170)). Eguares (70) describes the use of hyperimmune antisera, the test giving a precipitate in the presence of cows' milk. Earlier methods are reviewed by Bret (71). A series of papers describes the use of polyacrylamide gel electrophoresis (Pierre and Portmann (72)(73); Portmann and Pierre (74)). Adulteration of cows' milk with buffaloes' milk has been detected by starch gel electrophoresis (Majumder and Ganguli (75) and Majumder et al (76)). Guha et al (77) did not find the gel diffusion technique using Hansa test serum adequate to do this.

MILK FAT
(Gerber Method)

PRINCIPLE

The milk is mixed with H_2SO_4 and amyl alcohol in a special Gerber tube permitting solution of the protein present and release of the fat. The tubes are centrifuged and the fat rising into the calibrated part of the tube is measured as a percentage of the sample. The method is suitable as a routine or screening test.

APPARATUS

1. Gerber butyrometer tubes, with lock-stoppers and a key.

2. Gerber centrifuge, 50-cm diameter.

3. Milk pipette 10.75 ml. (See "Interpretation" section below.)

REAGENTS

1. Sulphuric acid Sp. gr. 1.815 $\pm$ 0.003 (about 90% m/m).

2. Amyl alcohol.

PROCEDURE

Measure 10 ml of sulphuric acid into a Gerber tube, preferably by use of an automatic dispenser, without wetting the neck of the tube. Mix the milk sample gently but thoroughly and fill the milk pipette above the graduation line. Wipe the outside of the pipette and allow the milk level to fall so that the top of the meniscus is level with the mark. Run the milk into the Gerber tube without wetting the neck, leave to drain 3 seconds and touch the pipette tip against the base of the neck of the Gerber tube.

Add 1 ml amyl alcohol. Close with a stopper, shake until homogeneous, inverting to complete admixture of the acid. Centrifuge for 4 minutes after the centrifuge has reached 1100 rpm. Allow the centrifuge to come to rest, remove the Gerber tubes and place in a water-bath at 65°C. Read off the percentage of fat after three minutes, adjusting the height in the tube as necessary by movement of the lock-stopper with the key.

The Gerber tubes must always be emptied without delay and the highly acid waste disposed of appropriately. The tubes may be cleaned with chromic acid.

INTERPRETATION

The ISO Standard 2446:1976 suggests that the volume of the pipette is chosen by carrying out both this and the Rose-Gottlieb determination on a large number of milk samples and, from a statistical analysis, computing the volume of sample to be taken appropriate to the national average fat content of milk in the country of use. This is probably not worthwhile in an enforcement laboratory where the Rose-Gottlieb method must be used on any doubtful samples. However, comparison of the results by the two methods on such samples over a period of time could be used to adjust the volume taken or compute a correction factor for use with the Gerber.

Volumes used in some countries are as follows:

- India, 10.75 ml (Ghouse and Jain (78))
- The Netherlands, 10.66 ml

- Hungary, 10.8 ml
- U.K., 10.94 ml
- U.S.A., 11.0 ml

An 11 ml pipette (which delivers about 10.9 ml milk) was used in the original Gerber method. The result is expressed m/m and the volume taken must correspond as closely as possible to 11.25 g as each unit graduation on the tube corresponds to 0.1125 g of fat.

The Gerber method does not work on milks preserved with formaldehyde. Stoppers of butyl and nitrile rubber are satisfactory, but new stoppers of natural rubber absorb fat to some extent. Homogenization causes the fat to separate with more difficulty and centrifuging more than once may be required. On the other hand, holding the tubes too long at 65°C results in esterification of the amyl alcohol with the consequent increase in the volume of the fat layer. To overcome this use a 40°C water-bath, centrifuge until a constant reading is obtained and multiply the reading by 1.02.

REFERENCES

Gerber, N., 1892. L'Industrie Laitiere 50, 397; 52, 413; 1893, 1,1; 6, 43.

ISO, 2446-78 and 488-93.

Marth, E.H., 1978. Standard Methods for the Examination of Dairy Products, American Public Health Association.

MILK FAT
(Rose-Gottlieb Method)

PRINCIPLE

The sample is treated with ammonia and ethanol, the latter to precipitate protein and the former to dissolve the precipitate, and the fat is extracted with diethyl ether and petroleum ether. The mixed ethers are evaporated and the residue weighed. This method is considered suitable for reference purposes. Strict adherence to detail is necessary in order to obtain reliable results.

APPARATUS

1. Extraction tube and siphon (see diagram).
2. 100-ml flat-bottomed flask with G/G joint.

REAGENTS

1. Ammonia SG 0.880. (six-fifths the amount of ammonia SG 0.910 should be used).
2. Petroleum ether BR 40 - 60°C.
3. Diethyl ether, peroxide free.
4. Denatured alcohol (95% ethanol).
5. Mixed ether. Equal volumes of petroleum and diethyl ethers.

PROCEDURE

Accurately weigh about 10 g of homogeneous sample into an extraction tube. This is conveniently done on a top-pan balance accurate to a milligram by standing the tube in a plastic beaker or other light container. For weighing on an analytical balance it may be necessary to attach a piece of wire to the neck of the tube and hitch this to the pan-hook of the balance.

Add 1 ml ammonia and mix thoroughly. Add 10 ml alcohol and again mix thoroughly. Add 25 ml of diethyl ether, close the tube with a wetted ground-glass stopper or well-fitting rolled cork, shake very gently and release the pressure without loss of ether, and repeat a couple of times until the tube can be shaken vigorously without risk of pressure build-up. Shake vigorously for one minute. Add 25 ml of petroleum ether, rinsing the stopper and neck with some of it, wet the stopper with water again and shake vigorously for half a minute.

Weigh a dried 100-ml flat-bottomed ground-glass flask. Leave the extraction tube to stand half an hour or more until the layers are clearly separated, insert the siphon tube so that the orifice is 2-3 mm above the aqueous layer and blow gently so that the ethereal

extract siphons into the weighed flask. Raise the siphon a little but do not remove it. Rinse the tip of it with about 5 ml of mixed ethers and, without shaking, siphon to the flask. Use a further 5 ml of mixed ethers to rinse the cork of the siphon and the neck of the tube and again transfer without shaking the tube, then remove the siphon from the tube. Rinse the tip of the siphon and the neck of the flat-bottomed flask. The evaporation of the solvent in the flask can be started while the second extraction is in progress.

Add 15 ml of diethyl ether to the tube and shake vigorously for one minute, taking the same precautions as before. Add 15 ml of petroleum ether and shake vigorously for half a minute, transfer the ethers and rinse as before. Extract a third time with 15 ml of each solvent and rinse as before. Evaporate all the solvent in the flask, completing the process on the water-bath and finally in the oven, drying to constant weight. Leave in the desiccator to cool at least an hour and do not wipe the flask just before weighing. (It may have been necessary to wipe it on removing from the water-bath).

Weigh, then add petroleum ether to dissolve the fat and carefully decant taking care to leave any sediment in the flask. Rinse the flask with petroleum ether until all the fat is removed, but any sediment remains. Dry in the oven and weigh as before. The difference in weights represents the weight of fat extracted from the milk.

For the most accurate work it may be checked that the residue from a fourth extraction is negligible and a blank extraction may be done using 10 ml of water in place of the sample.

CALCULATION

$$\% \text{ fat m/m} = \frac{\text{weight of fat}}{\text{weight of milk}} \times 100$$

INTERPRETATION

The average fat content of milk from adequately fed cows is about 3.5%, but varies widely depending on a number of factors including breed, feed, season, milking interval, etc. "European standard milk" contains 3.5% of fat, and in the U.K. below three percent is assumed to indicate adulteration or abstraction of fat until proved to the contrary. There is a federal minimum of 3.25% in the U.S.A., but some individual States set higher standards.

IDF, ISO and AOAC have jointly agreed upon a reference method, published also in CAC/M1-1973 of the Codex Alimentarius Commission. The wording here is slightly different. The IDF/ISO/AOAC method uses a different type of fat-extraction flask (ISO 1211:1970).

The NMKL version of the method (no. 10, 1977) recommends that coagulated samples be re-dispersed by adding a known amount of concentrated ammonia. If the fat has actually separated, re-emulsification is achieved by carefully shaking at 40°C with 0.5 ml of chloroform per 100 ml of milk or cream.

REFERENCES

ISO/R, 1211-70.

Official Methods of Analysis of the AOAC, 1984. 16.064.

Code of Principles Concerning Milk and Milk Products, International Standards Methods of Sampling and Analysis for Milk Products, 7th Ed., CAC/M1-1973.

TOTAL SOLIDS
(Rapid Method)

PRINCIPLE

The density of the milk is measured using a milk hydrometer (lactometer) and the total solids are calculated.

APPARATUS

1. Milk hydrometer.

2. Cylinder, at least 4 mm greater in diameter than the bulb of the hydrometer.

PROCEDURE

The milk should be warmed to and kept at 40-45°C for five minutes, carefully but thoroughly mixed, avoiding inclusion of bubbles, and then cooled to 20 $\pm$ 2°C and the density determined as soon as possible.

Fill the cylinder to overflowing by pouring the sample over the lactometer bulb, avoiding bubble formation. Allow the lactometer to float in the milk without permitting more than a few mm of the stem above the milk surface to become wetted. Read the scale at the top of the meniscus, the eye being horizontal to it, move the hydrometer a few mm vertically and check the reading. Remove the lactometer, record the reading and determine and record the temperature of the milk.

Warming the sample to 40-45°C is to ensure the fat is in a liquid condition. This can therefore be omitted if the sample has been warm for some time previously. Lactometer slide rules allow for density determinations between about 15° and 25°C.

CALCULATION

The formula SNF = 0.25D + 0.22F + 0.72 applies if the temperature of the sample is 20°C and if the fat of the milk is in the liquid state, where F = % m/m fat, D = (1000 x density - 1000) and SNF = solids-not-fat. If the milk was not at 20°C the density must be corrected before application of the formula, adding to "D" 0.24 for each degree above 20 and subtracting for each degree below.

For example, if the density reading is 1.032 at 18°C and the fat (Gerber) is 3.65%

Density at 18°C	= 1.032
D, (1000 x density - 1000)	= 32 at 18°C
D at 20°C	= 32 - (2 x 0.24) = 31.52
Therefore, solids-not-fat	= (0.25 x 31.5) + (0.22 x 3.65) + 0.72 = 9.39

INTERPRETATION

This method is rapid and is only approximate. It is convenient to use it in conjunction with the Gerber or Babcock methods for fat. Check any suspect samples by determination of the total solids gravimetrically and the fat by the method of Rose-Gottlieb.

There are two ways of improving the accuracy of the determination of total solids using this method. One way is to determine the gravity and also the weighed total solids on ten or twelve samples and to calculate the correction to be added to, or subtracted from, the gravity reading before the correction for temperature is made. This corrects for any error in the calibration of the lactometer. Another way is to carefully check the calibration of the lactometer against a solution of known gravity. B.S. 734, Part 2, 1959 uses a reference lactometer and solutions of sodium carbonate.

O'Keefe (79) has suggested that the above formula (SNF = 0.25D + 0.22F + 0.72) overestimates the solids-not-fat and that the older formula of SNF = 0.25D + 0.21F + 0.66 is more correct. The formula has also been discussed by Lunder (80) and Aparicio Gallego (81). See also Artem'ev and Panasenkov (82), Uzonyi and Varga (83), Dozet et al (84) and Slanovec et al (85).

REFERENCES

Official Methods of Analysis of the AOAC, 1984. 16.033.

British Standard, 734:1959.

TOTAL SOLIDS
(Weight Method)

PRINCIPLE

Milk is dried in the oven under standard conditions and the residue weighed.

APPARATUS

1. Dish and lid, metal, flat-bottomed, about 7-8 cm diameter by 1-2.5 cm deep.

2. Ventilated oven at 100°C.

PROCEDURE

Heat the clean dry empty dish and lid in the oven, cool in a desiccator and weigh. Add 3-4g milk, replace the lid and weigh again. Place the dish without the lid on a boiling water-bath until the water is evaporated from the sample, wipe the undersurface of the dish, and place in the oven at 102°C for 2-1/2 hours. Put the lid on the dish, cool in the desiccator and weigh. Continue heating and re-weighing at hourly intervals until successive weighings do not vary by more than 0.5 mg.

CALCULATION

$$\% \text{ total solids m/m} = \frac{\text{weight of residue}}{\text{weight of milk}} \times 100$$

REFERENCES

Official Methods of Analysis of the AOAC, 1984. 16.032.

British Standard, 1741:1963.

Society of Public Analysts, 1954. Analyst 70, 105-6; 1885, 10, 216.

FREEZING POINT

PRINCIPLE

The milk is supercooled, seeded with ice crystals if necessary and the freezing-point determined in the standard Hortvet apparatus, carefully following the exact instructions for use.

APPARATUS

Hortvet cryoscope (see diagram). The original equipment was ether-cooled, a procedure now considered unduly hazardous. Any refrigerated bath that can be kept at -3°C is suitable (e.g. that of Temple (86)); 20% ethylene glycol in water is a convenient refrigerant. Simple manual cryostats are still available commercially.

STANDARDIZATION OF THERMOMETER

The bore of the thermometer may not be uniform along its length and therefore requires calibration. Calibrated thermometers can be obtained from certain standards organizations, but uncalibrated ones must be checked against standard sucrose or salt solutions. There is a tendency now to use the salt although the Hortvet freezing-point tends to be held for a shorter time than with sucrose solutions.

Carefully prepare pure sucrose solutions, 7.0, 7.5, 8.0, 8.5 and 8.75% m/v at 20°C. Determine the freezing-point of each solution two or three times. The standard values are given in the following table:

% m/v sucrose at 20°C	Freezing-point depression, °C
7.0	-0.422
7.5	-0.454
8.0	-0.487
8.5	-0.520
8.75	-0.537

If the results differ from these values, the freezing-points of samples must be corrected by adding or subtracting this difference, interpolating if need be. For example, suppose the experimentally determined value for 8.0% sucrose is -0.489°C and for 8.5% sucrose is -0.524°C, and the freezing-point (uncorrected) of the samples is -0.504°C, 0.003 must be added to this giving a freezing-point (corrected) of 0.507°C.

If salt solutions are to be used, they may be prepared as follows:

NaCl g/kg water	Freezing-point °C
6.8920	-0.422
8.5978	-0.525
8.8772	-0.540
10.2060	-0.621

The salt should be dried by heating at 500°C before use, and cooled in an efficient desiccator. Note that although the sugar solutions are m/v the salt solutions are m/m. The weights of salt to be used have not been corrected for buoyancy but the error is not significant in practice.

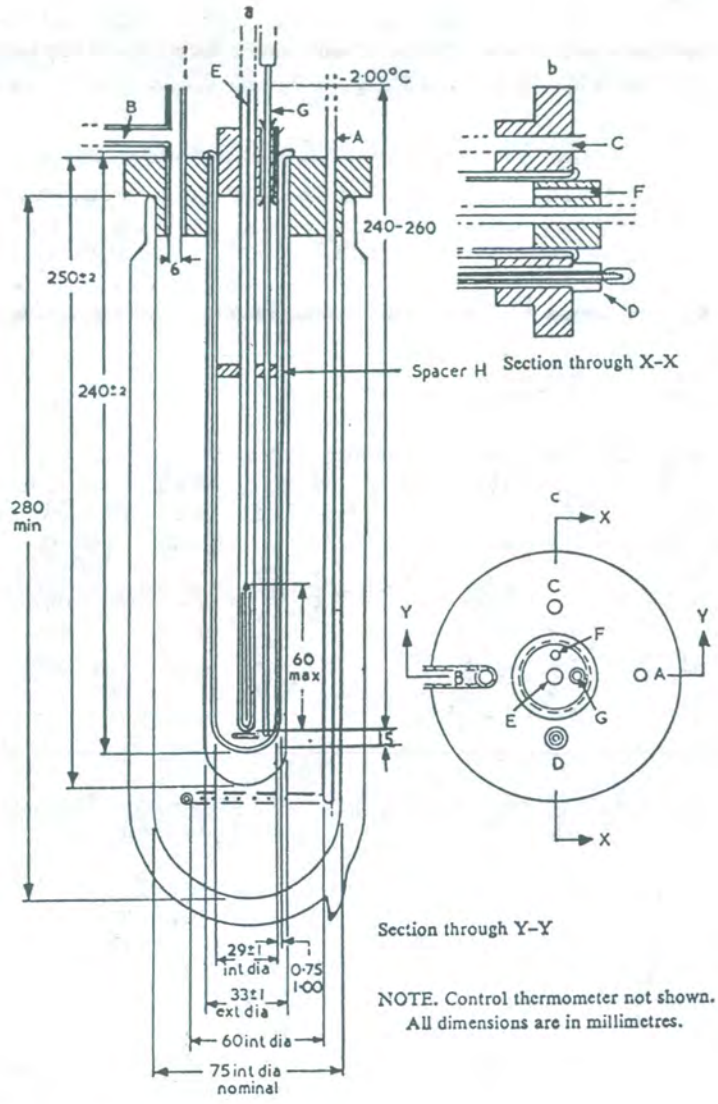


Diagram of Sample Tube and Sample Tube Holder of Hortvet Apparatus

PROCEDURE

Cool the apparatus by a suitable means in order to bring the temperature of the outer jacket to -2.5°C. Pour alcohol into the sample tube cavity so that the level is well above that of the milk in the sample tubes. Maintain the alcohol level during use as required.

Distil some de-ionized water from a little permanganate, or otherwise prepare high quality distilled water, rejecting the first part of the distillate, which may contain dissolved gases. The water may be kept in a stoppered flask in a deep freeze without its freezing-point altering.

Fill the sample tube with the high purity water to 12 mm above the top of the thermometer bulb, place the tube in the sample tube cavity and start the stirrer motor so that the stirring rate is about 30 strokes per minute (or stir by hand). The bung carrying the thermometer and the tube must be scrupulously clean as particles such as dust can act as nuclei for ice-crystal formation and prevent supercooling. For example, poor hygiene may result in mould growth in the stirrer channel and mould pieces falling into the sample tube.

Keeping the outer jacket at -2.5°C, supercool the water blank to -1.0°C to -1.2°C, stop stirring and seed with ice-crystals to induce the water to freeze. Momentary erratic stirring may be sufficient to start crystallization. In any case stop stirring as soon as the temperature begins to rise. It is convenient to keep a jagged-ended copper tube, or a wire with a cork handle, in the deep freeze to facilitate addition of ice to the tube.

As the temperature nears the expected freezing-point, stir (complete up and down motion) three times and sharply tap the thermometer alternative sides near the top of the mercury thread, seven times in all with a cork or rubber gavel. Take a reading. About thirty seconds after stirring, repeat the stirring, tapping and reading, and then a third time about half a minute later. The second and third readings should not differ by more than 0.002 - 0.003°C. If they do, or if the temperature begins to fall again, the result must be discarded.

The method relies on the heat produced by crystallization being compensated by the heat losses to the surrounding cooling jackets, hence the importance of adhering to the exact experimental procedure. The tapping of the thermometer is designed to prevent the mercury thread from getting stuck if there is any roughness on the inner glass surface at that point.

Some form of magnifier may be used to read the thermometer to the nearest 0.001°C by interpolation, but care must be taken with some of the heavier ones that can be attached to the thermometer that the latter is not moved from the vertical, (unless a spacer is used) bringing the bulb close enough to the side wall to alter the rate of heat loss mentioned above.

The blank and samples should be done at least in duplicate and preferably in triplicate. The blank will vary slightly, tending to rise with atmospheric pressure, changes in which are sufficient to slightly affect the volume of the bulb. The volume of the bulb also tends to change with age, thus affecting the blank.

Replace the water by a sample, adjust the cryostat so that as the milk supercools, the outer jacket is at -3°C. The work is hastened by using precooled milk (which must not have ice in it) and/or by allowing the jacket to be below -3°C during the initial stage of cooling the milk. Allow the milk to supercool 1.2° below the expected freezing-point (i.e., to about -1.65°C) and determine the freezing-point as for the water blank.

CALCULATION

$$\% \text{ m/m added water} = \frac{F_G - F_S}{F_G} \times (100 - T)$$

Where:

F_G = freezing-point of genuine milk (see "Interpretation" below).

F_S = freezing-point of sample.

T = total solids of sample (% m/m).

INTERPRETATION

The freezing-point of milk is usually at least 0.530°C below the freezing-point of water. This is spoken of as a freezing-point depression.

The F_G to be used in the formula above should be that of milk taken from the cow or herd by a supervised milking not more than 72 hours after the taking of the questioned sample. The milk from such a supervised milking is known as an appeal-to-cow or appeal-to-herd sample. The usual procedure is to notify the inspector as soon as possible and he will probably decide to take a sample from a supervised milking. He may be required by law to do it within a specified time (usually between 24 and 72 hours) and in any case it should be taken as soon as possible to avoid the possibility that other factors have affected the milk. If the freezing-point of the "appeal-to-cow" sample (which may in fact be an "appeal-to-herd" sample) is substantially the same as the original one, the milk is regarded as genuine even though the freezing-point depression is less than 0.530°C. What is meant by "is substantially the same" has to be defined. It is probably reasonable to regard a difference of 0.010°C or more as indicating the presence of added water in the sample showing the lower freezing-point depression. The precision and accuracy of the analyst's own results over a period of time may enable him to come to his own conclusions about what represents a signficant difference between two samples.

The absence of an "appeal-to-cow" sample should not mean that an adverse report cannot be made. It means that a minimum value for genuine milk must be chosen. The analyst should determine the average freezing-point depression for milk in his area.

Henningson (87) and his collaborators in the U.S.A. found that genuine milk from herds may have a freezing-point depression as low as 0.513°C and that 5.3% of the herd samples examined had freezing-point depressions less than 0.530°C. The spread of these results is similar to that from individual cows and it would appear that the conclusions of Wood (88) that the average freezing-point for bulked-milk is -0.544°C and the minimum -0.540°C, are valid as long as the bulked milk (perhaps better called pooled milk) is derived from enough herds to remove the effect of inter-herd variations. As the yield per cow and the size of herd have increased, the 200 gallons (originally regarded as the minimum volume representing bulked milk) seems to now be too small to be regarded as representative of pooled milk. Thus, the analyst must take these factors into account when choosing the appropriate value. When no "appeal-to-cow" sample is available, the regulatory analyst must choose an acceptable minimum freezing-point depression preferably based on at least a few hundred analyses from his area. When the freezing-point depression is less than about 0.510°C there is no doubt that water has been added, but the analyst still has to use a minimum value for genuine milk in order to calculate the approximate percentage of added water. For individual cows 0.530°C has frequently been taken while the AOAC 12th Edition used 0.525°C as a presumptive limit irrespective of size of herd. It is generally considered most appropriate to report the result as "not less than x % added water".

An automatic thermistor cryoscope is quicker than the Hortvet for routine purposes. Results for regulatory purposes may be checked against the latter, or it may be preferred to accept thermistor results as official. For details of its use see AOAC (13th Ed.) and Moore (89), Anon (90), and Hoffelner (91).

The effects of pasteurization and uperisation, which raise the freezing-point about 0.005°C are discussed by Dillier-Zulauf (92), and Dillier-Zulauf and Doyotte (93).

Milk samples for freezing-point tests may be preserved by the addition of 1.5 ml per 100 ml of a solution containing 1% m/v $HgCl_2$ and 0.85% m/v NaCl (Harding and Royal (4)) rather than formaldehyde, the presence of which invalidates the freezing-point test.

An increase in acidity lowers the freezing-point and hence tends to make samples appear to contain less added water. 0.0034°C is subtracted from the freezing-point depression for every 0.01% by which the acidity expressed as lactic acid exceeds 0.18%. If the acidity exceeds 0.30% the freezing-point test is invalid. Some care should be taken in interpreting the results if the acidity exceeds about 0.23 - 0.24%. Binder (94) suggests subtracting from the freezing-point depression 0.0002°C for each 0.1° Soxhlet-Henkel (0.00225 % lactic acid) above 7° Soxhlet-Henkel and adding that amount for each 0.1° S-H below. Panetsos and Georgakis (95) suggest 0.0008°C per 0.1° S-H and state that over 12° S-H (0.25% lactic acid) the corrections become unreliable. Binder's figure corresponds to 0.000888°C per 0.01% lactic acid and hence is about 4 times lower than that of Panetsos and Georgakis. The expression of milk acidity other than as % lactic acid is dealt with under "acidity".

Welboren and van der Velden (96) have suggested a screening method for added water. Prentice (97) reported an extensive study comparing the Hortvet apparatus with other cryoscopes.

For further data and discussion on the freezing-point determination, see: Luck and Dresner (98), Binder (99), Asperger (100), Bouchez and Waes (101), Henningson (102)(103)(104), Jamotte and Duchateau (105), Freeman et al (106), and Dermott (107).

REFERENCES

Official Methods of Analysis of the AOAC, 1984. 16.104.

Hortvet, J., 1922. Journal of the AOAC 5, 470-97.

British Standard Method, BS 3095, Parts 1-3, 1980 and 1981.

MILK ACIDITY

PRINCIPLE

The sample is titrated with sodium hydroxide to a phenolphthalein endpoint using milk containing rosaniline as a comparison standard.

APPARATUS

1. Two 100 ml poreclain evaporating dishes.

2. Burette, 5 ml.

REAGENTS

1. 0.1N sodium hydroxide solution.

2. Rosaniline acetate solution. Dissolve 0.12 g of rosaniline acetate in 95% ethanol, containing 0.5 ml of glacial acetic acid, and dilute to 100 ml. Store in the dark. Dilute 1 ml to 500 ml with 95% ethanol-water, 1+1.

3. Phenolphthalein. Dissolve 1 g phenolphthalein in 110 ml 95% ethanol and add 0.1 N sodium hydroxide dropwise until a faint pink colour is obtained. Dilute to 200 ml with distilled water.

PROCEDURE

Pipette 10 ml of milk into each evaporating dish. To one add 1 ml of the dilute rosaniline solution and stir with a glass rod. To the other add 1 ml of phenolphthalein solution and titrate with 0.1N sodium hydroxide, stirring the sample, until the colour is the same as that of the rosaniline comparison standard.

CALCULATION

$$\% \text{ m/v lactic acid} = \frac{\text{ml NaOH}}{1000} \times 0.1 \times 90 \times \frac{100}{10 \text{ (ml of milk taken)}}$$

$$= \text{ml NaOH} \times 0.09$$

where % m/v = percent mass in volume.

INTERPRETATION

The various standard methods for determination of acidity in milk give slightly different answers even if used by the same operator. These differences (discussed by O'Connor (108)) tend to be greater if no colour standard is used (e.g. AOAC method). The acidity of freshly drawn milk is mainly due to citric acid, phosphates, carbon dioxide and casein, and is about 0.13-0.14% expressed as lactic acid. The increase in acidity on ageing is due to the production of lactic acid as a result of bacterial action. Sourness begins to become noticeable when the acidity exceeds about 0.18% as lactic acid.

The acidity of milk is also often expressed in degrees, defined as follows:

Degrees Soxhlet-Henkel (°S-H) = ml of N/4 alkali required to neutralize 100 ml of milk (hence °S-H/44.4 = % of lactic acid).

Degrees Dornic (°D) = ml of 0.046N calcium hydroxide solution (saturated lime water at 15°C) required to neutralize 100 ml of milk.

Degrees English = ml of N/9 alkali required to neutralize 100 ml of milk.

Degrees Richmond (°R) = No. of ml of 0.1N alkali required to neutralize 100 ml of milk.

Hence 0.14% lactic acid = 30°D = 7°S-H = 17°R = 14° English.

Luck et al (109) have investigated the relation between acidity and pH. Stiles et al (110) compared a number of methods and found the one given above the most accurate. Citric acid may be determined by the IDF method for cheese (see Nowak and Laskowski (111)). The acidity of mastitis milk may be as low as 0.10% as lactic acid while very rich milks may contain as much as 0.16% before lactic fermentation starts. The acidity of pasteurized milk does not normally exceed 0.15% as lactic acid nor that of sterilized milk 0.16%.

REFERENCE

British Standard 1741:1963.

NITRATES IN MILK

PRINCIPLE

Under the conditions of test, diphenylamine is oxidized by nitrate to the intensely blue quinone-immonium salt via diphenyl benzidine. This is a qualitative test only.

APPARATUS

Note: Care must be taken to rinse all glassware to ensure the absence of even traces of nitrate. The test must not be conducted near sources of nitrous fumes such as reagent bottles of concentrated nitric acid. The filter papers used must also be checked for nitrates and washed prior to use if necessary.

REAGENTS

1. Diphenylamine solution - weigh 0.085 g diphenylamine and dissolve in 50 ml water. Slowly add 450 ml of concentrated sulphuric acid with shaking, keeping the solution cool.

2. Precipitating reagent - dissolve 20 g of mercuric chloride and 5 g of ammonium chloride in water, add 20 ml of concentrated hydrochloric acid and dilute to 100 ml with water.

PROCEDURE

To 5 ml of milk in a test-tube add 6 or 7 drops of the precipitating reagent and shake occasionally for about 2 minutes. Pipette 2 ml of the diphenylamine solution into the bottom of another test-tube without allowing any of the solution to touch the walls of the tube. Place a filter paper in this tube and incline it so that the filtrate from the precipitated milk runs gently down the side of the tube and forms a layer on top. When about 1 ml of filtrate has collected, remove the filter and examine the filtrate/diphenylamine interface over a white surface. In the absence of nitrates, there is no colour, and some yellow or brown colour may appear when the tube is rotated. In the presence of nitrates a blue colour develops either immediately or on rotation of the tube. Carry out a blank with genuine milk. The test detects down to about 0.1 micrograms/ml in the filtrate.

INTERPRETATION

It is generally accepted that nitrate does not occur in normal milk, but is often present in drinking water, so the test, when positive, serves as confirmation of the addition of water to milk. There is no reason to apply the test if nitrate is known to be absent from the local water supply. Remond (112) discusses the presence of nitrate in milk from other causes. Quantitative estimation of nitrate and nitrite in milk is not usually justified, but if needed, the method of Manning, et al (113) or Resmini and Volonterio (114) may be used. See also Ling (115) and Davis and MacDonald (1).

REFERENCE

This is a method of great antiquity, apparently due to Fuch. See for example:

Richmond, H.D., 1894. Analyst 19, 73-87.

Lorrigo, 1930. Analyst 55, 433.

PHOSPHATASE TEST

PRINCIPLE

Any phosphatase present in the milk splits the substrate, p-nitrophenyl phosphate, to give p-nitrophenol, which is highly coloured in alkaline solution.

APPARATUS

1. A Lovibond comparator with stand for work in reflected light.

2. A Lovibond comparator disc APTW or APTW7.

3. Two fused glass cells, 25 mm depth.

4. A water bath or incubator capable of being maintained at 37.5°C ± 0.5°C.

5. A pipette to deliver 5 ml.

6. A supply of 1.0 ml straight-sided pipettes.

7. A 1000 ml graduated flask.

8. A 100 ml measuring cylinder.

9. Test tubes nominal size 150/16 mm with rubber stoppers to fit.

CARE OF APPARATUS

1. After use, each test tube must be emptied, rinsed in water, well washed in hot water containing soda, rinsed in distilled water and finally dried.

2. If after this treatment a test tube does not appear to be clean, the treatment must be repeated and in addition after being rinsed in warm water it must be soaked in 50 percent hydrochloric acid and then rinsed again in warm water before being rinsed in distilled water and finally dried.

3. New glassware must be cleaned by soaking in chromic acid solution prepared by slowly and carefully adding 4 volumes of concentrated sulphuric acid to 5 volumes of 8 percent potassium dichromate. The solution must be kept covered and must be discarded when it becomes green. After cleaning in chromic acid solution, new glassware must be rinsed in warm water, rinsed in distilled water and finally dried.

4. Pipettes must be well rinsed in cold water and then cleaned by soaking for 24 hours in chromic acid solution in a 250 ml glass cylinder or other suitable container. The pipettes must then be well rinsed in warm water, rinsed in distilled water and finally dried.

5. Glassware used for the test must not be used for any other purpose and must be kept apart from other apparatus in the laboratory.

REAGENTS

1. Buffer solution: Dissolve 3.5 g of anhydrous sodium carbonate and 1.5 g of sodium bicarbonate in distilled water, and make up to 1 L with water. Store in a refrigerator and discard after one month.

2. Substrate: Disodium p-nitropohenyl phosphate. The solid substrate must be kept in the refrigerator.

3. Buffer-substrate solution: Weigh 0.15 g of the substrate into a 100 ml measuring cylinder, and make up to 100 ml with the buffer solution. The solution must be stored in a refrigerator and protected from light. It must give a reading of less than the standard marked 10 on the comparator disc APTW or APTW7 when viewed in transmitted light through a 25 mm cell in the comparator (distilled water being used for comparison). The solution must be discarded after one week.

PRECAUTIONS

The following precautions must be taken:

1. Milk which shows evidence of taint or souring should not be tested.

2. All glassware must be clean immediately before use.

3. A fresh pipette must be used for each sample of milk. Pipettes must not be contaminated with saliva.

4. The test must not be carried out in direct sunlight.

5. Distilled water must be used throughout.

6. The sample of milk should be examined as soon as possible after arrival at the laboratory. If not examined immediately, it must be kept at a temperature of between 3°C and 5°C until examined. The sample must be brought to room temperature immediately before being tested.

PROCEDURE

Pipette 5 ml of the buffer-substrate solution into a test tube, stopper the test tube and bring to a temperature of 37°C. Add 1 ml of the milk to be tested, replace the stopper in the tube and mix the contents well by shaking. Incubate for exactly 2 hours at 37°C. Incubate one blank prepared from boiled milk of the same type as that undergoing the test with each series of samples. (Where the samples consist of highly coloured milk, such as homogenized milk, prepare a separate blank of such milk). After incubation remove the test tube from the water bath and mix the contents of the tube. Place the blank on the left hand side of the comparator stand and the test sample on the right. Take readings in reflected light by looking down onto the two apertures with the comparator facing a good source of daylight (preferably north light). If artificial light is needed for matching, use a "daylight" type of illumination. Revolve the disc until the test sample is matched. Record readings falling between two standards by affixing a plus or minus sign to the figure for the nearest standard.

INTERPRETATION

The test is considered to be satisfied by milk which gives a reading of 10 µg or less of p-nitrophenol/ml of milk. Properly pastuerized milk will give no discernible colour.

REFERENCES

Aschaffenburg, R. and Mullen, J.E.C., 1949. Journal of Dairy Research 16, 58-67.

Official Methods of the AOAC, 1984. 16.111-.129.

The Milk (Special Designation) Regulations, 1963, S.I. 1571 of the U.K.

TURBIDITY TEST

PRINCIPLE

The heating required by sterilization together with ammonium sulphate effectively denatures and precipitates albumin as well as casein and the filtrate of a sample treated in this way is therefore clear.

APPARATUS

1. Conical flasks of 50 ml capacity.
2. Graduated cylinders of 25 ml capacity.
3. Test tubes, 150/16 mm.
4. Filter funnels, 6 cm diameter.
5. Beakers, 400 ml capacity.
6. 12.5 cm folded filter papers, Whatman No. 12 or equivalent.

REAGENTS

1. Ammonium sulphate, AR or equivalent purity.

PROCEDURE

Weigh 4 $\pm$ 0.1 g of ammonium sulphate into a 50 ml conical flask. Pipette 20 $\pm$ 0.5 ml of the milk sample into the conical flask and shake the flask for 1 minute to ensure that the ammonium sulphate dissolves. Leave the mixture for not less than 5 minutes and then filter through a folded filter paper into a test tube. When not less than 5 ml of a clear filtrate have collected, place the tube in a beaker of water which has been kept boiling and keep there for 5 minutes. Transfer the tube to a beaker of cold water, and when the tube is cool, examine the contents for turbidity by moving the tube in front of an electric light shaded from the eyes of the observer.

INTERPRETATION

The milk is considered sterilized when a sample treated as above gives a filtrate showing no sign of turbidity.

Unlike phosphatase, peroxidase once destroyed by heating (sterilization, boiling or heating more intensely than pasteurization) cannot be regenerated as far as is known and can thus be used as another test for sterilized milk or milk products. The method of detection employed by Guha and Roy (116) is as follows: Shake 5 ml milk, curd or milk products (dispersed in water) with 5-10 mg p-phenylenediamine and 2 drops of a 10 volume solution of H_2O_2. A violet colour indicates the presence of peroxidase.

REFERENCE

Aschaffenburg, R., 1950. Journal of the Society of Dairy Technology 3, 236.

2.2 DRIED MILK

COMPOSITION

Codex compositional standards for dried milk products are as follows:

1. **Milkfat and water:**

Whole milk powder	
Minimum milkfat content:	26% m/m
Maximum milkfat content:	less than 49% m/m
Maximum water content:	5% m/m
Partly skimmed milk powder	
Minimum milkfat content:	more than 1.5% m/m
Maximum milkfat content:	less than 26% m/m
Maximum water content:	5% m/m
Skimmed milk powder	
Maximum milkfat content:	1.5% m/m
Maximum water content:	5% m/m

2. **Additives:**

Stabilizers	Maximum level
Sodium, potassium and calcium salts of:	
hydrochloric acid	5000 mg/kg singly or in combination expressed as anhydrous substances
citric acid	
carbonic acid	
orthophosphoric acid	
polyphosphoric acid	
Emulsifiers in instant milk powders only	
Mono- and di-glycerides	2500 mg/kg
Lecithin	5000 mg/kg
Anticaking agents in milk powders intended to be dispensed in vending machines	
Tricalcium phosphate	10 g/kg singly or in combination
Silicates of aluminium, calcium, magnesium and sodium-aluminium	
Silicon dioxide (amorphous)	
Calcium carbonate	
Magnesium oxide	
Magnesium carbonate	
Magnesium phosphate, tribasic	

ROUTINE ANALYSIS

The most important determinations to assess the quality of dried milk are the bacteriological examination, moisture, solubility index, lactate, and acidity. Rancidity values should be determined on samples other than skimmed. The constituents other than moisture must be in the same proportions as in liquid milk and Vieth's ratio still applies. Roller-dried milk should be examined for burnt particles which may be inadvertently included in the product during manufacture. The bacteriological examination is more important than the chemical and should at least include tests for faecal Streptococci, total plate count and presumptive coliforms. If the latter is positive, tests should be

made for Staphylococci, Salmonella and Clostridia. Identity and composition may be checked from the fat, protein, lactose and ash and HAI or RPK on the fat.

The fat content may be determined by the method of Rose-Gottlieb, taking up to 1.5 g of sample depending on the expected fat content and diluting to 10 ml with water, then proceeding as for liquid milk. Patratii and Sochneva (117) discuss the various methods available.

Protein is calculated from the Kjeldahl nitrogen, using a factor of 6.38 for all milk products. Lactose can be determined on a cleared solution by a polarimetric, gravimetric or volumetric method.

About 2 g is taken for the ash determination. It is necessary to remove the dish from the muffle about an hour after smoke is no longer given off. Break up the ash with a glass rod, add water, evaporate, dry and continue ashing. The residue may be used to determine the alkalinity of the ash. 10 ml 0.2 N HCl is added and the mixture warmed to complete solution. After cooling 2 ml of 40% neutral calcium chloride solution and 0.5 ml of phenolphthalein are added and the mixture is titrated with 0.2 N NaOH to a faint pink end-point lasting for 30 seconds. The result is expressed as ml of 0.2 N HCl per 100 g of original powder.

Cans of dried milk are often packed under inert gas (which should not contain more than 5% oxygen) and this may result in a swelling of the aluminium foil seal if stored at a high temperature. This is not to be confused with gas production due to bacterial spoilage.

EQUIVALENCE

A claim may be made as to the number of pints or litres of liquid cows' milk to which the tin or container of dried milk is supposed to be equivalent. This is calculated from the analytical data assuming values for liquid milk.

Suggested assumed values for Standard Liquid Milk are:

Type of Dried Milk	% Fat	% Total Milk Solids	% Solids-Not-Fat	Density at 20°C
Full Cream	3.6	12.4		1.032
Three-quarters cream	2.7	11.6		1.033
Half cream	1.8	10.8		1.034
Quarter cream	0.9	9.9		1.034
Skimmed			9.0	1.0355

The equivalent pints may be calculated from the fat, total solids, solids-not-fat, or even from the protein or lactose provided the appropriate factor is calculated. For example, the label claims the contents of the can are equivalent to Z litres of half-cream milk.

1 litre of standard half-cream milk weighs 1,034 g.

This contains $\frac{1.8}{100}$ x 1,034 = 19.4 g of fat.

Fat in sample (by experiment) = 14.3%.

Contents of sample can = 507 g.

Then the can contains $\frac{14.3}{100}$ x 507 = 72.5 g

Equivalent to $\frac{14.3 \times 507}{19.4 \times 100} = \frac{14.3 \times 507}{1940}$ litres of liquid milk

Or in general: $\frac{P \times W}{400 \times P_1}$

where P = weight of the parameter in the whole contents of container.

W = weight of whole contents of container.

P_1 = weight of the parameter in the standard volume (litre or pint) of liquid milk.

DRIED MILK MOISTURE

PRINCIPLE

The sample is dried to constant weight at 102°C and the loss in weight reported as moisture.

APPARATUS

1. Metal dishes with close-fitting lids.

PROCEDURE

Dry a dish and lid in the oven and cool in the desiccator. Exactly weigh approximately 1 g of sample into the dish and dry in the oven 2 hours with the lid alongside. Place the lid on the dish, transfer to the desiccator and quickly weigh when the dish has completely cooled. Heat in the oven half-an-hour and re-weigh. Repeat until succeeding weights do not differ by more than 0.5 mg.

CALCULATION

$$\% \text{ moisture m/m} = \frac{\text{weight lost} \times 100}{\text{weight of sample}}$$

INTERPRETATION

Spray dried powders usually have a moisture content of about 3% and roller dried powders about 5%. The latter are less commonly found than formerly as an article of commerce. Results over 5% would justify further examination, as the moisture may be sufficient to sustain mould or bacterial growth.

Difficulties in obtaining consistent results may be due to small differences in oven temperature, oven design etc. These are discussed by Emmens et al (118), Morissey (119) and Anderson and Berlin (120) have shown that the total moisture, including the water of crystallization of alpha-lactose monohydrate may be determined by Karl Fischer or azeotropic distillation with toluene (method of Dean and Stark). Drying at 65°C for six hours under a vacuum of about 100 torr partial pressure gives a value corresponding to the "free" moisture. The AOAC method involves drying under vacuum at 100°C.

REFERENCE

International Dairy Federation, FIL-IDF 26:1964.

DRIED MILK SOLUBILITY

PRINCIPLE

The powder is shaken with water and the total solids of the suspension determined before and after centrifuging. The amount of powder remaining in suspension after centrifuging expressed as a percentage of the total amount in suspension is taken as a measure of the solubility.

APPARATUS

1. Centrifuge with 50 ml tubes.

PROCEDURE

Shake 4 g of powder with 32 ml water at 50°C for 10 seconds in a 50 ml centrifuge tube and keep the tube in water at 50°C for 5 minutes. Centrifuge the suspensions from half-cream and full-cream samples for 10 minutes at 2000 rpm. Cool in a refrigerator and remove the fat layer after prising it from the walls of the tube with a needle. Warm to room temperature, break up the deposit with a glass rod and shake vigorously until the suspension appears homogeneous.

For all types of sample, pipette 2 ml from the tube, weigh into a tared metal dish with lid and determine the total solids by drying on a waterbath and then in the oven 1-1/2 hours. Centrifuge for 10 minutes at 2000 rpm and determine the total solids of 2 ml of supernate.

CALCULATION

$$\% \text{ solubility} = \frac{100\ T_1\ S_2}{T_2\ S_1}$$

where T_1 = weight of suspension taken for total solids determination before centrifuging
T_2 = weight of suspension taken for total solids determination after centrifuging
S_1 = weight of dried solids remaining after evaporation of T_1
S_2 = weight of dried solids remaining after evaporation of T_2.

INTERPRETATION

Spray dried powders are almost completely soluble, roller dried to the extent of 80% or more. The results depend on the exact conditions of test and the acidity of the powder so it is advisable to compare doubtful samples with a spray dried powder known to be reasonably fresh. The powders become less soluble with age, thereby affecting the quality.

REFERENCES

Davis, J.G. and Mac Donald, F.J., 1953. Richmond's Dairy Chemistry, Griffin, London.

British Standard 1743:Part 2:1980.

Bonduelle, C. and Luquet, F.M., 1971. Technique Laitiere 718, 18-19.

DRIED MILK LACTATE

PRINCIPLE

The sample is reconstituted and clarified and lactate in the filtrate converted to acetaldehyde. The colour developed between the acetaldehyde and p-hydroxydiphenyl is determined spectrophotometrically and the lactate content calculated from a standard graph.

APPARATUS

1. Spectrophotometer reading at 570 nm.

2. Graduated pipette or burette accurate to ± 0.05 ml.

REAGENTS

1. Copper sulphate solution, 25% - dissolve 250 g of cupric sulphate pentahydrate in water and dilute to 1000 ml.

2. Acid copper sulphate solution - add 0.5 ml of 25% copper sulphate solution to 300 ml of concentrated sulphuric acid.

3. Calcium hydroxide suspension - grind in a mortar 300 g of calcium hydroxide with water to a total of 900 ml. Store in a tightly stoppered bottle.

4. p-Hydroxydiphenyl solution - dissolve 0.75 g of p-hydroxydiphenyl in 5 ml of 5% aqueous NaOH, shaking and warming as necessary. Dilute to 50 ml in a graduated flask. Store in the dark in a cool place and discard if discoloured or turbid.

5. Lithium lactate standard - dissolve 0.1067 g in water and dilute to 100 ml. 1 ml contains 0.1 mg equivalent lactate. Prepare fresh.

6. Fresh dried milk containing less than 30 mg lactic acid per 100 g of solids-not-fat.

PROCEDURE

For the test use a weight of sample equal to 1000/(SNF-10) where SNF (solids-not-fat) is the percentage calculated by subtracting fat and moisture from 100. Thus a little over 11 g of skimmed milk powder is usually used, weighed accurately to the nearest 0.1 g. Add this weight to 100 ml water and mix thoroughly. Pipette 5 ml into a 50 ml graduated flask and dilute to about 35 ml. Prepare a blank at the same time by adding about 35 ml of water to another flask. Add slowly, with swirling, 5 ml of 25% copper sulphate solution and leave to stand 20 minutes. Dilute to 50 ml, shake vigorously, filter and discard the first part of the filtrate. Pipette 1 ml of filtrate into a test tube (25 x 150 mm). Add exactly 6 ml of acid copper sulphate solution, heat in a boiling waterbath 5 minutes then cool in running water. Add 2 drops of p-hydroxydiphenyl solution and shake thoroughly. Keep in water at 30 ± 2°C for 15 minutes, in boiling water for 90 seconds and then cool to ambient temperature in running water. Measure the absorbance at 570 nm against the blank. If the reading is higher than the most concentrated standard, dilute an aliquot of the filtrate and repeat the test with 1 ml of this diluted solution.

PREPARATION OF STANDARDS

Mix a weight of fresh low-acid skimmed milk powder with 100 ml of water, the weight calculated from 1000/(SNF-10) as for samples. Pipette 5 ml of the reconstituted milk into each of 5 volumetric flasks and add 0, 1, 2, 3 and 4 ml respectively of standard lactate solution. The standards correspond to 0, 20, 40, 60 and 80 mg of added lactic acid per 100 g of solids-not-fat. Dilute the contents of each flask to about 35 ml with water and proceed as for a sample. Plot measured absorbance against mg of lactic acid. The standards must be measured against the same blank as the samples. The blank when measured against water should not give an absorbance reading corresponding to more than 20 mg of lactic acid per 100 g of solids-not-fat. Agreement of duplicate determinations should be better than 10%.

CALCULATION

The calibration curve gives the absorbances for 0 - 0.4 mg of lactic acid. 1 ml of filtrate is derived from 1/50 x 5/100 of the weight of powder taken.

Hence mg lactic acid per 100 g of solids-not-fat = mg in aliquot (read from graph) x 50/1 x 100/5 x 100/weight taken.

INTERPRETATION

The lactate content tends to decrease slightly on storage, but may generally be taken as an indication of the acidity of the milk before processing. A high lactate value would indicate the possibility that the dried milk was made using soured whole milk.

REFERENCE

ISO 3495:1975.

2.3 EVAPORATED AND CONDENSED MILK

COMPOSITION

Traditionally, evaporated milk refers to the unsweetened product and condensed to the sweetened, although there have been recommendations to discard this terminology.

Some typical analyses (taken from Pearson (2))

	Sweetened Condensed Milk				Evaporated Milk		
	Full Cream		Skimmed		Full Cream		Skimmed
	1	2	3	4	5	6	7
	%	%	%	%	%	%	%
Water	25.0	25.1	26.6	27.0	66.1	67.4	76.6
Milk Solids	33.3	32.9	27.2	26.3	33.8	32.5	23.3
Fat	10.6	9.6	0.2	0.2	9.2	9.1	0.7
Lactose monohydrate	12.2	13.0	14.9	14.2	13.3	12.7	12.5
Protein	8.5	8.4	9.6	9.5	9.1	8.7	8.3
Sucrose	41.5	41.9	46.1	46.5	-	-	-
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	1.30	-	-	-	1.08	1.08	-

The Codex Alimentarius Commission standards for evaporated and condensed milk are as follows:

	Evaporated Milk	Evaporated Skimmed Milk	Sweetened Condensed Milk	Skimmed Sweetened Condensed Milk
Minimum milkfat content % m/m	7.5	-	8.0	-
Minimum total milk solids content % m/m	25.0	20.0	28.0	24.0
Sodium, potassium and calcium salts of hydrochloric, citric, carbonic, orthophosphoric and polyphosphoric acid, expressed as anhydrous substances	2000 mg/kg singly, 3000 mg/kg in combination	2000 mg/kg singly, 3000 mg/kg in combination	2000 mg/kg singly, 3000 mg/kg in combination	2000 mg/kg singly, 3000 mg/kg in combination
Carrageenan	150 mg/kg	150 mg/kg	-	-

It is not suggested that these standards are necessarily the only compositional limits that need to be defined for the purposes of national legislation. Some national standards have somewhat higher values for milkfat and milk solids.

ROUTINE ANALYSIS

A proximate analysis - total solids, fat, lactose, protein, ash and sucrose in sweetened milks should be done on routine samples. The acidity should account for the remainder. If there appears to be a discrepancy in the analysis, it may be worthwhile to look for starch, or other fillers or thickeners, and if the protein is high in relation to the lactose and ash (Vieth's ratio), for gelatin.

The fat may be determined by the Rose-Gottlieb method, diluting 2 - 2.5 g, accurately weighed, with 8 ml of water and proceeding as for milk samples. A rapid result may be obtained by the Gerber method for liquid milk using 10.75 ml of a 20% m/v solution of the sample. Butgrometer reading x 5 = % fat m/m. It is convenient to use the 20% m/v solution so prepared for all of the proximate analysis. Livio (121) discusses use of the Mojonnier method.

Protein and ash may be determined by routine methods. Ashing of sweetened condensed milk may be facilitated by addition of a drop of oil.

Lactose may be determined by the Lane and Eynon titration, using 25 ml of mixed Fehling's solution and calculating the result as lactose monohydrate. 10-12 g is diluted with 200 ml of hot water in a 250 ml graduated flask and left to stand at least 30 minutes. After cooling, 4 ml of zinc acetate solution is added, mixed and this is followed by 4 ml of potassium ferrocyanide solution. The mixture is diluted to 250 ml and filtered and the titration carried out using the filtrate. The strength of the protein precipitants and the corrections for the volume of precipitate are the same as those used in the determination of sucrose in condensed milk.

Nowak and Laskowski (111) found that the ISO/IDF method for citric acid in cheese and processed cheese works equally well with other milk products. Phosphorus can be determined by the method given in IDF 42:1967.

EQUIVALENCE

The equivalent volume of a normal milk may be calculated and compared with any claim on the label. For the purpose of calculating the equivalent pints the can should be weighed unopened, the contents transferred to a sample jar with an airtight seal, the can washed and dried and reweighed, in order to obtain the exact weight of the contents. Any small slivers of metal produced during opening must be included in the weight of the empty can. Old tins may require warming to about 40° before opening to facilitate mixing the contents. The sample should also be examined for metallic contamination (tin and lead) if the can appears etched.

Next, dilute the contents of the can with the requisite amount of water to make milk of normal composition. The volume so obtained may be called by a phrase such as "equivalent pints". A claim may be made in the label relating to this volume and the accuracy of any such claim must be checked. The composition of "normal milk" is assumed, either by being stipulated in regulations, or by taking known average values. For the purpose of the worked example "normal milk" will be taken to contain 3.6% fat and 12.4% total milk solids. These figures have been calculated using TMS = 0.25D + 1.22F + 0.72 and densities of 1.029, 1.031 and 1.0325 (where TMS = total milk solids, D = 1,000 x density - 1,000 and F = % fat).

One pint ("Imperial" pint) of this milk = 20 fluid ounces x 1.032 = 20.64 ounces weight.

Total milk solids present = (12.4/100) x 20.64 = 2.559 ounces weight = 72.56 g.

Total milk solids in a can of condensed milk (TMS)

$$= \frac{\text{TMS} \times W}{100}$$ (where W is the weight of the contents in grams)

$$\text{Equivalent pints} = \frac{\text{TMS in can}}{\text{TMS in one standard pint}} = \frac{\%\ \text{TMS} \times W}{100/72.56} = \frac{\%\ \text{TMS} \times W}{7256}$$

By analogy, factors can be calculated for litres and American pints, if the claim is made in either of these units. Also, factors can be calculated using assumed values for fat, milk-protein, lactose or solids-not-fat. American and

Imperial fluid ounces are slightly different, but ounces weight are the same; One U.S. pint = 0.8327 "Imperial" pints.

Assumed Composition of Standard Milk

	Fat	Solids-not-fat	Total milk solids	SG
Full-Cream	3.6	8.8	12.4	1.032
Half-Cream (semi-skimmed)	1.8	9.0	10.8	1.034
Skimmed	-	9.0	9.0	1.0355

TOTAL SOLIDS
(Evaporated and Condensed Milk)

PRINCIPLE

The sample is dried to constant weight at 100°C, under standard conditions, which must be followed closely in order to obtain reproducible results.

APPARATUS

1. Flanged metal dish about 7.5 cm diameter and 2.5 cm deep with close fitting lid.

2. Glass stirring rod with a flattened end, the other end bent to rest over the dish edge.

3. Steam bath.

REAGENTS

1. Acid-washed sand, retained by an 85 mesh sieve but passing a 30 mesh one.

PROCEDURE

Place about 25 g of sand into a dish and dry to constant weight at 98-100°C. Dry a lid and stirring rod at the same time. Place the lid with the stirring rod on it, on the dish before removing it from the oven, cool in a desiccator and weigh after 45 minutes. Allow the sand to slide to one side of the dish and add about 3 g of well-mixed unsweetened or 1.5 g of sweetened milk and weigh rapidly. Add 3 ml of water for unsweetened, 5 ml for sweetened milk, mix with the stirring rod, and then mix the diluted milk with the sand. Leave the stirring rod in the dish, hooking the end over the edge of the dish. Place the dish on a steam bath such that the flange is at the level of the steam-bath cover but the two metal surfaces should be separated by supporting the flange of the dish on a porcelain or rubber ring. Stir the sand-milk mixture at first to avoid caking and to keep the mass aerated. Once the aqueous phase has evaporated, lay the stirring-rod on the sand. Leave the dish on the steam-bath twenty minutes in all, then transfer to an oven at 98-100°C, with the lid alongside. Leave in the oven 1-1/2 hours, replace the lid, cool in a desiccator 45 minutes and weigh. If possible cool each dish in a separate desiccator. (Ensure that the desiccant is effective.) Remove the lid and place both dish and lid in the oven for a further hour, cool and weigh as before. Repeat until successive weighings do not differ by more than 0.5 milligram.

CALCULATION

$$\text{Total Solids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

INTERPRETATION

This method is subject to errors similar to those mentioned under moisture determination in skimmed milk powder, due to the presence of lactose. It is for this reason that the method must be followed exactly in order to obtain reproducible results. Franzen (122) recommends 4-6 analyses per sample for reliable results.

REFERENCE

International Dairy Federation 15:1961.

SUCROSE
(Condensed Milk)

PRINCIPLE

The optical rotation of a cleared solution of the sample is measured before and after mild hydrolysis which inverts the sucrose but has almost no effect on lactose or other sugars. The percentage of sucrose present is calculated by use of a formula.

APPARATUS

1. Polarimeter with sodium or mercury green light (Mercury vapour lamp with prism or special Wratten Screen 66A) reading 0.05° of angle or better or saccharimeter with International sugar scale with white light passed through a filter of 1.5 cm of 6% potassium dichromate, or sodium light, and reading 0.1 cm or better on the International sugar scale. Both should be fitted with 2 dm tubes.

2. Waterbath at 60°C ± 1.

REAGENTS

1. Zinc acetate solution. Dissolve 21.9 g of the dihydrate in water, add 3 ml of glacial acetic acid and dilute to 100 ml with water.

2. Potassium ferrocyanide (II) solution. Dissolve 10.6 g of the trihydrate in water and dilute to 100 ml.

3. Dilute ammonia solution, 2N (3.5%).

4. Dilute acetic acid solution, 2N (12%).

5. Hydrochloric acid, 6.34N.

PROCEDURE

Accurately weigh approximately 40 g of well-mixed sample, add 50 ml of water at 80-90°C and mix. Transfer to a 200 ml volumetric flask, rinsing with quantities of water at about 60°C until the total volume is 120-150 ml. Mix, cool and add 5 ml of 2N ammonia solution. Mix and leave to stand 15 minutes. This completes lactose mutarotation. Add exactly the volume of 2N acetic acid required to neutralize the ammonia. Add 12.5 ml of zinc acetate solution, swirling at the same time and then 12.5 ml of potassium ferrocyanide solution, continuing swirling. Dilute to volume at 20°C. Avoid inclusion of air bubbles. If this inadvertently occurs, apply gentle suction. Shake, leave to stand a few minutes and filter through a dry 15 cm filter paper (e.g. Whatman No. 4), rejecting the first 25-30 ml of filtrate.

Determine the optical rotation of the filtrate at 20°C. There is no need to correct the reading for temperature as long as the solution is between 18° and 22°C.

Invert (hydrolyse) an aliquot by pipetting 40 ml into a 50 ml graduated flask, adding 6.0 ml of 6.34 N HCl and immersing the flask up to its neck in a waterbath at 60°C for 15 minutes. Mix by a rotary movement during the first five minutes. Cool to 20°C and dilute to 50 ml. Mix and leave to stand at 20°C for one hour. Determine the optical rotation. The temperature must be between 18 and 22°C. If it is not exactly 20°C use the correction factor 0.0037 x (T-20) where T is the experimental temperature, adding if the temperature is above 20°C and subtracting if it is below.

METHOD VALIDATION

Add 18.00 g of pure sucrose to 100 g of liquid milk (or 110 g of liquid skimmed milk). This corresponds to 40 g of condensed milk containing 45% sucrose. Calculate the sugar content using the weight of liquid milk and its protein and fat values to calculate the volume of precipitate, but using 40.00 g as the weight taken in the formula to calculate sucrose content. The result should be within 0.1% of 45%.

CALCULATION

Correction in ml for the volume of the precipitate formed during clarification,

$$v = (W/100)\ (1.08F + 1.55P)$$

where W = weight of sample in g
F = % fat in sample
P = % protein (N x 6.38) in sample

$$\% \text{ sucrose} = \frac{D - (5/4 \times I)}{Q} \times \frac{(V - v)}{V} \times \frac{V}{(L \times W)}$$

where D = polarimeter reading before inversion
I = polarimeter reading after inversion
V = volume in ml to which sample was diluted before filtration (200ml)
v = volume of precipitate as calculated above
L = length in dm of polarimeter tube (2 dm)
Q = the inversion divisor factor, which varies with wavelength of incident light, concentration and temperature

For sodium light and angular degrees

$$Q = 0.8825 + 0.0006\ (C - 9) - 0.0033\ (T - 20)$$

For mercury light and angular degrees

$$Q = 1.0392 + 0.0007\ (C - 9) - 0.0039\ (T - 20)$$

For white light and International sugar degrees

$$Q = 2.549 + 0.0017\ (C - 9) - 0.0095\ (T - 20)$$

(T = temperature of inverted solution when read)

C is the percentage of total sugars in the inverted solution as polarised, equal to 9.00 if exactly 40 g of condensed milk of normal composition is used. With this weight and the temperature at exactly 20°C, sodium light, angular degrees and a 2 dm tube, the formula simplifies to:

$$S = \left(D - \frac{5 - 1}{4}\right)\ (2.833 - 0.00612F - 0.008788)$$

The correction 0.0006 (C-9) is only accurate if C is close to 9, but the correction is small enough to be ignored with normal samples. Duplicates should agree within about 0.3%.

2.4 BUTTER AND GHEE (Butter Oil)

COMPOSITION

The fat content of butter is usually over 80% but may be a little under in salted butters. It is most conveniently determined by difference (100% less water, curds and salt). It can also be determined by direct ether or petroleum ether extraction of the residue from the moisture determination, filtration and evaporation. This requires more careful manipulation to ensure that no fat is left in the filter nor drawn to the outside of the dish by capillarity, especially if diethyl ether is used. The moisture is usually about 12-15% and some countries have an upper limit of 16%. The CAC recommends a minimum of 80% m/m fat, maxima of 2% milk solids other than milkfat and 16% water.

The CAC standard at present recommends that the colours annatto, beta-carotene and curcumin be permitted if added according to good manufacturing practice, and the neutralizing salts calcium hydroxide, and the carbonate, orthophosphate, bicarbonate and hydroxide of sodium may be added only for the purpose of pH adjustment up to a maximum, singly or in combination, of 2000 mg/kg expressed as anhydrous substances.

The ash is about 0.1% in unsalted butter and the salt is less than half of this, but may be 0.5 - 5% in salted butter. The amount of salt that may be added is limited by the solubility in the aqueous phase and cannot exceed 2-3% in countries that impose an 80% minimum fat content. If salt has been added, mention of this addition should be included in the name.

The value for free fatty acids as oleic acid is normally 0.2-0.3% in the fresh product, and the peroxide value less than 10 meq of oxygen per kilogram of fat. 0.5% and 20 meq/kg respectively may reasonably be taken as upper limits.

The CAC standard requires ghee (butter oil) to contain at least 99.3 m/m of butterfat and a maximum of 0.5% m/m of water. For the anhydrous product the corresponding figures are 99.8% and 0.1%. Butter oil for certain manufacturing purposes only (e.g. biscuit making), excluding use to prepare recombined milk or milk products, may contain propyl, octyl or dodecyl gallates in combination with BHA or BHT up to a maximum of 200 mg/kg in combination, but the gallates must not exceed 100 mg/kg.

The refractive index, specific gravity, titre, saponification value, iodine value, Valenta, and the Crismer and CTD tests all indicate adulteration if found to be outside the normal range for butter oil but the values for many other fats and fat mixtures also lie within this range, thus severely restricting the usefulness of these tests. The CTD test, favoured by Hart and Fisher, developed out of the Valenta and Crismer tests and may be found in the AOAC (13th edition, 1980). The methodology for the other tests is given in the section on oils and fats. Typical values for butter are as follows:

	Max	Min	Mean
Specific gravity at $\frac{37.8°C}{37.8°C}$	9.913	9.910	9.912
Refractive index at 40°C	1.4580	1.4522	1.4588
= Zeiss butryo scale	45.5	40.0	43.5
Saponification value	243.0	209.0	228.5
Iodine value	50.0	26.0	36.0
Mean molecular weight of fatty acids	267.0	258.0	260.0
Titre	variable, usually 32-38°C		

The refractive index used to be of value, but this is no longer true as many margarines have values in the same range. The various colour tests for particular oils given in the section on oils and fats may also be applied to butter and of course, if positive, indicate adulteration. The only proviso is that Halphen's test for cottonseed oil, and also the Villavecchia-Fabris test for sesame seed oil may be slightly positive for genuine butter samples if the animals have been feeding on these products. The feeding of unsaturated fats in micro-encapsulated form to cows facilitates the manufacture of a more spreadable butter with lower RPK, HAI and butryic acid values.

ROUTINE ANALYSIS

Butter should first be examined for moisture, salt, curds and fat. Butter oil (ghee) and the fat from butter should be checked for identity, rancidity and added antioxidants. The residual moisture in butter oil may be determined by drying in the oven or by Karl Fischer titration. It may also be necessary to look for trace metals (especially copper, but also Fe, Mn, Cr, Ni, etc. promote rancidity), added colour and undesirable preservatives such as boric acid. Ghee made from buffalo milk normally has a higher Reichert value.

For the means of checking the identity, and a more detailed examination if the identity is suspect, see the analysis sections on the detection of foreign fats in butterfat. There is very little information on the prevalence of the adulteration of butter, but a report from one country for the year 1970 showed that of 50 samples, 20 contained over 16% moisture, 10 contained less than 80% fat, 13 samples contained non-milk fat and two contained starch.

Tests for filth, sediment and pesticide residues are given in the AOAC. Details for the routine analysis of butter, including pH of serum, titratable acidity, extraneous matter, copper and iron are given in the Australian Standard AS 1939-1975 and in BS 769:1961. BS 5086:1974 describes the rapid methods. Timmen and Bluthgen (123) describe a photometric method for the determination of copper and iron in butterfat and Roschnik (124) describes an atomic absorption method for copper.

Parodi (125) found that examination of the ratios of fatty acids as determined by GLC was only partially successful in identifying adulterated butterfat samples. However, Parodi (126) found that a combination of fatty acid analysis, sterol analysis, triglyceride distribution patterns, softening point values and IR measurement of trans unsaturation was adequate to distinguish abnormal and those modified by fractionation, from adulterated samples. Hendrickx and Huyghebaert (127) discuss the examination of the sterols by GLC, TLC and IR, the fatty acids by GLC and the determination of the monoglyceride content for the characterisation of mixtures prepared from animal fats, vegetable oils and synthetic triglycerides including tributyrin such that the RPK and saponification values were similar to genuine butter. See also Hendrick and Huyghebaert (128), Kuzdal-Savoie (129) and Parodi (130) and (131).

To detect foreign fats in butterfat, two of the following three tests should be carried out: the hydroxamic acid index, GLC of the volatile fatty acids and the Reichert-Polenske-Kirschner procedure. If the butter is genuine by these tests, that may be considered adequate for routine purposes, but the possibility of adulteration is not rigorously excluded and it is preferable to also carry out at least a test for phytosterols.

A considerable amount of attention has been directed in recent years to triglyceride analysis. This is carried out on packed or capillilary GLC columns and is described by Timms (171), Burón Arias et al (172), Traitler and Prevot (173) and Wathelet et al (174).

MOISTURE IN BUTTER

PRINCIPLE

The weighed sample is dried to constant weight at 100°C, and the weight loss is calculated as moisture.

APPARATUS

1. Metal dish, flat-bottomed, about 7.5 cm diameter, 2.5 cm deep, preferably with a lip.

2. Oven at 100°C.

3. Analytical balance reading to 0.1 mg.

PROCEDURE

Keep the sample at 32-35°C in an airtight container and shake vigorously until a homogeneous lump-free emulsion is obtained.

It is convenient to put in the dish a glass rod with a flattened end and long enough so that the other end can rest on the rim. Dry in the oven, cool at least half an hour in the desiccator and weigh. Add 3-4 g of butter to the dish and rapidly and accurately weigh. Stir in a little alcohol to facilitate evaporation, leave the dish on a boiling waterbath, stirring occasionally, until no water is visible on the bottom of the dish. Wipe the outside of the dish and transfer to the oven. Dry to constant weight (less than 2 mg difference in successive weighings.

CALCULATION

$$\% \text{ moisture} = \frac{\text{Weight loss in oven}}{\text{Weight of sample}} \times 100$$

REFERENCES

FAO/WHO Code of Principles Concerning Milk and Milk Products, International Standards and Standard Methods of Sampling and Analysis for Milk Products.

Hunter, M., Kirch, G. and Hammond, H. 1973. New Zealand Journal of Dairy Science and Technology 3, 123.

FOREIGN FATS IN BUTTER
(Hydroxamic Acid Index)

PRINCIPLE

Fatty acid esters form hydroxamates and these give a red colour with ferric chloride. The hydroxamates of the lower fatty acid esters such as those of the butyrates found in butterfat are much more soluble in water than the hydroxamates of the higher fatty acids found in other oils and fats. The conditions of the test are arranged so that for pure butter the solution containing the water-soluble hydroxamates should have about the same color intensity as that containing an aliquot from all the hydroxamates.

APPARATUS

1. Graduated cylinders, 25 ml and 50 ml capacity, or glass tubes having graduations at 25 ml and 50 ml. Glassware commonly used in blood and urine analysis is suitable.

2. Photoelectric colorimeter, with 525 nm filter and absorption cells, or spectrophotometer.

3. Volumetric pipettes 20 ml, 10 ml, 1 ml, and 0.5 ml capacity.

REAGENTS

1. Hydroxylamine hydrochloride solution. Mix equal weights of hydroxylamine hydrochloride and water. Warm with stirring or swirling on a steam bath until salt is dissolved. Cool to room temperature. This reagent must be prepared with care. Use only the minimum amount of heat necessary to dissolve the salt. Prepare fresh daily.

2. Alcoholic potassium hydroxide solution. Add 6.25 g KOH pellets to 100 ml isopropanol. Swirl the mixture over a steam bath to dissolve the KOH. Cool to room temperature. This solution can be stored in a ground glass stoppered bottle in a refrigerator for several days, but should be discarded after it has become yellow. If a stored solution is used, warm it to room temperature before using in test.

3. Isopropanol-water-acetone solution. Mix equal volumes of isopropanol and water. Add 1% by volume of acetone to the mixture.

4. Acetic acid-chloroform solution. Dilute 6 ml glacial acetic acid to one litre with chloroform.

5. Iron solutions. (1) Dissolve 0.90 g anhydrous ferric chloride in 5 ml concentrated HCl by warming over a steam bath. Dilute to 100 ml with water and filter through a paper (No. 1 Whatman or equivalent). Store in a refrigerator. (This reagent can be stored successfully for two months under refrigeration).

Alternatively, dilute 1.6 ml of 60% solution with 5 ml HCl and dilute to 100 ml with water. (2) Dilute a portion of iron solution No. 1 with equal volume of 2.25% HCl, made by diluting 5 ml concentrated HCl to 100 ml with water. Dilute as needed.

PROCEDURE

Fat may be isolated from butter by churning and filtering procedures, or by ether extraction. Use ghee (butter oil) directly.

Place approximately 0.1 g melted fat in a 50 ml tube or cylinder, and add 0.50 ml hydroxylamine hydrochloride solution. While the fat is still melted, add 5 ml alcoholic KOH solution, mixing during addition. Place the tube in a 30°C water bath for 30 minutes.

At the end of the 30 minute reaction period remove the tube from the bath and dilute the contents to 50 ml with acetic acid-chloroform solution. Shake this mixture vigorously by placing the palm of the hand over the end of the tube and making ten up and down strokes in 5 seconds. After shaking, the contents of the tube will appear turbid, because of finely dispersed inorganic salt. Clarify the solution by inserting a ball of glass wool the diameter of the tube into the latter at the level of liquid and slowly pushing it to the bottom of the tube with a 1 ml pipette, while holding the index finger over the upper end of the pipette. Using this same 1 ml pipette transfer two 1 ml aliquots of the clarified solution to two 50 ml tubes (1 ml to each tube). Dilute each 1 ml aliquot to within a few ml of the 50 ml graduation with isopropanol-water-acetone solution. Put these tubes aside temporarily until the extract of the water-acetone soluble hydroxamic acids is prepared.

Transfer a 10 ml aliquot of the acidified reaction solution, from which the two 1 ml aliquots have already been removed, to a 50 ml tube. To this 10 ml add 20 ml acetic acid-chloroform solution and 20 ml distilled water, each from volumetric pipettes or burettes. Shake the tube vigorously 30 times in up and down strokes, in ten seconds, using the palm of the hand to stopper the tube. Set the tube aside to allow the aqueous layer to separate from the heavy organic layer. Tap the tube gently to shake down the droplets of chloroform clinging to the air meniscus of the aqueous layer. With a volumetric pipette transfer 10 ml of the clear aqueous upper layer to a 25 ml tube and dilute to within a few ml of the 25 ml graduation with isopropanol.

With a volumetric pipette, add 0.50 ml iron solution No. 1 to each of the two 50 ml tubes containing the 1 ml aliquots previously diluted to nearly 50 ml. Dilute to 50 ml with isopropanol-water-acetone solution. Add 0.50 ml iron solution No. 2 to the 25 ml tube containing the previously diluted 10 ml aqueous extract and dilute to 25 ml with isopropanol. Mix the contents of each of the three tubes by slowly inverting each tube three times, using the palm of the hand to stopper the tubes. Each of the three tubes should now exhibit about the same intensity of red colour if the sample being analyzed is pure milk fat. Adulterated milk fat or non-milk fats will yield an aqueous extract tube which is much lighter in colour than the two 50 ml tubes representing the total hydroxamic acids from the fat. If no milk fat is present in the sample the aqueous tube will exhibit no red colour. Place the coloured solutions in appropriate absorption cells and measure their absorbances in a colorimeter, using a 525 nm filter with distilled water as blank. (The iron solution should have been added to each of the tubes at about the same time, and the colorimeter readings should be made not more than 15 minutes apart.) Using the colorimeter readings, taken from the absorbance scale and not from the percent transmittance scale of the instrument, calculate the Hydroxamic Acid Index.

CALCULATION

$$\text{Hydroxamic Acid Index} = \frac{100 \times \text{colorimeter reading of water extractable acids}}{10 \times \text{average of two colorimeter readings of total acids}}$$

Example: $$HAI = \frac{100 \times 0.296}{10 \times \left(\frac{0.275 + 0.277}{2}\right)} = 10.7$$

The HAI is roughly equivalent to the molecular percentage of water-soluble fatty acids in the fat.

INTERPRETATION

The hydroxamic acid index is normally in the range 9-12. If the butter is diluted with other fats the index is lower and in the absence of butter the value is below 2 or 3. According to one source the test is not reliable if the proportion of butter present in the fat is below about 20%.

REFERENCE

Bassette, R. and Keeney, M., 1956. Journal of the AOAC 39 (2), 469-74.

FOREIGN FATS IN BUTTER
(Reichert-Polenske-Kirschner Values)

PRINCIPLE

Butter is distinguished from other fats by the presence of the glyceryl esters of relatively low molecular weight fatty acids, especially butyric but also caproic, capric, caprylic, lauric and myristic. These acids are wholly or partially steam-volatile and water-soluble.

The fat is saponified with sodium hydroxide, the melt acidified and distilled under standard conditions. The distillate is filtered and the soluble acids titrated (Reichert value). The insoluble acids are dissolved in alcohol and titrated (Polenske value). The titrated soluble acids are treated with silver sulphate and the filtrate is acidified and re-distilled and the distillate titrated (Kirschner value). The Reichert value reflects the amount of butryic and caproic acid present, the Kirschner, butryic alone and the Polenske, chiefly caprylic, capric and lauric with some contribution from myristic and even palmitic acids.

The procedure should be carried out without a sample in order to obtain a blank value, which is usually about 0.5 ml.

APPARATUS

1. Distillation apparatus. See diagram.

2. Burettes.

REAGENTS

1. Sodium hydroxide solution, 50%, m/m + glycerol (1+9)

2. Dilute sulphuric acid, 25 ml per litre, and adjust so that 40 ml exactly neutralizes 2 ml of the sodium hydroxide solution.

3. Pumice powder or anti-bumping granules.

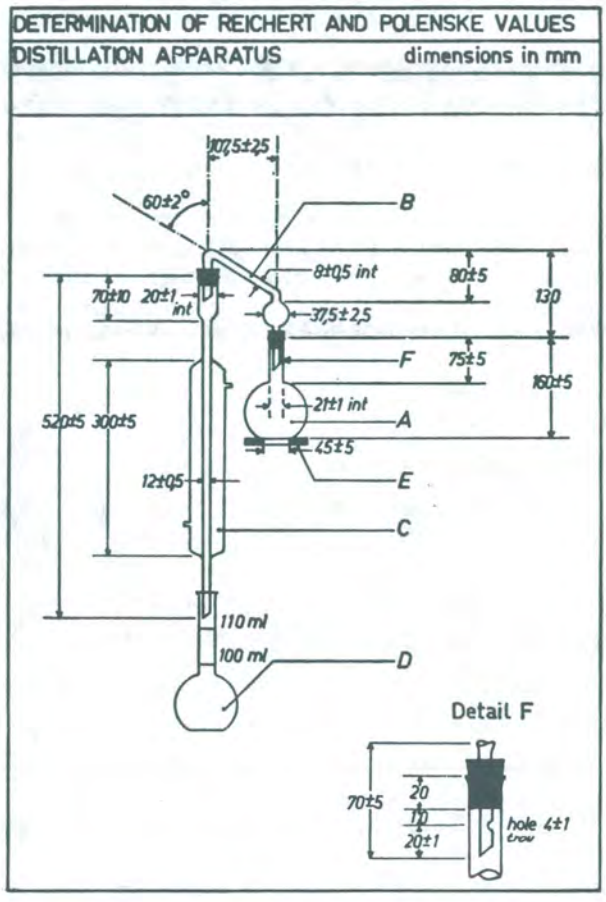
4. Phenolphthalein solution, 0.5% in denatured ethanol.

5. Barium hydroxide approximately 0.1 N, accurately standardized. Shake 20 g of the octahydrate with a litre of water until the crystals dissolve and leave a couple of days for the barium carbonate to settle out. Store in a bottle with a guard-tube containing powdered soda-lime to prevent ingress of carbon dioxide. Standardize the solution against 0.1 N hydrochloric acid or potassium hydrogen phthalate. (NaOH may be used instead of barium hydroxide if the Kirschner value is not going to be determined.)

6. Silver sulphate, finely powdered.

PROCEDURE

Weigh 5 g ($\pm$0.01) of the oil (obtained by melting the butter and filtering) into the distilling flask, and add 20 ml of the glycerol caustic mixture. The weighing may be done by attaching the flask to the pan hook of an analytical balance by a piece of wire and carefully adding the sample until the tare + 5 g is attained. Sapobify by gently heating over a small flame with constant swirling, until the liquid no longer foams and becomes clear. Allow the flask to cool to about 90°C, add 90 ml of recently boiled distilled water of about the same temperature and mix. The liquid should remain clear. Add 0.6 to 0.7 g of the pumice and then 50 ml sulphuric acid solution (1 N).



Connect the flask immediately to the distillation apparatus and warm it gently until the free fatty acids form a clear surface layer.

Start heating and regulate the flame so as to collect in the measuring flask 110 ml of distillate in 19-21 minutes, taking the moment when the first drop forms in the condenser as the beginning of the distillation period. Regulate the water flowing in the condenser so as to maintain the temperature of the water leaving the condenser at 20 $\pm$ 1°C.

If the temperature of the cooling water exceeds 20°C as in tropical and subtropical areas, and if no special arrangements can be made, the measuring flask should stay in the water-bath at 20 $\pm$ 1°C for about 1 hour. When exactly 110 ml of distillate have been collected, remove the burner immediately and substitute a small beaker for the measuring flask. Mix the contents of the measuring flask by gently shaking and immerse the flask in a water bath at 20 $\pm$ 1°C for 10 to 15 minutes, the 110 ml mark on the flask being 1 cm below the level of the water in the waterbath and the flask being turned from time to time.

Stopper the flask and mix by inverting it 4 or 5 times without shaking.

Filter the 110 ml of distillate through a dry medium speed filter paper (diameter 80-90 mm) which fits snugly into the funnel. The filtrate should be clear. The filter should be of such a size that 15 ml poured into it will fill it completely.

Determination of Total Soluble Fatty Acids (Reichert Value)

Pipette 100 ml of the filtrate into a conical flask of 300 ml, add 0.5 ml of phenolphthalein indicator solution and titrate with the standardized aqueous alkali solution to a pink colour persistent for 1/2 to 1 minute. Calculate the Reichert value according to the formula below. Retain the neutralized filtrate for the determination of the Kirschner value.

Conduct a blank test without fat and instead of saponifying over a naked flame, heat on a boiling water bath for 15 minutes. Not more than 0.5 ml of the standardized alkali solution should be required for the titration of the blank. If the volume exceeds this, prepare fresh reagent solutions.

Determination of Insoluble Volatile Fatty Acids (Polenske Value)

Rinse the filter with three successive 15 ml portions of distilled water at a temperature of 20 $\pm$ 1°C, each having previously passed through the condenser, the small beaker and the measuring flask.

Place the funnel and filter in the neck of a dry clean conical flask of 200 ml capacity.

Dissolve the insoluble fatty acids by repeating the washing procedure but using 15 ml portions of ethanol (95-96%, previously neutralized).

Titrate the combined ethanolic washings with the standardized aqueous alkali solution using 0.5 ml of phenolphthalein indicator solution, to a pink colour persistent for 1/2 to 1 minute. Calculate the Polenske value as outlined below.

Determination of Volatile Fatty Acids with Soluble Silver Salts (Kirschner Value)

Add 0.5 g of finely powdered silver sulphate to the neutralized solution from the Reichert determination. Leave the flask in the dark one hour with occasional shaking and filter the contents through a dry filter, in the dark. Transfer 100 ml of the filtrate to a dry Polenske flask, add 35 ml of cold recently-boiled distilled water, 10 ml of the dilute sulphuric acid solution and a little pumice powder or about 30 cm of aluminium wire about 1 mm thick wound into a coil about 5 mm across. Connect the flask to the standard distillation apparatus and distil as for a Reichert determination. Mix and filter, omitting immersion in a waterbath at 20°C. Titrate 100 ml of the filtrate with 0.1 N barium hydroxide solution. Calculate the Kirschner value as below.

CALCULATIONS

Reichert value (RV) = 1.1 x ml of 0.1 N barium hydroxide required for neutralization. The alkali will not normally be exactly 0.1 N, so the titre must be multiplied by a suitable factor after deduction of the blank titre. Report the result rounded to the first decimal.

Polenske value (PV) = ml of 0.1 N barium hydroxide required to neutralize the alcohol-soluble acids.

$$\text{Kirschner value} = \frac{\text{titre} \times 1.21 \times (100 + C)}{10{,}000}$$

where C is number of ml of 0.1 N barium hydroxide required in the Reichert titration.

REPEATABILITY OF RESULTS

The difference between results of duplicate determinations (results obtained simultaneously or in rapid succession by the same analyst) should not exceed 0.5 for the Reichert and Kirschner values and 0.3 for the Polenske value.

The results vary slightly with atmospheric pressure. The following formula may be applied to results obtained at an elevated altitude: (p = atmospheric pressure in mm of Hg)

$$\text{Corrected RV} = 10^{\frac{(RV - 10)\log 760}{\log p}}$$

$$\text{Corrected PV} = PV\left(\frac{760 - 45}{p - 45}\right)$$

INTERPRETATION

The Reichert value of butter is generally over 24, probably always so for butter produced in bulk from properly husbanded animals. Samples for which the value exceeds 28, with the Polenske in proportion, may be accepted as genuine. A value below 28 justifies further tests, and below 24 raises the suspicion that the sample is adulterated. A very small proportion of samples, less than 1%, may show values down to about 20 or even lower, but the Polenske and Kirschner values will be in proportion for such samples. Bulking of production of course tends to mask some of the natural variation. Conversely, butter from a small number of animals, or ones fed abnormally may have a composition outside the usual range (one of the problems of interpretation of the freezing-point of milk). For example, the Reichert value may be reduced by exposure of the animals to cold, and is lower in the milkfat towards the end of the lactation period. Cows fed on beetroot leaves or turnips appear to give milkfat with an abnormally high Polenske value.

Butter clarifies quickly during the saponification stage of the Reichert process, margarine usually more slowly. The acids from coconut oil distil as oily drops, those from palm oil as white flakes.

The relation between the three values, Reichert, Polenske and Kirschner accord fairly closely with the following table: (see Williams (128))

Reichert Value	Polenske Value	Kirschner Value
23.7	1.6	20.0
24.1	1.7	20.3
24.5	1.8	20.7
25.0	1.9	21.1
25.4	2.0	21.5
25.9	2.1	21.9
26.3	2.2	22.3
26.8	2.3	22.7
27.2	2.4	23.0
27.7	2.5	23.4
28.1	2.6	23.8
28.5	2.7	24.2
28.9	2.8	24.6
29.4	2.9	25.0
29.8	3.0	25.4
30.3	3.1	25.8
30.7	3.2	26.1
31.1	3.3	26.5
31.6	3.4	26.9
32.0	3.5	27.3

Read from the above table the Polenske value corresponding to the Reichert value obtained experimentally. If the Polenske value obtained experimentally is more than 0.5 above the value read from the table, the presence of coconut or palm kernal oil or their products may be assumed.

Next, read from the table the Polenske value corresponding to the Kirschner value obtained experimentally. The Polenske value obtained experimentally should not vary by more than 1.0 (either way) from that read from the table. The addition of less than 5% of coconut oil causes the Polenske value to fall outside this limit.

The following two tables taken from Davis and Macdonald (1) show how these values may vary in genuine butter.

Reichert-Polenske Values

Number of Samples	Reichert	Polenske		
		Average	Maximum	Minimum
15	22	1.50	1.7	1.2
22	23	1.60	1.8	1.4
43	24	1.65	2.0	1.4
56	25	1.70	2.3	1.4
35	26	1.90	2.4	1.5
26	27	1.95	2.9	1.6
22	28	2.05	3.1	1.6
15	29	2.20	2.9	1.8
26	30	2.10	2.9	1.7
30	31	2.25	3.2	1.6

Correlation of Reichert and Polenske Values

Polenske: \ Reichert:	36	35	34	33	32	31	30	29	28	27	26	25	24	23	Number of Samples:
1.3												1			1
1.4									1			1			2
1.5											1				1
1.6										1	2	2	1	1	7
1.7								3	4	2		1			10
1.8				1				2	8	4	5				20
1.9							1	5	6	12	2	1	1		28
2.0					4	1	3	9	17	9			1		44
2.1							1	10	15	6					32
2.2				1	2	1	5	10	15	1					35
2.3			1	3	3	3	7	16	11	2					46
2.4			3	3	3		12	9	4	1					35
2.5			1	4	1	3	6	8	7	1					31
2.6			1	2		4	6	7	2						22
2.7				2	8	6	4	6	6						32
2.8			1		5	8	5	5	5	1					30
2.9				1	7	8	10	4	6	1					37
3.0				4	7	7	5	17	2						42
3.1	1			1	9	4	6	13	5						39
3.2				3	8	8	11	13	4	1					48
3.3				3	3	7	8	11	5						37
3.4				1	2		6	8	3	1					21
3.5					3	1	2	9	2						17
3.6					2	1	2	1	2						8
3.7							2								2
3.8							1								1
3.9							1								1
	1	-	7	29	67	62	104	166	130	43	10	6	3	1	629

It is important to carry out the determination a few times to ensure that consistent results are obtained before any deduction is made as to the likelihood of adulteration, particularly in the case of borderline values. The result must be confirmed by tests for phytosterols, iso-oleic acid, etc.

Results on difficult samples must be interpreted with care. Addition of animal fats up to 10 or 20% may not lower the RPK values below the accepted minima. There is the possibility of addition of triacetin or tributyrin to increase the Reichert value and only extensive analysis would reveal that there was a discrepancy in the pattern of results. GLC of the intact triglycerides has been used (Breckenridge and Kuksis (133)) (Parodi (126)) and appears promising as a means of assessing the proportion of butterfat in fat mixtures. Reichert and Kirschner values of cultured butters with an artificially induced high acidity are lower than normal.

RPK values for the fat from the milk of non-bovine animals are not as well established as those for cow's milk. The values reported by various workers fall within the ranges given in the following tables: (See Williams (132)) (Note: 'a' indicates a single value and 'b' a typical value.)

	Reichert	**Polenske**	**Kirschner**
Goat	17 - 29	1.9 - 9.8	15.6[b]
Ass	about 14		
Buffalo	24 - 37	1.5 - 1.8	
Ewe	22.8 - 23.4	1.5 - 2.1	17.6[a]
Mare	6.2[a]	5.9[a]	2.6[a]

Typical analyses for some other oils are as follows:

	Reichert	**Polenske**	**Kirschner**
Palm Kernel	5.2 - 6.5	9.7 - 10.7	0.8 - 1.2
Coconut	6.5 - 8	15 - 17	1.6 - 1.9
Babassu	6.1[b]	11.4[b]	
	8.4[b]	15.9[b]	1.6[b]
Margosa or Neem oil (Azadriachta indica)	8.3[b]	0.25[b]	5.0
Shea nut oil, shea butter	2.6[b]	0.7[b]	
Macassar oil, Schleichera trijiga, "kusum" or "paka"	17.0	3.0	15.5

Other oils and fats may generally be assumed to give negligible values.

To distinguish cow butteroil from buffalo butteroil, Latif and Mazloumu (134)) used fractional crystallisation from acetone and determination of the Reichert, Polenske and Kirschner values on the fractions.

REFERENCES

IUPAC II.D.9. (IUPAC Method II.D.10. describes the determination of fatty acids with soluble magnesium and insoluble silver salts in order to calculate the properties of butter, coconut and palm kernel oils in mixtures).

International Dairy Federation 37:1966.

British Standard 769:1961.

Society of Public Analysts, 1936. Analyst 61, 404-8.

AOCS, Volume 1, Cd 5-40.

Garcia-Olmedo, R. and Hellin, B.C., 1971. Anales de Bromatologia 1, 41-59.

FOREIGN FATS IN BUTTER AS AN INGREDIENT
(Reichert-Polenske-Kirschner Values, Semi-Micro Method)

PRINCIPLE

See 'Foreign Fats in Butter'.

APPARATUS

1. Distillation apparatus. See diagram.

REAGENTS

As for macro-method, except for item 6, barium hydroxide, approximately 0.02N accurately standardised. Standardise and store as for 0.1 N.

PROCEDURE

Separate or extract the butterfat from sample and weigh exactly 1 g of the fat into the distilling flask, add 0.5 ml sodium hydroxide solution and 3.5 ml glycerol. Saponify by gentle heating over a micro-burner or small flame with constant swirling. Do not heat after saponification is complete, this being shown by the sudden change of the mixture to a clear single-phase liquid. It may require a little practice before this point is easily detected. If there is any sign of charring, reject and repeat with a fresh sample. Cover with a small watchglass. As soon as the melt is cool enough for water to be added without loss, add 19 ml of recently boiled hot water, initially drop by drop. Once the soap is dissolved, add 10 ml of dilute sulphuric acid and about 0.05 g powdered pumice.

Connect the flask to the distillation apparatus and heat gently to melt the fatty acids. Then heat strongly, adjusting the distillation rate to collect 21 ml of distillate in 5 to 7 minutes. Remove the burner and immediately substitute a small beaker for the 21 ml flask.

Reichert Value:

Stopper the flask, mix gently and stand in water at 15°C for 10 minutes. Filter through a small filter paper (e.g. 4.25 cm Whatman No. 4 or equivalent) and titrate 20 ml of the filtrate with 0.02 N barium hydroxide using phenolphthalein as indicator. Retain the neutralised filtrate.

Prepare and conduct a blank determination through the entire procedure.

Reichert value = 1.1 x (titre-blank)

Polenske Value:

Remove the splash-head of the distillation apparatus, and rinse the condenser with 3 ml of cold water, using it to also rinse the beaker, the 21 ml flask and the filter paper in turn and finally rejecting the filtrate. Repeat with a further 3 ml of water.

Pass 3 ml of neutral ethanol down the condenser, using it to rinse the beaker, the 21 ml flask and the filter-paper, collecting the filtrate in a small conical flask. Repeat with two further portions of 3 ml of neutral ethanol so as to collect all of the water-insoluble but alcohol-soluble acids in the conical flask. Titrate

the alcohol solution with 0.02 N barium hydroxide using phenolphthalein as indicator.

Polenske value = titre - blank

Kirschner Value:

To the neutral filtrate from the Reichert value, add 0.1 g of finely-powdered silver sulphate, and leave for one hour in the dark, shaking occasionally. Filter into a dry flask. Pipette 20 ml into a Reichert distillation flask, add 7 ml distilled water, 2 ml of dilute sulphuric acid and a little powdered pumice or short lengths of fine aluminium wire to reduce bumping. Distil 21 ml in 5 to 7 minutes, cool, mix and filter (4.25 cm Whatman No. 4 or equivalent) and titrate 20 ml of the filtrate with 0.02 N barium hydroxide using phenolphthalein as indicator.

$$\text{Kirschner value} = \frac{\text{titre} \times 1.21 \times (20 + C)}{20}$$

where C is the number of ml of 0.02 N barium hydroxide required for the Reichert value.

CALCULATION

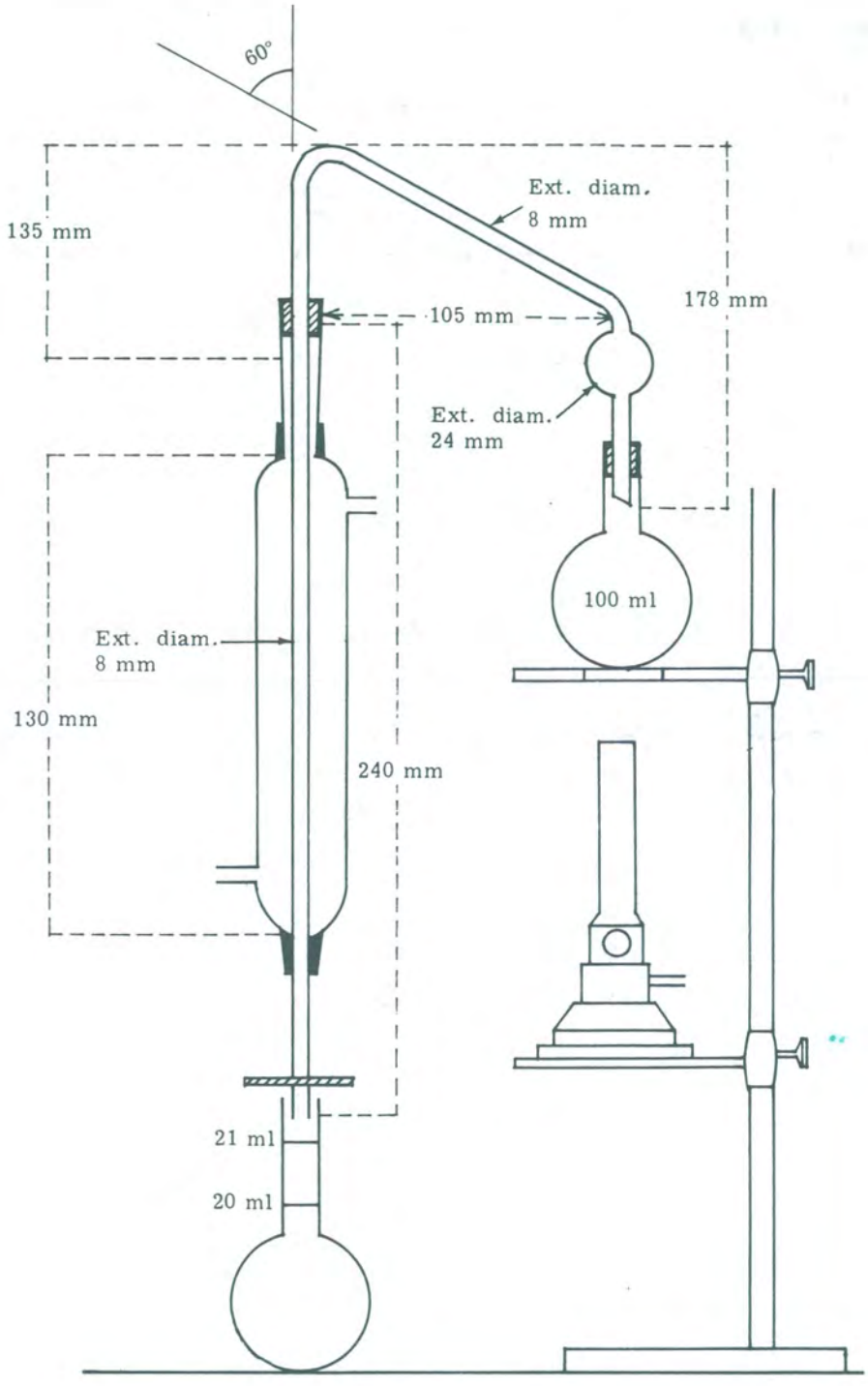
See the individual value calculations. The barium hydroxide solution will not usually be exactly 0.02 N so that the titre obtained must be multiplied by the appropriate factor before being used to calculate the above values.

INTERPRETATION

This test is intended for the examination of samples such as bread and butter, cream buns and items of flour, chocolate and sugar confectionery claiming to contain butter or milk derivatives, from which it may be difficult to obtain as much as 5 g of fat. The results, together with the HAI, are normally adequate to characterize the fat as butter or not. The interpretation given in the section on the detection of foreign fats in butterfat should be applied with a certain amount of caution as both methods are empirical. The original authors (Dyer et al (135) claim that the Reichert values agree with those by the macro-method, Polenske values are slightly lower by the semi-micro method. It is thus always preferable to use the macro-method if enough fat is available. There is no difficulty in interpretation if the only question is whether or not the sample is butter.

REFERENCE

Dyer, B., Taylor, G. and Hamence, J.H., 1941. Analyst 66, 355.



Distillation Apparatus for Semi-Micro Determination of Reichert-Polenske-Kirschner Values

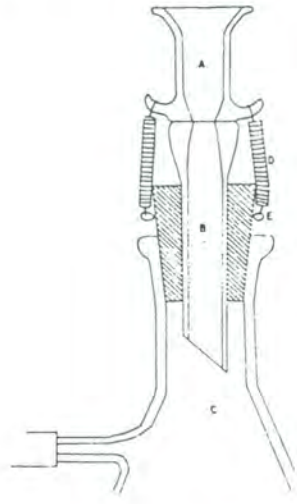
VEGETABLE FAT IN BUTTERFAT
(Thin-Layer Chromatographic Method)

PRINCIPLE

Sterols are precipitated as their digitonides from the saponified fat. The steryl acetates are prepared, separated by reverse-phase TLC and visualized using phosphomolybdic acid. The presence of phytosteryl acetates indicates that the sample contains vegetable fat.

APPARATUS

1. Micro filter such as:



Glass micro filter for sterol acetate precipitates
A: Top portion of filter, capacity 1 ml. B: Lower portion of filter. Ground surfaces between A and B hold filter pad. A and B are held together by springs, D. C: filter flask. E: wire twisted around stopper to hold lower end of springs.

2. TLC equipment (plates, tank, etc.)

3. Micropipettes or microsyringes, 10 µl.

4. Chromatography spray.

REAGENTS

1. Potassium hydroxide solution. Dissolve 400 g of KOH in 600 ml distilled water.

2. Digitonin solution. Dissolve 1 g digitonin in 100 ml of ethanol 95% v/v.

3. Ethanol, 95% v/v.

4. Ethanol, 80% v/v.

5. Diethyl ether.

6. Acetic anhydride.

7. Pentane or light petroleum ether (boiling range 40-60°C).

8. Copper sulphate solution. Dissolve 70 g of crystallized copper sulphate pentahydrate in water and dilute to 1 litre with water.

9. Anhydrous sodium sulphate.

10. Phosphomolybdic acid solution. (This reagent must be freshly prepared. If faint blue coloured bands are obtained when spraying the chromatoplate, the reagent must be discarded and a fresh solution prepared, preferably from phosphomolybdic acid, recrystallized in nitric acid (d=1.2) if not sufficiently pure.) Dissolve 10 g of phosphomolybdic acid $P_2O_5 \cdot 24\ MoO_3 \cdot nH_2O$ in 50 ml ethanol (95%, v/v). Contact of this solution with metallic objects, such as spatulas, etc., must be avoided.

11. Reference standards solution. This must be freshly prepared. Dissolve 98 mg cholosteryl acetate and 2 mg soybean oil phytosteryl acetates in 10 ml diethyl ether.

PROCEDURE

Prepare the acetic acid-acetonitrile mobile phase for TLC, as follows: Mix 100 ml glacial acetic acid and 300 ml acetonitrile and shake with 18 ml undecane (boiling range 190-220°C) in a separatory funnel. (Conduct the operation in a fume-cupboard as acetonitrile is toxic.) Leave the 2 layers to separate 16 hours at 22-23°C. The acetic acid-acetonitrile layer, saturated with undecane, is used as the mobile phase. (Note: mixtures already used for chromatographic separation must be discarded.)

Prepare the TLC tank by introducing enough of the acetic acid-acetonitrile (mobile phase) to obtain a layer of about 1 cm depth. Line the walls of the tank with filter paper and close the tank tightly with the lid. Let the tank equilibrate at 22-23°C for 24 hours.

Prepare the test samples as follows:

a. **Butter:** Melt about 50 g of the butter sample in a drying oven at a temperature below 50°C until the fat and water layers separate. Separate the fat layer by decantation and clarify the fat in the oven at a temperature of about 40°C by filtering it through a dry paper filter, taking care that the filter is not wetted by the aqueous phase.

b. **Milk and Cream:** Centrifuge the sample to obtain a cream of 40% fat. Churn the cream in a laboratory churn. Collect the butter lumps and proceed as described under butter.

c. **Cheese:** Rub the sample in a mortar with anhydrous sodium sulphate until a granular mass is produced. Extract the mass with pentane or light petroleum ether (a continuous extraction apparatus may be used) and evaporate the solvent in a boiling water-bath.

d. **Condensed Milk, Evaporated Milk, Ice Cream:** Add to the sample twice its volume of boiling water and heat the mixture on a boiling water-bath until the temperature is 75°C. Add an amount of copper sulphate solution equal to one-tenth of the volume of the mixture and continue heating until the precipitate coagulates. Filter the precipitate through a paper filter and wash it with warm water until the filtrate is colourless. Carefully drain the precipitate, rub it in a mortar with anhydrous sodium sulphate and proceed as described under cheese.

e. **Dried Milk:** Rub the sample in a mortar with some water so as to obtain a clotted mass. Allow it to stand for about 15 minutes. Then add anhydrous sodium sulphate and rub again until a granular mass is produced. Extract the mass with pentane or light petroleum ether (a continuous extraction apparatus may be used) and evaporate the solvent on a boiling water-bath.

Prepare steryl acetates as follows: Weigh to the nearest 0.1 gm about 15 g of the fat in a conical flask of 500 ml capacity. Add 10 ml of potassium hydroxide solution and 20 ml of ethanol (95% v/v). Attach an air condenser to the flask, heat it on a boiling water-bath, with swirling, until the solution has become clear, and continue boiling for half an hour. Add 60 ml of water and then 180 ml of ethanol (95% v/v), and raise the temperature to about 40°C. Add 30 ml of the alcoholic digitonin solution (1%), swirl and allow to cool. Place the flask in a refrigerator at about 5°C for about twelve hours or overnight.

Collect the precipitate of sterol digitonide by filtration through a medium speed filter paper in a Buchner funnel (diameter 8 cm). Wash the precipitate with water at about 5°C until the filtrate stops foaming, then once with 25-50ml of ethanol (95% v/v) and once with 25-50 ml of diethyl ether.

Dry the filter paper with the precipitate on a watch-glass in a drying oven at 102 $\pm$ 2°C for 10-15 minutes. Fold the filter paper in two, allowing the precipitate to come off as a pellicle and transfer the precipitate into a weighing bottle or other convenient container.

Transfer 100 $\pm$ 5 mg of the sterol digitonide to a test tube, add 1 ml of acetic anhydride and heat the tube in a glycerol bath at 130 - 145°C until the precipitate has dissolved. Do not use direct heat, since spattering may occur. Continue heating for two minutes and allow to cool to about 80°C. Add 4 ml of ethanol (95% v/v), mix, heat slightly to dissolve any steryl acetate which may tend to crystallize out. Filter the still warm solution through a small medium speed filter paper previously moistened with ethanol and collect the filtrate in another test tube. Carefully heat the filtrate in the test tube until it boils gently.

Keep the solution boiling and add drop by drop, from a pipette while shaking vigorously, 1 to 1.5 ml of water until the steryl acetate is just about to precipitate but still remains in solution. Avoid superheating. Add a few drops of 95% ethanol to redissolve any precipitated steryl acetate. Allow to cool in air for two hours and finally in ice-water for half-an-hour.

Filter the crystallized steryl acetates on a small disc of hardened fast filter paper by suction in a glass microfiltering device and rinse the crystals with 1 ml of ethanol (80% v/v). Dry the crystal cake on the paper in a drying oven first at about 30°C and then at 102 $\pm$ 2°C for 10-15 minutes. The cake may be stored in a desiccator ready for completion of the test the following day.

Dissolve the cake in about 2 ml of diethyl ether so as to give a concentration of about 10 µg/ µl. (Digitonin, MW 1229 forms an equimolar adduct with sterols and as the MW of cholesterol, for example, is 386, starting with 100 mg of adduct and assuming 80% of the acetate is recovered, the yield would be about 20 mg of cholesteryl acetate). This is the steryl acetate solution for spotting on the TLC plate. Prepare just before use.

Next, prepare the TLC plate as follows: Coat a glass plate with a slurry of diatomaceous earth in distilled water using a suitable spreader so as to obtain a layer of 0.18 mm uniform thickness. Prepare the slurry from diatamaceous earth containing 13% gypsum or use a proprietary powder (e.g. Kieselguhr G., Merck), using 1 part to 2 parts of water by weight.

After the plates have air-dried, activate by heating in a drying oven at 100°C for 25 minutes. Allow the plate to cool to room temperature.

Mark one of the sides of the plates, perpendicular to the direction of coating, as the bottom side by scratching a small sign in the layer.

Take the plate between thumb and forefinger of both hands, wearing rubber gloves, and dip it horizontally and carefully for some seconds in a shallow tray, containing a solution of 10% v/v undecane (BR 190-220°C) in petroleum ether (BR 40-60°C) or the undecane layer left from preparing the mobile phase. Alternatively, the plate may be sprayed directly with the undecane solution.

Remove the excess undecane solution by holding the plate in a vertical position, with the bottom side on top, over the tray for about ten seconds. The degree of impregnation should be 0.08 - 0.09 gram of undecane per gram of diatomaceous earth. Store the plate at a constant temperature of 20-24°C in a draught-free place for 50-60 minutes to evaporate the light petroleum.

During this evaporation period remove with a brush, pentagonal pieces from the thin layer and also strips of about 1.5 cm width along the edges of the plate in the same direction as used for applying the thin layer, using an appropriate template as indicated in the figure below, the pentagonal figures being situated on the bottom of the plate. Use the plate immediately.

(Note: The chromatoplates must be handled in a clean atmosphere, especially during heating procedures.)

Now, spot by means of a micro-pipette, 10 µl of the freshly prepared solution of the steryl acetates in diethyl ether containing 10 µg per µl, at the centre of the bridges of the chromatoplate as indicated in the figure. If more than 5% phytosteryl acetates are expected to be present in the steryl acetates sample, a smaller amount, e.g. 2.5 - 5 µl of the steryl acetates solution in diethyl ether, should be spotted on the starting point, otherwise the bands will not separate clearly.

Open the TLC tank and, in order not to disturb the vapour equilibrium, place the chromatoplate quickly in a vertical position in the vessel and close it immediately with the lid.

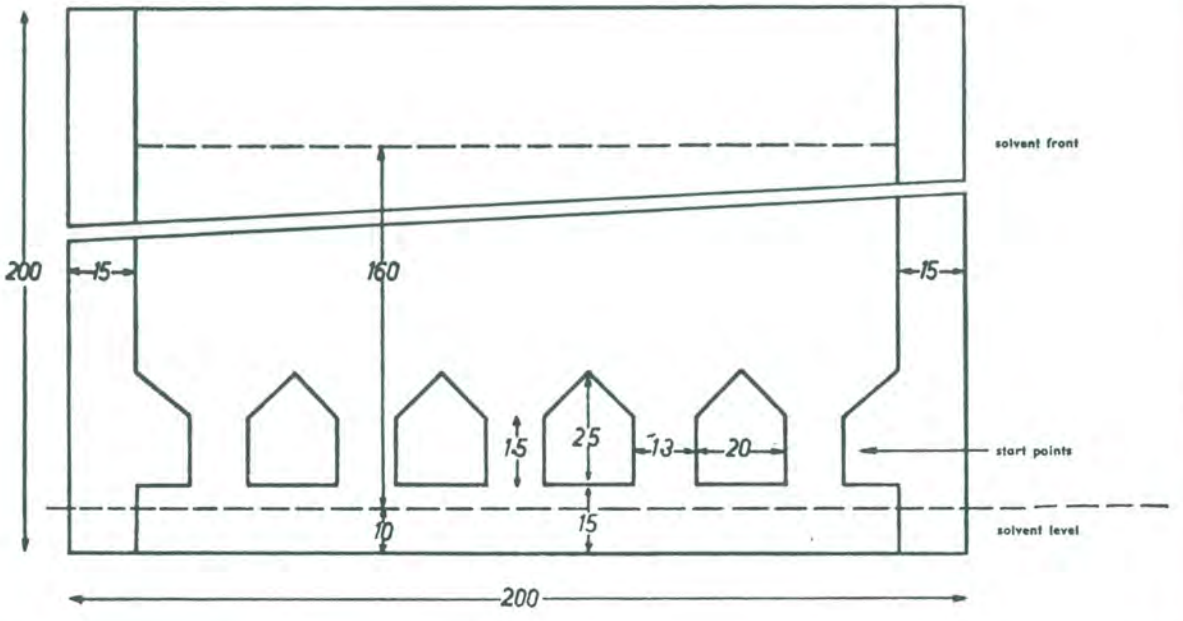
Develop by ascending chromatography at a temperature of 22-23°C for about 1.5 hours. Discontinue development when the solvent front has travelled to a height of about 16 cm, as measured from the baseline.

Dry the developed chromatoplate in air for 1-3 hours and then in a drying oven at 100°C for 45 minutes. Allow the chromatoplate to cool to room temperature and spray uniformly with the phosphmolybdic acid solution.

THE DETECTION OF VEGETABLE FAT IN MILK FAT by thin-layer chromatography of steryl acetates

CHROMATOPLATE TEMPLET —

dimensions in mm



The background of the chromatoplate should be yellow. A greenish colour may indicate interference of extraneous vapour. The cholesteryl acetate band will appear at a distance of 5-6,5 cm from the starting point. Heat the chromatoplate in a drying oven at 100°C for 5-10 minutes until the bands are coloured to maximum intensity.

A reference test must also be conducted simultaneously, on the same chromatoplate as used for the sample test, using a spot of 10 µl of the reference solution. A major band of cholesteryl acetate and a much smaller one of beta-sitosteryl acetate, with a lower migration rate, should be clearly discernible. If the acetates were not pure enough for an adequate separation, the digitonides should be recrystallised and the TLC repeated.

Recrystallisation may be carried out as follows: Redissolve the crystal cake by heating it over a micro-burner in a short Pyrex glass tube with 1 ml of ethanol (95% v/v). Allow to cool first in air for 15 minutes and then in ice-water for five minutes. Refilter the crystallized steryl acetates and dry as described above. Recrystallisation must be repeated up to four or five times as necessary to obtain a pure product.

INTERPRETATION

If a small band with the same migration rate as beta-sitosteryl acetate is observed on the sprayed chromatoplate, the presence of phytosteryl acetates is indicated and the fat sample under investigation, from which the steryl acetates have been obtained, is considered to contain vegetable fat.

The presence of at least 1% beta-sitosteryl acetate in steryl acetate mixtures can be demonstrated by this method.

The sensitivity of the detection of vegetable fat in milk fat cannot be given as this depends upon the nature of the fat added and especially upon the phytosterol content of such fat.

REFERENCE

International Dairy Federation 32:1965 and 38:1966.

VEGETABLE FAT IN BUTTERFAT
(Gas Chromatographic Method)

PRINCIPLE

Sterol digitonides prepared as given in the TLC method are dissolved in a mixture of formamide and dimethylformamide. The liberated sterols are extracted with pentane and separated by gas-liquid chromatography. If, on the chromatogram, a peak with the retention time of beta-sitosterol is obtained, the presence of vegetable fat in the fat sample under investigation is demonstrated. Peaks of other phytosterols may support this conclusion. (Note: Formamide is a suspected teratogen and must be handled carefully).

APPARATUS

1. Gas chromatograph, fitted with hydrogen flame ionization detector, silver or glass injection system, or direct-on-column injection device, and recorder.

2. Gas chromatographic column, glass, U-shaped or coiled, length 100-200 cm, inside diameter 3-4 mm. (Note: since some types of stainless steels cause false results by deterioration of sterols, only glass is recommended).

3. Micro-syringe, capable of delivering a volume of up to 5 or 10 µl.

REAGENTS

1. Mixture of equal volumes of formamide and dimethyl formamide. (See note under "PRINCIPLE").

2. n-Pentane.

3. Column packing: 2-4% loading of a methyl silicone gum rubber, stable up to at least 300°C, on a flux-calcined diatomaceous earth acid washed and silanized, mesh size 80/100 or 100/120.

4. Sensitivity test solution: 1 mg cholesterol in 1 ml n-pentane, freshly prepared.

5. Peak resolution test solution: 0.9 mg rape seed oil phytosterols and 0.1 mg cholesterol in 1 ml n-pentane, freshly prepared.

6. Reference test solution: 1 mg soyabean oil phytosterols in 1 ml n-pentane, freshly prepared.

PROCEDURE

Dissolve about 10 mg sterol digitonide, prepared as described in the TLC method for detection of phytosterols, in 0.5 ml of a mixture of equal volumes of formamide and dimethyl formamide in a small test tube, if necessary with gentle heating. Shake the solution, when cool, with 2.5 ml n-pentane. Let the layers separate and use the clear upper pentane layer (containing the liberated sterols) for gas chromatographic analysis.

Establish the gas chromatograph operating conditions as follows: set the column temperature at 220-250°C. Temperature of injection system, if it can be separately heated should be 20-40°C above column temperature. Nitrogen flow rate: 30-60 ml/min. Disconnect detector and equilibrate new columns under these conditions for 16-24 hours. Connect detector, ignite flame and regulate hydrogen and

oxygen or air flow rates so as to obtain appropriate flame height and detector sensitivity. Start the recorder at a suitable chart speed, adjust zero setting and attenuator. If the base line is steady the apparatus is ready for use.

Sensitivity test: Inject 3-5 µl of the sensitivity test solution. Only one peak of cholesterol will appear on the gas chromatogram (Figure 2.1). Adjust attenuator so as to obtain approximately full scale deflection on the recorder.

Figure 2.1

Peak resolution test: Inject 3-5 µl of the peak resolution test solution. Peaks of cholesterol, brassicasterol, campesterol and beta-sitosterol will appear on the chromatogram (Figure 2.2). Measure the retention distances (distance from sample injection to maximum peak height) of the peaks, d_{CH} for cholesterol, d_B for brassicasterol, d_C for campesterol and d_S for beta-sitosterol and the peak base widths (retention dimension between intersections of base line with tangents to the points of inflection on the front and rear sides of the peak) w_{CH} for cholesterol and w_B for brassicaste rol.

$$\text{Peak Resolution (PR)} = \frac{2\,(d_B - d_{CH})}{w_B + w_{CH}} \quad . \quad \text{PR shall be at least 1.}$$

Calculate the relative retention times (cholesterol = 1.00) for brassicasterol, campesterol and beta-sitosterol.

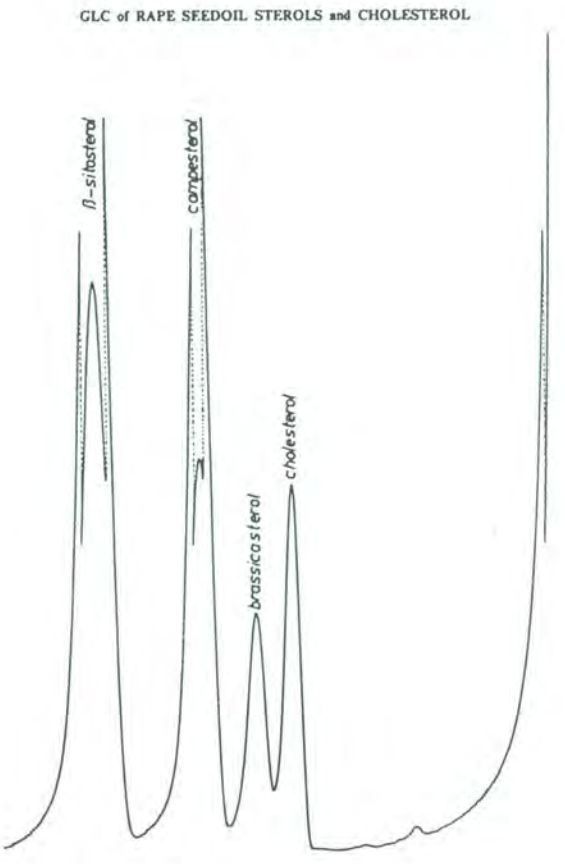


Figure 2.2

Reference test: Inject 3-5 µl of the reference test solution. Peaks of campesterol, stigmasterol and beta-sitosterol will appear on the chromatogram (Figure 2.3). Measure the retention distances of the peaks, d_C for campesterol, d_{ST} for stigmasterol, and d_S for beta-sitosterol.

Cholesterol	1.00 (about 15 minutes)
Brassicasterol	1.13 - 1.15
Campesterol	1.32 - 1.34
Stigmasterol	1.44 - 1.46
Beta-sitosterol	1.66 - 1.68

Next, inject 3-5 µl of the sample solution and switch the attenuator to a four times (usually two steps) lower attenuation factor. Record the chromatogram. If on the chromatogram a peak with the relative retention time of beta-sitosterol and a height of at least 2% of full scale is observed, the presence of beta-sitosterol is indicated and the fat sample under investigation from which the sterols have been isolated, is considered to contain vegetable fat. The presence, on the gas chromatogram, of peaks of other phytosterols such as campesterol or stigmasterol will support the conclusion.

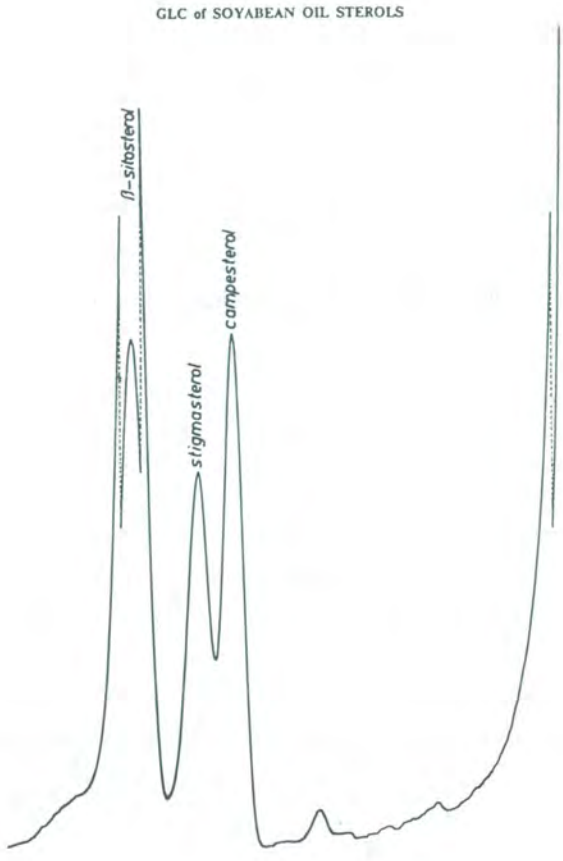


Figure 2.3

INTERPRETATION

The presence of at least 0.5% beta-sitosterol in sterol mixtures can be demonstrated by this method. The limit of detection of vegetable fat in milk fat cannot be given since this depends on the beta-sitosterol content of the fat used for admixture, i.e. upon the nature of the fat or mixture of fats added to the milk fats. Note that very small amounts of beta-sitosterol may be found in genuine butter, due to vegetable oil used as a diluent for added color.

Some papers relate GLC to RPK values. For example, Huyghebaert and Hendrickx (127)(128)(136). For interpretation of GLC data, the reader should consult the excellent review by Dickes and Nicholas (137) from which much of the following information is taken. The review by Kuzdal-Savoie (129) and the paper by Parodi (130) are also important.

A number of ratios of one group of fatty acids to another is given in the following table, compiled from Dickes and Nicholas's (137) data:

Ratio of Fatty Acids

	$C_4:C_{6+8}$	$C_{12}:C_{10}$	$C_{14}:C_{12}$	C_{18} unsat. : C_{15} sat.
Butterfat	1.8	1.0-1.6	3.2-6.0	2.4-3
Margarine		8-8.5		3
Coconut oil		7.9-8.2		
Palm oil		13.5-17.1		

In a GLC analysis, the esterification stage is critical. This has been examined by a number of workers, including Shehata et al (138), Hulstkamp and Stampbach (139), Tandan and Ganguli (140) and Schwartz and Bright (141).

The presence of added tributryin would have to be assessed from the chromatography of the glycerides themselves, without prior saponification. Kuksis and McCarthy (142) point out that addition of a judicious mixture of lard and palm or coconut oil could result in gas chromatographic results similar to those from butter, but of course such a sample should give a positive phytosterol test.

Separation of triglycerides by argentation-TLC prior to GLC has been used by Shehata et al (143). They later used a preliminary separation on silicic acid columns. Sebastian and Rao (144) have investigated TLC methods for detecting adulteration in butterfat.

Indicators are sometimes required to be put in butter not intended for human consumption and these have been discussed by Guyot in a series of papers (145)(146).

REFERENCES

International Dairy Federation 32:1965 and 54:1970.

IUPAC II.D.6.

2.5 ICE CREAM

COMPOSITION

It is important to maintain adequate standards of composition for ice-cream as it is often consumed by children. There are considerable differences among national standards.

The traditional product is made from milk, whether fresh or processed, sugar, and butterfat, with various emulsifiers and emulsion stabilizers added. The International Dairy Federation produced its own International Standard in 1969. This lays down compositional standards for ice-cream and milk ices (edible ices) produced from milk and milk products. This states that "edible ices produced from milk and milk produces are preparations, the solid or pasty state of which has been obtained by freezing and which are intended to be consumed in that state."

The IDF standards are as follows:

	Edible Ices	Fruit ice-cream (fruit or pulp 15%) (10% for lemon)	Milk Ices	Milk Ices with eggs	Ice-cream with eggs
Butter fat minimum	8% m/m	6% m/m	3% m/m	3% m/m	8% m/m
Total solids minimum	32% m/m	30% m/m	28% m/m	28% m/m	32% m/m
Liquid egg yolk or equivalent as dehydrated yolk				7% m/m	7% m/m

Water should only be used to reconstitute milk included as an ingredient. Milk of animals other than cows should be designated. The only other permitted ingredients are eggs, nutritive sweeteners, flavours and the following additives:

Alginic acid and alginates. Alginic acid and its sodium and calcium compounds with a total maximum content of 33% of mineral substances (sodium, potassium and calcium compounds of ortho- and pyro-phosphoric acid, tartaric acid and citric acid)	
Carrageenan Gelatin Pectin Agar Tragacanth gum Karaya gum Arabic gum Guar gum Carob seed gum Carboxymethylcellulose Propylene glycol alginate (for products containing fruit)	1%
Glycerine	1%
Glyceryl mono- and di-stearate Lecithin Sucrose esters	0.6%
Citric, tartaric, ascorbic, lactic and malic acids Starch and hydrolyzed starch derivates	3%

(Note: The percentages given are maximum for the item alone or in combination with other items in a bracket.

The over-run, defined as the ratio, volume in litres/ mass in kilograms, should not exceed 2 (2.25 for products containing over 35% total solids). Thatti, Gayakwad and Laxminarayana (147) have published the results of analysis of 270 samples from the Bombay market.

ROUTINE ANALYSIS

The bacteriological examination of ice-cream is very important as the product is implicated in outbreaks of food poisoning from time to time. Chemical analysis should be carried out on a separate sample, as the bacteriologist has to keep to a timetable for the methylene blue test and the sample is melted before testing. Samples for chemical analysis should be tested immediately on arrival or stored in the deep-freeze as low sucrose values may be obtained due to its conversion to dextran by Leuconostoc mesenteroides.

Ice-cream should be analyzed for composition, total solids, fat, sucrose, lactose, protein (N x 6.38), ash and for metallic contamination, particularly lead and zinc. It may also be necessary to test for the presence of preservatives, artificial sweeteners, antioxidants in the fat, starch, flavours and colours. The type of fat present may be ascertained in the first instance by determination of the hydroxamic acid index (HAI) or Reichert-Polenske-Kirschner (RPK) value. The iodine value of the fat is useful for indicating the presence of hydrogenated fat.

The level of milk solids other than milkfat can be calculated from the lactose, protein or calcium (1.88% as CaO in dry milk solids-not-fat) but the results must be interpreted with care as gelatin, lactose, calcium alginate thickener, etc., may have been added. This can be seen from the ratio between them if one of the figures is not in line. TLC is a convenient way of checking that sucrose and lactose are the only sugars present.

When ice-cream is manufactured, air is beaten into it to improve the texture. The volume is thus increased and the increase is expressed by the "over-run", defined as the percentage increase in volume, and calculable from the formula

$$\text{Over-run } (\%) = \frac{1/d - 1/c}{1/d} \times 100 = \frac{c - d}{c} \times 100$$

where c = SG of ice-cream before melting
d = SG of ice-cream, melted and deaerated.

For ice-cream sold by volume the overrun thus determines the weight sold per unit price and is therefore of importance to both manufacturer and consumer.

Most of the analyses can be carried out by slight modifications of standard methods and will not be described here in full. If the sample is taken from the deep-freeze, melt at below 45°C, shake and cool to room temperature. In certain mixed products the ice-cream portion may have to be separated before analysis.

Fat can be determined by Rose-Gottlieb, or Gerber methods. The various methods are discussed by Park and McKeon (148). For the Rose-Gottlieb method, use 5 g of sample, 2 ml ammonia and 8 ml of water. Maintain at 65°C for fifteen minutes, add 15 ml of 95% ethanol, mix and cool. The mixture should be free of lumps, but any remaining can usually be broken up with a glass rod. If this is impossible discard the determination and recommence with a fresh portion. 25 ml of diethyl ether is added to the homogeneous mixture and the analysis completed as for milk.

The Gerber method is most conveniently carried out using an ice-cream butyrometer, but those intended for milk, cream or cheese are suitable provided the correct amounts of sample and water are chosen.

For the routine screening of ice-cream for fat content, the Gerber or Babcock tube using Salwin reagent may be used. This reagent is prepared by mixing equal volumes of glacial acetic acid and 60% perchloric acid.

The procedure for the determination of total solids is essentially the same as that given in this Manual for evaporated milk.

Sugars can be determined before and after inversion by Lane and Eynon's volumetric method after clearing with zinc ferrocyanide or lead acetate and sodium oxalate. Mix 10 g with 150 ml water, clear and filter, wash the filter and dilute to 250 ml. Use an aliquot for inversion. If TLC has shown only sucrose and lactose to be present, calculate sucrose as follows:

% sucrose = (T - I) 0.95, where:

I = reducing sugars as invert sugar
T = total sugars as invert sugar

Use the lactose table to calculate the reducing sugars as % lactose. The liquid chromatography of sugars in ice cream is described by Warthesen and Kramer (149).

Protein may be determined by the standard Kjeldahl procedure, using a factor of 6.38. The method for total solids is described in detail.

To determine ash, dry about 10 g accurately weighed on the waterbath and incinerate at 550°C. Determine the calcium in the ash by oxalate precipitation, flame photometry or AAS.

For acidity, titrate 10 g of ice-cream in a porcelain dish with 0.1N NaOH using 1 ml phenolphthalein solution as indicator. Express the results as lactic acid. The mixture may then be used for the determination of protein by the formol titration (Crowhurst (150)).

2.6 CHEESE AND OTHER PRODUCTS

COMPOSITION

The Codex Alimentarius Commission has developed Recommended International Standards for the following cheeses:

Amsterdam	Gouda
Brie	Gruyere
Butterkase	Gudbrandsdalsost (Whey Cheese)
Camembert	Harzer Kase
Cheddar	Havarti
Cheshire	Herrgordsost
Cottage Cheese (including creamed Cottage Cheese)	Hushallsost
	Leidse (Leyden)
Coulommiers	Limburger
Cream Cheese	Maribo
Danablu	Norvegia
Danbo	Provolone
Edam	Romadur
Emmentaler	Saint Paulin
Esrom	Samsoe
Friese (Frisian)	Svecia
Fynbo	Tilsiter

Cheese standards typically prescribe minima for dry matter and for fat in the dry matter. The use of additives may be restricted to bacterial and mould cultures, rennet, salt, emulsifying salts such as phosphates or citrates of the alkali and alkaline-earth metals, natural colours and sometimes sorbate, nitrate or nitrite as preservatives.

The International Dairy Foundation has standards for cultured buttermilk, yoghurt, acidophilus milk, kefir and koumiss. Whether or not yoghurt as presented for sale should contain viable bacteria is discussed by Kroger (151) and Davis (152).

Different types of cream such as clotted, double, whipped, or sterilised are categorised by their fat content. Cream should consist only of that part of milk rich in fat and the aqueous phase should have the composition normal to skimmed milk, although in some countries some grades of cream may contain certain additives.

ROUTINE ANALYSIS

a. Cheese

The major constituents of cheese are moisture, butterfat, milk and protein and there are internationally agreed procedures to determine these.

Protein may be determined by the Kjeldahl process. Moisture can be determined by drying in the oven. Use of the Karl Fischer and Dean and Stark methods is discussed by Strange (153). Arentzen (154)(155) discusses sampling difficulties.

For routine purposes the fat may be determined by the Gerber or Van Gulik methods. Reference methods include the Rose-Gottlieb for whey cheeses and the Schmid-Bondzynski-Razlaff for other cheeses. The latter method is compared with the Weibull-Stoldt and Van Gulik methods by Winkler (156).

The IDF has developed a method for phosphatase activity in pasteurized stabilized cheese as a means of checking that pasteurization during processing was adequate.

The enzymic method of Bahl (157)(158) can be used for lactose. Novak and Laskowski (159) discuss the colorimetric methods of Marier and Boulet and Richards for small amounts (less than 100 micrograms) of lactose in cheese. Taylor (160) gives an enzymic method.

b. **Fermented Milk**

Methods of analysis generally applicable to milk products are suitable for fermented milks.

Ney and Wirotama (161) describe the electrophoretic detection of gelatin added to yoghurt. Quarg (known as tvorog in eastern Europe) is a fermented cheese-like product of relatively low total solids content. Papers on quality and composition include those by Solms (162), and Czulak and Hammond (163).

Khoa is another fermented cheese-like product, and may be analyzed by the same methods.

c. **Milk Formulae for Infants**

These also may be analyzed by methods usually applicable to milk products. The determination of lactose, sucrose and starch in infant milk formulae is dealt with the Laskowski and Jamiolkowska (164).

d. **Cream**

The analysis of cream does not present unusual difficulty. Butyrometer tubes for the determination of fat in cream by the Gerber method are available. The Rose-Gottlieb method can be used as a reference method for fat in cream. Davis (165) discusses the laboratory control of cream. A new method for the determination of added water has been reported (166).

e. **General Analysis of Dairy Products**

The level of lactose of another major milk consistuent may sometimes be taken as an adequate indication of the level of a milk product in a compound food.

The presence of whey powder in skimmed milk powder can be determined by estimating the sialic acid content (167).

Orotic acid (uracil-4-carboxylic acid) has been used as a measure of the proportion of non-fat milk solids present, occurring to the extent of 48 - 74.5 mg/100 g in non-fat milk solids (168).

PHOSPHATASE ACTIVITY
(In Dairy Products)

PRINCIPLE

Dilution of the liquid dairy product or the reconstituted liquid dairy product with a buffer at pH 10.6 containing disodium phenylphosphate and incubation at 37°C for one hour, liberates phenol if alkaline phosphatase is present in the product. The phenol is reacted with dibromoquinonechloroimide and the colour formed is measured photometrically.

APPARATUS

All glassware, stoppers and sampling tools must be carefully cleaned. It is desirable to rinse them with freshly boiled distilled water or to steam them. Certain types of plastic stoppers may cause phenolic contamination and their use must therefore be avoided. Use ware with glass stoppers when possible.

1. Water bath capable of being maintained at 37 $\pm$ 1°C.

2. Spectrophotometer, suitable for readings at a wavelength of 610 nm.

3. Test tubes, 16 or 18 mm x 150 mm, preferably graduated at 5 and 10 ml.

4. Pipettes, graduated 1 and 10 ml.

5. Glass funnels of convenient size, for example 5 cm diameter.

6. Filter paper.

7. Volumetric flasks for the preparation of standard solutions.

8. Litmus paper.

9. Analytical balance.

REAGENTS

All reagents should be of analytical reagent quality and water should be freshly boiled distilled water, or water of at least equal purity, free from CO_2.

1. Barium borate-hydroxide buffer: Dissolve 50.0 g of barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$), free from carbonate, in water to a volume of 1,000 ml. Dissolve 22.0 g of boric acid (H_3BO_3) in water to a volume of 1,000 ml. Warm 500 ml of each solution to 50°C, mix the solutions, stir, cool rapidly to about 20°C and adjust the pH if necessary to 10.6 $\pm$ 0.1 by addition of barium hydroxide or boric acid solution. Store the solution in a tightly stoppered bottle. Dilute the solution before use with an equal volume of water.

2. Colour development buffer: Dissolve 6.0 g of sodium metaborate ($NaBO_2$) or 12.6 g of $NaBO_2 \cdot 4H_2O$, and 20.0 g of sodium chloride (NaCl) in water and dilute with water to a volume of 1,000 ml.

3. Colour dilution buffer: Dilute 10 ml of the colour development buffer to 100 ml with water.

4. Buffer substrate: Dissolve 0.5 g of disodium phenyl-phosphate ($Na_2C_6H_5PO_4 \cdot 2H_2O$) in 4.5 ml of the colour development buffer, add 2 drops of the solution of 2, 6 - dibromoquinone-chloroimide and let

stand at room temperature for 30 minutes. Extract the colour so formed with 2.5 ml of butan-1-ol and let stand until the butan-1-ol separates. Remove the butan-1-ol and discard. Repeat this extraction if necessary. The solution may be stored in a refrigerator for a few days. Develop the colour and re-extract before use. Prepare the buffer substrate immediately before use by diluting 1 ml of this solution to 100 ml with the barium borate-hydroxide buffer.

5. Zinc-copper precipitant: Dissolve 3.0 g of zinc sulphate ($ZnSO_4 \cdot 7H_2O$) and 0.6 g of copper sulphate ($CuSO_4 \cdot 5H_2O$) in water to a volume of 100 ml.

6. 2, 6-dibromoquinonechloroimide solution (Gibbs reagent): Dissolve 40 ± 1 mg of 2, 6-dibromoquinonechloroimide (BQC) in 10 ml of 95% (v/v) ethanol. Store in a dark-coloured bottle in a refrigerator. Reject if discoloured or more than 1 month old.

7. Copper sulphate solution: Dissolve 0.05 g of copper sulphate ($CuSO_4 \cdot 5H_2O$) in water to a volume of 100 ml.

8. Sodium hydroxide, 0.5 N solution.

9. Phenol standard solutions: Weigh 200 ± 2 mg of pure anhydrous phenol, transfer to a 100 ml volumetric flask, fill to the mark with water and mix. (This stock solution remains stable for several months in a refrigerator. Dilute 10 ml of this stock solution to 100 ml with water and mix. 1 ml contains 200 µg of phenol.

PROCEDURE

Important notes:

1. Avoid direct sunlight during the determination.

2. Contamination with traces of saliva or perspiration can give false positive results and must be avoided. Pipetting in particular must be done with special care and not by mouth.

Preparation of Sample

Milk, buttermilk and whey: carry out the analysis preferably directly after sampling. Otherwise, keep the sample in a refrigerator, but not for more than 2 days. Mix the sample carefully, if necessary with moderate heating. The temperature of mixing must under no circumstances exceed 30°C.

Dried milk, buttermilk powder and whey powder: Dissolve 10 g of the product in 90 ml of water. The temperature applied in dissolving must under no circumstances exceed 35°C.

Neutralization of sour products: Add to a sour product dilute sodium hydroxide until the test solution is neutral to litmus paper.

Test Portion

Pipette into each of two test tubes 1 ml of the test sample, using one tube as a control or blank.

Determination

Heat the blank for 2 minutes in boiling water; cover the tube and the beaker of boiling water with aluminium foil to ensure that the entire tube will be heated. Cool to room temperature. From this point

treat the blank and the test sample alike. Add 10 ml of the butter substrate and mix. Immediately incubate in the water bath at 37°C for 60 minutes, mixing the contents occasionally. Heat in boiling water for 2 minutes in the same manner as for the blank. Cool to room temperature. Add 1 ml of the zinc-copper precipitant to each tube and mix thoroughly. Filter through a dry filter paper, discard the first runnings, refilter if necessary until the filtrate runs clear, and collect 5 ml in a test tube. Add 5 ml of the colour development buffer. Add 0.1 ml of the BQC solution, mix and allow the colour to develop for 30 minutes at room temperature. Measure the absorbance against the blank in a spectrophotometer at a wavelength of 610 nm.

Repeat the determination with an approximate dilution of the sample or the reconstituted sample if the absorbance exceeds the absorbance of the standard containing 20 µg of phenol per tube. Prepare this dilution by mixing 1 volume of the test sample with an appropriate volume of a part of the same test sample thoroughly boiled in order to inactivate the phosphatase.

Preparation of Standard Curve

Prepare a suitable range of diluted standards, starting from the standard phenol, containing 0 (control or blank), 2, 5, 10 and 20 µg of phenol per millilitre and pipette respectively 1 ml of water and 1 ml of the four phenol standard solutions into each of five test tubes.

Add to each tube 1 ml of the copper sulphate solution, 5 ml of the colour dilution buffer, 3 ml of water and 0.1 ml of the BQC solution, and mix. Allow the colour to develop for 30 minutes at room temperature. Measure the absorbance against the control or blank in the spectrophotometer at a wavelength of 610 nm.

Prepare the calibration curve by plotting the absorbances against the quantities of phenol in micrograms. The standard curve should be a straight line.

CALCULATION

Convert the absorbance to micrograms of phenol per millilitre of the liquid product or in the case of dried products, per millilitre of reconstituted liquid product, by means of the following formula:

$$\text{Phosphatase activity} = 2.4 \times A \times D$$

where: A is the quantity of phenol in micrograms obtained
D is the dilution factor of any dilution.
(In the case of no dilution, D = 1).

REPEATABILITY

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 2 µg of phenol. If a dilution is applied this limit refers to the results obtained on the diluted sample.

INTERPRETATION

A value of more than 1 is indicative of improper pasteurization or contamination with unpasteurized substances.

2.7 TEXT REFERENCES

1. DAVIS, J.G. & MacDONALD, F.J. 1953. Richmond's Dairy Chemistry, Churchill & Co.

2. PEARSON, D. 1981. The Chemical Analysis of Food 8th Ed. Livingstone-Churchill.

3. WEBB, B.H., JOHNSON, A.H. & ALFORD, J.A. 1974. Fundamentals of Dairy Chemistry, AVI.

4. HARDING, F. & ROYAL, J.H.L. 1974. XIX International Dairy Congress, Vol. 1E, 453.

5. KEMPINSKI, C. 1971. Deutsche-Milchwirtschaft, 22 (51/52) III-V.

6. HARDING, F. 1973. Dairy Industries, 38 (7) 311-315.

7. BERGMANN, J. 1976. Instrumental Methods Review, Tierzuchter, 28 (4) 156-8, 160-3.

8. CORRADINI, C. Milchwissenschaft, 30 (7) 413-416.

9. HAAVE, I.J.J. 1973. Meieriposten, 62 (49) 1078-1080.

10. HANSON, N.W. (Ed.) 1973. Standardised, Recommended & Official Methods of Analysis, 2nd Ed. The Society for Analytical Chemistry.

11. TILLMANS, J. & LUCKENBACH, W. 1925. Zeitschrift fur Untersuchungs der Lebensmitteln, 50 103.

12. TILLMANS, J. & LUCKENBACH, W. 1926. Milchwirtschaft Forschung, 3 225-267.

13. TILLMANS, J. & LUCKENBACH, W. 1930. Zeitschrift fur Lebensmittel-untersuchung und Forschung, 60 342.

14. WOIDRICH, K. & SCHMID, L. 1955. Zeitschrift fur Lebensmitteluntersuchung und Forschung, 102 167.

15. HANKINSON, D.J. & ANDERSON, E.O. 1939. Storrs Agricultural Experimental Station Bulletin, 234.

16. IWAIDA, M., EBINE, R. & TANIMURA, A. Journal of the Food Hygienic Society of Japan, 14 (3) 258-263.

17. DAVIDSON, J.J. 1949. Dairy Research, 209-216.

18. LAWRENCE, A.J. 1970. Australian Journal of Food Technology, 198.

19. HILLIG, F. 1942. Journal of the Association of Official Agricultural Chemists, 25 253.

20. STEFFEN, C. 1971. Schweizerische Milchzeitung, 67 1073, 1078.

21. BASAK, A. & ROY, B.R. 1975. Indian Journal of Dairy Science, 28 127.

22. MEHTA, R.S., BASSETTE, R. & WARD, G. 1974. Journal of Dairy Science, 57 (3) 285-289.

23. WALKER, N.J. & GRAY, I.K. 1970. Journal of Agricultural and Food Chemistry, 18 (3) 346-352.

24. KRATZER, D.D. 1967. Journal of Dairy Science, 50 1384 p 73.

25. DZHOROV, T., KOSTOV, L. & VITKOV, M. 1969. Veterinarno-medicinski Nauki, 6 (6) 113-119.

26. ZAGAEVSKII, I.S. 1974. Molochnaya Promyshlennost' No. 1, 42-43.

27. SCHALM, O.W., CARROLL, E.J. & JAIN, M.C. 1971. Bovine Mastitis, Lea & Febiger.

28. SCHULTZE, W.D., SMITH, J.W., JASPER, D.E., KLASTRUP, O., NEWBOULD, F.H.S., POSTLE, D.S. & ULLMAN, W.W. 1972. Journal of Milk and Food Technology, 35 (11) 639-645.

29. NEWSTEAD, D.F. & ORMSBY, J.E. 1970. New Zealand Journal of Dairy Science and Technology, 5 (2) 52-56.

30. ROWLAND, S.J. & ZEID-EL-DINE, M. 1939. Journal of Dairy Research, 10 108, 267.

31. WHEELER, S.M. 1979. Australian Journal of Dairy Technology, 34 (4) 169-175.

32. HAMANN, J., TOLLE, A., BLUTHGEN, A., & HEESCHEN, W. 1975. Milchwissenschaft, 30 (1) 1-7.

33. RYBINSKA, K. 1974. Roczniki Panstwowego Zakladu Higiney, 25 (2) 213, 215.

34. KUMAR, P., SUD, R.K. & GUPTA, K.G. 1973. Journal of Dairy Science, 56 (5) 553-557.

35. REINBOLD, G.W. 1971. Journal of Milk and Food Technology, 34 (12) 613-619.

36. AGGARWAL, P.K., SUD, R.K. & GUPTA, K.G. 1973. Journal of Dairy Science, 56 (12) 1562-1564.

37. KRAMER, J., CARTER, G.G., ARRET, B., WILNER, J., WRIGHT, W.W. & KIRSCHBAUM, A. 1974. Antibiotic Residues in Milk, Dairy Products & Animal Tissues: Methods, Reports and Protocols, National Center for Antibiotic & Insulin Analysis, Food & Drug Administration, Dept. of Health, Education & Welfare, Washington DC 20204.

38. FEAGAN, J.T. 1966. Dairy Science Abstracts, 28 (2) 53-60.

31. COX, D. & McNAMARA, C. 1975. Dairy Technology, 6 (2) 38-45.

40. RICHARD, J. & KERHERVE, L. 1973. Revue Laitiere Francaise, 306, 127, 129, 131.

41. NICKERSON, T.A., VUJICIC, I.F. & LIN, A.Y. 1976. Journal of Dairy Science, 59 (3) 386-390.

42. FERNANDEZ, F.J. & RAMIREZ, E.E. 1974. Revista de Medicina Veterinaria Argentina, 55 (3) 199-200, 203-204, 207-208, 211-212, 215-217.

43. WILSON, A.G., SWEETSUR, A.W.M. & WHITE, J.C.D. 1974. XIX International Dairy Congress, IE 455-456.

44. RUKINA, N.I., RASTEGAEVA, N.A. & NAUCHNYE TRUDY. 1974. Omskii Ordena Lenina Sel'skokhozyaistvennyi Institut imeni S.M. Kirova, 121 116-119, 136.

45. JAYNES, H.O. & ASAN, T. 1973. Journal of Milk and Food Technology, 36 (6) 333-336.

46. HOBBS, J.S. & LAWRENCE, J.G. Journal of the Science of Food and Agriculture, 23.

47. BAHL, R.K. 1972. Analyst, 97 (1156) 559-561.

48. VALDEHITA, M.T. & LOPEZ, M.L. 1971. Anales de Bromatologia, 23 (1) 35-40.

49. DOUGALL, B.M. & MORGAN, D.T. 1973. Proceedings of the Society for Analytical Chemistry, 10 (7) 181.

50. MADRID VICENTE, A. 1972. Revista Espanola de Lecheria, 83 19-22.

51. MITTAL, S.B. & ROY, M.K. 1974. XIX 1st Dairy Congress, 1E 472-3.

52. GUPTA, P.K. & MATHEW, T.V. 1969. Journal of the Institution of Chemists, India, 41 (2) 57-9.

53. DHAR, A.K., ROY, B.R., GUHA, K.C. & MITRA, S.N. 1970. Indian Journal of Dairy Science, 23 (1) 12-15.

54. JAMES, G.V. 1976. Journal of the Association of Public Analysts, 14.

55. JAOUEN, J.C. 1973. Le Technicien du Lait, July-Aug 7-13; Sept 7-25.

56. RAO, S.R. & BECTOR, B.S. 1980. Indian Journal of Dairy Science, 33 (1) 1-6.

57. PRUTHI, T.D., RAVAL, N.P. & BHAVSAR, N.K. 1974. Milchweissen-schaft, 29 (1) 21-23.

58. DAVIDE, C.L. & DOMINGO, L.E. 1971. Philippine Agriculturist, 54 (9/10) 432-438.

59. MINCIONE, B., SCUDIERO, A., ALBONICO, F. & FRANCISCIS, G. 1973. Scienza e Tecnologia degli Alimenti, 3 (6) 342-345.

60. SWAISGOOD, H. (Editor) 33pp. (undated) Urbana, Illinois, USA; American Dairy Science Association.

61. AL-SHABIBI, M., KARAM, H.A., ELIYA, J., JUMA, H., ABU-AL-MA'ALI, H.N. 1971. Alexandria Journal of Agricultural Research, 19 (1) 77-80.

62. WILLIAMS, A.P., BISHOP, D.R. & COCKBURN, J.E. 1976. Journal of Dairy Research, 43 (2) 325-9.

63. WAGNER, A. 1973. Acta Agronomica Academiae Scientiarum Hungaricae, 22 (1/2) 153-158.

64. RAO, M.B., GUPTA, R.C. & DASTUR, N.N. 1970. Indian Journal of Dairy Science, 23 (2) 71-78.

65. VAGHELA, L.H. 1976. Some Properties of Camel's Milk, M.Sc Thesis, Bombay University.

66. MONACELLI, R. & CANTAGALLI, P. 1975. Bolletino dei Laboratori Chimici Provinciali, 26 (10) 360-364.

67. CARINI, S. & BUSCA, M. 1975. Latte, 610-612.

68. FOISSY, H. 1976. Osterreichische Milchwirtschaft, 31 (3, Beilage 2) 5-8.

69. ASCHAFFENBURG, R. & DANCE, J.E. 1968. Journal of Dairy Research, 35 (3) 383-84.

70. EGUARAS, J.L. 1975. Alimentaria, 11 (65Z) 31-33.

71. BRET, G. 1968. International Dairy Federation Annual Bulletin, (7) 39 pp.

72. PIERRE, A. & PORTMANN, A. 1970. Annales de Technologie Agricole, 19 (2) 107-130.

73. PIRRRE, A. & PORTMANN, A. 1970. Annales de Technologie Agricole, 19 (2) 177-79.

74. PORTMANN, A. & PIERRE, A. 1971. Annales des Falsifications et de l'Expertise Chimique, 64 (696) 159-177.

75. MAJUMDER, G.C. & GANGULI, N.C. 1972. Indian Journal of Dairy Science, 25 (3) 189-190.

76. MAJUMDER, G.C., JOSHI, V.K. & GANGULI, N.C. 1972. Indian Journal of Animal Sciences, 42 (10) 779-784.

77. GUHA, K., DHAR, A.K. & ROY, B.R. 1972. Journal of Food Science and Technology (Mysore), 9 (2) 90-91.

78. GHOUSE, M. & JAIN, M.K. 1973. Indian Journal of Dairy Science, 26 (1) 43-47.

79. O'KEEFE, M.G. 1967. Journal of Dairy Research, 34 211.

80. LUNDER, T.L. 1970. Scienza e Tecnica Lattiero-Casearia, 21 (1) 1-18.

81. APARICIO GALLEGO, M. 1970. Revista Espanola de Lecheria, (78) 193-200.

82. ARTEM'EV, A.A. & PANASENKOV, N.S. 1969. Izvestiya Vysshikh Uchebnykh Zavadenii, Pischewaya Tekhnologiya, (1) 161-164.

83. UZONYI, G. & VARGA, G. 1973. Tejipar 22 (4) 89-91.

84. DOZET, N., STANISIC, M. & SUMENIC, S. 1973. Mljekarstvo, 23 55-62.

85. SLANOVEC, T., ARSOV, A., PLANKAR, S. & GALVAN, S. 1974. Mljekarstvo 24 (7) 156-160.

86. TEMPLE, P.L. 1937. Analyst, 62 709.

87. HENNINGSON, R.W. 1969. Journal of the Association of Official Analytical Chemists, 52 (1) 142-151.

88. WOOD, E.C. 1972. Journal of the Association of Public Analysts, 10 (3) 68-70.

89. MOORE, S. 1974. Milk Industry, 75 (3) 20-22.

90. ANON. 1971. New Zealand Journal of Dairy Science and Technology, 6 (1) 31.

91. HOFFELNER, K. 1968. Osterreichische Milchwirtschaft, 23 (15) 261-4.

92. DILLIER-ZULAUF, A. 1971. Deutsche Molkerei-Zeitung, 92 (40) 1704-1608 & 1710.

93. DILLIER-ZULAUF, A. & DOYOTTE, J.P. 1971. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene, 62 (1) 32-41.

94. BINDER, W. 1970. International Dairy Congress (18th Sydney), IE:87.

95. PANETSOS, A. & GEORGAKIS, S. 1970. Deutsche Milkerei-Zeitung, 91 (21) 870, 872-874.

96. WELBOREN, J.T. & VELDEN, H. van der. 1974. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene, 65 (1) 151-156.

97. PRENTICE, J.H. 1978. Analyst 103 1269-1273.

98. LUCK, H. & DRESNER, S. 1975. South African Journal of Dairy Technology, 7 (4) 221-227.

99. BINDER, W. 1974. Proceedings of the XIX International Dairy Congress, IE 465.

100. ASPERGER, H. 1974. Proceedings of the XIX International Dairy Congress, 465-466.

101. BOUCHES, D. & WAES, G. 1973. Revue de l'Agriculture, 26 (4) 799-812.

102. HENNINGSON, R.W. 1970. Journal of the Association of Official Analytical Chemists, 53 (3) 539-542.

103. HENNINGSON, R.W. 1972. Journal of the Association of Official Analytical Chemists, 53 (3) 504-506.

104. HENNINGSON, R.W. 1968. Journal of the Association of Official Analytical Chemists, 51 (4) 816-821.

105. JAMOTTE, P. & DUCHATEAU, J. 1973. Technicien du Lait, pp 17, 19, 21 in January issue and pp 25-27 and 29-34 in February issue.

106. FREEMAN, T.R., BUCY, J.L. & KRATZER, D.D. 1971. Journal of Milk and Food Technology, 34 (4) 212-214.

107. DERMOTT, B.J. 1971. Journal of Milk and Food Technology, 34 (3) 140-141.

108. O'CONNOR, C.B. 1970. Irish Agricultural and Creamery Review, 23 (5) 23-25.

109. LUCK, H., KRIEL, J.B., MOSTERT, J.F. 1973. South African Journal of Dairy Technology, 5 (3) 129-133.

110. STILES, M.E., BREDENKAMP, B.L.F. & ABBOTT, C.W. 1974. South African Journal of Dairy Technology, 6 (2) 123-130.

111. NOWAK, E. & LASKOWSKI, K. 1972. Roczinki Instytutu Przemyshu Mleczarskiego, 14 (3) 33-42.

112. REMOND, B. 1975. Lait, 55 (547) 390-395.

113. MANNING, P.B., COULTER, S.T. & JENNESS, R. 1986. Journal of Dairy Science, 51 (11) 1725-30.

114. RESMINI, P. & VOLONTERIO, G. 1973. Latte No. 9, 167-169.

115. LING, E.R. 1963. A Textbook of Dairy Chemistry Vol. II, Chapman & Hall.

116. GUHA, K.C. & ROY, B.R. 1973. Journal of the Science of Food and Agriculture, 24 1.

117. PATRATII, A.P. & SOCHNEVA, L.G. 1973. Trudy, Usesoyuznyl Nauchino-Issaldevatcl'skei, 30 108-110.

118. EMMENS, D.B. 1970. XIX International Dairy Conference, IE 523.

119. MORRISEY, P.A. 1974. Irish Agricultural & Creamery Review, 27 (7) 5-7.

120. ANDERSON, B.A. & BERLIN, E. 1974. Journal of Dairy Science, 58 (1) 25-29.

121. LIVIO, L.T. 1969. Latte, 43 (8) 561-64.

122. FRANZEN, K. 1947. Lebensmittelchemie und Gerichtliche Chemie, 28 (4) 139-144.

123. TIMMEN, H. & BLUTHGEN, J. 1973. Zeitschrift fur Lebensmitteluntersuchung und Forschung, 153 (5) 283-288.

124. ROSCHNIK, M.R. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene, 63 (2) 206-21.

125. PARODI, P.W. & DUNSTAN, R.J. 1971. American Journal of Food Technology, 26 60.

126. PARODI, P.W. 1972. Australian Journal of Food Technology, 27 (3) 90-94.

127. HUYGHEBAERT, A. & HENDRICKX, H. 1971. Milchwissenschaft, 26 (10) 613-617.

128. HUYGHEBAERT, A. & HENDRICKX, H. 1970. Milk Industry, 67 (3) 18-23.

129. KUZDAL-SAVOIE, S., LANGLOIS & TREHIN, J. 1975. Annual Bulletin of the International Dairy Federation, 86 180-184.

130. PARODI, P.W. 1973. Australian Journal of Dairy Technology, 28 (4).

131. PARODI, P.W. 1980. Australian Journal of Dairy Technology, 35 (1) 17-22.

132. WILLIAMS, K.A. Oils, Fats and Fatty Foods, p. 217.

133. BRECKENRIDGE, W.C. & KUKSIS, A. 1967. Symposium: Dairy Lipids & Lipid Metabolism AVI Westport, USA 233 pp.

134. LAFTI, A. & MAZLOUMU, K. 1969. Indian Journal of Dairy Science, 22 (3) 162-172.

135. DYER, B., TAYLOR, G. & HAMENCE, J.H. 1941. Analyst, 66 355.

136. HUYGHEBAERT, A. & HENDRICKX, H. 1970. Milchwissenschaft, 25 (99) 506-10.

137. DICKES, G.J. & NICHOLAS, P.V. 1972. Journal of the Association of Public Analysts, 10 (4) 87-98.

138. SHEHATA, A.Y., de MAN, J.M. & ALEXANDER, J.C. 1970. Canadian Institute of Food Technology Journal, 3 (3) 85-89.

139. HULSTKAMP, J. & STAMPBACH, H. 1970. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene, 61 (5-6) 388-394.

140. TANDAN, K.C. & GANGULI, N.C. 1972. Indian Journal of Dairy Science, 25 (3) 179-183.

141. SCHWARTZ, D.P. & BRIGHT, R.S. 1974. Analytical Biochemistry, 61 (1) 271-274.

142. KUKSIS, A. & McCARTHY 1964. Journal of the American Oil Chemists Society, 41 12-21.

143. KHALAFALLA, S.M., ELSADEK, G.M., SHEHATA, A.E. & EL-MAGDOUB, M.N.I. 1973. Egyptian Journal of Dairy Science, 1 (1) 13-20.

144. SEBASTIAN, J. & RAO, M.B. 1974. Milchwissenschaft, 29 (9) 533-34.

145. GUYOT, A.L. 1971. Bulletin des Recherches Agronomiques de Gembloux, 6 (3/4) 325-378.

146. GUYOT, A.L. 1972. Bulletin des Recherches Agronomiques de Gembloux, 7 (1/4) 91-113.

147. THATTI, B.L., GAYAKWAD, K.S. & LAXMINARAYANA, H. 1972. Indian Journal of Dairy Science, 25 (1) 9-13.

148. PARK, H.S. & McKEON, J.R. 1978. Journal of Food Protein, 41 424-426.

149. WARTHESEN, J.J. & KRAMER, P.L. 1979. Journal of Food Science, 44 (2) 626-627.

150. CROWHURST, B. 1956. Analyst, 81 123-4.

151. KROGER, M. 1975. Dairy & Ice Cream Field, 158 (5) 52, 54-55.

152. DAVIS, J.G. 1976. Cheese Manufacturing Methods Vol II J. Churchill, Edinburgh 965-1132.

153. STRANGE, T.E. 1970. Journal of the Association of Official Analytical Chemists 53 (4) 865-68.

154. ARENTZEN, A.G.J. 1972. Officieel Orgaan. Koninklijke Nederlandse Zuiveboud, 64 (42) 1003-6.

155. ARENTZEN, A.G.J. 1972. Officieel Orgaan. Koninklijke Nederlandse Zuiveboud, 65 (35) 825-28.

156. WINKLER, S. 1974. Milchwirtschaftliche Berichte aus den Bundesanstalten Wolfpassing und Rotholz, 40 161-200.

157. BAHL, R.K. 1971. Analyst, 96 88-92.

158. BAHL, R.K. 1972. Analyst, 97 559-561.

159. NOVAK, E. & LASKOWSKI, K. 1972. Roczinki Instytutu Przemyshu Mleczarskiego 14, (3) 33-42.

160. TAYLOR, G.G. 1970. Australian Journal of Dairy Technology, 25 (1) 7-9.

161. NEY, K.H. & WIROTAMA, I.P.G. 1970. Zeitschrift fur Lebensmitteluntersuchung und Forschung.

162. SOLMS, J. & GUIGOZ, Y. 1974. Lebensmittel-Wissenschaft & Technologie, 7 (6) 365-57.

163. CZULAK, J. & HAMMOND, L.A. 1973. CSIRO Food Research Quarterly, 33 (4) 73-74.

164. LASKOWSKY, K. & JAMIOLKOWSKA, J. 1970. Roczniki Instytutu Przemyslu Mleczarskieg, 12 35-45.

165. DAVIS, J.G. 1971. (May) Dairy Industries, 267-275.

166. LECHNER, E. 1975. Zeitschrift fur Lebensmitteluntersuchung und Forschung, 159 (1) 39-42.

167. SERRINI, G., LANZA-SERRINI, G. & MONFRINI, C. 1981. Determination of Free Sialic Acid in Skimmed Milk, Rennet Whey Powders and their Relative Mixtures, EUR 7598 EN Directorate-General, Information Market and Innovation, Batiment Jean Monnet, Luxembourg.

168. ARCHER, A.W. 1973. Analyst, 98 755.

169. GOMBOCZ, E., HELLWIG, E. & PETUELY, F. 1981. Zeitschrift fur Lebensmittaluntersuchung und Forschung, 172 178-181.

170. MITCHELL, G.E. & MIDDLETON. G. 1980. The Australian Journal of Dairy Technology, 35 (1) 15-16.

171. TIMMS, R.E. 1980. Journal of Dairy Research, 47 295-303.

172. BURON ARIAS, I., GARCIA TERESA, R. & VALENCIA DIAZ, F. 1980. Grasas y Aceites, 31 335-340.

173. TRAITLER, H. & PREVOT, A. 1981. Journal of High Resolution Chromatography and Chromatography Communications, 4 109-114.

174. WATHELET, J-P., DEROANNE, C. & SEVERIN, M. 1979. Revue Francaise des Corps Gras, 26 (4) 175-179.

Further Reading

AMERICAN DRIED MILK INSTITUTE, INC. 1971. Standards for Grades of Dry Milk including Methods of Analysis, Chicago, USA.

ARORA, K.L. & RAJORBIA, G.S. 1978. Review on the Determination of Fat in Milk, Indian Journal of Animal Research 12 (2) 59-70.

Challenges to Contemporary Dairy Analytical Techniques, Royal Society of Chemistry Special Publication No. 49. 1984. London, UK.

DAVIS, J.G. 1950. A Dictionary of Dairying and Supplement Leonard Hill.

DAVIS, J.G. 1965-1975. Cheese Vols I-IV, Churchill, London.

FOX. P.F. 1982 & 1983. Developments in Dairy Chemistry Vols 1 & 2 Applied Science.

MARTH, E.H. 1978. Standard Methods for the Examination of Dairy Products 14th Ed. American Public Health Association, Washington.

McGANN, T.C.A. 1979. Review of Analysis of Dairy Products in Carroll, D.M., Burns, D.T., Brown, D.A. & MacDaeid, D.A. Euroanalysis III Reviews on Analytical Chemistry, Applied Science Publishers.

SCOTT, R. 1981. Cheesemaking Practice Applied Science.

3. SUGARS AND HONEY

3.1 SUCROSE

COMPOSITION

CAC Standards for white sugar and white sugar products:

	White Sugar Spec.A	White Sugar Spec.B	Powdered (Icing) Sugar(a)	Soft Sugar Spec.A	Soft Sugar Spec.B
Polarization (min. 155°)	99.7°	99.5°	99.7°	-	-
Sucrose + Invert Sugar Content (min.)	-	-	-	88.0%	97.0%
Invert Sugar Content	0.04%	0.1%	0.04%	0.3-12.0%	0.3-12.0%
Conduct. Ash	0.04%	0.1%	0.04%	-	0.2%
Loss on Drying (3 hr at 105°C)(b)	0.1%	0.1%	0.1%	4.5%	3.0%
Color (ICUMSA Units)	60	150	60	-	60
Sulphur Dioxide (ppm)	20	70	20	40	40
Arsenic (ppm)	1	1	1	1	1
Copper (ppm)	2	2	2	10	10
Lead (ppm)	2	2	2	2	2
Sulphated Ash	-	-	-	3.5%	-

(Note: All single values are maxima unless otherwise noted.)

(a) Icing sugar may contain up to 5 percent starch as long as no other anti-caking agent is used. If starch is not present, it may contain any of the following, singly or in combination, up to a maximum of 1.5% - tribasic calcium phosphate, magnesium carbonate, magnesium stearate, silicon dioxide, amorphous (dehydrated) silica gel, calcium silicate, magnesium trisilicate, sodium calcium alumino-silicate.

(b) The limit for loss on drying does not apply to white sugar in lump or cube form, to crystal candy sugar (crystal korizato), or to rock sugar (korizato).

ROUTINE ANALYSIS

Methods of analysis suitable for powdered and crystalline sucrose are:

1. Loss on drying at 105°C for 3 hours (1).

2. Invert sugars by the Knight and Allen method, as recommended by ICUMSA. This depends upon titration of excess unreduced copper with EDTA using murexide as an indicator. If the invert sugar content exceeds 0.04%, the Berlin Institute method (1) should be used.

3. Polarization, conductivity ash and colour can be determined by ICUMSA methods reproduced in this chapter. ICUMSA have also recommended, among others, methods for SO_2, pH, arsenic, copper and lead. Except for determination of invert sugar, moisture and polarization, the above methods are suitable for other powdered and crystalline sugars. Moisture in anhydrous dextrose and dextrose monohydrate is determined by drying for 4 hours at 100°C and a pressure of 100 mm Hg. The ICUMSA method for sulphated ash is substantially the same as the general method. The copper reduction and polarimetric methods in general used for the determination of sugars in foods, corn syrup and other sugar syrups are also appropriate for assaying dextrose and fructose powders.

The brown sugar with a characteristic taste known as Demerara may have had the colour stabilized with stannous chloride, titanous chloride or artifial colour. The extraneous water-insoluble material in good quality white sugar is usually less than 25 mg/kg. It may be determined by making a solution of a large quantity of sample (e.g. 1 kg) up to 1,800 ml with boiling water, filtering and then washing with a litre of hot water and soaking in water on a sieve for an hour in order to remove traces of sugar from the periphery of the paper. The filter is then dried, weighed and examined under the microscope.

The lead content of sugars will usually be very considerably lower than the limit of 2 mg/kg. Trace metals can be determined directly on solutions of white sugars but brown sugars must be ashed in the presence of ash-aid for lead and digested or ashed according to standard methods for arsenic. Copper can be determined by extracting the diethyl carbodithioate into carbon tetrachloride.

Degrees Brix are defined as the percentage of sucrose by weight. For example, a sucrose solution of 50° Brix consists of equal weights of water and sucrose. Originally tables were constructed relating degrees Brix to specific gravity, a hydrometer was used to measure the latter and the Brix was read from the table. Later the refractometer was used and the Brix read from a similar table relating it to the refractive index. Strictly speaking, degrees Brix only apply to pure sucrose solutions, but in industry the refractive index of juices and fruit products generally is determined as a quality control measure and reported as "apparent Brix" or just "Brix". The analyst must treat these figures with some reserve, as the refractometer reading alters with temperature, acidity and the presence of other substances including other sugars and the "apparent Brix" may bear a modified relation to the actual sucrose content.

POLARIMETRY

Solutions of sugars alter the plane of polarised light, to an extent depending on concentration and on the sugar. Thus the concentration of a solution of a pure known sugar may be determined by comparison of the number of degrees of angle by which it turns the light (angular rotation) with the rotation caused by a standard solution. Note that any other substance capable of rotating polarised light, including any other sugars, will interfere. This does not prevent the use of the technique for the determination of sugars in mixtures. For example, sucrose may be determined in the presence of invert sugar by determination of the rotation before and after hydrolysis of the sucrose. The hydrolysis causes a change in rotation proportional to the percentage of sucrose present. If the reducing power of the mixture is also determined, the proportions of three sugars in admixture may be determined. The presence of salts of alkaline reaction decreases the reading by polarimetry and allowance for this is made in some of the formulas used to calculate results. For example, correction is made for the amount of salt produced by the neutralization of hydrochloric acid used for hydrolysis.

If a sugar exists in solution as two stereoisomers in equilibrium, but crystallises in only one form, then on re-solution it will require some time for the equilibrium to be re-established. If the two isomers have different

rotatory power, as they usually do, the optical rotation of the solution will be changing until equilibrium is attained. This phenomenon is called mutarotation. In practice, the solution is either left overnight before reading, or ammonia, which like any alkali accelerates the attainment of equilibrium, is added, or the neutral solution may be boiled.

In 1842, Ventzke devised a scale such that an approximately 26 percent m/v sucrose solution read in a 20 cm tube gave a reading of 100°. After a number of changes over the years, it is now agreed that the polarization of the normal solution (26.000 g of pure sucrose dissolved in 100 ml and polarized at 20°C in a 200 mm tube, using polarized light of the green ^{198}Hg line in vacuo (λ = 546.2271 nm) as defined by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA)), be accepted as the basis of calibration of the 100° point on the International Sugar Scale. The details have changed over the years. The present definitions are given in the ICUMSA methods (1). The rotation for 100°S (International Sugar Degrees) is equivalent to:

$$\alpha \begin{matrix} 20.00°C \\ 546.2271\ nm \end{matrix} = 40.765°$$

For practical polarimetry, wavelengths in the region of 540 to 590 nm are also permissible for fixing the 100 point. 100°S at the wavelength of yellow sodium light will be equal to a rotation of:

$$\alpha \begin{matrix} 20.00°C \\ 589.4400\ nm \end{matrix} = 34.616°$$

A saccharimeter is of somewhat different design to a polarimeter. Quartz crystals have a very similar rotatory power to sugars but in the opposite direction, hence by insertion of a quartz wedge into the light path, the rotation due to the sugar may be fixed and the percentage of sugar in saccharimeter degrees is read from the scale calibrated according to the distance the wedge has to be inserted in order to cancel the rotation of the sample. The instrument is scarcely sensitive to changes in the wavelength of the light used, unlike an ordinary polarimeter, because it works by this compensatory mechanism. Therefore, the use of white light is specified in the standard method and a dichromate filter is used to remove light from the violet end of the spectrum, giving better delineation.

The main factors affecting the rotation of sugars are the wavelength of the light used, the concentration of sugar in the solution, the concentration of acid and salt present, temperature, solvent, clarifying agent used and if hydrolysis (inversion) of a higher saccharide to a monosaccharide is involved, the exact conditions of temperature, time and hydrolysing agent used. Thus the many formulae found in methods of analysis relate to particular conditions and the method must be followed in every detail and the correct formula relating to that method used if accurate results are to be obtained. It should be noted that change of optical rotation with change of wavelength of the light used, like the other factors, is different for different sugars, being greatest for galactose and least for rhamnose.

The following table is taken from Pearson (2). The formulae given are for the calculation of the specific rotation of the indicated sugar:

Sugar	**Formula**
Sucrose	$(\alpha)_D^{20} = 66.462 + 0.0087c - 0.000235c^2$
Dextrose	$(\alpha)_D^{20} = 52.50 + 0.0188p + 0.000517p^2$
Dextrose	$(\alpha)_{5461A}^{20} = 62.032 + 0.04257c$

Sugar	Formula
Fructose	$(\alpha)_D^{20} = 138.475 - 0.01837p$
Invert sugar	$(\alpha)_D^{20} = -(19.415 + 0.07065c - 0.00054c^2)$
Invert sugar temperature correction	$(\alpha)_D^{t} = (\alpha)_D^{20} + (0.283 + 0.0014c)(t - 20°C)$

where

c = concentration in grams per 100 ml
p = percentage by weight
t = temperature (°C) during analysis

The Clerget-Herzfeld Formula is used to calculate the percentage of sucrose in a sample by measuring the rotation before and after inversion (hydrolysis) of a solution to glucose and fructose (invert sugar, an equimolecular proportion of the two). The determination was usually carried out using a saccharimeter and the results therefore had to be referred to the saccharimeter scale, based on a 26 percent m/v solution of the sample and the specific rotation of sucrose of about 66.5°. However, on inversion the specific rotation does not fall to zero but actually becomes negative, the specific rotation of invert sugar being about -20.4° and this must be multiplied by 1.053 as 100 g of sucrose give 105.3 g of fructose and dextrose on hydrolysis. Hence the total change is about 87.5° and the reading must be multiplied by 66.5/87.5 so that it can be read off as percentage sucrose on the saccharimeter scale. In the original Clerget formula the reading was divided by 87.5526/66.5 or 1.3166. As explained above, the analytical conditions affect the values and Herzfeld in 1888 closely standardized the procedure and derived a formula of the type:

$$S = \frac{100\ (P-I)}{133.2 + 0.0676\ (13-m) - 0.5\ (t-20)}$$

where 100/133.2 represents the factor converting to the saccharimeter scale, 0.0676 (13-m) corrects for the total solids (m) from the original solution in 100 ml of invert solution, P is the rotation of the normal weight before inversion and I the rotation after. (Thus if 26 g of anhydrous sample are taken the correction is zero, as the normal weight is dissolved in 100 ml and a 50 ml aliquot is inverted and diluted to 100 ml.) t represents temperature in degrees Centigrade.

The factors have been re-examined by Jackson and Gillis (3) and Jackson and McDonald (4) and from time to time by the succeeding Commissions for Uniform Methods of Sugar Analysis (1964)(1978). See also Norrish (5), International Critical Tables (6) and Brown and Zerban (7). These formulas apply if the exact methods given in official compendia such as those of the AOAC and ICUMSA are followed. In acid hydrolysis methods an amount of salt, equal to that produced as a result of the addition and subsequent neutralization of hydrochloric acid to the hydrolysed aliquot, is added to that portion which is not hydrolysed in order to cancel out the salt effect on the rotation.

The very accurate work of Jackson and McDonald (4) was done using saccharimeters and it is necessary to calculate the factors for use with an instrument reading in angular degrees. Since 1° on the International Sugar Scale = 0.34616° angular, the reading is simply divided by this figure and the result substituted in the formula above.

The Inversion Division Factor (Q) is an alternative method of calculation provided the angular degrees are read from a polarimeter illuminated by a sodium lamp. "Q" is defined as the change in the specific rotation of sucrose

on inversion. It can be calculated from saccharimeter formulas, e.g. for acid inversion at 60°C and readings at 20°C.

The value of Q is not only affected by the factors that influence the Clerget-Herzfeld formulas, but also the wavelength of light used (which is always white light with the saccharimeter) and the presence of other substances including the precipitant used. Values adopted by the SPA in 1930 were (8):

	Q (Zinc ferrocyanide precipitant)	Q (Phosphotungstic acid precipitant)
Sodium light	0.8825	0.8865
Mercury green light	1.0392	1.0439
White light (International Sugar Scale Light)	2.549	2.561

For impure products containing other polarizing substances such as raffinose, glucose, fructose, amino-acids etc. the determination of sucrose, by use only of a polarimeter, requires methods of multiple polarization of which the Clerget method is a particular example.

If the product does not contain raffinose, two polarizations are made: one direct and one after hydrolysis. The sucrose content is deducted from the change in polarization on hydrolysis, it being assumed that the polarizing impurities are not affected by the hydrolytic reagents and behave identically in both measurements.

If the product contains raffinose, this trisaccharide is partially hydrolysed to fructose and melibiose under the conditions required to hydrolyse sucrose, and a third polarization is required, either after complete hydrolysis of the raffinose (and sucrose), or under some other pH conditions so as to produce a predictable change in the optical activity of the sugars. The sucrose can be deducted from two simultaneous equations involving the three polarizations. Again it is assumed that the remaining polarizing impurities are otherwise unchanged by the hydrolytic reagents.

The fate of these impurities, in either case, may not correspond to the hypothesis for various reasons. Other optically-active compounds may be hydrolysed or their rotations may be changed by the hydrolytic reagents; the results obtained thus necessarily have an uncertain character. It is essential to take into account such factors as the nature of clarifying agent, the content of dry solids of the solution to be polarized and the temperature and conditions of hydrolysis. Since many optically-active compounds contribute to polarization, temperature corrections are necessarily uncertain and it is recommended that, if possible, all polarizations should be carried out at 20°C to avoid any temperature correction.

The simultaneous equations for three polarizations can be solved to deduce raffinose concentration, and formulas have also been proposed for deducing raffinose from two polarizations if the material is free from reducing sugars, or if the rotational contribution of the reducing sugars can be estimated independently. Since the raffinose content is very much less than the sucrose content, the uncertainties have a relatively greater effect on the raffinose value and none of these procedures is recommended for estimation of raffinose, which is best done by the method of Schiweck and Büsching (9,10).

WHITE SUGAR
(Polarization Method)

PRINCIPLE

An aqueous solution of the sugar sample (26 g, i.e. the normal weight of sucrose in 100 ml water) is polarized by means of a saccharimeter which is calibrated to read 100°S on the "International Sugar Scale" under specified conditions.

APPARATUS

1. Saccharimeter, calibrated with quartz plates.

2. Flasks (100 ml) conforming to ICUMSA class A, which are individually calibrated by weighing with water at 20 ± 0.1°C. Flasks whose contents fall within the range 100.00 ± 0.01 ml may be used without correction. Flasks whose contents fall outside this range must be used with the appropriate correction to 100.00 ml.

3. Polarimeter tube, length 200 mm, conforming to ICUMSA Class A, with cover glasses.

4. Water-bath, thermostatically controlled to 20.0 ± 0.1°C.

PROCEDURE

The normal weight (26 ± 0.002 g) of the sugar sample is weighed out and transferred to a flask (100 ml) by washing with distilled or de-ionized water (about 80 ml). The sugar is dissolved by agitation without heating and water added to just below the calibration mark.

The temperature of the sugar solution is adjusted to 20 ± 0.1°C by means of a water bath. The inside wall of the neck of the flask is dried with filter paper and the solution volume adjusted exactly to 100 ml with water (20 ± 0.1°C) using either a hypodermic syringe or a pipette with a drawn-out point. The flask is then sealed with a clean, dry stopper and its contents mixed thoroughly by hand-shaking.

The polarimeter tube is thoroughly rinsed twice with two-thirds its volume of sugar solution and filled with the sugar solution at 20 ± 0.1°C in such a way that no air bubbles are entrapped. The tube is placed in the saccharimeter and polarized at 20 ± 0.1°C. Five measurements are taken to 0.05°S or better. The average value is expressed to 0.01°S. In the same way the quartz control plate reading is determined to 0.01°S.

CALCULATION

Normally it is not convenient to adjust the temperature of the quartz-wedge compensator to 20 ± 0.1°C and consequently the following equation can be used to apply the necessary correction:

$$P^{20} = P\,[1 + 0.00014\,(t - 20)]$$

Where:

P^{20} is the polarization at 20 ± 0.1°C,

P is the observed polarization at 20 ± 0.1°C and

t is the temperature in °C of the quartz-wedge compensator.

If a flask correction is required then the polarization is corrected by adding the flask correction to the observed polarization.

Flask correction = Actual volume of the flask - 100.00

Highly coloured or turbid sugar solutions must be clarified with basic lead acetate before polarization.

The result is to be expressed as polarization in °S to an accuracy of 0.01°S.

REFERENCES

This is an ICUMSA tentative method (1979). ICUMSA, Report of the Proceedings of the 16th Session, 1974, Subject 19, Record 6, 268.

ICUMSA, Report of the Proceedings of the 16th Session, 1974, Subject 19, 265.

ICUMSA, Report of the Proceedings of the 15th Session, 1970, Subject 11, 95.

INVERT SUGAR IN WHITE SUGAR
(Knight and Allen Method)

PRINCIPLE

The method is suitable for the determination of low invert sugar contents (e.g. in white sugar up to 0.02%). A copper-complex solution containing sodium carbonate as the main alkaline agent is used. When heated in a boiling water-bath, the invert sugar reduces the cupric ions to cuprous oxide. After cooling, the residual cupric ions are titrated with EDTA, using murexide as indicator. The experimental conditions, including weight of sugar, volume of water, volume of alkaline copper solution and time of heating, are strictly standardised and the result is obtained from a calibration curve.

APPARATUS

1. Test tubes (150 x 20 mm).

2. White porcelain evaporating dishes, used for titration (to ensure better detection of the end-point).

3. Water-bath, maintained at boiling point.

REAGENTS

1. Alkaline copper solution: dissolve 25g anhydrous sodium carbonate and 25g sodium potassium tartrate tetrahydrate in 600 ml of distilled water containing 40 ml of N sodium hydroxide solution in a 1L volumetric flask. Dissolve 6g of cupric sulphate pentahydrate in about 100 ml of distilled water and quantitatively transfer to the alkaline tartrate solution and dilute to the mark and mix.

2. EDTA solution, 0.005N: dissolve 0.9306g EDTA dihydrate in distilled water and dilute to 1L in a volumetric flask.

3. Murexide indicator: since murexide solution is not very stable, the indicator is best prepared in a solid state. Grind 0.5g murexide, 0.15g methylene blue and 40g sodium chloride together using a pestle and mortar. To avoid caking, store in a desiccator over silica gel. Use approximately 0.1g solid indicator for each titration.

PROCEDURE

Dissolve 5 g of the sugar in 5 ml of cold distilled water in a test tube. Add exactly 2 ml of the alkaline copper solution. After thorough mixing place the tube in a boiling water-bath for exactly 5 min and then cool immediately in a cold water-bath. Transfer the solution and washings to a white evaporating dish and add approximately 0.1g of the indicator. Titrate the mixture with 0.005N EDTA solution.

The colour changes from green, through grey, to purple which is the end-point. The grey stage appears just before the end-point. When the purple colour is reached, it disappears slowly, owing to the oxidation of the cuprous oxide at a rate which depends to some extent on the amount of cuprous oxide present. The end-point should therefore be approached as rapidly as possible.

CALCULATION

Prepare a calibration curve giving ml 0.005N EDTA solution (abscissa) against % invert sugar (ordinate). The curve should be linear up to

0.02g invert sugar/100g sucrose. Read the amount of invert sugar directly from the calibration curve.

REFERENCES

Knight, J. and Allen, C.H. 1960. International Sugar Journal, 62, 344.

ICUMSA, Report of the Proceedings 15th Session, 1970, Subj. 14, Rec. 1 (c), 147.

Emmerich, A., 1967. Zucker, 20, 603. This is reproduced in ICUMSA Methods, 1979, (1).

INVERT SUGAR IN WHITE SUGAR
(Berlin Institute Method)

PRINCIPLE

This method is used for the determination of invert sugar in products containing not more than 10% invert sugar in the presence of sucrose. No defecation is required even in the case of dark-coloured solutions. To the solution containing invert sugar, Müller's solution is added (this contains sodium carbonate instead of the sodium hydroxide used in Fehling's solution). The mixture has a pH of about 10.4 (relatively low in comparison with other methods) and is heated in a boiling water-bath. Foaming caused by impurities during direct heating and boiling of the reaction mixture is thus avoided. The cupric ions are reduced by the invert sugar to cuprous oxide and, after cooling, the liquid is acidified and an excess of standardised iodine solution added. When all the cuprous oxide has reacted with the iodine, the excess iodine is back-titrated with a solution of sodium thiosulphate, using starch as indicator. The experimental conditions, including volume of test solution, volume of Müller's solution, time of heating and titre of iodine solution, are strictly standardised. Because the temperature is lower than in the other methods and because the pH of the reaction mixture is low, the sucrose correction is small and more closely controlled.

APPARATUS

1. Erlenmeyer flasks, 300 ml.

2. Water-bath, with vigorously boiling water (to ensure that immersion of flasks does not interrupt boiling). The flasks are placed in the water-bath so that the water level is 2 cm above the surface of the liquid in the flasks.

3. Burette stand equipped with two 50 ml burettes, with 0.1 ml graduations.

REAGENTS

1. Müller's solution: Dissolve 35g cupric sulphate pentahydrate in about 400 ml of boiling distilled water. In another beaker, dissolve 173g potassium sodium tartrate tetrahydrate and 68g anhydrous sodium carbonate in about 500 ml of boiling distilled water. Cool the two solutions and pour the sodium carbonate solution into the cupric sulphate solution with stirring. Dilute the combined solution to 1L. After vigorous shaking with 2g active carbon, filter the solution through hardened filter paper under vacuum. If cuprous oxide is precipitated on storage, refilter the solution.

2. Acetic acid, 5N.

3. Iodine solution, 0.0333N (this solution should not contain more than 6g potassium iodide/L).

4. Sodium thiosulphate solution, 0.0333N (this solution is stabilised by the addition of N sodium hydroxide solution, 3 ml/L).

5. Starch indicator: Dissolve 1g soluble starch in 100 ml of saturated sodium chloride solution.

PROCEDURE

Pipette or weigh an amount of sample (containing not more than 30 mg of invert sugar) into an Erlenmeyer flask and dissolve. Make the volume up to 100 ml. Add 10 ml of Müller's solution and mix. Place the flask in a boiling water-bath for 10 min ($\pm$ 5 sec.). After

heating, cool the flask rapidly, without agitation, under running water (a small beaker being placed over the mouth of the flask). Acidify the cold solution with 5 ml 5N acetic acid and add an excess of 0.0333 N iodine solution (20 to 40 ml) from the burette. Make both additions without agitation to avoid oxidation of cuprous oxide by air. Mix the solution. When the precipitate has completely dissolved, back-titrate the excess of iodine with 0.0333N sodium thiosulphate solution in the presence of a few drops of starch indicator which are added as the end-point is approached.

CALCULATION

The difference between the volume of the iodine and thiosulphate solutions used is the volume of 0.0333N iodine used in the reaction. This value is corrected by subtraction of the following three corrections:

1. Blank correction: the volume (ml) of the iodine solution required in a blank test with distilled water instead of sugar solution. This correction should not exceed 0.1 ml for pure reagents and should be determined for each new batch of Müller's solution.

2. Cold correction: the volume (ml) of iodine solution required by the sugar solution after standing 10 min at room temperature before acidification (this corrects for the effect of reducing substances present).

3. Sucrose correction: proportionate correction to allow for the reducing action of the sucrose (0.2 ml iodine solution/g sucrose).

After these corrections have been made, 1 ml of iodine solution is equivalent to 1 mg invert sugar.

REFERENCES

Spengler, O., Todt, F. and Scheuer, M. 1936. Z. Wirtschaftsgr. Zuckerind., 86, 130 and 322.

ICUMSA, Report of the Proceedings 15th Session, 1970, Subj. 14, Rec. 1(b), 147.

CONDUCTIVITY ASH

PRINCIPLE

The specific conductivity of a sugar solution of known concentration is determined. It is assumed that the conductivity has its own significance and the equivalent ash is calculated by the application of a constant factor. Two concentrations may be used, i.e. 28g/100g for white sugar and other products of very low conductivity and 5g/100 ml for all other products. The factors used for transforming measured conductivity into ash are purely conventional and applicable only to sugar solutions.

APPARATUS

1. Volumetric flasks, 100ml, 500ml and 1L.

2. Pipettes, 10ml.

3. Sugar ash bridge or null balance bridge with the following specifications:

Sugar ash bridge (a balanced bridge circuit with null point indicator):

a. Frequency: 50 to 2000 Hz.

b. Range: 0.001 to 0.1% ash for white sugars and 0.01 to 0.90% ash for raw sugars.

c. Accuracy of built-in standards: $\pm$ 1% or better.

d. Accuracy of measurement: $\pm$ 3% or better but, for low ash (conductivity) values, not less than 0.001% (0.5 µS/cm).

e. Indication: visual.

f. Scale units: ohms or siemens (=S) or ash units.

g. Temperature of solution: the standard temperature of measurement shall be 20°C.

h. Temperature compensation: shall have a temperature compensating mechanism.

Null balance bridge:

a. Frequency: 50 to 2000 Hz.

b. Range: 0 to 500 µS/cm.

c. Electrode voltage: 0.2 to 10V.

d. Accuracy of built-in standards: $\pm$ 1% or better.

e. Accuracy of measurement: $\pm$ 3% or better but, for low ash (conductivity) values, not less than 0.5 µS/cm.

f. Indication: visual.

g. Scale units: ohms or siemens (=S).

h. Electrodes: with fixed distance.

i. Cell construction: of glass or synthetic material.

j. Temperature measurement: means for measuring the temperature of the solution to be provided.

k. Cell constant: within the range 0.2 to 3 cm^{-1}.

REAGENTS

1. Purified water: twice distilled or de-ionized with a conductivity of less than 2 µS/cm.

2. Potassium chloride, 0.01N: weigh 745.5 mg of dried potassium chloride and dissolve in water. Make to 1L.

3. Potassium chloride, 0.0025N: Dilute 250 ml of the 0.01N solution to 1L. It has a conductivity of 328 µS/cm at 20°C.

4. Potassium chloride, 0.0002N: Dilute 10 ml of the 0.01N solution to 500 ml. It has a conductivity of 26.6 ± 0.3 µS/cm at 20°C after deduction of the specific conductivity of the water used.

PROCEDURE

Method for 5g/100ml solutions: Dissolve 5g of the sample in water in a 100 ml flask and make to volume at 20°C. In the event of the conductivity exceeding 500 µS/cm or the solids content of the solution being less than 5g, white sugar of low ash content must be added in such a way as to maintain the total solids concentration at 5g/100ml. After thorough mixing, transfer the solution into the measuring cell and measure the conductivity at 20 ± 0.2°C. Check the measurement using an appropriate potassium chloride reference solution.

Method for 28g/100g solutions: Dissolve 31.3 ± 0.1g of sugar in water in a 100 ml volumetric flask and make to volume at 20°C or dissolve 28.0 ± 0.1g of sugar in water to give a solution of 100.0g weight. In the case of liquids (syrups), the amount taken must be such that it contains 31.3 or 28.0 g of solids. After thorough mixing, transfer the solution into the measuring cell and measure the conductivity at 20 ± 0.2°C. Check the measurement using the 0.0002N potassium chloride solution.

If the determination cannot be made at the standard temperature of 20°C a temperature correction may be made to the final result as follows, provided that the range of ±5°C is not exceeded:

For the method at 5g/100ml, the correction is 2.3% per °C (to be added for temperatures below 20°C and subtracted for temperatures above 20°C.

For the method at 28g/100g, the correction is 2.6% per °C (to be added for temperatures below 20°C and subtracted for temperatures above 20°C).

CALCULATIONS

For the method at 5g/100ml:

$$C = M - 0.9W$$

where: C = corrected conductivity (in µS/cm)
M = measured conductivity (in µS/cm) at 20°C
W = specific conductivity (in µS/cm) of water at 20°C.

% conductivity ash = 0.0018C
(Note: Any white sugar added in the sample solution preparation must be taken into account.)

For the method at 28g/100g:

C = M - 0.35W

where: C, M and W are as above.

% conductivity ash = 0.0006C

REFERENCES

ICUMSA, Report of the Proceedings 15th Session, 1970, Subj. 16, Rec. 1 and 5, 171.

ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 16, Rec. 2,3 and 4, 222.

(Note: This method is only tentative for molasses.)

LOSS ON DRYING

PRINCIPLE

There is a considerable difference of opinion on terminology in sugar analysis. Loss of weight measurements have been recommended for the determination of what has variously been referred to as water content, moisture content, total solids content, dry substance content, dry matter content, loss of weight on drying, loss on drying, etc. In the light of current technological knowledge, the use of such terms as 'moisture content' and 'water content' in this context is erroneous. The Committee on Sugars of the Codex Alimentarius Commission has agreed upon the suitability of the term 'loss on drying' and this term has also been adopted by the EEC authorities. This method measures mainly 'surface moisture' by air oven drying with uniform conditions for cooling.

APPARATUS

1. Forced draught oven maintained at a temperature of 105 ± 1°C as measured 2.5 ± 0.5cm above the dishes in the test. The oven is to be ventilated and the circulation fan fitted with an interlock switch which opens when the oven door is opened.

2. Desiccator containing self-indicating silica gel.

3. Dishes with tight-fitting lids. These should have a diameter of 6 to 10 cm and a depth of 2 to 3 cm. Although they may be made of glass, platinum or nickel, aluminium is recommended. The thickness of the dishes is optional, except that due regard should be paid to the weight of the dish in relation to the weight of the sample and to the loss to be determined.

4. Surface pattern dial thermometer.

PROCEDURE

Preheat the oven to 105°C. Place the empty dishes, with lids open, in the oven for not less than 30 min. Remove the dishes from the oven, replace the lids and place in the desiccator. The contact thermometer is placed on top of one of the dishes. When the temperature of the dishes has fallen to ambient + 5°C, weigh as rapidly as possible to an accuracy of ±0.1mg. As rapidly as possible, place 20 to 30g of the sample in each dish, replace the lid, and weigh to an accuracy of ±0.1mg. (The depth of sugar in the dish must not exceed 1 cm).

Return the dishes, with the lids open, to the oven. Dry for 3 hours exactly. There must be no other materials in the oven during the drying period. Replace the lids and remove the dishes from the oven and replace in the desiccator with the contact thermometer on one of them. Cool the dishes until the thermometer indicates a temperature of ambient + 5°C. Weigh to an accuracy of ± 0.1mg. (No attempt should be made to dry to constant weight and care must be taken to ensure that there is no physical loss of sugar at any stage.)

CALCULATION

Loss in weight is expressed as a percentage of the original weight of the sample:

$$\text{Loss on drying} = \frac{100(W_2-W_3)}{W_2-W_1}$$

where: W_1 = weight of dish.
W_2 = weight of dish + sugar before drying and
W_3 = weight of dish + sugar after drying.

Duplicate results are acceptable if neither is outside the limits of $\pm$ 10% of the mean value for the test. Tests in which either duplicate exceeds this limit should be repeated.

REFERENCES

ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 19, Rec. 2. 268.

ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 19, 263.

ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 19, 252.

COLOUR INDEX

PRINCIPLE

The absorbance of a filtered solution of the sample is determined at 420 nm (560 nm for darker coloured products) using a spectrophotometer and the absorbance index is calculated and converted to ICUSMA colour units.

APPARATUS

1. Spectrophotometer with 10 cm absorption cells.

2. Membrane filters, 50 mm with poresize of 0.45 µ.

PROCEDURE

Prepare a solution of the sugar to be tested using distilled water. Use the following concentrations:

White sugar	50g/100g
Darker sugar	As high as practicable, consistent with reasonable filtration rates and cell depths
Liquors, syrups and juices	Original density, unless dilution is required to obtain reasonable filtration rates or cell depths

Filter the solution under vacuum. White sugar solutions and light coloured liquors should be filtered through a membrane filter of pore size 0.45 µ according to the mercury extrusion method. Darker solutions should be filtered with analytical grade Kieselguhr (1 percent on solids) over filter paper. The first portion of the filtrate should be discarded if cloudy. Adjust the pH of darker solutions to 7.0 $\pm$ 0.2 with dilute HCl or NaOH. Do not adjust the pH of white sugar solutions. Remove entrained air under vacuum, or in an ultrasonic bath (take care to minimise evaporation).

Place the solution in a 10-cm absorption cell (the cell length is chosen so that the instrument reading will be between 20 and 80 percent transmittancy). Determine the absorbance of the solution at 420 nm (or 560 nm) in a spectrophotometer or equivalent, using distilled water as a reference.

CALCULATION

$$\text{Absorbance index} = \frac{A}{bc} = \frac{-\log T}{bc}$$

Where: A = absorbance
T = transmittance
b = cell length in cm
c = concentration of total solids in g/ml

Absorbance index x 1000 = 'colour' in ICUSMA units.

REFERENCES

ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 22, Rec. 1 and 2, 303.

ICUMSA, Report of the Proceedings 15th Session, 1970, Subj. 22, Appendix 5, 255.

ICUMSA, Report of the Proceedings 14th Session, 1966, Subj. 22, 129.

3.2 SUGAR PRODUCTS

COMPOSITION

CAC Standards for reducing sugar products are:

	Dextrose (Anhydrous)	Dextrose (Monohyd.)	Powdered Dextrose (Icing)(a)	Glucose Syrup(b)	Dried Glucose Syrup(c)
d-glucose content (or equivalent)(min)	99.5%	99.5%	99.5%	20%	20%
Total solids (min)	98.0%	90.0%	-	70%	93.0%
Sulphated ash	0.25%	0.25%	0.25%	1.0%	1.0%
Sulphur dioxide (ppm)	20	20	20	40(d)	40(e)
Arsenic (ppm)	1	1	1	1	1
Copper (ppm)	2	2	2	5	5
Lead (ppm)	2	2	2	2	2

(Note: All values are maxima unless otherwise noted.)

(a) Icing dextrose may contain any of the following, singly or in combination up to a maximum of 1.5%: Tribasic calcium phosphate, magnesium carbonate, magnesium stearate, amorphous silicon dioxide (dehydrated silica gel), calcium silicate, magnesium trisilicate, sodium calcium aluminosilicate.

(b) Defined as a purified concentrated aqueous solution of nutritive saccharides obtained from starch.

(c) Defined as glucose syrup from which the water has been partially removed.

(d) In glucose syrup used only for manufacture of sugar confectionery, the limit is 400 ppm.

(e) When used in the manufacture of sugar confectionery only, the limit is 150 ppm.

ROUTINE ANALYSIS

Glucose syrup or corn syrup is also called liquid glucose, which is a term that should be discouraged. It is made by the hydrolysis of starch, which is frequently obtained from maize (corn), hence the term corn syrup. It consists of dextrose, maltose and higher saccharides. It is usually sold on the basis of its dextrose equivalent and density in degrees Baume. The dextrose equivalent is defined as the percentage of reducing sugars in the syrup, as determined by Lane and Eynon titration or another standard method, calculated as dextrose and expressed as a percentage of the total solids. Degrees Baumé is defined as the ratio of the total volume displaced in water by the hydrometer and the volume displaced by the unit scale length of the hydrometer stem. It is related to true specific gravity by

$$°Be' = 145 - \frac{145}{\text{true SG } 15.5°C/15.5°C}$$

For a product of any particular dextrose equivalent, the total solids in the sample may be deduced from degrees Baumé by use of a table.

Treacle, molasses and golden syrup are derived from the uncrystallisable residue left over after removal of sucrose during the processing of cane and beet sugar.

Invert sugar is obtained by the hydrolysis (inversion) of sucrose and therefore contains equimolecular proportions of glucose (dextrose) and fructose (laevulose). There are now enzymes available commercially which isomerise the glucose to the sweeter fructose.

The total solids of syrups are usually determined by drying at 70°C and a vacuum of about 25 mm Hg. The total solids (% m/m) may also be obtained from the refractive index at 20°C and use of a table. Correct for the presence of invert sugar (if the syrup is not a sucrose syrup) by adding 0.022 to the R. I. for every 1 percent invert sugar present. If crystals are present, dilute with an equal weight of water. Reducing sugars are usually determined by a copper reduction method such as the Lane and Eynon procedure and calculated as invert sugar or as the dextrose equivalent.

There are several qualitative chemical tests for sugars, such as Fehlings, Barfoed's or the reaction with anthrone. TLC does not take much more operator time and identifies the sugars present. This step is useful as it enables the analyst to choose the appropriate method for the quantitative analyses of mixtures of sugars such as polarimetry, gravimetry or titrimetry or a combination of them. These results may be used to calculate the proportions of known mixtures of sugars by use of simultaneous equations.

The analysis of sugars in food involves three steps: removal of interferences, hydrolysis (for all but reducing sugars) and final estimation. Interferences are usually removed either by extraction of food with 80 percent alcohol or by precipitation of protein and other material with clearing (defecating) agents or both. Extraction with 80 percent ethanol or 1-propanol dissolves sugars, amino acids, salts, organic acids and other small molecules, leaving polysaccharides and proteins undissolved. This is the method of choice for products containing starch and is necessary for the determination of sugars in fruit and vegetable products that contain polysaccharides precipitable by or insoluble in 80 percent ethanol. Organic acids and amino acids, colour and other small molecules are then removed by clarification with neutral lead acetate or by ion-exchange. Other clarifying agents such as alumina cream, basic lead acetate, activated charcoal, sodium tungstate and phosphotungstic acid are sometimes used; basic lead acetate and charcoal tend to absorb sugars. Zinc ferrocyanide is a very good clearing agent, precipitating protein (which takes down moderate amounts of fat with it) and is the agent of choice for dairy products. Fat can also be removed, if necessary, by prior extraction with petroleum ether.

The clarified solution is ready for determination of reducing sugars if present. Non-reducing sugars must first be hydrolysed with acid or enzymes. The conditions of hydrolysis must be adhered to strictly otherwise incomplete hydrolysis or the further breakdown of the sugars may result. If the final determination is by polarimeter, the conditions of hydrolysis affect the equation to be used to calculate the result.

The estimation on the prepared solution may be carried out by enzymic procedures, which are specific, as well as by polarimetry or reduction of Fehlings solution, with determination of the end-point by subsequent weighing of the precipitated cuprous oxide or by titration. Many other methods are available, but the above are probably the ones most commonly used in the food laboratory. Sugars may also be separated by column chromatography and determined by one of the above techniques, or determined by GLC (usually as the trimethysilyl derivatives), or by liquid chromatography.

SAMPLE PREPARATION - SUGARS
(Alcohol Extraction)

PRINCIPLE

The sample or filtered sample is mixed with ethanol so that the final concentration of the latter (taking into account the moisture in the sample) is 80 % v/v. Chalk is added to neutralize acidity. The mixture is boiled on the water bath, diluted to volume, filtered and the filtrate evaporated to remove alcohol. Any enzymes present are inactivated by the alcohol.

REAGENTS

1. Alcohol, 95 %.

2. Calcium carbonate, precipitated.

PROCEDURE

Add the ground or finely chopped and weighed sample to hot alcohol to which enough precipitated chalk has been added to neutralize acidity, using enough alcohol so that the final concentration, allowing for water in the sample, is 80 % v/v. Heat nearly to boiling on a waterbath for 1 hour, stirring frequently. The solution may be kept at this stage if it is not convenient to proceed immediately with the analysis. Decant the solution into a volumetric flask and mix the solids in a high-speed blender with 80 percent alcohol. Boil the blended material on a waterbath for 30 minutes, cool, transfer to the volumetric flask and dilute to volume with 80 % v/v ethanol at 20°C. Filter and evaporate an aliquot of the filtrate. Add water as necessary to prevent the solution evaporating to dryness. When the odour of alcohol disappears, add about 100 ml of water and heat to 80°C to soften gummy precipitates and break up insoluble masses. Filter through a thin mat of Celite on a filter paper in a Buchner funnel previously washed with water until the washings are clear. After passing the sample solution through the Celite, wash the latter with water and dilute the combined filtrate and washings to a suitable volume or use the entire filtrate, which should not be much in excess of 50 ml.

The solution is now ready for clarification by lead acetate or for hydrolysis and final determination if clarification is not necessary.

If it is necessary to determine starch in the product, boil the sample with 80 % alcohol initially only for 30 minutes, filter through a paper or extraction thimble, collecting the filtrate in a volumetric flask. Return any insoluble material to the beaker, boil an hour with 80 percent alcohol and filter through the same paper or thimble. If the extract is highly coloured, repeat a third time. Finally transfer the residue to the paper or thimble, leave to drain and then dry. Grind the residue to pass a 1 mm sieve, place thimble (or paper inside a thimble) in a Soxhlet extractor and extract 12 hours with 80 % alcohol. The combined alcohol extracts are evaporated as above and the residue may be used for the determination of starch.

SAMPLE PREPARATION - SUGARS
(Clarification)

PRINCIPLE

A solution or extract of the sample, free of polysaccharides, is treated with neutral lead acetate, removing colour, organic acids, amino acids and other small ionic molecules. Excess lead acetate is removed with oxalate.

REAGENTS

1. Neutral lead acetate, saturated solution in water.

2. Sodium or potassium oxalate crystals.

PROCEDURE

Weigh a quantity of sample appropriate for the final determination into a 250 ml volumetric flask. For example, the Lane and Eynon titration using 10 ml of mixed Fehling solution requires a quantity of sample containing 0.15 - 0.75 g of reducing sugars, the Berlin method requires 0.01 - 0.08 g of reducing sugars in 250 ml and the method of Somogyi requires 0.005 - 0.003 g in the same volume. If it was necessary to extract the sample with 80 percent alcohol, transfer to the 250 ml volumetric flask the aqueous extract of the sample after all alcohol has been removed by evaporation.

Dilute to 100 - 120 ml with distilled water and add by pipette enough saturated neutral lead acetate solution to produce a flocculent precipitate, shake thoroughly and leave to stand 15 minutes. Test tne supernate with a few drops of lead acetate solution and if a precipitate forms shake and leave to stand again. If no further precipitate forms, dilute to the mark with water, mix thoroughly and filter through a dry paper. Add enough solid sodium oxalate to the filtrate to precipitate all the lead and re-filter through a dry paper. Check for absence of lead in the filtrate by adding a little sodium oxalate.

Alternatively, add just enough lead acetate to the solution to cause complete precipitation. This point is reached when a drop of dilute sodium oxalate added to the supernate gives a precipitate. Then add the same volume of lead acetate solution again. Thoroughly shake and let the mixture stand a few minutes, then filter into a beaker containing an estimated excess of sodium oxalate crystals. Wash the filter until the filtrate no longer gives a precipitate with the oxalate. Check that oxalate is present in excess by adding one drop of lead acetate solution. Filter off and wash the lead oxalate precipitate, collecting the filtrate and washings in a volumetric flask. Dilute to the mark with water and mix. The solution is now ready for hydrolysis or final estimation of reducing sugars.

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 31.021(d).

SUGARS IDENTIFICATION
(Thin-Layer Chromatography)

PRINCIPLE

The sugars are extracted into water solution and separated and identified using thin-layer chromatography.

APPARATUS

1. Large shallow Pyrex tray.

2. TLC tank.

3. TLC plates - silica gel 60.

REAGENTS

1. 0.3N NaH_2PO_4, (12 g/L)

2. Sugar standard solutions: 1% aqueous solutions of sucrose, glucose, lactose, maltose and fructose.

3. Developing solvent: 40 ml Butan-1-ol + 50 ml acetone + 10 ml 0.3N NaH_2PO_4.

4. Spray reagent A: dissolve 2g diphenylamine and 2g aniline in acetone and make up to 100 ml.

5. Spray reagent B: orthophosphoric acid, 85%.

6. Zinc acetate solution, 21.6% w/v.

7. Potassium ferrocyanide solution, 10.6% w/v.

PROCEDURE

Line the tank with chromatography paper and pour in the developing solvent. Replace the lid and allow atmosphere in tank to saturate for about 30 minutes. Soak the TLC plate in 0.3 N NaH_2PO_4 solution in the Pyrex tray for one minute. Remove and dry in an oven at 100°C for 30 minutes. Meanwhile take a quantity of sample in a 50 ml beaker and add 8 ml water and 1 ml each of zinc acetate solution and potassium ferrocyanide solution. Mix well and filter. Spot the filtrate onto the prepared plate along with the standards (this can be done quantitatively with a micro-pipette or qualitatively with an extruded Pasteur pipette). Place the plate in the tank and allow to develop for 10 cm. Remove and dry in the oven for one minute. Take 20 ml spray reagent A in a beaker and add reagent B dropwise. The mixture goes cloudy and then slowly clears. Spray the dried plate with the clear spray reagent in a fume cupboard and dry in the oven for 1-2 minutes. The sugars show up as blue or brown spots. Each sugar is a slightly different colour and a slightly different R_f value. If the colours are faint spray again and dry.

Best separation is obtained by using the smallest possible spots (less than 1 mm) of a fairly concentrated solution (2-10%).

INTERPRETATION

This method will distinguish dextrose from glucose syrup (corn syrup), the latter showing the presence of various higher saccharides and a streak near the baseline. Glucose syrup gives an excellent series of spots on unbuffered silica gel using propanol:ethyl acetate:water as solvent.

SUGARS ANALYSIS
(Gas Chromatography)

PRINCIPLE

The sugars are extracted and silyl derivatives formed. The derivatized sugars are separated and quantitated using gas chromatography.

APPARATUS

1. Reactivial 3 ml with magnetic stirring elements.

2. 100 ml volumetric flasks, and beakers.

3. Thermostatically controlled water bath.

4. Drying oven at 80°C.

5. Vacuum oven at 70°C.

6. Ultrasonic bath.

7. Magnetic stirrer/hot plate.

8. Gas chromatograph with 3% OV-17 column and flame ionization detector. Operating conditions are typically:

Nitrogen carrier gas	-	30 ml/min
Hydrogen	-	30 ml/min
Air	-	300 ml/min
Column temperature	-	180°C

REAGENTS

1. Standard sugar samples: D(-) Fructose
   D(+) Glucose anhydrous
   Sucrose
   D(+) Lactose monohydrate
   Maltose monohydrate

2. Internal standard: meso-inositol.

3. Oximation reagent: dissolve 1.25g of Hydroxyammonium chloride in pyridine and make up to 50 ml.

4. Clearing agents: Carrez I (potassium ferrocyanide 10.6%)
   Carrez II (zinc acetate 21.6%)

5. Silylating agent: bis(trimethylsilyl)-trifluoroacetamide (BSTFA)

6. Propan-2-ol

PROCEDURE

Accurately weigh about 1 g of sample and 0.3 g of internal standard into a 100 ml beaker. Add 30 ml of hot distilled water and leave for 15 minutes in a hot water bath at 80°C to dissolve. Add 0.5 ml Carrez I and 0.5 ml Carrez II. Filter into a 100 ml volumetric flask, rinse and make up to volume with distilled water. Prepare a solution containing each of the reference sugars and internal standard (0.5 g of each sugar is a convenient amount), following the same procedure.

Pipette 0.5 ml of the solution into a 3 ml Reactivial containing a magnet. Evaporate on a magnetic stirrer/hot plate set on very low heat under a gentle stream of nitrogen. When nearly dry, add 0.5 ml propan-2-ol and continue evaporation. Place the vials in a vacuum oven at 70°C for 1.5 hours. Cool and add 0.5 ml of oximation reagent. Stopper tightly and place in an ultrasonic bath for two minutes to loosen magnet from the sides of the vial. Heat for 30 minutes at 80°C in an oven (time and temperature must be precisely controlled). Cool at room temperature. Add 1 ml BSTFA, stopper tightly and replace in the oven for a further 30 minutes. Cool at room temperature.

Inject 1 µl of the solution into a gas chromatograph, using the above operating conditions. Calculate the sugars by comparison of sample and standard chromatograms.

CALCULATION

If time and temperature of derivatisation are carefully controlled, the factors can be calculated once from a series of standard solutions.

Example: Calculation of the Sucrose factor (Ksu)

$$\text{Ksu} = \frac{\text{weight of Sucrose}}{\text{weight of m-inositol}} \times \frac{\text{Peak area m-inositol}}{\text{Peak area Sucrose}}$$

$$\text{Weight of Sucrose (mg)} = \text{Ksu} \times \text{mg of m-inositol added} \times \frac{\text{Peak area Sucrose}}{\text{Peak area m-inositol}}$$

$$\% \text{ Sucrose} = \frac{\text{wt of Sucrose (mg)} \times 100}{\text{wt of sample (mg)}}$$

INVERT SUGARS WITH ADDED SUCROSE
(Lane and Eynon Method)

PRINCIPLE

The sample solution is mixed with a known volume of Fehling's solution and water. After bringing to a boil, methylene blue indicator is added and the solution is titrated with a standard invert sugar solution. The final volume is kept constant. The sample invert sugar is calculated by difference. The titration is conducted in a boiling solution in order to eliminate air which would change the end-point. The experimental conditions must be strictly followed to gain reproducible results. As the final volume is kept constant by the addition of a predetermined amount of water, the titration always corresponds to the same amount of invert sugar and allows the use of a single formula instead of tables. If sucrose is present, the result must be multiplied by a sucrose correction factor.

APPARATUS

1. Heat-resistant glass flat-bottomed flasks of 300 to 400 ml capacity.

2. Burette, 50 ml, graduated in 0.1 ml for the sugar solution. The burette should have a pinch-cock instead of a glass tap and a bent outlet tube in order to keep the graduated section of the burette out of the steam while additions are made to the boiling mixture.

3. Pipettes, 10, 15, 20, 25 and 50 ml, class A.

REAGENTS

1. Fehling's solution (Soxhlet's modification): This solution does not keep indefinitely and the reagents are therefore dissolved in two separate solutions, A and B, which are mixed together immediately before use. This mixing is done by adding a volume of solution A to an exactly equal volume of solution B. It is essential that the mixing be carried out in this order, otherwise the precipitate of cupric hydroxide initially formed may not redissolve completely.

Solution A: cupric sulphate pentahydrate (69.28 g) is dissolved and diluted to 1000 ml in distilled water.

Solution B: sodium potassium tartrate tetrahydrate (346 g) and sodium hydroxide (100 g) are dissolved in distilled water and diluted to 1000 ml.

2. Invert sugar stock solution - 1 g invert sugar / 100 ml prepared as follows:

Dissolve 23.750 g pure sucrose in about 120 ml distilled water, add 9 ml concentrated hydrochloric acid and allow to stand at room temperature for 8 days. Make up the solution to 250 ml and check for completion of hydrolysis by a saccharimeter reading (-11.80 ± 0.055 at 20°C). Dilute 200 ml of the 10 percent solution of invert sugar thus obtained and add, with swirling, sufficient N sodium hydroxide (about 71.5 ml) so that the solution, if diluted to 2000 ml, would have an acidity of about 0.001 N with respect to hydrochloric acid. After this addition, add a solution of 4 g benzoic acid in warm water, cool the whole and make up to 2000 ml to give a 1 % solution of invert sugar in a 0.2 % solution of benzoic acid. This stable stock solution should be diluted immediately before use.

3. Standard invert sugar solution, 0.25 g/100 ml: Pipette 25 ml of the stock solution to a 100 ml volumetric flask and dilute to mark with water.

4. Methylene blue indicator, 1 g/100 ml: dissolve 1 g pure methylene blue and dilute to 100 ml using distilled water, and then filter.

PROCEDURE

The Fehlings solution must be standardized as follows: Add 15 ml water and 39 ml of the standard invert sugar solution to 20 ml of Fehlings. Titrate as below for sample, using the standard invert sugar solution. The total volume of the standard invert sugar solution required should be 40 ml (39 ml + titration volume). If necessary, adjust Fehlings and retitrate.

The concentration of the sample solution should be 250 - 400 mg invert sugar/100 ml.

A preliminary test should be carried out to ascertain the volume of water to be added to the 20 ml of Fehling's solution in order to obtain a final total volume of 75 ml when the end-point of the titration is reached. Using the sample titration procedure the following mixture is titrated: 20 ml of Fehling's solution, 25 ml of the test solution, and 15 ml of distilled water. The last two additions correspond to the 40 ml of dilute invert sugar solution.

If the reddish colour of the boiling solution persists after the addition of the methylene blue indicator, this indicates that the test solution is too concentrated; the test solution must then be discarded and a less concentrated solution used.

If more than 50 ml of test solution, added to 20 ml of the Fehling's solution, are required to attain the reddish colour, this indicates that a more concentrated solution should be used.

The volume of water to be added is calculated as 75 ml (total) - 20 ml (Fehling's solution) - volume (ml) of test solution = volume (ml) of water to be added.

The sample titration is conducted as follows:

Fehling's solution (20 ml) is pipetted into a glass flask; the volume of distilled water indicated by the preliminary test is then added. The burette is rinsed and filled with the test solution. The whole volume of the sample solution required in the preliminary test (less 1 ml) is run into the flask. A few fragments of pumice are added and the contents of the flask are well mixed by gentle swirling. The flask is placed on a wire gauze over a bunsen flame and heated to boiling.

The liquid is kept boiling moderately fast for precisely 2 minutes and then 3 or 4 drops of methylene blue indicator are added directly into the boiling mixture. The mixture should assume a distinctly blue colour. The titration is to be completed in 1 minute by the further addition of small increments, initially of 0.2 ml, then of 0.1 ml and finally of single drops until the end-point is reached. It is indicated by the disappearance of the blue colour of the indicator and the appearance of the reddish colour due to the precipitated cuprous oxide.

Note that the titration should be completed in 3 minutes from the commencement of boiling. The heating device used for boiling the reaction mixture during the titration is of prime importance when accurate results are to be guaranteed. During the whole time, the flask should remain on the wire gauze and boil at a moderate rate. The continuous emission of steam from the neck prevents atmospheric oxidation of the Fehling's solution or of the indicator. During additions of sugar solution to the boiling liquid, the main burette tube must be kept out of the steam while the jet is brought over the mouth of the flask.

CALCULATION

In the absence of sucrose:

If C is the concentration (g/100 ml) of the product (for example, molasses) in the test solution and V is the volume (ml) of test solution used in the titration, then:

$$\text{invert sugar, g/100 g product} = \frac{1000}{CV}$$

In the presence of sucrose:

If f is the correction factor deduced from the following table based on the amount of sucrose present, the invert sugar content is given by the formula:

$$\text{invert sugar, g/100 g product} = f \times \frac{1000}{CV}$$

For amounts of sucrose intermediate between two consecutive figures in this table, the correction factor is obtained by interpolation.

Sucrose Correction Factor Table

Sucrose in boiling mixture (g)	Correction factor (f)
0.5	0.985
1.0	0.972
1.5	0.964
2.0	0.956
2.5	0.949
3.0	0.942
3.5	0.936
4.0	0.930
4.5	0.924
5.0	0.918
5.5	0.913
6.0	0.908
6.5	0.903
7.0	0.898
7.5	0.895
8.0	0.891
8.5	0.887
9.0	0.883
9.5	0.880
10.0	0.876

INTERPRETATION

This method can be used for the determination of invert sugar, glucose, fructose, maltose and lactose and of invert sugar in the presence of sucrose. The range of sugar concentrations to which this method can be directly applied varies from 0.1 to 0.8 g/100 ml for glucose, fructose and invert sugar. For the determination of invert sugar in the presence of sucrose, this method is especially suitable for those products which have a relatively high reducing sugars content. This is due to the high value of the sucrose correction.

In any event, this method should never be used for the determination of the reducing sugars content of beet molasses because of the high colour of this product in relation to its low reducing sugars content.

Some non-sugars present in molasses influence the results. However, the removal of non-sugars by clarification and subsequent de-leading leads to irregular results and this procedure is not recommended.

Also, calcium present in molasses forms a complex with glucose and fructose, resulting in slower reaction rates and apparently low invert sugar results. the removal of calcium is therefore achieved by addition to the untreated molasses of a potassium oxalate solution followed by filtration. 1 ml or 2 to 4 ml of a potassium oxalate solution (5 g/100 ml) is added to 1 g of cane molasses. This procedure may be replaced by adding a complexing agent such as EDTA, which forms a stronger complex with calcium than do the hexoses, thus avoiding the filtration. An effective decrease in colour and an improved end-point are obtained. 4 ml of an EDTA solution (4 g/100 ml) per g of cane molasses is recommended.

REFERENCES

ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 14, Rec. 2, 189.

ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 14, Appendix 4, 180.

Emmerich, A., ICUMSA, Report of the Proceedings 16th Session, 1974, Subj. 14, Table 21, 182.

ICUMSA, Report of the Proceedings 10th Session, 1949, Subj. 4, 9.

Eynon, L. and Lane, J.H., 1949. International Sugar Journal, 51, 169.

3.3 HONEY

COMPOSITION

Honey is the sweet substance produced by honey bees from the nectar of blossoms or from secretions of or on living parts of plants, which they transform and combine with specific substances, and store in honey combs.

Honey consists essentially of different sugars, predominantly glucose and fructose. Besides glucose and fructose, honey contains protein, amino acids, enzymes, organic acids, mineral substances, pollen and other substances, and may include sucrose, maltose, melezitose and other oligo-saccharides (including dextrins) as well as traces of fungi, algae, yeasts and other solid particles resulting from the process of obtaining honey. The colour of honey varies from nearly colourless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallized. The flavour and aroma vary, but usually derive from the plant origin.

There are various subsidiary definitions of honey as follows:

Blossom or nectar honey is the honey which comes mainly from nectaries of flowers. **Honeydew honey** is the honey which comes mainly from secretions of or on living parts of plants. Its colour varies from very light brown or greenish to almost black. **Comb honey** is honey stored by bees in the cells of freshly built broodless combs and sold in sealed whole combs or sections of such combs. **Extracted honey** is honey obtained by centrifuging decapped broodless combs. **Pressed honey** is honey obtained by pressing broodless combs with or without the application of moderate heat.

The average composition of 490 samples of honey, and range values were (11):

	Average	Standard Deviation	Range
Moisture - - - - - - - - - percent	17.2	1.46	13.4-22.9
Fructose - - - - - - - - - do	38.19	2.07	27.25-44.26
Glucose - - - - - - - - - do	31.28	3.03	22.03-40.75
Sucrose - - - - - - - - - do	1.31	0.95	.25-7.57
Maltose - - - - - - - - - do	7.31	2.09	2.74-15.98
Higher Sugars - - - - - - do	1.50	1.03	.13-8.49
Undetermined - - - - - - do	3.1	1.97	0-13.2
pH - - - - - - - - - - - - - -	3.91	- - -	3.42-6.10
Free Acid - - - - - - - - meq/kg	22.03	8.22	6.75-47.19
Lactone - - - - - - - - - do	7.11	3.52	0-18.76
Total Acid - - - - - - - - do	29.12	10.33	8.68-59.49
Lactone/Free Acid - - - - - - -	0.335	0.135	0-.950
Ash - - - - - - - - - - percent	0.169	0.15	.020-1.028
Nitrogen - - - - - - - - - do	0.041	0.026	0-.138
Diastase Value - - - - - - - -	20.8	9.76	2.1-61.2

Honey must have no objectionable taint absorbed during processing or storage, it must not be fermenting or effervescing and must not have been heated enough to have seriously affected the enzymes present. The acidity must not have been changed artificially. No additives or additions should be permitted.

Honey should be heated as little as possible during processing. If the diastase activity is reduced and the content of 5-hydroxmethylfurfural (HMF) increased beyond the given limits it is considered that over-heating has taken place or the sample is adulterated. The use of the diastase activity to assess the degree of heating is discussed by Shirotori et al (12). Fini and Sabatini (13) found that Italian unprocessed honeys contained an average of 13 mg/kg of HMF (400 samples), while processed commercial samples averaged 59 mg/kg and 27% were above the Codex regional standard limit. Simonyan (14) examined 20

Russian honeys and found the HMF less than 10 mg/kg. High levels of HMF were originally taken to indicate adulteration with invert sugar (Fiehe's test) but honey from tropical areas may naturally contain over 40 mg/kg. Dalzell and Singers (15) report that some genuine New Zealand honeydew honeys did not comply with the Codex European regional standard. If it is desired to confirm that a honey is adulterated rather than that it merely has a high HMF level, a more extensive analysis is necessary. The effect on honey of heating and storage is discussed by Bergel and Stuwe (16), Hase et al (17) and Borukh and Panchenko (18).

Details of the composition of honeys from Australia are given by Chandler et al (19) and from Sicily by Fini and Sabatini (20). Minieri and Chiaramello (21) have published a review on honey which includes details of composition. Some examples of adulteration are reported by Katsuta and Nishikawa (22). The carbohydrate composition of honey is reviewed by Siddiqui (23) and that of Russian honey is described by Gensitskii (24).

ROUTINE ANALYSIS

Honey can be adulterated with other nutritive sweeteners. White (25) has suggested that adulterants are: conventional (acid or enzyme converted) corn syrup (CCS), high fructose corn syrup (HFCS) and invert syrups from cane (CIS) or beet (BIS). He recommends that tests should be carried out in the order listed below, using only as many as required to attain the objective. The limited availability of equipment for the carbon-13 isotope test reduces the practical value of this very useful indicator of adulteration.

Carbon-13 isotope test (26)

Values of less negative than -21.5°/oo (4s) are conclusive for the presence of corn or cane syrups, unless the sample is of citrus origin which can be ascertained by flavor and aroma or methyl anthranilate content (27). For citrus, the corresponding 4s value is -20°/oo. If values are between these limits and -23.4°/oo, the TLC method is required to confirm corn syrups.

Thin-layer chromatography (TLC) test (28)

A positive result shows about 5-7% HFCS or about 1-2% CCS. This test may be less sensitive to second or third generation HFCS.

Hydroxymethylfurfural (HMF) test (29)

Using the bisulfite method, then 20 mg/100g or more indicates probable adulteration with BIS or CIS. (Confirm by carbohydrate analysis). If 10-20 mg/100g then product may be heat or storage abused honey, or it could be adulterated. (Confirm with carbohydrate analysis).

Carbohydrate distribution analysis

The proportions of total monosaccharides, disaccharides and higher sugars are characteristic of honey and provide confirmation of the presence of most adulterants. At least one value outside the limits of 60-79% monosaccharides, 4-12% disaccharides and above 3.8% for higher sugars, is indicative of adulteration. Two or more values outside are conclusive.

Test for glucose, fructose and sucrose

Use HPLC or analyze the fractions from the distribution analysis, using glucose oxidase for glucose, fructose by difference, and sucrose by invertase hydrolysis. If glucose exceeds 40%, sample is not genuine honey; if 38-40%, pollen of cotton, blue curls, or manzanita must be present if a genuine honey; if absent, it is probably adulterated. Pure honey contains over 31% fructose, less than 38% glucose and less than 8% sucrose (unless freshly extracted citrus honey). Ratio of fructose to glucose is 1.00 or more.

Nitrogenous constituents analysis

All pure honey contains at least 15 mg proline per 100 g and at least 65 mg true protein per 100 g (determined after dialysis). Because average values are much higher, this is useful only to confirm gross adulteration.

Presence of honeydew

Honeydew may occur naturally in honey and is not an adulterant. It originates from extra-floral secretions on plants, gathered and stored by honeybees. It may contribute a molasses-like flavour and appear to respond positively to the TLC test. It may be differentiated as follows:

Determine constant direct polarization value. Values more positive than -2°S (not specific rotation) may indicate either honeydew or the presence of corn syrup or sucrose. Honeydew will contain over 5% melezitose, less than 8% sucrose, and may not conform to the normal distribution of sugars.

The methods given for the analysis of sugars in honey are adequate for routine purposes. Takahashi and Tokumura (30) describe the use of the Fehling-Lehmann-Schoorl and Furukolmen methods in conjunction with simultaneous equations to calculate the proportions of glucose, fructose, sucrose and maltose. Bose, Singh and Mukherjee (31) use an alkaline hypoiodite titration for estimation of glucose and total reducing sugars.

The use of GLC for the analysis of sugars in honey is described by Battaglini and Bosi (32) and Hadorn, Zurcher and Stracte (33). The sugars found in honey are discussed in a review by Doner (34).

The chemical analysis of honey has been reviewed by Iwaida (35) in relation to Japanese standards for the product and by Hase et al (17).

There are very occasionally reports of toxic effects due to the ingestion of honey and the cause is usually traced to toxic plants from which the bees have fed. Clinch and Turner (36) report the presence of a toxin, tutin, in some New Zealand honeys. The presence of grayanotoxins in some Canadian honey is reported by Scott, Coldwell and Wilberg (37) and an outbreak in Russia is described by Medveditskova (38).

The geographical origin of honey can be difficult to determine. Gilbert et al (39) has carried out statistical analysis on amino-acid values with some success. If bees have collected pollen from species of plants characteristic of a region, microscopical examination of the centrifuged sediment from the honey and identification of the characteristic pollen present permits the geographical origin of the honey to be determined. It also enables the microscopist to substantiate claims about the botanical origin - that it is heather honey, orange blossom honey and so on. In practice results have to be interpreted with extreme care. The proportion of pollen grains in the sample from different species of plant is in no way related to the extent to which the bees have fed from those plants. For example, citrus blossom sheds little pollen and although the bees may have derived the major part of the honey from citrus blossom the proportion of citrus pollen present may be extremely low. The method is still useful as there should be some citrus pollen present in honey from that source. The pollen of Eucalyptus spp has a characteristic morphology, but the trees are grown in many parts of the world and the presence of pollen is no guarantee that the honey originated from Australia, the only area in which Eucalyptus spp are indigenous. The vegetation from a particular area or region being characteristic, the pollen analysis in the honey from that region is also characteristic, even though the proportion of pollen grains of different types present is no reflection of the ecological pattern of the vegetation.

A great deal of useful information about the floral, geographical and topological sources of honey can be obtained from examination of the pollen. This requires considerable experience gained from examining many samples of known source. The origin of some honeys can be determined very quickly by an experienced microscopist, while other samples require very detailed work. A formal examination or an examination of a sample of disputed origin, should always be done according to the International Method (40). The literature on pollen analysis is very extensive. Some references are included at the end of this chapter.

SAMPLE PREPARATION - HONEY
(Clarification)

PRINCIPLE

A diluted sample of honey is clarified by treatment with alumina cream and filtering. The clarified sample can then be used for sugars analysis.

REAGENTS

Alumina cream - prepare a cold saturated solution of alum ($K_2SO_4Al_2(SO_4)_3.24H_2O$) in water. Add ammonium hydroxide with constant stirring until the solution is alkaline to litmus, let precipitate settle and wash by decantation with water until wash-water gives only slight test for sulphate with barium chloride solution. Pour off excess water and store residual cream in stoppered bottle.

SAMPLING

If the sample is liquid or strained honey and is free from granulation, mix thoroughly by stirring or shaking. If granulated, place the closed container in water-bath without submerging and heat 30 minutes at 60°C; then if necessary heat at 65°C until liquefied. Occasional shaking is essential. Mix thoroughly and cool rapidly as soon as sample liquefies. Do not heat honey intended for hydroxymethylfurfural or diastase determination. If foreign matter, such as wax, sticks, bees, particles of comb, etc. is present, heat sample to 40°C in water-bath and strain through cheese-cloth in hot-water funnel before sampling.

If it is comb honey, cut across top of comb (if sealed) and separate the honey completely from the comb by straining through a sieve with square openings of 0.5 mm by 0.5 mm. When portions of comb or wax pass through the sieve, heat sample as above and strain through cheesecloth. If honey is granulated in comb, heat until wax is liquefied; stir, cool and remove wax.

PROCEDURE

Transfer an accurately weighed sample of approximately 25 g from the well mixed honey to 100 ml volumetric flask, add 5 ml alumina cream, dilute to volume with water at 20°C and filter.

REFERENCE

Codex Alimentarius Commission Regional Standard for Honey, CAC/RS 12-1969.

MOISTURE IN HONEY

PRINCIPLE

The method based on refractometric examination of honey.

APPARATUS

1. Refractometer.

PROCEDURE

Determine the refractive index of the sample using a refractometer at a constant temperature near 20°C. Convert the reading to moisture content (percent m/m) using the table given below. If the determination is made at a temperature other than 20°C, convert the reading to standard temperature of 20°C, according to the temperature corrections quoted. The method used should be noted in the test report.

Table for the Estimation of Moisture Content

Refractive Index (20°C)	Moisture Content (percent)	Refractive Index (20°C)	Moisture Content (percent)	Refractive Index (20°C)	Moisture Content (percent)
1.5044	13.0	1.4935	17.2	1.4830	21.4
1.5038	13.2	1.4930	17.4	1.4825	21.6
1.5033	13.4	1.4925	17.6	1.4820	21.8
1.5028	13.6	1.4920	17.8	1.4815	22.0
1.5023	13.8	1.4915	18.0	1.4810	22.2
1.5018	14.0	1.4910	18.2	1.4805	22.4
1.5012	14.2	1.4905	18.4	1.4800	22.6
1.5007	14.4	1.4900	18.6	1.4795	22.8
1.5002	14.6	1.4895	18.8	1.4790	23.0
1.4997	14.8	1.4890	19.0	1.4785	23.2
1.4992	15.0	1.4885	19.2	1.4780	23.4
1.4987	15.2	1.4880	19.4	1.4775	23.6
1.4982	15.4	1.4875	19.6	1.4770	23.8
1.4976	15.6	1.4870	19.8	1.4765	24.0
1.4971	15.8	1.4865	20.0	1.4760	24.2
1.4966	16.0	1.4860	20.2	1.4755	24.4
1.4961	16.2	1.4855	20.4	1.4750	24.6
1.4956	16.4	1.4850	20.6	1.4745	24.8
1.4951	16.6	1.4845	20.8	1.4740	25.0
1.4946	16.8	1.4840	21.0		
1.4940	17.0	1.4835	21.2		

Temperature corrections to change Refractive Index:

Temperatures above 20°C: add 0.00023 per °C

Temperatures below 20°C: subtract 0.00023 per °C.

REFERENCES

Chataway, H.D., 1932. Canadian Journal of Research 6, 540 et seq.

Chataway, H.D., 1933. Canadian Journal of Research 9, 435 et seq.

Chataway, H,D., 1935. Canadian Bee Journal 43 (8), 215.

Wedmore, E.B., 1955. Bee World 36, 197 et seq.

(Note: The above method is the modification of Chataway's method by Wedmore. Also see White, J.W., 1969, Journal of the Association of Official Analytical Chemists 52 (4), 729-37 for a review of physical and chemical methods to determine moisture in honey. Other methods include drying honey on sand under vacuum (< 50 mm Hg) at 60°C.

DIASTASE ACTIVITY OF HONEY

PRINCIPLE

The rate of starch destruction is monitored by the intensity of the iodine blue color. Note - do not heat honey to be used for diastase activity.

APPARATUS

1. Water-bath at 40 ± 0.2°C.

2. Spectrophotometer to read at 660 nm.

REAGENTS

1. Iodine stock solution: dissolve 8.88 g of iodine analytical grade, in 30-40 ml water containing 22 g potassium iodide, analytical grade, and dilute to 1 litre with water.

2. Iodine solution about 0.0007 N: dissolve 20 g potassium iodine, analytical grade, in 30-40 ml water in a 500 ml volumetric flask. Add 5.0 ml iodine stock solution and make up to volume. Make up a fresh solution every second day.

3. Acetate buffer - pH 5.3 (1.59 N): dissolve 87 g sodium acetate trihydrate in 400 ml water, add about 10.5 ml glacial acetic acid in a little water and make up to 500 ml. Adjust the pH to 5.3 with sodium acetate or acetic acid as necessary, using a pH meter.

4. Sodium chloride solution 0.5 N: dissolve 14.6 g sodium chloride, analytical grade, in boiled-out distilled water and make to 500 ml. (The keeping time is limited by mould growth.)

5. Starch solution: weigh out that amount of starch which is equivalent to 2.0 g anhydrous starch. Mix with 90 ml of water in a 250 ml conical flask. Bring rapidly to the boil, swirling the solution as much as possible, heating over a thick wire gauze preferably with an insulated centre. Boil gently for 3 minutes, cover and allow to cool spontaneously to room temperature. Trasfer to a 100 ml volumetric flask, place in a water bath at 40°C to attain this temperature and make up to volume at 40°C. This is the starch solution to be used in the analysis. The starch used should have a "blue value" of between 0.5 - 0.55 using a 1 cm cell. If outside this range, adjust the weight of the starch taken.

The method of determining this is as follows: the amount of starch equivalent to 1 g anhydrous starch as prepared by the sample method is cooled and 2.5 ml acetate buffer added before making up to 100 ml in a volumetric flask. To a 100 ml volumetric flask add 75 ml water, 1 ml N hydrochloric acid and 1.5 ml of 0.02 N iodine solution. Then add 0.5 ml of the starch solution and make up to volume with water. Allow to stand for one hour in the dark and read in 1 cm cell using a spectrophotometer at 660 nm against a blank containing all the ingredients except the starch solution. The reading on the absorbance scale = "blue value".

PROCEDURE

Weigh 10.0 g honey into a 50 ml beaker and add 5.0 ml acetate buffer solution together with 20 ml water to dissolve the sample. Stir until dissolved. Do not warm. Add 3.0 ml sodium chloride solution to a 50 ml volumetric flask and transfer the dissolved honey sample

to this and adjust the volume to 50 ml. (Note: it is essential that the honey should be buffered before coming into contact with sodium chloride.)

Warm the starch solution to 40°C and pipette 5 ml into 10 ml of water at 40°C and mix well. Pipette 1 ml of this solution into 10 ml 0.0007 N iodine solution, diluted with 35 ml of water and mix well. Read the colour at 660 nm against a water blank using a 1 cm cell. The absorbance should be 0.760 ± 0.020. If necessary the volume of added water is adjusted to obtain the correct absorbance.

Pipette 10 ml honey solution into 50 ml graduated cylinder and place in 40°C ± 0.2°C water bath with flask containing starch solution. After 15 minutes, pipette 5 ml starch solution into the honey solution, mix, and start stop-watch. At 5 minute intervals remove 1 ml aliquots and add to 10.00 ml 0.0007 N iodine solution. Mix and dilute to standard volume (50 ml). Determine absorbance at 660 nm in spectrophotometer immediately using 1-cm cell. Continue taking 1 ml aliquots at intervals until absorbance of less than 0.235 is reached.

CALCULATION

The absorbance is plotted against time (minutes) on rectilinear paper. A straight line is drawn through at least three points on the graph to determine the time when the reaction mixture reaches an absorbance of 0.235. Divide 300 by the time in minutes to obtain the diastase number (DN). This number expresses the diastase activity as ml of 1% starch solution hydrolysed by the enzyme in 1 g of honey in 1 h at 40°C. This diastase number corresponds with the Gothe scale number.

Diastase activity = DN = ml starch solution (1 percent)/g honey/h at 40°C.

REFERENCES

Schade, J.E., Marsh, G.L. and Eckert, J.E., 1958. Food Research 23, 446.

White, J.W. and Pairent, F.W., 1959. Journal of the Association of Official Agricultural Chemists 42, 344.

Hadorn, H., 1961. Milleilungen aus dem Gebeite der Lebensmitteluntersuchung und Hygiene 52, 67.

ACIDITY AND LACTONE IN HONEY

PRINCIPLE

The sample is titrated with sodium hydroxide to obtain the free acidity. Excess sodium hydroxide is added to hydrolyse any lactose present and immediately back-titrated with hydrochloric acid. The total acidity is calculated as free acidity plus lactone in milliequivalents per kilogram.

APPARATUS

1. pH meter.

REAGENTS

1. 0.05 N NaOH

2. 0.05 N HCl

PROCEDURE

Dissolve 10 g of sample in 75 ml of carbon dioxide-free water in a 250 ml beaker. Stir with a magnetic stirrer, immerse the electrodes of the pH meter in the solution and record the pH. Titrate with 0.05 N NaOH at a rate of 5 ml/minute, until the pH reaches 8.5. Record the burette reading. Immediately add by pipette 10 ml of 0.05 N NaOH and immediately back-titrate with 0.05 N HCl from a 10 ml burette until the pH reaches 8.3. Also do a reagent blank.

CALCULATION

Free acidity = (ml of 0.05 N NaOH to bring solution to pH 8.5-blank) x 0.05 x 1000/10

Lactone = (10.00 - titre of 0.05 N HCl in ml) x 0.05 x 1000/10

Total acidity = free acidity + lactone (all results being expressed as milli-equivalents of acid per kilogram of honey).

REFERENCES

Codex Alimentarius Commission Regional Standard for Honey, CAC/RS12-1969.

Official Methods of Analysis of the AOAC, 1984, 31.168.

PROLINE IN HONEY

PRINCIPLE

Proline is determined spectrophotometrically after reaction with ninhydrin to form a coloured compound.

APPARATUS

1. Spectrophotometer.

REAGENTS

1. Formic acid

2. Ninhydrin - 3% soln (w/v) in methyl cellosolve

3. Proline Std. - in water (deionized) 40 mg to 25.0 ml; then 1 ml to 25.0 ml. (use 0.5 ml in procedure)

PROCEDURE

Dissolve 5.0 g honey in 50 ml water, quantitatively transfer to 100 ml volumetric flask, make to mark, stopper, and mix well. Use 0.5 ml per determination. Dilute sample (0.5 ml prepared above) and 0.5 ml of standard are placed in separate 20 ml screw-capped vials and 0.25 ml of formic acid and 1.0 ml of 3% ninhydrin solution are then added to each vial. The vials are tightly capped and placed in a boiling water bath for 15 minutes. The vials are then cooled in a water bath at 70° for 5 to 10 minutes. While cooling, add 5.0 ml of 1:1 isopropanol-water solution; continue cooling and develop colour for at least 1/2 hour. Scan spectra from 600 to 450 nm and take absorbance readings at maximum ca 512 nm. (Readings should be taken before an hour has elapsed). A blank using 0.5 ml water is also carried through the procedure.

CALCULATION

$$\text{mg Proline/100 g honey} = \frac{Au}{As} \times S \times \frac{16}{W}$$

Where: Au = absorbance of sample aliquot

As = absorbance of standard aliquot

S = mg std weighed = ca 40 mg (stock soln)

16 = product of dilution factors

W = g honey weighed = ca 5.0 g

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 31.124-.126.

DEXTROSE IN HONEY

PRINCIPLE

Dextrose is estimated iodometrically, total reducing sugars by a copper reduction method and the fructose obtained by difference.

APPARATUS

1. Burette.

REAGENTS

1. 0.1 N iodine

2. 0.2 N sodium bicarbonate/carbonate solution. Dissolve 16.8g sodium bicarbonate and 21.2 g sodium carbonate in water and dilute to 1 litre.

3. Sulphuric acid 25%, v/v.

4. Sodium thiosulphate 0.1 N.

PROCEDURE

Dilute 2 g of honey to 250 ml and estimate total reducing sugars by one of the standard procedures.

To determine dextrose, to 25 ml of the honey solution add by pipette a volume of 0.1 N iodine at least twice that required for the reaction followed by 100 ml of sodium bicarbonate/carbonate solution. Leave in the dark for 2 hours, acidify with 12 ml of 25% sulphuric acid and titrate with 0.1 N thiosulphate solution. Carry out a blank at the same time. The difference between the two titrations represent the dextrose.

CALCULATION

1 ml 0.1 N iodine = 0.009005 g dextrose

Fructose = total reducing sugars - dextrose.

REFERENCE

Pearson's Chemical Analysis of Foods, 1976, 7th Edition, 141.

APPARENT SUCROSE IN HONEY

PRINCIPLE

Sugars are determined by Fehling's titration before and after inversion. The sucrose is calculated by difference.

REAGENTS

1. Soxhlet modification of Fehling's solution. } See method for Invert Sugars with Added Sucrose
2. Standard invert sugar solution. } See method for Invert Sugars with Added Sucrose
3. Hydrochloric acid, 6.34 N.
4. Sodium hydroxide solution (5 N aqueous).
5. Methylene blue solution 2 g/L.

PROCEDURE

Prepare the honey sample as in "Sample Preparation-Honey". Dilute 10 ml of this solution to 250 ml with distilled water. Pipette 50 ml into a 100 ml graduated flask, together with 25 ml distilled water and heat to 65°C over a boiling water-bath. Cool the solution naturally for 15 minutes and then cool to 20°C, neutralize with 5 N sodium hydroxide, using litmus paper as indicator, cool again and adjust the volume to 100 ml (diluted honey solution). Continue as in the method for "Invert Sugars with Added Sucrose".

CALCULATION

Calculate percent invert sugar (g invert sugar per 100 g honey) before and after inversion using the same formula as for "Invert Sugars with Added Sucrose." Apparent sucrose content = (Invert sugar content after inversion minus invert sugar content before inversion) x 0.95. The result is expressed as g apparent sucrose/100 g honey.

REFERENCE

Walker, H.S., 1917. Journal of Industrial and Engineering Chemistry 2, 490.

HYDROXYMETHYLFURFURAL IN HONEY

PRINCIPLE

A colour is developed reacting HMF with p-toluidine and barbituric acid. HMF can be formed if honey is heated, so the sample must not be subjected to heat.

APPARATUS

1. Spectrophotometer to read at 550 nm.

2. Water bath.

REAGENTS

1. Barbituric acid solution: weigh out 500 mg barbituric acid and transfer to a 100 ml graduated flask using 70 ml water. Place in hot water bath until dissolved, cool and make up to volume.

2. p-Toluidine solution: weigh out 10.0 g p-toluidine, analytical grade, and dissolve in about 50 ml isopropanol by gentle warming on a water bath. Transfer to a 100 ml graduated flask with isopropanol and add to 10 ml glacial acetic acid. Cool and make up to volume with isopropanol. Keep the solution in the dark. Do not use for at least 24 hours.

3. Distilled water (oxygen free): Nitrogen gas is passed through boiling distilled water. The water is then cooled.

4. Hydroxymethylfurfural (HMF) standard solutions: dissolve 10 mg HMF in 1 litre water (stock solution). Pipette 10 ml, 20 ml, 30 ml and 40 ml into four 50 ml volumetric flasks. Make to volume with water (dilute solutions). HMF is very hygroscopic, so check the standard concentration by measuring the absorbance at 282.5 nm, assuming E = 21,215 (Turner, 1954).

PROCEDURE

Weigh 10 g of the honey sample and dissolve without heating in 20 ml oxygen-free distilled water. Transfer to a 50 ml graduated flask and make up to volume (honey solution). The solution sample should be tested after preparation without delay.

Pipette 2.0 ml of honey solution into each of two 25 mm diameter test tubes and add 5.0 ml p-toluidine solution to each. Pipette into one test tube 1 ml water and into the other 1 ml barbituric acid solution and shake both tubes. The one with added water serves as the water bank. The addition of the reagents should be done without delay and should be finished in about 1-2 minutes.

Read the absorbance of the sample against the blank at 550 nm using a 1 cm cell immediately the maximum value is reached.

Prepare a standard curve by pipetting 2 ml from each of the four dilute standard solutions into four test tubes. Add 5.0 ml p-toluidine to each and continue as with the sample.

CALCULATION

Find the amount of HMF (in micrograms) in the sample by reading from the standard curve. This figure divided by 10 equals ppm HMF in the sample.

INTERPRETATION

For cleanup, pass the dilute sample through a activated charcoal column.

HMF should not be present in genuine honey in large amount unless it has been abased by heating or has deteriorated due to improper or prolonged storage. The presence of HMF in significant amounts tends to indicate the presence of added invert sugars as an adulterant. 40 ppm is the legal limit in some countries.

REFERENCES

Winkler, O., 1955. Zeitschrift fur Lebensmitteluntersuchung und Forschung 102 (3), 166-7.

Turner, J.H., Rebers, P.A., Bamik, P.L. & Cotton, R.H. 1954. Analytical Chemistry 26, 898-901.

3.4 TEXT REFERENCES

1. SCHNEIDER, F. (Ed). 1979. ICUMSA Methods of Sugar Analysis.

2. PEARSON, D. 1981. The Chemical Analysis of Foods, 8th Ed. p145.

3. JACKSON, R.F. & GILLIS, C.L. 1920. The Double Polarization Method for Estimation of Sucrose and the Evaluation of the Clerget Divisor 375 US Dept Comm. Bur. Std. Scie. 125-194.

4. JACKSON, R.F. & McDONALD, E.J. 1939. Journal of the Association of Official Agricultural Chemists 22 580-588.

5. NORRISH, R.S. 1967. Selected Tables of Physical Properties of Sugar Solutions, Scientific and Technical Surveys 51 British Food Manufacturing Industries Research Association, Leatherhead, England.

6. BATES, F., PHELPS, F.P. & SNYDER, C.F. 1927. International Critical Tables 2 334-355.

7. BROWN, C.A. & ZERBAN, F.W. 1941. Physical and Chemical Methods of Sugar Analysis.

8. SOCIETY OF PUBLIC ANALYSTS. 1930. Analyst 55 115.55.

9. SCHIWECK, H. & BÜSCHING, L. 1969. Zucker 22 377.22.

10. SCHIWECK, H. & BÜSCHING, L. 1975. Zucker 28 242.

11. Composition of American Honeys. 1962. USDA Technical Bulletin 126.

12. SHIROTORI, T., IWAIDA, M. & KAWASHIRO, I. 1968. Journal of Japanese Society of Food Nutrition 21 (4) 261-264.

13. FINI, M.A. & SABATINI, A.G. 1972. Scienza e Tecnologia degli Alimenti 2 (6) 375-379.

14. SIMONYAN, T.A. 1972. Khlebopetarnaya i Konditerskaya Promysh-lennost 16 (3) 17-18.

15. DALZELL, K.W. & SINGERS, W.A. 1975. New Zealand Journal of Science 18 (3) 329-332.

16. BERGEL, C. & STUWE, G.G. 1972. Susswaren 16 (3) 77-78.

17. HASE, S., ODATE, M. & KURAYABASHI, A. 1973. Journal of Food Science & Technology (Japan) 20 (6) 257-264.

18. BORUKH, I.F. & PANCHENKO, G.N. 1975. Tovarovedenir 8 30-33.

19. CHANDLER, B.V., FENWICK, D., ORLOVA, T. & REYNOLDS, T. 1974. Technical Paper Div. Food Research 38 CSIRO 39.

20. FINI, M.A. & SABITINI, A.G. 1974. Scienza e Tecnologia degli Alimenti 4 (6) 349-355.

21. MINIERI, L. & CHIARAMELLO, S. 1975. Acta Medica Veterinaria 20 (5/6) 359-396.

22. KATSUTA, T. & NISHIKAWA. 1971. Annual Report Tokyo Metropolitan Research Laboratory Public Health 23 81-86.

23. SIDDIQUI, I.R. 1970. Advances in Carbohydrate Chemistry and Biochemistry 25 285-309. Food Research Institute Dept of Agriculture, Ottowa, Canada.

24. GENSITSKII, I.P. 1974. Voprosy Pitaniya 5 57-60 (In Russian).

25. WHITE, J.W. 1985. Honeytech Inc., P.O. Box 1059, Navasota, Texas, USA 77868 (Private communication).

26. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1984. Official Methods of Analysis 31.158 - 31.161.

27. WHITE, J.W. & ROBINSON, F.A. 1983. Journal of the Association of Analytical Chemists 66 (1) 1-3.

28. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1984. Official Methods of Analysis 31.156 - 31.157.

29. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1984. Official Methods of Analysis 31.153 - 31.155.

30. TAKAHASHI, T. & TOKUMURA, H. 1968. Annual Reports of the Federal Research Institute Aichi. Prefecture 9 125-128.

31. BOSE, S., SINGH, H. & MUKHERJEE, S. 1975. Indian Sugar 24 (10) 861.

32. BATTAGLINI, M. & BOSI, C. 1973. Scienza e tecnologia degli Alimenti 3 (4) 217-221.

33. HADORN, H., ZURCHER, K. & STRACTE, C. 1974. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene 65 (2) 198-208.

34. DONER, L.W. 1977. Journal of the Science of Food & Agriculture 28 443-456.28.

35. IWAIDA, M. 1970. Journal of Food Hygienic Society of Japan 11 (6) 486-489.

36. CLINCH, P.G. & TURNER, J.C. 1975. New Zealand Journal of Science 18 (3) 325-326.

37. SCOTT, P.M., COLDWELL, B.B. & WIBERG, G.S. 1971. Food and Cosmetic Toxicology 9 (2) 179-184.

38. MEDVEDITSKOVA, N.D. 1969. Voprosy Pitaniya 28 (6) 76-78.

39. GILBERT, J., SHEPHERD, M.J., WALLWORK, M.A. & HARRIS, R.G. 1981. Journal of Agricultural Research 20 (2), 125-135.

40. LOUVEAUX, J., MAURIZIO, A. & VORWOHL, G. 1978. Methods of Melissopalynology published in Bee World 59 (4) 139-157 and available as a reprint from the International Bee Research Association, Gerrards Cross, SL9 ONR, U.K.

Further Reading

Sugar

"Analysen-Methoden der Internationalen Vereinigung der Zuckerwaren-Fabrikanten".

Association Internationale des Fabricants de Confiserie edited by Benecke Verlag D-2000 Hamburg 22.

"International List of Serials dealing with Sugar and related Subjects". Institut fur Zuckerindustrie edited by Buchdruckerei Gruss D-1000 Berlin.

ICUMSA, 15th Session Proceedings 1970.

ICUMSA, 16th Session Proceedings 1974.

JUNK, W.R. & PANCOAST, H.M. 1973. Handbook of Sugars, AVI.

PLEWS, R.W. 1970. Analytical Methods Used in Sugar Refining, Elsevier.

SOUTHGATE, D.A.T. 1976. Determination of Food Carbohydrates, Applied Science.

Standard Analytical Methods of the Member Companies of Corn Industries Research Foundation, Corn Refiners Association Inc. 1001 Connecticut Avenue, N.W., Washington DC 20036.

Critical Data Tables of the Corn Refiners Association, Inc., 1001 Connecticut Avenue, N.W., Washington DC 20036.

LEE, C.K. & KING, R.D. 1978. Developments in Food Analysis Techniques Volume 1, Applied Science.

BIRCH, G.G. & PARKER, K.J. (Eds) 1982. Nutritive Sweeteners, Applied Science.

Honey

CRANE, E. 1979. Honey: A Comprehensive Survey, Bee Research Association UK.

Pollen Analysis

BARTH, O.M. 1970. Anales Academias Brasil Ciencia, Microscopical Analysis of Honey Samples:

1. Predominant Pollen 42 (2) 351-366.
2. Secondary Pollen 42 (3) 571-590.
3. Minor Pollen 42 (4) 747-772.
4. Pollen Spectrum of Some Honey Samples of the State Rio de Janeiro 30 (4) 575-582 (1970).
5. Honeydew in Honey of Bees 30 (4) 601-608 (1970).
6. Pollen Spectrum of Some Honey Samples of the States Bahia and Ceara 31 (4) 431-434 (1971).

BARTH, O.M. 1971. Apidologie 2 (2) 157-167 Mikroskopische Bestandteile Brasilianischer Honigtauhonige.

BATTAGLINI, M. & RICCIARDELLI D'ALBORE, G. 1971. Annali della Facolta di Agaraia Universita di Perugia 26 277-303 Pollen Spectrum of Some Silician Honeys.

BATTAGLINI, M. & RICCIARDELLI D'ALBORE, G. 1972. Apicultura Moderna 62 (4) 64-82 1. Botanical Origin of the Honeys of the Provincia di Perugia.

BATTAGLINI, M. & RICCIARDELLI D'ALBORE, G. 1972. Annali della Facolta di Argaria Universita di Perugia 27 219-224 Preliminary Studies on Italian Unifloral Honeys.

BEUG, J.J. 1961. Leitfaden der Pollenbestimmung Gustav Fischer Verlag, Stuttgart.

COWAN, A.C. & MITCHELL, T.J. 1964. The Analyst 89 (1056) 222-226 An Examination of the Occurrence of Honeydew in Honey.

ERDTMAN, G. 1960. Svensk Botanisk Tidskrift 54 561-564 The Acetolysis Method: A Revised Description.

FERRAZZI, P. 1974. Apicultura Moderna 65 (1/2) 21-26 Dandelion Honeys from Piedmont and Lombardy, 1. Melissopalynological Analysis.

FOSSEL, A. 1968. Zeitschrift fur Bienenforschung 9 (6) 252-258 Der Wasserknoterich (Polygonum Amphibium L.) Eine Wenig Beachtete Tracht-pflanze.

FOSSEL, A. 1973. Apidologie 4 (1) 81-85 Verbreitung und Pollen-analytische Bedeutung der Silberwurz (Dryas Octopetala).

FOSSEL, A. 1974. Mitt. Naturwiss. Ver. Steiermark 104 87-118 Die Bienenweide der Ostalpen, Dargestellt am Beispiel des Steirischen.

FOSSEL, A. & RUTTNER, F. 1966. Zeitschrift fur Bienenforschung 8 168-177 Die Kleine Wachsblume, Cerinthe Minor L., Eine Charakteristische Trachtpflanze Pannonischer und Alpiner Trockengebiete.

FOSSEL, A. & VORWOHL, G. 1974. Phyton (Austria) 16 (1/4) 49-55 Honigbienen und Fieberklee (Menyanthes Trifoliata).

GENIER, G. 1966. Annales Abeille 9 (4) 271-321 Le Pollen des Ericaceae dans les Miels Francais.

GRIEBEL, C. 1930/1931. Zeitschrift fur Untersuchung der Lebensmittel 59 (1) 63-79; (2/3) 197-211; (5) 441-471. Zur Pollenanalyse des Honigs 61 256-305.

HODGES, D. 1974. The Pollen Loads of the Honey Bee Bee Research Association, London.

SAWYER, R.W. 1975. Journal of the Association of Public Analysts 13 (2) 64-72 Melissopalynology in the Determination of the Geographical and Floral Origin of Honey.

SAWYER, R.W. 1981. Pollen Identification for Beekeepers University College Cardiff Press, Wales.

HYDE, H.A. & ADAMS, K.F. 1958. An Atlas of Airborne Pollen Grains Macmillan.

DEANS, A.S.C. 1957. Survey of British Honey Sources Bee Research Association UK.

LOUVEAUX, J. 1970. Annexes Microphotographiques aux Methodes Officielles D'Analyse, Tome III, Atlas Photographique D'Analyse Pollinique Des Miels, Service de la Repression des Fraudes et Du Controle de la Qualite, Paris, France.

HAYES, G. 1925. Nectar Producing Plants and their Pollen The British Bee Journal.

KAPP, R.O. 1969. Pollen and Spores Wm. C. Brown Publishers.

ERDTMAN, G. 1969. Handbook of Palynology Hafner.

MOORE, P.D. & WEBB, J.A. 1978. An Illustrated Guide to Pollen Analysis Hodder & Stoughton.

WODEHOUSE, R.P. 1965. Pollen Grains Hafner.

PRYCE-JONES, J. 1942-3. Proceedings of the Linnaen Society 155 Sess. 129-174.

MORRIS, N. & ROGERS, P.E.W. 1976. British Bee Journal Oct 152-153.

ALLEN, Rev M. Yate. 1937-38. The Microscope Dec 127-128, Feb Vol II No. 1 1-5, Mar Vol II No. 2 29-32, April Part III 82-84, May Part IV 92-95, June Part V 134-137, July Part VI 161-164, Aug Part VII 183-186, Nov-Dec Part VIII 260-263, Part IX 48-50, Part X 99-102, Part XI 228-231.

DEODIKAR, G.B., SHENDE, S.G., THAKAR, C.V. & VINCHURKAR, K.R. 1965. Indian Bee Journal 27 1.

LOUVEAUX, J. & VERGERON, Ph. 1964. Annales de 1'abeille 329-347.

KUBISOVA, S. & NEDBALOVA, V. 1976. Polnohospodarstvo 22 (10) 943-959.

KERKVLIET, J.D. & PUTTEN VAN DER A.P.J. 1975. Apidologie 6 (3) 195-206.

KROPACOVA, S. & NEDBALOVA, V. 1971. Acta Universitatis Agriculturae 19 (4) 703-710.

KROPACOVA, S. 1969. Acta Universatatis Agriculturae 17 (4) 793-797.

KROPACOVA, S. & NEDBALOVA, V. 1972. Acta Universatatis Agriculturae 20 (3) 455-461.

KUBISOVA, S., KROPACOVA, S. & NEDBALOVA, V. Acta Universitatis Agriculturae 22 (4) 719-729.

ZANDER, E. 1935. Pollengestaltung und Herkunft-sbestimmung bie Blutenhonig 3 vols Berlin.

HOWELLS, W.W. 1969. Journal of the Association of Public Analysts 7 (3) 88-93.

KROPACOVA, S. & NEDBALOVA, V. 1974. Acta Universitatis Agriculturae Facultas Agronomica Bruo 22 (1) 89-99.

PEREZ, B.S. & RODRIGUEZ, A.T. 1970. Anales de Bromatologia 22 (4) 377-406.

JAMES, G.V. 1969. Journal of the Association of Public Analysts 7 (4) 128-32.

4. FISH AND SHELLFISH

4.1 ADULTERATION

ROUTINE ANALYSIS

Fish and shellfish tend to accumulate heavy metals, other toxic elements and stable fat-soluble organic compounds such as organochlorine pesticides. The extent to which an element is accumulated varies with the species. Total mercury and organochlorine pesticides are contaminants which should be looked for routinely in fish. GLC procedures (Pearce et al (1), Teeny (2), Schafer et al (3), and Kacpurzak and Chvojka (4) can be used for the determination of methylmercury in fish, but 80-90 percent is present in this more toxic form so that total mercury determinations by cold-vapour atomic absorption spectrophotometry or the dithizone colorimetric procedure are adequate to assess this element for routine purposes. Elements that can be accumulated by some species of fish and shellfish include mercury, cadmium, zinc, copper and arsenic. Thrower and Eustace (5) describe the uptake of heavy metals by oysters and subsequent determination in the flesh.

In canned fish products, as other canned goods, the condition of the can should be checked. Contamination by toxic elements may be acquired during shelf life, or from deterioration of the can. Canned products should be examined for tin and lead. Uthe et al (6) have compared wet and dry ashing methods for the determination of arsenic. Gajewska et al (7) describe the determination of lead in fish by methods which include the use of dithizone. Nabrzyski (8) describes the determination of mercury, copper and zinc by dithizone.

Mirex residues in fish may be determined by the methods of Borthwick et al (9) and Hawthorne et al (10). Neff and Anderson (11) describe a UV method for determining naphthalenes in oil-contaminated fish.

Antibiotics have occasionally been used to delay bacterial spoilage of fish between catching and landing, a practice not generally recommended. Bethea and Hillig (12) describe a microbiological method reproduced in Hart and Fisher (13), for the determination of chlortetracycline in such fish. Perna (14) and McCracken et al (15) describe methods for some other antibiotics. Chloramphenicol is an antibiotic that has caused concern more recently.

The identity under which fish or shellfish has been sold may come into question. If the fish cannot be identified unequivocally from the morphology, microscopical examination of the scales and disc electrophoresis of the proteins are used. The AOAC gives a key, with photographs of scales, for the identification of canned salmon. For shrimps and prawns see the monograph by Fincham and Wickins (16) and the Report of the Government Chemist (U.K.) for 1975.

The CAC recommended standard for quick frozen gutted Pacific salmon gives the different species to which this trivial name may be applied. The standards for cod, haddock and ocean perch do likewise and specify that the additives phosphate and ascorbate should not be present in excess of 0.5 percent as P_2O_5 and 0.1 percent as ascorbic acid and their presence must be declared.

The drained weight for canned products should be determined if this is feasible. For example, the CAC standard for canned shrimps and prawns sets a minimum of 60 percent m/m drained weight (2 minutes on a sieve of 2.8 x 2.8 mm square holes). Alternatively, for products canned in oil, the amount of oil may be decanted and measured. The identity of the oil should be checked against any claim made on the label by carrying out the identity tests detailed under "oils and fats", as well as checking for rancidity. Semi-preserved fish may be examined for salt, total acidity and volatile acidity.

Aflatoxins have been reported in fermented fish products, dried fish and fish sauces.

The fish content of products is determined by the Stubbs and More method as for meat products, but the nitrogen content of many fish species varies considerably and the determination is correspondingly less accurate. Pearson gives the large number of nitrogen factors published by the U.K. Analytical Methods Committee of the Society for Analytical Chemistry (17).

The phospholipid content of the fat in fatty fish is fairly high and the chloroform-methanol method of Bligh and Dyer will therefore give higher results than an ether extraction. The chloroform-methanol extract is suitable for the determination of rancidity values if these are required. King and Ryan (18) have described a method for determining shrimp in shrimp cocktail.

Canned fish may contain crystals of struvite (magnesium ammonium phosphate) which is harmless, but looks like glass and can be the subject of complaint. The crystals will dissolve in dilute acid and give the usual tests of qualitative inorganic chemistry. Microscopic examination will demonstrate the presence of glass which does not show up between crossed polaroids. It may also be possible to heat a glass fragment sufficiently to melt an edge and confirm that this has taken place by reexamination under the microscope.

Fish may be examined for preservatives by the usual methods. Sulphur dioxide is often used to control oxidative blackening in the processing of lobster tails and shrimps (Barnett (19)). A limit of 30 mg/kg is being discussed by the relevant CAC committee. Canned shrimps and prawns may contain formaldehyde derived from the degradation of di- and trimethylamine and trimethylamine oxide.

Fish oil concentrates such as shark liver oil are sold as rich sources of vitamins A and D. Determination of vitamin A and the usual rancidity tests are normally adequate to assess quality and genuineness.

It is worthwhile to examine shrimps, prawns and smoked-cured fish for added colouring matters. Discolouration of skipjack tuna has been investigated by Yamanaka (20) and co-workers, and of fish jelly products by Fujita and Miyamoto (21). Caviar substitutes with added Black 7984 contain unidentified decomposition products of the colour (Dragoni and Cantoni (22)). Discolouration of crabmeat is reviewed by Boon (23).

The CAC standard for canned shrimps and prawns permits the following colours to be added up to a maximum of 30 mg/kg (singly or in combination) in the final product - amaranth, beta-carotene, erythrosine, Ponceau 4R, Sunset Yellow FCF, tartrazine. Calcium sodium EDTA is allowed up to 250 mg/kg and the addition of citric acid is not specifically limited.

The problem of "red tide", attributed to the growth of the dinoflagellata Gonyaulax tamarensis, is described by Ahles (24). The dinoflagellate produces saxitoxin, and shellfish which have fed on the organism in sufficient quantity are toxic to humans, causing "paralytic shellfish poisoning". The AOAC details a biological method for its determination using mice. TLC-fluorescence methods are described by Bates and Rapoport (25) and Buckley, et al (26).

Tetrodotoxin from pufferfish (Fugu spp) and ciguatera poison are the other likeliest causes of chemical poisoning from the ingestion of sea foods (NAS (27)). It was reported by Hashimoto et al (28) that the Chinaman fish, Glabrilutjanus nematophorus, is one of a fairly large number of species with which ciguatera poisoning is associated. All of these causes of chemical poisoning taken together account for no more than a few outbreaks per year of sufficient seriousness to be reported in the technical literature.

FISH SPECIES IDENTIFICATION

PRINCIPLE

An aqueous (for uncooked fish) 6 M or 10 M urea extract (for cooked fish) of fish muscle is prepared and an aliquot of the extract placed at one end of a tube of acrylamide gel which is polymerized in situ. The gel is subjected to an electric potential and then stained, excess stain removed and the pattern of stained protein layers compared with extracts from authenticated samples. Patterns of cooked and raw fish of the same species are not identical, as cooking denatures the protein.

APPARATUS

1. Analytical disc electrophoresis apparatus (6, 8 or 12 tube capacity)

2. Agla semi-micro syringe.

REAGENTS

1. TRIS-glycine Buffer (Stock Solution): Dissolve 28.8 g of glycine and 6.0 g of TRIS-(2-amino-2-(hydroxymethyl)-propane-1,3-diol) in 1 litre of distilled water and adjust the pH to 8.6 with 1 N hydrochloric acid.

2. TRIS-glycine Buffer Solution: Dilute 1 part of Stock Solution (1) with 13 parts of water for use as the solvent for the gel reagents and as the electrolyte for electrophoresis.

3. Gel reagents: e.g. (a) "Cyanogum 41": (A mixture of 95 percent of acrylamide monomer and 5 percent of bisacrylamide; supplied by B.D.H. Chemicals, Ltd. Poole, Dorset, U.K.); (b) Ammonium Persulphate Solution: Dissolve 0.2 g of ammonium persulphate in 100 ml of the TRIS-glycine buffer solution; (c) Beta-dimethylamino-propionitrile Solution (1.60 percent): Dissolve 1.83 ml of beta-dimethylamino-propionitrile in 100 ml of TRIS-glycine buffer solution.

4. Protein-staining Solution: 0.1 percent. Amido Black in 7 percent acetic acid solution.

5. Wash Solvent: Methanol/acetic acid/water (21:3:96).

6. Sucrose Solution: 40 percent sucrose in distilled water.

7. Urea Solution: A freshly prepared 10 M solution. Dissolve 480 g of urea in water and dilute to 1 litre. For 6 M dissolve 288 g of urea in water, dilute to 1 litre.

PROCEDURE

Preparation of Protein Extracts:

(a) Raw Fish: Extract the myogen proteins by homogenising the muscle (10-25 g) with an equal weight of distilled water. Centrifuge the homogenate at 3000 rpm for 20 minutes and decant off the clear supernatant solution. Dilute this solution with an equal volume of 40 percent sucrose solution and store at 0°C until required. (Note: Allow frozen muscle to thaw out before extracting the proteins as above. Where the fish is breaded as in fish portions and fish fingers, remove the outer layer of bread and cooked flesh. Extract only the inner raw portion).

(b) Cooked Fish: Break up 10-25 g of the cooked fish muscle and suspend in twice the volume (20-50 ml) of 10 M urea solution. Allow the mixture to stand overnight at room temperature and remove insoluble residue by centrifuging for 20 minutes at 3000 rpm. Use the supernatant solution directly for electrophoresis. (Note: When cooked fish are required as controls, prepare them by heating on a steam bath for 30 minutes in covered casseroles. Extract with urea solution as in (b) above).

Prepare the acrylamide gel rods for electrophoresis as follows:

(a) 6.0 percent acrylamide gel for myogen protein separation: Dissolve 2.40 g of "Cyanogum 41" in 20 ml of TRIS-glycine buffer solution. Add 10 ml of the 1.60 percent w/v -dimethylamino-propionitrile solution and 10 ml of the 0.20 percent ammonium persulphate solution. Mix, and transfer the solution quickly to the previously stoppered gelling tubes (7.5 x 0.5 cm i.d.) with a wide needle (19 G x 2 in.) syringe (10 ml) and adjust the levels to 6.5 cm. To obtain a flat surface at the top of the gels, apply a layer of water (2-3 mm) carefully on top of the acrylamide solution. Use an Agla syringe with the tip of its needle placed just above the solution. Set aside to polymerize at room temperature. It is important that polymerisation should take place within 20-30 minutes as diffusion of the water into the acrylamide solution will result in incomplete polymerisation and an uneven gel surface.

(b) 7.5 percent acrylamide gels for separation of urea extracts of cooked fish are prepared as in (a) above using 3.0 g of "Cyanogum 41".

After polymerisation is complete, decant off any unpolymerised monomer solution, remove the polythene stoppers and transfer the prepared acrylamide gel rods to the electrophoresis apparatus. Fill with the TRIS-glycine buffer electrolyte.

Carry out an electrophoresis pre-run for 20 minutes at 200 volts to remove persulphate ions. Then, by means of an Agla semi-micro syringe deliver 10-20 microlitres of the protein extracts to the tops of the gels. In this operation, dip the syringe through the upper electrolyte solution with the tip of the needle just above the surface of the acrylamide gel. (Normally, duplicate analyses are performed on each extract, allowing for 4 extracts to be examined simultaneously in one apparatus. If desired, a second disc electrophoresis apparatus can easily be connected to the transformer). Carry out the electrophoresis for 30 minutes at constant voltage (280 volts) at room temperature. (In some instances slightly improved separation, particularly for the urea extracts, can be obtained by running for 50 minutes at the same voltage in a chill-room near 0°C).

When the run has been completed, remove the upper reservoir, empty the buffer solution and pull out the tubes. Remove the gel columns from the tubes by an irrigation method using a syringe containing distilled water and fitted with a No. 1 size needle. Insert the needle between the gel and the wall of the tube, rotate the tube slowly and at the same time discharge water from the syringe. Repeat this operation at the other end of the tube. The gel should then slip out of the tube.

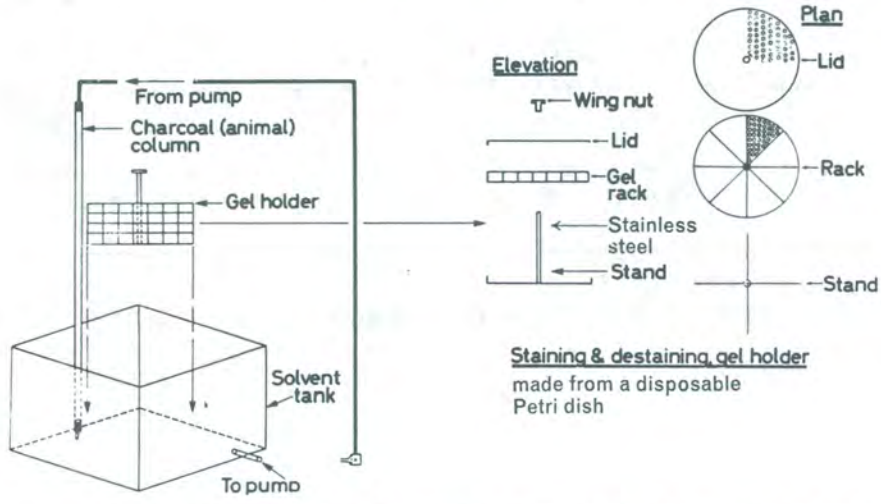
Stain the gel for 20 minutes in the Amido Black staining solution. Remove excess of dye by washing several times with the aqueous methanol solvent. After storing the gels overnight in the solvent, the protein zones should appear as dark blue discs against a water-clear background. Transfer the gels to glass test-tubes and store

them in the same solvent. Examine the gels against an illuminated white screen. This arrangement is also suitable for photographing the gels.

INTERPRETATION

This method only works on raw and partially cooked fish from which sufficiently undenatured proteins are still extractable. It does not work with fish cooked under pressure, such as that found in fish paste.

The procedure for cooked fish may be somewhat improved by preparing the 7.5 percent acrylamide gel in stronger buffer in 6 M urea (1 volume stock TRIS-glycine buffer + 7 volumes 6 M urea), and sufficient persulphate to produce polymerisation in 20-25 minutes. The tubes must be perfectly clean, e.g. by use of chromic acid, rinsing thoroughly and drying. The run may be extended from 30 to 45 minutes. After staining, the excess dye may be removed by repeated washing with 25-30 ml of the aqueous methanol solvent in 50 ml conical flasks. Alternatively, if samples are being examined daily, it may be more convenient to use the continuous destaining system shown below:



A recycling filtration system for destaining polyacrylamide disc electrophoresis gels.

In this system a pump continuously recirculates the solvent and on passage through the charcoal column the Amido Black is absorbed. Destaining by either method is normally achieved overnight. If rapid destaining (less than half hour) is required an electrolytic method can be used. In this latter system the unbound dye is removed by electrophoresis in dilute acetic acid.

REFERENCE

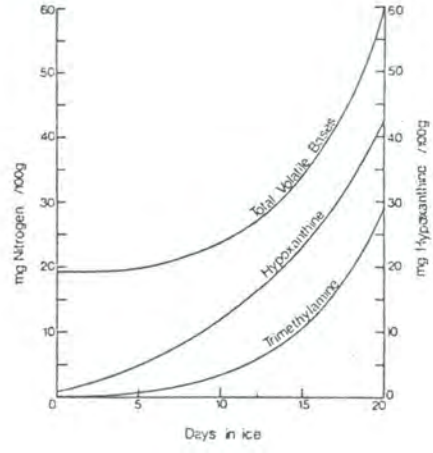
Mackay, I.M. 1972. Journal of the Association of Public Analysts 10, 18.

4.2 DECOMPOSITION

ROUTINE ANALYSIS

During storage of fresh fish a number of changes take place due to the action of bacteria and enzymes as well as purely chemical reactions. Among the chemicals produced are ammonia, trimethylamine, trimethylamine oxide, hypoxanthine and histamine. These, as well as those chemicals responsible for rancidity in fish oil, can be used to assess spoilage. The amounts of these substances formed depends upon species of fish, bacteria present, time, temperature, pH and probably other factors, so it is not possible to fix general limits for any of these quality parameters. Also, the presence of one or more of these substances are often a normal quality characteristic in certain products prepared by fermentation, semi-drying, etc. However, in fish sold as fresh, the concentration of these chemicals increases with time and results of their determination are used to support organoleptic analysis and physical appearance as to whether a sample is unfit for food.

Although the rate at which the spoilage chemicals are produced will vary according to the factors mentioned above, the general trend is as illustrated by the following graphs.



The changes in concentrations of total volatile bases, hypoxanthine and trimethylamine with the degree of spoilage of cod. The exact form of the changes differs for different species. From Connel "Control of Fish Quality". (29)

The results also vary with the exact method of analysis, and it is therefore advisable to determine each parameter on acceptable samples of the same species when fresh and again when the samples seem to be starting to decompose.

Histamine levels are sometimes high in scombroid fish such as mackerel and tuna after these have been kept some time. Shimizu et al (30) has suggested 100 mg/100 gm as a tentative limit. Histamine seems to be largely derived from the bacterial breakdown of histidine present in the fish muscle.

Other indices that have been used include volatile acid number, formic acid and acetic acid (Hillig et al (31)). Indole is suggested to assess spoilage of shrimps and ammonia to assess spoilage of crabmeat.

TOTAL VOLATILE BASES

PRINCIPLE

The spoilage of fish stored in ice is due to bacterial and enzyme action which results in the production of various volatile compounds such as trimethylamine (TMA), dimethylamine, ammonia and volatile acids. TMA is a reduction product of TMA oxide during spoilage and the ammonia is formed mainly as a product of protein breakdown. Total volatile bases (TVB) includes all volatile amines plus ammonia. TVB can be used as an index of spoilage of fish. This method steam distils all volatile nitrogenous compounds from an alkaline solution of the sample. These are collected in standard acid. The unreacted acid is titrated and the TVB calculated.

APPARATUS

1. Blender.
2. Centrifuge (optional).
3. Markham distillation apparatus.
4. Burette and pipettes.

REAGENTS

1. Trichloroacetic acid - 5% solution.
2. NaOH - 2N.
3. Hydrochloric acid - 0.01 N.
4. Rosolic acid indicator - 1% solution in 10% ethanol.
5. NaOH - 0.01 N.

PROCEDURE

Weigh 100 g sample and blend with 300 ml trichloroacetic acid. Filter or centrifuge to obtain a clear extract. Pipet 5 ml of the extract into the Markham apparatus. Add 5 ml 2N NaOH.

Steam distil into 15 ml standard 0.01N HCl containing 0.1 ml rosolic indicator. After distillation, titrate excess acid in the receiving flask using standard 0.01 N NaOH, to a pale pink end point.

Do a procedural blank using 5 ml trichloroacetic acid with no sample. Titrate as before.

CALCULATION

$$\text{TVB (mg/100 g sample)} = \frac{(N)\ (V_B - V_s)\ (14)\ (300 + W)}{(5)}$$

Where: V_B - ml NaOH used for blank titration.
W - Water content of sample in g/100 g
N - Normality of NaOH standard solution
V_s - ml NaOH used for sample titration

REFERENCE

Pearson's Chemical Analysis of Foods, 8th Ed, 1981, 414-5.

TRIMETHYLAMINE (TMA) IN FISH
(Colorimetric Method)

PRINCIPLE

Fish stored in ice spoils as a result of bacterial and enzyme action which results in the formation of volatile bases, including trimethylamine (TMA). TMA is the reduction product of trimethylamine oxide. The amounts of TMA and total volatile bases (TVB) present in the fish can be used as indices of fish spoilage.

This method involves the extraction of volatile bases with trichloroacetic acid. Bases other than TMA are complexed with formaldehyde. Toluene is used to extract the TMA from a basic medium and then reacted with picric acid to yield a coloured picrate salt. This is analyzed spectrophotometrically.

APPARATUS

1. Blender

2. Centrifuge

3. Pyrex glass-stoppered test-tubes

4. Spectrophotometer in the visible range.

REAGENTS

1. Trichloroacetic acid (TCA), 7.5% : 7.5 g to 100 ml with distilled water.

2. Toluene, anhydrous: To remove interferences, shake 500 ml toluene with 100 ml 1N H_2SO_4, distil, and dry with anhydrous Na_2SO_4.

3. Picric acid solutions:

a) Stock solution, 2% : Dissolve 2 g in 100 ml of anhydrous toluene. (Caution: Picric acid is highly sensitive to shock when in a dry state. In contact with metals and NH_3, it produces picrates which are more sensitive to shock than picric acid. It is readily absorbed through the skin and is irritating to eyes. Wear heavy rubber gloves and eye protection).

b) Working solution, 0.02% : Dilute 1 ml stock solution to 100 ml with anhydrous toluene.

4. Potassium Carbonate Solution, 50% w/w: Dissolve 100 g potassium carbonate (K_2CO_3) in 100 ml water.

5. Formaldehyde, 20% : Shake 1 litre 40% formaldehyde solution (HCHO) with 100 g magnesium carbonate ($MgCO_3$) until nearly colourless, and filter. Dilute 100 ml to 200 ml with water.

6. Hydrochloric acid (1+3) : 1 volume of concentrated HCl (S.G. 1.18) plus 3 volumes of distilled water.

7. Anhydrous sodium sulphate (Na_2SO_4).

8. Trimethylamine (TMA) standard solutions:

a) Stock solution 1 mg TMA/ml: Add 0.682 g trimethyl ammonium hydrochloride, to 1 ml HCl (1+3) and dilute to 100 ml with distilled water. Check basic N-content of 5 ml aliquot by adding 6 ml 10% NaOH solution, steam distilling into 10 ml 4% boric acid and

titrate with 0.1N H_2SO_4 using methyl red - methylene blue indicator as in a protein determination.

b) Working solution, 0.01 mg TMA - N/ml: Add 1 ml stock solution to 1 ml HCl (1+3) and dilute to 100 ml with distilled water.

PROCEDURE

Weigh 100 g minced or chopped, well-mixed sample into a blender. Add 200 ml of 7.5% TCA solution and blend. Centrifuge blended solution at 2000-3000 rpm until supernatant is practically clear.

Pipette aliquot (preferably containing 0.01-0.03 mg TMA-N) into a 20 x 150 mm Pyrex glass-stoppered test tube and dilute to 4.0 ml with distilled water. (Note: For fresh fish, use 4.0 ml aliquot of supernate, stale fish, use 1.0-3.0 ml aliquot of supernate.)

At the same time, prepare a blank and standards. For the blank, use 4.0 ml distilled water. For standards, use 1.0, 2,0 and 3.0 ml of working standard solution (0.1 mg TMA-N/ml) and dilute to 4.0 ml with distilled water.

To each tube (blank, standards, samples), add 1 ml HCHO (20%), 10 ml anhydrous toluene and 3 ml K_2CO_3 solution (100 g%). Stopper test-tube containing about 0.1 g anhydrous Na_2SO_4. Shake well to dry toluene. Pipette 5 ml toluene layer and add 5 ml picric acid working solution (0.02%). Mix and transfer to a spectrophotometric cell and measure absorbance at 410 nm against the blank. (Note: If original aliquot contains more than 0.03 TMA-N, dilute extract with TCA solution and repeat determination.)

CALCULATION

mg TMA-N/100 g sample

$$= \frac{A}{A^1} \times \frac{\text{mg TMA-N/ml std solution x ml std solution used x 300}}{\text{ml of aliquot of sample used}}$$

Where: A = absorbance of sample

A^1 = absorbance of standard nearest to absorbance of sample

300 = approximation total supernatant (ml) (100 g + 200 ml).

INTERPRETATION

Fresh fish gives TMA values of about 1 mg/100 g and spoiled samples over 8 mg/100 g. Values around 5 mg/100 g could be taken as indications of doubtful quality. In frozen-stored gadoid fillets, the TMA indicates the extent of microbial spoilage before the muscle was frozen.

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 18.031-.033.

INDOLE

PRINCIPLE

The Indole test is considered a reliable test for shellfish freshness. It is assumed that the odour of spoiled shellfish is caused in part by accumulation of indole, a breakdown product of tryptophan. The method involves steam distillation, followed by extraction of the indole into chloroform, colour development with p-dimethyl-aminobenzaldelyde in acid medium and re-extraction of the coloured product into the acidic phase. It is then quantitated spectrophotometrically.

APPARATUS

1. Blender.
2. Steam generator.
3. Distillation flask.
4. Condenser.
5. Flask.
6. Separatory funnels: 125 ml, 500 ml.
7. Spectrophotometer in the visible range.

REAGENTS

1. Colour reagent: Dissolve 0.4 g p-dimethyl-amino-benzaldhyde (use AR grade which should be pale yellow in colour) in 5 ml acetic acid and mix with 92 ml H_3PO_4 and 3 ml acetic acid.

2. Glacial Acetic acid, purified: If it turns pink with the colour reagent, purify as follows - add in order specified to 1 L round-bottom flask: 500 ml glacial acetic acid, 25 g $KMnO_4$ and 20 ml H_2SO_4. Distil in all glass still not more than 400 ml.

3. Dilute HCl, 5%: Dilute 5 ml HCl to 100 ml with water.

4. Indole standard stock solution 10 mg %: Accurately weigh 20 mg indole into a 200 ml volumetric flask and dilute to volume with alcohol. Keep refrigerated and discard after 2 weeks.

5. Indole standard working solution, 0.1 mg %: Pipette 1 ml of indole standard stock solution into a 100 ml volumetric flask and dilute volume to with alcohol.

6. Chloroform, AR Grade.

7. Saturated Na_2SO_4 solution.

8. Alcohol, 95%.

PROCEDURE

For oyster meats weigh 50 g. For drained crab meat or peeled prawns weigh 25 or 50 g (depending upon amount of indole expected).

Transfer weighed portion to a high-speed blender. Add 80 ml water (for oyster or crab meat sample) or 80 ml alcohol (if sample is shrimp) and blend for several minutes, until homogeneous. Quantitatively transfer mixture to distillation flask and rinse with

minimum amount of same solvent used for preparing mixture. Add glass-beads to distillation flask. Connect flask for steam distillation. Gently apply steam until distillation is well started, taking care not to pass in steam so vigorously as to cause excessive foaming. Collect 350 ml distillate in about 45 minutes (if alcohol was used in preparation of sample, collect 450 ml). Wash condenser with a small amount of alcohol and drain into receiving flask containing distillate.

Transfer distillate to a 500 ml separator and add 5 ml dilute HCl (5%) and 5 ml saturated Na_2SO_4. Extract with 25 ml $CHCl_3$. Shake vigorously for at least 1 minute each time. Allow the 2 layers to separate. Remove and save $CHCl_3$ layer. Re-extract the aqueous layer 2 more times with 20 ml and 15 ml portions of $CHCl_3$. In the final extraction, discard aqueous layer.

Combine the 25 ml and 20 ml $CHCl_3$ extracts in a 500 ml separator and wash with 400 ml water containing 5 ml saturated Na_2SO_4 and 5 ml dilute HCl (5%). Shake vigorously and allow the 2 layers to separate. Filter $CHCl_3$ layer through a cotton plug into a dry 125 ml separator. Wash the 15 ml $CHCl_3$ extract, using the same wash water as above. Filter through a cotton plug into the same 125 ml separator.

Add 10 ml colour reagent to the combined $CHCl_3$ extracts. Shake vigorously for exactly 2 minutes and let the acid layer separate as completely as possible. Transfer 9.0 ml acid layer to a 50 ml volumetric flask and dilute to volume with glacial acetic acid. Mix well.

Transfer a portion of the colour solution to a photometer cell and measure the absorbance at 560 nm against a reagent blank (blank consists of 9 ml colour reagent, diluted with glacial acetic acid to 50 ml). (Note: Colour solution may be diluted with glacial acetic acid containing 9 ml colour reagent/50 ml solution, provided blanks are determined at the same dilutions). Include a distillation blank (i.e., omitting addition of indole). Subtract absorbance of the distillation blank from the sample.

STANDARD CURVE

Pipette 0 ml, 1 ml, 3 ml, 5 ml, 7 ml of indole standard working solution (1 µg/ml) into separate distillation flasks. Add 80 ml alcohol and a few glass beads. Proceed as for sample. Plot absorbance at 560 nm against µg/ml indole.

CALCULATION

$$\text{Indole, } \mu g/100 \text{ g sample} = \frac{A_x}{A_s} \text{ (C) (V)} \quad \frac{100}{W}$$

Where:

A_x = absorbance of sample

A_s = absorbance of standard

C = concentration of standard solution (µg/ml)

V = volume of standard solution used (ml)

W = weight (g) of sample taken.

INTERPRETATION

If the indole value exceeds 25 µg/100 gm sample, the product is considered not to be fresh.

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 18.072-.074.

HISTAMINE

PRINCIPLE

Histamine is formed as a decomposition product in fish. Highest levels are often found in scombroid-type fish. Histamine can cause violent allergic reactions in sensitive persons. This method extracts the histamine into methanol, partitions it into benzene-butanol and isolates it using a cotton acid succinate column. The isolated histamine is then coupled to a diazotized aromatic amine to form a coloured complex. This is determined spectrophotometrically.

APPARATUS

1. Blender.

2. Volumetric flasks and pipettes.

3. Fine tip dropper with bulb.

4. Ice bath and water bath.

5. pH meter.

6. Spectrophotometer.

REAGENTS

1. Methanol.

2. Benzaldehyde (chloride-free).

3. Sodium hydroxide solution, 20%.

4. Benzene - butanol mixture, (3+2) v/v.

5. Cotton acid succinate (CAS): dissolve 5 g anhydrous sodium acetate (fused just before use) and 40 g succinic anhydride in 300 ml glacial acetic acid in a 500 ml erlenmayer flask. Cut 10 g asorbent cotton into strips and immerse in solution. Attach a drying tube containing a desiccant and heat at 100°C for 48 hours. Filter reacted cotton (CAS) from solution and wash with water, then (1+9) HCl, then water again and finally with alcohol. Dry the CAS in a vacuum oven at 100°C for 1 hour.

6. Ethanol, 95%.

7. Sulphuric acid, 0.40 N ($\pm$ 0.02N - standardized).

8. Diazonium reagent: dissolve 0.1 g p-nitroaniline in 0.1N HCl and dilute to 100 ml with 0.1 N HCl. Store in refrigerator. Dissolve 4 g sodium nitrite in water and dilute to 100 ml. Also store in a refrigerator. Prepare the diazonium reagent just before use by placing 10 ml of p-nitroaniline in an ice bath for 5 minutes, then add 1 ml of the $NaNO_2$ and mix. Let stand in the bath an additional 5 minutes. It is then ready to use.

9. Coupling buffer: dissolve 7.15 g sodium metaborate ($NaBO_2$) and 5.7 g sodium carbonate in water and dilute to 100 ml.

10. Borax ($Na_2B_4O_7.10H_2O$).

11. Methyl isobutyl ketone.

12. Barbital buffer: dissolve 10 g sodium barbital in 1 litre water and adjust to pH 7.7 using acetic acid and a pH meter. Store in a refrigerator to prevent mould growth.

13. Histamine standard solution - dry histamine · 2HCl 2 hours over H_2SO_4. Dissolve 0.1656 g in water and dilute to 100 ml (1 mg/ml). This is the stock solution. Prepare the working standard fresh weekly by diluting 10 ml of the stock to 100 ml with water, then dilute 5 ml of this plus 5 ml of methanol to 100 ml with water. This final working standard is 5 µg/ml. Store in a refrigerator.

(Note: all water must be distilled from glass. Do not use detergents to clean glassware. Use fresh chromic acid cleaning solution and rinse with distilled water).

PROCEDURE

Place 10 g minced sample in a small blender cup. Add 50 ml methanol and blend 2 minutes. Transfer to a 100 ml volumetric flask, rinsing with methanol. Heat in a water bath at 60°C for 15 minutes. Cool and dilute to volume. Mix and filter. Keep filtrate in a stoppered flask. This may be stored in a refrigerator several weeks. Pipette 5 ml of the filtrate into a 150 mm glass stoppered test tube and add 1 drop benzaldehyde and 0.1 ml of 20% NaOH. Stopper and shake vigorously at least 25 times. Let stand 5 minutes. (If an emulsion has formed, centrifuge to break it).

Prepare a CAS column by firmly placing a small plug of CAS (about 50 mg) in a micro column or a 15 ml centrifuge tube with the bottom cut off. Wash the plug with three 15 ml portions water and two 3 ml portions ethanol. Blow out last portion of each wash with a small amount of air pressure.

Transfer the upper organic layer in the tube, using a fine-tip dropper with bulb, to the CAS column. (Note: do not transfer any of the lower aqueous layer).

Add 15 ml benzene-butanol to the centrifuge tube and again shake 25 times. Let stand 5 min and transfer upper layer to the CAS column as before. Rinse inside of CAS column with small amount of ethanol and drain. Blow out last bit with mild air pressure. Add 3 ml ethanol, drain and blow out as before.

Repeat with two 3 ml portions of water. Discard all solvents and washings.

Elute histamine from the CAS by pipetting 2.0 ml of 0.4N H_2SO_4 into column. Drain and collect eluate. Add 3 ml water. Drain into same eluate and blow out last drop. Cool the eluate in an ice bath for 5-10 minutes. Add 0.5 ml cold diazonium reagent and let stand 5 minutes (still in ice bath). Pipette 0.5 ml coupling buffer while swirling. Let stand 5 minutes in the ice bath. Add 0.25 g powdered borax. Shake for 30 seconds and let stand 15 minutes in the ice bath. Pipette 5.0 ml methyl isobutyl ketone and shake vigorously 25 times.

Pour both layers (do not rinse) into 150 mm test tube. Let stand 10 minutes at room temperature. Transfer the upper organic layer using a fine-tip dropper, into a second 150 mm glass stoppered tube containing 5 ml of the barbital buffer. (Note: do not transfer any of the lower aqueous layer - the transfer need not be quantitative). Stopper and shake 25 times. Let stand 10 minutes.

Transfer upper organic layer with a fine-tip dropper, to a 1 cm cuvette. Determine absorbance at 475 nm against pure methyl isobutyl ketone.

Conduct a procedural blank using 5 ml methanol.

Conduct a standard determination using 5 ml of the 5 µg/ml working standard solution.

CALCULATION

$$\text{Histamine ppm} = \frac{Ax - A_B}{As - A_B} \quad (50)$$

Where: Ax = absorbance of sample

As = absorbance of standard

A_B = absorbance of blank

50 = factor: $\frac{25}{10} \times \frac{100}{5} = 50$

INTERPRETATION

There is no established maximum histamine level. However, some agencies have used a figure of 100 ppm as the level at which histamine would be of concern.

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 18.064-.066.

BORIC ACID

PRINCIPLE

Boric acid and borax were commonly used as preservatives prior to their prohibition by many countries. Boron preservatives are considered as less desirable substances in view of their cumulative nature and their possible use to mask incipient putrefaction. Boric acid and turmeric react to yield a characteristic red colour. The production of this colour forms the basis of the following test.

APPARATUS

1. Beakers, 200 ml.

2. Glass wool.

3. Pipettes.

4. Test tubes.

REAGENTS

1. Concentrated HCl.

2. Turmeric paper: Add 100 ml 80% alcohol to 1.5-2.0 g turmeric powder in 250 ml conical flask. Shake 5 minutes and filter. Dip sheets of Whatman No. 2 paper into the clear filtrate. Hang paper to dry. After 1 hour cut into 6x1 cm strips and store in tightly stoppered container protected from light.

3. Boric acid standard solution, (10 mg H_3BO_3/ml): Dissolve 1.0 g H_3BO_3 in water and dilute to 100 ml.

PROCEDURE

Heat 25 g minced sample with 50 ml of distilled water in a 200 ml beaker (do not overheat). Chill then filter through a plug of glass wool. Pipette 10 ml of the cooled filtrate into a test tube. Add 0.7 ml of concentrated HCl stopper and mix. Immerse strip of turmeric paper into the acidified filtrate. Let turmeric paper dry in the air.

If a red colour is formed on the turmeric paper, proceed as follows:

Transfer 0.0, 0.1, 0.2, 0.5, 0.75, 1.0, 2.50 and 5.0 ml of standard H_3BO_3 solution (20 mg/ml) to eight 15 ml test tubes. Dilute each to 10 ml with water and add 0.7 ml HCl. (These standards represent 0.0, 0.02, 0.04, 0.10, 0.15, 0.20, 0.50 and 1.0% H_3BO_3 in meat (based on 25 g sample extracted with 50 ml water and 10 ml aliquot used for test. Keep tubes tightly stoppered to prevent evaporation.

Make identification on end of eight pieces of turmeric paper and dip the unmarked ends into the series of standard solutions. Dry strips at room temperature (about 1 hour). Place dried standard strips about 1 cm apart on a white filter paper background and bring sample strip between adjacent standards for close colour matching. If colour falls between 2 standards, estimate the value. (Disregard streaks of colour that may develop at edge of test strip). If colour intensity of sample strip is beyond range of standards, repeat test with dilution of meat filtrate.

4.3 TEXT REFERENCES

1. PEARCE, I.D., BROOKS, R.R. & REEVES, R.D. 1976. Journal of the Association of Official Analytical Chemists 59 (3) 655-657.

2. TEENY, F.M. 1975. Journal of Agricultural and Food Chemistry 23 (4) 668-671.

3. SCHAFER, M.L., RHEA, U., PEELER, J.T., HAMILTON, C.H. & CAMPBELL, J.E. 1975. Journal of Agricultural and Food Chemistry 23 (6) 1079-1083.

4. KACPURZAK, J.L. & CHVOJKA, R. 1976. Journal of the Association of Official Analytical Chemists 59 (1) 153-157.

5. THROWER, S.J. & EUSTACE, I.J. 1973. Food Technology in Australia 25 (11) 546-553.

6. UTHE, J.F., FREEMAN, H.C., JOHNSTON, J.R. & MICHALIK, P. 1974. Journal of the Association of Official Analytical Chemists 57 (6) 1363-1365.

7. GAJEWSKA, R., NABRZYSKI, M., LIPKA, E. & GAJEWSKI, A. 1976. Bromatologia i Chemia Toksykologiczna 9 (1) 1-5, 7-12. FSTA 8 11R682.

8. NABRZYSKI, M. 1975. Bromatologia i Chemia Toksykologiczna 8 (2) 165-170 FSTA 8 4R182.

9. BORTHWICK, P.W., COOK, G.H. & PATRICK, J.M. Jr. 1974. Pesticides Monitoring Journal, 7 (3/4) 144-145.7.

10. HAWTHORNE, J.C., FORD, J.H. & MARKIN. G.P. 1974. Bulletin of Environmental Contamination and Toxicology 11 (3) 258-264.

11. NEFF, J.M. & ANDERSON, J.W. 1975. Bulletin of Environmental Contamination and Toxicology 14 (1) 122-128.

12. BETHEA, S. & HILLIG, F. 1965. Journal of the Association of Official Agricultural Chemists 48 1971.

13. HART, F.L. & FISHER, H.J. 1971. Modern Food Analysis Springer-Verlag.

14. PERNA, A. & CIEN, B. 1973. Atti della Societa Italiana delle Scienze Veterinarie 27 589-590.

15. McCRACKEN, A., FIDGEON, S., O'BRIEN, J.J. & ANDERSON, D. 1976. Journal of Applied Bacteriology 40 (1) 61-66.

16. FINCHAM, A.S. & WICKINS. 1976. Identification of Commercial Shrimps and Prawns. British Museum (Natural History) Publication No. 779. H.M.S.O., London.

17. ANALYTICAL METHODS COMMITTEE. 1966. The Analyst 91 540.

    ANALYTICAL METHODS COMMITTEE. 1971. The Analyst 96 744.

    ANALYTICAL METHODS COMMITTEE. 1973. The Analyst 98 456.

18. KING, F.J. & RYAN, J.J. 1976. Journal of the Association of Official Analytical Chemists 59 (3) 644-649.

19. BARNETT, D. 1976. Australian Fisheries 35 (2) 17-18.

20. YAMANAKA, H. 1975. Bulletin of the Japanese Society of Scientific Fisheries (Nihon Suisan Gakkai-shi), 41 (3) 357-363, 573-578 FSTA 8 2R100.

21. FUJITA, Y. & MIYAMOTO, M. 1975. Bulletin of the Japanese Society of Scientific Fisheries (Nihon Suisan Gakkai-shi) 41 (12) 1263-1269 FSTA 8 9R566.

22. DRAGONI, I. & CANTONI, C. 1975. Industrie Alimentari 14 (9) 126-128. FSTA 8 1R42.

23. BOON, D.D. 1975. Journal of Food Science 40 (4) 756-761. FSTA 8 1R28.

24. AHLES, M.D. 1974. American Journal of Public Health 64 (8) 807. FSTA 7 4R185.

25. BATES, H.A. & RAPOPORT, H. 1975. Journal of Agricultural and Food Chemistry 23 (2) 237-239.

26. BUCKLEY, L.J., IKAWA, M. & SASNER, J.J. 1976. Journal of Agricultural and Food Chemistry 24 (1) 107-111.

27. Toxicants occurring naturally in foods. 1973. National Academy of Sciences, Washington, D.C.

28. HASHIMOTO, Y., KAMIYA, H., KINJO, K. & YOSHIDA, C. 1975. Bulletin of the Japanese Society of Scientific Fisheries (Nihon Suisan Gakkai-shi) 41 (8) 903-905. FSTA 8 6R325.

29. CONNELL, J.J. 1976. Control of Fish Quality Fishing News Books Ltd., 1 Long Garden Walk, Farnham, Surrey, U.K.

30. SHIMIZU, W. & HIBIKI, S. 1955. Bulletin of the Japanese Society of Scientific Fisheries (Nihon Suisan Gakkai-shi) 21 (5) 357-360.

31. HILLIG, F., SHELTON, L.R. & LONGHREY, J.H. 1962. Journal of the Association of Official Agricultural Chemists 45 (30 724-731.

Further Reading

REPORT OF THE GOVERNMENT CHEMIST. 1975. H.M.S.O., London.

HATTULA, M.L. 1974. Bulletin of Environmental Contamination and Toxicology 12 (3) 331-337.

EGAN, H., KIRK, R.S. & SAWYER, R. 1981. Pearson's Chemical Analysis of Foods 8th Ed. Churchill Livingstone.

DREW, R.E. 1975. Journal of the Fisheries Research Board of Canada 32 (6) 813-816.

WADA, T., SAWADA, T., HASEGAWA, K. & YAMANAKA, H. 1974. Bulletin of the Tokai Regional Fisheries Research Laboratory (Tokai-ku Suisan Kenkyusho Kenkyu Hokoku), 78 59-66. FSTA 8 11R636.

ZITKO, V., HULZINGER, O. & CHOI, P.M.K. 1974. Bulletin of Environmental Contamination and Toxicology 12 (6) 649-653. FSTA 7 12R689.

WOLFRAM, J.H. 1975. Dissertation Abstracts International B 36 (3) 1205, Order No. 75-19879.

SCHUTZ, D.E., CHANG, G.W. & BJELDANES, L.F. 1976. Journal of the Association of Official Analytical Chemists 59 (6) 1224-1225.

Recommended International Standard for Quick Frozen Gutted Pacific Salmon. 1970. Codex Alimentarius Commission, Recommended Standard 36.

Recommended International Standard for Quick Frozen fillets of Cod and Haddock. 1971. Codex Alimentarius Commission, Recommended Standard 50.

Recommended International Standard for Quick Frozen fillets of Ocean Perch. 1971. Codex Alimentarius Commission, Recommended Standard 51.

Recommended International Standard for Canned Shrimps and Prawns. 1970. Codex Alimentarius Commission, Recommended Standard 37.

5. MEAT AND MEAT PRODUCTS

5.1 FRESH AND FROZEN MEAT

ROUTINE ANALYSIS

The quality of fresh meat is normally verified by veterinary inspection carried out at the abattoirs. Laboratory work includes examination for pesticide residues, drugs and anabolic agents. The identity and freshness of samples may be questioned.

Chemicals such as ascorbic, erythorbic and nicotinic acids are sometimes used to maintain a fresh appearance of the meat. Ascorbic and nicotinic acids are usually diluted with sucrose or glucose for sprinkling on meat, so examination of an extract for these sugars by TLC is a useful preliminary test. The method of Schmall et al (1)(2) may be used for the quantitative determination of ascorbic and erythorbic acids. Means of "tenderising" meat with proteinases such as papain (from papaya fruit), bromelain (from pineapples) and ficin (from figs), include injection into the circulation just before slaughter. Such tenderising increases the level of free tyrosine.

Diethylstilboestrol is a growth promoter in meat-producing animals and in food. Residues are not allowed in foods in countries such as the USA because of the carcinogenic potential of the drug. Residues in animal tissues may be determined by the GLC methods of Donoho et al (3) or Kohrman and MacGee (4). Simpler procedures include the qualitative paper chromatographic method of Smith and McNeil (5) and the TLC-fluorimetry procedure of Ponder (6). The paper chromatographic technique has a limit of detection of 10 ppb, which is above the carcinogenic level in mice. Mouse uterine assay was originally used (Umberger et al (7)(8)). Kling and Lazar (9) have developed a radioimmunoassay technique. The technique of Heffter et al (10) may also be used.

In problems of identity the veterinarian or meat inspector and the analytical chemist should work together. The veterinarian will often be able to identify bones and cuts of raw meat, obviating the necessity for laboratory work. If this is required for confirmation, precipitin tests and gel electrophoresis can be used on raw and frozen meat and the latter technique may give useful results as long as cooking has not been so severe as to denature completely all the proteins present. Identification of cooked meats can be difficult and the best approach is via GLC and U.V. of the fat. Pig fat contains less stearate than beef or sheep, but a little more linoleate. Horsefat contains more linolenate and linoleate than the other three fats mentioned.

Qualitative tests for colour, starch, sulphur dioxide and nitrate should be carried out on minced meat. It may be worthwhile to examine other fresh meat for preservatives. Minced meat may contain an excessive amount of fat, or gristle or offals such as brains, feet, gut, chitterlings (smaller intestines), manifolds, udders, sweetbreads (pancreas, sometimes thymus) tripe (rumen and reticulum) melts, lites (lungs), spinal cord, uteri, pigs' maws (stomach), calves vells (inner lining of fore stomach) and skirt (diaphragm).

Proximate analysis is of some assistance in assessing general quality, for example, minced meat should not contain more than a reasonable amount of fat. The analysis of the amino-acids making up the proteins may assist in determining the proportion of protein from animal or vegetable sources. 3-methyl histidine appears to occur only in protein from animal sources (Rangeley and Lawrie (11)). Addition of vegetable oils or fats to meat can be detected both in cooked and uncooked products by examination of the sterols, by GLC of the fats and by examination of the U.V. absorption.

Liver is high in iron so the iron may be used as an approximate indication of the amount of liver in a mixture, but the iron content has also been used to assess the amount of blood added to hamburgers (Hankin (12)). Liver and kidney, as part of their physiological functions, process and in some cases

accumulate exogenous substances and their metabolites. For example, analysis of animal kidneys and livers for heavy metals, especially cadmium, lead and arsenic, may be a useful guide to environmental contamination. These organs are appropriate ones to use when screening for residues of veterinary drugs and their metabolites.

Frozen chicken carcases may have been treated with salt and/or polyphosphates as during spin-chilling. This is said to improve the texture and also results in the retention of more water than in untreated carcases. The polyphosphate is quickly hydrolysed to orthophosphate, but the total level of phosphate (P) compared with nitrogen (N) appears a fairly reliable indication that the former has been added. P/N values do not exceed 0.1 in untreated birds (Hamence and Kunwardia (13)). The EEC method for water in frozen chicken is also of assistance in assessing quality. Extraction of phosphates from food is described by Doro and Remoli (14)). TLC can be used instead of PC (Iida and Yamabe (15)). See also Togonai and Tanaka (16), Pesino et al (17) and Van Hoof et al (18).

Freshness is measured by extract release volume and total volatile bases. The usual rancidity values on the fat may also be informative. The total volatile bases increase as the lean portion of the meat deteriorates. The TVB test is not reliable on smoked or cured products.

Hankin (12) describes the determination of iron to assess blood added to hamburger. A level of 2-3.5 percent may be added to mask the presence of excess fat, iron levels being significantly higher than in normal meat if added blood exceeds 1 percent. In the paper cited, it is suggested that iron is determined via the colour with thioglycollic acid but AAS would also be suitable. The determination of added blood in ground beef is described by Karasz et al (19).

TOTAL VOLATILE BASES

PRINCIPLE

The sample is distilled from magnesium oxide under standard conditions and volatile bases are titrated with boric acid.

APPARATUS

1. Kjeldahl macro-distillation unit.

REAGENTS

1. Magnesium oxide, solid.

2. Antifoam. Silicone preparation or octyl alcohol.

3. Boric acid, 2% aqueous solution.

4. Methyl red indicator. Dissolve 0.016 g methyl red and 0.083 g bromocresol green in 100 ml neutral denatured ethanol.

5. Sulphuric acid, 0.1 N.

PROCEDURE

Add 100 ml of water to 10 g of the minced sample in a food blender and homogenise for one minute. Wash into the distillation flask with a further 200 ml of water. Add 2g magnesium oxide and a drop or two of antifoam solution. Bring to the boil in exactly 10 minutes and distil for exactly 25 minutes, using the same rate of heating, into 25 ml of 2 percent boric acid solution with added indicator in a 500 ml conical flask. The tube on the end of the condenser should dip below the boric acid solution. Disconnect the splash-head, stop heating and wash the condenser down with distilled water and titrate the contents of the conical flask with 0.1 N H_2SO_4. Carry out a blank determination.

CALCULATION

Total volatile bases (as mg N per 100g flesh) = 14(titre-blank)

INTERPRETATION

The method is valid only for fresh or frozen meats and not for bacon and other cured meats. Meat should give a value of less than 20 mg percent calculated on a fat-free basis, values over 30 mg percent are considered to correspond to staleness and appreciable production of trimethylamine. However, it is important to compare with values obtained from satisfactory samples of the same meat.

REFERENCE

Pearson's, The Chemical Analysis of Foods, 7th Ed., 1976, 376-386, Churchill Livingstone.

THIOBARBITURIC ACID (TBA) VALUE

PRINCIPLE

Oxidized lipids are formed as fats become rancid. Thiobarbituric acid will react with these lipids to form a red-colored complex which can be determined spectrophotometrically. Malonaldehyde is one of the end products of oxidative rancidity, and is believed to be involved in the reaction with TBA. Therefore, the TBA value is expressed as mg malonaldehyde per kg sample. The TBA test is applicable to fatty foods (e.g. meat) as well as fats and oils.

APPARATUS

1. Distillation apparatus (flask, condenser, receiver).
2. Glassbeads.
3. Electric mantle.
4. Pipette.
5. Glass stoppered test tube.
6. Spectrophotometer.

REAGENTS

1. Hydrochloric acid - 4N.
2. Antifoam liquid.
3. Thiobarbituric acid reagent - dissolve 0.2883 g in 100 ml of 90% glacial acetic acid.

PROCEDURE

Macerate 10 g of minced sample with 50 ml water for 2 minutes and then transfer to distillation flask, using 47.5 ml water for washing. Add 2.5 ml 4N HCl. (pH should be 1.5). Add antifoam and a few glass beads. Distil at a rate so that 50 ml of distillate is collected in 10 minutes from the time boiling commences.

Pipette 5 ml of the distillate into a glass-stoppered tube. Add 5 ml TBA reagent. Shake and heat in boiling water for 35 minutes.

Prepare a blank similarly, using 5 ml water, for 35 minutes.

Cool the sample and blank tubes and measure the absorbance of the sample against the blank at 538 nm using 1 cm cells.

CALCULATION

TBA value (as mg malonaldehyde per kg sample) = 7.8 x A

where A = absorbance of sample vs blank

(Caution: the method must be followed exactly for the 7.8 factor to be valid).

INTERPRETATION

The TBA value increases as the fat in meats becomes rancid. The value for fresh meats, however, will vary. It is therefore necessary to establish TBA

values for fresh meats and for those meats found to be going rancid by organoleptic tests. These base values can then be used to evaluate results on samples.

REFERENCE

Tarladgis, B.G., Watts, B.M., Younathan, N.T. and Dugan, L. 1960. Journal of the Association of the American Oil Chemists Society 37, 44.

FREE FATTY ACIDS AND PEROXIDE VALUE

PRINCIPLE

Fat spoilage can be assessed by estimating the free fatty acids (FFA) and peroxide value on a common chloroform extract.

The FFA in the sample extract is diluted with alcohol and neutralized by titration with sodium hydroxide. The FFA are expressed as % oleic acid on the extracted fat. This method may be used for determining FFA in lard (edible pig fat) and dripping (edible beef and mutton fat), as well as tallow and fats extracted from fresh meats.

The analysis for peroxide value depends on the reaction of potassium iodide in acid solution with the peroxide oxygen followed by titration of the liberated iodine with sodium thiosulphate. The amount of iodine liberated is expressed as milli-equivalents of peroxide oxygen per kg extracted fat. This method determines all substances which oxidize potassium iodide under the conditions of the test. These substances are generally assumed to be peroxides or other similar products of fat oxidation. The test may be used to determine the peroxide value of fats extracted from fresh meat.

APPARATUS

1. Erlenmeyer flasks, 250 ml.
2. Filter paper, Whatman No. 1 or equivalent.
3. Water-bath with temperature regulator.
4. Drying oven maintained at 100° $\pm$ 2°C.
5. Glass desiccator, charged with any efficient desiccant.

REAGENTS

1. Chloroform.
2. Anhydrous sodium sulphate.
3. Sodium hydroxide standard solution (0.002N)
4. Glacial acetic acid.
5. Potassium iodide, saturated solution. Freshly prepared.
6. Sodium thiosulphate standard solution(0.01N)
7. Phenolphthalein indicator, 1.0% solution in 95% ethanol.
8. Starch solution as indicator, 1% (m/v) freshly prepared.
9. Ethanol, 95% neutralized with 0.1 N sodium hydroxide using 1% phenolphthalein solution as indicator.

PROCEDURE

Trim as much fat as possible from the sample. Macerate 50 g of the fat mechanically with 200 ml chloroform, filter. Re-filter through a paper containing anhydrous sodium sulphate and keep the filtrate in a stoppered flask.

Pipette 20 ml of the filtrate into a tared evaporating dish. Evaporate off the chloroform on a water-bath and then dry in an air oven at 100°C for 3 hours. Cool the dish in desiccator before weighing. (This is to determine the fat content).

Determine the FFA as follows: Pipette 20 ml of filtrate into a 250 ml conical flask. Add 20 ml of neutralized ethanol. Titrate with 0.02N sodium hydroxide solution using phenolphthalein as indicator. Shake vigorously during the titration.

Next, determine the peroxide value as follows: Pipette 20 ml of filtrate into a 250 ml glass-stoppered Erlenmeyer flask. Add 15 ml glacial acetic acid and 0.5 ml of saturated potassium iodide. Allow the solution to stand with occasional shaking for exactly 1 minute and then add 30 ml of distilled water. Titrate with 0.01 N sodium thiosulphate adding it gradually and with constant and vigorous shaking. Continue the titration until the yellow colour has almost disappeared. Add about 0.5 ml of starch indicator solution. Continue the titration, shaking the flask vigorously near the end point to liberate all the iodine from the chloroform layer. Add the thiosulphate dropwise until the blue colour has just disappeared.

Conduct a blank determination of the reagents daily. The blank titration must not exceed 0.5 ml of the 0.01N sodium thiosulphate solution.

CALCULATION

Fat content in 20 ml of chloroform extract:

$$M = M1 - M2$$

Where: M1 = mass in grams of dish and contents
M2 = mass in grams of empty dish

FFA (as oleic acid on extracted fat), % (m/m):

$$\% \text{ FFA} = \frac{V1 \times N \times 28.2}{M}$$

Where: V1 = volume in millilitres of sodium hydroxide
N = normality of sodium hydroxide
28.2 = milliequivalent weight of oleic acid (include factor of 100 for %)

(Note: FFA are frequently expressed in terms of acid value instead of % oleic acid. The acid value is defined as the number of mg of KOH necessary to neutralize 1 gram of extracted fat. To convert % oleic acid to acid value, multiply the former by 1.99).

Peroxide value, milliequivalents of peroxide oxygen per kg of extracted fat:

$$PV = \frac{V2 \times N2 \times 1000}{M}$$

Where: V2 = volume in millilitres of sodium thiosulphate solution used

N2 = normality of sodium thiosulphate solution used.

INTERPRETATION

Animal fats largely consist of glycerides which are esters of the trihydric alcohol glycerol with fatty acids of various types. These acids are of the long-chain variety, having 14 to 18 carbon atoms in each chain. They may be fully saturated, such as stearic and palmitic acid, or unsaturated, such as oleic acid and linoleic acid where one or more reactive double bonds occur in the side chain.

Unsaturated fatty acids are much more unstable chemically than are saturated acids as oxidation can readily occur at the site of the double bonds causing the fatty acid chain to break down into fragments. This causes the development of rancid off-odours and off-flavours. Such oxidation is caused by atmospheric oxygen and increases with increasing temperature. It is catalysed by metals such as copper and iron. The extent to which rancidity has developed in a fat is measured by its peroxide value. Most fresh beef samples give a peroxide value of 0-1. A value of 5 could be considered a maximum acceptable level.

Animal tissues contain enzymes called lipases which have the ability to hydrolyse fats, splitting fatty acid molecules from the glycerol molecule. The extent to which this occurs can be determined by measuring the free fatty acid content. In a good quality product, the free fatty acid content should not exceed 1.2%, expressed as oleic acid in the extracted fat.

REFERENCES

Sully, B.D., 1954, Analyst 79, 86.

IUPAC Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th Ed., Pergamon Press, 1979, Method 11.D.1.

5.2 MEAT PRODUCTS

ROUTINE ANALYSIS

The determination of the meat content of a meat product is made on the assumption that a specific lean defatted meat has an average nitrogen content, referred to as the nitrogen factor. For example, lean defatted raw beef is taken to have an average nitrogen content of 3.55 percent. If a sample is found to contain 1.775 percent of nitrogen, the lean defatted beef content is taken to be 50 percent. Addition of the amount of fat in the sample gives its total meat content.

Other attempts to assess meat content include determination of particular amino-acids derived from protein such as 3-methylhistidine (Rangeley and Lawrie (11)) and methionine. The reliability of these values as assessments of meat content is not yet well-established.

Meat products may have to be examined for preservatives, especially SO_2, nitrite and nitrate. Other constituents that may have to be determined or looked for are salt, added colour, starch, cereal and other fillers, textured vegetable protein, dried milk, soya bean meal, added phosphates and ascorbic acid. Sometimes, products contain excessive amounts of gristle or connective tissue. The level of hydroxyproline present can be used to estimate the proportion of rind and gristle in a meat product. Protein from collagen and skin contains 11-14 percent. The EEC Intervention Board uses the formula hydroxyproline x 8 = gristle/collagen. Canned products should be examined for lead, tin and arsenic. The lead content of meat products such as corned beef in tins sealed by spot soldering may be high near the seal only. Tests that are routine for canned foods such as vacuum, water capacity of the can, headspace and drained weight (where appropriate) may also be made on canned meat products.

Bacterial spoilage due to Clostridium species, Streptococcus faecalis, etc. may be due to a mixture of poor hygiene and inadequate pickling. Analysis for salt, pH, nitrate and nitrite may be helpful.

The AMC (20) considered that the proportion of creatine and creatinine was a suitable parameter for assessment of the quality of a meat extract and that in addition the moisture, ash, chloride and total nitrogen should be determined in routine analysis. The report gives a method for the estimation of creatine and creatinine, republished in Official, Standardized and Recommended Methods of Analysis.

The presence of offal and textured vegetable proteins in comminuted meat is investigated mainly by histological techniques at the present time (Hole et al (21)). Most textured vegetable proteins contain more magnesium (around 6000 mg/kg) than meat (300-3000 mg/kg). Soya bean protein isolate from North America is required to contain titanium diobxide as a marker but soya bean products of lower protein content are not. The immunochemical techniques of double diffusion, single radial diffusion, haemagglutination and immunoelectrophoresis suffer from the disadvantage of requiring soya specific agglutinin and tend to show cross-reactions with some other legumes. They are screening, qualitative methods. Polyacrylamide gel electrophoresis and isoelectric focusing of extracts prepared with urea, 2-mercaptoethanol or sodium dodecyl sulphonate work well on uncooked products but less well if the meat is cooked.

Soya products used in meat include defatted flour (about 50 percent protein), protein concentrate (70-73 percent protein) and soya protein isolate (over 90 percent protein). Soya flour may be detected microscopically by looking for "beaker cells", but these will be absent from protein isolate. The proportion of soya flour present may be assessed from the hemi-cellulose level (Bennett (22)), or pentoses and pentosans (Fredholm (23)), or by the levels of fibre, manganese and magnesium (Formo, Honold and MacLean (24)) or by gel

electrophoresis (Parsons and Laurie (25), Guy et al (26)). The validity of a method of analysis may be affected by the type of soya product present and by the other constituents of a meat product.

Formo, et al (24) report the following figures for textured soy flour (50 percent protein) and ground beef:

	Average Concentration		Standard Deviation		Coefficient of Variation	
	Textured soyflour	Ground beef	Textured soyflour	Ground beef	Textured soyflour	Ground beef
Magnesium	2049 mg/kg	151 mg/kg	186 mg/kg	7.4 mg/kg	6.3%	4.9%
Manganese	33.4 mg/kg	0.125 mg/kg	3.4 mg/kg	0.025 mg/kg	10.1%	20%
Fibre	2.03%	0.01%	0.17%	-	13.3%	-

MEAT CONTENT

The moisture, fat, protein and ash are determined and the carbohydrate or cereal filler obtained by difference. The amount of nitrogen derived from the filler (assumed to be present herein to the extent of 2 percent, or 12.5 percent as protein) is subtracted from the total nitrogen and the rest assumed to be derived from the meat. The total meat content is then calculated from the meat-derived nitrogen and fat contents, as follows:

$$\text{Lean defatted meat} = \frac{\text{nitrogen}}{\text{nitrogen factor}} \times 100$$

The nitrogen factor is the nitrogen content of lean defatted meat of the species present in the sample under analysis. Commonly accepted figures are as follows (Meat Products Sub-Committee of the Society for Analytical Chemistry, U.K. (27)(28)(29)(30)(31)(32)(33)):

Beef	3.55	Chicken, breast	3.9
Veal	3.35	dark meat	3.6
Pork	3.45	whole	3.7
Tongue (Ox and Pig)	3.0	Turkey, breast	3.9
		dark meat	3.5
Ox Liver	3.45	whole	3.65
Pig Liver	3.65	Blood	3.2
Kidney	2.7		

Total meat = lean defatted meat plus fat.

Alternatively, it may be considered that lean meat contains 10 percent of fat (interstitial). Therefore, lean meat = 1.1 x lean defatted meat and the remaining fat is regarded as free fat. The amount of interstitial fat in lean meat varies considerably but 10 percent is taken as an average for the purposes of the calculation.

There are a number of considerations to be borne in mind when using this calculation. The topic has been reviewed by Pearson (34). Incorporation of a certain amount of meat extract or other nitrogen sources may lead to an

erroneously high assessment of the meat content from the nitrogen figure. Monosodium glutamate, sodium guanylate and similar flavour enhancers increase the nitrogen content in this way.

The nitrogen factors apply to the fresh meat with the normal amount of accompanying water. Pressed and cured meats such as canned beef, cured pates and salami ingredients have had water removed during processing and therefore contain a higher proportion of protein. For example, a factor of 4.7 has been suggested for traditional corned beef.

It is advisable to carry out qualitative tests for carbohydrate such as adding iodine to detect the presence of starch. It may be possible to make assumptions about the nitrogen content of carbohydrate filler. The calculation is modified accordingly. For example, if rusk of an assumed or known nitrogen content of 2 percent (equivalent to 12.5 percent protein) is present:

Carbohydrate (C) = 100 - (moisture + fat + protein + ash)

Nitrogen attributable to filler = 0.02C

$$\text{Lean defatted meat} = \frac{(N - 0.02C)}{\text{nitrogen factor}} \times 100$$

Meat analysis for the presence of adulteration by other protein sources (e.g. soya flour, textured vegetable protein (TVP)) which give an elevated apparent meat content has been an area of great analytical interest in recent years. A review of soya protein determinations was carried out by Olsman and Hitchcock (35). Soya can be detected and quantified microscopically, using histological staining techniques. (Flint and Meech (36)). A more recent method, the ELISA technique (Enzyme-linked Immunoassay) is becoming more widely accepted as a routine quantitative test for Soya Protein (Hitchcock et al (37), Crimes et al (38), Griffiths et al (39)).

The addition of offals to meat products can be detected histologically (Hole et al (21), Flint and Meech (36)) and this method can provide a semi-quantitative assessment. Gel electrophoresis methods can be used to identify offals but more research is required for quantitative analysis.

Foreign meats (i.e. other meat types present in meat products) has for many years relied upon Unlenluths test (Castledine and Davis (40)) which is a qualitative method for uncooked products and is non-specific in many cases. Electrophoresis methods have become more widely used for identifying meat types (Hoyem and Thorson (41), Coduri and Rand, (42)). Quantification of foreign meat in meat products by this type of method is as yet in its early stages of development.

HYDROXYPROLINE

PRINCIPLE

The sample is hydrolysed in a hydrochloric acid solution containing stannous chloride. After neutralization, filtration and dilution, the hydroxyproline is oxidized by chloramine-T, followed by the formation of a red compound with 4-dimethylaminobenzaldehyde. This is measured spectrophotometrically.

APPARATUS

1. Mechanical meat mincer, laboratory size, fitted with a plate with holes not exceeding 4 mm in diameter.

2. Round or flat-bottomed hydrolysis flask, capacity about 200 ml, wide-necked, equipped with an air-cooled or water-cooled condenser.

3. Electric heating device (for example heating mantle, hotplate or electrically heated sand bath).

4. Filter paper discs, diameter 12.5 cm (e.g. S and S No. 187 is suitable).

5. pH meter.

6. Aluminium or plastics foil.

7. Water bath, thermostatically controlled at 60 $\pm$ 0.5°C.

8. Spectrophotometer, capable of being used at a wavelength of 558 $\pm$ 2 nm.

REAGENTS

1. Stannous chloride solution: Dissolve 7.5 g of stannous chloride dihydrate ($SnCl_2.2H_2O$) in water, dilute to 500 ml and add 500 ml of hydrochloric acid.

2. Hydrochloric acid, 6N solution: Mix equal volumes of hydrochloric acid and water.

3. Sodium hydroxide, 10N solution: Dissolve 40 g of sodium hydroxide in water. Cool and dilute to 100 ml.

4. Sodium hydroxide, 1N solution: Dissolve 4 g of sodium hydroxide in water. Cool and dilute to 100 ml.

5. Buffer solution, pH 6.0: Dissolve in water: 50 g of citric acid monohydrate, 12 ml of acetic acid, 120 g of sodium acetate trihydrate, 34 g of sodium hydroxide, and dilute to 1000 ml with water. Mix this solution with 200 ml of water and 300 ml of propan-1-ol. This solution is stable for several weeks at 4°C.

6. Chloramine-T reagent: Dissolve 1.41 g of N-chloro-p-toluenesulphonamide, sodium salt (chloramine-T) in 10 ml of water and successive add 10 ml of propan-1-ol and 80 ml of the buffer solution pH 6.0. Prepare this solution immediately before use.

7. Colour reagent: Dissolve 10.0 g of 4-dimethylaminobenzaldehyde in 35 ml of 60% perchloric acid solution and then slowly add 65 ml of propan-2-ol. Prepare this solution on the day of use. (Note: Purification of the 4-dimethylaminobenzaldehyde is necessary. Proceed as follows: Prepare a saturated solution of the 4-dimethylaminobenzaldehyde in hot 70% ethanol. Cool, first at room

temperature, and finally in a refrigerator. After about 12 hours filter on a Buchner funnel. Wash with a little 70% ethanol. Again dissolve in hot 70% ethanol. Add cold water and agitate thoroughly. Repeat this procedure until a sufficient quantity of milk-white crystals has been formed. Place in the refrigerator overnight. Filter on the Buchner funnel, wash with 50% ethanol and vacuum dry over phosphorus pentoxide.

8. Hydroxyproline standard solution: Prepare a stock solution by dissolving 100 mg of hydroxypyrrolidine-alpha-carbonic acid (hydroxyproline) in water. Add 1 drop of hydrochloric acid solution and dilute to 100 ml with water. On the day of use, dilute 1 ml of the stock solution to 100 ml with water in a volumetric flask. Then prepare four standard solutions by diluting 10, 20 and 40 ml of this solution to 100 ml with water to obtain hydroxyproline concentrations of 1, 2, 3 and 4 µg/ml respectively.

PROCEDURE

Prepare the sample as follows: Raw meat and raw meat products: Reduce intact meat to small cubes (approximately 0.5 cm^3) by cutting it while it is cold (just below 0°C) using a sharp knife. Either place the sample in a container and seal the latter hermetically, or vacuum pack the sample in a heat-resistant plastic film; then heat so as to maintain a temperature of at least 70°C for at least 30 minutes in the geometrical centre of the sample; cool and proceed as follows: (Note: in this way, the raw connective tissue is softened and less resistant to homogenization by mincing). Cooked meat and cooked meat products: Make the sample homogeneous by passing it at least twice through the meat mincer and mixing. Keep the homogenized sample in a completely filled, airtight, closed container and store it in such a way that deterioration and change in composition are prevented. Analyse the sample as soon as possible, but always within 24 hours.

Weigh, to the nearest 1 mg about 4 g of the prepared sample into the hydrolysis flask. Ensure that none of the sample adheres to the side-wall of the flask. Add some boiling chips and 100 $\pm$ 1 ml of stannous chloride solution. Heat to gentle boiling and maintain for 16 hours under reflux (conveniently overnight). (Note: if desired the hydrolysis may alternatively be accomplished in two periods, each of 7 to 8 hours on consecutive days. This alternative procedure has been proved experimentally to yield results that are not significantly different from those obtained with a single hydrolysis period of 16 hours).

Filter the hot hydrolysate through filter paper into a 200 ml volumetric flask. Wash the filter three times with 10 ml portions of hot hydrochloric acid solution and add the washings to the hydrolysate. Make up to the mark with water. (Note: the hydrolysate can be kept at this state for at least one week under refrigeration).

Using a pipette, transfer into a beaker a volume V ml of the hydrolysate so as to obtain a hydroxyproline concentration within the range 1 to 4 µg/ml after dilution to 250 ml. (Note: In most cases, V will be of the order of 5 to 25, depending on the amount of connective tissue present in the sample). With the aid of the pH meter, adjust the pH to 8 $\pm$ 0.2 by dropwise addition first of 10 N sodium hydroxide solution then, when approaching the required pH, of 1 N sodium hydroxide solution. Remove the tin hydroxide precipitate by filtering the solution through a filter paper washing the precipitate on the filter at least twice with 50 ml portions of water and collecting the filtrate and washings in a 250 ml volumetric flask. Make up to the mark with water and mix.

Transfer 4.00 ml of this solution into a test tube and add 2.00 ml of the chloramine-T reagent. Mix and leave at room temperature for 20 ± 1 minute.

Add 2.00 ml of the colour reagent, mix thoroughly and cap the tube with aluminium or plastics foil. Transfer the tube quickly into the water bath, controlled at 60±0.5°C and heat for exactly 20 minutes. Cool under running tap water for at least 3 minutes. Measure the absorbance at 558 ± 2 nm in a glass cell using the spectrophotometer. Carry out in duplicate the same procedure, substituting water for the diluted hydrolysate. (Note: if the absorbance of the blank exceeds 0.040, a fresh colour reagent should be prepared and, if necessary the 4-dimethylaminobenzaldehyde should be repurified).

Carry out the same procedure again but substituting 4.00 ml of each of the four diluted standard hydroxyproline solutions for the diluted hydrolysate. Plot the measured absorbance values, corrected for the blank value, against the concentrations of the standard hydroxyproline solutions and construct the best fitting straight line through the plotted points and the origin.

CALCULATION

Calculate the hydroxyproline content, H, of the sample, as a percentage by weight, from the formula:

$$H = \frac{5h}{w \times V}$$

Where:

h = hydroxyproline concentration, in micrograms per millilitre, of the diluted hydrolysate

w = weight, in grams, of the test portion

V = volume, in millilitres, of solution taken for dilution to 250 ml.

INTERPRETATION

Duplicate determinations should be run to determine the repeatability of the analysis. The values on duplicate determinations should not differ by more than 5% of the arithmetic mean of the two values.

The presence of hydroxyproline is an indication of excess collagen-containing skin and connective tissue in the meat product. Pearson's Chemical Analysis of Foods, 8th Ed., gives the following table of hydroxyproline content of tissue:

Tissue (fat free)	**Hydroxyproline Content (%)**
Collagen	13.4 - 14.5
Tendon	11.2 - 13.2
Tendon with muscle	12.3 - 13.3
Cooked skin	11.0 - 12.0
Skeletal muscle	0.002- 0.07
Plant materials	none

NITRATE AND NITRITE

PRINCIPLE

Sodium nitrite in a product produces the characteristic red colour commonly associated with cured meats. The nitrite itself combines with the red meat pigment, myoglobin, to form the relatively stable pink compound which is carried through the smoking and cooking operation.

Nitrate is added to provide a reservoir of nitrite. The nitrate is active only after conversion to nitrite. This conversion is brought about by certain bacteria normally present in meat.

An aromatic primary amine will react with an acidified solution of nitrite to produce a diazonium salt. If this salt is then condensed or coupled with another primary aromatic amine (N-1-naphthylethyenediamine·2 HCl) an aminoazo compound (a red dye) is formed which is measured in a spectrophotometer at 538 nm.

This method determines nitrite directly and the nitrate indirectly, after reduction of the nitrate to nitrite using a cadmium column.

APPARATUS

1. Beakers.

2. Glass column and glass wool.

3. Volumetric flask.

4. Pipettes.

5. Spectrophotometer.

REAGENTS

1. Zinc rods, ca 15 cm x ca 6 mm - 3 to 5 rods are needed per cadmium column.

2. Cadmium sulfate solution, 30 g/L - dissolve 37 g of $(CdSO_4)_3.8H_2O$ in water and dilute to 1 litre.

3. Hydrochloric acid, 0.1 N.

4. Ammonium buffer solution, pH 9.6 - 9.7. Dilute 20 ml concentrated HCl with 500 ml water. Add 10 g Na EDTA dihydrate and 55 ml concentrated NH_4OH. Dilute to 1 litre. Check pH.

5. Sodium nitrate standard solution - dissolve 1.2329 g $NaNO_3$ in water and dilute to 100 ml in a volumetric flask. This is the stock solution. Pipette 5 ml of this solution into a litre volumetric flask. Dilute to volume with water. This is the working solution (61.6 µg/ml) and must be prepared on the day of use.

6. Sulphanilamide solution, 0.2%. Dissolve 2 g sulphanilamide in 800 ml water, warming if necessary. Cool and add 100 ml of concentrated HCl. Dilute to 1 litre.

7. Coupling reagent, 0.01%. Dissolve 0.25 g N-1-napthyl-ethylenediamine dihydrochloride in water and dilute to 250 ml. Store in tightly closed brown-glass bottle. Solution may be kept in a refrigerator for up to one week.

8. Hydrochloric acid, (1+1).

9. Borax solution. Dissolve 50 g $Na_2B_4O_7.10H_2O$ in 1 litre warm water. Cool before using.

10. Activated charcoal.

11. Potassium ferrocyanide solution, 1%. Dissolve 10 g $K_4Fe(CN)_6.3H_2O$ in water and dilute to 1 litre.

12. Zinc acetate solution, 21.6%. Dissolve 216 g zinc acetate dihydrate and 30 ml glacial acetic acid in water and dilute to 1 litre.

13. Sodium nitrite standard solution - dissolve 1.0 g of $NaNO_2$ in water and dilute to 100 ml (1% solution) in a volumetric flask. This is the stock solution. Pipette 5 ml of this into a litre volumetric flask and dilute to volume with water (50 µg/ml). This is the working solution and must be prepared fresh each day.

PROCEDURE

Preparation and pre-treatment of cadmium column:

a. Place 3 to 5 zinc rods in the cadmium sulphate solution contained in a beaker (1 litre of cadmium sulphate solution is sufficient for preparing one cadmium column).

b. Remove the spongy metallic cadmium deposit from the zinc rods every 1 or 2 hours by swirling them in the solution or rubbing them against each other.

c. After 6 to 8 hours, decant the solution and wash the deposit twice with 1 litre of water, taking care that the cadmium is continuously covered with a layer of liquid.

d. Transfer the cadmium deposit with 400 ml of hydrochloric acid solution to a laboratory mixer, blend for 10 seconds and return the cadmium slurry to the beaker.

e. Occasionally stir up the cadmium deposit with a glass rod. Leaving it overnight in hydrochloric acid.

f. Stir to remove bubbles and decant the solution. Wash the cadmium slurry twice, each time with 1 litre of water. (Keep the cadmium under liquid).

g. Fit a glass wool plug to the bottom of the glass column intended to contain the cadmium.

h. Wash the cadmium into the glass column with water until the height of the cadmium bed is about 17 cm.

i. Drain the column occasionally during filling, taking care not to allow the level of the liquid to fall below the top of the cadmium bed. Eliminate inclusions of gas (by stirring with a wire or thin glass rod).

j. Fill the reservoir completely with water and check the flow of the liquid. It should flow out at a rate not exceeding 3 ml per minute to avoid the risk of incomplete reduction.

k. Wash the cadmium column successively with (1) 25 ml of 0.1N HCl; (2) 50 ml of water; and (3) 25 ml of the ammonia buffer solution. The column is now ready for use.

Check of cadmium column reducing efficiency:

a. Pipette 20 ml of sodium nitrate working standard solution (61.6 µg/ml) and simultaneously add 5 ml of ammonia buffer solution into the reservoir on top of the cadmium column. (Note: 61.6 µg/ml nitrate is equivalent to 50.0 µg/ml nitrite). Collect the effluent in a 100 ml volumetric flask.

b. When the reservoir is nearly empty (about 20 min), wash the walls with about 15 ml of water; repeat the same treatment with another 15 ml portion of water (takes about 5 min, each time). After this portion has run into the column as well, completely fill the reservoir with water.

c. After nearly 100 ml of effluent has been collected, remove the flask from under the column and dilute to the mark with water. Pipette 10 ml of the eluate into a 100 ml volumetric flask.

d. Add 10 ml sulphanilamide solution followed by 6 ml of (1+1) HCl, mix and leave the solution for 5 minutes at room temperature in the dark. Add 2 ml coupling reagent, mix and leave the solution for 3 to 10 minutes at room temperature in the dark. Dilute to the mark with water.

e. Measure the absorbance of the solution in a 1 cm cell using a spectrophotometer at a wavelength of 538 mm. Obtain the nitrite concentration of the eluate from the calibration curve.

f. If the nitrite concentration of the eluate as determined from the calibration curve is below 0.9 µg of sodium nitrite per millimeter (i.e. 90% of the critical value), the cadmium column should be rejected.

Preparation of calibration curve:

a. Pipette the following amounts of the $NaNO_2$ working standard (50 µg/ml) into six 100 ml volumetric flasks: 1 ml, 2 ml, 4 ml, 5 ml, 10 ml, 20 ml. Make each flask to volume using water.

b. Pipette 10 ml of each of the above into six 100 ml volumetric flasks. In a seventh flask, pipette 10 ml water. (These represent $NaNO_2$ concentrations of 0, 0.5, 1.0, 2.0, 2.5, 5.0 and 10.0 µg/ml, respectively).

c. Add water to all seven flasks to a volume of about 60 ml. Add 10 ml of sulphanilamide solution, followed by 6 ml of (1+1) HCl to each flask, mix and leave the solution for 5 minutes at room temperature in the dark. Add 2 ml of coupling reagent, mix and leave the solution for 3 to 10 minutes at room temperature in the dark. Dilute each flask to the mark with water.

d. Prepare a calibration curve by plotting absorbance against concentration (µg/ml).

Prepare the sample by finely mincing and mixing. (Start analysis as soon as possible after preparation - the minced sample may be stored only overnight in a closed, well-filled container in a refrigerator).

Weigh, to the nearest 1 mg, 10 g of the sample into a 200 ml volumetric flask with the aid of a funnel and 100 ml of hot water. Add 5 ml of borax-solution and 0.5 g activated charcoal. Heat, with repeated agitation, for 15 minutes on a boiling water bath. Allow the flask and its contents to cool to room temperature.

After not less than an hour, add successively 2 ml each of potassium ferrocyanide and zinc acetate solutions. Mix thoroughly after each addition. Dilute to 200 ml with water, mix and allow the flask to stand for 30 minutes at room temperature. Carefully decant the supernatant liquid and filter it through a No. 44 filter paper to obtain a clear filtrate.

Pipette 20 ml of the filtrate into the reservoir on top of the column and simultaneously add 5 ml of ammonia buffer solution. Collect the effluent from the column in a 100 ml volumetric flask.

When the reservoir is nearly empty, wash the walls with about 15 ml of water; repeat the same treatment with another 15 ml portion of water. After this portion has run into the column as well, completely fill the reservoir with water. After nearly 100 ml of effluent has been collected, remove the flask from under the column and dilute to the mark with water.

Pipette an aliquot of the eluate (but not more than 25 ml) into a 100 ml volumetric flask. At the same time, pipette 25 ml of sample filtrate (containing less than 100 µg NO_2) into a 50 ml volumetric flask.

For both, proceed as specified above under, "preparation of calibration curve".

CALCULATION

Reducing efficiency of Cd column:

$$\% \text{ efficiency} = \frac{C \times 50 \times 1.23 \times 100}{S}$$

Where: C = µg/ml nitrite from calibration curve
S = µg/ml nitrate working standard (61.5 µg/ml)
1.23 = factor to convert nitrite to nitrate

Na nitrite ($NaNO_2$) without reduced nitrate ($NaNO_3$)

$$\text{ppm } NaNO_2 = \frac{C \times 100 \times 200}{S \times V}$$

Where: C = µg/ml nitrite from calibration curve
S = sample weight in grams
V = ml aliquot of filtrate taken

Na nitrite ($NaNO_2$) including reduced nitrate ($NaNO_3$)

$$\text{ppm } NaNO_2 + \text{reduced } NaNO_3 = \frac{C \times 100 \times 200 \times 5}{S \times V}$$

Where: C = µg/ml total nitrite from calibration curve
S = sample weight in grams
V = ml aliquot of eluate taken

Na nitrate ($NaNO_3$)

ppm nitrate = ($NaNO_2/NaNO_3$ calculation - $NaNO_2$ calculation) x 1.23

Where: 1.23 = factor to convert nitrite to nitrate.

REFERENCES

ISO 2918:1975, Meat and Meat Products - Determination of Nitrite Content.

ISO 3091:1975, Meat and Meat Products - Determination of Nitrate Content.

5.3 TEXT REFERENCES

1. SCHMALL, M., PIFER, C.W. & WOLLISH, E.G. 1953. Analytical Chemist 25 1486.

2. SCHMALL, M., PIFER, C.W. & WOLLISH, E.G., DUSCHINSKY, R. & GAINER, H. 1954. Analytical Chemist 26 1521.

3. DONOHO, A.L., JOHNSON, W.S., SIECK, R.F. & SULLIVAN, W.L. 1973. Journal of the Association of Official Analytical Chemists 56 (4) 785-792.

4. KOHRMAN, K.A. & MACGEE, J. 1977. Journal of the Association of Official Analytical Chemists 60 5-8.

5. SMITH, W.G. & McNEIL, E.E. 1972. Analytical Chemist 44 1084-1087.

6. PONDER, C. 1974. Journal of the Association of Official Analytical Chemists 57 919-923.

7. UMBERGER, E.J., GASS, G.H. & CURTIS, J.M. 1958. Endocrinology 63 806-15.

8. UMBERGER, E.J., CURTIS, J.M. & GASS, G.H. 1959. Journal of Animal Science 18 221-6.

9. KLING, R.E. & LAZAR, A. 1976. Food & Drug Administration By-Lines No. 2 Sept.

10. HEFFTER, A., VOCKS, R. & WALTHER, G. 1972. Deutsche Lebensmittel-Rundschau 68 (11) 367.

11. RANGELEY, W.R.D. & LAWRIE, R.A. 1976. Journal of Food Technology 11 143-59.

12. HANKIN, L. 1965. Journal of the Association of Official Agricultural Chemists 48 1122-26.

13. HAMENCE, J.H. & KUNWARDIA, G.H. 1974. Polyphosphates and Moisture in Meat Products Journal of the Association of Public Analysts 12 85-92.

14. DORO, B. & REMOLI, S. 1963. Bolletino Laboratorio Chimico Provinciali 14 (1) 23.

15. IIDA, T. & YAMABE, T. 1971. Journal of Chromatography 54 413.

16. TOGONAI, Y. & TANAKA, K. 1974. Shokukin Eiseigaku Zasshi 15 (5) 331-6 (Japan).

17. PESINO DE BATTISTIS, P. & MARZADORI, F. 1974. Acta. Medica Veterinaria 20 (1-2) 41-7.

18. VAN HOOF, J., MOLLAERT, P. & VANDENBRANDE, G. 1974. Vlaams Diergeneeskundig Tijdschrift 43 (5) 260-73.

19. KARASZ, A.B., ANDERSEN, R. & POLLMAN, R. 1976. Journal of the Association of Official Analytical Chemists 59 (6) 1240-3.

20. AMC Analyst 1951 76 329-333.

21. HOLE, N.H., ROBERTS, M.P. & BRYCE JONES, K. 1964. Analyst 89 332-337.

22. BENNETT, O.L. 1948. Journal of the Association of Official Agricultural Chemists 31 513-9.

23. FREDHOLM, H. 1967. Food Technology 21 93-4.

24. FORMO, M.W., HONOLD, G.R. & MACLEAN, D.B. 1974. Journal of the Association of Official Analytical Chemists 57 (4) 841-6.

25. PARSONS, A.L. & LAWRIE, R.A. 1972. Journal of Food Technology 7 (4) 455-62.

26. GUY, R.C.E., JAYARAM, R. & WILLCOX, C.J. 1973. Journal of Scientific Food Agriculture 24 (12) 1551-63.

27. Analyst 1961 86 557-60.

28. Analyst 1963 88 422-3, 583-4.

29. Analyst 1964 89 630 1.

30. Analyst 1965 90 256-7, 579-80, 581.

31. Analyst 1966 91 538-9.

32. Analyst 1967 92 326-7.

33. Analyst 1968 93 476-7, 478-9.

34. PEARSON, D. 1975. The Examination of Meat Products with Special Reference to the Assessment of the Meat Content. Analyst 100 73-81.

35. OLSMAN, W.J. & HITCHCOCK, C. Development in Food Analysts Techniques, Vol 2 225-60 (Ed. R.D. King) Applied Science Publishers, 1980.

36. FLINT, F.O. & MEECH, M.V. 1978. Analyst 103 252.

37. HITCHCOCK, C.H.S., BAILEY, F.J., CRIMES, A.A., DEAN, D.A.G. & DAVIS, P.J. 1981 Journal of Scientific Food Agriculture 32 157.

38. CRIMES, A.A., BAILEY, F.J. & HITCHCOCK, C.H.S. 1981. Analytical Procedures 164.

39. GRIFFITHS, N.M., BILLINGTON, M.J. & GRIFFITHS, W. 1981. Journal of the Association of Public Analysts 19 113.

40. CASTLEDINE, S.A. & DAVIES, D.R.A. 1968. Journal of the Association of Public Analysts 6 39.

41. HOYEM, T. & THORSON, B. 1970. Journal of Agricultural and Food Chemistry 18 737.

42. CODURI, R.J. & RAND, A.G. 1972. Journal of the Association of Official Analytical Chemists 55 461.

Further Reading

LAWRIE, R.A. 1979. Meat Science Pergamon.

WIGGINS, G.S. & WILSON, A. 1976. A Colour Atlas of Meat & Poultry Inspection Wolfe Medical Publications Ltd.

WILSON, N.R.P. (Ed) 1981. Meat & Meat Products : Factors Affecting Quality Control Elsevier.

6. VEGETABLES AND FRUITS

6.1 FRESH VEGETABLES AND FRUITS

ROUTINE ANALYSIS

Although fresh produce is among the commonest of foods, regulatory control does not usually involve compositional analysis. Visual inspection is adequate to detect gross adulteration, so routine laboratory analysis of the fresh article is restricted to testing for contaminants in most cases. Vegetables and fruits may be examined for potentially toxic elements such as copper, lead, cadmium, arsenic and pesticides. Mycotoxins can also occur in these products (e.g. aflatoxin in figs, patulin in apples). Analysis for such contaminants should preferably be done as part of systematic contaminants survey. The determination of starch and sugars is often of relevance to dietary surveys.

Potatoes at certain stages of their growth occasionally contain an undesirably high level of solanine, which is not entirely destroyed by cooking. If the tuber has been exposed to light while growing it will be green due to the presence of chlorophyll, but this is not necessarily accompanied by a high solanine content. Baker, Lampitt and Meredith (1) describe a method for the determination of solanine.

The composition and analysis of onions are described by Sherratt (2). The yellow patches occasionally seen on onions show under the microscope as needle crystals. They are composed of quercetin and are generally considered to denote prolonged storage.

The proximate composition of vegetables change greatly with ripening. The proximate composition also varies due to variety, climate, soil, stage at harvest and many other factors so it is often very difficult to relate composition to quality parameters of interest to the regulatory chemist. The quality of peas and canned corn is judged by the percentage of alcohol-insoluble solids.

Fresh fruit should be examined for pesticides, especially fungicides, including dithiocarbamates, biphenyl, 2-hydroxybiphenyl, thiabendazole and benomyl. Compounds of copper, arsenic and lead may also have been used as fungicides on some crops. Citrus fruits and dried fruits may be treated with mineral oil, which can be removed with solvent, which is then evaporated and the unsaponifiable matter weighed. Fruits are sometimes treated with colouring matter in an attempt to improve the appearance.

Proximate analysis of fruit and vegetable products is generally carried out by standard methods. Homogeneity of the sample can be difficult to achieve and it may be necessary to blend or otherwise homogenise quite large amounts. Reports should always state how the sample was prepared, whether it was peeled, ready-to-eat, cooked or uncooked, etc. For the determination of total solids, ISO/R 1026-1969 recommends drying at 70°C and 20-25 mm mercury to 'constant weight' (change of less than 0.001 g/hr).

For the vacuum drying method, a slow current of air (about 40 L/hr at NTP), dried by sulphuric acid, is recommended. Determinations on 10 percent sucrose solution and 1 percent lactic acid are suggested as checks on the method.

6.2 CANNED VEGETABLES AND FRUITS

COMPOSITION

The various Codex recommended standards for canned fruits generally set limits for drained weight, minimum fill, cut-out strength of the syrup and quality criteria such as maxima for blemished, broken or otherwise defective fruit. The standards also indicate acceptable labeling and in some cases limits for additives such as firming agents, flavours, acidifying agents, anti-foaming agents, colouring matters, anti-clouding agents and antioxidants. Some standards recommend a maximum for tin of 250 mg/kg. Codex standards for canned fruit packing syrups are:

Minimum Cut-out Strength of Syrup in Degrees Brix

	Slightly sweetened syrup	Extra light syrup	Light syrup	Heavy syrup	Extra heavy syrup
Peaches	-	10	14	18	22
Grapefruit	12	-	16	18	-
Pineapple	-	10	14	18	22
Plums	-	11	15	19	25
Raspberries	-	11	15	20	26
Pears	-	10	14	18	22
Strawberries	-	10	14	18	22
Mandarin oranges	-	10	14	18	22
Fruit Cocktail	-	10	14	18	22

The following drained weight standards have been recommended by the Codex Alimentarius Commission:

Product	Minimum % drained weight		
Canned mushrooms	53 (27.5 if packed in sauce)		
Canned mature processed peas	60		
Canned green peas	60		
Canned sweet corn (whole kernel)	61		
Canned green beans and canned wax beans	50 for "whole" and "sliced lengthwise" style; 55 for other styles		
Canned asparagus, peeled	60 for long shoots; 58 for all other styles		
Canned asparagus, unpeeled	57 for long shoots and shoots; 55 for all other styles		
Canned tomatoes	50		
Canned peaches	In extra heavy and heavy syrup	In light and extra light syrup	Solid pack
Clingstone type	57	59	84
Freestone type	54	56	82

Product	Minimum % drained weight
Canned grapefruit	50
Canned pineapple	58 for all styles other than crushed or chips; 63 for crushed or chips style regular pack; 73 for crushed or chips style heavy pack; 78 for crushed or chips style solid pack.
Canned plums, whole style	50 for whole; 55 for halves
Canned raspberries	37
Canned pears	50 for whole; 60 for diced; 53 for all other styles
Canned strawberries	35
Canned mandarin oranges	55 for whole segments; 58 for broken segments and pieces

ROUTINE ANALYSIS

Salt, sugars and "conditioners" such as the phosphates, sulphate, citrate or chloride of calcium or double sulphates of aluminium with an alkali metal may be added to vegetables during canning and mention of this addition on the label may be required. Since calcium and, at a lower level alumimium, mostly occur naturally in food products, careful comparison of the normal range for the untreated article with the results of analysis would be necessary before any statement could be made that addition without declaration had taken place. The proportion of total solids and alcohol-insoluble solids in canned processed peas are higher than in canned garden peas and therefore these parameters are used to check label claims.

The syrup strength of the fill liquor is of importance. The final syrup strength, known as the "cut-out" strength, may be determined by refractometer or by density, e.g. with a Westphal balance. Generally, the syrup used in canning will have a higher osmotic potential than the cellular liquid in the fruit and water will therefore be drawn out of the fruit, diluting the syrup. The drained weight of the fruit will thus tend to be lower than the "filled weight", that is, the weight of fruit put into the can at the time of filling. The degree to which these changes take place depends on the strength of the syrup used, the type of fruit, its ripeness and succulence and the ratio of fruit to syrup. The cut-out strength and drained weight from fixed initial syrup strength and filled weight will therefore vary over a considerable range. Conversely, the canner has to put in substantially more fruit in order to be sure of conforming to a minimum drained weight. It is therefore important to take the average drained weight of at least ten cans. Codex recommended standards include definitions of different syrups in terms of degrees Brix.

If erythrosine has been used as a colour, the can contents should be examined for the presence of fluorescein, formed by interaction between erythrosine and the tin and iron present.

Canned grapes may contain argol crystals (Kagan et al (3)), crude potassium bitartrate, which can be mistaken for glass by the layman. Similarly, calcium tartrate has been reported in cherries (Dickinson and Fowler (4)). Orange spots on mandarins may be narirgin. The mould Byssochlamys fulva may withstand processing and will then break down the pectin in fruits, e.g. strawberries, causing their disintegration.

CAN EXAMINATION

Samples may be submitted because the condition of the can leads to a suspicion that the contents are spoiled. In such a case, it is important to have several cans, as bacteriological examination may require half a dozen and it is much easier to carry out chemical tests if two or three extra cans are available.

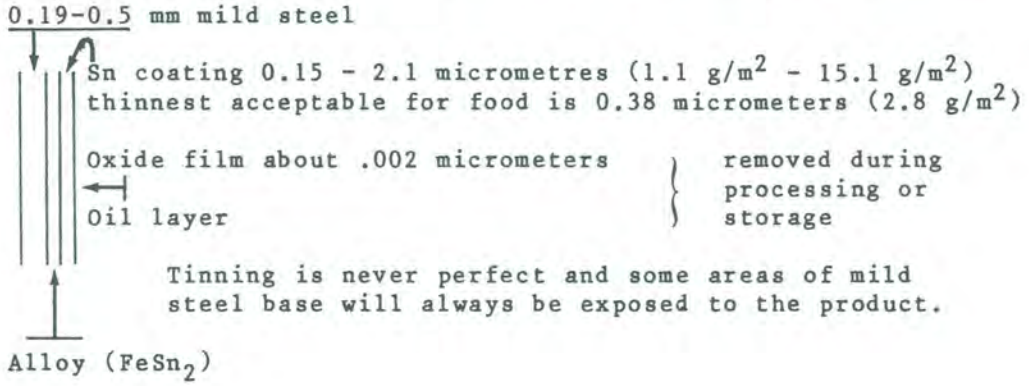
The can is first tested for vacuum, which should be about 200-300 mm below atmospheric pressure and never less than 75 mm below atmospheric pressure. If it is zero, the contents should be tested for tin. A positive pressure as in a swollen can is likely to be due to hydrogen derived from chemical action on the can itself or to carbon dioxide due to product decomposition. Hydrogen lights with a popping noise. Carbon dioxide may be absorbed into lime water or into sodium hydroxide solution. Identification of the gas may be used to obtain confirmation of bacteriological results. Once the can is open, the can coating is inspected to see if it is intact. The can must be checked for pin-holes, especially if there is rust on the internal surface. Cans with pin-holes should always be removed from sale, as they could become contaminated at any time, if not already so. Outside, rust tends to be around the seams and where the label is glued. Complete detinning is indicated if the inside of the can rusts quickly after emptying and being left to dry.

The bacteriological examination is the most important in the case of swollen (blown) cans but the contents should also be examined for tin, lead and pH. Interpretation of bacteriological results is beyond the scope of this book. Suffice it to say that the swell may be "hard" or "soft", and hard swells are almost invariably due to bacteriological contamination, which may be due to Clostridium spp and therefore such cans should only be opened under the supervision of a competent bacteriologist. Slight swelling may also be due to overfilling of the can, so swollen cans should not be reported as unsatisfactory without proper examination. Overfilling is a quality control problem of more interest to the canner than inspection authorities. On the other hand, the volume of gas in the can, called the "headspace", should not be excessive. At least 90 percent fill (headspace maximum 10 percent) is a reasonable general standard but there are exceptions.

Sound cans will usually be examined for headspace and vacuum and the contents for drained weight, pH, acidity and specific gravity of the liquor and metallic contaminants, most importantly lead, arsenic and tin but also cadmium, zinc, mercury and copper. In the absence of national legislation, more than 250 mg/kg of tin or 20 mg/kg of copper might be considered excessive and occasionally products containing less than these amounts may be of bitter or metallic taste. Limits for tin in certain canned foods have been recommended by the Codex Alimentarius Commission.

Most metal food cans (other than aluminium) are made of mild steel with a tin alloy and a tin coating on the inside surface.

Schematic Representation of the Structure of Tinplate



For beer and beverages, acidified beetroot, berry fruits, etc. the tin is lacquered, the underlying mild steel having a low corrosion resistance. Tin may be lacquered for many other products or just for effect. Detinning by acid foods takes place more slowly on tin with a more complete alloy layer.

Lacquers are usually sprayed on at the rate of 6-9 g/m^2 (which needs two sprayings) when the tin is flat and, where it is important that the product does not interact with the can (beer and beverages), the seam is given a "wipe" after can fabrication. There are often lines on tinplate which can be used to identify its type. The tinned mild steel sheet may be used as it is or coated with lacqueur, enamel or plastic. Cans coated with these last three but without tinning are also used for certain products.

The three main types of reaction of food with tinplate are:

1. Corrosion of the tin coating.

2. Pitting of the mild steel base.

3. Staining.

The various problems encountered with tinned food cans are discussed below:

Corrosion

Corrosion of the tin is essential because it provides electrochemical protection to the minute areas of base steel that are exposed to the product through pores and unavoidable scratches in the tin coating. Normally, etching should occur evenly over the wetted internal surface of the can; in the first month or so the mirror surface of the tin coating should change to one in which the shape of the individual tin crystals may be seen with the eye. Definition of the crystal boundaries in the tin should become clearer over the first year at temperatures of about 25°C, but no detinned areas should be visible. Detinned areas are identified by their grey surface, by resistance to scratching with a needle and by a tendency to rust rapidly when exposed to water and air. Detinned areas should not be seen in cans stored for less than 1-1/2 to 2 years.

Examination, with a lens or microscope, of cans undergoing uniform etching should show no evidence of corrosion of the base steel until detinning is extensive. However, localized areas of detinning may occur around the spots of solder that are sometimes found near the side seams of cans. These detinned areas should not be confused with pitting corrosion and they do not cause early failure of the can.

Rapid Detinning

Unusually rapid detinning in plain cans will result in the accumulation of unacceptable levels of tin and iron in the product and sometimes in the early development of hydrogen swells. Rapid detinning is caused by the use of plate with a tin coating mass that is too light, or by a product that is intrinsically too corrosive or contains corrosion accelerators. Nitrate in products with pH >6 has been implicated in incidents of rapid detinning, but dissolved oxygen, some dyes, anthocyanins and amine oxides also accelerate corrosion. During the detinning process these compounds (termed depolarizers) are chemically reduced, so loss of colour is evidence of the depolarizing action of some substances. Sulphur dioxide may affect the can and therefore should be absent from fruit used for canning. If it is desired to try to establish the cause of unexpectedly corroded cans, it may be worthwhile to look for sulphur dioxide.

Plain cans are unsuitable for foods containing active depolarizers. If possible, such foods should be packed in lacquered cans or the product should be modified to remove or inactivate the depolarizer.

Waterline Detinning

Uneven detinning is another result of product-container incompatibility or of unsatisfactory canning practice. If the can is detinned at the interface between product and headspace within a week or so of processing, it is likely that the can contained excessive amounts of oxygen when it was closed. The headspace volume may be large, the vacuum may be poor or the product may have been inadequately deoxygenated. If the cans are leaky, air will enter the headspace. Alternatively, the tin coating may be too light.

Waterline detinning (as this condition is often called) will usually be followed by even etching of the rest of the can once the oxygen has been consumed. However, detinning will be somewhat faster because of the increased area of exposed steel and the shelf life of the cans will be marginally shortened. Red rust is sometimes found at the headspace end of cans closed with excessive levels of residual oxygen, but, should the can be shaken before examination, the rust may be dislodged or dissolved by the product. The only evidence of the previous existence of rust will be isolated areas of detinning with shallow pits in the base steel.

Side-seam Detinning

Preferential detinning of the side seam usually occurs in cans made from plate given cathodic dichromate passivation treatment (the most common). The passive surface of the plate resists the attack of the product except where the tin coating was reflowered and the passive film disrupted by the heat of soldering; this area is then preferentially detinned. This condition is usually seen with mildly corrosive products, such as tomato soup. It has caused very large commercial losses. The problem may be overcome by using plate passivated by the sodium dichromate dip treatment which gives even etching of the wetted surfaces of the can, or by using lacquered cans.

Pitting Corrosion

If the tin does not corrode for one reason or another, then the spots of the mild steel base that are exposed to the product will probably corrode instead. This may occur due to protective substances in the product being preferentially absorbed on to the tin. This can happen with sauerkraut. The resulting pits in the steel can usually be seen by the aid of a hand-lens or low-power microscope. They may appear to be covered by an intact layer of tin, but scratching with a sharp needle will usually show that this is no longer plated to the layer beneath. This type of corrosion produces hydrogen which may have resulted in swelling of the can. The manufacturer may have to use lacquered cans in order to overcome this problem.

Sulphide Staining

Black sulphide staining due to FeS usually occurs at isolated points on the can mainly in the headspace region. It can be avoided by using lacquered cans, or cans with lacquered ends. It looks objectionable and therefore may give rise to consumer complaints but is in fact harmless. Iron sulphide stains rub off very easily.

Tin sulphide stain is also harmless and is blue-black (sometimes brown) and adheres firmly to the tinned surface. It can be removed by rubbing with a wet eraser or by cathodic treatment in 5% Na_2CO_3 at 6 volts. This serves to distinguish this type of staining from detinning. Localized sulphide staining may be seen in meat products.

Reaction Between Lacquered Cans and Products

If the lacquer layer is not continuous, the acidic food usually processed in this type of can will soon attack the underlying metal. This will be apparent from the appearance of the lacquer film. Objection need only be taken if

swelling has occurred, the product has acquired a metallic taste, the tin level is excessive, the can has rusted inside and contaminated the contents, or pin-holes have formed.

TOMATO PRODUCTS

Tomato concentrates are usually marketed canned or in tubes. The Recommended Codex International Standard for Processed Tomato Concentrates designates those of 8-14% natural tomato soluble solids as puree and those more concentrated as pastes. Under this standard, a lot will be considered as meeting the applicable minimum natural tomato soluble solids requirement when: (a) the average of the values from all containers or sub-samples tested meets at least the minimum percentage requirement for the concentration as declared or as required for the product name or description; and (b) individual test values are at least 92.5% of such minimum declared or required percentage of concentration. The recommended standard permits addition of seasoning such as salt, spices, onion and lemon juice as an acidulent but not sugars or other sweeteners. Sodium bicarbonate may be added to reduce acidity or citric, malic, L-tartaric and lactic acids added to increase it, provided the final pH is not more alkaline than 4.3. Tin must not exceed 250 mg/kg. The product must be of proper colour, texture and flavour and not otherwise defective. The percentage of natural tomato soluble solids must be declared and the term 'concentrated tomato puree' may only be applied to products containing at least 18 percent of such solids.

The determination of soluble solids is by refractive index. Determination of potassium and lycopene can be of assistance in verifying that the soluble solids are derived from tomato. Pearson's Chemical Analysis of Foods states that the dry solids of tomato purees and pastes contain about 9.5 percent ash, 13.8 percent protein, 50-65 percent total sugar (as invert sugar), 5.8-13.4 percent total acidity (as citric acid) and 4.8 percent potassium (as K_2O). Darbishire (28) reported a mean value of 1420 mg/kg of lycopene in the dry solids of various tomato purees but the natural variation is quite wide. There should be no dark specks or scale-like particles sufficient to noticeably affect the appearance. The total solids may be determined by drying at 70°C and less than 50 mm mercury. The metal contaminant of most importance is copper although it may also be useful to determine lead, tin and arsenic. Sugars are determined by boiling the sample gently with water, diluting to volume and filtering. Hydrolysis and titration of the filtrate follows standard procedures. The sucrose content is usually very low. The papers of Bigelow et al (5), Williams (6) and Goose and Binsted (7) give useful additional information. Jarvis (8) has suggested a chemical method for the estimation of mould in tomato products. The method is based on the estimation of chitin, which is hydrolysed to chitosan, the latter deaminated to 2, 5-anhydromannose which is estimated colorimetrically. To determine lycopene, vigorously shake 50 ml of an 0.2% aqueous suspension of puree with 25 ml of petroleum ether (BR 80-100°C) and then shake mechanically for 15 minutes. Measure the extinction of the organic phase at 505 nm.

$$E_{1\%}^{1cm} \text{ lycopene} = 2820.$$

DRAINED WEIGHT

PRINCIPLE

The sample is drained on a standard mesh sieve. The weight of material remaining in the sieve is expressed as a percentage of the can contents.

APPARATUS

Sieve with square openings 2.8mm x 2.8mm (no. 6 B.S.). Use a sieve with square openings 11.2 x 11.2mm for canned tomatoes. Use a 20cm sieve if the total weight of the produce is under 1.5 kilos and a 30cm sieve if it is over.

PROCEDURE

Weight the full can, open and pour the entire contents on a circular sieve for which a tare has been established. Without shifting the product, incline the sieve so as to facilitate drainage. In the case of products with a cavity such as peach halves, invert if necessary so that liquid can drain from the cavity but otherwise the product should not be touched. Drain 2 minutes, weigh either drained solids or free liquid direct, and weigh the dried empty can.

For products in canned sauce, it is usual to determine the "washed drained weight". Use a sieve with square openings 0.3 x 0.3mm. Wash the contents of the can on to the sieve, and rinse with running cold water and then running hot water until free of adhering substances. Spread the product out on the sieve, leave it to drain 5 minutes, dry the underside of the sieve and weigh.

REFERENCES

CAC/RM 36/37 1970.

CAC/RM 44 1972.

FILL OF CONTAINER

PRINCIPLE

This method determines the percent total volume of a container occupied by the contained food. It is designed primarily for cans but can be used for wide mouth glass containers also.

APPARATUS

Head space gauge. One can be conveniently made from a straightedge and a small ruler. Place the straightedge across the top of the opened container, resting on the container edge. Use the ruler to measure the distance from the bottom of the straightedge to the top of the food in the container.

PROCEDURE

Open the container (use a can opener for cans and remove lid for jars) and measure the distance from the container top to the food using the headspace gauge. This is usually done at the center, but if the food surface is uneven, then make several measurements at different points and average them.

Pour out and discard the food. Wash, dry and weigh the container.

Fill the container with water to within 5 mm of the top (using the headspace gauge). Weigh the container and water.

Next, draw off water from the container until the water is at the same level as measured for the food. Again weigh container and water. (Note that the water temperature should be the same during both weighings).

CALCULATION

$$\text{The \% fill of container} = \frac{W2-T}{W1-T} \times 100$$

Where: T = tare weight of the container
W1 = container plus water, first weight
W2 = container plus water, second weight

REFERENCE

USA Code of Federal Regulations, revised April 1, 1984. Title 21, Part 130.12.

SOLUBLE SOLIDS
(Tomato Products)

PRINCIPLE

The sample is treated with pectic enzyme, filtered and the refractive index of the filtrate determined. The result is expressed as percentage of sucrose at 20°C.

APPARATUS

1. Refractometer, sensitive to 0.0001.

2. Filters. Cut stems off 75mm ID glass or plastic funnels about 1cm from the apex at 90° angle and firepolish the ends. Place the funnels in 150ml jars, about 55mm ID. If 150ml beakers are used, close the pouring spout with tape to prevent evaporation.

REAGENTS

1. Pectic enzyme, in a diatomaceous earth base e.g. Pectinol R-10 (Rohm & Hass Co.) Klerzyme® analytical (Wallerstein Co.) or Spark-L® (Miles Laboratories Inc.). Prepare a 0.4-1% aqueous solution, mix thoroughly and allow to settle. Use the clear supernate.

PROCEDURE

Weigh 100g sample at room temperature and add a weighed amount (0.2-1.0g) of The dry enzyme preparation. Mix with a spatula immediately to avoid evaporation and transfer to a filter containing a 12.5cm paper (Whatman 2V or equivalent). Tamp so that the sample is in close contact with the paper and cover with the top or bottom half of a petri dish to form a loose seal with the top of the funnel. Discard samples that take more than an hour to filter. For those, mix 0.2-1g dry enzyme with 100g fresh sample and incubate 30-60 minutes at about 40°C in a closed container. Cool nearly to room temperature before opening, re-mix and transfer to the filter. Samples that still filter too slowly should be filtered after dilution.

For samples that contain 35% solids or more and those slow to filter, add 100g enzyme solution to 100g sample and mix with a spatula immediately to avoid evaporation, or in a sealed blender. Alternately blend and shake to dislodge and break up lumps sticking to container. Examine the mixture carefully for lumps and continue mixing until homogeneous. Transfer to the filter and cover with a petri dish.

Check that the refractometer reads 1.3330 with water at 20°C. Transfer a drop of the substantially clear filtrate to the refractometer prism. If it is not possible to read at 20°C or if condensation occurs at this temperature, read at room temperature and correct according to the table. Read as percentage sucrose. Repeat the determination with another drop of filtrate. Readings should agree within 0.1% sucrose. If not, repeat readings on successive portions of filtrate until agreement is obtained. Erratic readings indicate evaporation of sample or faulty mixing and/or filtration.

Determine the refractive index of a 1% solution of dry enzyme on the refractometer as % sucrose.

CALCULATION

Soluble solids of sample = % sucrose (test reading) - 1.15xBxC

Where: 1.15 = correction for insoluble solids in the sample assuming 12% of the total solids to be insoluble

B = % enzyme preparation added to sample

C = reading as sucrose obtained on 1% enzyme solution

If a dilute sample is used:

Soluble solids of sample = 2 [% sucrose (test reading) - 0.55 x D x C] + E

Where: 0.55 = correction for insoluble solids, as above

D = % enzyme preparation to sample and E is a correction factor according to the following table

Natural tomato soluble solids as % sucrose, corrected for enzyme x 2 (that is, 2 (test reading - 0.55 x D x C))	Correction, E
25.0	0.3
30.0	0.4
35.0	0.5
40.0	0.7
45.0	0.8
50.0	0.9

Note that correction for salt may be made if the sample contains added salt and use of the following formula leads to a lower figure:

Soluble solids as % increase at 20°C (corrected for salt)

= (R-N) 1.016 where R is the uncorrected value and N is % total chloride expressed as NaCl.

REFERENCES

Official Methods of Analysis of the AOAC, 1984, 32.023-.025.

Lamb, F.C., 1969. Journal of the Association of Official Analytical Chemists, 52, 1050-1054.

Lamb, F.C., 1972. Journal of the Association of Official Analytical Chemists, 55, 809-810.

6.3 JUICES

COMPOSITION

Orange juice is defined by the Codex as the unfermented but fermentable juice, intended for direct consumption, obtained by a mechanical process from the endocarp of sound, ripe oranges (Citrus sinensis Osbeck). The juice may contain up to 10% m/m of mandarin juice (Citrus reticulata Blanco). The juice may have been concentrated and later reconstituted with water, provided the quality of the reconstituted product is equivalent to that of pure juice.

The soluble solids exclusive of added sugar shall not be less than 10% m/m as determined by refractometer at 10°C, uncorrected for acidity and read as °Brix on the International Sucrose Scale. Sucrose, dextrose or dried glucose syrup may be added as long as the total added sugars do not exceed 5%. The product shall have the characteristic colour, aroma and flavour of orange juice. Natural volatile orange juice components may be restored to any orange juice from which natural orange juice components have been removed. The addition of concentrate to juice is permitted. Only concentrates from orange and mandarin may be used. There shall be only traces of volatile acids present, the ethanol content shall not exceed 3 g/kg nor the essential oils content 0.4 ml/kg. Limits for toxic elements are as follows:

Metal	Maximum level
Arsenic (As)	0.2 mg/kg
Lead (Pb)	0.3 mg/kg
Copper (Cu)	5 mg/kg
Zinc (Zn)	5 mg/kg
Iron (Fe)	15 mg/kg
Tin (Sn)	250 mg/kg
Total metal content precipitable by potassium hexacyanoferrate (II)	20 mg/kg, expressed as Fe

The product must occupy not less than 90% v/v of the water capacity of the container. The complete list of ingredients must be declared on the label in descending order of proportion, and mention must be made of reconstitu-tion if preparation involved that process. For juice reconstituted from the concentrate, soluble solids must be at least 11% m/m.

The contents of sugars as a percentage of total sugars expressed as invert are usually in the following ranges in genuine orange juice:

Sucrose:	30.6 - 56.0%
Glucose:	22.6 - 33.0%
Fructose:	18.1 - 37.2%

The sucrose/invert sugar ratio does not exceed 1.3/1. The amino-acid pattern and carboxylic acid patten are useful indications of adulteration. Synthetic carotenoids and glycine are possible additives used to hide adulteration with added water and sugar. Addition of peel and pulp is indicated by an abnormally high chloramine value and pentose equivalent. Beet-sugar is a possible source of betaine. Serine averages about 38 mg/100g in juice and levels in pulp and peel are similar.

ROUTINE ANALYSIS

The sugars present in fruit juices may be detected by TLC and a suitable combination of the routine methods devised for the quantitative estimation of those present. Acidity is determined by titration to pH 8.2, using phenolphthalein or a pH meter. The result is expressed as percentage m/m of citric acid. The organic acids present may be detected by paper chromatography. Ethanol may be determined as in beer, but the sample should be neutralized first.

Fruit juices should be examined for preservatives, sulphur dioxide being the preservative most usually present, and for vitamin C and trace metals. As extensive a compositional analysis as possible should be carried out. Acidity, determined by titration to phenolphthalein or a potentiometric end-point, is expressed as the acid characteristic of the fruit - citric in citrus, malic in apples and pears, tartaric in grape products, etc. Adulteration can be extremely sophisticated and the analyst is strongly advised not to report adversely on samples without careful methods validation and a thorough understanding of the interpretation of the results of fruit juice analysis. Analysis reviews include those of Herrmann (9), Wallrauch (10) and Wucherpfennig (11).

The review of Mears and Shenton (12) and the paper by Sawyer (13) are very useful as an introduction to the problems of the detection of adulterations of orange and grapefruit juices. There is evidence of the manufacture of products specifically intended for the adulteration of orange juice (Kefford (14), Anon (15), Koch and Hess (16)). More recent papers on the adulteration of this juice include those of Benk (17)(18), Benk and Bergmann (19), Rother (20), Katsouras (21), Weitz et al (22), van Gils and van den Bergh (23) and the review of Koch (24).

Generally speaking, a single analytical parameter is not reliable as an indication of adulteration and it is for this reason that as many constituents as possible should be determined. The difficulty is accentuated by the wide natural variation. Variation is less in fruits of specified variety, locality and soil characteristics, but these details are rarely available to the enforcement analyst. For example, in genuine orange juice the analytical parameters show relationships within certain limits. The data is therefore susceptible to statistical examination, either by use of the X^2 distribution (Lifschitz, Stepak and Brown (25)) or regression analysis (Coffin (26)). Brown (27) discusses the use of combined non-independent, one sided tests of significance.

FRUIT CONTENT
(Formol Number Method)

PRINCIPLE

By the addition of formaldehyde, one H^+ is liberated per molecule of amino-acid. It is titrated with alkali. The secondary amino-group of histidine does not react; those of proline and hydroxy-proline react to about 75%. Tertiary nitrogen and guanidine-groups undergo no reaction.

APPARATUS

1. pH meter.

REAGENTS

1. Sodium hydroxide, 0.25N.

2. Formaldehyde solution: pure formalin of at least 35% is brought exactly to pH 8.1 with dilute sodium hydroxide as determined by means of the pH meter.

3. Hydrogen peroxide, pure, 30%.

PROCEDURE

25 ml fruit juice (for lemon juice 10 ml + 10 distilled water) or the corresponding amount of concentrate diluted to this volume are neutralized in a beaker with 0.25 N sodium hydroxide to pH 8.1 on the pH meter. 10 ml of the formaldehyde solution is then added. After ca. 1 minute the solution is titrated potentiometrically to pH 8.1 with 0.25N sodium hydroxide.

If more than 20 ml 0.25N sodium hydroxide are required, the titration is to be repeated using 15 ml formaldehyde solution instead of 10 ml. When sulphur dioxide is present the sample is treated with a few drops of 30% hydrogen peroxide before neutralization.

CALCULATION

The amount of alkali used in the titration, expressed as ml 0.1 N alkali and referred to 100 ml fruit juice or 100 g concentrate is equal to the formol number of the sample under test. Calculate to whole numbers (without decimals).

INTERPRETATION

As fruits ripen the formol number of the juice tends to decrease, as a rule. Conversely, on storage of the juice a slight increase may be noted. Various factors can lead to a lowering of the formol number of a fruit juice, e.g. treatment with ion-exchangers or addition of ascorbic acid.

In the literature the formol number may also be found defined as ml N alkali for each 100 ml sample, which corresponds to values 10 times smaller than those given by the preceding method of calculaion.

For orange juice, the % fruit is $\frac{1.05F}{1.4}$, where F is the formol number.

REFERENCES

Ayres, A.D., Charley, V.L.S. & Swindells, R., 1961. Food Processing and Packaging 30, 413.

Schröder, H., 1956. Zeitschrift fur Lebensmitteluntersuchung und Forschung 104, 386.

ORGANIC ACIDS

PRINCIPLE

The organic acids present in fruit juices can be separated by descending paper chromatography. After drying the paper the acid spots obtained are sprayed with an acridine solution. Under the UV-lamp they can be detected by their yellow fluorescence. By additional spraying of the paper with an alcoholic solution of bromophenol blue they can also be detected as yellow spots on a blue background. For the identification of unknown acids it is recommended to use developing solvents of varying composition.

APPARATUS

1. Glass chromatography tank with two solvent troughs, 40 cm long, 30 cm wide and 55 cm high, sealable.

2. Micro-pipette, 20 microlitre capacity.

3. Spray assembly.

4. Chromatography paper, Schleicher and Schuell, No. 2043b or Whatman equivalent.

5. Warm air drier.

6. Ultraviolet lamp (spectral range: 280-360 nm).

REAGENTS

1. Solvent mixture I: mix together 7.5 parts n-butanol, 2.5 parts tert. amyl alcohol, 3 parts formic acid (98%, anhydrous), and 3 parts water. This is to be made at least 24 hours before use in a separating funnel. The lower aqueous layer is separated and kept. It will be used later for equilibrating the chromatography tank.

2. Solvent mixture II: mix together 95 parts n-butanol (saturated with water) and 5 parts formic acid (98%, anhydrous).

3. Solvent mixture III: mix together 500 g phenol, 6.7 ml formic acid (98%, anhydrous) and 167 ml water.

Note: all three solvent mixtures are stable for one week.

4. Spray reagents:

   a. Alcoholic bromophenol blue solution, 0.4 g/L (this solution is to be neutralized with 0.1 N NaOH until the appearance of the first blue-green colouration).

   b. Acridine solution: 250 mg acridine dissolved in 200 ml ethanol.

5. Cation exchanger Dowex 50, 12% cross-linked, 50-100 mesh BSS, H^+ form.

6. Standard acids (tartaric acid, malic acid, citric acid, lactic acid, succinic acid, etc., each 5 g/L).

PROCEDURE

10 ml of the fruit juice is vigorously shaken three times, each for 1 minute and each time with 1g cation exchanger Dowex 50 (H^+), without intermediate filtration. Filter and use the filtrate for spotting.

Sugar in fruit juices can interfere in the separation if present in too high a concentration. It is therefore recommended that, in such cases, 10-20 µl, at the most 40 µl, of the filtrate be spotted on the paper.

(Note that chromatography papers are very sensitive to contact with acids and alkalis. Stringent cleanliness is therefore to be observed when cutting and marking papers, e.g. wash hands, use clean supports, etc.).

The width of paper chosen can be varied according to the number of samples or standard acids to be examined. For example, with a width of 20 cm, 8 starting points can be applied. A distance from the margin of 3 cm on each side should invariably be observed whatever the width of the paper.

Spot the individual sample and standard solution using a blood-sugar pipette. 10-20 µl of each solution is applied to the starting point in 4 portions of about equal size (ca 7 cm from the upper edge). After each application dry the moist spot on the paper using a warm-air drier.

Line the chromatography tank with filter paper on the inner side. Add 10-20 ml of the aqueous phase to the tank bottom.

If especially repeatable chromatograms are required, and if time permits, it is recommended to hang the spotted chromatogram in the empty chromatograph trough overnight. Next day the solvent is introduced. Develop by the descending technique. The time of running is at least 10-15 hours according to the choice of solvent.

Remove the developed chromatogram from the tank and dry for 3-4 hours in a stream of cold air (chamber with ventilator). When the solvent contains phenol, the drying is to be carried out for one hour with a hot-air drier at 70°C.

Spray the dried chromatogram on each side with acridine solution. After a short drying-off period, view under the UV-lamp. The acid spots can be distinguished as sharply defined, brightly glowing yellow spots. It is recommended that the outlines of the spots be lightly marked with a hard pencil.

In order to make the acid spots also visible by ordinary light, it is recommended that the chromatogram be additionally sprayed on both sides with bromophenol blue solution. The acid spots can then be distinguished as yellow zones on a blue background.

INTERPRETATION

The RF-values serve as an aid in the characterization of the individual acids. These values are, however, influenced by many factors, so that they are not to be taken as absolute values, but as reference points in the characterization. Unequivocal results will be obtained if the acids to be identified are run together with the standard acids presumed present. The approximate RF-values for the different acids run in solvent mixtures I, II and III can be taken from the following tables.

Acids	Solvent I (n-butanol: tert-amylalcohol :formic acid: water) RF-Values	Solvent II (n-butanol, H_2O saturated: formic acid) RF-Values	Solvent III (phenol: formic acid: water) RF-Values
Galacturonic acid	0.04	0.08	0.23
Gluconic acid	0.05	-	-
Quinic acid	0.14	0.22	0.52
Tartaric acid	0.18	0.32	0.20
Ascorbic acid	0.28	0.32**	0.41
Citric acid	0.32	0.48	0.24 and 0.28
Malic acid	0.39	0.55	0.42
Chlorogenic acid*	0.41	0.58	0.71
-Ketoglutaric acid	0.56	0.69	0.60
Lactic acid	0.67	0.78	0.72
Succinic acid	0.67	0.80	0.66
Caffeic acid*	0.69	0.82	0.62
Glutaric acid	0.75	0.85	0.79
Fumaric acid	0.83	0.89**	0.63

* These acids can be distinguished by their blue fluorescence if the unsprayed chromatogram is viewed under the UV-lamp

** Visible in UV-light as dark violet spots before spraying.

Note that if acids present only in traces are to be detected (provided that the sugar content is below 20 g/L) juice volumes up to 50 µl may be applied directly to the paper.

The ion exchange treatment described also sets free inorganic acids that may be present; after development they are usually found near the starting point. This fact must be taken into account in the identification of unknown acids.

Volatile acids such as formic acid, acetic acid, etc. cannot be separated by acid solvents, they volatilise during the sample application.

For semi-quantitative determinations suitable standard acids are applied at different concentrations. Conclusions as to the amount present can then be drawn by a comparison of the spot size of the test substance with those of the concentration series. More accurate values can be obtained if the spots are marked out with pencil under the UV-lamp, and then cut out and weighed. Recording spot weights on the one hand and concentrations on the other in a system of co-ordinates, gives a curve from which the concentration of the required acid can be derived with good accuracy.

As an alternative to paper chromatography, thin-layer chromatography can also be applied. In the latter case plates may be prepared from cellulose powder. The various solvent mixtures can be employed in the separation.

REFERENCES

Tanner, H. 1959. Scheweizerische Zeitschrift fur Obst und Weinbau 68, 270-273.

Tanner, H. 1964. Scheweizerische Zeitschrift fur Obst und Weinbau 73, 150-154.

Tanner, H. & Rentschler, H. 1958. Mitteilungen Klosterneuberg VIII A, 113-120.

Lehongre, G., Tanner, H. & Rentschler, H. 1957. Mitteilungen aus der Gebiete der Lebensmitteluntersuchung und Hygiene 48, 40-46.

Rentschler, H. & Tanner, H. 1954. Mitteilungen aus der Gebiete der Lebensmitteluntersuchung und Hygiene 45, 142-158.

VITAMIN C

PRINCIPLE

This method was designed for fruit juices but can be applied successfully to most liquid samples, or samples that can be easily dissolved. The exception is blackcurrant juice which is too densely coloured.

REAGENTS

1. Standard indophenol solution - dissolve 0.05g 2,6-dichlorophenol-indophenol in water, dilute to 100 ml and filter. Prepare freshly.

2. Standard ascorbic acid solution - dissolve 0.0500g of pure ascorbic acid in 60 ml 20% metaphosphoric acid and dilute with water to exactly 250 ml.

3. 20% metaphosphoric acid.

4. Acetone.

PROCEDURE

Standardize the indophenol solution as follows: Pipette 10 ml of standard ascorbic acid solution into a small flask and titrate with indophenol solution until a faint pink colour persists for 15 seconds. Express the concentration as mg ascorbic acid equivalent to 1 ml of the dye solution (i.e. 10 ml ascorbic acid solution = 0.002g ascorbic acid).

If 0.002g ascorbic acid requires V ml dye solution to neutralize it then 1 ml dye solution = $\frac{0.002}{V}$ g ascorbic acid.

Pipette 50 ml of unconcentrated juice (or the equivalent of concentrated juice) into a 100 ml volumetric flask, add 25 ml of 20% metaphosphoric acid as stabilizing agent and dilute to volume. Pipette 10 ml into a small flask and add 2.5 ml acetone. Titrate with the indophenol solution until a faint pink colour persists for 15 seconds. (The acetone may be omitted if sulphur dioxide is known to be absent.)

CALCULATION

Vitamin C (mg/100 ml juice) = 20 (V)(C)

Where: V = ml indophenol solution in titration
C = mg Vitamin C/ml indophenol.

REFERENCES

Official Methods of Analysis of the AOAC, 1984. 43.064-.068.

Deutsch, M.J. 1967. Journal of the Association of Official Analytical Chemists, 798-806.

6.4 TEXT REFERENCES

1. BAKER, L.C., LAMPITT, L.H. & MEREDITH, O.B. 1955. Journal of the Science of Food & Agriculture 6 197-202.

2. SHERRATT, J.G. 1943. Analyst 200-202.

3. KAGAN, I.S., GRISHINA, I.P. & ZYABKO, L.P. 1964. Izvestiya Vysshikh Uchebnykh Zavedenii (5) 172-4.

4. DICKINSON, D. & FOWLER, H.D. 1955. Analyst 80 706.

5. BIGELOW, W.D., SMITH, H.R. & GREENLEAF, C.A. 1950. Tomato Products: Research Laboratory Bulletin 27-L, National Canners Association, Washington.

6. WILLIAMS, H.A. 1968. Journal of the Association of Public Analysts 6 69 and 1972 10 103.

7. GOOSE, P.G. & BINSTED, R. 1973. Tomato Paste and Other Tomato Products, Food Trade Press.

8. JARVIS, B. 1977. Journal of Food Technology 12 581-91.

9. HERRMANN, K. 1971. Ernahrungs-Umschau 18 (10) 410-415.

10. WALLRAUCH, S. 1975. Flussiges Obst 42 (6) 225-9.

11. WUCHERPFENNIG, K. 1976. Flussiges Obst 43 (6) 214-6, 218, 220-6.

12. MEARS, R.G. & SHENTON, A.G. 1959. Journal of Food Technology 8 357-89.

13. SAWYER, R. 1963. Journal of Scientific Food Agriculture 14 302.

14. KEFFORD, J.F. 1969. Food Preservation Quarterly 29 65.

15. ANON. 1972. Gordian 72 15.

16. KOCH, J. & HESS, D. 1971. Deutsche Lebensmittel-Rundschau 67 185.

17. BENK, E. 1971. Industrielle Obst und Gemusevrerwertung 56 (23) 692-694.

18. BENK, E. 1971. Mineralbrunnen 21 (9) 278 & 280.

19. BENK, E. & BERGMANN, R. 1971. Industrielle Obst und Gemuseverwetung 56 10 282-284.

20. ROTHER, H. 1971. Mineralbrunnen 21 390, 392 & 394.

21. KATSOURAS, K.G. 1971. Hemika Hronika 36 (8/9) 185-90.

22. WEITZ, J., BROEKE, R. ten, GODDIJN, J.P., GORIN, N. & SCHUTZ, G.P. 1971. Zeitschrift fur Lebensmitteluntersuchung und Forschung 145 (6) 335-38.

23. GILS, W.F. Van & BERGH, J.A. Van den. 1974. Zeitschrift fur Lebensmitteluntersuchung und Forschung 152 (5) 288-91.

24. KOCK, J. 1975. Flussiges Obst 42 (6) 217-24.

25. LIFSCHITZ, A., STEPAK, Y. & BROWN, M.B. 1971. Journal of the Association of Official Analytical Chemists 54 1266.

26. COFFIN, D.E. 1968. Journal of the Association of Official Analytical Chemists 51 1199.

27. BROWN, M.B. 1975. Biometrics 31 (4) 987-92.

28. DARBISHIRE, O.B. 1965. Analyst 90 439-40.

Further Reading

KEFFORD, J.F. & CHANDLER, B.V. The Chemical Constituents of Citrus Fruits.

Methods of Analysis of the International Federation of Fruit Juice Producers, 10 Rue de Liège, 5-75009, Paris 9e, France.

NAGY, S. & ATTAWAY, J.A. 1980. Citrus Nutrition & Quality ACS Symposium 143 American Chemical Society.

7. CEREALS, CEREAL PRODUCTS AND PULSES

7.1 WHOLE GRAIN (UNMILLED) PRODUCTS

ROUTINE ANALYSIS

As cereals, pulses and their products form the larger part of the diet of most people, it is most important that the quality of these foods is maintained. For unmilled products this is more a matter of inspection than analysis, but samples received at the laboratory should be examined for filth (including uric acid), rodent hairs and excreta, infestation, damage (breakage, shrivelling, fungal attack, insect damage), moisture, pesticide residues, mycotoxins, fumigant residues and heavy metals. Rodent urine on grain shows fluorescence under ultraviolet light. Mercury compounds are still used as seed dressings and dressed seed occasionally become available for human consumption. Cadmium and zinc are absorbed from the soil by rice and other cereal and root crops and may reach undesirable levels if the plants are grown in soils containing high-levels of cadmium or zinc. Selenium levels can be high in the soil in certain areas and in such areas crops may need to be analysed for selenium.

Some pulses contain toxicants naturally, such as cyanogenetic glycosides (lima bean), trypsin inhibitors (e.g. soybean, lima bean, navy bean, black-eyed pea), goitrogens (soybean) and heamagglutinins (Phaseolus vulgaris, soybean). The latter three toxicants are proteinaceous and are therefore denatured and rendered harmless by heat. Cyanogenetic glycosides occur in a wide range of plants, including almonds and other fruits of the Rosaceae, sorghums and Kaffir corns, cassava and elderberry (Sambucus nigra). Added organic colouring material can be extracted from pulses or pulse flour with 80 percent ethanol. After evaporation of the ethanol, the identification may be completed in the usual way.

The general methods of ISO for oleaginous seeds may be used for soya. ISO 664 describes preparation of the sample for analysis. ISO 659-1968 describes determination of the oil by hexane extraction using a soxhlet or similar extractor, grinding with sand after 4 hours and six hours of a total eight hour extraction time. ISO 658 describes the determination of impurities by sieving and hand-picking. Moisture and volatile matter is determined as loss in weight at 103°C (665-1968) after reduction of the seeds to below 2 mm.

The ISO basic reference method for the determination of moisture in cereals and cereal products requires that the sample be first ground so that particles are 1.7 mm or less, less than 10 percent over 1 mm and over 50 percent less than 0.5 mm. Drying is at 50°C and 10-20 mm mercury in the presence of phosphorus pentoxide until constant weight is achieved (over 100 hours). The routine method requires drying at 130°C for 2 hours (1-1/2 hours for flours). It is important to allow the dish to cool completely before weighing (30-45 minutes).

When examining samples for contaminants it can be preferable to wash the whole grain (with organic solvents for pesticide residues or with dilute nitric acid for mercury) rather than use a milled sample. A few samples of washed grains should be ground and analyzed to check the effectiveness of the washing. Methods for the determination of residues of fumigants are given by Malone (1) and a panel of the U.K. (2). Aluminium phosphide is finding increasing use as a source of phosphine for fumigation. Muthu, Kashi and Majumder (3) describe a method of analysis.

GLYCOSIDIC CYANIDE

PRINCIPLE

The glycosides are hydrolysed and the cyanide steam distilled. The cyanide is determined by argentimetric titration.

APPARATUS

1. Mechanical grinding mill, easy to clean, enabling samples to be ground without becoming heated and without appreciable change in moisture content.

2. Sieve with 1 mm apertures.

3. Analytical balance.

4. Incubator, adjusted to operate at 38 $\pm$ 2°C.

5. Steam distillation apparatus, provided with a 1 litre flask with G/G neck. This removable flask should be able to be stoppered hermetically with a G/G stopper. The end of the condenser should be provided with an extension drawn out to a point.

REAGENTS

1. Orthophosphoric acid,concentrated.

2. Sodium hydroxide pellets.

3. Silver nitrate, 0.01 N standard volumetric solutions.

4. Potassium iodide (KI) solution, 50 g/L.

5. Ammonia solution, approximately 6 N obtained by diluting concentrated ammonia solution with an equal volume of water.

PROCEDURE

Grind about one twentieth of the laboratory sample in the previously well cleaned mechanical grinding mill (in order to complete the cleaning of the mill), and reject these grindings. Then grind the rest to particles which will pass through the sieve completely. Collect the grindings, mix thoroughly and carry out the determination without delay.

Weigh, to the nearest 0.1 g, approximately 20 g of the prepared sample. Transfer to the 1 litre distillation flask and add 200 ml of distilled water and 10 ml of orthophosphoric acid. Stopper hermetically, mix well and leave the flask for 12 hours (overnight) in a incubator at 38°C.

Fit the flask to the distillation apparatus and distil into 20 ml water containing 0.5 g NaOH. Collect 100-120 ml of distillate.

Transfer the distillate to a 250 ml volumetric flask and dilute to the mark with distilled water. Pipette 100 ml into a beaker. Add 2 ml of potassium iodide solution and 1 ml of ammonia solution.

Titrate with silver nitrate solution until permanent turbidity appears. For the easy recognition of the end point of the titration, it is recommended that a black background should be used. Make a

second titration with another 100 ml portion of distillate and take the mean of the two titrations. Carry out two complete determinations on the same prepared sample.

Carry out a blank test under the same conditions as in the determination but replacing the distillate by distilled water.

CALCULATION

Under the conditions of the reaction: 1 ml of 0.01 N silver nitrate solution corresponds to 0.54 mg of hydrocyanic acid (HCN).

The content of glycosidic hydrocyanic acid, expressed in milligrams of HCN per 100 g of sample, is equal to

$$0.54\ (V_o - V_1) \times \frac{250}{100} \times \frac{100}{m} = \frac{135\ (V_o - V_1)}{m}$$

Where:

m is the mass, in g, of the test portion

V_o is the volume, in ml, of 0.1 N silver nitrate solution used for the determination.

V_1 is the volume, in ml, of 0.1 N silver nitrate solution used for the blank test.

INTERPRETATION

If the amount of HCN determined is less than 1 ml of titrant volume, consider the sample practically free from glycosidic cyanide.

REFERENCE

ISO 2164:1975.

TALC ON RICE OR BARLEY

PRINCIPLE

The talc is floated off, filtered, digested, ignited and weighed.

REAGENTS

1. 10% ammonia solution.

2. Hydrogen peroxide, 3%.

3. Hydrochloric:chromic acid mixture. Carefully dissolve 10 g of chromic trioxide in 100 ml of water and add to 900 ml of concentrated hydrochloric acid.

PROCEDURE

Shake 20 g of sample with the dilute ammonia and dilute hydrogen peroxide solutions. Heat to about 60°C so that the gas formed causes the particles of talc to come away from the surface. Decant off the liquid containing the talc, wash the grains several times with water and add these washings to the decanted liquid. Heat the liquor with the hydrochloric/chromic acid mixture to oxidize suspended meal, filter off the talc, wash, ignite and weigh.

INTERPRETATION

In unpolished rice, the talc residue does not normally exceed 0.025%.

REFERENCE

Kozizan, R. 1906. Zeitschrift Untersuchung Nahrung Genussmittel xi, 641-650.

7.2 FLOURS AND MILLED PRODUCTS

ROUTINE ANALYSIS

The identity of any flour or other milled cereal product should be checked by microscopical examination, comparing the sample with authentic specimens.

The quality of flour can usually be adequately assessed by determination of moisture, ash, acid-insoluble ash, acidity, nitrogen, gluten (for wheat flour) and filth. The proportion of fibre is an indication of the extraction rate, the higher the rate of extraction (80 percent, 85 percent, etc.) the closer the flour is to wholemeal (100 percent). In the case of wheat flour the lowest extraction (i.e., whitest flour) is normally 72 percent. Flour may be fortified with iron, chalk and vitamins such as thiamine and nicotinic acid. Self-raising flour is made by addition of an acid phosphate and sodium bicarbonate. The baking process may be assisted by the addition to flour of bleaching agents, improvers and other additives including ammonium persulphate, ammonium chloride, cysteine, acetone peroxide, sulphur dioxide, azodicarbonamide, potassium bromate, nitrosyl chloride, chlorine, chlorine dioxide, benzoyl peroxide, potash alum, magnesium carbonate, sodium aluminium sulphate, calcium sulphate and di- and tri-calcium phosphate. The use of some of these additives is under review and subject to legislation in many countries.

The main sources of methods of analysis for flours are the International Association for Cereal Chemistry (ICC), the American Association of Cereal Chemists, Kent-Jones and Amos (1967) and Pearson (4). Among the many ICC methods may be mentioned numbers 110 and 111 for nicotinic acid, 117 and 119 for thiamine, and 122 and 123 for starch.

Flours commanding a higher price may be admixed with cheaper ones. The methods of Griffiths (5), Tillmans, Holl and Janivala (6) and Tillmans (7) and the AACC method 06-10 may be used for the detection of rye flour in wheat flour. The presence of soy flour is usually detected by the urease test, this enzyme being peculiar to soya beans among the flours of commerce. While a positive test certainly indicates the presence of soya, the enzyme may have been inactivated during processing and hence it is preferable also to carry out a microscopical examination, the flat "hour-glass" cells of the epidermis of soya being very characteristic.

Sometimes rice and barley are "faced" with materials such as talc and glycerol or oil containing a trace of blue pigment to improve the appearance. The effect is solely cosmetic and the practice should be discouraged. The total ash of rice is usually between 0.2 and 0.4 percent.

MICROSCOPIC IDENTIFICATION

This is a useful physical analytical procedure to identify the source of flours or starches. The starch grains may be examined in mounts in water but some grains may distort in time. This can be avoided by mounting in alcohol. Addition of a drop of 2 percent aqueous solution of chromium trioxide makes the striations of starch grains more easily seen. The presence of starch in a powder such as flour may be confirmed by adding a drop of very dilute (say 0.001 N) iodine to the slide, the grains staining an intense blue. Different characteristic patterns of starch grains can be seen between crossed polaroids.

The microscopist should be aware of the form of the various layers in the entire grain so that he can recognize the various elements in the flour and pick out any that are foreign. A grain may be softened by soaking in water or glycerol:methanol (1:1) overnight and then sections made as thin as possible by embedding the grain in pith and cutting with a razor. The diagram of the wheat caryopsis typifies the morphological elements of cereal grains. On another

portion the starch is solubilized with hydrochloric acid or diastase or by boiling with chloral hydrate or glacial acetic acid and the residue centrifuged and examined, mounted in dilute glycerol or chloral hydrate.

The following descriptions and illustrations will assist the microscopic identification of cereal and pulse starches, flours and some grains:

1. **Wheat Grains**

A transverse section of a grain of wheat, examined under the microscope, exhibits the following layers (Figure 7.1):

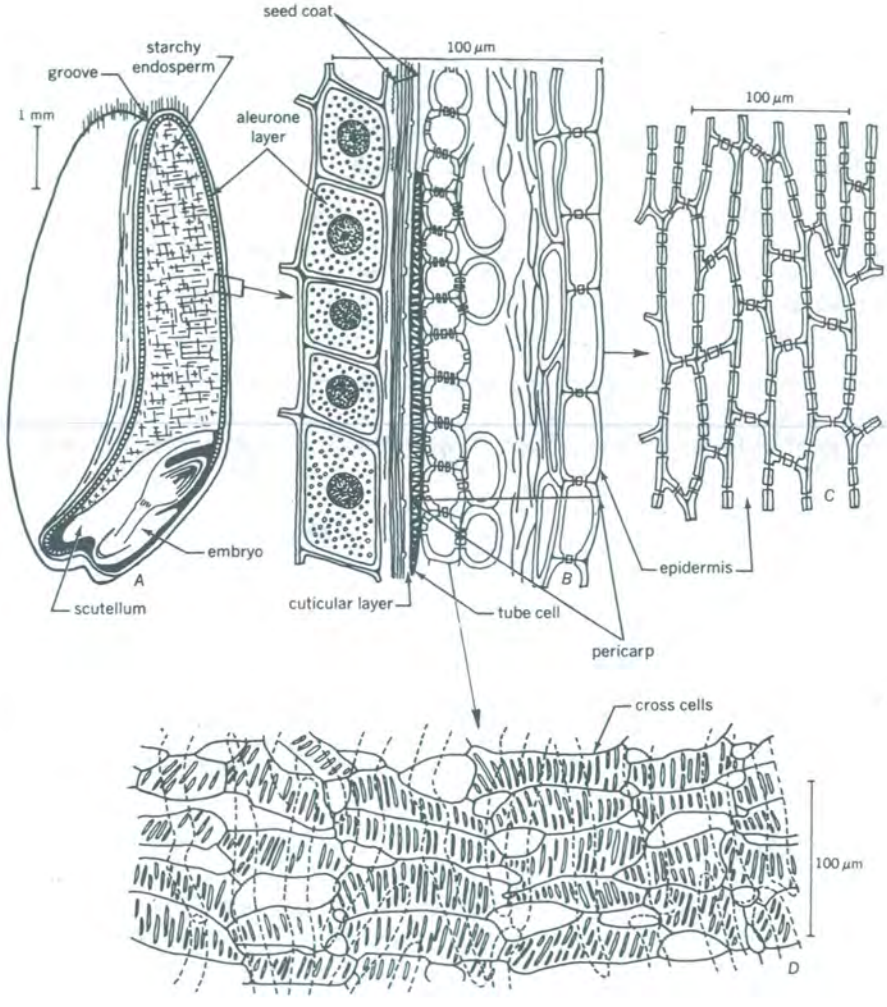


Figure 7.1. Caryopsis (A) of Wheat (Triticum) and Parts of its Pericarp in Longitudinal Section (B) and Surface Views (C,D)

(a) Outer epidermis of the pericarp, composed of tabular cells which, in surface view, are polygonal, elongated and have thickened, pitted walls. In the upper part of the grain it bears simple, unicellular, conical hairs, the lumen of which is somewhat abruptly enlarged at the base.

(b) Hypoderma, consisting of cells which, towards the exterior, closely resemble those of the outer epidermis, but in the inner part vary in form and often lignify.

(c) A layer of transverse cells; these are tangentially elongated and have thickened, pitted walls.

(d) Inner epidermis of the pericarp, consisting of small cells with rounded section, but elongated in surface view; their tubelike appearance has gained for them the name of tubular cells. These four layers constitute the pericarp of the grain.

(e) Seed-coat or brown layer composed of two layers of cells closely applied to one another, and of a yellow or yellowish-brown colour.

(f) Hyaline layer: a layer of rectangular cells with small, narrow lumen.

(g) Proteid or aleurone layer; a single layer of cubical cells with very thick walls, and filled with a granular substance.

(h) Endosperm, consisting of polygonal cells filled with starch, the characters of which have already been described.

2. Wheat Flour

To whatever degree of fineness the flour may have been reduced it always contains portions of the pericarp and seed-coats in addition to the starch, although the latter, of course, constitutes by far the greater portion.

The diagnostic characters of wheat flours are (Figure 7.2):

(a) The shape and size of the large starch grains.

(b) The hairs, with lumen enlarged at the base, but in the upper part rather narrower than the wall.

(c) The thick-walled, pitted cells of the hypoderma.

(d) The thick-walled, pitted transverse cells.

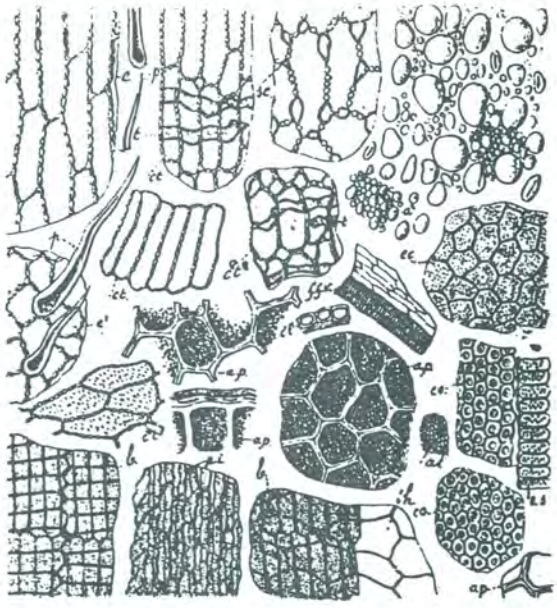


Figure 7.2. Wheat Flour

3. Wheat Starch

Wheat starch (Figure 7.3) is obtained from the fruits of several species of Triticum, as, for instance, T. sativum, Lam., etc. Like some other starches derived from cereal grasses it consists principally of a mixture of a large number of very small grains with others of much larger size; intermediate grains are comparatively rare.

The small grains very from 2 μ to 8 μ in diameter, averaging about 6 μ or 7 μ ; they are rounded or oval in outline, seldom polygonal or pointed.

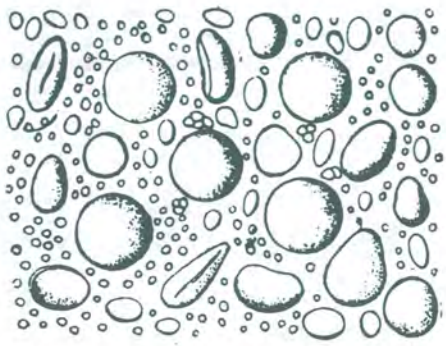


FIGURE 7.3. Wheat Starch

The large grains in surface view appear sometimes rounded, sometimes slightly irregular or oval, but when, by touching the coverslip with the needle they are made to present their edges to an observer, they are seen to be flattened or lenticular in shape. They seldom exhibit any concentric striae or evident hilum. They may attain as much as 45 μ in diameter, but they average only 25 to 35 μ . In side view they are elliptical or sometimes spindle-shaped, and exhibit a longitudinal line that is always simple and usually straight or slightly wavy.

4. Rye Flour

The anatomical structure of the rye grain closely resembles that of wheat. The principal diagnostic features of the flour are to be found in the appearance of the hairs and in the size and characters of the starch grains. The hairs of rye have about the same shape as those of wheat; they differ, however, in the lumen, for in the rye this gradually enlarges from apex to base, whereas in the wheat it is nearly linear in the upper part, and then suddenly enlarges and becomes bulb-shaped at the base. There is also a difference in the shape of the lignified cells of the hypoderma; these are usually longer than the transverse cells, whereas in wheat they are shorter. The transverse cells are also more frequently rounded at the ends, and have thinner walls than they have in wheat.

The diagnostic characters of rye flour are (Figure 7.4):

(a) The hairs with less abruptly enlarged lumen and thinner walls than in wheat.

(b) The transverse cells which have thinner walls and fewer pits, they are mostly shorter than the hypoderma cells, and often rounded at the ends, where the walls are also rather thicker.

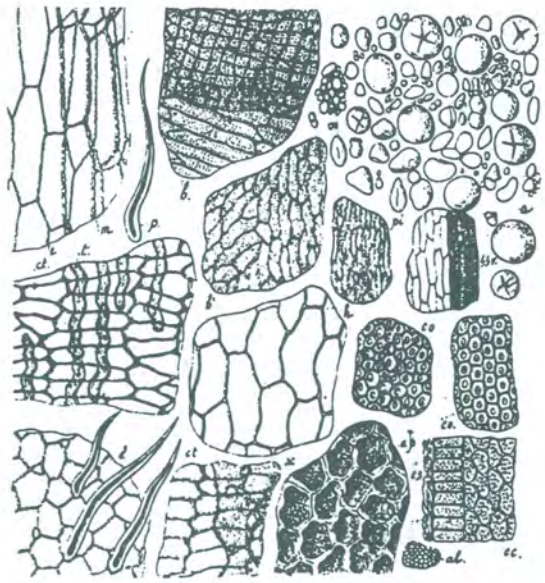


Figure 7.4. Rye Flour

(c) The starch grains which are rather larger than those of wheat and often show a stellate hilum and concentric striae.

5. Rye Starch

Rye starch (Figure 7.5) is contained in the fruits of the rye, Secale cereale, Linn. Like wheat starch it consists of a mixture of very small grains and large ones, together with a certain number of intermediate size. The large grains are discoid in shape, and often exhibit irregular protuberances, in consequence of which their side view is often less regularly fusiform or elliptical than it is in wheat starch. They average 40 μ in diameter, but may attain 50 and are therefore larger than the grains of wheat starch. Sometimes the concentric striae are indistinct, sometimes they are easily visible. In the centre there is often a cavity with from three to five rays and in such a case the helum is said to be stellate. Amongst the grains of medium and small size, hat-shaped and bell-shaped ones are to be found; these are very seldom seen in wheat starch. The small grains of rye starch are also rather larger than the corresponding grains of wheat starch; they vary from 3 to 10 μ in diameter.

Figure 7.5. Rye Starch

6. Barley Flour

In many respects barley resembles wheat and rye in its anatomical structure. One of the principal differences is to be found in the hairs which have a larger lumen than those of either wheat or rye. The walls of the epidermal cells of the pericarp are not pitted, nor are they so thick as those of the two latter grains. The aleurone layer invariably consists of two or three rows of cells, whereas in the wheat and rye there is only one row.

The grains of barley starch are smaller than those of wheat and much smaller than those of rye. They are less regular in shape, and frequently reniform, but these characters are difficult even for an expert to determine, and the detection of barley flour when mixed with wheat or rye flour is very difficult. There is, however, one peculiarity that may facilitate the identification of barley flour. The barley grain is enclosed between two paleae, and these adhere so firmly to the pericarp of the fruit that it becomes very difficult to effect their complete removal, especially from the groove on the ventral surface of the grain. The result is that barley flour often contains traces of the debris of the paleae, and these are easily identified by the very remarkable sinuous walls of the outer epidermal cells, and by the little hairs on the inner epidermis. The double or triple layer of aleurone cells should also be searched for.

The diagnostic characters of barley flour are (Figure 7.6):

(a) The epidermal cells of the paleae with thickened, sinuous walls.

(b) The hairs on the inner epidermis of paleae.

(c) The thin-walled epidermal cells of the pericarp, which are not pitted.

(d) The aleurone layer of two or three rows of cells.

(e) The starch grains, which are rather smaller than those of wheat, and often more irregular in shape.

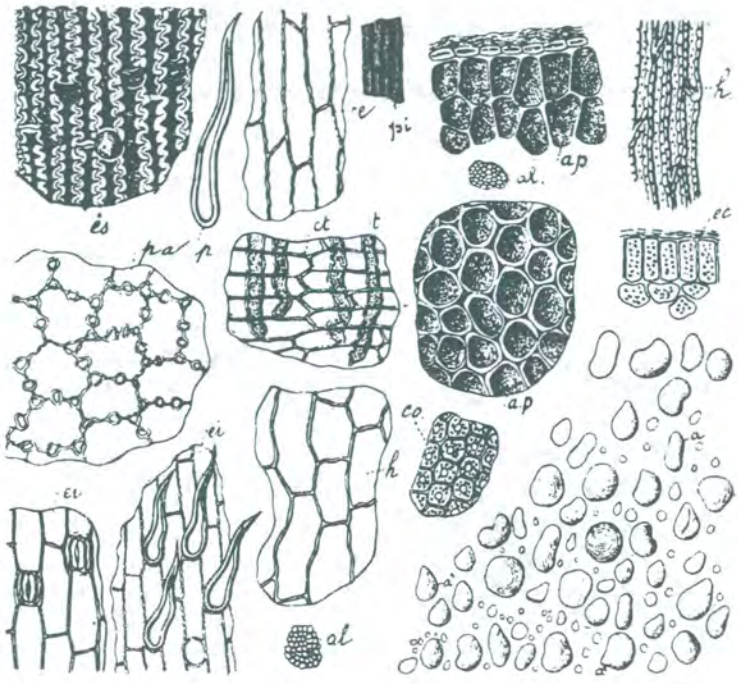


Figure 7.6. Barley Flour

7. Barley Starch

Barley starch (Figure 7.7) may be obtained from the fruits of Hordeum distichon, Linn. and other species of Hordeum.

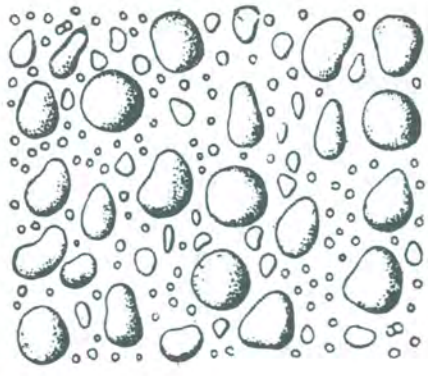
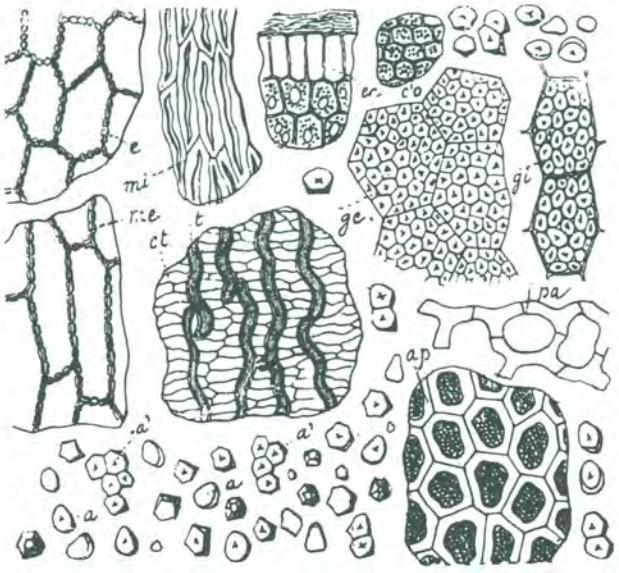


Figure 7.7. Barley Starch

Barley starch consists of a mixture of large and small grains, with a few of intermediate size. They are rather smaller than the grains of wheat starch, and are also distinguished by their outline, which is less regular and often bears protuberances. In surface view the large grains are seldom round; they are more often slightly elongated or elliptical, sometimes reniform, bulb- or pear-shaped. In diameter they vary from 20 μ to 35 μ, many being between 20 μ and 25μ. They have no apparent hilum, but some of them exhibit concentric striations. They are seldom fissured at the hilum and when that is the case the fissure is much less conspicuous than it is in rye starch, and never stellate. The grains of medium size vary from 10 μ to 15 μ in diameter, the small ones are about the same as those of wheat or rye-starch.

8. Maize Flour

The anatomy of the grain of maize is analogous to that of the fruit of other cereal grasses. It is characterized by the following particulars: Below the epidermis of the pericarp are two layers of cells that exhibit marked differences, one having pitted and relatively slightly thickened walls, those of the other being smooth and relatively strongly thickened. Within these layers is one of irregular cells with lacunae (transverse cells), and next to this the tubular cells that form the inner epidermis of the pericarp. The tubular cells are smaller, more numerous, and closer together than they are in wheat. During the ripening of the grain the seed-coats disappear almost entirely.



The diagnostic characters of maize flour are (Figure 7.8):

(a) The characteristic starch grains.

(b) The hypoderma.

(c) The numerous small tubular cells.

Figure 7.8. Maize Flour

9. Maize Starch

Maize starch (Figure 7.9) is obtained from the fruits of Zea mays, Linn. The grains of maize starch exhibit a certain difference in shape depending whether they are derived from the mealy centre of the endosperm or from the translucent horny periphery.

Those from the centre of the grain, having been subjected in a less degree to mutual pressure, are irregularly rounded in shape, or at least not markedly angular; some are nearly round, others are elongated, oval or pear-shaped. The hilum is always rather large and conspicuous; the grains measure from 10 μ to 25 μ in diameter, the average being about 13 μ to 15 μ .

Figure 7.9. Maize Starch

The grains from the horny part of the endosperm exhibit an angular contour due to the mutual pressure to which they have been subjected. Their appearance under the microscope varies considerably according to the position in which they lie. They are always easily recognized by their regular shape, angular outline, more or less uniform size, and by the presence of a distinct hilum, which is sometimes rounded, but more often fissured or stellate. The diameter of these grains averages from 14 μ to 15 μ but may sometimes reach 25 μ or 26μ.

10. Rice Flour

In the rice flour of commerce there is only a very small proportion of the seed-coats of the grain, and the chief characters are therefore to be found in the size and shape of the grains of starch of which it almost entirely consists. These are as follows: (1) Small simple polyhedral grains. (2) Large or small compound grains, oval or rounded in shape, and varying in size according to the number of constituent grains. (3) Fragments of the above of varying shape. (4) Masses of starch from the cells of the endosperm, or masses of several cells together (Figure 7.10):

The vegetable debris to be found in rice flour consists of only a few very narrow tubular cells closely attached to a layer of very small elongated cells. A knowledge of these characters is very desirable as the adulteration of wheat flour with rice flour is frequent at times.

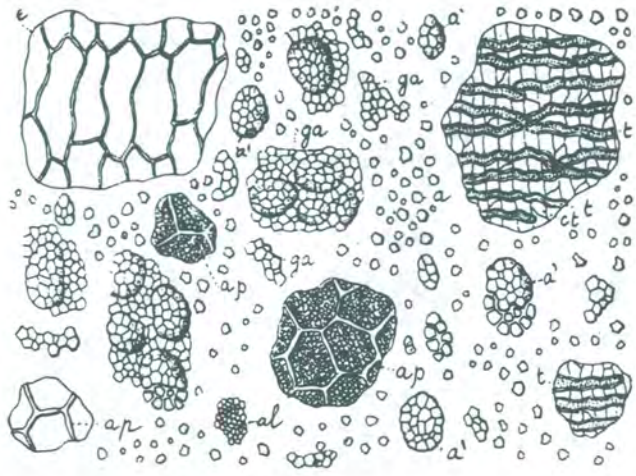


Figure 7.10. Rice Flour

11. Rice Starch

Rice starch (Figure 7.11) is obtained from the fruits of Oryza sativa, Linn. Like oat starch, it consists of both simple and compound grains. The simple grains are tolerably uniform in size and shape; they range from 4 μ to 6 μ, sometimes reaching 8 μ, and are generally angular. The compound grains are ovoid or rounded in shape, but vary very much in size, according to the number of constituent grains that they contain.

Rice starch closely resembles oat starch. The grains are, however, uniformly rather smaller and never spindle- or lemon-shaped. When treated with water the compound grains are readily dissociated into their constituent grains, and so it happens that the former are seldom found in the rice starch of commerce.

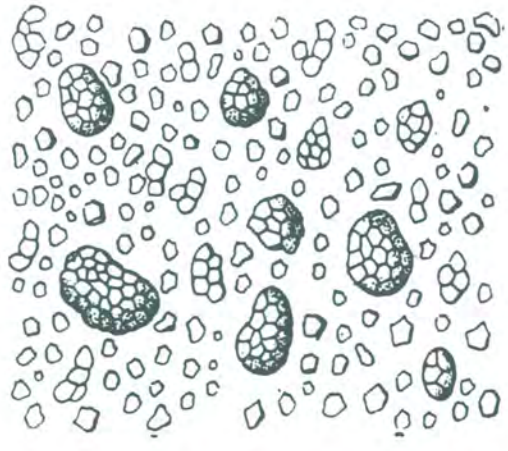


Figure 7.11. Rice Starch

12. Oat Flour

The oat grain is also enclosed between two paleae which may furnish valuable means of identifying the flour. The grain may also be distinguished from the three foregoing grains by the following characters:

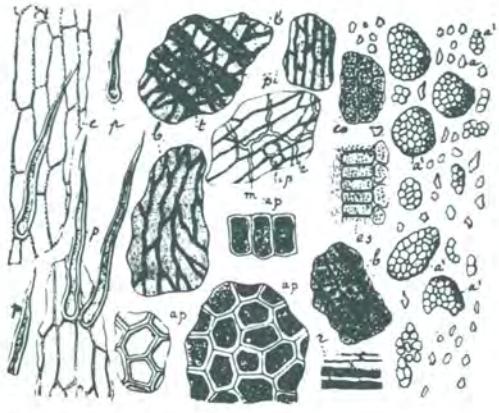


Figure 7.12. Oat Flour

(a) By the elongated shape of the hairs which are also often geminate. (b) By the cells of the outer epidermis of the pericarp, which have very thin walls and fairly numerous pits. (c) By the irregular polygonal shape of the cells of the hypoderma, of which, however, there is but little to be found. (d) By the cells of the seed coat, of which there is only a single row; they are polygonal or fusiform in shape, pale yellowish-brown in colour: their walls are smooth and seldom pitted, and the cells often exhibit an irregular arrangement. (e) By the starch which consists of small simple rounded grains associated with a number of large oval compound grains and the isolated angular constituent grains of the latter (Figure 7.12).

13. Oat Starch

Oat starch (Figure 7.13) is contained in the fruits of Avena sativa, Linn. It consists of two kinds of grains, simple and compound. The simple grains average about 10 μ in diameter. They are mostly rounded in outline, very few are angular, but some are spindle-shaped or lemon-shaped. The latter should be specially noted as they form a distinctive feature of oat starch.

The compound grains are oval or rounded and more or less regular in shape, ranging usually from 35 μ to 45 μ in length, but attaining as much as 50 μ . They consist of a varying number (5 to 200) of grains compacted together. The constituent grains vary in shape according to the position they have occupied in the compound grain. Those from the centre are angular, whilst those from the periphery are curved on one side and angular on the other; they are generally rather smaller than the simple grains.

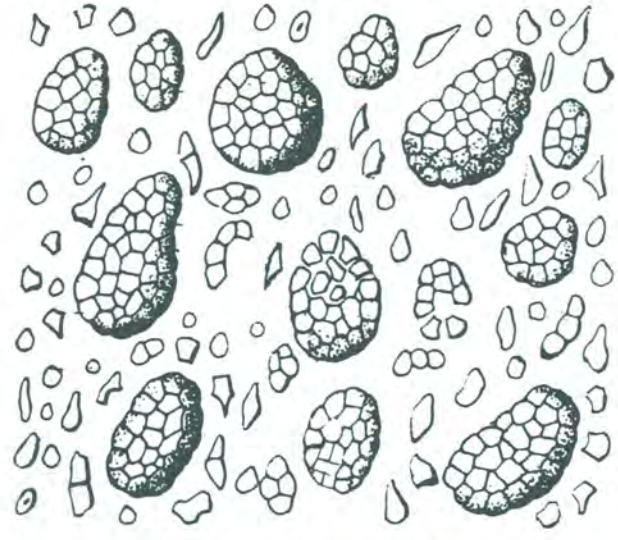


Figure 7.13. Oat Starch

14. Pea Flour

The pea (Pisum sativum, Linn.) much resembles the lentil in structure, but differs in the shape of the palisade cells, which are square at the apex instead of conical. These are thickened by similar bars visible in surface sections viewed from above, but not when viewed from below (size of the cells, 60 μ or more in length, 12 μ to 15 μ in width). The parenchyma of the seed-

coat is composed of cells similar in shape to those of the lentil, but exhibiting conspicuous intercellular spaces. The cells of the epidermis of the cotyledons are elongated, but in varying directions instead of parallel to one another as in the lentil. The starch grains are rather larger than those of the lentil (30 μ to 47 μ) and many of them bear rounded protuberances. The hilum is comparatively seldom fissured, and even then the fissure is not branched as it is in bean starch; the concentric striae are less regular, and often indistinguishable.

The diagnostic characters of pea flour are (Figure 7.14):

(a) The palisade cells with square ends.

(b) The characteristic hypodermal cells.

(c) The epidermal cells of the cotyledons not parallel.

(d) The starch grains with rounded swellings, less distinct unbranched hilum and less evident striae.

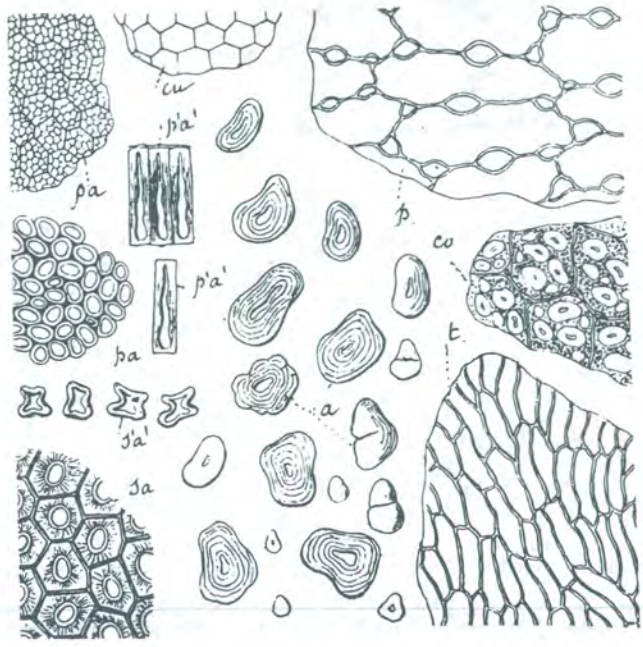


Figure 7.14. Pea Flour

15. **Lentil Flour**

The lentil (Lens esculenta, Moench), resembles most leguminous seeds in structure. The seed-coat is composed of the three following layers:

1. An epidermis consisting of a layer of palisade cells (about 40 μ by 10 μ) with a lumen that gradually tapers toward the cuticle; towards the upper part the wall is thickened by nearly vertical bars, the sections of which are distinctly seen in the surface view. The outer end of the cell is not flat but shortly and bluntly conical.

2. A layer of parenchymatous cells (about 15 μ by 15 μ); these are contracted in the middle, and hence assume the shape of an hour-glass; they differ from the hypodermal layer of the bean in not containing calcium oxalate crystals.

3. A layer of irregular parenchymatous cells with thin walls; this layer varies very much in the extent to which it is developed.

The cotyledons are covered with an epidermis consisting of polygonal cells, all of which are elongated in the same direction. The cells of the cotyledons themselves are polygonal, their walls are thin and occasionally exhibit small pits. They are filled with starch and aleurone grains. The former occupy a position that is intermediate between bean and pea starch as regards their shape and appearance. Many are ovoid but less regularly so than bean starch; many exhibit a fissured hilum and distinct concentric striae, but in others the hilum is not to be seen nor are the striae distinct; size of the larger grains 30 μ to 40 μ.

The diagnostic characters of lentil flour are (Figure 7.15):

(a) The palisade cells with conical ends.

(b) The hour-glass cells without calcium oxalate.

(c) The nearly parallel epidermal cells of the cotyledons.

(d) The thin-walled cells of the cotyledons.

(e) The starch intermediate in character between pea starch and bean starch.

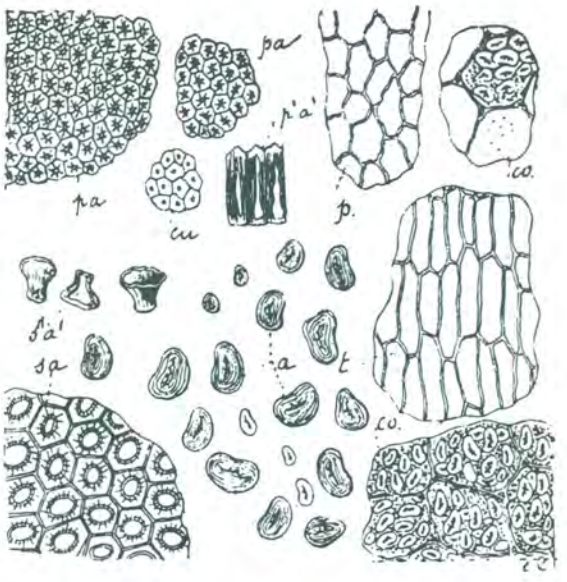
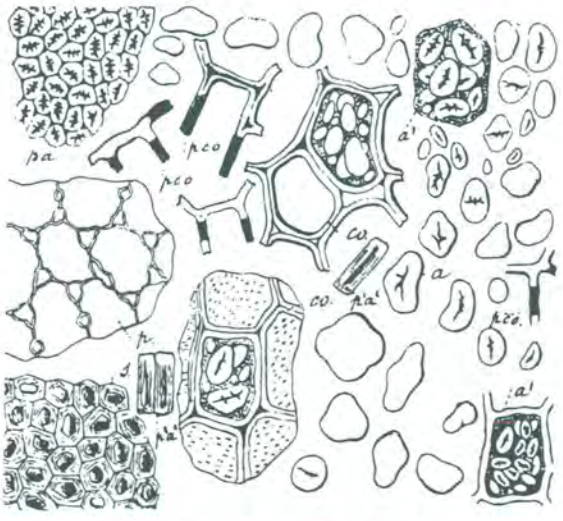


Figure 7.15. Lentil Flour

16. **Haricot Bean Flour**

The haricot bean, Phaseolus vulgaris, Linn., possesses an anatomical structure resembling that of other leguminous seeds. In the seed-coat the following layers can be distinguished: (1) An epidermis consisting of prismatic cells, radically arranged, like palisade cells; the walls of these cells are much thickened and possess slit-like pits. (2) A layer of nearly square, or rectangular cells, each containing a large prismatic crystal of calcium oxalate. These cells are often called "bearer cells", and are found in all varieties of beans, but in many other leguminous seeds they are contracted in the middle (in transverse section) and free from calcium oxalate. (3) A layer of parenchymatous tissue. The cells of the cotyledons are polygonal and exhibit at their angles either a collenchymatous thickening or more or less conspicuous intercellular spaces.

The starch of the haricot bean is in ovoid grains, seldom rounded, sometimes reniform, or exhibiting one or more protuberances. The hilum, which is usually very distinct, is elongated or fissured. In size the large grains vary from 30 μ to 75 μ ; however, many smaller ones are to be found.



The diagnostic characters of the flour of the haricot bean are (Figure 7.16):

(a) The remarkable palisade cells.

(b) The bearer cells with crystals of calcium oxalate.

(c) The cells of the cotyledons.

(d) The characteristic starch.

Figure 7.16. Bean Flour

17. **Buckwheat Flour**

The fruit of the buckwheat (Fagopyrum esculentum, Moench) is an achene. The pericarp is composed of (1) An epidermis consisting of a single layer of prismatic cells with thickened walls. (2) A fibrous hypoderma consisting of four or five layers of polygonal cells with thickened walls. (3) A layer of brown cells. (4) An inner epidermis of very long, flattened cells.

The seed is enveloped in three coats: (1) An outer coat composed of cells with very sinuous walls. (2) A middle coat of cells with lacunae. (3) An inner coat of elongated cells. Within these coats is an aleurone layer consisting of a single row of cubical cells; then the endosperm filled with starch.

The grains of starch are simple, and either isolated or agglomerated into masses. The isolated grains are bluntly or sometimes sharply angular, or often rounded. They may attain 10 μ or 12 μ in diameter, but average about 4 μ to 6μ. Buckwheat starch always contains a number of abnormal grains larger than the others; they are irregularly enlarged, often bearing some resemblance to an hour-glass.

The diagnostic characters of buckwheat flour are (Figure 7.17):

(a) The characteristic starch grains.

(b) The epidermis of the seed-coat, the cells of which have very sinuous walls.

(c) The middle layer, the cells of which exhibit lacunae.

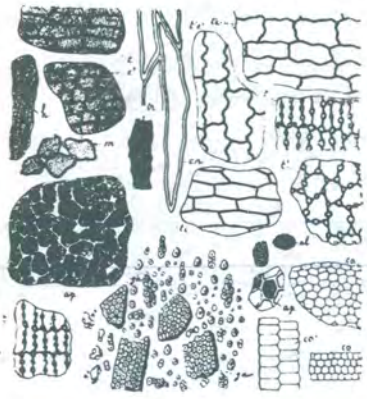


Figure 7.17. Buckwheat Flour

18. **Curcuma Starch (East Indian Arrowroot)**

Curcuma starch (Figure 7.18) is obtained from the rhizomes of Curcuma angustifolia, Roxb., C. leucorrhiza, Roxb. and other species of Curcuma.

The grains of this starch are oval, elliptical, almost rectangular or rounded in outline. At one of their extremities they usually terminate in a short obtuse point in which the very eccentric punctiform hilum is situated surrounded by concentric striae. The grains are so thin that when viewed on their edges they appear to be extremely narrow; several may often be seen in this position adhering together by their flat sides. Mixed with these larger grains are smaller ones of similar shape.

Curcuma starch grains average from 30 μ to 60 μ in length, 25 μ to 35 μ in breadth, and 7 μ to 8 μ in thickness. The length of the smallest grains scarcely exceeds 15 μ to 25 μ but the largest grains from C. leucorrhiza may attain as much as 140 μ .

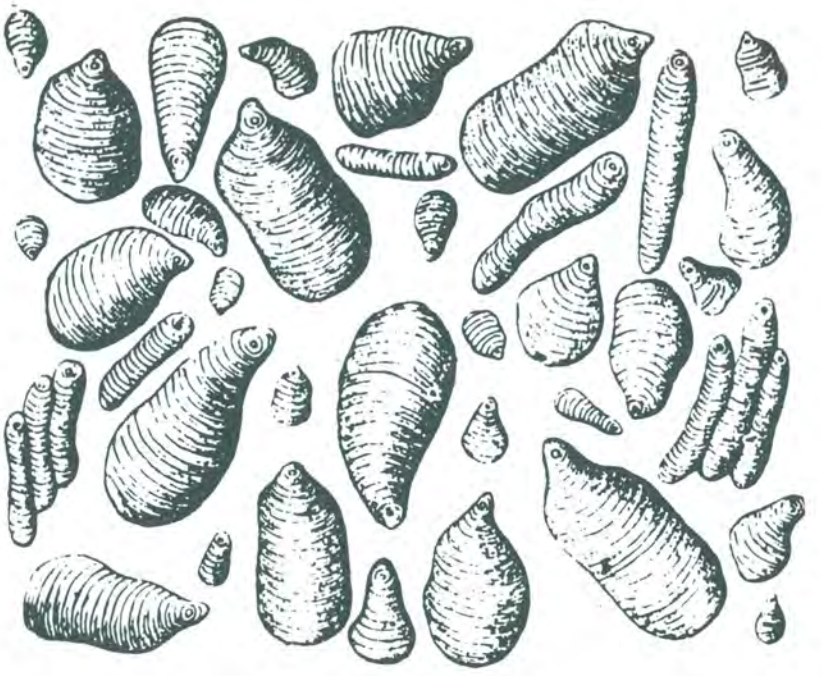


Figure 7.18. Curcuma Starch

19. **Tous les Mois Starch (Queensland Arrowroot)**

This starch (Figure 7.19) is obtained from the rhizomes of Canna edulis, Linn., and other species of Canna. The grains of which it consists are so large as to impart a satiny-white appearance to the starch. The majority are seldom less than 60 μ or 70 μ in length, whilst the largest occasionally reach 110 μ to 130 μ. They are usually simple and are elliptical, slightly oval, conchoidal, or sometimes reniform in outline; they are flattened and often prolonged at the narrower end to a short obtuse point in which the rounded hilum is situated, surrounded by concentric striae.

Figure 7.19. Tous les Mois Starch

20. Yam or Dioscorea Starch (British Guiana Arrowroot)

This variety of starch (Figure 7.20) is obtained from Dioscorea alata, Linn., and other species of Dioscorea. The largest of the grains of which it is composed measure 45 μ to 90 μ in length and 25 μ to 60 μ in breadth, while the smaller vary from 15 μ to 30 μ in length and about half that in breadth. In outline they are very variable, being often oval or elliptical, three-sided, with rounded angles, or sometimes curved. The larger extremity is often truncate and in the opposite narrower extremity the hilum is situated; this is rounded, eccentric and surrounded by concentric striae.

Figure 7.20. Dioscorea Starch

21. Banana (or Plantain) Starch

This is obtained from the unripe fruits of Musa sapientum, Linn., and has also been offered in commerce as Guiana arrowroot. The grains are simple and show a great variation in outline: some are oval, ellipsoidal or elongated, whilst others are curved, bottle-shaped, bean-shaped, etc. They are always flattened, and therefore appear narrow and sausage-shaped when presenting their edges to the observer. The hilum is rounded, situated near one extremity and surrounded by concentric striae. The largest grains measure 45 μ to 65 μ , the smallest about 7 μ , intermediate grains from 22 μ to 34 μ (Figure 7.21).

The fruits from which the starch is prepared are often distinguished as plantains, and the plant yielding them is sometimes distinguished by the name Musa paradisiaca, Linn.

Figure 7.21. Banana Starch

22. Manihot Starch (Cassava)

This variety of starch (Figure 7.22) is obtained in large quantities from the tubers of Manihot utilissima, Pohl, and other species as Manihot. It is also known under the name of cassava starch, mandioc starch, Brazilian, Bahia, Rio or Para arrowroot, etc. The majority of it is converted into tapioca before exportation.

The grains are originally compound, consisting of two, three or four component grains, and are occasionally found intact. Most of them, however, have been separated into their component grains. They are seldom quite round. Most of them exhibit one or two flat surfaces where other of the constituents of the compound grain have been attached, and are in consequence muller-shaped, cap-shaped, or shortly conical, curved on one side and irregular on the other, etc., some are even polygonal. The majority possess a distinct rounded linear or stellate hilum and delicate concentric striations. The largest measure 25μ to 35 μ in length, the smallest 5 μ to 15 μ ; many range from 15 μ to 25 μ .

Figure 7.22. Manihot Starch

23. **Tapioca**

This substance is prepared from manihot starch, by heating and stirring the moist starch until it agglomerates into the little irregular, rugged masses that are known in commerce as tapioca; it is usually exported in this form and constitutes an important article of food. The granules of tapioca soften when soaked in water for a few hours, and a small portion, taken preferably from the whiter and more opaque part, can be broken up with a needle in a drop of water and covered with a coverslip, a little pressure being applied if necessary. Many of the grains will be seen to have preserved their original shape, and exhibit a distinct hilum; in many the hilum is stellately fissured; in others the central part of the grain is a translucent mass, but the outline is still recognizable; whilst finally many have swollen into a shapeless, unrecognizable mass. These are the various stages in the gelatinisation of the starch by heat in the presence of moisture(Figure 7.23).

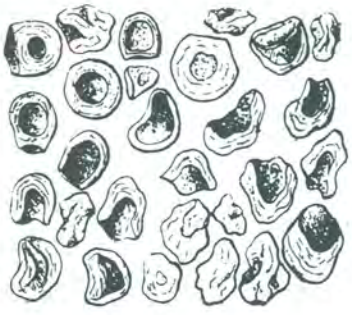


Figure 7.23. Tapioca

24. **Tacca Starch (Tahiti Arrowroot)**

It is obtained from the tubers of Tacca pinnatifida, Linn. The grains vary both in size and shape. Typical ones are rounded, oval or even lenticular; some are elliptical, almost triangular, etc. The hilum is generally fissured situated near the centre of the grain, and surrounded by concentric striae. The larger grains measure 38 μ to 50 μ , the smaller 15 μ to 25 μ (Figure 7.24).

Figure 7.24. Tacca Starch

25. Sago Starch

Sago starch is obtained from the stem of the sago palm, Metroxylon sagu, Rottb. and allied trees. The form of the grains of sago starch varies according as they are simple or compound. Simple grains are oval, rounded, etc. but the compound grains have a very remarkable shape. Each of these usually consists of a large grain to which 1, 2 or 3 small ones are attached. The large grain is conical or muller-shaped, and frequently bears one or two projections, to the flat ends of which the smaller grains have been attached; sometimes these two flat surfaces meet to form an angle. The largest grains measure 50 μ to 65 μ in length, the smallest 10 μ to 20 μ . The hilum is very distinct, linear, transverse or oblique, sometimes stellate and usually surrounded by distinct striae (Figure 7.25). Commercial sago starch often contains debris of vegetable tissue, etc. left in it by the imperfect washing it has undergone. Sclerenchymatous cells, hairs and crystals may thus be found in it.

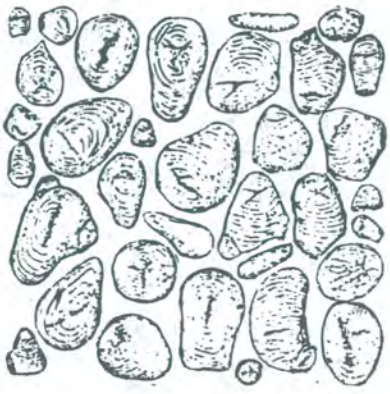


Figure 7.25. Sago

26. Pearl Sago (East Indian Sago)

Genuine or East Indian sago (pearl sago) is largely prepared in Singapore from sago starch (Figure 7.26). The starch is converted into sago by heating it whilst moist, as described under tapioca. Several varieties occur in commerce, differing in source and appearance. Pearl sago, when examined under the microscope, exhibits starch grains in various stages of transformation, induced by the heat to which they have been subjected. Some of them have preserved their original shape and can be easily identified. Many are more or less altered; in some the central portion has been gelatinised and is transparent and homogeneous; others have swollen to an unrecognizible gelatinous mass.

Figure 7.26. Pearl Sago

27. Potato Starch

Potato starch (Figure 7.27) is obtained from the tubers of Solanum tuberosum, Linn. It is composed of grains of variable size, some being so large as to be visible to the naked eye. Typical grains of this starch are flattened, and have an oval, ovate, ellipsoidal or conchoidal outline. The hilum is punctiform, eccentric, and generally situated in the narrow end of the grain; it is surrounded by numerous distinct concentric striations, some few of which are much more conspicuous than the others. In addition to these typical grains there are a few others, smaller in size and rounded in outline, or rounded on one side and flattened on the are sometimes attached by their flat sides in twos or threes. The largest grains vary in length from 75 μ to 110μ, those of the medium size from 45 μ to 65 μ and the smaller ones from 15 μ to 25μ.

Figure 7.27. Potato Starch

28. **Maranta Starch**

Maranta starch (Figure 7.28) is obtained from the rhizomes of Maranta arundinacea, Linn. and other species of Maranta. It is commonly known in commerce as "arrowroot", a term however, which is also applied to the starches of other and widely different plants.

The different varieties of arrowroot are distinguished by their geographical sources. Maranta starch is known as Bermuda, St. Vincent, West Indian or Natal arrowroot, according to the country in which it is prepared.

The grains of the Maranta starch are simple and rather large. They are irregular in shape, being rounded, ovoid, pear-shaped or sometimes almost triangular; the smallest ones are nearly spherical. The largest bear numerous fine concentric striations, and a conspicuous rounded, linear or stellate, eccentric hilum. In some varieties of arrowroot (Natal), the rounded hilum predominates, in others (St. Vincent) the linear or stellate; it often resembles the wings of a poised bird. They average about 30 μ to 40 μ in length, but may attain to 45 μ , 60 μ , or even 75 μ as, for instance, in Bermuda arrowroot; the smaller grains vary from 7 μ to 15 μ .

Figure 7.28. Maranta Starch

ACIDITY IN FLOUR
(Water Extract)

PRINCIPLE

The acidity of an aqueous extract prepared under standard conditions is determined by titration and calculated as lactic acid.

APPARATUS

1. Waterbath at 40°C.

REAGENTS

1. 0.1 N NaOH.

PROCEDURE

Weigh 18 g of flour into a 500 ml conical flask and add 200 ml of carbon dioxide-free distilled water. Stand the flask in a waterbath at 40°C for 1 hour so that the flask is covered to just about the level of liquid. Swirl occasionally to ensure complete mixing. After 1 hour, filter and titrate 100 ml of the filtrate with 0.1 N NaOH.

CALCULATION

$$\text{Acidity, \% lactic acid} = \frac{\text{ml of 0.1 N NaOH}}{1000} \times 0.1 \times 90 \times \frac{100}{9}$$

$$= \frac{\text{ml of 0.1 N NaOH}}{10}$$

(90 = equivalent weight of lactic acid).

INTERPRETATION

Under tropical conditions, there have been adverse reports on consignments of white wheat flour as landed if the acidity was over 0.35%. Samples containing over about 0.45% almost invariably taste stale and bitter and above 0.35% the taste of white wheat flour correlates fairly well with the acidity. Higher extraction flours have a naturally higher acidity.

REFERENCE

Egan, H., Kirk, R.S. and Sawyer, R., 1981. Pearson's Chemical Analysis of Foods, 8th Ed., Churchill Livingstone.

ACIDITY IN FLOUR
(Alcohol Extract)

PRINCIPLE

The sample is shaken with ethanol and the extract titrated with standard alkali.

REAGENTS

1. Ethanol, 90%, v/v, neutralized to phenolphthalein.

2. Phenolphthalein, 1% in neutral ethanol.

3. 0.05 N Sodium hydroxide solution.

PROCEDURE

Weigh 5.0 g of sample into a conical flask, add 50 ml of 90% ethanol, stopper the flask, shake and leave to stand overnight. Decant the extract through a filter, wash the residue with 20 ml of 90% v/v neutral ethanol and decant through the same filter. Titrate the combined filtrate with 0.05 N sodium hydroxide solution to the phenolphthalein end-point.

CALCULATION

$$\% \text{ acidity (as sulphuric acid)} = \frac{\text{titre}}{1000} \times .05 \times 49 \times \frac{100}{5}$$

INTERPRETATION

Recently milled flours have alcohol extract acidity of 0.03 - 0.04% as sulphuric acid.

REFERENCE

Egan, H., Kirk, R.S. and Sawyer, R., 1981. Pearson's Chemical Analysis of Foods, 8th Ed., Churchill Livingstone.

ASH IN FLOUR

PRINCIPLE

A portion of flour is ashed and the residue weighed.

APPARATUS

1. Muffle furnace.

2. Analytical balance.

PROCEDURE

Weigh 3-5 g flour in a tared silica dish. Ignite in a muffle furnace at 600°C to constant weight.

CALCULATION

$$\% \text{ ash} = \frac{\text{wt ash (g)}}{\text{wt flour (g)}} \times 100$$

INTERPRETATION

The outer portions of grains have a higher mineral content than the inner. Therefore the ash of white flour will be less than that of wholemeal flour. For wheat flours, the ash gives an indication of grade. 'Patent' flour gives an ash of 0.3 - 0.4%, 'Straight Run' (72% extraction) gives about 0.45% and 'Wholemeal' gives 1.2 - 1.8%. However, if chalk has been added to the flour, then ash figures are invalid.

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 14.006.

IRON IN FLOUR

PRINCIPLE

Iron is often added as a nutrient to enriched flours, usually as ferrous sulphate, ferric ammonium citrate or iron powder. This method involves ashing the flour to remove organic material, reducing ferric ion to ferrous with sulphur dioxide and reacting with o-phenanthroline. The resultant red compound is determined spectrophotometrically.

APPARATUS

1. Muffle furnace.

2. Steam bath.

3. Hot plate.

4. Pipettes and volumetric flasks.

5. Spectrophotometer (visible range).

REAGENTS

1. Glycerol-alcohol mixture (1+1).

2. Nitric acid, concentrated.

3. Hydrochloric acid, 5 N.

4. Hydrochloric acid, dilute (1 ml acid to 100 ml with water).

5. Sulphur dioxide solution, 2%. Dissolve 3.25g sodium meta-bisulfite in 100 ml water.

6. Sodium acetate solution, 2 N.

7. Congo red indicator paper.

8. o-Phenanthroline solution, 0.25% in water.

9. Ferrous ammonium sulphate hexahydrate standard - dissolve 0.7024 g in water, add 2 drops HCl and dilute to 1 litre. Dilute 50 ml of this to 1 litre (1 ml = 0.005 mg Fe).

PROCEDURE

Weigh an amount of flour containing up to 0.4 mg iron into a silica ashing dish. (Note that iron is often added to flour at 1.6 mg/100 g). Add 10 ml of the glycerol-alcohol mixture. Ash overnight at 600°C (after initial heating with an infrared lamp or at the mouth of the open muffle).

Cool and add 1 ml concentrated nitric acid. Evaporate and re-ash 1 hour. Cool and add 5 ml 5 N HCl. Heat on a steam bath 15 minutes and filter through hardened filter paper into a 100 ml volumetric flask.

Add 3 ml dilute HCl to the silica dish, bringing to boil on a hot plate and filter using the same filter and flask as above. Repeat this process four more times. Then wash the dish and filter with hot water to make the filtrate flask to the mark.

Mix and pipette 10 ml into a 25 ml volumetric flask. Add 1 ml 2% sulphur dioxide solution. Add 2 N sodium acetate solution by burette until the solution changes congo red paper from blue to pink. Next, add 2 ml 0.25% o-phenanthroline solution and make to the mark.

Let stand overnight to develop the red colour. Read the absorbance in a 4 cm cell at 520 nm versus a blank prepared the same way as the sample.

Prepare a standard curve by pipetting 0, 2, 5, 7 and 10 ml of the dilute standard solution (0.005 mg Fe/ml) into five 25 ml volumetric flasks. Add reagents as with the sample and read the absorbances. Plot the absorbance versus the mg iron.

CALCULATION

Find the mg iron corresponding to the sample absorbance using the standard curve. This is 'w'.

$$\text{mg iron/100 g flour} = \frac{w \times 1000}{\text{g sample taken}}$$

REFERENCE

This method is adapted from Official Methods of Analysis of the AOAC, 1984, 14.011-.013. It differs primarily in the reducing agent used.

7.3 BREAD

ROUTINE ANALYSIS

Bread may be enriched by addition (to the flour) of thiamine, riboflavin, niacin, iron, vitamin D and calcium. Preservatives such as propionic acid and emulsifiers and stabilizers are sometimes used in bread making. Propionic, sorbic and benzoic acids may be determined by the GLC procedure of Graveland (8).

The fibre content gives an indication of whether the bread was made from wholemeal flour. The residue from the fibre test may be examined microscopically to assess the amount of filth in the ingredients. The fibre may be determined by a number of methods, including the dichromate method of van de Kamer and van Grinkel (9).

Starch is determined by titration of reducing sugars after hydrolysis with acid or by an enzymic method. The latter depends on conversion of autoclaved starch to glucose by glucamylase, oxidation of the glucose to gluconic acid with liberation of hydrogen peroxide and reaction of the latter with o-dianisidine to form an orange-red colour. Milk solids in bread are assessed from the levels of lactose or orotic acid. The determination of lactose in bread requires the prior fermentation of other sugars present. Normally, the bread is air dried, a portion taken for moisture and analytical figures are expressed as a percentage of the dry matter since the moisture content of fresh bread varies considerably. Recommended conditions of temperature and pressure for drying vary (100°C and 25 mm Hg to constant weight or 130°C for 1 hour, or 50°C and 10-20 mm Hg to constant weight or 130°C for 2 hours, or 105°C to constant weight).

MOISTURE IN BREAD

PRINCIPLE

The loaf is weighed, cut and air-dried, the air-dried product is weighed and ground and the moisture determined on a small portion. The total moisture is calculated as a percentage of the whole loaf as received. The method is not applicable to bread containing fruit.

PROCEDURE

Accurately weigh loaf of bread immediately upon receipt, using scales sensitive to at least 0.2 g. If impossible to weigh accurately at this time, seal sample in air-tight container and weigh accurately as soon thereafter as is practicable. Preserve sample in such a manner that no loss of bread solids can occur whereby loss would be calculated as moisture.

Cut bread into slices 2-3 mm thick. Spread slices on paper, let dry in warm room (15-20 hours) and when apparently dry, break into fragments. If bread is not entirely crisp and brittle, let it dry longer - until it is in equilibrium with moisture of air - so that no moisture changes occur during grinding.

Weigh, grind and weigh again to check absence of grinding losses. Mix well and determine the moisture content on a small weighed portion (about 2 g) by drying 1 hour at 130°C.

CALCULATION

$$\% \text{ moisture in bread} = \% \text{ moisture in dry ground sample} \times \frac{\text{weight of air-dried slices}}{\text{weight of fresh loaf}}$$

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 14.087.

7.4 TEXT REFERENCES

1. MALONE, B. 1969. Journal of the Association of Official Analytical Chemists 52 (4) 800-805.

2. PANEL ON FUMIGANT RESIDUES IN GRAIN, REPORT. 1974. Analyst 99 570-576.

3. MUTHU, M., KASHI, K.P., & MAJUMDER, S.K. 1978. Chemistry and Industry 4 18.2.78. 129-131.

4. PEARSON, D. 1981. The Chemical Analysis of Foods, 8th Ed. Churchill Livingstone.

5. GRIFFITHS, J.G.A. 1937. Analyst 62 510.

6. TILLMANS, J., HOLL, H. & JANIVALA, L. 1928. Zeitschrift fur Lebensmitteluntersuchung und Forschung 56 26.

7. TILLMANS, J. 1929. Analyst 44 43.

8. GRAVELAND, A. 1972. Journal of the Association of Official Agricultural Chemists 55 (5) 1024.

9. VAN de KAMER, J.H. & Van GRINKEL, L. 1952. Cereal Chemistry 29 239-51.

Further Reading

BUSHUK, W. 1976. Rye: Production, Chemistry and Technology AACC.

POMERANZ, Y. 1971. Wheat: Production, Chemistry and Technology AACC.

HOUSTON, D.F. 1972. Rice: Production, Chemistry and Technology AACC.

TSEN, C.C. 1974. Triticale, First Man-Made Cereal AACC.

KENT-JONES, D.W. & AMOS, A.J. 1957. Modern Cereal Chemistry 5th Ed. The Northern Publishing Co.

AACC Approved Methods, American Association of Cereal Chemists, St. Paul, Minnesota, USA.

ICC-Standards, International Association for Cereal Chemistry Schmidgasse 3-7, A-2320 Schwechat, Austria.

8. HERBS AND SPICES

8.1 HERBS

COMPOSITION

The following table is taken in part from Pearson's Chemical Analysis of Foods, 8th Ed., 1981, and represents analyses of various herbs. (Note that single values represent one analysis. All other values are ranges.)

Herb	Total Ash %	Acid Insoluble Ash %	Volatle Oil %	Stalks etc. %
Basil	11.5	0.3	1.8	-
Bay	2.8 - 4.1	0.2 - 0.8	0.7 - 2.3	10
Marjoram	8.9 -12.0	0.4 - 2.7	1.2 - 2.5	2-12
Mint	10.0 -14.4	0.3 - 2.2	0.5 - 3.0	4-15
Parsley	12.4 -18.4	1.0 - 6.0	6.6	2
Rosemary	-	-	0.9	-
Sage	5.4 -14.3	0.5 - 3.5	0.6 - 1.5	4
Savory	6.3 - 9.9	0.1 - 0.8	0.9 - 1.5	5-15
Thyme	7.0 -19.2	0.8 - 9.8	0.4 - 2.5	2- 8

8.2 SPICES - SEEDS

COMPOSITION

Umbelliferous spices are called cremocarps and are a variety of splitting fruit which divides into one-seeded parts known as mericarps. Some ranges of composition of these spice seeds are as follows:

Spice	Ash %	Non-volatile extract %	Volatile Oil %	Crude Fibre %
Anise	-	8 - 20	1.5 - 4.0	-
Caraway	4.8 - 7.6	8 - 20	2.5 - 5.9	17 - 22
Celery	0.1	15 - 20	1.5 - 3.0	-
Coriander	-	12 - 20	0.3 - 1.0	-
Cummin	0.8	10 - 14	2.0 - 4.0	-
Dill	-	15 - 18	2.0 - 4.0	-
Fennel	-	12 - 20	0.8 - 4.0	-

The composition of other spice seeds are:

Spice	Moisture %	Ash %	Non-volatile extract %	Volatile Oil %	Crude Fibre %
Cardamom	< 1.3	< 9.2	-	> 4	< 8
Fenugreek	-	< 6.0	-	-	-
Mace	3.5 - 7.0	1.6 - 2.5	24 - 33	4 -15	4.7 - 8.0
Mustard	4.8 - 7.0	3.7 - 4.5	24 - 39	0.5- 1.0	1.4 - 4.2
Nutmeg	4.8	1.8 - 4.5	30 - 40	5 -15	2.0 - 3.7

ROUTINE ANALYSIS

Spices should be of normal pungency, usually assessed from the volatile oil and the non-volatile ether extract. They should also be free from extraneous material (assessed from the ash, acid-insoluble ash and examination for filth, hairs, insects and insect fragments and rodent droppings). The identity of ground spices must be checked by a careful microscopical examination. It is common to determine lead and arsenic in spices.

The International Organization for Standardization (ISO) has been active in standardizing methods and compositional limits for spices. ISO R 676:1968 gives the botanical, French, Russian and English names of 68 spices and condiments. ISO 2825:1974 recommends that in the preparation of samples for analysis, they are gound to approximately 1 mm, avoiding undue heating and as far as possible contact with the outside air. They should then be placed in clean, dry, air-tight containers so as to nearly fill them.

The quality of spices is assessed from a number of values in addition to the volatile and fixed oil contents. Other values include crude fibre, water-soluble ash, alcohol extract, cold water extract and total nitrogen. Nitrogen is determined by the routine Kjeldahl procedure. Moisture is determined by the Dean and Stark entrainment method, except in the case of mustard. Filth and insect fragments can be determined by the AOAC procedures. Added salt may be found in powdered spices. Whole spices may be faced with mineral oil or added colour in an attempt to improve the appearance.

Mixtures of spices also must be analyzed from time to time. Curry powder is one of the most common mixtures. This is usually composed of a few of the following - capsicum, cassia, cinnamon. coriander, ginger, turmeric, fenugreek, fennel, mustard, pepper and pimento. Leaves of Bay and Murraya Koenigii, cumin, dill, mace and cardamom are also possible though the last two are more expensive than the others.

IDENTIFICATION

Anise

Anise is the fruit of Pimpinella anisum, Linn. (Umbelliferae). The fruits are about 3 mm long, greenish-grey to brown in colour, ovoid and somewhat laterally compressed. The volatile oil contains about 90% of anethole.

The transverse section of the fruit exhibits the following structure:

a. An outer epidermis composed of flattened cells and provided with stomata as well as with numerous single hairs. In surface view the epidermal cells appear polygonal and strongly striated; the hairs are short, conical, thick-walled warty, and usually one-celled.

b. The parenchymatous tissue next to the epidermis is made up of polygonal cells, and is traversed by secretory ducts. The number of the latter is variable, but always considerable, and they are placed close together. This tissue is also traversed by a number of fibro-vascular bundles surrounded by sclerenchymatous tissue of varying extent, the cells of which are polygonal and have thickened pitted walls.

c. An inner epidermis, consisting of a single row of cells, all of which are elongated in the same direction.

d. A seed-coat, which is represented by a single row of brown flattened cells; in surface view these appear polygonal and isodiametric.

e. An endosperm, composed of polygonal cells containing fixed oil and aleurone grains; in the latter a globoid or a rosette of calciun oxalate may be found.

The diagnostic characters of powdered anise fruit are (Figure 8.1):

(1) The short, stout, conical hairs.

(2) The numerous, narrow, brown oil-ducts.

(3) The sclerenchyma of the pericarp.

(4) The contents of the endosperm cells.

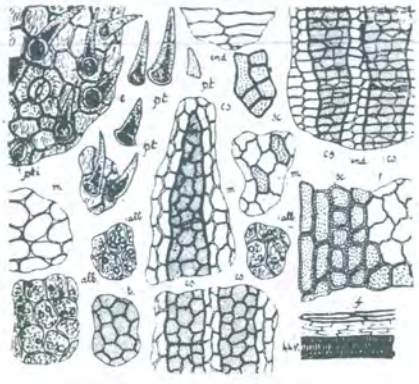


Figure 8.1. Anise

Caraway

Caraway is the fruit of Carum carvi, Linn. (Umbelliferae). The fruits are about 6 mm long with a brown, glabrous surface and slightly curved. The volatile oil contains about 50% carvone. The slight brown Levant (or Mogador) caraway and also Indian dill have been substituted for genuine caraway.

The fruit exhibits the following structure:

a. An outer epidermis, composed of axially elongated cells with striated cuticle and pitted walls; here and there a stoma is visible, but it offers no remarkable features.

b. A narrow layer of parenchymatous tissue, consisting of irregular polygonal cells; this tissue is traversed by fibro-vascular bundles, which are situated in the ridges of the fruit, and are supported by strands of sclerenchymatous cells; the latter possess pitted walls, but vary greatly in size and shape. Six large brown vittae also occur in this tissue.

c. An inner epidermis, composed of polygonal, thin-walled cells, which are all tangentially elongated and exhibit a very regular arrangement.

d. The seed-coat, consisting of a single layer of small polygonal cells of a rather dark brown colour.

e. The endosperm, made up of rather thick-walled cells containing aleurone grains and fixed oil.

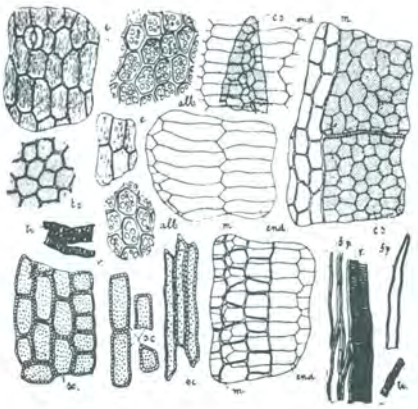


Figure 8.2. Caraway

The diagnostic characters of powdered caraway are (Figure 8.2):

(1) The abundant sclerenchymatous tissue.

(2) The absence of hairs and of spiral and reticulate cells.

(3) The striated epidermis.

(4) The large cells of the inner epidermis and their regular arrangement.

(5) The small aleurone grains.

Celery

Celery is the fruit of Apium graveolens. The "seeds" are brown, sub-spherical and very small. The most outstanding feature of the microscopic structure are the elongated crossing cells similar to fennel.

Celery salt is usually a mixture of salt and ground celery seed. Canada requires a minimum of 20% celery seed, for example. Celery salt usually contains magnesium carbonate and may also contain calcium stearate.

Coriander

This is the fruit of Coriandrum sativum, Linn. (Umbelliferae).

The dorsal portion exhibits in transverse section:

a. An outer epidermis composed of tabular cells which in surface view are seen to be polygonal, and have slightly thickened, pitted walls. It is often partially thrown off, especially from the intercostal regions; it is provided with stomata, and in some of the cells a prismatic crystal of calcium oxalate may be observed.

b. A tissue, corresponding to the mesocarp, which has undergone considerable differentiation, and in which the following layers can be distinguished: (1) an outer layer of tangentially elongated parenchymatous cells; (2) a well-developed layer of sclerenchyma, traversed by fibro-vascular bundles, and forming a continuous and very thick protective tissue throughout the entire dorsal portion of the mericarp; the cells of which this layer is composed are elongated, have thick, pitted walls and cross in different directions; (c) one or two rows of flattened thin-walled cells; (d) two or three rows of large, irregular polygonal cells with very thick, pitted walls.

c. An inner epidermis of flattened, tangentially elongated cells which in surface view are seen to be rectangular, four or five times as long as they are broad, and all elongated in the same direction.

d. A seed-coat consisting of a single layer of pale yellow polygonal cells with slightly wavy walls.

e. An endosperm made up of thick walled polygonal cells containing aleurone grains, fixed oil, and small rosette-crystals of calcium oxalate.

The structure of the commissural portion of the fruit is slightly different from that of the dorsal portion; the sclerenchymatous layer is absent, and the mesocarp is traversed by two large secretory ducts (vittae).

The diagnostic characteris of powdered coriander fruit are (Figure 8.3):

(1) The epidermal cells with prismatic crystals.

(2) The fibrous sclerenchymatous layer of the pericarp.

(3) The large sclerenchymatous cells in the inner part of the pericarp, to which the inner epidermis is often attached.

(4) The large secretory ducts.

(5) The minute rosettes of calcium oxalate in the endosperm.

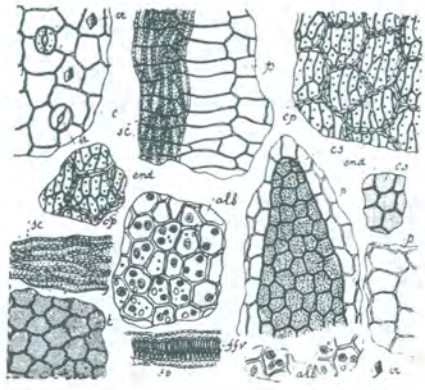


Figure 8.3. Coriander

(The last two characters are found in other umbelliferous fruits.)

Cummin

The fruit of Cuminum cyminum, Linn. (Umbelliferae).

The fruit exhibits the following structure:

a. An outer epidermis composed of polygonal cells and provided over the secondary ridges with conical, pluricellular, pluriserial hairs.

b. A tissue, corresponding to the mesocarp, traversed by five fibro-vascular bundles situated below the primary ridges. This tissue also contains six vittae, four of which are placed below the secondary ridges and the remaining two on the commissural surface. In this tissue and near the fibro-vascular bundles sclerenchymatous cells of varying shapes are to be found; some are polygonal and elongated, others sinuous, etc., but all of them have thick, pitted walls. The bundles themselves are accompanied by sclerenchymatous fibres with lignified walls.

c. An inner epidermis composed of tolerably regular polygonal cells all elongate in the same direction.

d. A seed-coat consisting of brown polygonal cells.

e. An endosperm with thick-walled cells in which aleurone grains, fixed oil and small rosette crystals of calcium oxalate are contained.

The diagnostic characters of powdered cummin fruit are (Figure 8.4):

(1) The pluricellular, pluriserial hairs.

(2) The sclerenchymatous cells from the mesocarp.

(3) The large oil-ducts.

(4) The contents of the cells of the endosperm.

Figure 8.4. Cummin

Dill

Dill is from Anethum graveolens. The mericarps are about 4 mm x 2.5 mm, flat and glabrous. The main constituent of the volatile oil is carvone as is also the case with caraway. The main microscopical features are the striated cuticle of the epidermis, the fixed oil and the aleurone grains enclosing a microcrystal of calcium oxalate.

Fennel

The fruit of Foeniculum capillaceum, Gilib. (Umbelliferae).

The transverse section exhibits the following characters:

a. An outer epidermis, composed of polygonal cells with straight walls and furnished with stomata.

b. Parenchymatous tissue (mesocarp) composed of irregular polygonal cells; many of these are characterized by their reticulate or spiral thickening; they are either isolated or form groups in the ridges of the fruit, near the fibro-vascular bundles. There are six large vittae, easily distinguished by the brown colour of their walls; four are situated on the dorsal surface of the fruit, and two on the commissural. The bundles are composed of tracheids with a few bast cells, supported by a mass of sclerenchymatous fibres with pitted walls.

c. An inner epidermis, composed of a single layer of narrow, elongated cells; these cells are arranged in groups of some six or more, with their long axes parallel to one another, but at right angles or obliquely to the long axes of the cells of other groups.

d. A seed coat; this consists of a single layer of brown polygonal cells.

e. An endosperm made up of rather thick walled polygonal cells, containing aleurone grains, fixed oil, and protoplasm. Some of the aleurone grains contain a rounded globoid, others a small rosette of calcium oxalate.

The diagnostic characters of powdered fennel are (Figure 8.5):

(1) The spiral and reticulate cells of the mesocarp.

(2) The narrow cells of the inner epidermis and their characteristic arrangement.

(3) The absence of hairs.

(4) The thick-walled endosperm cells.

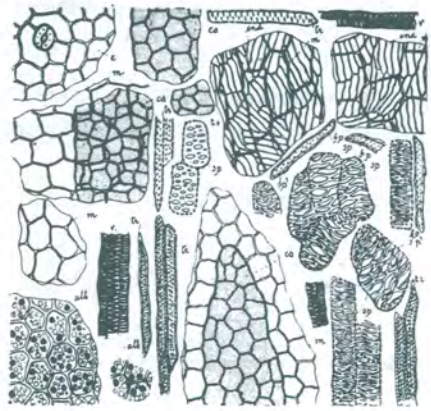


Figure 8.5. Fennel

Cardamom

The fruits of Elettaria cardamomum, Maton (Scitamineae).

The pericarp of the fruit presents the following tissues:

a. An outer epidermis consisting of a single row of irregular polygonal cells with straight, smooth walls.

b. A rather thick layer of parenchyma traversed by numerous fibro-vascular bundles, and containing scattered cells filled with brownish oleoresin. The fibro-vascular bundles are supported by a mass of fibres, most of which have thickened, pitted walls.

c. An inner epidermis, resembling the outer in structure but usually more or less collapsed.

The arillus is very thin and composed of several rows of elongated, yellowish, more or less collapsed, cells, containing small rounded or oval droplets of oil.

The seed is composed of the following tissues:

a. An epidermis, consisting of cells which appear rectangular in transverse section, but in surface view are seen to be much elongated and taper towards the ends; they are furnished with slightly thickened, undulating walls.

b. A single row of smaller cells, also elongated in shape but crossing the cells of the epidermis at right angles.

c. A single row of large rectangular oil-cells.

d. A narrow layer composed of several rows of cells, the structure of which is not distinctly visible.

e. An inner epidermis, consisting of a single row of brown or yellowish-brown, radially elongated cells with very thick walls, the cavity being shallow and almost entirely filled with a nodule of silica.

f. A largely developed perisperm, the cells of which have thin walls, and are packed with minute starch grains; in the centre of each cell there is a prismatic crystal of calcium oxalate.

g. An endosperm and embryo, the cells of which contain proteid matter.

The diagnostic characters of the powdered pericarps are (Figure 8.6):

(1) The parenchyma with empty cells and scattered resin cells.

(2) The fibres from the bundles.

Powdered seeds are identified by:

(3) The characteristic epidermis.

(4) The sclerenchymatous layer.

(5) The fragments of perisperm with small starch grains and calcium oxalate crystals.

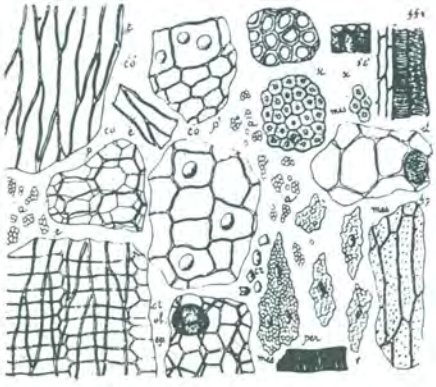


Figure 8.6. Powdered Cardamom

Fenugreek

Fenugreek is from Trigonella foenum-graecum. The seeds range in size up to 6.5 mm long, 3 mm wide and 2.5 mm thick. They are hard, yellowish brown, irregularly rhomboidal and flattened.

They have a depression in one of the long, narrow sides. The seeds contain trigonelline and choline and are about 18% mucilage. Microscopically there are epidermal palisade cells which are about five times longer than they are wide, plus hour-glass cells with bar-like thickenings, aleurone grains and starch. Fenugreek is a principal constituent of many curry powders.

Mace

Mace is the fleshy arillus surrounding the seeds of Myristica fragrans, Houtt. (Myristicaceae). It consists principally of parenchymatous tissue containing numerous oil cells, and traversed by fibro-vascular bundles. The cells of the parenchyma (pa) are polygonal and isodiametric. They contain a remarkable substance, known as amylodextrin, embedded in a fatty mass. Amylo-dextrin occurs in grains of very irregular shape. Sometimes they are discoid or rounded but more often they are angular. Solution of iodo-potassium iodide colours them red. The oil-cells contain either yellow volatile oil or reddish-brown oleo-resin.

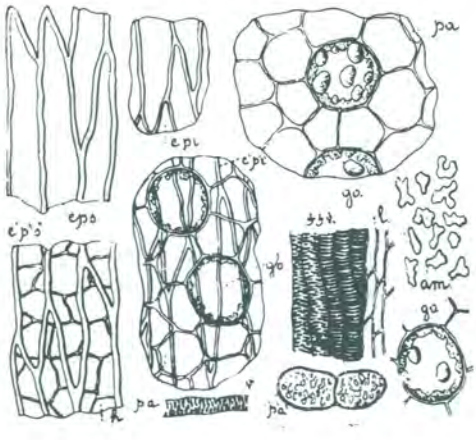


Figure 8.7. Powdered Mace

The epidermis of both surfaces is covered with a thick cuticle. In surface view the epidermal cells (eps, epi) are strongly elongated axially; they are often fusiform, and have thick walls. Below the upper epidermis there is a collenchymatous hypoderma; this, however, is not continuous or uniform, but disappears in some places, whilst in others it is composed of two rows of cells.

The diagnostic characters of powdered mace are (Figure 8.7):

(1) The large, pointed, thick-walled cells of the epidermis.

(2) The oil-cells, many of which may be broken.

(3) The grains of amylo-dextrin in the parenchymatous cells.

Mustard

ISO 1237-1974 specifies that mustard seed must be the dried clean seeds of Sinapis alba (white or yellow mustard), Brassica nigra (black mustard) or Brassica juncea (Indian mustard). The mustard seeds shall be whole and mature and shall not contain more than 2% of extraneous matter or other vegetable material. Extraneous seeds include charlock (Sinapis arvensis Linnaeus), rape (Brassica napus Linnaeus) and Melilotus species. The proportion of damaged or shrivelled mustards seeds shall not exceed 2%.

Black Mustard Seeds: The seeds of Brassica nigra, Koch (Cruciferae). In the seed-coats the following layers can be distinguished:

a. An epidermis (am) composed of large thin-walled cells containing mucilage.

b. A single layer of large parenchymatous cells, the walls of which are not collenchymatous, as those of white mustard seeds are. These cells are generally collapsed, and lie closely pressed on to the next layer of cells. In the powdered form they are not easy to see.

c. A single layer of dark brown sclerenchymatous cells (sc) which, in transverse section, exhibit the very characteristic thickening (s'c') shown in the illustration. Some of these cells at regular intervals are longer than the others, and thus produce the pitted appearance of the seed as well as the peculiar, polygonal network seen in the surface view of the seed-coats.

d. A thin membranous layer (cm) consisting of large, polygonal, flattened cells, containing a brownish amorphous substance. This layer is closely applied to the sclerenchymatous layer, and in the powder generally remains firmly adhered to it, producing, in part, the characteristic colour of the seed.

e. Within the seed-coat is an aleurone layer (ap) consisting of rather thick-walled, polygonal cells, containing aleurone grains. Next to this row of cells is a layer composed of several rows of collapsed parenchymatous cells, the cavities of which are only indistinctly visible as faint lines.

f. The cotyledons (co) are covered by a transparent epidermis, consisting of polygonal cells. They contain small irreglular aleurone grains, in each of which numerous minute globoids can be detected.

The powdered seeds contain very numerous fragments of the delicate tissue of the cotyledons and radicle; in glycerin these exhibit the characteristic aleurone grains, which may also be found scattered over the preparation. Examined in chloral hydrate globules of fixed oil are very conspicuous. Fragments of the seed-coat are easily recognized by their brown colour; they usually present their surface view and exhibit the polygonal network alluded to above. Colourless, transparent fragments of the epidermis may also be found as well as portions of the aleurone layer.

The diagnostic characters of powdered mustard seeds are (Figure 8.8):

(1) The dark, yellowish-brown sclerenchymatous layer.

(2) The polygonal network exhibited by the upper surface of that layer.

(3) The small aleurone grains, each with numerous minute globoids.

(4) The mucilaginous cells of the epidermis.

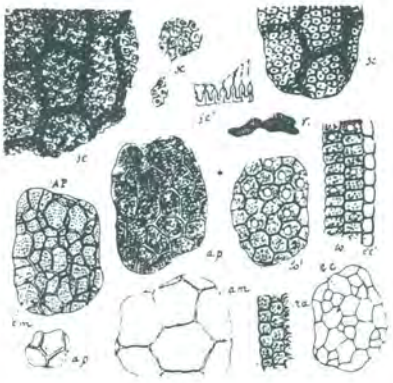


Figure 8.8.
Powdered Black Mustard Seeds

White Mustard Seeds: The seeds of Brassica alba, Linn. (Cruciferae). The seeds are yellow in colour, and so minutely pitted that they appear smooth to the naked eye. In the seed-coats the following layers can be distinguished:

(a) An epidermis (am) made up of large cells containing mucilage which swells very rapidly and very considerably in contact with water.

(b) A collenchymatous layer (col) composed of two rows of polygonal cells, the walls of which are thickened, particularly in the angles.

(c) A sclerenchymatous layer (sc) consisting of a single row of cells, the lateral and inner walls of which are thickened and pale yellow in colour. The cells are tolerably uniform in size and arrangement.

The above three layers represent the outer seed-coat.

(d) A membrane layer (cm) composed of two or three rows of strongly flattened cells. These are free from the dark brown pigment which is contained in the corresponding cells of black mustard seed.

Within the seed-coats is the embryo surrounded by the aleurone layer (ap); the latter consists of a single row of isodiametric polygonal cells which have uniformly thickened walls and granular contents. The epidermis of the cotyledons (cc) is composed of irregular empty cells amongst which groups of two or three smaller ones may be observed; these are stomata in process of formation. The cells of the cotyledons themselves (co) are filled with aleurone grains and fixed oil. The aleurone grains are small and irregular in shape; they contain numerous minute globoids but no crystalloids.

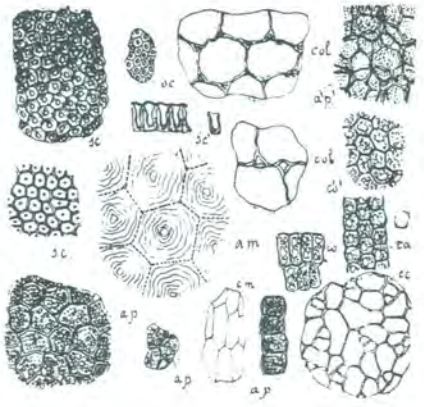


Figure 8.9.
Powdered White Mustard Seeds

The powder consists, like that of black mustard, largely of fragments of the cotyledons and radicle. The portions of the seed-coat are pale yellow in colour, and hence easily distinguished from the red-brown fragments of black mustard. The polygonal network that is so easily seen on the upper surface of the sclerenchymatous layer of black mustard is much less conspicuous.

The diagnostic characters of powdered white mustard are (Figure 8.9):

(1) The pale yellow sclerenchymatous layer.

(2) The epidermal cells with striated mucilage.

(3) The collenchymatous hypodermal layer.

(4) The small irregular, aleurone grains containing numerous minute globoid.

Nutmeg

Nutmeg is from Myristica fragrans and consists of the kernel of the seed. (Mace is from the same, but is the dried extra seed coat or aril).

Nutmegs are ovoid with the Banda nutmegs measuring about 2 x 2.5 cm. They are light brown, finely pitted and have reticulate marking.

Bombay nutmegs (M. malabaria), and Macassar nutmegs (M. argentea) are longer, narrower and do not have the aroma of the Banda (M. fragrans).

Microscopically, powdered nutmeg shows solid fat, starch grains and brown cells in the perisperm.

UMBELLIFEROUS SEEDS
(TLC Identification)

PRINCIPLE

Petroleum ether extracts are developed by TLC and visualized with 2, 4-dinitrophenylhydrazine.

APPARATUS

1. TLC equipment.

2. UV light source, 365 nm.

3. Prepare plates 0.25 mm thick from 25 g silica gel G in 50 ml of 0.05% aqueous fluorescein sodium. Heat at 105°C for 30 minutes before use.

REAGENTS

1. Petroleum ether BR 60-80°C.

2. Chloroform - benzene (1:1) developing solvent.

3. Bromine.

4. 2,4-dinitrophenylhydrazine, saturated solution in 1N hydrochloric acid.

5. Sulphuric acid containing 1% vanillin.

PROCEDURE

Shake 0.5 g powdered sample with 5 ml of petroleum ether. After the suspended matter has settled, apply 0.03 ml of extract to the TLC plate, allowing the spot to become up to 1 cm in diameter. Apply extracts of authentic specimens for comparison. Develop the chromatogram for about 15 cm. Examine under UV light and lightly outline any quenched areas. Treat briefly with bromine vapour (converting fluorescein to eosin) and examine under UV light for persisting fluorescein fluorescence due to unsaturated substances. Spray with the 2,4-dinitrophenylhydrazine solution. Aldehydes and ketones appear as orange spots. Air dry and spray with 1% vanillin in sulphuric acid. Khellin, thymol and similar substances give coloured spots rapidly, fenchone and some others may take several hours.

INTERPRETATION

Betts found the following R_f values for Umbelliferous seeds:

Anise	0.17, 0.39
Caraway	0.43
Coriander	0.26
Cummin	0.58, 0.55
Dill	0.42
Fennel	0.72, 0.57, 0.46, 0.41, 0.38

REFERENCE

Betts, T.J., Quarterly Journal of Pharmacy and Pharmacology (Supplement) 16 131T.

NON-VOLATILE EXTRACT

PRINCIPLE

The spice material is extracted with diethyl ether, the ether is distilled off and the non-volatile residue is weighed.

APPARATUS

1. Soxhlet or similar extraction apparatus.

2. Oven at 110 $\pm$ 1°C.

REAGENT

1. Diethyl ether, anhydrous.

PROCEDURE

Extract a weighed 2 g sample portion (ground to pass a 1 mm sieve) in a continuous extraction apparatus with anhydrous diethyl ether for 18 hours. Remove the ether from the extract by distillation, followed by blowing with a stream of air, with the flask kept on a boiling-water bath, and dry the flask in the oven at 110 $\pm$ 1°C until the loss in weight between two consecutive weighings is less than 0.005g. Shake the residue in the flask with 2 to 3 ml of anhydrous diethyl ether at laboratory temperatures, allow to settle and decant the ether. Repeat the extraction until no more of the residue dissolves. Dry the flask again until the loss in mass between two successive weighings is less than 0.005g.

CALCULATION

The percentage of non-volatile ether extract in the sample, on the dry basis, is equal to

$$(M_1 - M_2) \times \frac{100}{M_0} \times \frac{100}{100 - H}$$

Where:

M_0 = g of sample portion

M_1 = g of the residue obtained after drying at 110°C

M_2 = g of the final residue.

H = moisture content, as a percentage by weight, of the sample as received.

INTERPRETATION

This determination is also referred to as the fixed oil or fixed ether extract. The condenser water may be too hot for the refluxing of diethyl ether, in which case the method as written cannot be carried out. Di-isopropyl ether is readily available and of higher boiling-point. The author is not aware of any report comparing results using the two solvents but it seems likely that any difference would be small.

REFERENCE

ISO 1108 - 1980.

VOLATILE OIL

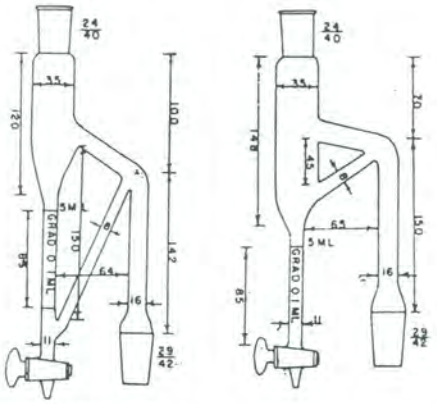
PRINCIPLE

The determination of volatile oil in a spice is made by distilling the spice with water, collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of volatile oil is expressed as a percentage v/w.

APPARATUS

1. Flask, distilling, 1 litre capacity, preferably with magnetic stirrer.

2. Volatile oil traps, Clevenger type.



Dimensions in mm. It is essential to wash the apparatus with acetone and water and then leave to stand in chromic-sulphuric acid mixture with complete rinsing prior to use.

PROCEDURE

Grind the spice to pass a number 20 (850 micron) sieve. Weigh 20g of spice or enough to yield 2-4 ml of oil if possible and place in the flask with glass beads or porous earthenware pieces if a magnetic stirrer is not used. Add about 300 ml of water and a drop of antifoam if necessary. Fill the trap with water. Place an efficient water-cooled condenser on top of the trap and heat the flask with good stirring or agitation until boiling starts and continue boiling moderately briskly but so that the lower part of the condenser remains cold. Set the apparatus so that the condensate will not drop directly on the surface of the liquid in the trap but run down the side walls. Rotate the flask occasionally to wash down any material adhering to the upper part of the walls. Distil until 2 consecutive readings taken at 1 hour intervals show no change in oil content (>6 hr). Remove the source of heat and read the volume of oil ten minutes or so later. Calculate as v/w.

If the oil separates in the graduated portion of the trap or clings to the walls, add several drops of a saturated aqueous detergent solution through the top of the condenser. Repeat if necessary although once is usually sufficient. Distil for at least 10 minutes after adding detergent in order to wash it out of the trap.

Some oils (e.g. cassia) have a density close to 1 or separate into 2 fractions in the trap (allspice, nutmeg). For these, prior to adding the sample to the flask, add 1.0ml xylene to the trap and distil without sample for at least half an hour. Cool and more than ten minutes later read the volume of xylene. Add the sample and distil for up to 6 hours as described above. Subtract the volume of xylene from the total volume of the organic layer in the trap. Calculate as before.

The oil obtained (without use of xylene) may be recovered, dried with a very small amount of sodium sulphate and its characteristics, such as density, RI etc. determined.

REFERENCES

Sage, C.E. and Fleck, H.R., 1934. The Analyst 59, 614.

Official Methods of Analysis of the AOAC, 1984, 30.020-30.027.

Note: The apparatus of Sage and Fleck can be constructed from ordinary laboratory glassware. Results by either method may be a little lower than the true value, due to the slight solubility of some oils in water. The results are also affected by the particle size of the sample.

8.3 SPICES - PODS AND FRUITS

COMPOSITION

All red pod peppers are species of Capsicum and are found throughout the world. The whole pods are usually called 'chilies' and vary dramatically in size, colour and pungency.

Dried and ground Capsicum of many species are used as a spice. The two more common are cayenne and paprika. Cayenne can be made from C. frutescens L., C. baccatum L. or other small-fruited species. Paprika is much less pungent and is usually made from C. annuum.

The pungency (or hotness) of capiscums is due to capsaicin ($CH_3OC_{17}H_{24}.NO_2H$) in the pepper oil. About 0.05% of cayenne pepper is capsaicin, for example.

Some composition data for cayenne and paprika are as follows:

Spice	Moisture %	Total Ash %	Non-Volatile Extract %	Volatile Oil %
Cayenne	3.7 - 9.0	5.1 - 6.4	15 - 22	0.7 - 2.6
Paprika	7.0 - 9.5	5.6 - 7.6	7 - 12	0.3 - 1.5

Other spice fruits include black and white pepper and allspice (pimento). Some compositional data are as follows:

Spice	Moisture %	Total Ash %	Non-Volatile Extract %	Volatile Oil %
Allspice	9.4 -10.5	4.1 - 4.8	4.3 - 7.7	3.0 - 5.2
Black Pepper	8.7 -13.0	3.1 - 6.4	6.1 -10.7	0.5 - 2.5
White Pepper	9.5 -14.5	0.6 - 2.5	6.2 - 9.7	0.5 - 1.8

IDENTIFICATION

Capsicums

The fruit of Capsicum annuum L. (Paprika) has a pericarp composed of the following tissues:

a. An epidermis of tabular cells, which are seen in surface view to be polygonal and to have thickened, pitted, yellow walls.

b. Next to the epidermis is a hypoderma consisting of four or five rows of tangentially elongated cells with collenchymatous and suberized walls; these cells contain red chromoplasts and droplets of oil. The hypoderma is followed by parenchymatous tissue made up of thin-walled, polygonal cells, and traversed by numerous bicollateral bundles.

c. Lastly, an inner epidermis composed of cells with thickened and pitted walls, which in surface view are seen to be irregularly sinuous. These thick-walled cells are interrupted at intervals by bands of polygonal thin-walled cells, the whole forming an extremely characteristic tissue.

d. The calyx possesses on its lower surface an epidermis bearing stomata, and composed of rectangular cells, which in surface view are polygonal and elongated. The epidermis of the upper surface is formed of irregular polygonal

cells with pitted walls, and bears short unicellular conical hairs as well as bicellular and pluricellular stalked glands of varying size.

e. The epidermis of the seed-coat is very characteristic. In surface view the cells of which it is composed are seen to be very large and provided with very thick sinuous walls, but in transverse section the outer wall is thin, whilst the radial and inner walls are thickened; immediately below the epidermis is a layer of parenchymatous tissue made up of polygonal cells with thin, pitted walls, next to which there is a thicker layer of polygonal, isodiametric cells. The cells of the endosperm are polygonal and contain small aleurone grains.

f. The placenta is covered with an epidermis of polygonal cells with pitted walls. Below the cuticle of these cells oily drops are secreted in which the active constituent, capsaicin, is contained; the latter may sometimes be observed in lamellar crystals. Next to the epidermis is a parenchymatous tissue composed of smaller irregular cells and traversed by fibro-vascular bundles.

The diagnostic characters of powdered capsicums are (Figure 8.10):

(1) The inner epidermis of the pericarp with thick-walled cells interrupted by bands of thin-walled cells;

(2) The epidermis of the seed-coat with large, thick-walled, sinuous pitted cells;

(3) The droplets of yellow or orange-coloured fixed oil;

(4) The thickened cells of the outer epidermis of the pericarp.

Figure 8.10
Powdered Capsicum Fruits

Black Pepper

Black pepper is the unripe fruit of Piper nigrum, not to be confused with pimento (allspice), the fruit of Pimenta officinalis (an obvious difference is that the calyx and style still remain in the latter) or pimiento, a variety of Capsicum or red pepper.

A transverse section of black pepper exhibits the following structure:

a. An outer epidermis consisting of small cells with brown contents and a rather thick cuticle. In surface view these cells appear polygonal, and here and there a stoma may be seen; many of them contain small prismatic crystals of calcium oxalate.

b. An outer sclerenchymatous layer abutting upon the epidermis or separated from it by a single row of parenchymatous cells. This layer is not continuous, but is interrupted at intervals by thin-walled parenchymatous cells. The sclerenchymatous cells vary somewhat in shape, but most of them are radially elongated, and contain a brown substance; their walls are thick and pitted.

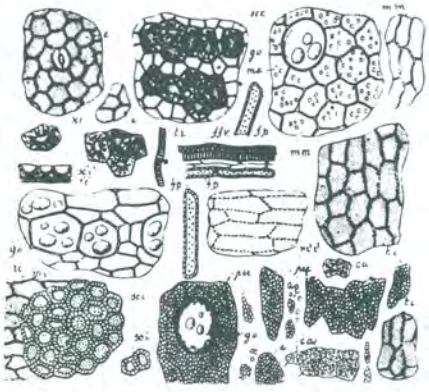
c. Parenchymatous tissue corresponding to the mesocarp, and constituting the bulk of the pericarp. The outer layers of this tissue consist of large polygonal cells, amongst which an occasional still larger oil-cell may be seen; the former contain a few small starch grains, the latter globules of volatile oil. The inner layers of parenchymatous cells have lignified walls and are more strongly tangentially elongated or even flattened so as to present a well marked line of demarcation, which is accentuated by the presence of fibro-vascular bundles. Oil-cells are more numerous in this inner part of the parenchymatous tissue than they are in the outer.

d. An inner sclerenchymatous layer consisting of a single row of cells thickened on their radial and inner tangential walls; in surface view these cells are seen to be isodiametric, polygonal, and to have moderately thick, pitted walls; their cavities are colourless and larger than those of the outer layer of sclerenchymatous cells. This layer of cells is generally adherent to the brown seed-coat.

e. A brown and a yellow layer of collapsed cells to which is firmly attached a colourless layer of collapsed cells; these last three layers constitute the seed-coat.

The kernel of the seed consists almost entirely of perisperm. The outer two or three rows of cells are polygonal and contain aleurone grains, but the others are elongated and are packed with minute grains of starch. Scattered throughout the perisperm are cells containing yellowish volatile oil.

The diagnostic characters of powdered black pepper are (Figure 8.11):



(1) The outer epidermis, together with the subjacent interrupted sclerenchymatous layer.

(2) The inner sclerenchymatous layer.

(3) The starch grains, often in compact masses.

(4) The oil-cells, the contents of which are coloured red by sulphuric acid.

Figure 8.11. Powdered Black Pepper

White Pepper

This consists of the dried whole ripe berries of Piper nigrum from which the outer part of the pericarp has been removed by abrasion after previously fermenting or soaking in water. The quality may be assessed from the level of crude fibre, (0.5 - 5.0%).

CAPSAICIN

PRINCIPLE

The powdered spice is extracted with methanol and the capsaicin separated by an ether-alkali partition extraction procedure. The capsaicin is determined by a spectrophotometric difference method.

APPARATUS

1. Soxhlet type continuous extraction apparatus.

2. Separatory funnels.

3. Spectrophotometer and 1 cm cuvettes.

REAGENTS

1. Carbon, purified. Shake 10 g of activated carbon with 100 ml of dehydrated methanol, filter through a sintered-glass funnel, and dry the residue at 105°C.

2. Ether. Diethyl ether, anhydrous peroxide-free.

3. Light petroleum. Boiling range 80° to 100°C.

4. Methanol, anhydrous.

5. Sodium chloride.

6. Hydrochloric acid, 0.1 N. Reagent solution. Dilute 9 ml of concentrated hydrochloric acid, to 1 litre with water.

7. Hydrochloric acid, 0.05 N. Dilute 4.5 ml of concentrated hydrochloric acid, to 1 litre with water.

8. Methanol, 60%. Dilute 3 volumes of methanol with 2 volumes of water.

9. Sodium hydroxide solution, 0.1 N. Prepare freshly as required.

10. Phenol red indicator solution. Warm 20 mg of phenol red with 1.1 ml of 0.05 N sodium hydroxide and 2 ml of alcohol (90% v/v) and when solution is complete, dilute the solution to 100 ml with alcohol (20% v/v).

PROCEDURE

Transfer about 5 g (accurately weighed) of the well mixed sample, ground to pass a 30-mesh sieve, to a continuous extraction apparatus, extract with dehydrated methanol for not less than 6 hours, or until the sample is exhausted, and dilute the extract to 100 ml with dehydrated methanol.

To 10 ml of this solution add 15 ml of dehydrated methanol, 15 ml of water, 2 g of sodium chloride and 5 ml of 0.1 N sodium hydroxide, mix, and extract with three successive 10 ml portions of light petroleum (boiling range 80° to 100°C). Wash the combined extracts with two successive 5 ml portions of 60% methanol and discard the light petroleum extract; filter the combined aqueous solution and washings through cotton wool, washing the filter with 10 ml of 50% methanol.

Evaporate the combined filtrate and washings on a water-bath until the volume is reduced to about 5 ml, dilute the solution to about 50 ml with water, adjust the pH to 7.0 to 7.5 with 0.1 N hydrochloric acid, using either a pH meter or phenol red solution as indicator and extract with six successive 20 ml portions of ether, ensuring that the correct pH is maintained during the extraction. Wash the combined extracts with 10 ml of water, and discard the aqueous solution and washings. Add 20 ml of dehydrated methanol and evaporate on a water-bath in a fume cupboard until the volume is reduced to about 1 ml.

Dilute the residue to 100 ml with dehydrated methanol, add 0.05 g of decolourising charcoal, shake and filter through a fine grade filter-paper, discarding the first 20 ml of filtrate. To 10 ml of this solution add 5 ml, accurately measured, of 0.1 N sodium hydroxide, cool and dilute to 25 ml with dehydrated methanol. To a further 10 ml add 5 ml, accurately measured, of 0.05 N hydrochloric acid, cool, and dilute to 25 ml with dehydrated methanol.

Measure the extinction of the alkaline solution against the acid solution at the maxima at 248 and 296 nm.

Using dehydrated methanol as solvent, dilute 5 ml, accurately measured, of 0.1 N sodium hydroxide to 25 ml and dilute 5 ml, accurately measured, of 0.05 N hydrochloric acid to 25 ml; measure the extinction of the alkaline solution against the acid solution at the maxima again at 248 and 296 nm.

CALCULATION

From the extinctions at the two maxima given by the solutions containing the sample, deduct the corresponding extinctions given by the blank solutions. For purposes of calculation, use a value of 313 for the $E^{1\%}_{1cm}$ of capsaicin at 248 nm under these conditions and of 127 for that at 296 nm; calculate the proportion of capsaicin in the sample from the extinction at each wavelength. If the two results so obtained differ by not more than 5%, the content of capsaicin is given by the mean of the two results.

INTERPRETATION

Capsaicin is the substance which gives capsicums their pungency. As example, cayenne pepper powder should contain about 0.5% capsaicin.

REFERENCES

British Pharmaceutical Codex, 1973, 119.

Hartman, K.J., 1970. Journal of Food Science 35, 543.

Masada, Y., Hashimoto, K., Inoue, T. and Suzuki, M., 1971. Journal of Food Science 36, 858.

Note: Hartman and Masada et al describe gas chromatographic methods. The Scoville Index, defined as the greatest dilution expressed as the denomination of the dilution fraction, at which a pungent sensation from chillies is perceived, under conditions of the method as described, may be determined by ISO 3513-1977.

8.4 SPICES - ROOTS, BARKS AND FLOWERS

COMPOSITION

Spice	Moisture %	Total Ash %	Non-Volatile Extract %	Volatile Oil %
Roots				
Ginger	8.4 -13.9	3.2 - 9.3	2.8 - 8.1	0.9 - 3.1
Tumeric	8.0 -10.0	6.5 - 7.5	7.0 - 9.0	3.0 - 5.0
Barks				
Cassia	-	-	-	-
Cinnamon	7.8 -10.5	4.1 - 5.7	1.3 - 1.7	0.7 - 1.4
Flowers				
Cloves	5.0 -11.0	4.7 - 7.0	6.2 -10.1	14 - 20
Saffron	8.0	5.0 - 8.0	-	-

Cassia is the bark of Cinnamomum cassia and the usual cinnamon of commerce is the bark of Cinnamomum zeylanicum. They differ from each other and other Cinnamomum species as follows:

	Cinnamomum cassia	Cinnamomum zeylanicum (Cinnamon)	Cinnamomum Burmanni	Cimmamomum loureirii	Cinnamomum pedatinervium & Cinnamomum sintok
Cinnamaldehyde	+	+	+	+	-
Eugenol	-	+	-	-	+
Diameter of fibres (microns)	> 40	> 30	> 30	-	> 40
Calcium oxalate	minute prisms	Acicular crystals	small prisms	-	Acicular crystals
Mucilage	9.5-10.9	1.9-2.1			
Ash of mucilage	6.1- 6.4	20.6-24.7			
Appearance	cork present	thin, no cork			

IDENTIFICATION

Ginger

Ginger is the scraped and dried rhizome of Zingiber officinale. There are a number of different types of ginger used commercially throughout the world. All are scraped rhizomes of a different species such as Z. mioga used in Japan.

Limed ginger is ginger treated with lime to soften the skins before peeling. The calcium content of limed ginger usually exceeds 1% (as CaO) while unlimed is less than 0.5%.

Crude fiber is a useful means to determine if the degree of scraping has been sufficient. The crude fiber of normal ginger is usually in the range of 1.7 - 6.5%. Excessive crude fiber would indicate possible insufficient scraping.

Microscopically, ginger shows:

a. Starch grains which are sack-shaped, ovoid or simple, are faintly striated, have an eccentric hilum (length 12-50 microns), and exhibit an asymmetric cross when examined using polarized light.

b. Thin-walled parenchymatous cells.

Turmeric

Turmeric is the rhizome of Curcuma domestica. The primary rhizome is pear shaped and is called 'bulb' turmeric. The secondary rhizome is cylindrical and is usually known as 'finger' turmeric.

The turmeric rhizomes are usually boiled or steamed during preparation, so the yellow starch usually appears gelantinized.

Turmeric is most often used for its colour only and is considered inferior as a flavouring agent. It is used in most curry powder preparations and in some ground mixed spices.

Cassia Bark

Cassia is the bark of Cinnamomum cassia, Blume. (Laurineae). The bark presents the following structure:

a. Cork, in which layers of thin-walled, tabular cells alternate with layers of cells with thickened, brown walls.

b. Cortex, which is moderately wide and characterized by the abundance of sclerenchymatous cells contained in it. Some of these cells have very thick walls with branching pits; others have comparatively large cavities and walls that exhibit a more or less conspicuous one-sided thickening. They occur either isolated or in small groups in the primary cortex, and also form a sclerenchymatous ring, which is interrupted at intervals by small groups of parenchymatous cells, and bears on the outer margin scattered bundles of pericyclic fibres.

c. Bast ring, constituting the greater part of the bark. It is traversed by medullary rays two cells wide, and contains numerous secretion cells as well as bast-fibres and sclerenchymatous cells. The secretion cells are mostly larger than the cells of the bast parenchyma, and are axially elongated; they may contain either mucilage or volatile oil, or a mixture of both. The bast fibres are either isolated, or occur in groups of two or three; they are larger but less numerous than those of cinnamon bark. The sclerenchymatous cells are also either isolated or in small groups. The cells of the bast parenchyma contain starch grains which are considerably larger than those of cinnamon bark. Many cells, especially those of the medullary rays, contain numerous minute prismatic crystals of calcium oxalate. The sieve-tubes are narrow, and have small, transverse sieve-plates.

Typical specimens of cassia bark may be distinguished from typical specimens of cinnamon bark by the presence of cork, by the larger, thicker, bast fibres, and by the larger starch-grains, but the lower grades of cinnamon bark are often difficult to distinguish from cassia.

The diagnostic characters of cassia bark are (Figure 8.12):

(1) The cork, some of the cells of which are thick walled.

(2) The isolated bast fibres.

(3) The sclerenchymatous cells, many of which are more strongly thickened on one side than on the other.

(4) The secretion cells, containing oil or mucilage.

(5) The minute prismatic crystals of calcium oxalate.

Figure 8.12. Powdered Cassia Bark

Cinnamon Bark

Cinnamon is usually the bark of Cinnamomum zeylanicum, Breyne (Laurineae). The bark, which is deprived of the cork and of the majority of the cortex, presents the following structure:

a. Cortex, represented by two or three rows of tangentially elongated polygonal cells.

b. Bast ring, separated from the remains of the cortex by a continuous ring of sclerenchymatous cells, with thickened pitted walls, the thickening being often more strongly developed on one side than on the others. On the outer margin of this ring bundles of pericyclic fibres may be detected at intervals, as in the case of cassia bark, but the sclerenchymatous ring of cinnamon differs from that of cassia in being continuous instead of interrupted. The bast ring is traversed by medullary rays, which are very narrow near the canbium, but enlarge towards the periphery. It contains secretion cells similar to those of cassia bark, and bast fibres that have very thick walls and are most isolated. The sieve-tubes are arranged in tangential groups, which in the outer portions of the bast ring are collapsed and exhibit traces only of cavities; they are narrow and have transverse sieve-plates. Many of the cells of the cortical parenchyma and medullary rays contain small starch grains or numerous minute crystals of calcium oxalate.

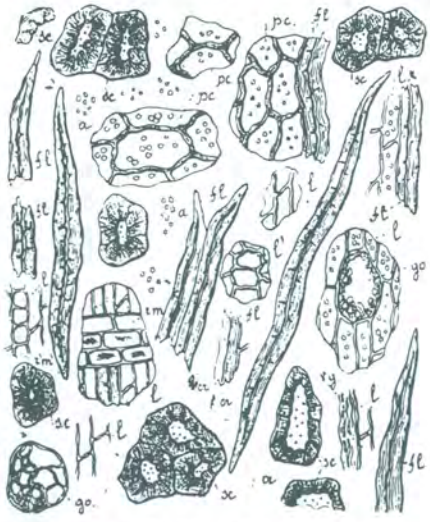


Figure 8.13 Cinnamon

The diagnostic characters of cinnamon bark are (Figure 8.13):

(1) The absence of cork.

(2) The sclerenchymatous cells, sometimes thickened on one side more than on the others.

(3) The secretion cells containing oil or mucilage.

(4) The minute crystals of calcium oxalate.

(5) The small sieve-tubes with transverse plates.

Cloves

Cloves are the dried flower-buds of Eugenia caryophyllata, Thunb. (Myrtaceae). Each clove consists of a somewhat quadrangular stem-like portion slightly contracted at the base; this part is sometimes interrupted as a calyx-tube, sometimes as the solid lower part of the ovary. It is crowned by four thick, divergent, suboval calyx-teeth in the centre of which there is a small rounded body consisting of four imbricated petals enclosing the dried stamens and style.

The transverse section of the lower part of the stem-like portion exhibits the following characters: The epidermis is composed of polygonal cells covered with a rather thick cuticle, and provided with stomata. Below the epidermis is parenchymatous tissue well differentiated into three layers, viz. an outer one with radially elongated cells, and numerous fibro-vascular bundles in which thick sclerenchymatous fibres are conspicuous elements, and an inner, lacunous layer. In the centre there is a moderately large fibro-vascular bundle. Both the teeth of the calyx and of the petals consist chiefly of parenchymatous tissue in which there are numerous oil-glands. The anthers are composed of small cells with pitted walls, and larger cells with spiral thickenings. The powder also contains numerous pollen grains as well as pericyclic fibres derived from the bundles in the lower part of the clove.

Powdered cloves possess no well-marked diagnostic characters; the following features, may, however, be mentioned (Figure 8.14):

(1) Epidermis of lower part of ovary, with thick cuticle.

(2) Epidermis of calyx-teeth and corolla.

(3) Fragments of the oil-glands.

(4) Parenchyma of the corolla with numerous rosettes of calcium oxalate. Powdered cloves should not contain sclerenchymatous cells (clove stalks) or starch (mother-of-cloves).

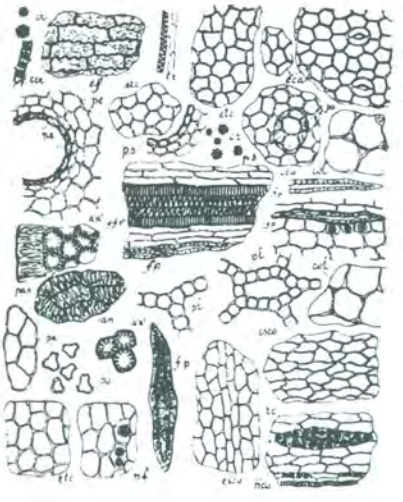


Figure 8.14. Powdered Cloves

Saffron

Saffron consists of the stigmata and upper parts of the styles of Crocus sativus Linn. (Irideae). It forms a loosely matted mass of dark, reddish-brown flattened threads, amongst which a few narrower yellow ones can be distinguished. The upper, enlarged part of the flattened threads is the stigma of the flower, the lower narrower portion is the style.

The stigma is composed of polygonal or rounded, thin-walled, parenchymatous cells containing mucilage and an orange-red colouring matter composed of crocin and crocetin. This tissue is traversed by small fibro-vascular bundles, which appear rounded in transverse section. It is covered by a slightly thickened, easily detached cuticle, which on the upper surface of the stigma bears small protuberances (pr.). Near the apex the stigma is furnished with large

papillae. The yellowish lower part of the threads of saffron is provided with an epidermis consisting of slightly sinuous cells, and is traversed by a small fibro-vascular bundle.

The diagnostic characters of powdered saffron are (Figure 8.15):

(1) The orange-red colouring matter in the cells; it is soluble in water, but insoluble in fixed oil.

(2) The upper epidermis of the stigmata with small papillose protuberances.

(3) The large pollen grains.

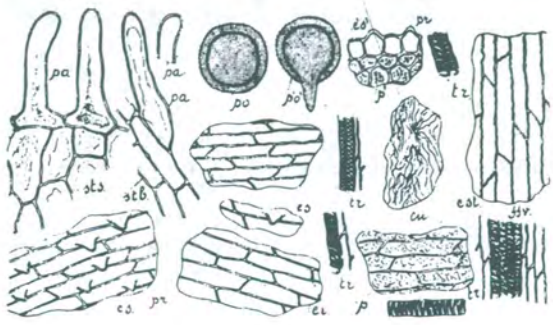


Figure 8.15. Powdered Saffron

COLOURING POWER OF SAFFRON

PRINCIPLE

Dilute a cold water soluble extract, so as to bring the concentration to about 4 mg of saffron per 100 ml of solution. Measure the absorbance at 440 nm in a 1 cm cell.

APPARATUS

1. Pipette, 1 ml.

2. Volumetric flask, 500 ml.

3. Spectrophotometer (or any other apparatus capable of measuring absorbance at 440 nm).

4. Quartz or glass cells for spectrophotometry, transparent to light at 440 nm, with optical path length of 1 cm.

PROCEDURE

Extract 2 g of saffron with 100 ml cold water. Pipette 1 ml of the supernatant into a 500 ml volumetric flask and dilute to the mark with distilled water so as to obtain a solution containing about 4 mg of saffron per 100 ml of solution.

Measure the absorbance of the final dilution at 440 nm in a cell, using distilled water as reference.

CALCULATION

Note the absorbance measured at 440 nm and calculate the absorptivity $E^{1\%}_{1\ cm}$.

INTERPRETATION

The colouring power of saffron is defined as the absorptivity of a 1% extract at 440 nm. $E^{1\%}_{1cm}$ should be 110 for filaments and 150 for powder.

REFERENCE

ISO 3632:1975.

8.5 TEXT REFERENCES

None

Further Reading

HART, F.L. & FISHER, H.J. 1971. Modern Food Analysis Chapter 14 Springer-Verlag.

PARRY, J.W. 1962. Spices, their Morphology, Histology & Chemistry Food Trade Press, London.

April 1956 - March 1957 inc. Articles entitled Food, Microscopy parts 4-15.

JACKSON & SNOWDON, 1974. Powdered Vegetable Drugs S. Thornes.

AMERICAN SPICE TRADE ASSOCIATION. 1968. Official Analytical Methods 580 Sylvan Avenue, PO Box 1267, Englewood Cliffs, N.J. 07632.

PRUTHI, J.S. 1976. Spices and Condiments National Book Trust, New Delhi, A5 Green Park, New Dehli, 110016.

ROBERTS, L.A. 1968. GLC of Chlorinated Hydrocarbon Contaminants in Oleoresins, Journal of the Association of Official Analytical Chemists (4) 825-28.

STRAUSS, D. 1969. Microscopy of Tamarind, Deutsche Lebensmittel-Rundschau (4) 121-22.

STAHL, W.H., SKARZYNSKI, J.N. & VOELKER, W.A. 1969. GLC & TLC of Oregano, Journal of the Association of Official Analytical Chemists (6) 1184-9.

GERHARDT, U. 1969. Typical Analytical Values for Ginger, Cinnamon, Garlic and Onion, Fleischwirtschaft (10) 1356-8.

GERHARDT, U. 1969. Typical Analytical Values for Nutmeg and Mustard, Fleischwirtschaft (9) 1191-3.

GERHARDT, U. 1970. Typical Analyses for Herbs, Fleischwirtschaft (2) 192-4 and 197.

SHANKARANARAYANA, M.L., NAGALAKSHMI, S. & NATARAJAN, C.P. 1970. Colorimetric Determination of Pungent Constituents of Pepper, Flavour Industry (3) 173-5.

ANON. 1970. Cultivation, Histology, Constituents and Adulterants of Caraway, Flavour Industry (8) 524-26.

ANON. 1970. Cultivation, Histology, Constituents and Uses of White and Black Mustard, Flavour Industry (9) 596-8.

ANON. 1970. Cultivation, Histology, Constituents and Uses of Peppermint, Flavour Industry (7) 452-3.

ANON. 1970. Cultivation, Histology, Constituents and Uses of Anise and Star Anise, Flavour Industry (7) 446-8.

SHANKARACHARYA, N.B. & NATARAJAN, C.P. 1971. Cardamon, Chemistry, Technology and Uses, Indian Food Packer (5) 28-36.

FORREST, J.E., HEARCOCK, R.A. & FORREST, T.P. 1972. Identification of Major Components of Essential Oil of Mace, Journal of Chromatography 69 (1) 115-21.

SHANKARACHARYA, N.B. & NATARAJAN, C.P. 1972. Fenugreek, Composition and Use, Indian Spices (1) 2-12.

SHANKARACHARYA, N.B. & NATARAJAN, C.P. 1971. Cummin, Composition and Use, Indian Food Packer (6) 22-8.

SHANKARACHARYA, M.L., RAGHAVAN, B. & NATARAJAN, C.P. 1972. Mustard, Varieties, Chemistry and Analysis, Lebensmittel-Wissenschaft + Technologie (6) 191-7.

PEYRON, L., SENAUX, S., ACCHIARDI, M.J. & BROUA, M. 1972. Adulteration of Basil Essence with Ylang-Ylang, Annales des Falsification et de l'Expertise Chimique (701) 247-54.

ANON. 1972. Production, Composition and Use of Indian Spices, Indian Spices (3/4) 1-13.

HARTMAN, C.P., DIVAKAR, N.G. & RAO, U.N.N. 1973. Adulteration of Pepper with Papaya Seeds, Journal of Food Science and Technology India 10 43.

PRUTHI, J.S. & KULKARNI, B.M. 1969. Detection of Papaya Seeds in Black Pepper Berries, Indian Food Packer 23 51.

KARASZ, A.B., COCCO, F de & BOKUS, L. 1973. Rapid Detection of Turmeric in Foods, Journal of the Association of Official Analytical Chemists (3) 56 626-8.

OSISIOGU, I.U.W. 1973. Adulteration in Ginger Galenicals, Journal of Chromatography (1) 200-3.

SEN, A.R., SARDAR, P.K., SIL, S. & MATHEW, T.V. 1973. Coriander, Composition and Analysis, Journal of Food Science and Technology, India 10 126-7.

SEN, A.R., GUPTA, P.S. & DASTIDAR, N.G. 1974. Detection of Curcuma Zedoaria and Curcuma Aromatica in Curcuma Longa, Analyst 99 (1176) 153-5.

SUD'EVA, N.G., SENTICH, V.Ya., POPOVA, S.A. & POLYAKOV, A.F. 1973. Composition of Aniseed, Izvestiya Vysshikh Uchebnykh Zavedenii, Pischchevaya Tekhnologiya No. 2 131-2.

SEN, A.R., SARDAR, P.K., SIL, S. & MATHEW, T.V. 1973. Analysis and Composition of Celery Seed, Journal of Food Science and Technology India 10 187-8.

BHALLA, K. & PUNEKAR, B.D. 1975. Adulteration of Asafoetida with Colophony and Adulteration of Black Pepper, Indian Journal of Nutrition and Dietetics (7) 216-22.

HUMPHREY, A.M. 1973. TLC and GLC of Spice Oils, Tropical Products Institute Conference Papers 123-6.

MITRA, S.N., GUPTA, P.S. & SEN, A.R. 1971. Studies on the Composition of Curry Powder. Journal of the Institute of Chemists, India 43 (2) 81-2.

SEN, A.R., SARDAR, P.K. & SENGUPTA, P. 1977. Journal of the Association of Official Analytical Chemists (1) 60 235 describe a simple TLC method for distinguishing Caraway Seeds from those of Black Caraway (Carum bulbocastanum Koch).

SEN, A.R., SENGUPTA, P., SARDAR, P.K. & ROY, B.R. 1977. Describe a TLC Method to distinguish true cardamom from greater cardamom. Journal of the Association of Official Analytical Chemists (1) 60 307-8.

HEATH, H. 1974. Herbs and Spices a Bibliography, IX, Tarragon, Thyme, Tumeric. Flavour Industry 5 (5/6) 123-4.

SENGUPTA, P., SEN, A.R., BOSE, A. & MATHEW, T.V. 1973. Ajowan & Celery. Journal of the Association of Official Analtyical Chemists 56 1510.

SEN, A.R., SENGUPTA, P., MONDAL, A. & ROY, B.R. 1974. Cumin, Caraway & Coriander. Journal of the Association of Official Analytical Chemists 57 763.

BALLARIN, C. & BALLARIN, J. 1972. Fennel & Aniseed. Pharmazie 27 544.

ISO 1237:1981. Determination of Allyl Isothiocyanates in Mustard.

RAGHAVAN, B., SHANKARANANYAMA, M.L., NAGALAKSHMI, S. & NATARAJAN, C.P. 1972. Colorimetric Method for Allyl Isothiocycanates. Mikrochimica Acta No. 6 818-22.

SHANKARANARAYANA, M.L., NAGALAKSHMI, S., RAGHAVAN, B. & NATARAJAN, C.P. 1972. Method for Allyl Isothiocyanates using Chloramine-T. Flavour Industry (2) 75-77.

CHIKKSPUYYAIAH, K.S., SHANKARANARAYANA, M.L. & NATARAJAN, C.P. 1971. A Rapid Method. Flavour Industry (10) 591-3.

ISO 5567:1982. Dehydrated Garlic - Determination of volatile organic sulphur compounds.

ISO 3632:1975. Thin-layer chromatography of saffron pigments.

PARVANEH, V. 1972. Saffron and Safflower. Journal of the Association of Public Analysts 10 31.

DHAR, D.N. & SURI, S.C. 1974. Saffron Adulterants. Journal of the Institute of Chemists (India) 46 (4) 130-2.

HOHMANN, B. 1969. Microscopical Identification of Cinnamon Leaves in Curry Powder and Points of Difference from Laurel Leaves. Zeitschrift fur Lebensmitteluntersuchung und Forschung (3) 152-7.

LAWRENCE, B.M. 1969. Botanical Origins of Cinnamon. Canadian Institute of Food Technology Journal (4) 178-80.

LAWRENCE, B.M. & HOGG, J.W. 1974. Safrole in two Cinnamonum spices. Planta Medica (1) 1-5.

STAHL, W.H., SKARZYNSKI, N.N. & VOELKER, W.A. 1969. Mucilage Determination to Distinguish Cinnamon and Cassia. Journal of the Association of Official Analytical Chemists 741.

DUTTA, A.B. 1961. Distinguishing Cinnamon from Cassia by Chemical Analysis. Journal of the Association of Official Agricultural Chemists 44 (4) 639-40.

MARTINDALE, The Extra Pharmacopoeia, 1955. The Pharmaceutical Press London, 54-5 (Asafoetida).

COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH, NEW DEHLI. 1953. The Indian Pharmaceutical Codex, Vol. 1. (Asafoetida).

UNITED STATES PHARMACOPOIEA, 1936. (Asafoetida).

9. OILS & FATS

9.1 VEGETABLE OILS

COMPOSITION

Codex Alimentarius Commission recommended standards for vegetable oils:

Vegetable Oil	Density 20°/20°	Refractive Index	Saponification Value	Iodine Value	Unsaponifiable matter (maximum)	Acid value (mg KOH/g oil)(max)	Peroxide value (meq O_2/kg oil) (max)
Soya Bean	0.919-0.925	1.466-1.470	189-195	120-143	15	0.6	10
Arachis	0.914-0.917	1.460-1.465	187-196	80-106	10	virgin 4 not " 0.6	10
Cottonseed	0.918-0.926	1.458-1.466	189-198	99-119	15	0.6	10
Sunflower	0.918-0.923	1.467-1.469	188-194	110-143	15	virgin 4 not " 0.6	10
Rapeseed	0.910-0.920	1.465-1.469	168-181	94-120	20	virgin 4 not " 0.6	10
Maize	0.917-0.925	1.465-1.468	187-195	103-128	28	virgin 4 not " 0.6	10
Sesame(*)	0.915-0.923	1.465-1.469	187-195	104-120	20	virgin 4 not " 0.6	10
Safflower	0.922-0.927	1.467-1.470	186-198	135-150	15	0.6	10
Mustard-seed	0.910-0.921	1.461-1.469	170-184	92-125	15	virgin 4 not " 0.6	10

(*) Also known as Gingelly, Benne, Ben, Till and Tillie oil.

The Codex has also recommended maximum levels for various antioxidants commonly used with vegetable oils. These are:

Antioxidants	Maximum level of use
Propyl, octyl and dodecyl gallates	100 mg/kg individually or in combination
Butylated hydroxyanisole (BHA) Butylated hydroxytoluene (BHT)	200 mg/kg individually or in combination
Any combination of gallates with BHA or BHT, or both	200 mg/kg but gallates not to exceed 100 mg/kg
Ascorbyl palmitate Ascorbyl stearate	200 mg/kg individually or in combination
Dilauryl thiodipropionate	200 mg/kg

The Codex permits vegetable oils also to contain beta-carotene, annatto, curcumin, canthaxanthin, beta-apo-8'-carotenal, and methyl and ethyl esters of beta-apo-8'-carotenoic acid and flavours of no known toxic hazard which may be added for the purpose of restoring or standardizing colour or flavour as long as the consumer is not thereby misled. Dimethylpolysiloxane, singly or combined with silicon dioxide, may be added as antifoam up to a limit of 10 mg/kg and oxystearin as crystallization inhibitor up to 1250 mg/kg.

ROUTINE ANALYSIS

Note that the analytical values for most oils are broadly similar, a reflection of similar triglyceride compositions. It is important to check that the values for a sample lie within the accepted range, since if they do not, it is reasonable to assume adulteration until the contrary is proved. However, climatic and other factors do influence these values. For example, it is considered in Italy that olive oil has an iodine value between 79 and 88, rather than the range of 75-94 given in the Codex standard, while in North Africa even higher values may be found and Tunisia has discussed introduction of an upper limit of 97. Thus, although these determinations are valuable they are not adequate by themselves. Further tests to establish identity and detect adulteration include the classical tests for specific oils (e.g. Fitelson's test for teaseed oil), examination of the unsaponifiable matter (e.g. tests for phytosterols), and detailed analysis of the triglycerides themselves or the component fatty acids. The latter technique is often refined by measuring the ratio of the proportion of one acid to another, as this only varies within a small range in some cases. The Codex Committee on Fats & Oils has decided that the inclusion of fatty acid composition in Codex standards is not advisable at the present time. Nevertheless, determination of this composition by GLC and the comparison with the normal range may be of considerable assistance in establishing the identity of a sample.

Water in oil is generally determined by the method of Dean and Stark using xylene. The stability of fats and oils is usually assessed by the Swift test (IUPAC method 2.506 (1979)).

The American Oil Chemists Society (AOCS), Deutsche Gesellschaft fur Fettwissenschaft and the International Union of Pure and Applied Chemistry (IUPAC) publish standardized methods for the analysis of oils and fats (1,2). The books by Christie (3) and Bockenoogen (4) in particular are useful introductions to some of the more recent techniques in oil and fat analysis such as argentation chromatography and GLC. The paper by Parodi (5) describes the use of these techniques with some margarines, cooking oils and fat.

The squalene content of olive oil is higher than that of other oils. Hart and Fisher (6) report values of 1360-7080 mg/kg in olive oil. Cottonseed, peanut, corn, soy, sunflower seed, teaseed, sesame seed and rapeseed oils showed values in the range 30-490 mg/kg. Rapeseed oil naturally contains high levels of erucic acid glycerides. Varieties have now been bred which give oil containing only a few percent of erucic acid glycerides (canbra oil). A method for monitoring erucic acid levels may therefore be required. It may be convenient to carry out a general screening test for docosenoic acids (Conacher and Chadha (7), Conacher (8)) and if necessary follow a procedure such as that of Ackman et al (9) for the quantitative determination of erucic acid.

There is sometimes a need to assess the degree of hydrogenation to which a oil has been subjected. The iodine value and refractive index fall and the melting and solidification points rise as a result of hydrogenation. This is of little assistance if the untreated oil is not available for comparison and advantage was therefore taken of the effects of the isomerization which occurs during hydrogenation. For example, part of the naturally occurring cis-oleic acid is converted to the trans-isomer, elaidic acid. This problem was first investigated several decades ago before the introduction of infra-red spectrophotometry and gas-liquid chromatography. The procedure of Cocks, Christian and Harding (10) measured the so-called iso-oleic acids, taken to

mean those isomers of oleic acid with a melting-point of 20°C or over. Since these have lead salts almost insoluble in alcohol and ether, it was usually assumed that all unsaturated acids precipitated as insoluble lead salts, other than a small amount due to incomplete separation of soluble lead salts from insoluble ones, were isomers of oleic acid having the same iodine value as that acid. The iso-oleic acid values obtained are generally in the range 5-30 percent depending on the iodine value of the original fat and the extent and conditions of hydrogenation.

The advent of infra-red spectrophotometry provided a much easier means of assessing the extent of hydrogenation from the proportion of trans-isomers present. Elaidic acid is the standard normally used. Both sample and standard are methylated to remove interference by the free carboxyl group. The standard I.R. procedure is given in AOCS Cd 14-61 and in Cocks and Van Rede (11). The procedure of Allen (12) may also be used but in that case the standard curve must be prepared with material very similar to that to be analyzed (Huang and Firestone (13)). IUPAC method 2.208. describes the separation of trans-octadecenoic acids by TLC and quantitation by GLC.

The test of Synodines and Kovitas (14) appears to reliably distinguish virgin oils from refined oils. The section by Gracian in Boekenoogen (4) or the paper by Matarese (15) are recommended reading before relying on the results of the test.

The refractive index is taken at 20°C for oils liquid at that temperature and at 40°C, 60°C or 80°C for those that are solid at 20°C. The D lines of sodium are the usual light source. The temperature of measurement should be ± 2°C within the selected temperature. If a correction is necessary, 0.00035/° is added to readings taken above 20°C to correct to 20°C and the same amount subtracted if readings are taken below 20°C. The factor becomes 0.00036/° at 40° and above. If condenser water of a suitable temperature is not available for circulating around the prism, a cold water supply may be fed into and out of a closed conical flask by tubes that reach nearly to the bottom of the flask and then connected to the instrument. The conical flask is heated and suitable adjustment of the tap and the heating makes it possible to pass water of the desired temperature through the refractometer.

The relative density of oils and fats is determined by gravity bottle or pycnometer according to a general procedure. It is conventional to record the relative density of oils at 20°C or, if they are not entirely liquid at that temperature, at 40°C, 60°C or higher as appropriate. If the temperature of measurement differs from the standard temperature by up to 5°C, IUPAC method 2.101 (1976) recommends that corrections be made according to the following formulae:

$$\rho t = \rho t_1 + (t_1 - t) \times 0.00068 \text{ if } t_1 > t$$

$$\rho t = \rho t_1 - (t - t_1) \times 0.00068 \text{ if } t_1 < t$$

If the correct coefficient of expansion of the fat under examination is known, this figure should be substituted for 0.00068.

SAPONIFICATION VALUE

PRINCIPLE

The saponification value denotes the weight in mg of potassium hydroxide (KOH) required to saponify one gram of oil.

APPARATUS

Glass flask, resistant to alkalis, of about 200 ml capacity, which can be fitted to a reflux condenser.

REAGENTS

1. 1% w/v solution of phenolphthalein in 95% ethanol.

2. Approximately 0.5 N ethanolic KOH solution. This is stored in a brown or yellow glass bottle having a rubber stopper and then decanted before use. The solution should be colourless or straw yellow.

3. 0.5 N hydrochloric acid solution (standardized).

PROCEDURE

Weigh into the flask about 2 g of oil to within 0.001 g and add exactly 25 ml of 0.5 N ethanolic KOH solution. Attach the flask to the condenser. Boil gently, mixing from time to time.

After 60 minutes, stop heating. (Note: For certain fats which are difficult to saponify it is necessary to heat for longer than 60 minutes.) Add 4 to 5 drops of phenolphthalein solution. Titrate the hot soap solution with the 0.5 N hydrochloric acid solution.

Carry out a reagent blank test on the ethanolic potassium hydroxide solution.

CALCULATION

$$\text{Saponification value} = \frac{56.1\ N\ (a - b)}{p}$$

Where:

a = ml of hydrochloric acid solution used in blank test
b = ml of hydrochloric acid solution used for the sample
N = exact normality of the hydrochloric acid solution used
p = weight in g of sample

REFERENCE

IUPAC 2.202.

IODINE VALUE
(Hanus Method)

PRINCIPLE

The unsaturated glycerides of an oil have the ability to absorb a definite amount of iodine, especially when aided by a carrier such as bromine, and thus form saturated compounds. The quantity of iodine absorbed is a measure of the unsaturation of an oil or fat. The iodine value is generally expressed as the number of grams of iodine absorbed by 100 g of the oil.

The iodine value is often the most useful and easily determined figure for identifying an oil or at least placing it into a particular group. It should also be noted that for natural oils and fats the less unsaturated fats with low iodine values are solid at room temperature, or conversely, oils that are more highly unsaturated are liquids (showing there is a relationship between the melting points and the iodine values). In general, the greater the degree of unsaturation (i.e., the higher the iodine value), the greater is the liability of the oil or fat to become rancid by oxidation.

REAGENTS

1. Hanus Iodine Solution - Measure 825 ml acetic acid and dissolve 13.615gI in it with the aid of heat. Cool, and titrate 25 ml with 0.1N sodium thiosulphate. Measure another portion of 200 ml acetic acid and add 3 ml Br. To 5 ml of this solution add 10 ml 15% KI solution and titrate with 0.1 N sodium thiosulphate. Calculate volume of Br solution required to double halogen content of remaining 800 ml I solution as follows:

$$X = \frac{A}{B}$$

Where:

X = ml of Br solution to be added to 800 ml I solution.
A = 800 x thiosulphate equivalent of 1 ml Br solution.
B = thiosulphate equivalent of 1 ml Br solution.

If necessary, reduce mixed solution to proper concentration by dilution with acetic acid.

2. Chloroform

3. 15% potassium iodide solution (freshly prepared)

4. Standard 0.1 N sodium thiosulphate solution.

5. 1% starch solution as indicator.

PROCEDURE

Weigh accurately about 0.1 g of oil into a 500 ml stoppered conical flask. Dissolve oil in 10 ml chloroform. Add 25 ml Hanus iodine solution from bulb pipette. Keep in the dark for 30 minutes, shaking occasionally.

Add 10 ml 15% potassium iodide and 100 ml water (washing down any free iodine on stopper). Titrate with 0.1N $Na_2S_2O_3$, using 1% starch solution as indicator. (Note: toward end of titration, stopper bottle and shake vigorously, so that any iodine remaining in solution in $CHCl_3$ may be taken up by KI solution.) Conduct 2 blank determinations along with determination on sample.

CALCULATION

Iodine number = [(B - C) x N x 12.69]/g sample (oil)

Where:

B = average blank titration volume in ml

C = sample titration volume in ml

N = normality of $Na_2S_2O_3$ solution

12.69 = equivalent of I_2 $(\frac{126.9}{1000} \times 100)$

INTERPRETATION

The following are typical iodine value ranges for selected oils and fats.

Product	Range (Iodine Value)
Beef tallow	35 - 42
Butter fat	26 - 28
Coconut oil	6 - 10
Corn oil	111 - 128
Lard oil	62 - 82
Lard	47 - 66
Mutton tallow	32 - 61
Palm oil	49 - 59
Peanut oil	85 - 100
Sesame oil	103 - 117

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 28.021-.022.

UNSAPONIFIABLE MATTER

PRINCIPLE

The unsaponifiable matter is defined as the substances soluble in an oil which after saponification are insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, alcohols and hydrocarbons as well as any foreign organic matter non-volatile at 100°C (mineral oils) which may be present. Light petroleum or diethyl ether is used as a solvent but in most cases the results will differ according to the solvent selected and, generally, the use of diethyl ether will give a higher result.

The method is applicable to all oils. It is, however, only approximate for certain oils having a high content of unsaponifiable matter. The percentage of unsaponifiable matter is usually determined on the oil as received since, for trade purposes, it often has to be added to the percentage moisture and impurities to determine the net amount of oil.

APPARATUS

1. 150 ml flask fitted with a reflux condenser;

2. 500 ml separating funnels;

3. Oven regulated at 103°C (± 2°C).

REAGENTS

1. 2 N potassium hydroxide solution in ethanol (dissolve 120 g KOH in 95% ethanol and make up to 1 litre). The reagent must not be darker than straw yellow.

2. Light petroleum ether (BR 40-60°) free from residue.

PROCEDURE

Weigh about 5 g of fat to within 0.01 g into the flask. Add 50 ml of approximately 2 N ethanolic KOH solution. Attach the condenser. Boil gently for an hour. Stop heating. Add through the top of the condenser 50 ml of distilled water and shake.

After cooling, transfer to a separating funnel and rinse the flask several times using in all 50 ml of light petroleum. Shake vigorously for a minute. Let it stand until there is complete separation of the two phases, and draw off the soap solution into a second separating funnel. If an emulsion should form, break it by adding small quantities of ethanol or of concentrated potassium hydroxide solution.

Extract the soap solution twice more, using 50 ml light petroleum each time. Combine the 3 petroleum extracts in one separating funnel, wash 3 times with 50 ml portions of 50% ethanol.

Pour off the petroleum extract quantitatively, if necessary in instalments through the top of the funnel into a tared 250 ml flask. Rinse the funnel with small quantities of light petroleum. Evaporate the solvent by distillation over a boiling-water bath. Dry the residue in an oven at 103°C for 15 minutes, placing the flask in a horizontal position. Weigh after cooling in a desiccator.

Repeat the drying for successive 15-minute periods until the loss of weight between two successive weighings is less than 0.1 percent. If a constant weight is not obtained after 3 operations, the

unsaponifiable matter is probably contaminated. To ascertain whether unsaponifiable matter is free from soap, ignite the residue and titrate the ash with an aqueous solution of 0.1 N hydrochloric acid in the presence of methyl orange.

CALCULATION

$$\text{Percentage of unsaponifiable matter} = \frac{100a}{p}$$

Where: a = weight of residue in g
p = weight of sample in g

In the case where the residue has been ignited and titrated with the aqueous solution of 0.1 N hydrochloric acid:

$$\text{Percentage of unsaponifiable matter} = \frac{100\ (a - 0.32Nb)}{p}$$

Where: a = weight of residue in g
p = weight of sample in g
b = number of ml of 0.1 N hydrochloric acid used
N = exact normality of the aqueous solution of HCl

REFERENCE

IUPAC 2.401. The use of diethyl ether as an alternative solvent is described in the method.

ACID VALUE

PRINCIPLE

The acid value is defined as the weight in milligrams of potassium hydroxide required to neutralize one gram of oil. It is a relative measure of rancidity as free fatty acids are usually formed during decomposition of oil glycerides.

REAGENTS

1. 0.5 N or 0.1 N ethanolic solution of potassium hydroxide (standardized). This solution is stored in a brown or yellow glass bottle furnished with a rubber stopper and then decanted for use. The solution should be colourless or straw yellow. A stable colourless solution can be prepared in the following manner: reflux 1 L of ethanol with 8 g of potassium hydroxide and 5 g of aluminium pellets for one hour, then distil immediately. Dissolve the required amount of potassium hydroxide in the distillate. Allow the whole to stand for several days and decant off the clear supernatant liquid from the deposited potassium carbonate. The solution can also be prepared without distillation in the following manner: add 4 ml of aluminium butylate to 1 L ethanol and allow the mixture to stand for several days. Decant the supernatant liquid and dissolve therein the necessary amount of potassium hydroxide. This solution is ready for use.

2. 1% w/v solution of phenolphthalein in 95% ethanol.

3. 95% ethanol containing 5 drops of phenolphthalein solution in 100 ml. Neutralize immediately before use by means of the 0.1 N ethanolic KOH solution.

PROCEDURE

Weigh 5 to 10 g of oil to within 0.01 g into a 250 ml Erlenmeyer flask. Dissolve in 50-150 ml of a 1:1 v/v mixture of ethanol and diethyl ether neutralized to phenolphthalein.

Titrate, while shaking, with the 0.5 N ethanolic KOH-solution (or 0.1 N for acidities less than 2 percent by weight) until the colour of the indicator changes.

CALCULATION

$$\text{Acid Value} = \frac{(56.1)(a)(N)}{p}$$

Where: a = number of ml of the ethanolic KOH solution used
N = exact normality of the ethanolic KOH solution used
p = weight of sample in g

INTERPRETATION

If mineral acids are present, special procedures may be necessary.

REFERENCES

IUPAC 2.201 (1979).

Lowry, R.R. and Tinsley, I.J., 1976. Journal of the American Oil Chemists Society, 53 (7), 470-472 (Describes a colourimetric method).

PEROXIDE VALUE

PRINCIPLE

The sample is added to boiling chloroform-acetic acid containing dissolved potassium iodide. After cooling, the mixture is diluted with water and the liberated iodine titrated with sodium thiosulphate. The amount of iodine liberated by the sample is a measure of the active oxygen present.

APPARATUS

1. 100 ml round-bottomed flask with a ground-glass joint connecting to a tube about 750 x 9 mm, the upper 150 mm cooled by a water condenser.

2. Microburner.

REAGENTS

1. Chloroform.

2. Glacial acetic acid, high purity.

3. Potassium iodide, low in iodate.

4. Standard sodium thiosulphate solution 0.002 N.

5. Starch solution, about 1 percent.

PROCEDURE

Boil 10 ml of chloroform and 10 ml acetic acid in the flask until the mixture refluxes. Pour a solution of 1 g of potassium iodide in 1.3 ml of water down the column sufficiently slowly that refluxing continues without interruption. This ensures that absence of oxygen in the flask is maintained. Redissolve any precipitated potassium iodide by adding of not more than about 6 drops of boiled distilled cooled water. Add about 1 or 2 g of sample, accurately weighed, down the column without interrupting the steady refluxing. It is convenient to add the sample to the flask via the column and reweigh the beaker plus any residual oil, obtaining the weight taken for the determination by difference. Turn off the cooling water in order to raise the condensation level and so ensure that all the sample is washed into the flask. Boil the solution with sample added for 3-5 minutes, rapidly cool, dilute with 50 ml of water and titrate the liberated iodine with 0.002 N sodium thiosulphate solution using starch as indicator.

CALCULATION

$$\text{Peroxide Value} = \frac{\text{ml 0.0002 N thiosulphate used}}{\text{g oil taken}}$$

INTERPRETATION

Herzinger and Pazlar (16) have compared iodometric and colorimetric methods of peroxide determination. The Kreis test may also be used (Mehlenbacher (17)). Williams (18) describes a method for ketonic rancidity. The thiobarbituric acid value (TBA) is also favoured by some workers (Tarladgis et al (19)). This method is thought to give more consistent results than many others. Codex standards for oils and fats require that IUPAC method II.D.13 be used. Results from different methods should not be regarded as necessarily equivalent.

REFERENCES

Sully, B.D., 1954. The Analyst 79, 86-90.

Lea, C.H. 1945. Journal of the Society of Chemical Industry 64, 106.

Lea, C.H. 1946. Journal of the Society of Chemical Industry 65, 286.

Stuffins, C.B. and Weatherall, H., 1945. The Analyst 70, 403.

TITRE

PRINCIPLE

The titre is the solidification point of the water-insoluble fatty acids, prepared as described and left to crystallize in a desiccator at laboratory temperature for about 24 hours before completing the determination. The method is that of Dalican. Water-insoluble fatty acids are those obtained after saponification of a fat and decomposition of the soap formed according to the method described. Because of the possible presence of fatty acids which are partially soluble in water, the details of this method must be strictly followed.

APPARATUS

1. Round-bottomed basin of about 1500 ml capacity.

2. Sand-bath.

3. Glass test tube, length 12 cm, internal diameter exactly 2.75 cm.

4. Flat circular cork having a central hole just big enough to support the tube.

5. Wide-necked jar, height 13 cm, external diameter 10 cm, into which the cork and the tube are fitted.

6. Thermometer accurately calibrated, graduated in 0.1 or 0.2°C up to 70°C. The bulb is 2 cm long and 0.6 cm in diameter.

REAGENTS

1. Ethanolic potassium hydroxide solution: dissolve 18 g KOH in 20 ml distilled water and dilute with 50 ml of 95% ethanol.

2. Dilute sulphuric acid: add conc. acid (SG = 1.84) to 4 times its volume of distilled water.

3. 10% w/v sodium chloride solution.

PROCEDURE

Weigh 50 g of the fat into a 1500 ml basin. Heat slowly and progressively up to 115-118°C. Add in one single operation the requisite quantity of the ethanolic KOH solution. Stir vigorously with a spatula. Continue to heat gently while constantly stirring and scraping the mass with the spatula until the soap forms fragments which no longer adhere to the spatula when lightly pressed under it.

Pour 1 litre boiling distilled water onto the soap. Maintain the soap solution at boiling point for 45 minutes in order to drive off the ethanol and obtain a clear soap solution. Stop heating. Replace the evaporated water by cold water approximately weight for weight, then carefully pour in 70 ml of the dilute sulphuric acid. Do not allow undecomposed soap particles to adhere to the surface or rim of the basin.

Bring to the boil and maintain at the boiling point until the free fatty acids float to the surface in a clear layer (in the special case where the fatty material contains lauric acid glycerides, heat over a boiling-water bath and not by boiling the aqueous layer).

Wash the fatty acids twice, each time using 500 ml of boiling aqueous sodium chloride solution. After each washing draw off the aqueous layer as completely as possible.

Transfer the fatty acids to a flask. Add anhydrous sodium sulphate and filter through a dry filter paper. Allow the fatty acids to crystallize in a desiccator at laboratory temperature for about 24 hours before determining the titre.

Bring the temperature inside the jar to 20-25°C below that of the expected titre by immersing the jar in a heated or cooled water bath. Melt the fatty acids at a temperature of about 10°C above the titre expected. Pour the fat into the tube to a height of about 5.5 cm. Support the tube in the cork so that a length of about 3 cm projects above it. Suspend the thermometer carefully in the centre of the tube so that the base of the bulb is 1 cm from the bottom of the tube.

The mercury column falls rapidly at first and then more slowly. Simultaneously, the fatty acids crystallize firstly at the bottom of the tube, gradually covering the base of the thermometer bulb. When the mercury column appears to be stationary during four observations made at 5 second intervals, stir the fatty acids with a rapid, circular movement of the thermometer three times to the right and three times to the left, thus breaking up the crystals formed. Replace the thermometer immediately in the centre of the tube and take further readings.

The mercury column, which fell sharply during the agitation, now rises again and attains a maximum before falling again. This maximum is the titre.

Carry out two determinations and if the difference between them does not exceed 0.2°C, take the mean. If a difference exceeds 0.2°C, repeat the determinations until the readings agree to within 0.2°C. Take the mean of two acceptable values.

INTERPRETATION

Some typical titre figures for various oils are: cottonseed 33°, olive 23°, sesame 23°, teaseed 14°, arachis 30°. These figures are guides only. For accurate comparison, authentic oils should be tested using this method.

REFERENCE

IUPAC 2.121 (1979).

SOAP TEST IN EDIBLE OILS

PRINCIPLE

Detection of alkalinity using bromophenol blue as indicator.

APPARATUS

1. 150 mm x 15 mm test tube.

REAGENTS

1. Solution of 0.1% of bromophenol blue in 95% v/v ethanol.

2. Acetone, analytical grade containing 2% v/v water.

(A few drops of the solution of bromophenol blue should give a yellow to yellow-green colour to the 2% water in acetone.)

PROCEDURE

Place 10 ml of the acetone and 1 drop of the bromophenol blue solution in a test tube. The solution should have a yellow colour. (If not, rinse the test tube with acetone until the blue colour disappears). Add 10 g of the oil to the test tube, stopper with a clean stopper, shake and allow to settle. The presence of blue colour in the upper acetone layer indicates the presence of soap.

INTERPRETATION

The result is expressed as positive or negative. The test is applicable to any edible oil.

REFERENCE

British Standard 684:Section 2.5:1977. Determination of Dissolved Soap (Alkalinity).

ARACHIS (GROUNDNUT) OIL TEST

PRINCIPLE

This test is designed for use with arachis oil, alone or in admixture with liquid fatty oils. It is not applicable to mixtures of arachis oil with solid fats. It is not suitable for the evaluation of the purity of arachis oil.

Arachidic acid is precipitated from the saponified oil sample, crystallized from 90% ethanol and dissolved in diethyl ether. The solution is then evaporated and the residue dried and weighed, after the confirmation of its identity by the determination of its melting point which should be 71°C or over.

REAGENTS

1. Potassium hydroxide, 1.5 N solution in 95% (v/v) ethanol.

2. Acetic acid, 33% (m/m) solution.

3. Ethanol, 70% (v/v).

4. Ethanol 90% (v/v).

5. Hydrochloric acid, concentrated.

6. Diethyl ether.

PROCEDURE

Boil 5 g of the oil in a 250 ml conical flask with 25 ml of the potassium hydroxide solution for 10 minutes under a reflux condenser. Add to the hot solution 7.5 ml of the acetic acid solution and 100 ml of the 70% ethanol to which 1 ml of HCl has been added. Maintain the temperature of the mixture for 1 h at 12°C to 14°C. If no precipitate forms arachis oil is absent.

If there is a precipitate, filter the solution and wash the precipitate with the same mixture of 70% ethanol and HCl at 17°C to 19°C, breaking up the precipitate occasionally by means of a platinum wire bent into a loop. Continue the washing until the washings give only a faint turbidity with water.

Note the total volume of the 70% ethanol used for the crystallization and washing. Dissolve the precipitate in the smallest possible quantity of the 90% ethanol (25 to 70 ml) and cool the solution to 15°C for 3 hours. If no precipitate forms arachis oil is absent.

If any crystals appear, filter the solution and wash with about half the volume of 90% ethanol used for crystallization at the same temperature and then with 50 ml of the 70% ethanol. Dissolve the crystals in warm diethyl ether and filter into a tared flask, washing the filter well with the ether and adding the washings to the solution in the flask. Evaporate the ether and dry the residue at 100°C and weigh. Determine the melting point of the crystals, preferably in a closed capillary melting point tube and, if it is found to be lower than 71°C, recrystallize from 90% ethanol, transfer to a tared flask, dry, weigh as before, and determine the melting point.

If the melting point is below 71°C arachis oil should be assumed to be absent. If the melting point is 71°C or over add to the weight thus found (from Tables 1 and 2 respectively), corrections for the solubility of the crystals (a) in 90% ethanol and (b) in 70% ethanol,

in the latter case calculating the correction from the total quantity of 70% ethanol used in precipitating and washing - including the 100 ml used in the first instance.

Table 1

Weight of crystals obtained	Correction to be added per 70 ml of 90% (v/v) ethanol at 15°C
g	g
0.05	0.021 7
0.10	0.025 2
0.15	0.029 5
0.20	0.033 6
0.25	0.036 4
0.30	0.038 5
0.35	0.040 7
0.40	0.042 7
0.45	0.044 0
0.50	0.045 7

Table 2

Melting point of crystals	Correction to be added per 100 ml 70% (v/v) ethanol at 50°C	Factor for converting corrected proportion of arachidic acid to arachis oil (*)
°C	g	
71.0	0.013 0	17.0
71.5	0.009 9	18.6
72.0	0.008 0	20.0
72.5	0.006 7	21.1
73.0	0.006 0	22.0

(*) Not needed for calculation

CALCULATION

Results are calculated applying the appropriate corrections, and expressed as g arachidic acid/kg oil.

g arachidic acid/kg oil = corrected mass of dried crystals obtained (g) x 200

Note: If desired, the apparent % m/m of arachis oil present can be evaluated by applying the appropriate factor in Table 2, Column 3. The results so obtained are not suitable for the evaluation of the purity of arachis oil.

REFERENCE

CAC/RM-1969, which is based on BS 684:1958, Methods of Analysis of Oils and Fats, p. 97 and updated as BS 684:Section 2.31:1978, Arachis Oil Test (Evers).

COTTONSEED OIL TEST

PRINCIPLE

Based on the red colour developed by cyclopropenoic acids (such as sterculic and malvalic acids) under the conditions of test in the presence of sulphur. Hydrogenation and deodorisation wholly or partly destroy the chromogens and a positive reaction is not given by an oil heated to 225°C or above.

APPARATUS

1. 30 ml screw-capped bacteriological media bottle or other strong glass container which can be stoppered. Alternatively, use test-tubes, 150 x 25 mm.

2. Water-bath, boiling or heating bath at 110°-120°C such as an oil bath, heating block or bath of saturated salt solution.

REAGENTS

1. Sulphur reagent. Mix equal volumes of amyl alcohol and a solution of 1% precipitated sulphur in carbon disulphide.

PROCEDURE

Add 2.5 ml oil and 2.5 ml reagent to the bottle or test-tube. If a bottle is used, lightly screw on the cap. Sensitivity is improved by retarding evaporation of the carbon disulphide and by carrying out under moderate pressure. If a test-tube is used, the cork may be inserted firmly and kept in place by tying a piece of linen over it. Although this enhances sensitivity, it may be omitted if it appears to be hazardous. If a bottle is used, keep in the boiling water-bath for two hours. If a test-tube is used, keep at 70 - 80°C for 30 minutes, loosen the stopper and transfer to a boiling water-bath or preferably a heating apparatus at 110°C for one and a half hours.

INTERPRETATION

The presence of 2% or more of cottonseed oil is indicated by a rose-red colour. K'apok seed oil (Eriodendron anfractuosum), baobab or fony oil, the oil of Sterculia foetida and some other oils not normally found in commerce also react. The butter, lard, etc. of animals fed on cottonseed may give a faint positive reaction.

REFERENCES

Halphen, G., 1897. Analyst 22, 326.

CAC/RM 23-1970.

Official Methods of Analysis of the AOAC, 1984, 28.120-.123, obtains a quantitative result by using a standard preparation of cyclopropene fatty acids in cottonseed oil methyl esters.

SESAME OIL TEST

PRINCIPLE

A colour is produced from a reaction with sesamin in the oil.

APPARATUS

1. Separator, cylindrical type, 25 ml. Mark at 10 ml and 15 ml.

2. Porcelain dish, flat-bottomed, about 60 mm diameter.

REAGENTS

1. Concentrated sulphuric acid.

2. Furfural, 0.035% in acetic anhydride. Use freshly distilled furfural. Store in the refrigerator in a dark bottle with a well-fitting ground-glass stopper. The solution keeps several months.

PROCEDURE

Pour 10 ml of oil followed by 5 ml of furfural reagent into the separator. Stopper and shake vigorously approximately one minute. Leave to separate. Run 1-2 ml of the lower layer into the porcelain dish, add 5-6 drops of sulphuric acid and mix by swirling.

INTERPRETATION

For quantities of sesame seed oil higher than 1 percent, a dark blue coloration with a slight greenish tint, develops immediately. For lesser amounts the blue colour is progressively paler and the period required for the complete development of the colour is increased. 0.25 percent in olive oil gives a bluish grey colour which takes 5-10 minutes to develop. The test is generally applicable.

REFERENCES

Pavolini, L., 1934. Olii Minerali, Grasse Saponi, Colori Vernici 12, 41. Also, see the section by Gracián in Bockenoogen (4). The Bandouin-Villavecchia-Fabris test is less dependable.

TEASEED OIL TEST

PRINCIPLE

Based on the Fitelson (modified Lieberman-Burchard) test, i.e. red colour developed by acetic anhydride in the presence of sulphuric acid in chloroform solution of the oil.

APPARATUS

1. 150 mm x 15 mm test tube.

2. 2 ml pipette, graduated to 0.1 ml.

3. Dropper so calibrated that 7 drops of oil weigh approximately 0.22g.

4. Water bath at 5°C.

REAGENTS

1. Chloroform.

2. Concentrated sulphuric acid.

3. Acetic anhydride.

4. Diethyl ether, anhydrous, stored over sodium and peroxide-free.

PROCEDURE

Pipette into a test-tube 0.8 ml of acetic anhydride, 1.5 ml of chloroform and 0.2 ml of sulphuric acid. Cool to 5°C, then add approximately 0.22 g (7 drops) of oil. If any turbidity appears, add acetic anhydride drop by drop with shaking until the solution becomes clear. Keep at 5°C for 5 minutes. Add 10 ml of diethyl ether previously cooled to 5°C. Stopper the test tube and mix immediately by inverting it twice. Return the test tube to the bath at 5°C. An intense red colour which develops about a minute after the addition of the ether, reaches a maximum and disappears, indicates pure teaseed oil. A less intense colour suggests the presence of teaseed oil, but caution must be exercised in interpreting results in the presence of olive oil. The test is generally applicable, but some olive oils yield a pink colour and the test is therefore not reliable for the detection of less than about 15% of teaseed oil in olive oil.

REFERENCES

Fitelson, J., 1936. Journal of the Association of Official Agricultural Chemists 19, 493.

CAC/RM 24-1970.

IDENTIFICATION OF OILS AND FATS
(GLC of Fatty Acid Methyl Esters)

PRINCIPLE

The methyl esters are formed using boron trifluoride or methanol and alkali and separated by gas-liquid chromatography using a flame ionization detector. The methyl esters are also suitable for analysis by TLC and IR. The pattern of methyl esters can be compared to authentic oils for identification.

APPARATUS

1. Gas liquid chromatograph with the following characteristics:

a. Injection system heated to a higher temperature (20 to 50°C than that of the column.

b. Oven: the oven should be capable of heating the column to at least 220°C and of maintaining the desired temperature to within 1°C. If temperature programming is to be employed, an apparatus with a twin column is recommended.

c. Packed column: columns may be glass or stainless. However, glass is preferred as the steel may decompose polyunsaturated fatty acids having more than 3 double bonds. Some successful column packings are given below with the column length, internal diameter and operating temperature indicated:

(1) 12-15% ethylene glycol succinate on 100/120 mesh Gas-Chrom P (2 m x 4 mm, 180°C).

(2) 2-10% Apiezon-L on 80/100 mesh Chromosorb W or Celite (2 m x 4 mm, 220°C).

(3) 10% butan-1-4-diol succinate on 80/100 mesh chromosorb W or Celite (2 m x 4 mm, 175°C).

(4) 3% SE-30 on 100/120 mesh Chromosorb G, silanised (2 m x 3 mm, 190°C).

(5) 10% Silar-10C on 100/120 mesh Gas-Chrom Q (2 m x 4 mm, from 130-220°C at 4°C/min.).

(Note that column lengths may have to be shortened to determine long chain, C20, fatty acids.)

Condition a newly prepared column by disconnecting the detector and heating the column in the oven at the normal operating temperature, while running the carrier gas at a rate of 20-60 ml/min. Condition for about 16 hours.

d. Detector: Flame-ionization detector capable of being heated to a temperature above that of the column. Hydrogen flow should be about half of the carrier gas and oxygen flow should be 5 to 10 times the hydrogen.

2. Syringe: Maximum capacity 10 µl, graduated in tenths of a microlitre.

3. Recorder: If the recorded curve is to be used to calculate the composition of the mixture analyzed, an electronic recorder of high precision is required. The characteritics of the recorder should be:

a. Rate of response below 1.5 second, preferably below 1 second.

b. Width of the paper: 25 cm minimum.

c. Paper speed: 25 to 150 cm/hr.

4. Integrator (optional): Rapid and accurate calculation can be performed with the help of an electronic integrator. This must give a linear response with adequate sensitivity, and the correction for deviation of the base-line must be satisfactory.

5. 50 and 100 ml boiling flasks with ground joints.

6. Reflux condenser, 20 to 30 cm effective length, with ground joint.

7. Boiling chips (fat-free).

8. Inlet tube for nitrogen.

9. Graduated pipette, capacity at least 10 ml, fitted with a rubber bulb, or automatic pipette.

10. Test tubes with ground stoppers.

11. 250 ml separating funnels.

REAGENTS

1. Carrier gas: inert gas (nitrogen, helium, argon) thoroughly dried and containing less than 10 mg/kg of oxygen. (Use at about 40-60 ml/min for 4 mm ID columns).

2. Auxiliary gas: hydrogen (99.9 percent min. purity) free from organic impurities; air or oxygen, free from organic impurities.

3. Reference standards: a mixture of methyl esters, or the methyl esters of an oil of known composition, preferably similar to that of the fatty matter to be analyzed.

4. Methanolic sodium hydroxide solution, approximately 0.5 N: Dissolve 2 g of sodium hydroxide in 100 ml methanol containing not more than 0.5 percent m/m of water. When the solution has to be stored for a considerable time, a small amount of white precipitate of sodium carbonate may be formed; this has no effect on the preparation of the methyl esters.

5. Methanolic solution of boron trifluoride, 12 to 15 percent m/m. 14 and 50 percent solutions are available commercially. **CAUTION**: Boron trifluoride is poisonous. For this reason, it is not recommended that the analyst prepare the methanolic solution of boron trifluoride from methanol and boron trifluoride. However, if it is quite unavoidable to prepare a solution of boron trifluoride from gaseous boron trifluoride, the recommended method is: Weigh 2 L flask containing 1 L of methanol. Cool in ice bath, and with flask still in bath, bubble BF_3 from cylinder through a glass tube into the methanol until 125g BF_3 is absorbed. Perform operation in fume hood. BF_3 must be flowing through the glass tube before it is placed in and until it is removed from methanol to prevent liquid from being drawn into gas cylinder valve system. Gas should not flow so fast that white fumes emerge from flask. Reagent is stable two years.

Also note that methanolic boron trifluoride solution may produce adventitious peaks on the graph in the region of C_{20} - C_{22} acids. Consequently, any new batch of reagent should be checked by preparing the methyl ester of pure oleic acid, and chromatographing; if an extraneous peak appears, the reagent should be rejected. The various reagents must not give peaks interfering with those of methyl esters of fatty acids during the gas liquid chromatography. The methanolic solutions of boron trifluoride must be stored in a refrigerator.

6. Heptane, chromatographic quality (Note that if C_{20} or higher fatty acids are absent, hexane may be substituted.)

7. Redistilled light petroleum (BR 40-60°C), bromine value less than 1, residue-free: or hexane.

8. Anhydrous sodium sulphate.

9. Saturated aqueous solution of sodium chloride.

10. Methyl red, 1 g/L solution in 60% v/v ethanol.

PROCEDURE

Prepare the methyl esters of the fatty acids. The method using boron trifluoride gives good results on a wide range of samples and is preferable to alternative methods which may be used in case boron trifluoride is not available.

Because of the toxic character of boron trifluoride, the following operations are best performed under a ventilated hood. All glassware must be washed with water immediately after use.

If the oil or fatty acids include fatty acids containing more than 2 double bonds, it is advisable to purge the air from the methanol and the flask by passing a stream of nitrogen into the methanol for a few minutes.

Transfer about 350 mg of clear oil (filtered if necessary) to a 50 ml conical flask, add 6 ml of the 0.5 N methanolic sodium hydroxide solution, 7 ml of methanolic boron trifluoride solution and a boiling chip. Fit the condenser to the flask. Boil under reflux until the droplets of fat disappear (this may take 5 to 10 minutes, but in exceptional cases it may require more than 10 minutes). Add the appropriate amount of methanolic boron trifluoride solution from the bulb or automatic pipette through the top of the condenser to the boiling liquid. Continue boiling for 2 minutes.

Add 2 to 5 ml of heptane (the precise amount does not affect the reaction) to the boiling mixture through the top of the condenser. Continue boiling for 1 minute. Withdraw the source of heat, and then remove the condenser. Add a small portion of saturated sodium chloride solution to the flask in order to bring the level of liquid into the neck of the flask. Transfer about 1 ml of the upper layer (heptane solution) into a test-tube with a ground-glass neck and add a little anhydrous sodium sulphate to remove any trace of water. This solution will contain about 5-10 percent of methyl esters and may be injected directly into the column for gas liquid chromatography.

If the sample consists of fatty acids, not triglycerides, the former may be methylated directly by proceeding as above, but omitting the methanolic sodium hydroxide solution.

The amount of sample methyl esters injected should be adjusted to fit the operating conditions and to be within the linear range of the detector and electrometer.

Carry out the analysis of a mixture of methyl stearate and oleate in about equivalent proportions (e.g. methyl esters from cocoa butter). Choose the size of the sample, the temperature of the column and the carrier gas flow so that the maximum of the methyl stearate peak is recorded about 15 minutes after the solvent peak, and reaches about 3/4 of full scale deflection.

The sample for examination should be 0.1 to 1 µl of the heptane solution of methyl esters. In the case of esters not in solution, prepare an approx. 10 percent solution in heptane and inject 0.1 to 2 µl of this. If the search is for constituents present only in trace amounts, the sample size may be increased (up to tenfold).

As a rule, the operating conditions will be those defined above. Nevertheless, it is possible to work with a lower column temperature where the determination of acids below C_{12} is required, or at higher temperature when determining fatty acids above C_{20}. If possible, it is recommended that analysis be made on two columns of different polarity to check for the absence of coincident peaks, for example in the case of fish oils, or in the case of the simultaneous presence of $C_{18:3}$ and $C_{20:0}$ or of $C_{18:3}$ and conjugated $C_{18:2}$. On occasion, it is possible to employ temperature programming. If the sample contains methyl esters of fatty acids below C_{12}, it is necessary to inject the sample at 100°C (or at 50-60°C if butyric acid is present), and to raise the temperature at 4-8°C/min. In some cases the two procedures can be combined: after the programmed heating, continue the elution at a constant temperature until all the components have been eluted. If a temperature programming facility is not provided, work at two fixed temperatures between 100°C and 195°C.

Note that it is preferable to analyze the methyl esters as soon as possible. If necessary, the heptane solution containing the methyl esters may be stored under an inert gas in a refrigerator. In the case of prolonged storage, it is desirable to protect the methyl esters from autoxidation by adding to the solution an antioxidant in such concentration as will not interfere with the subsequent analysis, e.g. 0.005 percent m/v of BHT (2,6-di-tert-butyl-toluene). If necessary, the dry and solvent-free methyl esters may be stored 24 hours under inert gas in a refrigerator, or longer in a sealed tube under vacuum in a freezer.

EXPRESSION OF RESULTS

Analyse a reference standard mixture of known composition under the same operating conditions as those employed for the sample, and measure the retention distances (or retention times) for the constituent fatty acids. Using semi-logarithmic paper, construct the graphs showing the logarithm of the retention distance (or retention time) as a function of the number of carbon atoms; in isothermal conditions, the graphs for straight chain acids of the same degree of unsaturation should be straight lines, and lines for homologous series of different degrees of unsaturation should be approximately parallel. Identify the peaks in the sample from these graphs, if necessary by interpolation. It is necessary to avoid conditions such that "masked peaks" exist, i.e., where the resolution is insufficient to separate two acids.

The fatty acid composition of common oils and fats is given in the tables below. The values are in percent and single values represent maxima (that value or less). Maximum values of less than 1% are not shown. (Table information is from Pearson's Chemical Analysis of Foods, 8th Ed.).

Fatty Acid	Coconut	Palm	Palm Kernel	Cotton-seed	Safflower	Sesame	Soyabean
C 6:0	1.2	-	-	-	-	-	-
C 8:0	3.4-15	-	2.4-6.2	-	-	-	-
C10:0	3.2-15	-	2.6-7.0	-	-	-	-
C12:0	41-56	1.2	41-55	-	-	-	-
C14:0	13-23	0.5-5.9	14-20	0.4-2.0	1.0	-	-
C16:0	4.2-12	32-59	6.5-11	17-31	2.0-10	7.0-12	7.0-14
C16:1	-	-	-	0.5-2.0	-	-	-
C18:0	1.0-4.7	1.5-8.0	1.3-3.5	1.0-4.0	1.0-10	3.5-6.0	1.4-5.5
C18:1	3.4-12	27-52	10-23	13-44	7-42	35-50	19-30
C18:2	0.9-3.7	5-14	0.7-5.4	33-59	55-81	35-50	44-62
C18:3	-	1.5	-	0.1-2.1	1.0	1.0	4.0-11
C20:0	-	1.0	-	-	-	1.0	1.0
C20:1	-	-	-	-	-	-	1.0

Fatty Acid	Ground Nut	Maize	Sunflower	Olive	Rapeseed
C16:0	6.0-16	8.0-19	3.0-10	7.5-20	2.5-6.0
C16:1	1.0	-	1.0	0.3-3.5	-
C18:0	1.3-6.5	0.5-4.0	1.0-10	0.5-3.5	0.9-2.1
C18:1	35-72	19-50	14-65	56-83	50-66
C18:2	13-45	34-62	20-75	3.5-20	18-30
C18:3	1.0	2.0	-	1.5	6.0-14
C20:0	1.0-3.0	1.0	1.5	-	0.1-1.2
C20:1	0.5-2.1	-	-	-	0.1-4.3
C22:0	1.0-5.0	-	1.0	-	-
C22:1	2.0	-	-	-	5.0
C24:0	0.5-3.0	-	-	-	-

Fatty Acid	Lard and Rendered Pork Fat	Edible Tallow and Premier Jus
C14:0	0.5-1.5	1.4-7.8
C14:1	-	0.5-1.5
C15:0	-	0.5-1.0
C15:iso	-	1.5
C16:0	20-32	17-37
C16:1	1.7-5.0	0.7-8.8
C16:2	-	1.0
C17:0	-	0.5-2.0
C17:1	-	1.0
C17:iso	-	1.5
C18:0	5.0-24	6.0-40
C18:1	35-62	26-50
C18:2	3.0-16	0.5-5.0
C18:3	1.5	2.5
C20:all	4.0	-

INTERPRETATION

It is possible to quantitate from the methyl ester chromatograms by comparison to authentics. This method, however, is best used for identification of pure oils or detection of adulteration of an expensive oil by a cheaper one. For example, soyabean oil can be detected as an adulterant of sesame oil by the presence of C18:3 fatty acid. Also, animal fat adulterants in vegetable oils can be detected by C14:0, C16:1 and the large amount of C18:0.

Note that methylation with boron trifluoride may lead to erroneous results with the following compounds:

a. Compounds having secondary oxygen groupings (hydroxy, hydroperoxy, keto, epoxy);

b. Compounds containing cyclopropane and cyclopropene groups;

c. Conjugated polyunsaturated compounds and acetylenic compounds;

d. Waxes.

For these it is preferable to use one of the alternative methods unless the amounts present are small (e.g. as in cottonseed oil).

REFERENCE

IUPAC 2.301, 2.302 (1979).

ALTERNATIVE METHYL ESTER PREPARATION METHOD
(Neutral Oils and Fats)

PRINCIPLE

This method is alternate to the boron trifluoride esterification procedure, for use with neutral oils and fats (i.e., those having an acid value of less than 2). It involves methyl esterification of the fatty acids in an alkaline medium.

APPARATUS

1. High speed stirrer, with heater (e.g. magnetic stirrer and hot-plate.
2. 100 ml ground-necked conical or round-bottomed flask.
3. Inlet tube for passing nitrogen.
4. Reflux condenser to fit the flask.
5. Boiling chips (fat-free).
6. 125 ml separating funnels.
7. 50 ml narrow-mouthed conical flask.

REAGENTS

1. Methanol, containing not more than 0.5% of water.
2. Methanolic potassium hydroxide solution, approx. 1N. Dissolve 5.6 g of potassium hydroxide in 100 ml of methanol containing not more than 0.5 % m/m of water.
3. Heptane, chromatographic quality.
4. Anhydrous sodium sulphate.
5. Nitrogen, containing less than 5 mg/kg of oxygen.

PROCEDURE

If the oil includes fatty acids containing more than 2 double bonds, it is advisable to purge the air from the methanol and the flask by passing a stream of nitrogen into the methanol for a few minutes. Transfer about 4 g of clear sample oil into a 100 ml round-bottomed or conical flask. Add about 40 ml of methanol, 0.5 ml of potassium hydroxide solution and a boiling chip. Fit under the reflux condenser, stir and bring to the boil. The solution should become clear. The reaction is usually complete after 5 to 10 minutes. (Oils such as castor oil may not become completely clear).

Cool under running water and transfer the contents to a 125 ml separating funnel, rinsing the flask with 20 ml heptane. Add about 40 ml of water, shake and allow to separate. The esters pass into the upper heptane layer. Separate. Extract the aqueous layer again with 20 ml heptane. Combine the two extracts and wash them with several 20 ml portions of water. Separate and dry the ester solution over anhydrous sodium sulphate. Filter through cotton wool into a 50 ml conical flask and evaporate the solution down to approximately 20 ml over a water-bath, while passing a stream of nitrogen. Note that

there is some risk of losing part of the most volatile methyl esters if the solvent evaporation is prolonged or if the current of nitrogen is too vigourous.

REFERENCE

IUPAC 2.301, 2.302 (1979).

ALTERNATIVE METHYL ESTER PREPARATION METHOD
(Acidic Oils and Fats)

PRINCIPLE

This method is an alternative to the boron trifluoride esterification procedure, for use with acidic oils and fats (i.e., those having an acid value greater than 2). It involves neutralization of previously formed free fatty acids, alkaline methanolysis of the glycerides and esterification of the fatty acids in acid medium.

APPARATUS

1. High speed stirrer and heater (e.g. a magnetic stirrer and hot-plate).

2. 250 ml ground-necked conical or round-bottomed flask.

3. Inlet tube for passing nitrogen.

4. Reflux condenser to fit the flask.

5. Boiling chips (fat-free).

6. 250 ml separating funnels.

7. 100 ml narrow-necked conical flask.

REAGENTS

1. Sodium methoxide solution. Dissolve 1g of sodium in 100 ml of methanol containing not more than 0.5% m/m of water.

2. Methanolic solution of anhydrous hydrochloric acid, approx. N: Gaseous hydrochloric acid can easily be prepared in the laboratory by simple displacement from the commercial solution by dripping concentrated sulphuric acid on to the hydrochloric acid solution. The liberated gas is dried by bubbling through concentrated sulphuric acid. Since hydrochloric acid is very rapidly absorbed by methanol, it is advisable to take the usual precautions in dissolving it, i.e., introduce the gas through a small inverted funnel with the rim just touching the surface of the liquid. Large quantities of methanolic hydrochloric acid solution can be prepared in advance, as it keeps well in glass-stoppered bottles stored in the dark.

3. Heptane, chromatographic quality.

4. Anhydrous sodium sulphate.

5. Nitrogen, containing less than 0.5 mg/kg of oxygen.

PROCEDURE

If the acid oil includes fatty acids containing more than 2 double bonds, it is advisable to purge the methanol and the flask by passing a stream of nitrogen into the methanol for a few minutes. Transfer about 4 g of clear sample oil into a 250 ml conical or round-bottomed flask. Add 40 ml of the sodium methoxide solution. Fit the reflux condenser, stir and bring to the boil. The solution should become clear, which usually occurs in about 10 minutes. The reaction is normally complete after 15 minutes. Note that in the case of very acid oils and fats precipitation of sodium chloride occurs, owing to the relatively high quantity of sodium methoxide. This may lead to

bumping during the subsequent boiling. The precipitate may be filtered off, but this is usually unnecessary owing to the short period of boiling prescribed.

Add at least 50 ml of the hydrochloric acid solution to the flask, and boil again for 10 minutes. Cool under running water, add 100 ml of water to the flask, then pour the contents into a 250 ml separating funnel and add 30 ml of heptane. Shake vigorously and let settle until the 2 phases have separated. Collect the heptane extracts and wash them several times with water until neutral. Separate and dry over anhydrous sodium sulphate. Filter through cotton wool into a 100 ml flask and finally evaporate the solution down to 20 ml over a boiling water-bath, while passing a stream of nitrogen.

REFERENCE

IUPAC 2.301, 2.302 (1979).

9.2 MARGARINE

COMPOSITION

The Codex Standard CAC/RS 32-1969 recommends that margarine contain not less than 80% fat and not more than 16% water.

Vitamins A and esters, D, E and esters and other vitamins may be added and it is recommended that maximum and minimum levels be established under national legislation. Sodium chloride, sugars and suitable edible proteins may also be added and milk or milk products may be used in manufacture. The following may be added, the amount not being limited, otherwise than by good manufacturing practice: beta-carotene, annatto, curcumin, canthaxanthin, beta-apo-8'-carotenal, methyl and ethyl esters of beta-apo-8'-carotenoic acid, natural flavours and their identical synthetic equivalents (except those which are known to represent a toxic hazard), other synthetic flavours approved by Codex Alimentarius Commission, mono- and diglycerides of fatty acids, citric diglycerides of fatty acids, citric and lactic acids and their potassium and sodium salts, L-tartaric acid and its sodium and sodium-potassium salts, sodium hydrogen carbonate, sodium carbonate and sodium hydroxide, natural and synthetic tocopherols.

Recommended limits have been set for the following added substances:

Additives	Maximum level of use
Mono- and diglycerides of fatty acids esterified with the following acids: acetic, acetyltartaric, citric, lactic, tartaric and their sodium and calcium salts	10 g/kg
Polyglycerol esters of fatty acids	5 g/kg
1,2-propylene glycol esters of fatty acids	20 g/kg
Esters of fatty acids with polyalcohols other than glycerol, such as sorbitan monopalmitate, sorbitan monostearate, sorbitan tristearate	10 g/kg
Sucrose esters of fatty acids	10 g/kg
Preservatives	
Sorbic acid and its sodium, potassium and calcium salts Benzoic acid and its sodium and potassium salts	1,000 mg/kg individually or in combination.
Antioxidants	
Propyl, octyl, and dodecyl gallates Butylated hydroxytoluene (BHT) Butylated hydroxyanisole (BHA)	100 mg/kg individually or in combination
Ascorbyl palmitate Ascorbyl stearate	200 mg/kg individually or in combination
Antioxidant Synergists	
Isopropyl citrate mixture	100 mg/kg

Metal Contaminants	Maximum Level
Iron (Fe)	1.5 mg/kg
Copper (Cu)	0.1 mg/kg
Lead (Pb)	0.1 mg/kg
Arsenic (As)	0.1 mg/kg

ROUTINE ANALYSIS

Margarine may be analyzed by the methods used for butter. Determination of the RPK values will enable assessment to be made of the amount of butterfat added, if any. (See methods under 2.4 of this Manual).

CAC/RS 32-1969 details the AMC (1959) method for vitamin E, using paper chromatography to separate the different tocopherols. The method of Christie et al (20) is easier to use, although the chromatography materials specified must be used. Interference by dimers is unusual in practice.

Rancidity can be assessed by determining the free fatty acids (FFA) in the fat portion of the margarine. Freshly manufactured margarine should have FFA values of about 0.16% calculated as oleic acid.

MONOGLYCERIDES IN MARGARINE

PRINCIPLE

This method determines the amount of 1-monoglycerides, conventionally expressed as glyceryl monostearate, present in a fatty material such as margarine. Oxidation of the monoglycerides by periodic acid is the basis of the method, which is valid in the presence of free glycerol. Dry filtered fat should be taken for the examination.

APPARATUS

1. Graduated flasks, 100 ml

2. Stoppered flasks, 500 ml

3. Stoppered flask, 2 L.

REAGENTS

1. Periodic acid solution: weigh out 5.4 g of periodic acid into a 2 L glass-stoppered flask and dissolve in 100 ml of distilled water; add to this aqueous solution 1900 ml of glacial acetic acid. Mix thoroughly and keep away from the light.

2. Aqueous citric acid solution: 2% w/v citric acid crystals dissolved in water.

3. Chloroform.

4. Aqueous solution of potassium iodide, 15% w/v.

5. Aqueous sodium thiosulphate solution 0.1 N accurately standardized.

6. Aqueous starch indicator solution, 1% w/v.

PROCEDURE

The sample must be homogeneous and to ensure homogeneity the fat should be melted and vigorously shaken. If the sample is solid but apparently homogeneous it should be liquified at a temperature not exceeding 10°C above its melting point. Do not take a portion for analysis until the whole of the sample has been liquified and well shaken. The amount to be taken for analysis can be determined in the light of its probable 1-monoglyceride content as shown in the table below:

Presumed content of 1-monoglyceride present in the sample (% w/w)	Weight of the portion to be taken for assay (g)
100	0.3
75	0.4
50	0.6
40	0.7
30	1.0
20	1.5
10	3.0
5	6.0
3 or less	10.0

Weigh out the quantity for analysis with an accuracy of 0.1 percent, and transfer it quantitatively into a 100 ml graduated flask using successive small quantities of chloroform to aid the transfer until the volume reaches about 50 ml. Add 25 ml of aqueous citric acid solution in order to decompose any soap that may be present, shake vigorously for 1 minute and allow it to stand.

After the mixture has separated into two phases remove the supernatant aqueous phase as completely as possible and transfer it to another 100 ml graduated flask. (The washing of the chloroform solution may be effected by a suitable contrivance which will allow the washing to be carried out with ease and without any risk of losing any of the aqueous or chloroform phases.) Repeat the washing of the chloroform phase twice more, either with 25 ml of water or with 25 ml of the aqueous citric acid solution when emulsions are formed. Combine the aqueous solutions in the second 100 ml flask and fill up to the 100 ml mark with distilled water.

Make up a chloroform solution remaining in the first graduated flask to the 100 ml mark with chloroform. Pipette exactly 50 ml of periodic acid solution and 50 ml of the chloroform solution into a 500 ml glass-stoppered flask. Shake for a few minutes, stopper the flask and allow to stand away from light at room temperature for 30 minutes. Carry out a blank test under the same conditions using 50 ml of the periodic acid solution and 50 ml of chloroform.

After the 30 minute period add about 20 ml of aqueous potassium iodide solution both to the sample and to the blank. Allow them to stand for a further minute. Add about 100 ml of distilled water to each. Titrate with the 0.1 N sodium thiosulphate solution while shaking continuously in order to ensure thorough mixing. Towards the end of titration, add about 2 ml of starch solution as indicator and then continue titrating drop by drop with vigorous agitation until the end-point is reached.

The number of ml used to titrate the sample should not be less than 80 percent of the number of ml required for the titration of the blank. If this condition is not fulfilled, repeat the determination using a smaller quantity for analysis, to ensure that the oxidation of the 1-monoglycerides is complete.

CALCULATION

The percentage of 1-monoglycerides (calculated as glyceryl monostearate, MW 358)

$$= \frac{35.8\ (a-b)\ N}{p}$$

Where:

a = number of ml of 0.1 N sodium thiosulphate solution utilized for the blank test

b = number of ml of 0.1 N sodium thiosulphate solution utilized for the test sample

N = the exact normality of the aqueous sodium thiosulphate solution

p = weight in g taken for the determination.

REFERENCE

IUPAC 2.322 (1979).

VITAMIN A IN MARGARINE

PRINCIPLE

The margarine is saponified and the unsaponifiable material is extracted and eluted through an adsorption column. This separates the Vitamin A and carotenes which are determined spectrophotometrically.

APPARATUS

1. Spectrophotometer capable of readings at 325 nm and 450 nm.

2. Chromatographic tubes 12 mm OD x 90 mm long with stem on lower end. The tube can be fitted with a medium porosity glass frit at the bottom, or a pledget of glass wool can be used.

3. Vacuum source (a water aspirator vacuum would suffice.)

4. Long wavelength (300 nm) ultraviolet light source for viewing bands (use with caution).

REAGENTS

1. Potassium hydroxide solution, 50% w/w (780 g/L).

2. Ethanol, absolute and 95%. Check spectral purity by determining absorbance at 300 nm in a 1 cm cell against water. It should not exceed 0.05.

3. Ethyl ether, peroxide-free. Distil fresh and discard first and last 10% of distillate.

4. Petroleum ether (BR 30-60°). Check transmittance (T) at 300 nm in a 1 cm cell against air. T should be greater than 85%.

5. Eluting solution I - 16% ethyl ether in petroleum ether.

6. Eluting solution II - 25% ethyl ether in petroleum ether. (Note: dry both I and II with anhydrous sodium sulphate and store over bright copper metal turnings to prevent peroxide formation).

7. Eluting solution III - 10% absolute ethanol in petroleum ether.

8. Sodium sulphate, anhydrous. Check acidity by dissolving 10% in water. The solution must not be acidic to methyl red indicator.

9. Alumina. Check mesh size as follows: All should pass a 60 mesh sieve, up to 20% can be retained on a 100 mesh, about 50% should be retained by a 160 mesh and the rest should pass through. Activate the alumina by heating for 3 hours at 600°C. After cooling add water dropwise to a weighed amount until the alumina contains 3% m/m water. Keep in a tightly closed container.

10. Alkaline alumina - mix a portion of the dry activated alumina with an equal weight of 10% w/w KOH solution in a dish. Decant and discard the excess liquid. Dry overnight at 100°C. Crush dried material and pass through a 60 mesh sieve. Take a weighed amount and add water dropwise until 3 % m/m water has been added. Store in a tightly capped bottle.

PROCEDURE

Weigh 10 ± 0.1 g margarine. Add 75 ml absolute ethanol and 15 ml 50% KOH solution. Saponify by boiling 5 minutes. Let cool for 20 minutes.

Transfer the cooled solution to a separatory funnel using about 100 ml total of water, for washings. Add 100 ethyl ether and shake to extract. Transfer the aqueous phase to another separatory funnel and re-extract with four 50 ml portions of ethyl ether. Combine all of the ether extracts and wash with two 100 ml portions of water. Discard washings. Dry the combined ether extracts with anhydrous sodium sulphate.

Evaporate the combined dried ether extract on a steam bath to about 25 ml. Transfer to a 50 ml beaker and continue evaporation until a viscous, oily material remains.

Remove from the steam bath and evaporate the last traces of solvent using nitrogen gas. Dissolve the oily material in 5 ml petroleum ether and transfer to a 10 ml volumetric flask. Make to the mark with petroleum ether. This is the sample solution.

Prepare a chromatographic column by packing (gravity and slight tapping on the sides) 1 cm of the damp alumina, then 2 cm of alkaline alumina and finally another 4 cm of the damp alumina. Place the column exit stem through a stopper into a vacuum flask with side arm. Attach to a vacuum source and control using a pinchcock.

Apply vacuum to the column and add 5 ml petroleum ether. Next add by pipette 5 ml sample solution and another 5 ml petroleum ether. As the last of the petroleum ether enters the column top, add 5 ml of eluting solution I. Continue adding 5 ml portions of I until all of the carotene has eluted from the column. This can be noted when the eluate is no longer yellowish. Combine all of the carotene eluate and save for further analysis.

Next, continue adding 5 ml portions of eluting solution I and monitor the band of Vitamin A as it progresses down the column, using the UV lamp (Vitamin A is fluorescent under UV). Discard the eluate after the carotene and before the Vitamin A begins to move. The Vitamin A should elute in less than 20 minutes.

Continue elution until no fluorescence is noted in 1 ml of the eluate. Combine all Vitamin A eluates.

Evaporate the carotene and Vitamin A eluates separately on a steam bath to about 2 ml each. Cool and remove the remaining solvent from each using nitrogen or a vacuum. Dissolve the carotene in 5 ml petroleum ether and transfer to a 10 ml volumetric flask. Make to the mark with petroleum ether. Dissolve the Vitamin A in 5 ml absolute ethanol. Transfer to a 10 ml volumetric flask and make to the mark with absolute ethanol.

Determine the absorbance of the carotene solution in a 1 cm cell at 450 nm using petroleum ether as the reference.

Similarly, determine the absorbance of the Vitamin A solution at 310 nm and 325 nm using absolute ethanol as the reference. The ratio of the absorbances at 310 and 325 should be 1 or less.

CALCULATION

$$\text{Vitamin A in } \mu/g = \frac{A_{325} \times 5.5}{\text{g sample}}$$

$$\text{Vitamin A in Intnl. Units/g} = \frac{A_{325} \times 18.3}{\text{g sample}}$$

$$\text{Carotene in } \mu/g = \frac{A_{450} \times 4.17}{\text{g sample}}$$

$$\text{Carotene in Intnl. Units/g} = \frac{A_{450} \times 6.95}{\text{g sample}}$$

REFERENCES

Analtyical Methods Committee, 1964. The Analyst 89, 7.

Official Methods of Analysis of the AOAC, 1984. 43.001 - .007.

9.3 ANIMAL FAT

COMPOSITION

The recommended International General Standard for Edible Fats and Oils (CAC/RS 19-1969) defines edible oils and fats as: foodstuffs composed of glycerides of fatty acids of vegetable, animal or marine origin. Fats of animal origin must be produced from animals in good health at the time of slaughter and be fit for human consumption as determined by a competent authority recognized in national legislation. They may contain small amounts of other lipids such as phosphatides, of unsaponifiable constituents and of free fatty acids naturally present in the fat or oil.

The Codex recommended standards for animal fats are:

Animal Fat	Density 20°/20°	Refractive Index	Saponification Value	Iodine Value	Unsaponifiable matter (max)	Acid Value mg KOH/g (max)	Peroxide value meq O/kg (max)
Lard (40°-20°)	0.896-0.904	1.448-1.460	192-203	45-70	10	1.3	10
Rendered pork fat (40°-20°)	0.894-0.906	1.448-1.461	192-203	45-70	12	2.5	16
Edible tallow (40°-20°)	0.893-0.904	1.448-1.460	190-202	32-50	12	2.5	16
Premier Jus. (40°-20°)	0.893-0.898	1.448-1.460	190-200	32-47	10	2	10

The titres of animal fats must be within the following ranges:

Fat	Titre
Lard	32 - 45°C
Rendered Pork Fat	32 - 45°C
Edible Tallow	40 - 49°C
Premier Jus	42.5 - 47°C

Lard may contain refined lard, lard stearine and hydrogenated lard provided this is declared on the label, but the recommended standard does not apply to refined lard. Rendered pork fat may contain fat from bones, ears, tails, etc. which lard may not. The recommended standard does not apply to refined rendered pork fat, but the article may contain refined lard or rendered pork fat, hydrogenated or not, lard stearine and rendered pork fat stearine as long as these are declared on the label. Edible tallow (synonym: dripping) is the product obtained by rendering the clean, sound fatty tissues (including trimming and cutting fats), attendant muscles and bones of bovine animals (Bos taurus) and/or sheep (Ovis aries) in good health at time of slaughter and fit for human consumption as determined by an authority recognized as competent in national legislation. Premier Jus (Synonym: Oleo Stock) is the product obtained by rendering at low heat the fresh fat (killing fat) or heart, caul, kidney and mesentery collected at time of slaughter of bovine animals (Bos taurus) in good health at time of slaughter and fit for human consumption as determined by an authority recognized as competent in national legislation. The raw material does not include cutting fats.

FREE FATTY ACIDS

PRINCIPLE

Animal tissues contain lipase enzymes which have the ability to hydrolyze fats, splitting fatty acids from the glycerol molecules. The extent to which this has occurred can be determined by the free fatty acid (FFA) content of the animal fat by placing in solution and titration.

APPARATUS

1. Steam bath.
2. Drying oven.
3. Burette.

REAGENTS

1. Chloroform.
2. Ethanol, 95%.
3. 0.02 N sodium hydroxide solution, accurately standardized.
4. Phenolphthalein solution 1%.
5. Anhydrous sodium sulphate.

PROCEDURE

Weigh 50 g of fat and macerate with 200 ml chloroform. (Do not use heat). Filter through a paper containing anhydrous sodium sulphate. Collect the filtrate in a stoppered flask.

Pipette 20 ml of the filtrate and evaporate the chloroform on a steam bath. Dry in an oven at 100°C for 3 hours. Cool in a desiccator and weigh the fat. (This determines the fat content of the filtrate solution).

Pipette 20 ml of the filtrate into a 250 ml flask. Add 20 ml of neutralized ethanol. Titrate with 0.02 N sodium hydroxide solution using phenolphthalein indicator.

CALCULATION

$$\text{FFA (as \% oleic acid in the fat)} = \frac{V \times N \times 28.2}{W}$$

Where: V = ml of NaOH used
N = normality of NaOH
W = g fat per 20 ml filtrate

INTERPRETATION

In good quality animal fat the FFA should not exceed 0.5% as oleic acid.

REFERENCE

Official and Tentative Methods of the American Oil Chemists' Society, Ca 5a-40.

THIOBARBITURIC ACID VALUE

PRINCIPLE

Oxidized lipids are formed as fats become rancid. Thiobarbituric (TBA) acid will react with these lipids to form a red-coloured complex which can be determined spectrophotometrically. Malonaldehyde is one of the end products of oxidative rancidity, and is believed to be involved in the reaction with TBA. Therefore, the TBA value is expressed as mg malonaldehyde per kg sample. The TBA test is applicable to fatty foods (e.g. meat) as well as fats and oils.

APPARATUS

1. Distillation apparatus (flask, condenser, receiver).
2. Glassbeads.
3. Electric mantle.
4. Pipette.
5. Glass stoppered test tube.
6. Spectrophotometer.

REAGENTS

1. Hydrochloric acid, 4N.
2. Antifoam liquid.
3. Thiobarbituric acid reagent: dissolve 0.2883 g in 100 ml of 90% glacial acetic acid.

PROCEDURE

Macerate 10 g sample with 50 ml water for 2 minutes and then transfer to the distillation flask, using 47.5 ml water for washing. Add 2.5 ml 4N HCl. (pH should be 1.5). Add antifoam and a few glass beads. Distil at a rate so that 50 ml of distillate is collected in 10 minutes from the time boiling commences.

Pipette 5 ml of the distillate into a glass-stoppered tube. Add 5 ml TBA reagent. Shake and heat in boiling water for 35 minutes.

Prepare a blank similarly, using 5 ml water, for 35 minutes.

Cool the sample and blank tubes and measure the absorbance of the sample against the blank at 538 nm using 1 cm cells.

CALCULATION

TBA value (as mg malonaldehyde per kg sample) = 7.8 x A

(A = absorbance of sample against blank)

(Caution: The method must be followed exactly for the 7.8 factor to be valid.)

REFERENCE

Tarladgis, B.G., Watts, B.M., Younathan, M.T. and Dugan, L., 1960. Journal of the American Oil Chemists' Society 37, 44.

9.4 TEXT REFERENCES

1. AMERICAN OIL CHEMISTS SOCIETY. Official & Tentative Methods of the American Oil Chemists' Society 508 S. 6th Street, Champaign, Illinois 61820.

2. IUPAC. 1979. Standard Methods of the Analysis of Oils, Fats and Derivatives 6th Edition, Butterworths, London.

3. CHRISTIE, W.W. 1973. Lipid Analysis Pergamon Press.

4. BOCKENOOGEN, H.A. 1964. Analysis & Characterisation of Oils, Fats and Fat Products Vol I and II and 1968 Interscience.

5. PARODI, P.W. 1976. Journal of the American Oil Chemists Society 53 530-534.

6. HART, F.L. & FISHER, H.J. 1971. Modern Food Analysis, Springer-Verlag.

7. CONACHER, H.B.S. & CHADHA, R.K. 1974. Journal of the American Oil Chemists Society 57 (5) 1161.

8. CONACHER, H.B.S. 1975. Journal of the American Oil Chemists Society 58 (3) 488.

9. ACKMAN, R.G. et al 1977. Technical Report No. 577, Fisheries and Marine Service Halifax Laboratory, Halifax, Nova Scotia.

10. COCKS, L.V., CHRISTIAN, B.C. & HARDING, G. 1931. Analyst 56 368.

11. COCKS, L.V. & Van REDE, C. 1966. Laboratory Handbook for Oil and Fat Analyses Academic Press 1966.

12. ALLEN, R.R. 1969. Journal of the American Oil Chemists Society 46 552.

13. HUANG, A. & FIRESTONE, D. 1971. Journal of the Association of Official Analytical Chemists.

14. SYNODINES, E. & KOVITAS, 1958. 408 Chim Chronia 23 21.

15. MATARESE, L. 1962. Rivista Italianna delle Sostanze Grasse 39 74 and 40 429 1963.

16. HERZINGER, V. & PAZLAR, M. 1975. Prumysl Potravin 26 (8) 473-474.

17. MEHLENBACHER, V.C. 1960. The Analysis of Fats and Oils The Garrard Press, Champaign, Illinois.

18. WILLIAMS, K.A. 1966. Oils, Fats and Fatty Foods Churchill, London.

19. TARLADGIS, B.G., WATTS, B.M. & YOUNATHAN, M.T. & DUGAN, L. 1960. Journal of the American Oil Chemists' Society 37 44.

20. CHRISTIE, A.A., DEAN, A.C. & MILBURN, B.A. 1973. Analyst 98 1164.

Further Reading

Dietary Fats and Oils in Human Nutrition, FAO Food & Nutrition Paper No. 3 1977.

PATTERSON, H.B.W. 1983. Hydrogenation of Fats & Oils. Applied Science.

HAMILTON, R.J. & BHATI, A. 1980. Fats & Oils: Chemistry & Technology, Applied Science.

ALLEN, J.C. & HAMILTON, R.J. 1983. Rancidity in Foods, Applied Science.

PERKINS, E.G. (Ed) 1978. Analysis of Lipids & Lipoproteins.

WITTING, L.A. (Ed) 1978. Glycolipid Methodology AOCS.

Official & Tentative Methods of Analysis, AOCS, 508 South 6th St., Champaign, Illinois 61820 U.S.A.

10. BEVERAGES

10.1 ALCOHOLIC - DISTILLED

COMPOSITION

The five most common distilled spirits are brandy, gin, rum, vodka and whisky. Brandy is distilled from the fermented juice of grapes or other fruits. Gin is a diluted spirit flavored with juniper berries or other plant extracts. The basic spirit is usually made from maize and rye with malted barley. Vodka is a diluted spirit usually made from potato, wheat or other grains, with no added flavouring. Rum is a spirit distilled from fermented sugar cane juice or molasses. Whisky is generally meant to be Scotch Whisky but does include the U. S. bourbon whiskies.

Composition data for some distilled spirits are as follows (note that these date are ranges or approximations only, as composition can vary widely): (Data compiled from Pearson's Chemical Analysis of Food, 8th Ed.)

Spirit	Total Solids%	Ash %	Acidity % (as acetic)	Total Esters (*)	Furfural (*)	Alde-hydes (*)	Higher Alcohol (*)
Brandy	0.1-1.9	.004-.012	.041-.072	1.1-5.8	.02-.06	.24-.76	1.5-7.8
Gin	.01-.09	.008-.009	.015-.020	-	-	-	-
Rum	.29-.42	.025-.041	.082-.102	1.9-2.7	.07-.10	.24-.35	1.6-1.9
Whisky**	-	-	.030-.070	0.1-0.3	0.1-0.2	1.0-2.7	0.7-4.5

* expressed as g/L of ethanol
** Scotch whisky.

ROUTINE ANALYSIS

Distilled spirits should be examined for ethanol and total solids (extract). Ethanol is normally over 37 percent v/v in spirits. It may also be necessary to examine for ash, acidity, tannins, esters, furfural and other aldehydes, ketones, higher alcohols, methanol and isopropanol. Hart and Fisher (1) give some analysis for American whiskies. See also "Standard Methods of Whisky Analysis" published by Scotch Whisky Association, U.K. and the Reports of the Research Committee on the analysis of Potable Spirits.

Liqueurs are usually sweetened and flavoured distilled spirits. The flavouring components and other materials may interfere in the estimation of ethanol, in which case one of the methods of the BP. 1968, p. 1278 may be used. The presence of interfering substances is inferred when the refractive index reading of the distillate does not correspond to the specific gravity according to the following table:

Specific Gravity 20°/20°	Refractive Index 20°	Specific Gravity 20°/20°	Refractive Index 20°
0.9710	1.34661	0.9860	1.33842
0.9720	1.34605	0.9870	1.33796
0.9730	1.34549	0.9880	1.33751
0.9740	1.34493	0.9890	1.33705
0.9750	1.34437	0.9900	1.33663
0.9760	1.34380	0.9910	1.33620
0.9770	1.34324	0.9920	1.33578

Specific Gravity 20°/20°	Refractive Index 20°	Specific Gravity 20°/20°	Refractive Index 20°
0.9780	1.34267	0.9930	1.33540
0.9790	1.34211	0.9940	1.33501
0.9800	1.34154	0.9950	1.33466
0.9810	1.34098	0.9960	1.33432
0.9820	1.34044	0.9970	1.33397
0.9830	1.33991	0.9980	1.33362
0.9840	1.33942	0.9990	1.33331
0.9850	1.33892	1.0000	1.33300

The BP methods require that the sample be distilled and diluted to four times its volume (quadruple bulk), so the use of the table will require dilution of most liqueur distillates. Both determination of SG and RI must be done at 20°C (or corrected thereto) and the RI should not differ by more than 0.00007 from the value corresponding to the specific gravity. If necessary, the RI at temperatures other than 20°C can be related to the SG via AOAC tables. Liqueur chocolates should be steam-distilled prior to the determination of ethanol.

ETHANOL

PRINCIPLE

The sample is distilled and the specific gravity of the distillate measured, the proportion of alcohol being calculated from tables.

APPARATUS

1. Distillation flask with an efficient condenser, such as 'revenue' or West condenser.

2. Specific gravity bottle or pyconometer.

PROCEDURE

General note: The distillation must be carried out under conditions that do not incur loss of alcohol. For example, the American Society of Brewing Chemists method specifies that the condenser water must be 25°C and the receiving flask should be surrounded with ice or ice and water. All determinations of gravity are most conveniently and accurately carried out at 20°C and therefore the volume of sample taken initially should be at 20°C but if this is impossible, temperature corrections must be made. It is essential that distillates be diluted to volume (or a multiple of it) at the same temperature as that at which the sample was measured initially.

Spirits; 50 ml of sample is washed into a 100 ml distillation flask with not more than 5 ml of water, distilled and the distillate diluted to 50 ml. The specific gravity is determined. (See RCAPS (2)). The specific gravity determined without distillation does not give seriously erroneous results for products such as vodka in which the total solid matter is small. The solid matter increases the gravity and therefore the alcohol percentage calculated from the specific gravity without distillation is lower than the true value. This difference between the apparent and true alcohol values is referred to as the 'obscuration'. This may be determined by dilution of the alcohol-free residue remaining from distillation to the original sample volume and determination of the specific gravity. Subtract 1 from the S.G. and subtract the result from the specific gravity of the sample (obtained without distillation). This gives a result corresponding to the true alcohol content. Instead of carrying out the determination, it is sometimes assumed that each 1 percent m/v of extract (total solids by drying to constant weight at 100°C) increases the specific gravity by 0.0041.

In contrast to pure spirits, some liqueurs may contain sufficient volatile matter other than alcohol to give an incorrect value. In cases of doubt, the specific gravity and refractive index are compared and the distillate accepted as of adequate purity if the values correspond to the same percentage of alcohol. If not, proceed by the method of the British Pharmacopoeia (1968).

Wines: The OIV reference method requires that a 1 L flask and a 20 cm condenser should be used. The equipment chosen must be capable of giving 99.8 percent recovery of alcohol distilled from it. De-gas the wine if necessary by shaking in a flask of about twice the volume of liquid. Measure 200 ml or other suitable volume into the distillation flask, rinsing the graduated flask with 4 x 5 ml water. Add 10 ml of a 12% suspension of calcium oxide. For very acid wines add extra lime suspension until the wine becomes alkaline as shown by phenolphthalein used as an external indicator. Add porous pot and distil into the flask in which the sample was measured after placing about 10 ml of water in the flask. Ensure that the outlet to the

condenser is inserted well into the flask. Distil about three-quarters of the initial volume. Wash out the distillation flask, and return the distillate to it, rinsing with 4 x 5 ml of water as before. Add porous pot and 1 ml of 10 % sulphuric acid and re-distil into the same volumetric flask containing about 10 ml of water. Dilute the distillate to the mark, ensuring that the temperature is within 2°C of that at which the wine was measured initially.

Beer: De-carbonate by pouring from one beaker to another several times. Measure 100 ml of beer and place in the distillation flask, rinsing the graduated flask with a total of 50 ml of water used in several portions. Distil 96 ml at the uniform rate in 30-60 minutes. Dilute to volume, mix well and determine the specific gravity.

Determination of Specific Gravity of the Distillate

Weigh an empty specific gravity bottle or pycnometer, fill to the mark with distillate avoiding inclusion of air and carefully wiping the outside of the bottle if necessary, and weigh. Adjust the temperature to 20°C and ensure that the bottle or pycnometer is exactly full or at the graduation mark at that temperature, or adjust to the mark and determine the temperature. Replace the distillate with water and weigh, adjusting the temperature or determining it as for the distillate.

If both distillate and water were measured at the same temperature, then:

$$\text{Apparent SG} = \frac{\text{weight of volume of distillate}}{\text{weight of equal volume of water}}$$

The percentage of alcohol v/v, m/m or m/v at 20°C from determinations carried out at 20°C may be calculated from tables such as that of the American Society of Brewing Chemists or by AOAC. U.K Customs and Excise Tables relate values at 20°C to those at 15.5°C.. The OIV tables enable determinations at other temperatures to be calculated to 20°C and those of the AOAC enable determinations at other temperatures to be calculated to 15.56°C.

It is generally desirable to carry out the determinations with the temperature of the distillate and of the water at the same temperature. If this is not done, the apparent specific gravity of the water may be adjusted to the temperature at which the distillate was weighed, according to the water density table below:

e.g. weight of gravity bottle = 49.0000 g
weight of gravity bottle + distillate at 18°C = 100.4320 g
weight of distillate at 18°C = 51.4320 g
weight of gravity bottle + water at 22°C = 101.3330 g
weight of water at 22°C = 52.3330 g
density of water at 22°C = 0.997801
density of water at 18°C = 0.998625

water required to fill gravity bottle at 18°C would weigh

$$52.3330 \times \frac{0.998625}{0.997801} = 52.3762$$

$$\text{apparent specific gravity of distillate at } 18°C = \frac{51.4320}{52.3762}$$

$$= 0.9810$$

alcohol % v/v at 20°C (OIV tables) = 12.70

Water density table (from G.S. Kell, Handbook of Chemistry and Physics, 49th Ed.)

°C	Density (g/ml)	°C	Density (g/ml)
10	0.999728	23	0.997569
11	0.999634	24	0.997327
12	0.999526	25	0.997075
13	0.999406	26	0.996814
14	0.999273	27	0.996544
15	0.999129	28	0.996264
16	0.998972	29	0.995976
17	0.998804	30	0.995678
18	0.998625	31	0.995372
19	0.998435	32	0.995057
20	0.998234	33	0.994734
21	0.998022	34	0.994403
22	0.997801	35	0.994063

One degree Gay-Lussac corresponds to one litre of ethanol in 100 litres of wine, both measured at 15°C. The International degree (Convention Internationale, 1957) is the percentage by volume expressed at 20°C. Jaulmes and Marignan (3) showed that there was no simple relation between the two and suggested use of the following table:

15°/15°	20°/20°	20°/20°-15°/15°	15°/15°	20°/20°	20°/20°-15°/15°
6	6.03	0.03	18	18.07	0.07
7	7.03	0.03	19	19.07	0.07
8	8.04	0.04	20	20.07	0.07
9	9.04	0.04	21	21.08	0.08
10	10.04	0.04	25	25.08	0.08
11	11.05	0.05	30	30.09	0.09
12	12.05	0.05	40	40.08	0.08
13	13.05	0.05	50	50.07	0.07
14	14.06	0.06	60	60.07	0.07
15	15.06	0.06	70	70.06	0.06
16	16.06	0.06	80	80.05	0.05
17	17.07	0.07	90	90.03	0.03

REFERENCES

OIV (Office International de la Vigne et du Vin) Receuil des Methodes d'Analyse, 1978. (Note: When methods are stated to follow OIV procedures, the description of the details of the procedure is not to be considered an official translation from the French. The original French text should be referred to for the definitive text of OIV recommended methods).

Methods of Analysis of the American Society of Brewing Chemists, 1976.

Bee, H.M., 1970. Journal of the Association of Public Analysts 8, 97.

Customs and Excise Spirit Tables, U.K., current edition.

METHANOL

PRINCIPLE

Methanol is oxidised to formaldehyde and the colour developed by reaction with chromotropic acid is measured at 570 nm.

APPARATUS

1. Spectrophotometer.

REAGENTS

1. Chromotropic acid, 4,5-dihydroxy-naphthalene-2,7-disulphonic acid, or the disodium salt. If it is necessary to purify it, dissolve 10 g of acid or salt in 25 ml water, adding 2 ml of concentrated sulphuric acid in the latter case, add 50 ml of methanol, boil and filter. Add 100 ml isopropanol, allow to cool and filter off the crystals. Dissolve 0.05 g chromotropic acid or salt in 35 ml water, cool to 0°C and slowly with stirring add 75 ml of sulphuric acid. Prepare fresh.

2. Standard methanol, 0.05% in 5% v/v ethanol in water.

3. Phosphoric acid, 50% m/v.

4. Potassium permanganate 5% m/v.

5. Sodium sulphite 2% m/v. Check the strength by titration with standard iodine solution.

PROCEDURE

Dilute the distillate from the determination of ethanol so that it contains 5 percent ethanol. To 0.5 ml of diluted distillate in a test-tube add 1 drop of 50% phosphoric acid and 2 drops of 5% potassium permanganate. Shake and leave to stand 10 min. Decolourize the potassium permanganate with sodium sulphite solution, avoiding an excess. Add 5 ml of 0.05% chromotropic acid solution and leave 20 minutes at 70°C.

In a series of 50 ml flasks add 2.5, 5, 10, 15, 20 and 25 ml of the standard 0.05% methanol solution. Dilute to 50 ml with 5% ethanol in water. These solutions contain 0.025, 0.05 etc. g methanol per litre. Treat 0.5 ml of each as for the sample. Determine the absorbance at 570 nm using the 0.5 ml of each as for the sample. Determine the absorbance at 570 nm using the solution prepared from 0.5 ml of 5% ethanol in water as a blank.

CALCULATION

From a standard curve, calculate the g of methanol per litre of distillate diluted to 5 percent.

g/L of methanol = g/L in 5% distillate x (% ethanol in sample)/5

REFERENCE

Official Methods of Analysis of the AOAC, 1984, 9.102-.105. This is the OIV (4) routine method. The reference method uses GLC.

HIGHER ALCOHOLS

PRINCIPLE

The sample is distilled after adjustment of the ethanol level to 40 percent. The higher alcohols are reacted wiyh 4-hydroxybenzaldehyde-3-sulphonic acid in the presence of sulphuric acid (the Komarowsky reaction) and the colour compared with standards.

APPARATUS

1. Distillation apparatus.

2. Spectrophotometer.

REAGENTS

1. Sodium 4-hydroxybenzaldehyde-3-sulphonate, 4% m/v in water.

2. Mixed pentanols. Isoamylalcohol, AR or equivalent (mainly 3-methylbutan-1-ol).

3. Standard higher alcohol solutions.

4. Prepare the following mixtures:

   7.5 ml of 2-methylpropan-1-ol and 2.5 ml of mixed pentanols
   5.0 ml of 2-methylpropan-1-ol and 5.0 ml of mixed pentanols
   2.5 ml of 2-methylpropan-1-ol and 7.5 ml of mixed pentanols

From each of these foregoing mixtures, and also from 2-methylpropan-1-ol and from mixed pentanols prepare solutions of 5 g of (total) higher alcohols in 100 ml of 40% v/v aqueous ethanol. Further dilute each of the five solutions so obtained with 40% v/v aqueous ethanol to give five sets of dilute standard solutions of (total) higher alcohols, each set containing .25, .50, .75, 1.25 and 2.50 g of (total) higher alcohols per litre of solution.

PROCEDURE

If necessary, dilute the sample with water or ethanol to an ethanol concentration of 40 percent $\pm$ 1% v/v. To 50 ml of the diluted sample add about 20 ml of water and distil. Collect at least 47 ml in a 50 ml flask, mix, dilute to volume with water and mix well. Transfer 0.10 ml of each dilute standard of a set, and also of a 40% v/v aqueous ethanol solution as a blank, to a series of six 10 ml volumetric flasks and then add in order: 0.20 ml of sodium 4-hydroxybenzaldehyde-3-sulphonate solution, and 2.0 ml of concentrated sulphuric acid. Mix by swirling.

Place the flasks in a cold water bath and heat the water to boiling. Boil for 30 minutes. Remove the flasks and allow them to cool. Make the volume to 10 ml with concentrated sulphuric acid. Mix well. Measure the absorbance at 445 nm and at 560 nm, using the blank as a reference in a 1 cm cell. Repeat for each of the other four sets of dilute standards. Plot absorbance at 445 nm against the higher alcohol concentration for each set, to give a group of five straight calibration lines. Measure the slopes of the lines as grams of higher alcohols per litre of solution per unit of absorbance. Determine the average ratio of absorbance at 560 nm to absorbance at 445 nm for each set of standards. Plot the slopes against the absorbance ratios. (Note that the absorbance at 560 nm may be much higher. If so, dilute and re-read, and correct the final calculation for this dilution).

To estimate the total higher alcohols in the sample, proceed as in the 'blank test' using duplicate 0.10 ml aliquots of prepared sample and of any one of the 25 dilute standard higher alcohol mixtures. Use 0.10 ml of 40% v/v aqueous ethanol solution as the blank. Calculate the average of the results of each pair of measurements.

CALCULATION

Let A = absorbance at 445 nm of the prepared sample

B = absorbance at 560 nm of the prepared sample

P = absorbance at 445 nm of the standard when measured at the same time as the prepared sample

R = absorbance at 445 nm of the standard when measured at the time the standard lines were established.

Calculate the ratio B/A and from the plot of the slopes in the 'blank test' find the slope corresponding. Let this slope be S. The higher alcohol content of the sample, H, is given by H = .025 (SAR/P) grams per litre of ethanol in the sample.

If the original sample contains V% v/v of ethanol, where V is less than 40, and ethanol has been added to raise the strength, then H = (SAR/VP) grams per litre of ethanol in the sample.

For some products the proportion of 2-methylpropan-1-ol in the higher alcohols is fairly constant and can be matched by a single set of standards, i.e. the slope, S, can be taken always to be the same. Typical ratios of pentanols to 2-methylpropan-1-ol are: malt whisky, 1.7:1; blended whisky, 1:1; rum 3:1; cognac brandy, 2.6:1.

REFERENCE

Research Committee on the Analysis of Potable Spirits (RCAPS), 1970. Journal of the Association of Public Analysts 8, 81. The RCAPS reports give four GLC methods. The 1984 AOAC determines "fusel oil" colorimetrically and "higher alcohols" by GLC, although the two terms are essentially synonymous.

ACIDITY

PRINCIPLE

Titration of acidity with alkali, detecting the end-point potentio-metrically or using phenolphthalein or bromothymol blue.

APPARATUS

1. Steam-distillation apparatus.

REAGENTS

1. Sodium hydroxide, 0.1N.
2. Bromothymol blue, 0.1% in neutral ethanol.
3. Phenolphthalein, 0.1% in 60% neutral ethanol.

PROCEDURE

Total Acidity

Spirits: Dilute six-fold with water adjusted to pH 7.8 and titrate to pH 7.8. Calculate as both acetic and tartaric acids. It is preferable to use a pH meter, otherwise bromothymol blue.

Wines: Boil 25 ml under reflux for 20 minutes to expel CO_2. Wash down the condenser with water and titrate to pH 7.8. Calculate as milliequivalents/L or as tartaric acid.

Beer: Dilute with water and titrate potentiometrically or to phenolphthalein.

Fixed Acidity

Spirits: Evaporate 50 ml on a water-bath for 30 minutes after evaporation is complete. Add 50 ml water (at pH 7-8), stir and titrate. Calculate as tartaric acid.

Wines: Calculate by difference, Total-Volatile, as tartaric acid or as milliequivalents/L.

Beer: Evaporate 20 ml to dryness, add water, evaporate and repeat this process 3 or 4 times. Add water and titrate with 0.1N alkali to phenolphthalein. Calculate as lactic and as acetic acids.

Volatile Acidity

Spirits: Calculate as g acetic acid/100 L of alcohol in sample from

$$\frac{1200\ (V_1-V_2)}{S}$$

where S = % v/v ethanol in the sample, V_1 and V_2 are ml of 0.1N alkali required to neutralize total or fixed acidity respectively.

Wines: Boil 50 ml of sample under reflux for 20 minutes, wash down the condenser with water and steam distil. Adjust the heat so that the volume in the distillation flask is about 25 ml and collect at least 200 ml of distillate. Titrate to phenolphthalein and calculate as milliequivalents per litre or as acetic and tartaric acid.

Beer: Calculate as acetic acid from (Total-Fixed).

REFERENCES

Official Methods of Analysis of the AOAC, 1984, 9.062-.064.

EEC Regulation 1539/71, Annex.

OIV Methods (4).

10.2 ALCOHOLIC-FERMENTED

COMPOSITION

Fermented beverages include both wines and beers. Wines are the fermented products of grapes or fruit juices. Table wines usually contain 9 to 14% ethanol derived from the original fermentation only. Fortified wines (such as sherry, port, marsala, etc.) have added distilled spirits usually to about 20% total ethanol. Sparkling wines (such as Champagne) have carbonation from a second in-bottle fermentation or from added pressurized carbon dioxide.

The deep colour of red wines is due to pigments extracted from the grape skins during the fermentation. Red wines also contain higher amounts of grape tannins which give them their characteristic astringency. White wines, on the other hand, are made from the expressed juice alone with little or no grape skin contact during fermentation.

A correct acid balance is very important for wines. Too little acid and the wine will taste flabby and not age well. Too much acid and the wine will be unpalatable. Most wines have a fixed acidity of 0.3 to 0.55% calculated as tartaric acid, and a volatile acidity of 0.03 to 0.35% as acetic acid. If this latter exceeds 0.14%, the wine may be unacceptable. Often crystals are found in a bottle of wine, or adhering to the bottom of the cork. These are merely tartrate crystals and do not detract from the wine.

Residual sugar is another important feature of a wine. Dry wines usually have from 0 to 0.3% residual sugar, although most persons cannot organoleptically detect sugar until it is about 1% or greater. The amount of residual sugar in a wine depends on the sugar content of the original juice and whether or not the fermentation was stopped before completion.

The traditional manufacture of beer is from barley, which is allowed to sprout, enzymes being present which convert starch to sugars. Yeast is added to the infusion obtained from the sprouted barley. The fermentation converts a considerable proportion of the sugars to ethanol. Bitters such as those from hops are added at some stage and the ferment is cleared and filtered.

The specific gravity of the infusion before fermentation is greater than unity due to the presence of dissolved solids, mainly sugars. Fermentation lowers the gravity both because sugars are removed and because ethanol, which has a lower gravity than water, is produced. The higher the specific gravity ("original gravity") of this unfermented infusion or beer wort the stronger the beer from it if the fermentation is allowed to proceed to its completion. For this reason the original gravity has been used traditionally for taxation purposes. It is determined experimentally by removal of the alcohol from the finished article by distillation and determination of the alcohol from the gravity of the distillate. From this may be calculated the concentration of sugar from which this alcohol was derived and what contribution that sugar would make to the gravity of the beer wort. This is added to the gravity of the residue adjusted for volume, the result being an estimation of the original gravity of the beer wort prior to fermentation.

Beers vary widely in composition, depending on ingredients and type of fermentation. Some composition data examples are given in the following table (from Pearson's Chemical Analysis of Foods, 8th Ed.):

Type of Beer	Alcohol %	Total Solids %	Acidity % (as Acetic)	Ash %
Pale Ale	4.30	4.95	0.15	0.26
Mild Ale	3.15	3.55	0.08	0.20
Bock	4.50	6.80	0.12	0.29
Lager	3.20	5.38	0.17	0.20
Stout	4.30	5.70	0.17	0.23

ROUTINE ANALYSIS

The more important determinations on wine include ethanol, sulphur dioxide, other preservatives, glycerol, methanol and total solids. It may also be necessary to determine ash, acidity (total, fixed and volatile), higher alcohols, sugar, added colour, isopropanol, artificial sweeteners, acetaldehyde, tartaric acid and diethyl pyrocarbonate.

OIV official methods (4) include procedures for salicylic, sorbic, p-hydroxybenzoic (and esters), p-chlorobenzoic and benzoic acids and monochloroacetic and monobromoacetic acids all of which are possible preservatives. All except sorbic and possibly hydroxybenzoic acids are generally considered undesirable. Both the OIV and AOAC give GLC methods for diethyl pyrocarbonate (DEPC). The OIV also describes methods for sulphur dioxide, acetaldehyde, sugars, glycerol and 2,3,-butanediol, sorbitol and mannitol and tartaric acid, among others.

Beer should be analyzed for alcohol and original gravity, acidity, preservatives and toxic elements such as arsenic, lead and tin in canned beer. Cobalt has been added as a froth stabilizer and one instance of toxicity arising from this has been reported. In addition, beer may be examined for carbon dioxide, extract, ash, chloride, glycerol, tannin, bitter substances and diethyl pyrocarbonate.

Sources of analytical methods for beer include methods of analysis of the American Society of Brewing Chemists, the European Brewery Convention, the Institute of Brewing Analysis Committee (5) and Hudson (6). The ASBC methods include quality criteria such as colour, taste, foam collapse rate and turbidity.

For excise purposes, the specific gravity of the beer wort before fermentation is of importance, the complete fermentation of worts of higher specific gravity leading to stronger beers. The gravity before fermentation is referred to as the extract of the original wort or the original gravity. The ASBC gives formulae from which the extract and the real and apparent degrees of fermentation may be calculated. Essentially the same calculation but using different terminology may be carried out as follows. The alcohol is distilled and the specific gravity of the distillate at 15.5°C determined. 1000 (1-SG) is called the spirit indication. The residue in the flask is diluted to the original volume of sample taken and the gravity of the solution determined at 15.5°C or corrected to that temperature (specific gravity of the extract SG_1).

Suppose SG = 0.9946, SG_1 = 1.0197, then 1000(1-SG) = 5.4

If the total acidity exceeds 0.1 percent as acetic acid, a correction must be made to the spirit indication according to the following table:

CORRECTIONS TO BE MADE TO SPIRIT INDICATION OF BEER FOR EXCESS ACID

Acetic	Corresponding degrees of spirit indication									
Acid	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

Suppose the acidity = 0.35%, the excess = 0.25 and therefore 0.33 must be added to the spirit indication, which becomes 5.4 + 0.33 = 5.73. From the following table, this is equivalent to gravity of 25.18 lost by fermentation.

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	12.40
3	12.90	13.34	13.78	14.22	14.66	15.10	15.54	15.98	16.42	16.8
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	38.44	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 above table is reproduced from The Beer Regulations, 1952 (SI 1952 No. 2232) U.K.)

This figure is added to the gravity of the extract (x 1000) giving 1019.7 + 25.18 = 1044.9 (usually expressed in this way but may also be expressed as a specific gravity of 1.0449).

Essery and Hall describe a quick means of calculation. Light beers may have original gravities of around 1030-1040 and very strong beers up to 1070 or 1080.

Total solids may be calculated from the formula:

$$TS(\%\ m/v) = \frac{1000\ (SG - 1)}{3.86} - 1.1\ x\%\ ash$$

or determined by drying 25 ml at 100°C to constant weight.

10.3 TEA

COMPOSITION

Tea is a beverage made by infusing a vegetable substance with boiling water. Herbs and many other substances can be used to make "teas", but traditional tea is the prepared leaves of various species of Camellia thea, including T. assamica, T. bohea, T. sinensis and T. viridis.

There are three general types of teas, black, green and oolong. Black tea is fully fermented, green is unfermented and oolong is partially fermented. Fermentation is a step in the process of tea manufacture which produces changes in the tannin and other constituents.

Some legal standards for tea from a few countries are as follows:

	Canada	Australia	Switzerland	USA	Iraq
Total ash (%)	8 (max)	-	-	4-7	5-7.5
Water-soluble ash (%)	2.75 (min)	-	-	-	3 (min)
Extractives (%)	30 (min)	30 (min)	-	-	30 (min)
Stalks (%)	-	-	22 (max)	-	10 (max)
Caffeine (%)	-	-	-	-	1.0 (min)

Composition data for black and green tea are as follows: (compiled from Pearson's Chemical Analysis of Foods, 8th Ed.)

Constituent	Black Tea	Green Tea
Moisture %	3.9 - 9.5	6.1 - 9.2
Total ash %	4.9 - 6.5	5.2 - 7.2
Water soluble ash %	3.0 - 4.2	2.6 - 4.1
Alkalinity % (as K_2O)	1.2 - 2.0	1.2 - 1.6
Extractives %	30 - 50	33 - 45
Caffeine %	1.9 - 3.6	1.5 - 4.3
Tannin %	7.3 -15.1	-
Crude fibre %	14 - 18	9 - 15

ROUTINE ANALYSIS

The most important determinations on tea are a microscopical examination, moisture, water-soluble extractive, filth test and the proportion of stalks. Samples containing over 11% moisture may have attracted mould growth. If an infusion of the tea smells musty it may be examined for mould with a hand-lens or binocular microscope and tested for mycotoxins if tentative or positive identification of the mould suggests this is warranted. The total ash and acid-insoluble ash are useful indications of the presence of any extraneous mineral matter. This is confirmed by the test for heavy filth. A low water-soluble ash and alkalinity of it indicate the presence of spent leaves. These also yield low water-soluble extractive and low caffeine figures. Unless the

presence of spent leaves is suggested by the extractive and ash values, the determination of caffeine is not particularly valuable in routine testing.

Although tea-chests are now lined with aluminium, not lead, the lead content of tea can occasionally be higher than desirable. It is therefore important to test for this element, and also worthwhile to examine for copper and arsenic. At one time Prussian blue was added to green tea to improve its appearance. The presence of other added colour would probably be suspected by the appearance of a cold-water infusion. Tea may be examined for filth by the general tests for herbs and spices.

The ash of tea should be determined by heating to as low a temperature as possible for ashing so that the colour of it is grey or brown, not green because of excessive oxidation. A green ash usually gives rise to a pink filtrate on extracting with water, and makes it difficult to see the end-point when titrating the alkalinity. Either titrate to pH 3.7 using a pH meter, use screened indicator, or evaporate the extract/ash mixture until the pink colour (due to manganese compounds) is no longer produced, before diluting to about 25 ml and filtering.

MICROSCOPIC EXAMINATION OF TEA

PRINCIPLE

The tea is cleared with chloral hydrate and bleached with hypochlorite to prepare for examination.

APPARATUS

1. Microscope and lamp.

REAGENTS

1. Chloral hydrate. Dissolve 7 g in 5 ml water.

2. Sodium hypochlorite solution, 5 %. If only bleaching powder is available prepare as follows: Dissolve 75g of crystalline sodium carbonate in 125 ml of distilled water; rub down 50g of chlorinated lime in a mortar with 375 ml of distilled water, added a little at a time. Mix the two fluids and shake occasionally during three or four hours; filter.

3. Phloroglucinol. 1 % in 90 % ethanol.

4. Concentrated hydrochloric acid.

PROCEDURE

Allow the leaves to remain in chloral hydrate solution for several hours. Pour off the chloral hydrate and replace with hypochlorite solution. Once the leaves are adequately bleached, replace the bleaching solution with phloroglucinol solution. Allow this to become nearly dry by evaporation, then add a drop of concentrated hydrochloric acid. The sclerenchymatous matter of which the hairs and the idioblasts largely consist is stained pink. Mount and examine under low power. Apart from the two elements mentioned, note the stomata and calcium oxalate. Check that there are no elements present that are not characteristic of tea.

A microscopic description of tea is as follows:

The upper epidermis is composed of cells with undulating walls and covered with a rather thick cuticle. The lower epidermis consists of smaller cells and is alone provided with stomata; the latter are surrounded by three or four tangentially elongated cells. Simple hairs occur on both surfaces of the leaf, but they are more abundant on the lower; the number, however, varies with the variety of tea, and with the age of the leaf; they are unicellular, tapering and rather thick walled, varying very much in length, but often attaining 500-700 μ .

The mesophyll is heterogeneous and asymmetrical. It is characterized by the presence of a large number of sclerenchymatous idioblasts. These are more or less branched and warty and often extend transversely from the upper to the lower epidermis. They vary very much in shape and in the thickness of the walls. The cells of the spongy parenchyma contain cluster crystals of calcium oxalate.

The midrib is biconvex. Under each epidermis there is a layer of collenchyma of varying thickness. The wood is arched and the bast contains crystals of calcium oxalate. The meristele is surrounded by a pericycle consisting of slightly lignified cells arranged in a circle. The cortical tissue contains idioblasts which are usually rather larger and more branched than those of the mesophyll.

The little fragments of the stems, which are often to be found in ordinary tea, have a slightly different structure. The wood in them forms a circle within which there is a pith containing branched idioblasts; these have comparatively thin, pitted walls.

CAFFEINE IN TEA

PRINCIPLE

The caffeine is extracted with ethanol, the ethanol removed, the residue taken up in water and extracted into chloroform. The chloroform is evaporated and the residue weighed.

APPARATUS

1. Soxhlet extraction apparatus or equivalent.
2. Separator, 500 ml.

REAGENTS

1. Ethanol, absolute.
2. Magnesium oxide, heavy.
3. Sulphuric acid, 10% v/v.
4. Sulphuric acid, 0.5% v/v.
5. Chloroform.
6. Potassium hydroxide solution, 1%.

PROCEDURE

Moisten 10g of sample, ground fine enough to pass a 30 mesh (0.5mm) sieve, with ethanol in an extraction thimble. Transfer to a Soxhlet and extract with ethanol eight hours. Completeness of extraction may be checked by using a fresh flask and fresh ethanol, extracting a further two hours, evaporating and checking that the residue is negligible. Transfer the ethanolic extract with hot water to a porcelain dish containing 10g heavy magnesium oxide suspended in 100 ml water. Evaporate slowly on the steam bath with frequent stirring to a dry, powdery mass. Rub the residue with a pestle into a paste with boiling water and transfer with hot water to a filter, cleaning the dish with a policeman. Collect the filtrate in a 1 litre conical flask marked at 250 ml and wash with boiling water until the filtrate reaches the mark. Add 20 ml of 10 % sulphuric acid and boil gently 30 minutes, with a funnel in the neck of the flask to reduce evaporation. Cool, filter through a moistened double paper into the separator and wash with small portions of 0.5 % v/v sulphuric acid. Extract with 6 x 25 ml of chloroform. Wash the combined chloroform extracts with 5 ml of 1 % potassium hydroxide solution. Filter the chloroform into a tared flask (100 ml is suitable). Wash the potassium hydroxide solution with 2 x 10 ml chloroform, pour these through the filter paper and wash the filter paper with chloroform, filtering all of these washings into the flask containing the filtered chloroform extract. Evaporate or distil the chloroform on a steam-bath, evaporating the last 10-15ml carefully to avoid loss of residue, dry 30 minutes at 100°C and weigh.

CALCULATION

Weight of caffeine x 100/10 = % caffeine in sample. Determine the nitrogen on the residue by Kjeldahl procedure, N x 3.464 = caffeine.

The caffeine residue can also be dissolved in water, and the absorbance measured at 274nm, taking $E^{1\%}_{1cm}$ = 525 for pure caffeine.

REFERENCES

Official Methods of Analysis of the AOAC, 1965, 14.019 (Power-Chestnut Method). The 1984 AOAC method gives the modified Bailey-Andrew Method.

TLC has also been employed for detection and quantitative estimation of caffeine. See Sengupta, P., Mondal, A., Sen, A.R. and Roy, B.R., 1975. International Flavour and Food Additives 6, 340.

EXTRACTIVES FROM TEA

PRINCIPLE

A known weight of dried tea is boiled with water and an aliquot of the extract is evaporated and the residue weighed. The result is expressed as a percentage of the dried tea.

APPARATUS

1. Metal dish, flat-bottomed.
2. 250 ml round-bottomed flask and reflux condenser.
3. 250 ml volumetric flask.
4. Metal evaporating dish, round bottom.
5. Waterbath.
6. Oven.

PROCEDURE

Dry approximately 2g tea, accurately weighed, in the oven in a tared flat-bottom dish for 5 hours at 100°C. Remove the dish from the oven, cool and weigh. Calculate the weight of tea taken for the test.

Transfer the dried tea to a 250 ml round-bottom flask, add 100ml distilled water, and reflux for one hour. Filter into a 250 ml graduated flask. Return the filter paper with the residue in it to the reflux flask, add a further 100 ml water and reflux for a further hour. Filter into the volumetric flask, rinse the reflux flask with hot water and pass the rinsings through the filter. Wash the filter with hot water until the volumetric flask is filled nearly to the mark. Cool, dilute to 250 ml, mix and pipette 50 ml into a weighed metal evaporating dish. Evaporate the solution on a waterbath, and finally dry in the oven. Cool and weigh.

CALCULATION

% water-soluble extractives

$$= \text{weight of residue} \times \frac{250}{50} \times \frac{100}{\text{weight of dried tea}}$$

INTERPRETATION

Manley found black tea to contain 25.5-46.4% extractives and green teas 29.6-41.4%. Several countries have a legal limit of 30%. Spent tea leaves, i.e., those that have been infused, dried and re-used give values of less than 10 %.

REFERENCE

Manley, 1965. Journal of the American Pharmaceutical Association 3, 101.

STALK IN TEA

PRINCIPLE

The sample is boiled with water, spread out and the stalk separated by use of forceps. The stalks and leaf fractions are dried separately and weighed.

PROCEDURE

Boil 5 g of the sample with 500 ml of water for 15 minutes. Pour into a basin or other suitable receptacle with a white interior and pick out the stalks with the aid of tweezers or forceps. Take care not to confuse stalk with leaf midrib, or leaf fragments that have remained rolled. Use of a hand-lens will enable foreign material to be detected and examined separately. Dry stalk and leaf portions separately for 5 hours at 100°C and weigh.

CALCULATION

$$\text{Percent stalk} = \frac{\text{weight of dried stalk} \times 100}{\text{wt. of dried stalk} + \text{wt. of dried leaf}}$$

INTERPRETATION

Over 25 % may be regarded as excessive but some countries have considerably lower limits. Crude fibre is also an index of stalk. While usual tea with its normal quota of stalks has a fibre content of around 10 %, and the regulatory limit is usually 15 % (max) on a dry weight basis, stalky tea contains much more and hard stalks are up to 60 % of crude fibre.

10.4 COFFEE

COMPOSITION

Coffee is sold as the raw green bean or after roasting and grinding. Roasting produces carbon dioxide which may continue to be released after packaging so a swollen hermetically sealed container is usually not an indication of spoilage. "French coffee" is a mixture of coffee and chicory. "Viennese coffee" is a mixture of coffee and fig seasoning or flavouring. No other substances should be present in either product.

Some compositional data for raw and roasted coffee, roasted chicory and instant coffee are as follows: (all figures are percents)

	Raw Coffee			Roasted Coffee			Roasted Chicory			Instant Coffee
	min	max	aver	min	max	aver	min	max	aver	aver
Moisture	8.2	13.8	10.3	0.3	5.6	2.2	2.5	12.0	5.5	3.98
Total Ash	3.0	4.5	4.0	3.4	4.9	4.3	4.0	6.7	5.0	11.21
Water-soluble ash	-	-	2.9	3.0	3.6	3.2	1.6	3.3	2.8	-
Ditto (as % of total ash	-	-	-	65	85	75	-	-	5.5	-
Alky. of sol. ash as K_2CO_3)	-	-	-	1.9	3.2	2.4	-	-	-	-
Acid insoluble ash (as % of total ash)	-	-	-	-	-	-	10	35	20	-
Crude fibre	-	-	-	10.5	15.3	13.0	-	-	6.9	0.0
Tannin	-	-	9.0	-	-	4.6	-	-	-	-
Total nitrogen	-	-	2.7	2.1	3.3	2.6	-	-	1.4	2.24
Caffeine	1.1	1.8	1.3	0.9	1.8	1.2	-	-	Nil	3.4
Starch	-	-	-	0.9	3.5	2.3	-	-	2.1	-
Ether Extract	11.4	13.7	12.2	8.0	14.2	13.5	0.9	3.9	2.1	-
Extractives of dry sample	25	34	-	23	33	-	70	78	-	100
Dextrose	-	-	-	-	-	-	-	-	-	2.13
Maltose	-	-	-	-	-	-	-	-	-	4.44

ROUTINE ANALYSIS

Materials likely to be mixed with coffee generally have a higher proportion of soluble matter ("extractives"). (One exception is exhausted coffee from the preparation of instant powders). This determination, together with a microscopical examination, are the most important tests. The moisture content of ground coffee should be less than 5 %, determined by drying 5g at 100°C to constant weight. Caffeine content usually lies between 0.9 and 1.8 %, and may be determined by the two-column method.

In addition to microscopical examination, the presence of chicory may be shown simply by sprinkling the powder onto water in a measuring cylinder. Coffee floats while chicory particles start sinking within a few seconds, and leave behind a brown trail of caramel. The proportion of chicory is usually determined by the amount of extractives present. It is best to adhere to an exact procedure as the results vary somewhat with the method used. That of the European Decaffeination Association is as follows:

Weigh 10g sample into 500 ml erlenmeyer. Place a 20 cm glass stirring rod into the flask, add 200 ml water and weigh. Bring to boil while stirring and boil exactly 5 minutes. Cool, reweigh, and add water to bring weight back to original. Filter, evaporate 25 ml. Dry in air oven at 105°C and weigh the residue. Continue the drying until constant weight is obtained.

Chicory contains inulin, which hydrolyses to laevulose. Coffee contains no inulin. The presence of chicory is shown by a positive reaction with Seliwanoff's reagent (0.05 % resorcinol in 33% V/V hydrochloric acid). Clarify a 2 % aqueous extract of coffee with a little neutral lead acetate and filter. To 5 ml of the clear filtrate add 5 ml of Seliwanoff reagent followed by 1 ml of concentrated hydrochloric acid. Boil for one minute. A distinct red colour produced on standing shows the presence of chicory in coffee.

Roasted cereals such as barley, oats and wheat and also soya and other products may be mixed with coffee, or coffee and chicory as coffee substitutes. Careful microscopical examination will reveal the presence of these products.

Samples of ground coffee have been examined, with coats, stems, roasted maize, sugar, soil, sand and spent coffee the commonest adulterants.

Low ash, nitrogen and caffeine in instant coffee could indicate the presence of added dextrin, maltose or dextrose.

Coffee extracts should be analyzed for moisture, caffeine, insoluble residue, sulphur dioxide and solvent residues. Moisture of dry coffee extracts is determined by drying at 70°C under a pressure of about 25 mm of Hg. Liquid coffee extracts are dried at 70°C and about 100 mm pressure of Hg for 16 hours. EEC discussion documents suggest the Schilling-Gall GLC method for solvent residues. Insoluble matter may be determined by U.S. Federal Specification method HHH-C-57CC of 10/7/74.

MICROSCOPIC EXAMINATION OF COFFEE

PRINCIPLE

Ground coffee is boiled with water, cleared and stained for examination.

APPARATUS

Microscope, with low power objective (high-power objective may be needed occasionally).

PROCEDURE

Boil about 0.5g of sample with water so that most of the colour is extracted. Drain and replace the volume of water used with chloral hydrate. Heat until the material is sufficiently cleared. Wash out the chloral hydrate and stain with phloroglucinol/hydrochloric acid.

Coffee is characterized by longitudinal and transverse sclerenchymatous fibres (from the pericarp).

Chicory has very large vessels up to 115 microns across which have rather short pits. Clearing with boiling 5 % sodium hydroxide solution may also be used.

CAFFEINE IN COFFEE

PRINCIPLE

The method described follows the Levine procedure. It has been chosen from amongst numerous methods, studied comparatively for general applicability, reproducibility, specificity, ease of application and rapidity. The method is sensitive to disturbances and small variations while the analysis is proceeding. It applies to green coffee, decaffeinated green coffee, roasted coffee, decaffeinated roasted coffee, extracts of coffee, both dried and liquid and decaffeinated extracts. The lower limit of detection is 0.02% caffeine. The caffeine is extracted from the sample, in an ammoniacal medium. After successive purifications with diethyl ether on two chromatographic columns, the caffeine is estimated spectrophoto-metrically in the ultra-violet range.

APPARATUS

1. Chromatographic columns, 250 mm long, 21-25 mm interior diameter, with stopcocks.

2. Ultra-violet spectrophotometer with 1 cm quartz cells.

3. Beaker, 100 ml.

4. Boiling water bath.

5. One-mark volumetric flasks 50 ml, 100 ml and 1000 ml.

6. One-mark pipettes, 2 and 5 ml.

7. Analytical balance.

8. Coffee mill (e.g. domestic) for roasted coffee beans.

9. Toothed disk mill with cooling jacket or analytical mill with sparecutter and cooling jacket or similar mill for green coffee beans.

10. Sieve, woven wire cloth or perforated plates, aperture size 600 microns or 630 microns.

REAGENTS

1. Sulphuric acid solution.

2. Sodium hydroxide, 2N solution.

3. Celite 545.

4. Ammonia solution (1 volume of concentrated ammonia + 2 volumes of water).

5. Diethyl ether, repurified by chromatography on a column of basic aluminum oxide of activity grade 1. Pass 800 ml of diethyl ether through a column filled with 100 g of aluminum oxide. The diethyl ether thus purified should be kept in a dark bottle until used.

6. Caffeine, pure, anhydrous, $C_8H_{10}N_4O_2$.

7. Chloroform pure, repurified by chromatography according to the method described for diethyl ether.

PROCEDURE

If necessary, mill the sample until it passes a sieve of 600 or 630μ aperture size. (See ISO Recommendation R 565 - Woven wire cloth and perforated plates in test sieves. Nominal size of apertures). A part of the sample thus prepared should be taken for the determination of dry matter.

For green coffee and roasted coffee, weigh, to the nearest 0.1 mg, about 1 g of the prepared sample. Transfer it to a 100 ml beaker, add 5 ml of ammonia solution and warm for two minutes on a boiling water bath. Allow to cool, then transfer to a 100 ml volumetric flask and adjust to volume with distilled water. Take 5.0 ml of this (turbid) solution, add 6 g of Celite and mix carefully.

For decaffeinated green coffee and roasted coffee weigh, to the nearest 0.1 mg, about 1 g of the prepared sample. Transfer to a 100 ml beaker, add 5 ml of ammonia solution and warm for 2 minutes on a boiling water bath. Add 6g of Celite and mix carefully.

For dried and liquid coffee extract, proceed as for green and roasted coffee, above, except weigh portions of 0.5 g and 20 g respectively. For the liquid extract, also use only 2 ml of the turbid solution and 3 g of Celite.

For decaffeinated coffee extract follow the same procedure as decaffeinated coffee, above, except use a 0.5 g portion.

Prepare a two layer alkaline column as follows:

Layer A: Mix carefully, by kneading with a flexible spatula blade, 3g of Celite and 2 ml of sodium hydroxide solution until homogeneous. A slightly wet powder is obtained. Transfer this powder, in portions, into a chromatographic column, the lower part of which is packed with a wad of cotton or glass wool. Tamp down the mixture, without force, with a glass rod, one end of which is flattened to the diameter of the column until a perfectly homogeneous and compact layer is obtained. A small wad of cotton or glass wool can then be placed on the top of layer A.

Layer B: Transfer the Celite sample mixture to the column on top of layer A. Dry the beaker with about 1 g of Celite using it twice. Tamp down to obtain a homogeneous mass and place a wad of cotton or glass wool on the top.

Prepare an acid column as follows:

Place in a second column, the lower part of which is packed with a small wad of cotton or glass wool, 2g of Celite and 2 ml of sulphuric acid solution carefully mixed. Place a wad of cotton or glass wool on the top of the layer to keep it in place.

Mount the columns one above the other so that the effluent of the alkaline column can drip directly on the acid column. Pass 150 ml of diethyl ether through the two columns. Keep the stopcock in the alkaline column open. Adjust the stopcock of the acid column so that a quantity of supernatant liquid remains above the layer. Pass 50 ml of diethyl ether through the acid column, using the initial portion to wash the top of the alkaline column. Use this portion also for the acid column. Discard the effluent from the acid column. Remove alkaline column. Pass a stream of air, from the top to the lower part of the acid column (e.g. using an inflated rubber bulb), until no more diethyl ether drips from the column and the air flow from the

stopcock carries only a weak smell of diethyl ether. Elute the acid column with 45-50 ml of chloroform. Collect the eluate in a 50 ml volumetric flask, adjust to volume with chloroform and mix carefully. The flow rate of diethyl ether and chloroform should not exceed 1.5-2 ml per minute.

Measure the absorption of the solution of caffeine in chloroform in quartz cells against chloroform at 276 nm (absorption maximum). Measure also the absorption at 246 nm (absorption minimum) and at 306 nm in order to verify the purity of the caffeine obtained. If the absorption at 276 nm exceeds 1.3, repeat the measurement with the diluted chloroform solution. In this case the dilution factor must be included in the calculation.

Prepare a reference solution of caffeine in the following manner: Weigh to the nearest 0.1 mg, about 100 mg of pure anhydrous caffeine. Place in a 1000 ml graduated flask. Dissolve in chloroform, and adjust to volume. With a graduated pipette, take 5 ml of this solution and adjust to a volume of 50 ml with chloroform. Measure the absorption of this solution. The corrected absorption of the reference solution should be in the region of 0.4. Carry out at least two determinations on the same prepared sample.

CALCULATION

For green or roasted coffee, the caffeine content, in grams per 100 grams of dry matter of the sample is equal to:

$$\frac{50 \times 10^6 \times C \times X}{5 \times A \times m \times ms}$$

Where:

C = the concentration of caffeine and the reference solution in g/ml.

X = the corrected absorption of the purified extract, which is absorption at 276 nm - 1/2 (absorption at 246 nm + absorption at 306 nm).

A = the corrected absorption of the reference solution of caffeine, which is: absorption at 176 nm - 1/2 (absorption at 246 nm - absorption at 306 nm).

m = the mass, in grams, of the test portion.

ms = the content of dry matter, percentage by mass, of the sample.

For all decaffeinated products, the caffeine content, in grams per 100 g of dry matter of the sample, is equal to:

$$\frac{5 \times 10^5 \times C \times X}{A \times m \times ms}$$

Where: C, X, A, m, ms are as above.

For dried and liquid coffee extracts, the caffeine content, in grams per 100 g of dry matter of the sample, is equal to:

$$\frac{25 \times 10^6 \times C \times X}{A \times m \times ms}$$

where: C, X, A, m, ms are as above.

REFERENCE

ISO 4052:1983.

10.5 TEXT REFERENCES

1. HART, F.L. & FISHER, H.J. 1971. Modern Food Analysis, Springer-Verlag.

2. RESEARCH COMMITTEE ON THE ANALYSIS OF POTABLE SPIRITS (RCAPS), 1970. Journal of the Association of Public Analysts 8 81. 1972. 10 49. 1974. 12 40,45.

3. JAULMES, P. & MARIGNAN, R. 1953. Annales des Falsifications et Fraudes 46 336.

4. OIV Receuil des Methodes Internationales d'Analyse des Vins.

5. INSTITUTE OF BREWING ANALYSIS COMMITTEE. 1971. Journal of the Institute of Brewing 77 181-226.

6. HUDSON, J.R. 1960. Development of Brewing Analysis - A Historical Review, London, Institute of Brewing.

Further Reading

RIBEREAN-GAYON, J. & PEYNAUD, E. 1982. Analyse et Controle des Vins. Dunod.

BIRCH, G.G. & LINDLEY, M.G. 1985. Alcoholic Beverages, Elsevier Applied Science.

Methods of Analysis of the American Society of Brewing Chemists, 1976, 3340 Pilot Knob Road, St. Paul, MN 55121, USA.

JOHNSON, H. 1977. The World Atlas of Wine, Mitchell Beazley.

AMERINE, M.A. & OUGH, G.S. 1980. Methods for Analysis of Musts & Wines, Wiley.

GLENISTER, P.R. 1975. Beer Deposits, Miles Laboratories Inc.

Food Standards Committee Report on Beer, 1977, HMSO, London.

INTERNATIONAL STANDARDS ORGANIZATION (ISO), Methods for analysis of tea:

1572 - 1980	-	Preparation of ground sample of known dry matter content
1573 - 1980	-	Loss in mass at 103°C
1574 - 1980	-	Water extract
1575 - 1980	-	Total ash
1576 - 1975	-	Water-soluble ash and water-insoluble ash
1577 - 1980	-	Acid-insoluble ash
1578 - 1975	-	Alkalinity of water-soluble ash
1839 - 1980	-	Sampling
3103 - 1980	-	Preparation of liquor for use in sensory tests
3720 - 1981	-	Black tea - Specifications
6078 - 1982	-	Black tea - Vocabulary
6770 - 1982	-	Instant tea - Determination of free-flow and compacted bulk derivatives.

APPENDIX

Abreviations Used in the Manual

Units of Measure

A	=	absorbance
g	=	gram
kg	=	kilogram (10^{3} g)
mg	=	milligram (10^{-3} g)
µg	=	microgram (10^{-6} g)
ng	=	nanogram (10^{-9} g)
L	=	litre
ml	=	millilitre (10^{-3} L)
µl	=	microlitre (10^{-6} L)
m	=	metre
cm	=	centimetre (10^{-2} m)
mm	=	millimetre (10^{-3} m)
in	=	inch(es) (25 mm)
hr	=	hour(s)
min	=	minute(s)
sec	=	second(s)
rpm	=	revolutions per minute
$^{\circ}$C	=	degrees Celsius (centigrade)
R_f	=	ration of: $\frac{\text{distance spot moved}}{\text{distance solvent moved}}$

Descriptive Units

AR	=	analytical reagent (grade)
BR	=	boiling range
EDTA	=	ethylene diamine tetracetic acid
G/G	=	ground glass
IU	=	International Units
MW	=	molecular weight
%	=	parts per hundred (percent)
ppm	=	parts per million
ppb	=	parts per billion
max	=	maximum
min	=	minimum
ave	=	average
M	=	molar
N	=	normal
ID	=	interior diameter
OD	=	outside diameter

m/m	=	mass in mass
m/v	=	mass in volume
v/v	=	volume in volume

Analytical Techniques

AAS	=	atomic absorption spectrophotometry
GLC	=	gas-liquid chromatography
HPLC	=	high performance liquid chromatography
PC	=	paper chromatography
TLC	=	thin-layer chromatography
RI	=	refractive index
SG	=	specific gravity
IR	=	infrared
UV	=	ultraviolet
vis	=	visible

Organizations and Agencies

AACC	=	American Association of Cereal Chemists
AOAC	=	Association of Official Analytical Chemists
AOCS	=	American Oil Chemists Society
BP	=	British Pharmacopoeia
BS	=	British Standards
CAC	=	Codex Alimentarius Commission
EEC	=	European Economic Community
ICC	=	International Association for Cereal Science and Technology
ICUMSA	=	International Commission for Uniform Methods of Sugar Analysis
ISO	=	International Standardization Organization
IUPAC	=	International Union of Pure and Applied Chemistry
OIV	=	Office International de la Vigne et du Vin

ISBN 92-5-102412-X ISSN 0254-4725

9 789251 024126

M-87 W6530E/2/11.97/200