FAO FOOD AND NUTRITION PAPER 14/8

manuals of food quality control

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

FOOD AND AGRICULTURE ORGANIZATION
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food analysis: quality, adulteration, and tests of identity

prepared by fao with the support of the swedish international development authority (sida)

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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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:

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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.