

**manuals
of food quality control**

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



**manuals
of
food quality control**

**food analysis:
quality, adulteration, and tests
of identity**

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

The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

M-87
ISBN 92-5-102412-X

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying or otherwise, without the prior permission of the copyright owner. Applications for such permission, with a statement of the purpose and extent of the reproduction, should be addressed to the Director, Publications Division, Food and Agriculture Organization of the United Nations, Via delle Terme di Caracalla, 00100 Rome, Italy.

© FAO 1986

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	1
2.	MILK AND DAIRY PRODUCTS.	2
2.1	Whole Milk	
	Discussion - Composition	2
	- Routine Analysis	3
	- Detection of Adulteration	3
	- Off-flavors	4
	- Mastitis	5
	- Antibiotics	6
	- Sugars	6
	- Non-bovine Milk	7
	Analysis - Milk Fat (Gerber Method)	8
	- Milk Fat (Rose-Gottlieb Method)	10
	- Total Solids (Rapid Method)	12
	- Total Solids (Weight Method)	14
	- Freezing Point	15
	- Milk Acidity	20
	- Nitrates in Milk	22
	- Phosphatase Test	23
	- Turbidity Test	26
2.2	Dried Milk	
	Discussion - Composition	27
	- Routine Analysis	27
	- Equivalence	28
	Analysis - Dried Milk Moisture	30
	- Dried Milk Solubility	31
	- Dried Milk Lactate	32
2.3	Evaporated and Condensed Milk	
	Discussion - Composition	34
	- Routine Analysis	34
	- Equivalence	35
	Analysis - Total Solids	37
	- Sucrose	38
2.4	Butter and Ghee (Butter Oil)	
	Discussion - Composition	41
	- Routine Analysis	42
	Analysis - Moisture in Butter	43
	- Foreign Fats in Butter (Hydroxamic Acid Index)	44
	- Foreign Fats in Butter (Reichert-Polenski-Kirschner Values).	47
	- Foreign Fats in Butter as an Ingredient (R-P-K Semimicro Method)	54
	- Vegetable Fat in Butterfat (TLC Method)	57
	- Vegetable Fat in Butterfat (GLC Method)	63

2.5	Ice Cream			
	Discussion	-	Composition	68
		-	Routine Analysis	69
2.6	Cheese and Other Products			
	Discussion	-	Composition	71
		-	Routine Analysis	71
	Analysis	-	Phosphatase Activity (in Dairy Products)	73
2.7	Text References			77
3.	SUGARS AND HONEY			85
3.1	Sucrose (White Sugar)			
	Discussion	-	Composition	85
		-	Routine Analysis	85
		-	Polarimetry	86
	Analysis	-	White Sugar (Polarization Method).	90
		-	Invert Sugar in White Sugar (Knight and Allen Method).	92
		-	Invert Sugar in White Sugar (Berlin Institute Method).	94
		-	Conductivity Ash	96
		-	Loss on Drying	99
		-	Colour Index	101
3.2	Sugar Products			
	Discussion	-	Composition	103
		-	Routine Analysis	103
	Analysis	-	Sample Preparation - Sugars (Alcohol Extraction)	105
		-	Sample Preparation - Sugars (Clarification)	106
		-	Sugars Identification (TLC Method)	107
		-	Sugars Analysis (GLC Method)	108
		-	Invert Sugars with Added Sucrose	110
3.3	Honey			
	Discussion	-	Composition	114
		-	Routine Analysis	115
	Analysis	-	Sample Preparation - Honey (Clarification)	118
		-	Moisture in Honey	119
		-	Diastase Activity of Honey	121
		-	Acidity and Lactone in Honey	123
		-	Proline in Honey	124
		-	Dextrose in Honey.	125
		-	Apparent Sucrose in Honey.	126
		-	Hydroxymethylfurfural in Honey	127
3.4	Text References			129
4.	FISH AND SHELLFISH			134
4.1	Adulteration			
	Discussion	-	Routine Analysis	134
	Analysis	-	Fish Species Identification	136

4.2	Decomposition		
	Discussion	- Routine Analysis	139
	Analysis	- Total Volatile Bases	140
		- Trimethylamine (TMA) in Fish	141
		- Indole	143
		- Histamine	146
		- Boric Acid	149
4.3	Text References		151
5.	MEAT AND MEAT PRODUCTS		154
5.1	Fresh and Frozen Meat		
	Discussion	- Routine Analysis	154
	Analysis	- Total Volatile Bases	156
		- Thiobarbituric Acid Value	157
		- Free Fatty Acids and Peroxide Value.	159
5.2	Meat Products		
	Discussion	- Routine Analysis	162
		- Meat Content	163
	Analysis	- Hydroxyproline	165
		- Nitrate and Nitrite	169
5.3	Text References		174
6.	VEGETABLES AND FRUITS		176
6.1	Fresh Vegetables and Fruits		
	Discussion	- Routine Analysis	176
6.2	Canned Vegetables and Fruits		
	Discussion	- Composition	177
		- Routine Analysis	178
		- Can Examination	179
		- Tomato Products	182
	Analysis	- Drained Weight	183
		- Fill of Container	184
		- Soluble Solids (Tomato Products)	185
6.3	Juices		
	Discussion	- Composition	187
		- Routine Analysis	188
	Analysis	- Fruit Content (Formol Number Method)	189
		- Organic Acids	191
		- Vitamin C	194
6.4	Text References		195

7.	CEREALS, CEREAL PRODUCTS AND PULSES	197
7.1	Whole Grain (Unmilled) Products	
	Discussion - Routine Analysis	197
	Analysis - Glycosidic Cyanide	198
	- Talc on Rice or Barley	200
7.2	Flours and Milled Products	
	Discussion - Routine Analysis	201
	- Microscopic Identification	201
	Analysis - Acidity in Flour (Water Extract)	218
	- Acidity in Flour (Alcohol Extract)	219
	- Ash in Flour	220
	- Iron in Flour	221
7.3	Bread	
	Discussion - Routine Analysis	223
	Analysis - Moisture in Bread	224
7.4	Text References	225
8.	HERBS AND SPICES	226
8.1	Herbs	
	Discussion - Composition	226
8.2	Spices - Seeds	
	Discussion - Composition	227
	- Routine Analysis	227
	- Identification	228
	Analysis - Umbelliferous Seeds (TLC Identification)	237
	- Non-volatile Extract	238
	- Volatile Oil	239
8.3	Spices - Pods and Fruits	
	Discussion - Composition	241
	- Identification	241
	Analysis - Capsaicin	244
8.4	Spices - Roots, Barks and Flowers	
	Discussion - Composition	246
	- Identification	246
	Analysis - Colouring Power of Saffron	251
8.5	Text References	252

9.	OILS AND FATS	255
9.1	Vegetable Oils	
	Discussion - Composition	255
	- Routine Analysis	256
	Analysis - Saponification Value	258
	- Iodine Value (Hanus Method)	259
	- Unsaponifiable Matter	261
	- Acid Value	263
	- Peroxide Value	264
	- Titre	266
	- Soap Test in Edible Oils	268
	- Arachis (Groundnut) Oil Test	269
	- Cottonseed Oil Test	271
	- Sesame Oil Test	272
	- Teaseed Oil Test	273
	- Identification of Oils and Fats (GLC of Fatty Acid Methyl Esters)	274
	- Alternative Methyl Ester Preparation Method (Neutral Oils and Fats)	280
	- Alternative Methyl Ester Preparation Method (Acidic Oils and Fats)	282
9.2	Margarine	
	Discussion - Composition	284
	- Routine Analysis	285
	Analysis - Monoglycerides in Margarine	286
	- Vitamin A in Margarine	288
9.3	Animal Fats	
	Discussion - Composition	291
	Analysis - Free Fatty Acids	292
	- Thiobarbituric Acid Value	293
9.4	Text References	294
10.	BEVERAGES	296
10.1	Alcoholic - Distilled	
	Discussion - Composition	296
	- Routine Analysis	296
	Analysis - Ethanol	298
	- Methanol	301
	- Higher Alcohols	302
	- Acidity	304
10.2	Alcoholic - Fermented	
	Discussion - Composition	306
	- Routine Analysis	307

10.3 Tea		
Discussion	- Composition	309
	- Routine Analysis	309
Analysis	- Microscopic Examination of Tea	311
	- Caffeine in Tea	313
	- Extractives from Tea	315
	- Stalk in Tea	316
10.4 Coffee		
Discussion	- Composition	317
	- Routine Analysis	317
Analysis	- Microscopic Examination of Coffee	319
	- Caffeine in Coffee	320
10.4 Text References		324
APPENDIX - Abbreviations Used in the Manual		325

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:

	<u>Richmond(1)</u>	<u>Davis(1)</u>	<u>Pearson(2)</u>	<u>Webb et al(3)</u>
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 ₂ O	CaO	Na ₂ O	MgO	Fe ₂ O ₃	P ₂ O 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 CO₂. 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 + 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 CO₂ 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.