GUIDELINES FOR THE PRESERVATION OF RAW MILK BY USE OF THE LACTOPEROXIDASE SYSTEM

CAC/GL 13-1991

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INTRODUCTION

Milk is an easily perishable raw material. Contaminating bacteria may multiply rapidly and render it unsuitable for processing and/or unfit for human consumption. Bacterial growth can be retarded by refrigeration, thereby slowing down the rate of deterioration. Under certain conditions refrigeration may not be feasible due to economical and/or technical reasons. Difficulties in applying refrigeration are specially a problem for certain areas in countries setting up or expanding their milk production. In these situations, it would be beneficial to have access to a method, other than refrigeration, for retarding bacterial growth in raw milk during collection and transportation to the dairy processing plant.

In 1967 the FAO/WHO Expert Panel on Milk Quality concluded that the use of hydrogen peroxide might be an acceptable alternative in the early stages of development of an organized dairy industry, provided that certain conditions were complied with. However, this method has not achieved any general acceptance as it has several drawbacks, most important of which is the difficulty of controlling its use: it may be misused to disguise milk of basic hygienic quality produced under poor hygienic conditions. The toxicological aspects of the use of relatively high concentrations of hydrogen peroxide in milk have also been questioned.

A chemical method for preserving milk would still be of great advantage in certain situations. The search for such a method has therefore continued. Interest has recently been focused on the indigenous antibacterial systems in milk to determine if these could be applied practically to preserve raw milk. During the last decade, basic and applied research has demonstrated that one of these systems, the lactoperoxidase/thiocyanate/hydrogen peroxide system (LP-system) can be used successfully for this purpose.

1. SCOPE

1.1 This Code of Practice describes the use of the lactoperoxidase system for preventing bacterial spoilage of raw milk (bovine and buffalo) during collection and transportation to a dairy processing plant. It describes the principles of the method, in what situations it can be used, its practical application and control of the

method. It should be stressed that this method should be utilized when refrigeration of the raw milk is not feasible.

2. PRINCIPLES OF THE METHOD

2.1 The lactoperoxidase/thiocyanate/hydrogen peroxide system is an indigenous antibacterial system in milk and human saliva. The enzyme lactoperoxidase is present in bovine and buffalo milk in relatively high concentrations. It can oxidise thiocyanate ions in the presence of hydrogen peroxide. By this reaction, thiocyanate is converted into hypothiocyanous acid (HOSCN). At the pH of milk HOSCN is dissociated and exists mainly in the form of hypothiocyanate ions ($OSCN^-$). This agent reacts specifically with free sulphydryl groups, thereby inactivating several vital metabolic bacterial enzymes, consequently blocking their metabolism and ability to multiply. As milk proteins contain very few sulphydryl groups and those that are present are relatively inaccessible to $OSCN^-$ (masked), the reaction of this compound is in milk quite specific and is directed against the bacteria present in the milk.

2.2 The effect against bacteria is both species and strain dependent. Against a mixed raw milk flora, dominated by mesophilic bacteria, the effect is bacteriostatic (predominantly inhibitory). Against some gram-negative bacteria, i.e. pseudomonads, *Escherichia coli*, the effect is bactericidal. Due to the mainly bacteriostatic effect of the system it is not possible to disguise poor quality milk, which originally contained a high bacterial population, by applying this method.

2.3 The antibacterial oxidation products of thiocyanate are not stable at neutral pH. Any surplus of these decomposes spontaneously to thiocyanate. The velocity of this reaction is temperature dependent, i.e. more rapid at higher temperatures. Pasteurisation of the milk will ensure a complete removal of any residual concentrations of the active oxidation products.

2.4 Oxidation of thiocyanate does not occur to any great extent in milk when it has left the udder. It can, however, be initiated through addition of small concentrations of hydrogen peroxide (see Section 4). The high concentrations of hydrogen peroxide used to preserve milk (300-800 ppm), destroy the enzyme lactoperoxidase and thereby preclude the oxidation of thiocyanate. With this method the antibacterial effect is thus an effect of hydrogen peroxide itself.

2.5 The antibacterial effect of the LP-system is, within certain limits, proportional to the thiocyanate concentration in the milk (provided that an equimolar amount of hydrogen peroxide is provided). The level thiocyanate in milk is related to the feeding of the animals and can thus vary. The practical use of the method consequently requires addition of some thiocyanate to ensure that a level necessary to achieve the desired effect, is present in the milk.

2.6 The levels of thiocyanate resulting from this treatment are within the physiological levels reported to occur in milk under certain circumstances and feeding regimes. They are also far below the thiocyanate levels known to exist in human saliva and certain common vegetables, e.g. cabbage and cauliflower. In addition, results from clinical experiments have clearly demonstrated that milk treated according to this method will not cause any interference of the iodine uptake of the thyroid gland, neither in persons with a normal iodine status nor in cases of iodine deficiency.

3. INTENDED UTILIZATION OF METHOD

3.1 This method should only be used in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities for maintaining the quality of raw milk. Use of the LP-system in areas which currently lack an adequate infrastructure for collection of liquid milk, would ensure the production of milk as a safe and wholesome food, which otherwise would be virtually impossible.

3.2 The method should not be used by the individual farmers but at a suitable collecting point/centre. These centres must be equipped with proper facilities for cleaning and sanitising the vessels used to hold and transport milk.

3.3 The personnel responsible for the collection of milk should be in charge for the treatment of the milk. They should be given appropriate training, including training in general milk hygiene, to enable them fulfil this in a correct way.

3.4 The dairy processing the milk collected by use of the lactoperoxidase system should be made responsible for ensuring that the method is used as intended. This dairy should set up appropriate control methods (see Section 5) to monitor usage of the method, raw milk quality and quality of the milk prior to processing.

3.5 The method should primarily be used to prevent undue bacterial multiplication in raw milk during collection and transportation to the dairy processing plant under conditions stated in 3.1. The inhibitory effect of the treatment is dependent on the temperature of the stored milk and has been found to act for the following periods of time in laboratory and field-experiments carried out in different countries with raw milk of an initial good hygienic standard:

Temperature, °C	Time, h
30	7 - 8
25	11 - 12
20	16 - 17
15	24 - 26

3.6 The use of the lactoperoxidase method does not exclude the necessity of pasteurization of the milk before human consumption. Neither does it exclude the normal precautions and handling routines applied to ensure a high hygienic standard of the raw milk.

4. PRACTICAL APPLICATION OF THE METHOD

4.1 The lactoperoxidase system can be activated in raw milk to give the above stated antibacterial effect by an addition of thiocyanate as sodium thiocyanate and hydrogen peroxide in the form of sodium percarbonate by the following procedure:

14 mg of NaSCN is added per litre of milk. The milk should then be mixed to ensure an even distribution of the SCN^-. Plunging for about 1 minute with a clean plunger is normally satisfactory.

Secondly, 30 mg of sodium percarbonate is added per litre of milk. The milk is then stirred for another 2-3 minutes to ensure that the sodium percarbonate is completely dissolved and the hydrogen peroxide is evenly distributed in the milk.

4.2 It is essential that the sodium thiocyanate and sodium percarbonate are added in the order stated above. The enzymatic reaction is started in the milk when the hydrogen peroxide (sodium percarbonate) is added. It is completed within about 5 minutes from the addition of H_2O_2; thereafter, no hydrogen peroxide is present in the milk.

4.3 The activation of the lactoperoxidase system should be carried out within 2-3 hours from the time of milking.

4.4 Quantities of sodium thiocyanate and sodium percarbonate needed for the treatment of a certain volume of milk, for example 40 or 50 litre milk churns, should be distributed to the collecting centre/point in prepacked amounts lasting for a few weeks at a time. The technical specifications of the thiocyanate and sodium percarbonate which should be used are stated in Appendices I and II.

5. CONTROL OF USAGE

5.1 The use of the lactoperoxidase system for preserving raw milk must be controlled by the dairy processing plant receiving the milk. This should be a combination of currently used acceptance tests, e.g. titratable acidity, methylene blue, resazurin, total viable count and analyses of the thiocyanate concentration in the milk. Since the thiocyanate is not consumed in the reaction, treated milk arriving at the dairy plant would contain approximately 10 mg above the natural amount of thiocyanate (the latter can be determined by

analysing untreated milk from the same area) per litre of milk. The analytical method for SCN^- is described in Appendix III Testing should be undertaken at random. If the concentration of thiocyanate is too high (or too low), investigation must be carried out to determine why the concentration is outside specification. The dairy processing plant should also be responsible for the control of the chemicals to be used at the collection centre for the activation of the lactoperoxidase system.

5.2 Analysis of the bacteriological quality of the milk (methylene blue, resazurin, total plate count) should also be carried out to ensure that good hygienic standards are not neglected. Since the effects of the system are predominantly bacteriostatic, an initial high bacterial population in the milk can still be revealed by such tests.

APPENDIX I: TECHNICAL SPECIFICATION OF SODIUM THIOCYANATE

Definition

Chemical name	Sodium thiocyanate
Chemical formula	NaSCN
Molecular weight	81.1
Assay content	98-99%
Humidity	1-2%

Purity (according to JECFA specification)*

Heavy metals (as Pb)	< 2 ppm
Sulphates (as SO_4)	< 50 ppm
Sulphide (S)	< 10 ppm

* Joint FAO/WHO Expert Committee on Food Additives.

APPENDIX II: TECHNICAL SPECIFICATION OF SODIUM PERCARBONATE

Definition

Chemical name	Sodium percarbonate (*)
Chemical formula	$2Na_2CO_3 \cdot 3H_2O_2$
Molecular weight	314.0
Assay content	85%

Commercial available sodium percarbonate recommended to be used has the following specification:

Sodium carbonate peroxyhydrate	> 85%
Heavy metals (as Pb)	< 10 ppm
Arsenic (as As)	< 3 ppm

(*) For information where sodium percarbonate could be obtained commercially, please apply to IDF General Secretariat, 41 Square Vergote, B-1040 Brussels, Belgium.

APPENDIX III: ANALYSIS OF THIOCYANATE IN MILK

Principle

Thiocyanate can be determined in milk, after deproteinisation, with trichloroacetic acid (TCA), as the ferric complex by measuring the absorbance at 460 nm. The minimum level of detection by this method is 1 to 2 ppm of SCN^-.

Reagent Solutions

1. 20% (w/v) trichloroacetic acid: 20 g TCA is dissolved in 100 ml of distilled water and filtered

2. Ferric nitrate reagent: 16.0 g $Fe(NO_3)_3.9H_2O$ is dissolved in 50 ml 2 M HNO_3* and then diluted with distilled water to 100 ml. The solution should be stored dark and cold.

* 2M HNO_3 is obtained by diluting 138.5 ml 65% HNO_3 to 1000 ml with distilled water.

Determination

4.0 ml of milk is mixed with 2.0 ml of 20% TCA solution. The mixture is blended well and then allowed to stand for at least 30 minutes. It is thereafter filtered through a suitable filter paper (Whatman No. 40). 1.5 ml of the clear filtrate is then mixed with 1.5 ml of the ferric nitrate reagent and the absorbance measured at 460 nm. As a blank, a mixture of 1.5 ml of ferric nitrate solution and 1.5 ml of water is used. The measurement must be carried out within 10 minutes from the addition of the ferric nitrate solution as the coloured complex is not stable for any length of time. The concentration of thiocyanate is then determined by comparison with standard solutions of known thiocyanate concentration, e.g. 10, 15, 20 and 30 µg/ml of thiocyanate.