BULLETIN OF THE INTERNATIONAL DAIRY FEDERATION N° 234/1988

 ISSN 0250 - 5118

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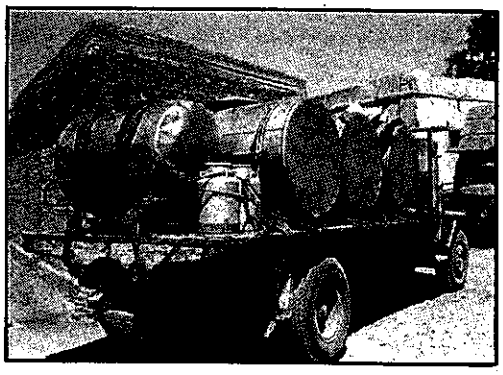

Milk collection in a tropical country (Photo: Bath Symposium)

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CODE OF PRACTICE FOR THE PRESERVATION OF RAW MILK BY THE LACTOPEROXIDASE SYSTEM

CONTENTS

PREFACE

At the 21st Session of the FAO/WHO Milk Committee (Committee of Government Experts on the Code of Principles concerning Milk & Milk Products) in Rome (2-6 June 1986), the technical advice of IDF regarding the use of the lactoperoxidase system for the preservation of raw milk was submitted and discussed.

The decision reached at that meeting was to ask IDF to prepare a Code of Practice for raw milk preservation using the lactoperoxidase system. A draft for such a Code of Practice was prepared by Group F19 (Indigenous antibacterial systems in milk) on the initiative of Dr L. Björck of the Swedish University of Agricultural Science.

The first draft (D-Doc 150) was submitted to IDF Commission D at the 70th Annual Sessions of the IDF in The Hague in September 1986, and subsequently amended by the author in the light of the discussion.

Commission D then decided to circulate this amended draft to all NC with the following questions (questionnaire 1787/D):

1. Please give general comments on the Code of Practice.

2. Is it, in your opinion, advisable that the method should be used at individual farms or should it be used only at collection centres as stated in the present text ?

3. Are you aware of any other antibacterial system that can be used for the preservation of raw milk ? Please send details, literature, references, etc.

Replies were received from 19 countries and the replies were considered and analyzed by the Group. In this light, it was concluded that the following amendments should be introduced in the first draft:

1. In Section 1 - Scope

The following sentence should be added: "It should be stressed that this method should only be utilized when refrigeration of the raw milk is not feasible".

2. In Section 3 - Intended utilization of method

In para. 3.6 the following sentence should be added: "Neither does it exclude the normal precautions and handling routines applied to ensure a high hygienic standard of the raw milk".

3. Section V - Control of usage

To be added in this paragraph: "The dairy processing plant should also be responsible for the control of the chemicals to be used at the collection centres for the activation of the lactoperoxidase system".

4. Technical specification of sodium percarbonate - Appendix II

The following footnote should be inserted:

"Information where sodium percarbonate can be obtained commercially can be obtained from the IDF General Secretariat, 41, Square Vergote, B-1040 Brussels, Belgium".

5. Analysis of thiocyanate - Appendix III

The following should be added: "The minimum level of detection by this method is 1 to 2 ppm of SCN^-.

..... should be stored dark and cool (in refrigerator). It is then stable for a minimum of 30 days".

It was also concluded that, regarding the second question, all answers were in favour of the method being used at collection centres as stated in the suggested text.

It was also obvious from the answers that any other antibacterial system to be used for preservation of raw milk is not known.

As a next step, the draft Code was revised and the revised version was submitted by Dr L. Björck as paper D-Doc 157 to Commission D at its next Session in Helsinki (Finland) in September 1987 and the following is taken from the minutes of that meeting:

In reply to Dr J. Nichols (US), who had drawn attention to the difficulties encountered in USA resulting from the formation of histamine in cheese consequent upon protein breakdown mediated by the addition of significant amounts of hydrogen peroxide to the cheese milk, Dr Björck remarked that when the lactoperoxidase system (LPS) was used the very small quantity of hydrogen peroxide added reacted in its entirety with the enzyme (lactoperoxidase) and the proteins were not affected. In reply to Mr I.M.V. Adams (GB), who asked if there was further evidence in relation to the activity of LPS against pathogens, Dr Björck explained that LPS was not intended as a substitute for pasteurization and furthermore there was no evidence that the system favoured the development of pathogens - it had a negative effect on all common pathogenic bacteria occurring in milk, which should be pasteurized.

The Commission approved the Code of Practice appended to D-Doc 157 for publication.

The thus amended Code of Practice is appended. It was also submitted to the FAO/WHO Codex Committee on Food Hygiene (CCFH) meeting in March 1988 and the following is quoted from the minutes of that CCFH meeting:

The following views were presented at the 23rd Session of the Codex Committee on Food Hygiene (ALINORM 89/31, paras. 74-80) in consideration of a Code of Hygienic Practice for Raw Milk Preservation by the Use of the Lactoperoxidase System.

> ***74.*** *The Observer from IDF introduced a report, document CX/FH 88/12 (Conference Room Document No. 9), on a Code of Practice for the Preservation of Raw Milk by the Lactoperoxidase System. He informed the Committee that the 21st Session of the Joint FAO/WHO Committee of Government Experts on the Code of Principles Concerning Milk and Milk Products had considered the technical advice IDF had given on the use of the Lactoperoxidase System for the preservation of raw milk. That Committee had requested IDF to prepare the draft of a Code of Practice. The draft document had been amended at the 70th Annual Session of IDF (The Hague, 1986) and subsequently circulated to IDF members for comments. Comments had been received from 19 countries and had been incorporated in a revised text of the draft code of practice for submission to the IDF Session in Helsinki (1987) and to the Milk Committee (IDF Working Paper D-Doc 157/1987).*
>
> ***75.*** *The representative of IDF noted that the additional comments received from Thailand and Denmark had been taken into consideration during the preparation of the present wording of the Code.*
>
> ***76.*** *Several delegations inquired about the implications of elaborating a Code of Practice which might be used to discourage efforts to improve the use of refrigeration of raw milk. The delegation of Cuba expressed the view that, supported by such a Code adopted at the international level, the lactoperoxidase system might be used on a continuous basis for an indefinite period of time and questioned whether that was the intent of the Committee. The delegation of Australia questioned whether the holding of raw milk at ambient temperatures had effects on the chemical composition and*

nutritional qualities of the milk. Concern was also expressed about adequate control of the use of the chemical additives under practical conditions.

77. *The Observer of IDF indicated that IDF had considered nutritional and safety aspects of the lactoperoxidase system especially in regard to the use of other chemicals which may be used to preserve milk. He stated that the final goal for good hygienic practices remained the use of refrigeration. He informed the Committee that the process was being introduced in certain areas of India and China, and that the draft document contained adequate provisions for the control of the chemicals used. The delegation of the United Kingdom stated the lactoperoxidase system operated naturally in raw milk and that the proposed application was an enhancement of the naturally-occurring bacteriostatic action.*

78. *It was proposed that the draft Code should be given only the status of a Guideline, in view of the opinion of several delegations that the procedure should be used only under specified conditions, and that this should also be indicated in the title and in the introductory sections. The Committee agreed that the following alternative title:*

"Draft Guidelines for the Preservation of Raw Milk by Use of the Lactoperoxidase System where Refrigeration is Virtually Impossible"

should be included in the document, and that both titles should be placed in square brackets for further government comments.

79. *Concerning the status of the Code/Guidelines within the Codex Procedures, it was agreed to advance the draft document to Step 3 for government comments and to request the Commission to decide whether further elaboration of the Code/Guidelines should be referred to this Committee in view of the extended period of time between sessions of the Milk Committee. In the meantime, the Secretariat was requested to seek authorization from the Executive for obtaining comments at Step 3. The document distributed for comments would carry references to both the Committee on Food Hygiene and the Milk Committee.*

80. *The Committee expressed its appreciation to IDF for the extensive work carried out in relation to these agenda items.*

The Code/Guidelines was issued by the Codex Alimentarius Commission (CAC) to governments and International Organizations for comments, in August 1988 (CL 1988/22 - FH/MDS) - Comments were invited for 31 March 1989.

The General Secretariat was informed that the lactoperoxidase system was approved by the National Expert Committee on Food Additives in the People's Republic of China as "an acceptable preservative used for milk preservation" in certain localities in China. Experiments on the use of the system appear to being done in several countries: India, Mexico, Fidji. Field trials have been published from Kenya, India, Pakistan, Mexico, Sri Lanka, Egypt, People's Republic of China, Poland.

In IDF, consideration is being given to the development of a standardized method for the determination of lactoperoxidase activity. IDF Group F19, Chairman, Dr L. Björck, is following up on international developments in relation to indigenous antibacterial systems in milk and a copy of the report (F-Doc 96) of the Group, issued in 1983, on "temporary preservation of raw milk by activation of the lactoperoxidase system" is attached, for information.

Finally, attention should be called to the publication in 1985, of the Proceedings of a Symposium on "Antimicrobial Systems in Milk" held in Bath (UK) in September 1985 under the joint auspices of IDF, BSPP and FEMS. A copy of the Proceedings is available from IDF Brussels for £15.-

IDF General Secretariat
November 1988.

CODE OF PRACTICE FOR THE PRESERVATION OF RAW MILK BY USE OF THE LACTOPEROXYDASE SYSTEM

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 inferior 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. SECTION I – SCOPE

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 only be utilized when refrigeration of the raw milk is not feasible.

2. SECTION II – PRINCIPLES OF THE METHOD

The lactoperoxidase/thiocyanate/hydrogen peroxide system is an indigenous antibacterial system present in milk and human saliva. The enzyme lactoperoxidase is present in bovine and buffalo milk in relatively high concentrations. It can oxidize 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 inacessible to $OSCN^-$ (masked), the reaction of this compound is in milk quite specific and is directed against the bacteria present in the milk.

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.

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. Pasteurization of the milk will ensure a complete removal of any residual concentrations of the active oxidation products.

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 IV). 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.

The antibacterial effect of the LP-system is, within certain limits, proportional to the thiocyanate concentration in the milk (provided than an equimolar amount of hydrogen peroxide is provided). The level of 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.

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. SECTION III – INTENDED UTILISATION OF METHOD

3.1 The method should 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 sanitizing the vessels used to hold and transport milk.

3.3 The personnel responsible for the collection of the 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 to fulfill 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 V) 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, hrs
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. SECTION IV – 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 Appendix I and II.

5. SECTION V – CONTROL OF USAGE

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 3. 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.

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.

TECHNICAL SPECIFICATION OF SODIUM THIOCYANATE — APPENDIX I

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 (SO_4)	<	50 ppm
Sulphide (S)	<	10 ppm

* *Joint FAO/WHO Expert Committee on Food Additives*

TECHNICAL SPECIFICATION OF SODIUM PERCARBONATE — APPENDIX II

DEFINITION

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

Commercially 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.

ANALYSIS OF THIOCYANATE IN MILK — APPENDIX III

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 $Fe(NO_3)_3 . 9 H_2O$ 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.

3. 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 colored 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 mg/ml of thiocyanate.

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

APPENDIX IV

TEMPORARY PRESERVATION OF RAW MILK BY ACTIVATION OF THE LACTOPEROXIDASE SYSTEM

(Report F-Doc 96 submitted by Group F19 to the IDF Sessions in Oslo, July 1983)

1. INTRODUCTION

In many countries which are in the process of starting up and/or expanding their milk production, the collection and transportation of raw milk to processing centres present many problems. Frequently, it is impossible to refrigerate the milk during collection and transportation, which often take place at high ambient temperatures, with the result that the milk often has an inferior hygienic quality when it arrives at the dairy plant. It is well-known that large quantities of milk are spoiled in this way. Although the long-term objective of these countries is to set up and maintain adequate refrigeration facilities, this will, for both practical and economic reasons, take a number of years to accomplish. In the meantime, an alternative method of preserving raw milk would certainly be of advantage and enable larger intakes of raw milk with better hygienic quality.

In countries with modern dairying, the collection and storage of raw milk is today more or less totally dependent on the supply of electricity for the cooling equipment. Consequently, a widespread power failure will have a dramatic effect on the hygienic quality of raw milk due to insufficient cooling. In such situations, access to an alternative way of preserving the raw milk would be beneficial also to developed countries.

Today, the only alternative method available is the use of hydrogen peroxide as a preservative. The hydrogen peroxide treatment of raw milk was approved by the FAO in 1957 (1): "when technical and/or economic reasons do not allow the adoption of cooling facilities for maintaining the quality of raw milk, hydrogen peroxide may be an acceptable alternative in the early stages of development of an organised dairy industry". The hydrogen peroxide treatment of raw milk is today officially used only in a few countries. The method has not achieved general acceptance since it involves a number of disadvantages, which mainly are due to the high concentrations, i.e. 300-800 ppm, of hydrogen peroxide required to obtain the necessary preservative effect.

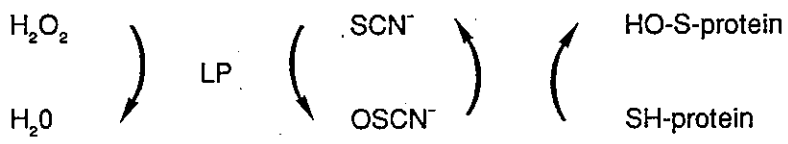
2. THE LACTOPEROXIDASE SYSTEM

In recent years the idea of using an antibacterial system in milk to prevent bacterial spoilage has been put forward (2, 3). The antibacterial system used is the so-called lactoperoxidase / thiocyanate / hydrogen peroxide system (LP-system). This system is not only present in milk (4, 5), but has also been found to be active in saliva (6, 7). In saliva it is believed to be a factor that regulates the bacterial metabolism but serves also as a mechanism that protects the cells of the mucous membrane in the oral cavity against hydrogen peroxide toxicity (8).

In the following only a brief presentation of the basic mechanism of the LP-system and its use as a temporary milk preservative will be given, since several review articles have recently covered this in detail (9, 10, 11).

2.1 Basic function of the LP-system

The antibacterial effect of the LP-system is mediated by short-lived oxidation products of thiocyanate (12, 13). These are formed by the lactoperoxidase catalysed oxidation of thiocyanate by hydrogen peroxide, and have been identified as $OSCN^-$ (14, 15) and O_2SCN^- (16, 17). These oxidation products react rather specifically with free SH-groups in proteins, thereby oxidising them to the corresponding sulfenyl derivatives, which in turn undergo hydrolysis to yield sulfenic acids (18). The over-all reaction can be illustrated by the following reaction:



Functional SH-groups are destroyed in this way by the LP-system, thereby causing an interference with the metabolism of the bacteria. In some bacteria, such as streptococci and lactobacilli, this results in a temporary inhibition, after which the bacteria recover (19, 20). In other bacteria, such as most strains of *Escherichia coli,* Salmonella and Pseudomonads spp, it leads to an irreversible inhibition, i.e. the killing of the bacteria (21, 22).

The antibacterial effect is proportional to the formation of the oxidation products of thiocyanate. This is, in turn, dependent on the available concentrations of thiocyanate and hydrogen peroxide. Lactoperoxidase is always present in milk in non-limiting concentrations.

2.2 Preservation of milk by the LP-system

Activation of the LP-system in raw milk in order to achieve a temporary preservative effect is accomplished by supplementing the thiocyanate level to about 15 ppm, i.e. an addition of about 10 ppm thiocyanate, and an addition of 8 to 9 ppm of hydrogen peroxide. This initiates the enzymatic reaction and the antibacterial agents are formed *in situ.* It is important to note the following:

- the added hydrogen peroxide is consumed within minutes in the enzymatic reaction - it does not persist in the milk
- the active oxidation products of thiocyanate formed are unstable compounds that decompose spontaneously or during pasteurization of the milk.

The length of the antibacterial effect achieved by activation of the LP-system is inversely related to the storage temperature of the milk. The following approximate length of time has been reported (23):

storage temperature °C	duration of effect hours
30	7 - 8
25	11 - 12
20	16 - 17
15	24 - 26

At still lower storage temperatures the effect is prolonged, viz. at 10°C-48 hours; at 5°C-96 hours.

Field experiments in Kenya (23) and Sri Lanka (24) have demonstrated that a substantial improvement of the hygienic quality of the raw milk can be achieved during collection and transportation after an activation of the LP-system at the collecting point or collecting centre. An example of the improvements that can be achieved is shown in Table 1 (24).

Table 1. Analyses of milk samples from Giriulla & Siringapatha collection centres. Cows were milked at 4.6 a.m. and samples were stabilized at 7:30-8 a.m. Ambient (= samples) temperature: 30-32°C.

Quality test	Treatment [1]	Percentage accepted samples at				
		10 a.m.	12 noon	2 p.m.	4 p.m.	6 p.m.
10 min. resazurin	LP	100	100	70	50	30
	C	80	60	10	0	0
Acidity [2]	LP	100	100	80	60	50
	C	70	60	20	0	0
Alcohol stability	LP	100	100	90	60	50
	C	70	60	30	10	10
Clot-on-boiling	LP	100	100	100	100	80
	C	100	100	90	30	30

[1] LP = Samples stabilized by activation of the LP-system; C = Controls
[2] Samples with an acidity > 0.16% recorded as rejected.