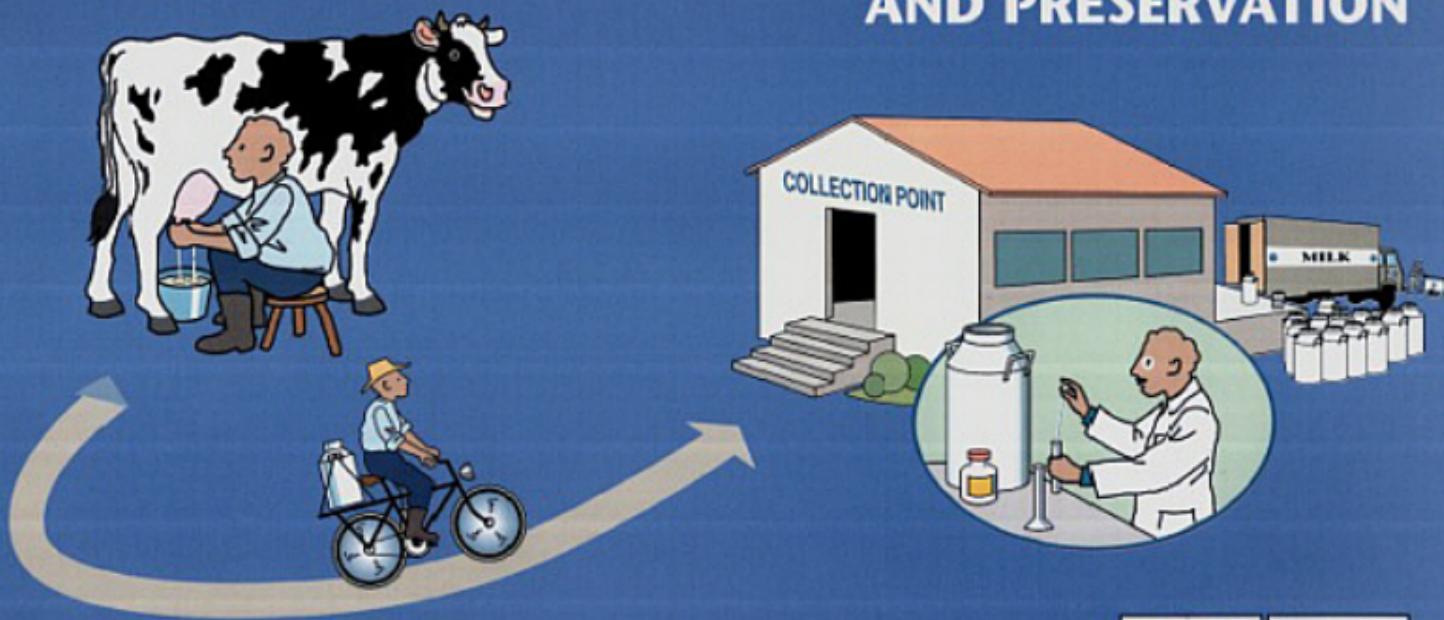




Global
Lactoperoxidase
Programme

MANUAL ON THE USE OF THE LP-SYSTEM IN MILK HANDLING AND PRESERVATION



Food
and
Agriculture
Organization
of
the
United
Nations

MANUAL ON THE USE OF THE LP-SYSTEM IN MILK HANDLING AND PRESERVATION

Animal Production Service
FAO Animal Production and Health Division

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 1999

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ABOUT THIS MANUAL

FAO, with the support of the Government of Sweden, is overseeing demonstrations of the lactoperoxidase (LP-) system of milk preservation in some 80 countries. Focusing on areas where milk collection is difficult, FAO is providing prepacked bags of the necessary chemicals for use by trained personnel at milk collection points. This manual explains the background to this natural and harmless antibacterial system, its benefits and its correct application.

The biological background of the LP-system

Milk is the last nutritional link between a mammalian mother and her offspring. As well as providing a complete and well-balanced diet for the newborn calf, kid or human baby, milk also contains antibacterial agents to protect the suckling young from various infectious diseases.

The knowledge that milk, particularly the first milk after parturition – the colostrum – contains immune factors essential for the survival of offspring is not new. Thousands of years ago herdsmen knew that newborn kids, lambs and calves needed colostrum if they were to survive.

The antibacterial properties of milk

Science has now caught up with the knowledge of those herdsmen and we know that milk contains several antibacterial factors. The most well known are the immunoglobins. Colostrum has a particularly high immunoglobin content for an immediate immunization of the newborn.

Milk also contains other non-specific factors such as lysozyme, lactoferrin and peroxidase. This peroxidase, which is called lactoperoxidase, is identical to the peroxidase present in saliva and gastric juice.

Lactoperoxidase has no antibacterial effect on its own. But, combined with oxidized thiocyanate (also present in milk as well as in saliva and gastric juice) and hydrogen peroxide, the resulting chemical reaction creates antibacterial compounds as follows:



These antibacterial compounds interfere with the metabolism of bacteria. Some bacteria, such as streptococci and lactobacilli, which belong to the normal gut flora, are temporarily inhibited, and later recover. Other, more harmful, bacteria including most strains of *Escherichia coli*, *salmonella* and *Pseudomonas* spp. are killed.

The antibacterial effect is proportional to the formation of the oxidation products of thiocyanate. This in turn depends on the available concentrations of thiocyanate and hydrogen peroxide in the milk. Lactoperoxidase is always present in milk in concentrations adequate for the antibacterial action.

Bovine milk contains about 30 mg per litre of lactoperoxidase and the concentration is fairly constant throughout the lactation. The amount necessary for the antibacterial activity is just 1 mg per litre.

The concentration of thiocyanate is more variable as it is dependent on the feeding of the cow. Most of the thiocyanate is derived from various glucosides in the feed. Concentrations in the range 4-5 ppm are generally found. Human saliva, however, is reported to contain 50-300 ppm. And human gastric juices, into which thiocyanate seems to be actively secreted, contain 40-50 ppm, thus supplementing the too low thiocyanate content of the milk.

The third component of the system, hydrogen peroxide, is not normally detected in milk and its source *in vivo* is not quite clear. But traces in newly drawn milk have

been found and are possibly derived from the metabolism of the mammary tissue and leucocytes. Another source seems to be xantine oxidase in the milk reacting with some substrate, e.g. hypoxanthine.

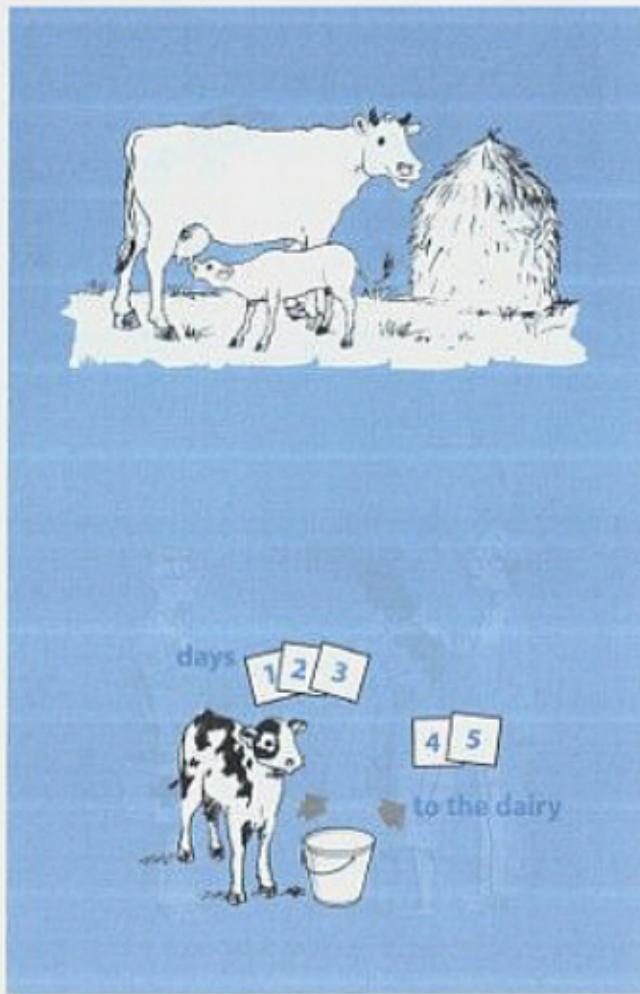
The combination of these three provides the mechanism for the bacteriostatic properties of newly drawn milk, which last one or two hours, while the milk is suckled and during the stay in the stomach of the young. Studies have also shown that gastric juice contains the same peroxide forming system, thus maintaining the antibacterial action against gut infections.

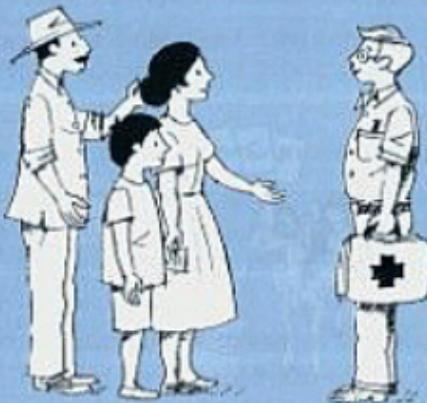
Lactic acid streptococci in the intestine produce hydrogen peroxide which can complete the LP-system and similar sources of the chemical seem to exist in saliva, where the system appears to be an anticaries factor. Thus the LP-system in milk from healthy udders is also active in the digestive tract of the suckling calf.

The importance of the natural LP-system for suckling calves, kids, etc.

Milk contains several antibacterial factors and systems to protect the newborn suckling calf against infections which could cause illness and digestive disturbances.

In the first few days after calving, the mother's milk is rich in immunoglobulins for the immediate immunization of the newborn.



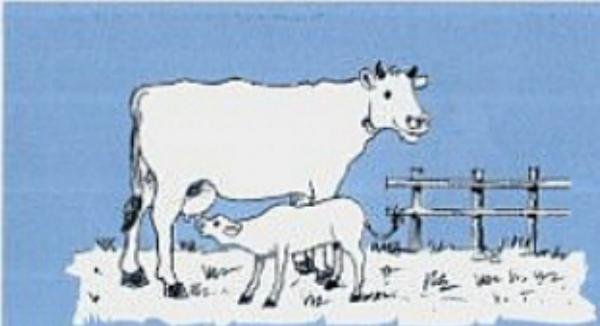


One specific system protecting the stomach and intestine of the newborn against bacterial infection is the lactoperoxidase/thiocyanate/hydrogen peroxide system – the LP-system.

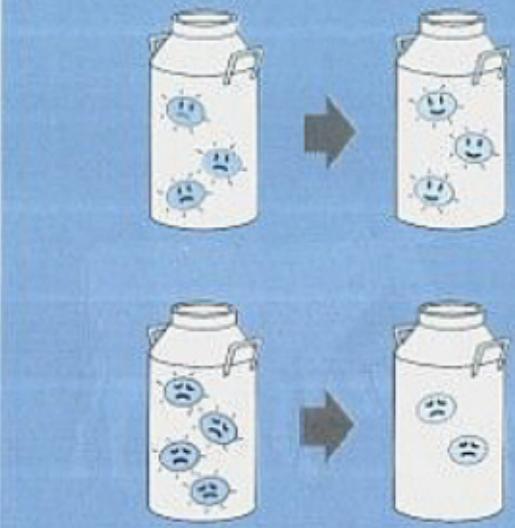
The LP-system consists of three components:

- The enzyme *lactoperoxidase* which is abundant in milk.
- *Thiocyanate*, the content of which is suboptimal and varying in milk.
- *Hydrogen peroxide*, of which milk contains only a trace.

When the young calf or kid is suckling, the milk is mixed with saliva and gastric juice supplementing low levels of thiocyanate and hydrogen peroxide, and creating an antibacterial reaction.



The normal bacterial gut flora, e.g. streptococci and lactobacilli, are only temporarily inhibited and then recover. This is called a bacteriostatic effect.



Other bacteria, some of them harmful, such as *Escherichia coli*, *Salmonella* and *Pseudomonas* spp. are killed. This is called a bactericidal effect.

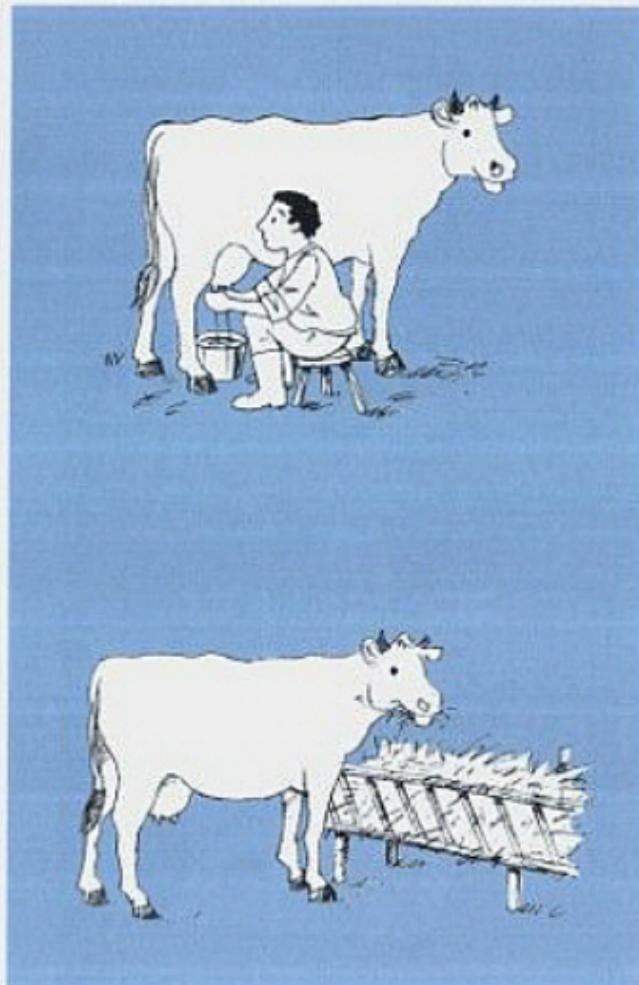
THE USE OF THE LP-SYSTEM IN MILK HANDLING AND PRESERVATION

Following fundamental studies on the natural LP-system, a method using the system to preserve milk at ambient temperatures was developed in Sweden. Field tests were carried out in Kenya, Sri Lanka and several other warm countries, with good results. Following toxicological tests and consideration by the Joint FAO/WHO Expert Committee on Food Additives, the method was finally approved by the Codex Alimentarius Commission in 1991 for field implementation. Guidelines for implementation of the Code of Practice for Preservation of Raw Milk by the Use of the Lactoperoxidase System were adopted (see Appendix I and II for technical specifications of sodium thiocyanate and sodium percarbonate).

Recreating the natural LP-system to preserve milk

In drawn milk the antibacterial activity is rather weak and lasts for only one or two hours because the milk contains only low levels of thiocyanate and hydrogen peroxide.

The content of thiocyanate in the fresh milk varies depending on the type of fodder consumed.





Feeds such as cabbages of various types are rich in sulphur-containing components, which are the source of thiocyanate in milk.



It is now possible to reactivate the antibacterial property by adding extra thiocyanate and hydrogen peroxide, supplementing these components in the same way as the saliva and gastric juice of a sucking calf.



Before the LP-system is used to preserve milk the original thiocyanate content should be analysed by the dairy plant collecting the milk, or by the local health authority. The method described in Appendix III should be used.

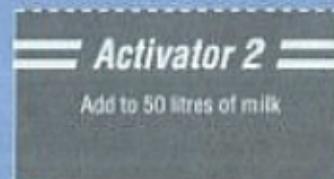
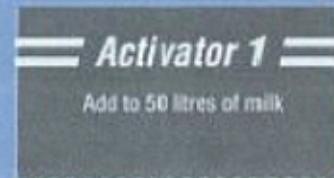
Based on these results the thiocyanate content of the milk is adjusted to 15 ppm by adding the preweighed amount of thiocyanate in solution. Usually the thiocyanate content of milk is about 4.5 ppm, and the addition would then be 10 ppm.

The thiocyanate is used as Activator 1 (A1) and is usually distributed as a liquid.

Activator 2 (A2) is the hydrogen peroxide, usually distributed as a granulated sodium carbonate peroxyhydrate which yields hydrogen peroxide equal to 8.9 ppm when dissolved in milk.

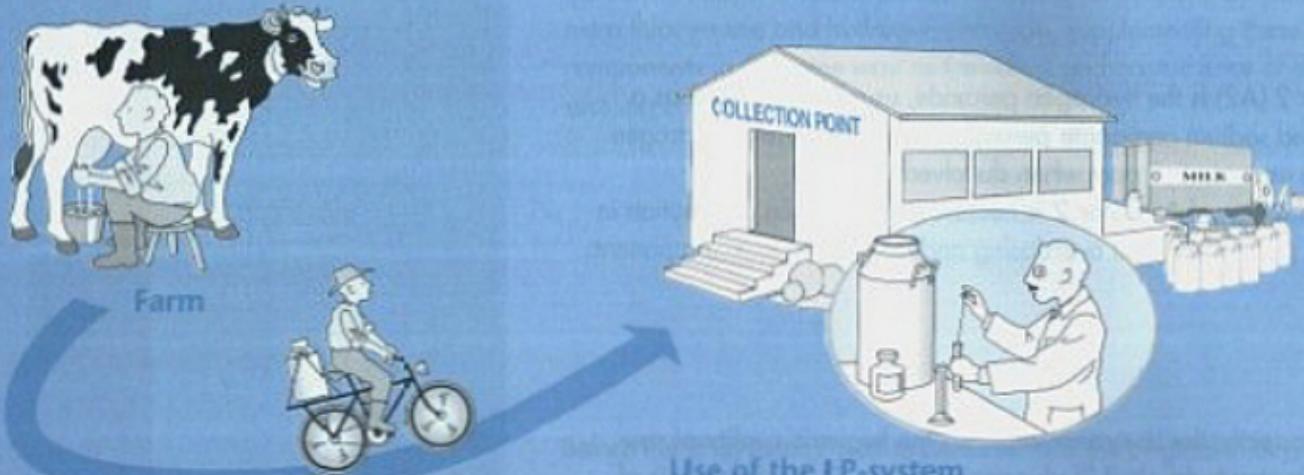
The granulation of Activator 2 is necessary for an even distribution in the milk, preventing local overdosing and damage to milk components.

If used correctly, the LP-system improves the hygienic quality of raw milk and extends the shelf-life by several hours. This extra time is of great benefit to small farmers, particularly those living in areas far from a large dairy.



PROCEDURE FOR LP-SYSTEM ACTIVATION IN CHURNS

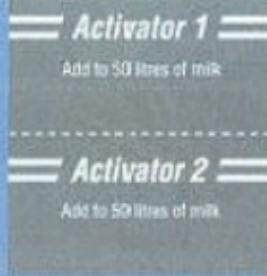
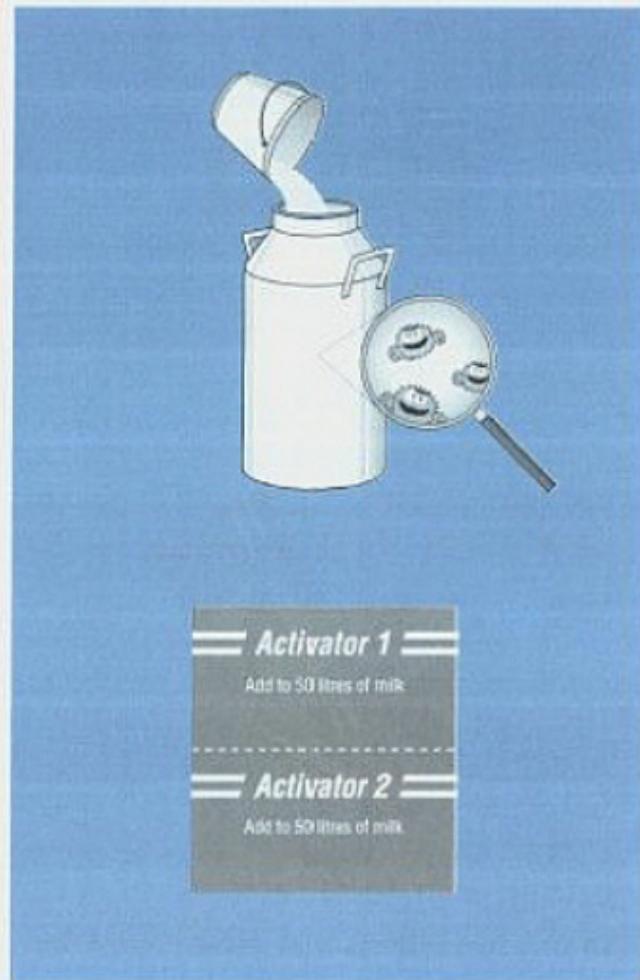
**Remember! The lactoperoxidase system should be used
by trained personnel (milk collectors),
at the collection point**



Use of the LP-system

1. Fill up the churn.

Separate the two prepacked bags for the correct churn size (20-30 or 40-50 litre). It is very important that thiocyanate, Activator 1, is added first.

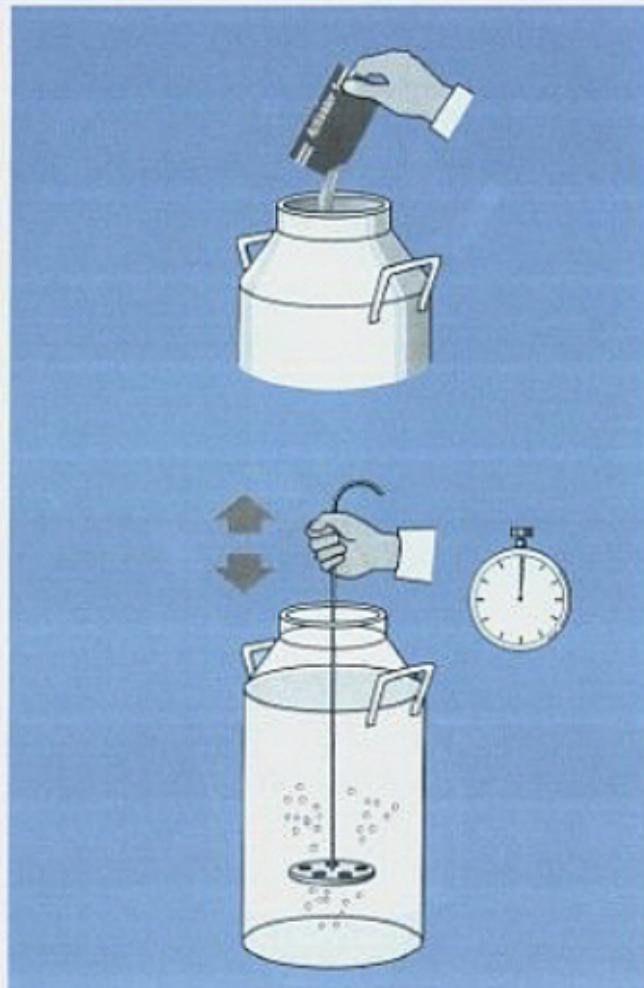


2. Take bag 1.



3. Open with scissors.

4. Empty the liquid into the milk by squeezing the bag between your fingers. Empty the liquid completely into the milk.



5. Stir for 30 seconds with a clean plunger.



6. Take bag 2.

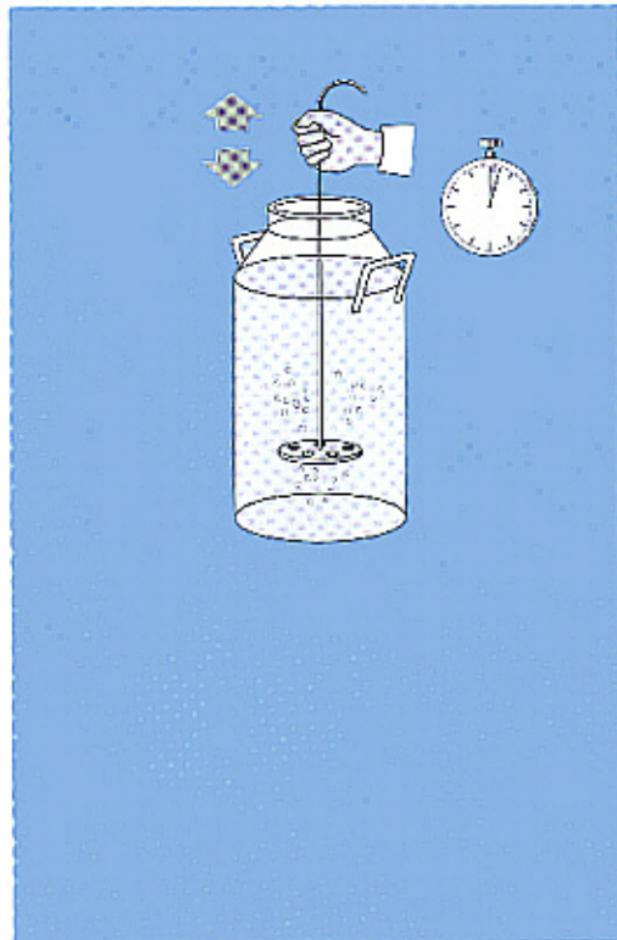


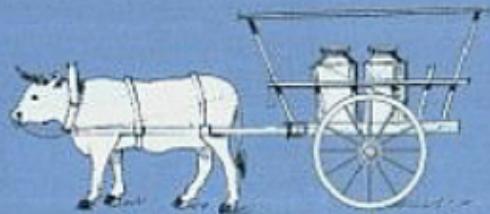
7. Shake it so that the contents are at one end and cut the opposite end with the scissors.

8. Empty the contents into the milk. Make sure that the bag is completely emptied.

9. Stir the milk for about two minutes.

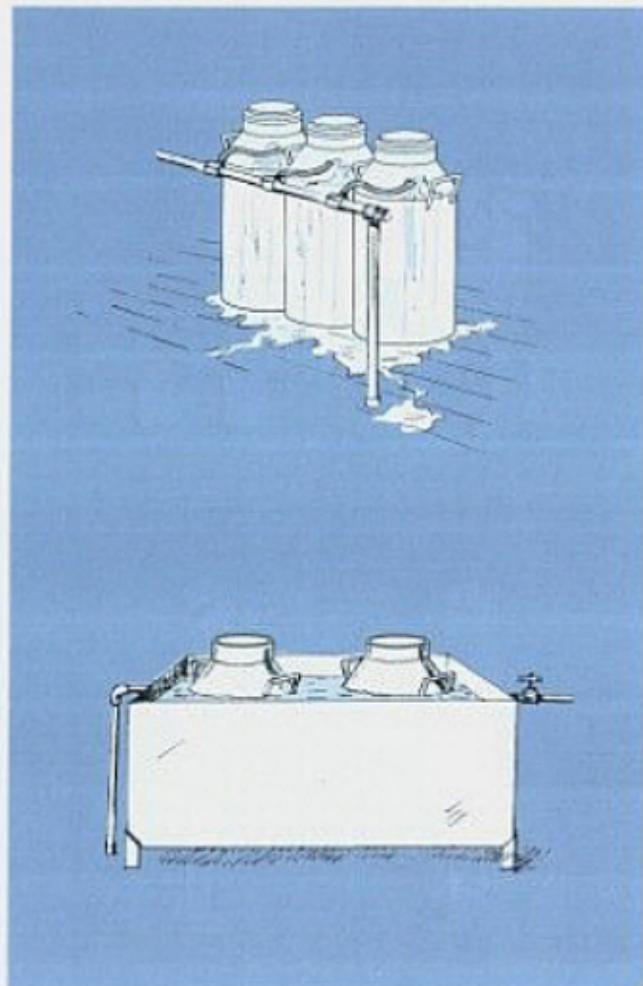
This starts the enzymatic reaction. The reaction is completed within about five minutes. After that, no hydrogen peroxide is present in the milk.





If you keep the milk in the shade or in a dark well-ventilated place at about 30°C the effect of the LP-system of preservation lasts for seven to eight hours.

If you can cool the milk to 15-20°C the LP-system keeps the evening milk preserved overnight, allowing for the collection of milk only once a day.





According to the Code of Practice for the use of the LP-system in the preservation of milk, "the method should not be used by the individual farmers but at a suitable collecting point/centre". There are good reasons for this, depending on the size of the farms.

Many farmers produce only a small quantity of milk which may differ from day to day.

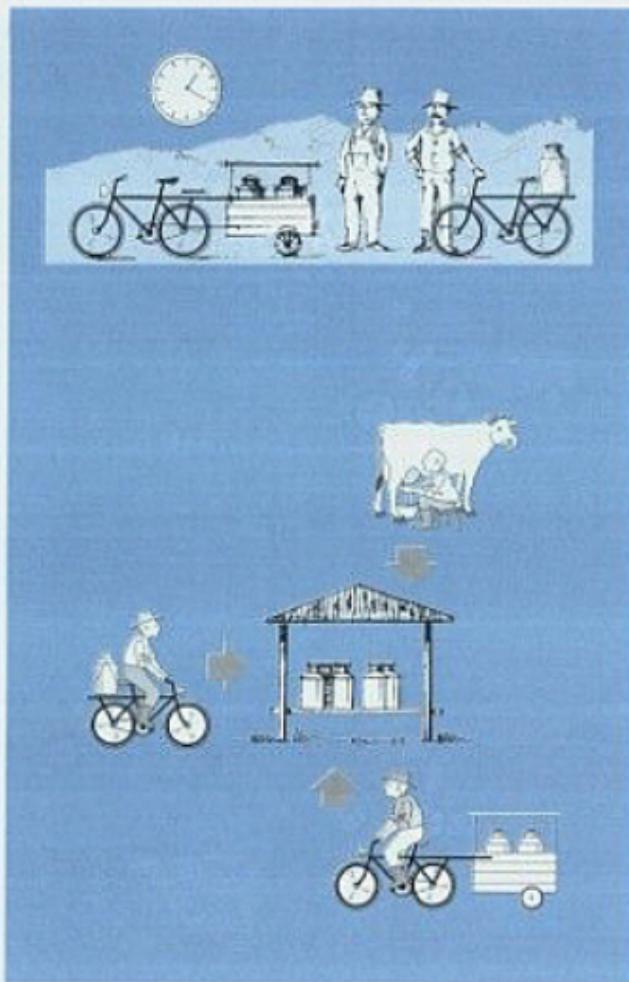
It is not possible to prepare prepacked activators for varying small amounts of milk.

It may also be difficult for many small farmers to handle the method in the correct way. This means that the results may vary, with negative effects on the milk quality.

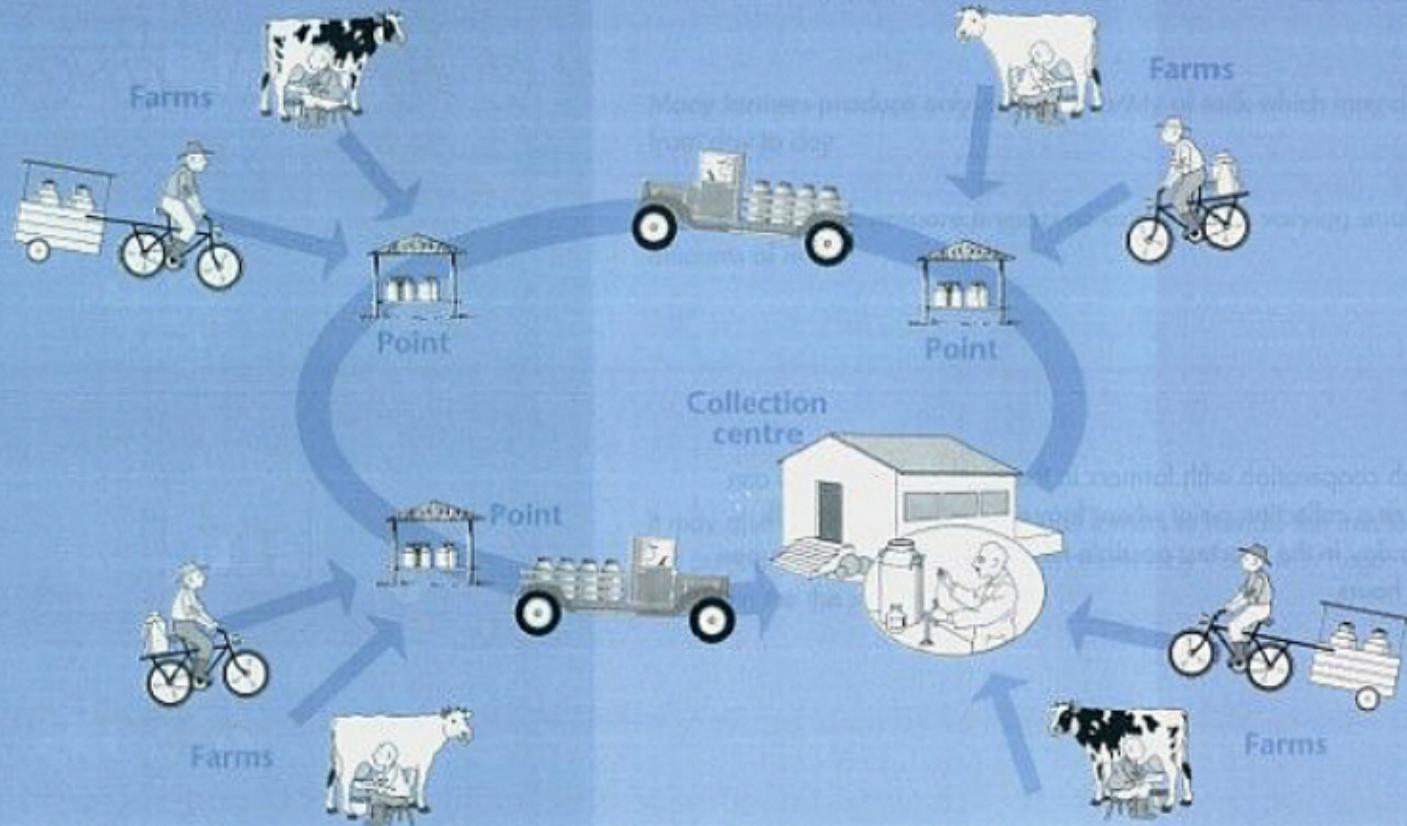
MILK COLLECTION

Often the dairy is far away and farmers must bring their milk to the nearest road and wait for the dairy truck to collect it.

Through cooperation with farmers in the neighbourhood you can organize a collection point where farmers can bring their milk twice a day in the shortest possible time after milking – within one or two hours.



It is better to have more small collection points that are easy to reach in a short time than a few large collection points far away from the small farmers.



MILK HYGIENE QUALITY TESTS AT COLLECTING POINTS

There are three simple tests for milk quality:

- Sensory tests
- The clot-on-boiling test
- The alcohol test

Sensory tests

The milk in each transport vessel is assessed with regard to appearance, colour, cleanliness and smell.

If there is visible dirt such as straw or manure in the milk, the milking and handling have not been carried out in accordance with good hygienic practice. This should be made clear to the producer.

There is a difference between the colour of the milk of some species. Cow and sheep milk is slightly yellowish-white, while milk from buffaloes and goats is completely white.





Deviation from the normal colour indicates damage to the udder; reddish - blood, or yellow - pus.

In cases where the smell is not normal, taste and flavour should also be checked.

If the smell and taste are slightly sour, milk should not be accepted, since it cannot be heat-treated at the dairy.

Sensory tests are quick and cheap and, with trained personnel, very reliable.

Besides sensory tests there are some more "objective" tests, which can be carried out at the collecting points.

The clot-on-boiling test

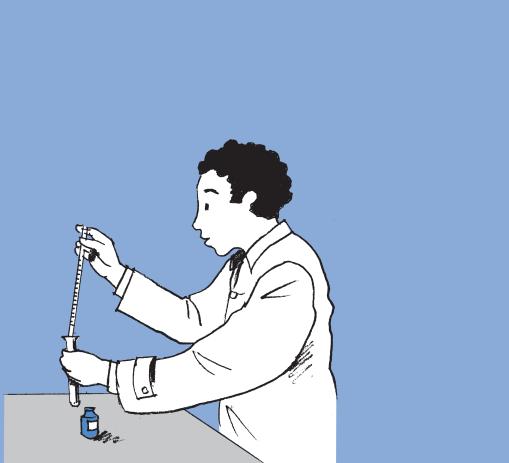
This means that a small volume of milk is heated to boiling point to check whether it clots or not.

If it clots, it is sour and cannot be heat-treated any more (for example, by pasteurization) and must be rejected.

The clot-on-boiling test is simple, quick and cheap, and can be carried out in the presence of the milk producers, who then understand and accept the results.

The test should always be carried out in combination with sensory tests.





The alcohol test

This test is based on the fact that milk with increased acidity (souring) flocculates when mixed with an equal or double volume of alcohol (68 percent volume per volume – v/v).

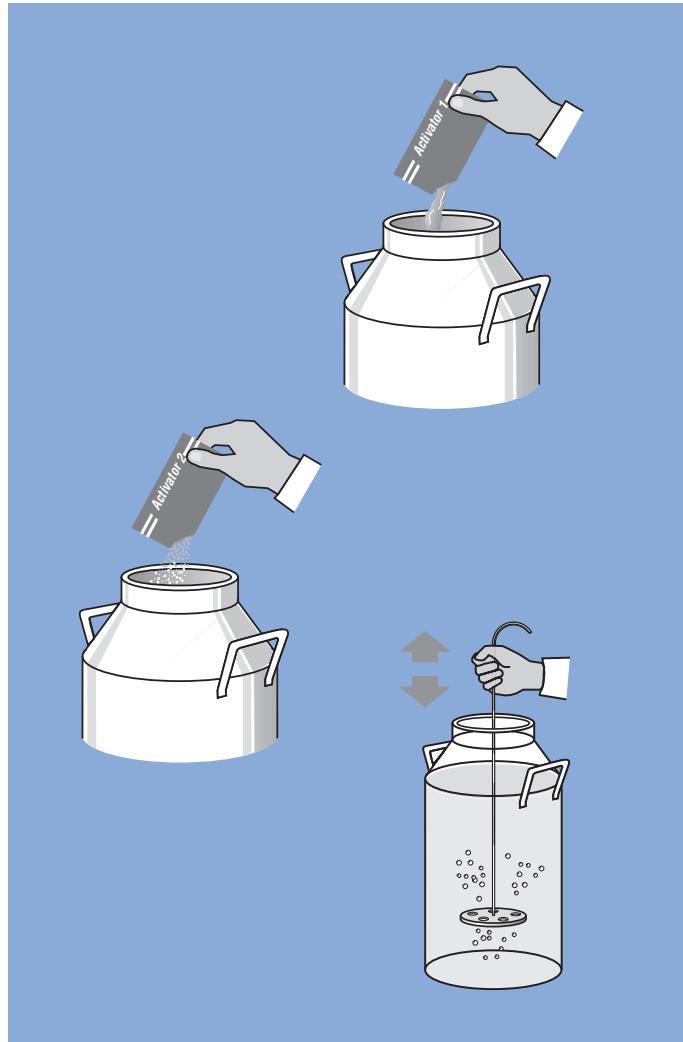
Fresh milk can be diluted with alcohol without flocculation. If it flocculates when mixed with an equal amount of alcohol, there is an increase in acidity. This milk may not clot on boiling, but has to be heat treated as soon as possible. If it flocculates on adding double the quantity of alcohol, it is sour.

The alcohol test is quick, cheap and easy to carry out. In combination with sensory tests and the clot-on-boiling test it confirms the hygienic quality of the producer's milk when delivered to the collecting point.

After quality control the volume (or weight) of the milk is measured and it is poured into (40-50 litre) transport churning.

These churning usually belong to the collecting organization, i.e. the dairy, and should be clean and available at the collecting point before any milk is received.





As soon as the churn is full, the activators should be added as described.

CONCLUSION

Preservation of milk by activation of the lactoperoxidase system: advantages

Improved bacterial and chemical quality of collected milk = improved quality of dairy products.

More economic milk collection.

Milk production possible in areas with high ambient temperature and no or poor cooling facilities.



Appendix I

TECHNICAL SPECIFICATIONS 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 SPECIFICATIONS OF SODIUM PERCARBONATE

Definition

| | |
|------------------|---|
| Chemical name | Sodium percarbonate* |
| Chemical formula | $2\text{Na}_2\text{CO}_3\text{H}_2\text{O}_2$ |
| 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 can be obtained commercially, please apply to the 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 deproteinization, 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% (weight per volume = w/v) trichloroacetic acid: 20 g TCA is dissolved in 100 ml distilled water and filtered.
2. Ferric nitrate reagent: 16.0 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ 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 then filtered through a suitable filter paper (Whatman No. 40) and 1.5 ml of the clear filtrate is mixed with 1.5 ml

* 2M HNO_3 is obtained by diluting 138.5 ml 65% HNO_3 to 1 000 ml with distilled water.

of the ferric nitrate reagent and the absorbence 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 ten 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 determined by comparison with standard solutions of known thiocyanate concentration, e.g. 10, 15, 20 and 30 mg of thiocyanate.

Secretariat of the Global Lactoperoxidase Programme

Secretary: Dr J.C. Lambert, Senior Officer, AGAP, FAO, Rome

Assistant to Secretary: Mr R. N'Gatta, Dairy Consultant, AGAP, FAO, Rome

Tel.: (+39) 06 5705 4701/06 5705 3003

Fax: (+39) 06 5705 5749

E-mail: JeanClaude.Lambert@fao.org/Regis.NGatta@fao.org

This manual has been prepared as a practical reference and training document for the Sweden-sponsored Global Lactoperoxidase Programme. The programme, which has been developed by FAO, the International Dairy Federation, the World Health Organization and Uppsala Agricultural University, Sweden, will be implemented in 80 countries worldwide. The programme consists of practical demonstrations of the lactoperoxidase system in the field in collaboration with preappointed national and regional focal points and dairy research institutions.

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