Benefits and Potential Risks of the Lactoperoxidase System of Raw Milk Preservation

Report of an FAO/WHO technical meeting

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Declarations of interest

Ing. Ponce: As a researcher at the National Centre for Animal and Plant Health (CENSA), he is the author of a patent on a product based on the activation of the Lactoperoxidase system. He does not have rights for its commercial exploitation nor profits derived from it as established by Cuban Laws of Intellectual Property.

Abbreviations

CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
COMESA	Common Market for Eastern and Southern Africa
FAO	Food and Agriculture Organization of the United Nations
GEMS	Global Environment Monitoring System
GLP	The FAO Global Lactoperoxidase Experts Group
JECFA	Joint FAO/WHO Expert Committee on Food Additives
HTST	High Temperature Short Time
IDD	Iodine Deficiency Disease
IGAD	Inter-Governmental Authority on Development
LP-s	Lactoperoxidase system
ppm	Parts per million
SADC	Southern African Development Community
UHT	Ultra-high temperature (sterilization) / Ultra heat treated (milk)
WHO	World Health Organization

EXECUTIVE SUMMARY

This technical meeting was jointly organised by the Animal Production and the Food Quality and Standards Services of the Food and Agriculture Organization of the United Nations (FAO), in cooperation with the Department of Food Safety, Zoonoses and Foodborne Disease of the World Health Organization (WHO) to obtain the best available scientific advice on issues related to the use of the lactoperoxidase system (LP-s) in raw milk preservation.

After reviewing the available scientific information (References, Appendix A and B), the technical meeting concluded that the LP-s is a safe method of preventing milk losses due to microbial spoilage when used according to the Codex guidelines either alone or in combination with other approved procedures. The LP-s is particularly suitable for application in situations where 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-s does not preclude or replace the need for the pasteurization of raw milk to improve safety for human consumption.

Post harvest losses are a major issue in dairying in developing countries. Smallholder dairy farmers could increase their participation in worldwide milk production, processing and marketing if they could reduce their losses using any approved milk preservation method. Refrigeration is the preferred means of milk preservation but does require high capital investment and can incur high running and maintenance costs. The LP-s provides a cost effective method to increase the availability of milk that contributes to income generation, household food security and nutrition in developing countries.

The LP-s elicits antimicrobial activity against a wide variety of milk spoilage and pathogenic microorganisms including bacteria, HIV-1 virus, moulds, yeasts, mycoplasma and protozoa. Furthermore, the LP-s does not promote the growth of pathogenic microorganisms after completion of the bacteriostatic effect[1]. The activated LP-s is effective in raw milk of different species, the overall activity being primarily bacteriostatic[2], depending on the initial total bacterial load, species and strains of contaminating bacteria and the temperature of milk.

Observations from laboratory and field studies indicate that the LP-s does not induce any significant adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. Under practical conditions the activated LP-s cannot be used to disguise milk of poor microbiological quality.

None of the components of the LP-s presents a significant toxicological risk to public health at the levels proposed. Where iodine deficiency is common, public health measures to rectify the iodine deficiency are needed whether or not the LP-s is used.

[1] Under laboratory conditions.
[2] The LP-s is classified as a 'microbiostatic' in the Codex Code of Hygienic Practice for Milk and Milk Products (CAC/RCP/57 – 2004) (CAC, 2004b).

In adopting the "Guidelines for the preservation of raw milk by use of the lactoperoxidase system" in 1991, the Codex Alimentarius Commission agreed to emphasise that the LP-s should not be used for products intended for international trade. This provision is considered a major obstacle to the adoption of the system, limiting both regional and international trade in LP-s treated milk and dairy products.

Based on the available data and an assessment thereof, the technical meeting considered the LP-s to be a safe method of raw milk preservation when implemented according to established Codex guidelines. The meeting concluded that this report provides a scientific basis for Codex to reconsider the provision related to the limitation on the international trade of LP-s treated milk and dairy products.

RECOMMENDATIONS

In making its recommendations, the meeting reiterated the safety of the Lactoperoxidase system of raw milk preservation when used according to the existing guidelines (CAC, 1991b), recommending its use in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities. Based on its deliberations the following specific recommendations were made.

To Codex

Consider expanding the guideline for the use of this system with regard to temperature of application of the LP-s to also include the temperature range from 31 ºC to 35 ºC for 4–7 hours and down to 4 ºC for 5–6 days.

Develop milk and dairy product standards that can be easily adopted at regional or national level through the encouragement and support of active participation of a representative range of country members in the development of standards.

Remove the current provision regarding the restriction on the use of LP-s in milk or dairy products intended for international trade as the meeting found no scientific or technical basis or economic justification for the provision.

To member countries, FAO, WHO, Codex, NGO's and the dairy industry

Acknowledge the LP-s as an effective and feasible method of raw milk preservation that does not display a negative impact on the further processing of milk.

Owing to its bacteriostatic effect, give consideration to the application of the LP-s as part of a programme to improve milk hygiene and safety along the milk chain.

Consider the application of the LP-s to complement cooling in order to extend the keeping quality of raw milk and halt proliferation of milk spoilage and pathogenic microorganisms.

Use the LP-s to improve the quality of processed products based on its proven bacteriostatic effect from milk collection to final processing and in particular to extend milk collection distances in developing countries, thereby increasing the amount of milk available for marketing. This can have significant direct benefits for both milk producers and consumers.

Recognise that the use of the LP-s is an economically viable option (either standalone or in combination with refrigeration) to significantly reduce milk losses and increase milk availability.

In addition to recommendations specific to the use of the LP-s a number of other related issues were discussed, based on which the technical meeting made the following recommendations.

Promote the consumption of milk as a valuable source of human nutrition contributing to healthy development and growth.

Promote the contribution of small-scale dairying to household nutrition, food security, and poverty alleviation.

Implement measures to rectify iodine deficiency in recognised IDD areas accompanied by appropriate monitoring of its prevalence. Milk can also be a valuable source of iodine, providing there is adequate iodine in the diet of the milk-producing animals.

1. INTRODUCTION

This technical meeting was jointly organised by the Animal Production and the Food Standards and Quality Services of the Food and Agriculture Organization of the United Nations (FAO), in cooperation with the Department of Food Safety, Zoonoses and Foodborne Disease of the World Health Organization (WHO) to get the best available scientific advice on issues related to the Lactoperoxidase system (LP-s). The LP-s consists of the addition of sodium thiocyanate and hydrogen peroxide to reactivate the existing lactoperoxidase enzyme in milk that maintains the initial quality of the milk without refrigeration until the milk can be processed or pasteurized.

FAO and WHO recognise the important role smallholder dairy producers play in supplying milk and dairy products to markets in developing countries. Their continued participation in these markets is encouraged. Milk is an important commodity that contributes to household nutrition and health, and can also provide an income. Therefore, approaches for enhancing the availability of safe milk and dairy products are important for the continued improvement of household nutrition and health.

This meeting was part of the FAO/WHO activities on the provision of scientific advice to Codex and to their member countries. The Codex guidelines (CAC /GL 13 – 1991(CAC, 1991b)) for the preservation of raw milk by use of the LP-s were adopted in 1991 at which time the Codex Alimentarius Commission (CAC) also "agreed to emphasise that the lactoperoxidase system not be used for products intended for international trade" (CAC, 1991a). Since then many member countries have raised concerns over this provision. In this regard, FAO and WHO have been asked to provide scientific advice based on comprehensive and relevant information in order to support appropriate decision-making within the Codex system on the use of the LP-s (CAC, 2004a).

Experts from five regions – Africa, Asia, Europe, North and Latin America, and the Caribbean – participated in the meeting in their independent professional capacities and not as representatives of their governments, employers, or institutions. The meeting was supported by a number of submitted papers following an open call for information and data from member countries on issues relating to the LP-s. In particular, issues related to microbiological effects and performance, human health and nutrition, processing and technology, and economic value and trade were addressed. These documents, as listed in Appendix A, were distributed to the experts prior to the meeting. Additional materials consulted and provided by participants during the meeting are included in the Reference section and Appendix B of this report.

1.1 Background

Lactoperoxidase is an enzyme that is naturally present in milk. One of its unique biological functions is a bacteriostatic effect in the presence of hydrogen peroxide and thiocyanate. Both of these substances are naturally present in milk in varying concentrations. The method of activating the LP-s in milk is to add about 10 ppm (parts per million) of thiocyanate (preferably in powder

form) to the raw milk to increase the overall level to 15 ppm (around 5 ppm is naturally present). The solution is thoroughly mixed for 30 seconds and then an equimolar amount (8.5 ppm) of hydrogen peroxide is added (generally in the form of a granulated sodium carbonate peroxyhydrate). The activation of the lactoperoxidase has a bacteriostatic effect on the raw milk and effectively extends the shelf life of raw milk for 7–8 hours under ambient temperatures of around 30 ºC or longer at lower temperatures. This allows adequate time for the milk to be transported from the collection point to a processing centre without refrigeration.

There are several ways in which the spoilage of milk may be controlled, including refrigeration, heat treatment (pasteurization in bulk or in pouch), microfiltration (with or without pasteurization), bactofugation, high-pressure treatment and use of chemical preservatives (including salting at levels of 3–12%). Some of these procedures require expensive equipment and are not widely applicable, particularly in small-scale dairy production and processing systems in developing countries where up to 80% of the milk produced may enter the informal market.

The FAO Global Lactoperoxidase Experts Group (GLP) was set up in July 1998. The main objective of this group was to promote the LP-s and carry out demonstrations in specific regions in the world where refrigeration is difficult. The partners involved in this group were the Lund University of Sweden, WHO, the International Dairy Federation, and FAO, with support from the Governments of Sweden, France, Hungary and Ireland. The strategy of the GLP was to inform countries and assess their interest in these issues, to identify regional partner institutions, national institutions and experts, conduct national training and demonstrations in collaboration with the relevant ministries and follow-up through national experts and governments. The outputs from the GLP included posters and manuals on the use of the LP-s in English, French and Spanish, the printing and distribution of Field Manuals, the implementation of training and demonstrations in 35 countries, annual meetings and the Bushmilk *(Lait de brousse*) programme in West Africa.

Codex adopted the "Guidelines for the preservation of raw milk by use of the lactoperoxidase system" in 1991 (CAC, 1991a, b). Issues concerning the LP-s of raw milk preservation have been raised in numerous Codex meetings, most recently during the meeting of the Codex Alimentarius Commission in Geneva in 2004 (CAC, 2004a). Issues related to the guidelines have also been raised as a concern by numerous FAO member countries.

In 2002, the GLP requested that the Codex Committee on Milk and Milk Products (CCMMP) consider amendments to the guidelines (CAC, 2002a). Highlighting the need for a scientific basis for any amendments, the committee referred the issue to the Codex Executive Committee later in 2002, which agreed that this might be of particular interest to developing countries and invited Regional Committees to consider the issues (CAC, 2002b). It was recognised that all relevant health aspects of this complex issue should be considered to ensure that any revision of the guidelines would be based on sound science and risk analysis.

In 2002, the Codex Coordinating Committee for Africa supported these decisions and maintained that until uncertainties related to the process were resolved the provisions on the use of this

system should be maintained (CAC, 2002c). The Codex Coordinating Committee for North America and South West Pacific (CCNASWP) in 2002 also recommended that further reviews by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of the chemical and microbiological safety of the LP-s should be undertaken before revising the guidelines (CAC, 2002d). In 2003, the Codex Committee on Food Hygiene (CCFH) concluded that the current provision excluding the use of the lactoperoxidase system for products intended for international trade should continue to be applied and that there was no need for the revision of the existing Guidelines in the framework of Codex or a JECFA review (CAC, 2003).

The issue was raised again in the CCFH in 2004 when the committee was informed that new data were being generated. It was also discussed at the 27th session of the Codex Alimentarius in 2004 in the course of the adoption of the Draft Code of Practice for Milk and Milk Products, during which the following text was added to the code, "The use of the lactoperoxidase system for milk and milk products in international trade will be re-examined by the Committee on Food Hygiene (CCFH) after completion of an expert review by FAO and WHO of available data and considering the FAO Lactoperoxidase Expert Group report about potential risks and benefits of lactoperoxidase system. CCFH will then review the issue in 2006" (CAC, 2004a).

1.2 Scope and purpose of the technical meeting

The current meeting was implemented to respond to member country concerns and to provide scientific advice to the next session of the CCFH in 2006 on the benefits and possible risks associated with the LP-s for raw milk preservation and any dairy products derived from treated milk.

The objective of the technical meeting was to determine the benefits (economic and nutritional) and the level of health risks, if any, posed by the application of the LP-s, advise on the safety of the LP-s treated milk and derived milk products, and to address the issue of the limitation on the use of LP-s treated milk or derived products intended for international trade.

The group agreed to discuss these issues under the following four headings and a chairperson and rapporteur was assigned for each of the four subject areas.

1. The microbiological effects and performance of the LP-s
   Chairperson: C. Michiels
   Rapporteur: H. Korhonen

2. The effects of LP-s treated milk and dairy products on human health and nutrition
   Chairperson: J. Vanderveen
   Rapporteur: R. Walker

3. Milk processing, technology and preservation

Chairperson: J. P. Ramet
Rapporteur: A. Grandison

4. Economic value and trade of LP-s treated milk and dairy products
Chairperson: H. G. Muriuki
Rapporteur: O. C. Emata

This report summarises the deliberations, findings and conclusions of the meeting.

2. MICROBIOLOGICAL EFFECTS AND PERFORMANCE OF THE LACTOPEROXIDASE SYSTEM

The effectiveness of the LP-s in maintaining the hygienic quality of raw milk for a limited period of time has been established in many experimental and field studies conducted in different geographical regions. The method can be applied to preserve raw milk from different species. The effectiveness depends on the initial amount and type of microbiological contamination and the temperature of milk during the treatment period. The LP-s exerts primarily a bacteriostatic effect in raw milk. Experimental data and experience from practice indicate that the LP-s can be applied beyond the temperature limits (15–30 ºC) referred to in the 1991 Codex guidelines (CAC, 1991b). At the lower end of the temperature scale, several studies indicate that activation of the LP-s can delay growth of psychrotrophic milk bacteria and thus delay milk spoilage for several days compared to what can be achieved with refrigeration alone.

It is important to emphasise that the purpose of the use of the LP-s is not to render milk safer for consumption but to preserve its initial quality. Good hygienic practices in milk production are critical to the efficacy of the LP-s and to the microbiological quality of the milk. The safety of milk is only achieved through a combination of good hygienic practices and heat treatment of milk, independent of the LP-s. This effectiveness of the LP-s under various conditions and against a range of microorganisms is addressed below.

2.1 Effectiveness of the lactoperoxidase system for preventing spoilage of raw milk

a) Effectiveness under conditions as specified in the Codex guidelines

The Codex guidelines focus on the application of the LP-s for preventing spoilage of raw milk (bovine and buffalo) during collection and transportation to a dairy processing plant, under conditions where adequate refrigeration is not feasible. The guideline is based on a number of scientific papers from the late 1970s elucidating the working principles of the method and providing proof of concept (Björck, Claesson and Schulthess, 1979; Reiter *et al.*, 1976; Björck, 1978).

Since the adoption of the Codex guidelines, a substantial amount of data on the effectiveness of the LP-s has accumulated, not only from laboratory and field studies, but also from experience with the large-scale adoption of the system in commercial milk production in some countries. During the meeting, summary reports showing results from many countries, for example Cuba, Colombia, Peru, Venezuela, Cameroon, Kenya, Uganda and Pakistan, covering a wide range of different production conditions, were presented and have been reviewed (Björck, Claesson and Schulthess, 1979; Bibi and Bachmann, 1990; Ponce *et al.,* 2005; Albujar, Ludena and Castillo, 2004; Siirtola, 2005; Fonteh, Grandison and Lewis, 2005). Overall, these data confirm the effectiveness of the LP-s for preventing spoilage of non-refrigerated raw milk within the framework defined in the Codex guidelines, i.e.:

- The principles of good hygienic practice in milk production must be respected in order to guarantee a good initial microbiological quality of the raw milk (see below)

- The inhibitory effect of the treatment is dependent on the storage temperature of LP-s treated milk as follows (Table 1):

Table 1: Extension of milk keeping quality by the LP-s at different temperatures

Temperature (ºC)	Time (hours)	Reference
31–35	4–7	Ponce *et al*., 2005
30	7–8	CAC, 1991b
25	11–12	CAC, 1991b
20	16–17	CAC, 1991b
15	24–26	CAC, 1991b
4	5–6 days	Zapico *et al.,* 1995; Lin and Chow, 2000

It should be emphasised that these spoilage delay times should be considered indicative, because they are affected to a great extent by the initial bacterial load (see below).

b) Effectiveness under different ambient conditions

The temperature dependence of the effectiveness of the LP-s as shown above, and as already specified in the original CAC guidelines (CAC, 1991b), illustrates that with respect to prevention of spoilage of raw milk, the LP-s can be complementary to refrigeration. In other words, it can compensate for a lack of refrigeration whenever the latter cannot be supplied. However, the efficacy of the LP-s persists for a limited period of time, which decreases as the ambient temperature increases. This temperature dependence of the effectiveness of the LP-s was defined only in a range between 15 and 30 ºC in the original Codex guidelines. However, milk storage temperatures may exceed 30 ºC during daytime, and may fall below 15 ºC during night-time in some regions without refrigeration facilities. Therefore, the effectiveness of the LP-s at temperatures outside this range is a relevant issue.

Temperature is one of the most important factors influencing microbial growth. The role of refrigeration and the cold chain in maintaining the quality and safety of both raw and pasteurized milk is well recognised. Many bacteria are mesophilic, growing best at temperatures of 30 ºC to 40 ºC. However, psychrotrophic and psychrophilic bacteria can grow at low temperatures, with some strains capable of surviving and growing at temperatures down to 0 ºC. *Listeria monocytogenes* is an example of a pathogenic bacterium that can grow at very low temperatures. However, in products such as milk that has a diverse microflora, it would normally

be outgrown by the psychrotrophic spoilage bacteria, such as members of the genera, *Pseudomonas*, *Bacillus* and *Micrococcus*.

Some recent field studies that have been carried out with raw milk treated by the LP-s and stored at 30–35 ºC showed a consistent inhibition of microbial growth for 4–7 hours (Ponce *et al.*, 2005).

Effectiveness of the LP-s may also be relevant to microbial quality and safety issues in relation to extended storage of raw milk under refrigerated conditions. Current issues of concern with regard to low temperature storage include the formation of heat stable proteases by psychrotrophic *Pseudomonas* spp. and the outgrowth of psychrotrophic pathogens such as *Listeria monocytogenes* and some *Bacillus cereus* spp.. At this end of the temperature scale, several studies indicate that activation of the LP-s can delay growth of psychrotrophic milk bacteria and thus delay milk spoilage for several days compared to what can be achieved with refrigeration alone. For example, studies in Taiwan indicated a six-day extension of the spoilage-free storage period of raw milk at 4 ºC upon activation of the LP-s (Lin and Chow, 2000). Another study showed that the LP-s prevented the growth of psychrotrophic *Pseudomonas fluorescens* for five days at 4 ºC and for three days at 8 ºC (Zapico *et al*., 1995). A summary table comparing LP-s, refrigeration and the combination of LP-s with refrigeration is included as Appendix C.

c) Effectiveness in milk of different species (bovine, buffalo, sheep, goat, camel)

The lactoperoxidase enzyme is present in the milk of all mammals. Although there are variations at the species and even at the individual animal level (Fonteh, Grandison and Lewis, 2002), the enzyme levels in the milks that are used for human consumption are not believed to be a limiting factor for the effectiveness of the LP-s. In general, the available studies show that the time/temperature combination as outlined for cow and buffalo milk are also applicable to goat and sheep milk. In camel milk, the activation of the LP-s may induce a longer-lasting bacteriostatic effect than in cow's milk due to the presence of higher levels of other indigenous antimicrobial components (Ramet, 2001). Less information is available for milk from other species.

d) Effectiveness in relation to principles of hygienic milk production

The Codex guidelines state that, "*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*", and, "*The use of the LP-s does not exclude the necessity of pasteurization of 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*" (CAC, 1991b).

Microbiological studies conducted over the last 10 to 15 years support this view. Invariably, the antibacterial efficacy of the LP-s is found to be inversely correlated to bacterial cell density. The antibacterial efficacy of the LP-s is low at high bacterial concentrations, primarily bacteriostatic at intermediate concentrations and primarily bactericidal at low concentrations. This follows from both laboratory observations with pure cultures of pathogenic or spoilage bacteria suspended in

buffer or broth (El-Shenawy, Garcia and Marth, 1990; Garcia-Graells *et al.,* 2003), and from field studies in milk with its natural mixed microflora (Ponce, 2005; Albujar, Ludena and Castillo, 2005). Consequently, safeguarding a high bacteriological milk quality before application of the system by adopting good hygienic practices is critical to its efficacy. In this respect, the use of the LP-s for preserving the quality of the milk before pasteurization does not differ from use of refrigeration for the same purpose. It is important to emphasise that the purpose of both methods is to prevent (microbiological) deterioration of the milk after milking and before pasteurization, not to render the milk safer for consumption, which is achieved by subsequent pasteurization of milk.

2.2 Effectiveness of the lactoperoxidase system against pathogenic microorganisms

The antimicrobial activity of the LP-s in milk, whey and synthetic media has been demonstrated against a wide range of microorganisms, including bacteria, HIV-1 virus, moulds, yeasts, mycoplasma and protozoa (for reviews see Korhonen, 1980; Reiter and Härnulv, 1984; IDF, 1991; Wolfson and Sumner, 1993; Stadhouders and Beumer, 1994; de Wit and van Hooijdonk, 1996; van Hooijdonk, Kussendrager and Steijns, 2000; Seifu, Buys and Donkin, 2005). These microorganisms cover non-pathogenic starter cultures and spoilage bacteria as well as pathogenic organisms that cause gastrointestinal infections in humans and udder infections in cows. However, considerable differences have been found in the sensitivity of different bacteria to the LP-s. Depending on the bacterial species or even the strain of the organism, the effect can be either bactericidal or bacteriostatic even under identical conditions. The LP-s has been found to be less effective against some non-pathogenic streptococci and lactococci.

The variations in sensitivity between strains may be explained by different cell wall structures and inhibitory compounds generated by the organisms concerned. Lactic acid bacteria, for example, are deficient in the catalase enzyme, and many species metabolically produce H_2O_2, which is accumulated in the growth medium. This H_2O_2 can activate the LP-s and lead to the self-inhibition of bacterial growth. Many dairy cultures are sensitive to the LP-s, and while some reports indicate interference with the fermentation processes (Wright and Tramer, 1958; De Valdez, Bibi and Bachmann, 1988; Seifu, Buys and Donkin, 2003), the impact is not consistent. This issue is also addressed in section 4.4. Most Gram-negative bacteria possess the catalase enzyme, which decomposes any generated H_2O_2. These bacteria, therefore, are not self-inhibited in milk through the LP-s and, to activate the system**,** H_2O_2 has to be supplied from an exogenous source, e.g. by the addition of sodium percarbonate. Under such conditions Gram-negative pathogenic and spoilage bacteria can be killed or their growth arrested for a certain period of time (Reiter *et al.,* 1976; Sandholm *et al.,* 1988; Dionysius, Grieve and Vos, 1992).

A number of studies on the impact of the LP-s on some of the most common milk-borne pathogens and other microorganisms causing infections in humans and domestic animals have been undertaken. Some of those on common milk-borne pathogens, namely *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Brucella melitensis* are summarised in Table 2. In various experimental

studies, the bacteriostatic or bactericidal effect of the LP-s has been demonstrated against several other human pathogenic microorganisms, such as *Streptococcus mutans* (Carlsson, Iwami and Yamada, 1983), *Aeromonas hydrophila* (Santos *et al.,* 1995), *Candida albicans* (Lenander-Lumikari, 1992) and *Helicobacter pylori* (Shin *et al.,* 2002). Also, the LP-s has been shown to inhibit the reverse transcriptase enzymatic activity of HIV-1 virus (Wang, Ye and Ng, 2000). Furthermore, a recent study by Armenteros *et al.,* (2005) has shown that the activation of the LP-s in raw milk does not exacerbate the presence of human pathogens including *E. coli* O157: H7, *L. monocytogenes, S. aureus* and *S.* Typhimurium when introduced into raw milk under laboratory conditions.

The LP-s is considered as one of the body's natural defence mechanisms against microbial infections. Increased concentrations of lactoperoxidase and thiocyanate ions are found in milk from mastitic cows as compared to milk from healthy animals. In general, the same applies to other major antimicrobial factors occurring in milk, e.g. immunoglobulins, lactoferrin, lysozyme and phagocytic cells (Korhonen *et al.,* 1977; Reiter, 1978; Reiter, 1985; Reiter and Perraudin, 1991; Korhonen, 2002). The LP-s has been shown to be bactericidal or bacteriostatic *in vitro* against many microorganisms that cause udder infections, e.g. *E. coli*, *S. aureus*, *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis* and *Pseudomonas aeruginosa* (Mickelson, 1966; Reiter *et al.,* 1976; Marshall, Cole and Bramley, 1986; Sandholm *et al.,* 1988). Many of these bacteria also pose a potential risk to human health. There is some experimental data to show that the LP-s in mastitic milk is not as effective as in milk from healthy cows because of a higher concentration of reductive agents and higher catalase enzyme activity present in mastitic milk (Sandholm *et al.,* 1988). No studies have so far been reported on the antibacterial activity of the LP-s against antibiotic-resistant mastitis organisms or coagulase-negative staphylococci. These organisms are frequently isolated from mastitic udders.

Table 2: Summary of studies on the impact of the LP-s on some common milk-borne pathogens

Pathogen	Effect of LP-s	Demonstrated in	Reference
Escherichia coli, including *E. coli* O157:H7	Bactericidal Reduced gastrointestinal colonization rate of coliform bacteria Bacteriostatic	Raw cow milk, buffer solution and synthetic medium Infected calves and piglets Raw cow, goat and camel milk, culture medium and infant formula	Reiter *et al.,* 1976; Reiter, Marshall and Philips, 1980; Earnshaw *et al.*, 1990; Farrag, El-Gazzar and Marth, 1992a; Grieve, Dionysius and Vos, 1992; Zapico *et al.*, 1995; Kangumba, Venter and Coetzer, 1997; Heuvelink *et al.*, 1998; Bosch, van Doormen and De Vries, 2000; Seifu, Dunkin and Buys, 2004
Salmonella Typhimurium *Salmonella typhi*, other *Salmonella* spp.	Bactericidal and bacteriostatic (dependent on number of organisms) Bactericidal	Raw milk Culture medium, infant formula and fresh cheese	Reiter *et al.,* 1976; Purdy *et al.*, 1983; Earnshaw *et al.,* 1990; Pitt, Harden and Hull, 2000
Campylobacter jejuni (various strains)	Bactericidal	Cow milk	Borch *et al.*, 1989; Beumer *et al.*, 1985
Staphylococcus aureus (several strains)	Bactericidal and bacteriostatic	Cow, goat and camel milk	Kamau, Doores and Pruitt, 1990; El-Agamy *et al.,* 1992; Kangumba, Venter and Coetzer, 1997; Pitt, Harden and Hull, 2000; Seifu, Donkin and Buys, 2004
Listeria monocytogenes (several strains)	Bactericidal and bacteriostatic (activity depending on temperature, length of incubation and strain)	Raw cow and goat milk, UHT milk, soft cheese and in synthetic medium	Dennis and Ramet, 1989; Siragusa and Johnson, 1989; Bibi and Bachmann, 1990; El-Shenawy, Garcia and Marth, 1990; Gaya, Medina and Nuñez, 1991; Zapico *et al.,* 1993; Pitt, Harden and Hull, 1999; Seifu, Donkin and Buys, 2004; Gay and Amgar, 2005
Yersinia enterocolitica	Bactericidal	Cow milk	Beumer *et al.,* 1985; Farrag, El-Gazzar and Marth, 1992b
Brucella melitensis	Bactericidal	Goat milk	Seifu, Donkin and Buys, 2004;

2.3 Possible consequences of the long-term use of the lactoperoxidase system on its antimicrobial efficacy

The issue of whether long-term use of the LP-s would result in any microbiological risks, e.g. development of LP-s resistant, antibiotic-resistant or toxin-producing bacteria was considered.

Some studies show that the efficacy of the LP-s could be interfered with by residues in milk of certain antibiotics used in the treatment of mastitis (Ali-Vehmas, Vikerpuur and Sandholm, 1994). Mutants of *Escherichia coli* with increased tolerance to the LP-s have recently been isolated in the laboratory and characterised (De Spiegeleer *et al.*, 2005). For one category of such mutants (*waaQ* and *waaO*), LP-s tolerance was linked to a deficiency in the outer core polysaccharide of the lipopolysaccharides, which causes a reduced permeability of the outer membrane for the hypothiocyanate anion ($OSCN^-$) due to a reduced porin content in the outer membrane. This type of mutation also causes a slightly elevated resistance to some penicillins (Nikaido, 2003). However, LP-s tolerant mutants have never been isolated from LP-s treated milk, which may be due to a reduced fitness under these conditions. For example, the *waaQ* mutation mentioned above causes a so-called rough phenotype, which is also associated with enhanced sensitivity to lactoferrin and lysozyme, two other important antimicrobial factors in milk. Thus, the available data indicate that adoption of the LP-s is not likely to stimulate the development of resistance to the LP-s itself or antibiotic-resistant microorganisms. However, as with all antimicrobial systems and due the ability of microorganisms to adapt the meeting considered that ongoing monitoring and research in this area is warranted.

2.4 Conclusions and recommendations

The LP-s elicits antimicrobial activity against a wide variety of milk spoilage and pathogenic microorganisms including bacteria, viruses, moulds, yeasts, mycoplasma and protozoa. The overall activity is primarily bacteriostatic[3], depending on the initial total bacterial load, species and strains of contaminating bacteria and the temperature of milk. While its effectiveness against well-known milk spoilage and pathogenic microorganisms is well established, further studies would be useful on the efficacy of the LP-s against milk-borne viruses and emerging pathogenic microorganisms.

The activated LP-s is effective in raw milk of different species and available studies also indicate that the same time-temperature as outlined in the Codex guidelines (CAC, 1991b) can be applied to goat and sheep milk.

The LP-s does not promote the growth of pathogenic microorganisms after completion of the bacteriostatic effect and there is no evidence to show that the long-term use of the LP-s would lead to any such microbiological risks, e.g. development or accumulation of toxin-producing bacteria.

[3] The LP-s is classified as a 'microbiostatic' in the Codex Code of Hygienic Practice for Milk and Milk Products(CAC/RCP/57 – 2004) (CAC, 2004b).

Under practical conditions the activated LP-s cannot be used to disguise poor microbiological quality of milk. Good hygienic practices in milk production are critical to the efficacy of the LP-s.

LP-s is effective in refrigerated raw milk. Experimental and field studies have demonstrated that the activated LP-s is effective in prolonging the keeping quality of raw milk both for up to 5–6 days in refrigerated milk (+4 ºC) and up to 4–7 hours at high ambient temperatures (from 31 to 35 ºC).

The application of the LP-s is not likely to stimulate the development of resistance to the LP-s itself or other antimicrobial agents but due to the dynamic nature of microorganisms ongoing monitoring of the situation would be reasonable.

Based on the above the meeting recommended that:

- When refrigeration is not technically feasible or economically viable the LP-s be applied to raw milk to halt proliferation of milk spoilage and pathogenic microorganisms.
- The application of the LP-s should be considered as part of a programme to improve milk hygiene and safety along the milk chain, owing to its bacteriostatic effect.
- Consideration be given to the application of the LP-s to complement cooling in order to extend the keeping quality of raw milk.
- Codex consider expanding the guideline for the application of the LP-s with regard to temperature of application to also include the temperature range from 31 to 35 ºC for 4–7 hours and down to 4 ºC for 5–6 days.
- Monitoring for the development of resistance be undertaken to detect the development of any resistant microorganisms.

3. HUMAN HEALTH AND NUTRITION

Milk has an important nutritional role in the diet, particularly for growing children, throughout the world and not just in developing countries. It represents a major source of protein, calcium, phosphorus, magnesium, and fat-soluble vitamins and may make a significant contribution to dietary intakes of some other vitamins and minerals including iodine. Milk can also be a useful vehicle for supplementation of nutrients such as vitamins A and D (WHO, in press). Lactose in milk is involved in regulating osmotic pressure but an additional role in facilitating calcium absorption in infants has been suggested (Abrams, Griffin and Davila, 2002; Garrow, James and Ralph, 2000).

There is a negative correlation between milk consumption and morbidity and mortality from childhood diseases and in this respect the provision of school milk programmes has been effective in improving childhood health and nutritional status (Scrimshaw and San Giovanni, 1997).

While the condition of lactose intolerance may limit the amounts of milk that can be consumed without adverse effects by some individuals/populations, up to one cup of milk (approx. 200ml) is generally tolerated. Furthermore, lactose serves as a substrate in lactic fermented milk products, leading to a reduction in the levels in such products and yeast fermentation results in hydrolysis of lactose by microbial β-galactosidase. Considering the important role of milk in human nutrition and health this section addresses the impact of the application of the LP-s for raw milk preservation from a public health and nutrition perspective.

3.1 The lactoperoxidase system in context

The LP-s differs uniquely from other preservation systems in that it is a natural biological protective system in the biology of animals. It functions as a protective antimicrobial mechanism in mucosal tissue, including in the oral cavity and lung (Tenovuo, 2002; Geiszt *et al.,* 2003). In this regard, the LP-s does not introduce substances into milk that are not normal human metabolites.

The LP-s can be applied to reduce spoilage of milk where refrigeration is not immediately available. However, the use of LP-s is not exclusive and may be combined with other procedures (e.g. refrigeration) to reduce losses of milk both in the formal and informal markets. The safety evaluation of the use of the LP-s in milk by JECFA at its 35th meeting (see below) was restricted by the terms of reference to the application of the system "when refrigeration is virtually impossible", and by the Guidelines drafted by the Joint FAO/WHO Committee of Government Experts on the Code of Principles concerning Milk and Milk Products. It is recognised that the safety issues concerning the broader application of the LP-s in conjunction with other methods for controlling spoilage, including refrigeration, were not addressed at that time.

3.2 Potential health issues associated with the use of the lactoperoxidase system: toxicological aspects

As noted above, the components or metabolites of the LP-s, namely lactoperoxidase, the thiocyanate ion and hypothiocyanate have been detected in animal and human tissues and secretions, including milk. The levels of hydrogen peroxide introduced into the milk via sodium percarbonate are lower than those previously considered acceptable by the 24th meeting of the JECFA (WHO, 1980) and are, therefore, not of concern.

The use of the LP-s does not require the addition of further lactoperoxidase above the levels of the enzyme occurring in raw milk. As there is no change to the enzyme concentrations naturally present in milk, this component is not considered of toxicological significance.

Hypothiocyanate has been detected in human saliva (Thomas, Bates and Jefferson, 1980) and has a very short half-life in milk, so that residual levels in milk treated with the LP-s do not pose a toxicological risk. The breakdown products are considered innocuous.

In the earlier evaluation, at its 35th meeting in 1990 the JECFA concluded that "*when used according to the draft guidelines, the lactoperoxidase system does not present a toxicological hazard and, furthermore, that the system should be used in preference to hydrogen peroxide alone for the preservation of raw milk, though only where absolutely necessary i.e. in the absence of adequate refrigeration facilities*". Very few new data on the toxicology of thiocyanate have become available since the previous JECFA evaluation.

The present group examined the potential toxic effect of thiocyanate, which was considered to interfere with iodine metabolism and uptake by the thyroid (WHO, 1990). The mode of action of the goitrogenic effect is via competitive inhibition of iodine and tyrosine oxidation leading to lower levels of thyroxine (T4) and inhibition of uptake by the thyroid. However, this effect occurs at relatively high plasma thiocyanate concentrations (60–80 micromolars or 4.8-6.4 milligram/litre) whereas at lower levels (0.5–1.0 μmolar) there is a stimulatory effect by interacting with thyroid peroxidase (Green, 1978).

At high plasma thiocyanate concentrations there is an increased excretion of iodine and a reduced iodine uptake by the thyroid gland, resulting in a low thiocyanate/iodine (SCN/I) excretion ratio. The value of the threshold level for this ratio seems to be three (Delange and Ahluwalia, 1983) after which endemic goitre appears. This phenomenon can occur only when the iodine intake is below about 100 micrograms per day. At SCN/I ratios of lower than two there is a risk to cognitive function and development (Erman *et al.,* 1983). A low ratio leads to abnormal levels of the thyroid stimulating hormone (TSH) and low thyroxine (T4). Ayangade, Oyelola and Oke (1982) found that in pregnant women the thiocyanate level of the cord blood was proportional to the maternal serum thiocyanate level, indicating that thiocyanate can cross the placental barrier and affect the foetus. However, there is very little thiocyanate in breast milk indicating that the mammary gland does not concentrate thiocyanate and so breast-fed infants are not affected.

In this context, in clinical studies on sodium thiocyanate in milk, negative effects on iodine metabolism were only observed at concentrations of 200–400 milligrams/litre (Vilkki and Piironen, 1962). Furthermore, in studies in normal euthyroid individuals no significant effects on thyroid function (T4, T3, TSH) resulted from consumption of 8 milligrams of thiocyanate in milk daily for 12 weeks (Dahlberg *et al.,* 1984) although serum and urinary levels increased. Conversely, the group with a (presumed) daily consumption of milk containing about 45 milligrams/litre had higher serum levels of T4 and lower T3 and TSH levels than a control group (Banerjee *et al.,* 1997). It should be noted that this last study was published only as a short communication and the level of reporting did not allow the group to conduct a critical evaluation.

From the foregoing it can be concluded that the groups likely to be at highest risk from thiocyanate exposure are iodine-deficient subjects. However, in one study in which iodine-deficient adults were given milk containing 19 milligrams thiocyanate/litre (controls 3.6 milligrams/litre) leading to an additional daily intake of 4.75 milligrams, there was no apparent effect on thyroid function (Dahlberg *et al.,* 1985). The milk used in this study contained iodine at a concentration of 100 micrograms/litre.

There were no experimental data available on the effects of dietary thiocyanate on reproductive function or on the genotoxicity of thiocyanate. Plasma thiocyanate concentrations can reach 100 milligrams/litre during sodium nitroprusside therapy, but toxicity often occurs at concentrations above 120 milligrams/litre. Plasma concentrations in the order of 200 milligrams/litre have been reported in fatalities.

A two-year chronic toxicity/carcinogenicity bioassay of sodium thiocyanate (alone or in combination with sodium nitrite) has been conducted in F344 rats. The animals received sodium thiocyanate at a level of 3.2 grams/litre in drinking water. The results of this study led to the conclusion that sodium thiocyanate is not carcinogenic to rats (Lijinsky and Kovatch, 1989).

The clinical symptoms of overt iodine deficiency during pregnancy as manifested in foetal development and growth of children have been known for more than eighty years. These include stillbirth, abortion and congenital anomalies (Hetzel, 1983; Mastovinovic, 1983). In recent years, research has revealed that iodine deficiencies during pregnancy, even in which overt maternal symptoms are lacking, can have an effect on the growing child, such as hearing deficits (Wang and Yang, 1985).

The normal levels of thiocyanate in milk depend on the levels of thiocyanate and its precursors in the animals' diet, including thioglycosides (glucosinolates) and cyanogenic glycosides. Concentrations have been reported to vary between 2.3 and 35 milligrams/litre in milk from individual cows and to be around 8 milligrams/litre in bulked milk (Ponce *et al.*, 2005). Higher levels occur in colostrum and in mastitis milk. Similar results were obtained for cow milk (6–12 milligrams/litre; mean 8.5 milligrams/litre) and goat milk (6.6–8 milligrams/litre; mean 7 milligrams/litre) (Fonteh, Grandison and Lewis, 2002). When used according to the Codex

guidelines, the level of supplementation of sodium thiocyanate in activating the LP-s is 10–15 milligrams/litre so that overall levels in activated bulk milk would be in the order of 20 milligrams/litre, a factor of 10–20 lower than those reported to lead to detected effects on iodine metabolism. A study of the thiocyanate concentrations in milk mixtures under practical conditions of the American tropics indicates that they oscillate between 5.8 and 8.12 milligrams/litre, although the levels in milk of individual cows vary widely, ranging from 2.9 to 34.8 milligrams/litre. That is why the total content of thiocyanate, once the LP-s is activated in a milk mixture, does not surpass the natural maximal concentration in any particular cow milk (Ponce *et al.*, 2005). Evidence of undesirable effects were not observed in the populations consuming milk activated with the LP-s for more than 10 years (Fernandez, Marrero and Capdevila, 2005).

Thiocyanate is found in animal and human tissue and fluids where it is part of the defensive system (e.g. high in colostrums and in milk of cows with mastitis) and is a metabolite of the detoxication process of cyanogenic glycosides. Thiocyanate is also present in foods of plant origin and it is formed in the human or animal body from substances in plants such as glucosinolates (in brassica an average 100 milligrams/kilogram) or cyanogenic glycosides. Thiocyanate is present in raw lima beans (100–3100 milligrams/kilogram), raw cassava tubers (10–462 milligrams/kilogram), raw cassava leaves (68–468 milligrams/kilogram), dried cassava root cortex (2450 milligrams/kilogram), almonds (6.2 milligrams HCN/bitter almond), bamboo shoots tips (8000 milligrams/kilogram), stone fruits and sorghum (2500 milligrams/kilogram) (FAO, 1990). Cyanides readily decompose upon heating, and cooked foods contain little or no cyanide, e.g. cooked cassava tubers had 1-10 milligram/kilogram depending on the cooking method and the initial content. Glucosinolates and glucosinolate breakdown products are hydrophilic, and as much as 63% of the glucosinolate content of a vegetable may leach into the cooking water during boiling (WHO, 1993).

The additional intake of sodium thiocyanate from one cup (200 ml) of LP-s treated milk would correspond to 3 milligrams of sodium thiocyanate which is also present in 30 grams of raw cabbage, 1 gram of raw lima beans or 8 grams of raw cassava tuber. When applying the food supply of the 13 GEMS/Foods regional diets (See Appendix D), exposure to sodium thiocyanate is estimated to be in the range of 2.8 to 9.5 milligrams/day. If all milk were treated with the LP-s the exposure would increase to 5.9 to 21.2 milligrams/day.

The highest potential risk from thiocyanate would arise with infants because of the high need for energy per kilogram bodyweight and the unitary diet. As an example, in a 10 kilogram infant, 500 millilitres of LP-s treated milk would result in 1 milligrams/kilogram body weight of sodium thiocyanate compared to 0.3 milligrams/kilogram body weight from untreated milk. The LD_{50} dose of orally administered sodium thiocyanate in rats, a measure of acute toxicity, is reported to be 764 milligrams/kilogram body weight (FAO/WHO, 1965). Clearly, acute toxicity is not a relevant aspect of exposure through the LP-s treated milk.

In non-smokers, plasma thiocyanate concentrations range from 0.1 to 0.4 milligrams/litre, while in heavy smokers concentrations typically range from 5 to 20 milligrams/litre (WHO, 1995). Thiocyanate is concentrated in other human body fluids, notably saliva and gastric juice, where levels typically range from 10 to 300 milligrams/litre (Björck, Claesson and Schulthess, 1979; Korhonen, 1980; Reiter and Härnulv, 1984; Farrag and Marth, 1992, Food Standards Australia and New Zealand, 2002).

3.3 Nutritional effects

The LP-s reduces losses of milk through microbial spoilage and can thus increase the volume of milk available as an important nutritional component of the diet. Although a reduction in folate levels in milk may occur as a result of LP-s treatment, milk is not considered to be a significant dietary source of folate and the overall dietary impact is not considered important.

3.4 Effects on milk-borne pathogens

Although LP-s may be effective to a limited degree against some pathogens, it should not be considered as an alternative to pasteurization in this regard. The effects on a number of pathogens are dealt with in more detail in section 2. There are no available data on the effects of the LP-s on milk-borne viruses, although some research has been undertaken on the impact of the LP-s on HIV-1 (Wang, Ye and Ng, 2000).

3.5 Conclusions and recommendations

Overall, the meeting considered the LP-s to be a safe method of preventing losses of milk owing to microbial spoilage when used according to the guidelines (and with an extended temperature range as recommended under 2.5) either alone or in combination with other approved procedures.

It was concluded that the advantages of the LP-s mainly result from significantly reduced spoilage losses of milk and thus improved availability of milk as a good nutrient source in the diet and benefiting both milk producers and consumers.

Milk improves health and reduces morbidity and mortality from childhood disease. Therefore, the application of the LP-s could be considered as part of a system to improve public health by increasing the availability and safety of milk.

Based on the available scientific information the meeting concluded that none of the components of the LP-s presents a significant toxicological risk to public health at the levels proposed. Nevertheless, where iodine deficiency is common, public health measures to rectify the iodine deficiency are needed whether or not the LP-s is used.

Based on the assessment, the LP-s is a safe method of raw milk preservation when implemented according to established guidelines (with an extended temperature range as recommended under 2.5); it can reduce milk losses which is a major benefit for both milk producers and consumers.

Based on the above the meeting recommended that:

- The LP-s be considered safe, when used according to the Codex guidelines, for use in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities and that it be applied as part of an integrated programme to improve milk production and quality.
- Milk consumption be promoted because of its value in human nutrition for healthy development and growth.
- Measures to rectify iodine deficiency be implemented in recognised IDD areas accompanied by appropriate monitoring of its prevalence. It was noted that milk could also be a valuable source of iodine, providing there is adequate iodine in the diet of the milk-producing animals.

4. PROCESSING AND TECHNOLOGY

Milk is recognised as a highly nutritious food and valuable source of vitamins and minerals. It is, however, highly perishable and has, in its raw state, a relatively short shelf-life. There are numerous processes for prolonging the shelf-life of milk and dairy products and an increasing array of technologies that can be applied to improve the safety and quality of milk.

While refrigeration and heat treatment of raw milk are also highly effective in and widely used for extending shelf-life, more advanced physical treatments are also evolving and being applied such as microfiltration and high pressure processing. The cost of these processes and associated technologies is relatively high as compared to the combination of heating and cooling such as in pasteurization processes (high temperature short time or low temperature long time). Also in many rural areas even the cost of cooling remains prohibitively high. The use of LP-s is not designed to replace adequate heat treatment, which kills harmful bacteria, but has the potential to increase the quality and quantity of raw milk available for further processing into dairy products.

The LP-s is one of the growing families of biostatics that can have beneficial effects in the processing of milk by extending the shelf life and improving the quality of milk collected or preserved. This section reviews the LP-s activation/inactivation and examines potential risks and benefits of the system.

4.1 Methods of activating the lactoperoxidase system

Addition of thiocyanate/peroxide

Thiocyanate ions (in the form of sodium or potassium salt) are the substrate for lactoperoxidase and are normally added to milk at a level of approximately 14 milligrams/litre, although this could be adjusted in relation to variation in levels in milk. This is followed by addition of peroxide, either in the form of hydrogen peroxide or sodium percarbonate.

Hydrogen peroxide would be added at a level of 1-10 milligram/litre. This dose is difficult to achieve accurately and could lead to detrimental overdosing. Hydrogen peroxide is unstable and also reacts with proteins, although the latter is unlikely to cause processing problems at this concentration. Therefore sodium percarbonate (30 milligrams/litre) is recommended by Codex as the source of peroxide ions, as it leads to slower release of the active agents.

Activation kits consisting of sachets of thiocyanate and percarbonate can be obtained from a range of companies at a cost of treatment of US$0.0025–0.01 per litre of milk, and are recommended for administration by trained personnel only. It should be noted that the majority of the cost arises from packaging that limits the range of package sizes, especially for small volumes of milk. Most kits are designed for use with 50 litre batches of milk, although kits for treatment of 500 to 10,000 litres are commercially available. The major problems associated with these materials are as follows:

i) thiocyanate is hygroscopic and may deteriorate with time, although this problem may be obviated by the use of coatings or hermetically sealed containers;
ii) some sources of thiocyanate do not comply with accepted quality standards[4];
iii) percarbonate may produce oxygen leading to 'blown' packets of activator.

Addition of glucose oxidase (1–2 milligrams/litre) to milk, following thiocyanate ions, has been demonstrated on a laboratory scale to activate the LP-s by conversion of glucose to gluconic acid and peroxide. There is usually sufficient glucose present in raw milk as a result of β-galactosidase action, particularly derived from yeasts, although addition of 2–3 grams/litre exogenous glucose is a further possibility. It is an expensive method and dose control at such low levels of addition would be very difficult.

Addition of lactic starter bacteria (catalase negative) could be used in milk for cheesemaking in cases where chemical additions were unacceptable. Use of 10^4–10^5 cells/millilitre is effective, for instance in combating psychrotrophic organisms.

Addition of microorganisms (introduced deliberately or inadvertently) such as yeasts or Corynebacteria can activate LP-s. The use of the latter following surface rinsing with thiocyanate has been shown to be effective in controlling *Listeria* on the surface of soft cheese. Autoinhibition by contaminating microorganisms may contribute to shelf-life extension in pasteurized milk and milk products where significant levels of activity remain following heat treatment (see section 4.2 below).

Leucocytes may activate LP-s through production of hydrogen peroxide, although their presence is obviously undesirable, reflecting mastitic infection.

Hydrogen peroxide residues from disinfectant solutions following cleaning of milk containers may also activate the system.

4.2 Thermal inactivation of the lactoperoxidase system

The kinetics of thermal inactivation of the lactoperoxidase enzyme are well established (e.g. Ramet, 2004; Barrett, Grandison and Lewis, 1999). In practical terms, batch pasteurization (e.g. 65 ºC/30 minutes) has little effect on enzyme activity, HTST pasteurization (72 ºC/15 seconds) results in retention of approximately 70% lactoperoxidase while treatment at 80 ºC or more (including conventional or UHT sterilisation) leads to complete destruction of the enzyme. It has been suggested (Marks, Grandison and Lewis, 2001) that this residual activity explains the fact that milk pasteurized at 72 ºC has a longer shelf life than milk subjected to 80 ºC, which has implications in cases where the milk industry may contemplate increasing severity of

[4] Purity criteria of thiocyanate has been specified by the Joint FAO/WHO Expert Committee on Food Additives (available from the JECFA database for food additives http://www.fao.org/ag/agn/jecfa-additives/search.html?lang=en)

pasteurization conditions. In fact, it is possible that residual lactoperoxidase plays a role in the keeping quality of pasteurized milk and dairy products generally.

Lactoperoxidase activity could be used as a marker enzyme for effectiveness of HTST heat treatment because of its similarity to phosphatase in terms of thermal inactivation. The official method involves estimation of phosphatase, although this is not useful in camel milk (Ramet, Abeideirrahmane and Ould Mohammed, 2004) where phosphatase remains active following heat treatment at 82–86 ºC for two minutes. A lactoperoxidase assay would clearly be more appropriate as a marker in the latter case.

4.3 Other approved methods of milk preservation

The major approved methods of milk preservation are refrigeration and/or heat treatment, although both methods have limitations with respect to processing.

Refrigeration

While refrigeration[5] is clearly very effective in inhibiting growth of bacteria, limited negative physical and chemical effects occur which could have small effects on processing parameters. The most important are solubilisation of β-casein, solubilisation of minerals, changes to fat crystallisation and alteration of the balance of bacteria in milk, with an increase in psychrotrophic organisms. Residual proteolytic and lipolytic enzyme activity coming from psychrotrophs following processing gives rise to problems including rancid or bitter off-flavours in products (especially cheese), gelation in UHT milk and gelation in reconstituted calf-feeding powders.

In some countries refrigeration is not feasible at some production sites because of the prohibitive cost (in terms of both initial investment and running costs), but also because of technical problems, such as the absence or unreliability of an electricity supply. The LP-s could be used as a complementary treatment where a power supply is unreliable.

Heat treatment

Obviously heating is the most effective way of destroying microorganisms and is applied to milk in treatments of varying severity (thermisation, pasteurization, sterilisation). Several negative chemical effects occur in products depending on severity of treatment. Whey protein denaturation leads to changes in functionality which can lead to problems owing to reduced syneresis of cheese curd, although high heat treatments are necessary to produce satisfactory yoghurt texture, where syneresis is undesirable. Attachment of β-lactoglobulin to κ-casein on the casein micelle surface at high temperatures results in milk with reduced ability to coagulate with clotting enzymes. Hence rennet cheese-making from sterilised milk is not possible. Heating of milk leads to the Maillard reaction (between proteins and reducing sugars) giving rise to browning reactions as a result of melanoidin formation, and also to 'cooked' off-flavours. Heating of milk gives rise to insolubilisation of calcium phosphate (and complexes with proteins) which leads to fouling of

[5] According to the Codex guidelines, milk for further processing should be cooled within two hours to or below 6 °C when collected on a daily basis, or to or below 4 °C when not collected every day (CAC, 2004b).

processing surfaces, and may require the heated milk to be supplemented with calcium salts before cheesemaking.

It should be noted that heat treatment is more effective if the initial cell counts are minimised before processing, hence application of the LP-s prior to heating provides a complementary, possibly synergistic, combination.

4.4 Effects of the lactoperoxidase system on organoleptic quality of milk and the manufacture of products

It can be surmised that use of the LP-s might lead to limited chemical changes to the milk – e.g. through oxidation of fat and proteins. Subsequent physical effects, combined with microbiological changes could lead to negative effects on organoleptic quality of milk and milk products, and the manufacture and texture of some products. However, a report from Ponce *et al.* (2005) indicates that such effects have not been observed in practice.

It has been found that enrichment of raw milk with reagents used for LP-s activation does not modify sensory properties of the treated milk compared to control milk (Ramet, 2004). The flavour of fermented goats' milk and cheese may actually be improved as a result of the action of the LP-s changing the balance of microflora (Seifu, Buys and Donkin, 2005).

There is a clear potential for inhibition of lactic starters due to lactoperoxidase activity, resulting in reduced acid production and coagulation problems with acid-gelated products. In addition, interaction of lactoperoxidase with sulphydryl groups of proteins could alter texture of gelled products – e.g. reduction in β-lactoglobulin/κ-casein interaction in yoghurt. Evidence for these phenomena is mixed. Evidence from Latin American studies suggests that the LP-s has no negative effects on the quality of cheese and fermented products when milk has been subjected to adequate heat treatment following the use of the LP-s (Ponce *et al.*, 2005). Ozer *et al.*, (2003) reported some limited effects of LP-s activation on yoghurt gel texture, while Revol-Junelles and Milliere (2005) and Seifu, Buys and Donkin (2005) reviewed the topic and found some evidence of slower rennet clotting and weaker gels in cheese, and lower acid production in yoghurt. However, the effects were generally very limited and reports are not consistent.

The sensitivity of the lactic acid starter bacteria to LP-s action mainly depends on the susceptibility of the specific strains. Susceptibility can be categorised into three groups as follows (Seifu, Buys and Donkin, 2005; Guirguis and Hickey, 1987):

- The most sensitive group of organisms which generate hydrogen peroxide, e.g. *Lactobacillus acidophilus, Lactobacillus bulgaricus*;
- Organisms that are sensitive but do not have the ability to generate hydrogen peroxide and thus require an exogenous source of hydrogen peroxide e.g. *Lactobacillus helveticus, S. thermophilus*;
- Organisms resistant to inhibition e.g. *Lactococcus lactis*.

In summary, it is concluded that any effects of LP-s activation on processing of milk are quite limited. There is no evidence that LP-s activation results in any serious negative effects. It should be emphasised that using the LP-s to maintain the microbiological status of milk for processing should lead to superior product quality and this has been borne out by some of the FAO field trials on different fermented products (FAO, 2004a).

4.5 Other methods of microbiological control

Microfiltration is used in some countries to reduce bacterial populations prior to pasteurization. It is feasible that it could be used as a "stand-alone" technique in the future. The process has the benefit that it is a purely physical treatment based on membrane filtration, which could circumvent many of the disadvantages of heat treatment. A disadvantage is that the diameters of fat globules and microorganisms are similar such that microfiltration is limited to skimmed milk, which can subsequently be remixed with heat treated fat-rich streams, if required. Microfiltration has also been proposed as an alternative solution to the health risks in manufacture of cheese from raw milk. However, it is unlikely that microfiltration will be adopted at present in countries where refrigeration is not routinely carried out because of technical complexities and higher costs.

High-speed centrifugation has been applied to reduce bacterial cell and spore counts in milk prior to hard and semi-hard cheesemaking. Again this is a physical process, but is unlikely to be adopted at present in developing countries because of technical complexities.

High pressure processing (400–800 MPa) has the potential to inactivate microorganisms in milk and alter the protein functionality. This has not been applied commercially.

Addition of lysozyme chlorohydrate (derived from eggs) is a permitted treatment to prevent "blowing" because of outgrowth of clostridium spores during ripening of hard and semi-hard cheeses. However, this is a limited application.

Addition of high levels of sodium chloride (3–12%) reduces water activity (A_w) of milk sufficiently to arrest bacterial growth. The technique is employed in some middle-eastern countries in the traditional manufacture of local brined cheese. Although it is a traditional process, there are many negative effects including very salty taste, micelle disruption, coagulation problems and corrosion of processing equipment. Hence the application is extremely limited.

4.6 Impact of the adoption of the lactoperoxidase system on the use of non-approved methods of milk preservation

A number of non-approved milk preservation methods are applied in some countries, including:

- Addition of high (300–800 milligrams/litre) levels of hydrogen peroxide, which leads to a direct bactericidal effect, but causes problems in processing because of disruption of proteins, and from a nutritional perspective it reduces the levels of vitamin A and carotenoids.
- Direct addition of antibiotics.
- Addition of ice (from water which may be contaminated), which clearly dilutes the milk.
- Transfer of chemicals from burnt wood containers to the milk.
- Alkalisation with sodium hydroxide or calcium dihydrate.
- Addition of other chemicals, including formalin or chlorine.

It is clear that lack of, or limited effectiveness of quality control procedures in developing countries leads to lack of detection of these non-approved methods. While adoption of the LP-s has the potential to reduce the use of these non-approved methods, and hence reduce potential risk to consumer health, there is currently little available evidence to illustrate this. However, evidence from extensive studies in Cuba and Latin American countries (Ponce, 2005) suggests that use of LP-s activation has reduced the utilisation of some of the non-approved practices mentioned above.

4.7 Conclusions and recommendations

The meeting concluded based on numerous observations from laboratory and field studies that the LP-s does not induce adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. Therefore, LP-s is an efficient alternative for preservation of raw milk that will be subjected to further processing. It does not preclude the need for pasteurization and does not negatively impact on, or interfere with, subsequent processing.

The LP-s can be used alone when refrigeration is not available, or in synergy with cooling or chilling and can be considered to be an efficient tool to improve the quality and quantity of milk and dairy products by maintaining the microbiological quality of raw milk.

Considering that the LP-s is technically considered as an effective method of milk preservation for further milk processing the meeting recommended that:

- The LP-s be considered as suitable to extend milk collection distances particularly in developing countries and thereby increase the amount of milk available for further processing and subsequent marketing.
- The LP-s is be used to improve the quality of processed products because of its proven bacteriostatic effect from milk collection to final processing.

5. ECONOMIC VALUE AND TRADE

In addition to the nutritional benefits of milk and its contribution to household food security, particularly in developing countries, dairying can also provide a major contribution to income generation. This is particularly important in areas where up to 80% of the total milk marketed goes through informal channels.

Refrigeration is the preferred means of milk preservation but requires high capital investment and can incur high running and maintenance costs. Use of LP-s is a reliable and economical method of preserving raw milk as compared to cooling in small-scale dairy enterprises, coupled with good hygiene and sanitation.

There is increasing regional and international trade in milk and dairy products from countries which were, in the past, net milk importers. Regional standards and equivalence are therefore of increased importance, particularly due to regional trade blocks and global trade agreements.

5.1 Current Situation

In 2004, the total world milk output was 613 million metric tons of which 263 million metric tons was produced by developing countries – contributing about 30% share of the total world milk production, with small dairy farmers contributing about 70% of the total (NDA, 2004). The small farmer contribution to milk production may be conservative considering their share of the informal market. There was a 10.4% growth in global sales of milk and milk products recorded in 2003 (NDA, 2004). The group noted that one of the contributing factors is the rapid growth of emerging markets such as China, the Philippines and Saudi Arabia.

In 2003, FAO conducted a rapid appraisal of milk post harvest losses in five countries, including the Near East and Eastern Africa (FAO, 2004b). In Kenya, for example, the study found that a total of 15.4% of milk was lost at the farm and market level. The total national loss was estimated at 95 million litres, valued at about US$22.4 million. The losses at farm level are equivalent to US$15.4 million. Viewed against the poverty level where almost 60% of the population survive on less than US$1 a day, the loss at farm level alone is equivalent to the annual salary for 32,000 rural wage earners on US$40 per month (FAO, 2003).

Although milk production costs are low in developing countries, there can be high milk losses where ambient temperatures are high and the milk market chain lacks infrastructure and resources for refrigeration, and where there are problems with electricity supply. The World Bank estimates that 20% of milk in developing countries is wasted. The opportunity to increase milk production and create additional income to farmers is also constrained by limited capacity for market absorption, lack of facilities to store milk (morning and evening milk) and difficulties to deliver milk on time to processing plants/collection centres.

Milk prices range from US$13 to US$50 per 100 kilograms, with a total cost of production from US$18 to US$28 per 100 kilograms of milk (IFCN, 2002). Due to low input production systems and the exchange rates, cost of milk production and milk prices are lower in developing countries. According to the FAO Dairy Outlook (FAO, 2002) the farm gate prices of milk were highest in Japan and lowest in developing countries such as Kenya, Malawi, Pakistan and Colombia (Table 3).

Table 3. Farm gate prices (cows milk) in US$/kilogram (October, 2002)

Range US$	Country (Price US$ per kilogram)
0.61 – 0.70	Japan (0.62)
0.51 – 0.60	Switzerland (0.53)
0.41 – 0.50	Mauritania (0.42)
0.31 – 0.40	Malta (0.37), Canada/Italy/Mauritius (0.35) France/Ireland/Germany (0.33), Sweden (0.31)
0.21 – 0.30	Costa Rica/Thailand/USA (0.28), Philippines/UK (0.27) Ecuador/Netherlands (0.26), Egypt (0.24), Nepal (0.22)
0.11 – 0.20	Kenya/Malawi (0.20), Pakistan/Colombia (0.18)

Source: *Calculation from FAO Dairy Outlook* (Muriuki, 2002)

Preserving milk using the most practical and economical method while maintaining its initial quality is deemed necessary to increase total milk production and marketing. This is especially relevant to developing countries through the reduction of post harvest losses of milk, promoting afternoon milking collection and the capture of more milk volume from informal markets.

5.2 The cost of refrigeration and the lactoperoxidase system

When considering the cost effectiveness of the LP-s, it should be borne in mind that it is difficult to compare with other methods applied throughout the world because costs, such as energy, vary widely and have increased significantly in recent years. It is important that such an evaluation be done on a case-by-case basis.

In the Philippines, initial investment in small-scale chilling equipment is between US$3000 and US$5000, and with the on-going cost of electricity it would not be viable to operate such equipment in a cooperative society with a 100 litre per day collection. In 1994, the total cost to cool 100 litres of milk was approximately US$0.5 compared to US$0.35 if the LP-s is applied. LP-s preservation is cheaper and does not require a large outlay for equipment and cooling facilities (Barraquio *et al.*, 1994).

In Kenya, the cost of cooling a litre of milk ranged from US$0.017 (large scale coolers) to US$0.032 (small scale) while LP-s application was lower at US$0.014 (Wanyoike *et al.*, 2005). However, large scale milk cooling is not a solution to the problem considering the high cost of equipment, from US$197,000 to US$4 million, in addition to maintenance costs and the costs of milk collection.

In Cuba, more than 50% of the milk is not refrigerated due to, among other reasons, the high cost of cooling equipment and lack of electricity. However, the use of the LP-s has allowed significant quantities of milk, valued at US$100 million over 13 years, which would otherwise have been lost, to enter the food chain. The LP-s has proved to be effective in the dairy chain in maintaining the initial quality of the milk from the farm level through to the dairy plant. In Latin America, 30 million litres of milk was activated using the LP-s between 2000 and 2005. Fifty percent of the milk that would otherwise be lost is saved through the LP-s, amounting to a value of around US$3 million. In the Latin American region the cost of cooling a litre of milk can range from US$0.05 to US$0.1 per litre compared to a cost of US$0.0025 to US$0.05 per litre for LP-s application, again without considering the large capital outlay for investing in the cooling equipment and its maintenance.

The cost of using the LP-s compares favourably with that of cooling, particularly for smallholder dairy farmers. It has been shown that the LP-s is more cost effective than cooling in areas where milk quantities are small or there is irregular or no power supply. This is also the best way to improve the flow of milk from the farm to markets thereby creating additional income for dairy households.

5.3 International trade

Although milk production costs in the developing countries are lower than in developed countries, the developing countries have been net importers of milk and dairy products. However, this is slowly showing signs of change with some development of regional trade, for example among a number of the regional trade blocks in Africa including the East African Community (EAC), Common Market for Eastern and Southern Africa (COMESA), Inter-Governmental Authority on Development (IGAD) and the Southern African Development Community (SADC). Due to increased international trade in countries like Kenya in the EAC and South Africa in the SADC area, there is need for harmonisation in milk and dairy product standards to facilitate trade. Most of these countries have their national standards based on the Codex standards. It is therefore easy to harmonise their standards, although it is important that in the development of Codex standards, regional differences are taken into consideration if the standards are to continue to be of relevance to those countries.

It is difficult to estimate the loss in trading opportunities as a result of the Codex provision that the LP-s should not be used for products intended for international trade. However, the issue is not only related to trade, but also that the LP-s is not adopted in the first place because of a fear of being excluded from international markets. If products treated with the LP-s are not considered

suitable for international trade then this raises doubts as to whether it is appropriate and safe to use for milk and dairy products in the domestic market. Despite this, the LP-s is applied in some countries where it is the most practical option for raw milk preservation. Kenya, for example, exports dairy products worth over US$4 million (2003 estimate) to the immediate region, and this is rising. This is the trade that could potentially be lost if they were to officially adopt the use of the LP-s and abide to the condition of not trading the milk treated with the LP-s. The meeting noted that it is likely that similar situations exist in Africa, Latin America and other developing countries.

5.4 Dairy standards, policy and the lactoperoxidase system

The standards developed by the Codex Alimentarius Commission are, under the WTO SPS agreement, the recognised international benchmark standards for food safety. Codex has developed a number of standards for milk and dairy products. These standards inform many of the dairy standards adopted in both developed and developing countries. National governments adopt or modify these standards depending on their national needs and dairy development policy and implementation strategies. It is important that developing world conditions are borne in mind in standard development. This would contribute to the ease with which standards are understood and can be adopted by governments and adapted under prevailing conditions within the national legal framework governing the dairy industry and milk and dairy products.

Smallholder dairy farmers play an important role in the supply of fresh milk and dairy products to growing urban centres in developing countries. To ensure the supply of the quantity of milk needed, dairy development policies need to have a choice of suitable options for milk preservation, which can be adopted by the national milk industry (Muriuki *et al.*, 2003). There are currently only two Codex approved means of preserving raw milk, i.e. refrigeration and the lactoperoxidase system of raw milk preservation. The LP-s is recognised as a cost efficient means of raw milk preservation and can be effective in reducing milk losses and expanding milk collection systems. In addition, it also appears to have significant potential for use with refrigeration as a complementary means of milk preservation. The consideration of the use of the LP-s within a national dairy development policy and strategy is therefore essential to meet the needs of producer groups, milk collectors and processors, particularly in developing and transitional countries where refrigeration is not an immediately feasible and practical option.

5.5 Economic value and impact

The World Bank reported that in West Africa approximately 5 million litres of milk is thrown away annually due to spoilage. Cuba has reported that the use of LP-s system has produced a wide range of benefits over a 13-year period. It has enabled them to get total volumes exceeding 1000 million litres of milk into the market. A conservative estimate indicated that the use of the LP-s has prevented the loss of approximately 50,000 tons of milk, which is equivalent to the annual dairy imports for the country in foreign currency. In addition it has led to the creation of

employment and improvement in dairy farmers incomes (P. Ponce, personal communication, 2005).

A functional system of raw milk preservation can stimulate increased milk production to be benefit of both producers and consumers. In a country like Kenya, milk production fluctuates between seasons and, mainly only the morning milk gets into the market chain. During a high production season, there are very high milk losses due to collection logistics, exacerbated by lack of preservation systems. Evening milk is not collected due to a lack of feasible preservation systems. It has been estimated that the total amount of marketed milk would increase by about 30% through collection of evening milk. This would translate to an annual increase of over 100 million litres. An FAO study (FAO, 2005) however estimated a lower level of losses. A conservative estimate by Muriuki (H. Muriuki, personal communication, 2005) is that there would be an increase of 68 million litres of milk from market growth.

Milk markets usually pay a premium for quality milk. In Kenya, the processors pay about US$0.06 per litre for high quality milk over the going standard milk price. An increase in marketed milk, especially from the smallholder sector, would also improve livelihoods through employment, increased incomes and improved nutrition. Other issues that will need to be addressed with an increase in marketed milk include whether this will take milk away from home consumption and whether it will shift incomes from women to men. In some communities, income from milk sold within the immediate neighbourhood is controlled by women and the income from the formal sector is controlled by men.

5.6 Availability of the lactoperoxidase system components

Most countries with pharmaceutical facilities have the capacity to produce activators as long as they meet the specifications stipulated in the Codex guidelines and account for the purity and hygroscopic nature of percarbonate. Currently, only a few countries produce the LP-s activators, such as Sweden, Cuba and France. It would be expected that the LP-s would be more economical if the activators were made in the countries applying the system. The cost of packaging also needs to be considered given that the package alone constitutes around 40–60% of the total cost of the product.

5.7 Conclusions and recommendations

Economic benefits of dairying include household income generation that can be a major contribution to regular income and household food security and nutrition, particularly for vulnerable groups, e.g. children and women, in developing countries. Small-scale dairy production, collection, processing and marketing are a major source of off-farm rural employment. Nevertheless, post harvest losses are a major issue in dairying in developing counties. Smallholder dairy farmers could increase their participation in worldwide milk production, processing and marketing if they could reduce their losses using any approved milk preservation method. The potential increase in the quality and shelf life of milk and dairy

products may have a considerable social and economic benefit at local level. While refrigeration is the preferred means of milk preservation it does require high capital investment and can incur high running and maintenance costs for expensive equipment. Thus the use of the LP-s provides a reliable and economical alternative for preserving raw milk, particularly in small-scale dairy enterprises when coupled with good hygiene and sanitation. Its economically viablity, either as a standalone system or in combination with refrigeration, and its potential to significantly reduce milk losses and thereby increase the amount of milk collected leads to direct benefits for both milk producers and consumers.

There is increasing regional and international trade in milk and dairy products from countries which were, in the past, major milk importers. With an increasing demand and milk production growth in developing and transitional countries, regional standards are of growing importance coupled with proper hygiene and sanitation practices along the dairy chain. Such standards are often based on Codex standards as these are considered the benchmark standard under WTO for foods in international trade. However, the provision relating to the use of the LP-s makes this somewhat of an exception and is an important limitation to the adoption of the system because of the potential of being shut out of regional and international trade in these products.

Based on these conclusions the meeting recommended that:

- Small-scale dairying be promoted given its contribution to household nutrition, food security, and poverty alleviation.
- Codex Alimentarius develop milk and dairy product standards that can be easily adopted at regional or national level. Active participation of a representative range of country members should be supported in the development of standards.
- The current Codex limitation related to the use of LP-s in milk or dairy products intended for international trade be removed.

6. OVERALL CONCLUSIONS AND RECOMMENDATIONS

The meeting sought to take a holistic approach to its review of the LP-s as a system of raw milk preservation taking into consideration the relevant microbiological, human health and nutrition, processing and technology and economic value and trade aspects.

The antimicrobial activity of the LP-s against a wide variety of milk spoilage and pathogenic microorganisms including bacteria, viruses, moulds, yeasts, mycoplasma and protozoa has been well documented in both laboratory and practical settings. The overall activity is primarily bacteriostatic, the extent of which is dependent on the initial total bacterial load, species and strains of contaminating bacteria and the temperature of milk. While its effectiveness against well-known milk spoilage and pathogenic microorganisms is well established, it was concluded that further studies would be useful on the efficacy of the LP-s against milk-borne viruses and emerging pathogenic microorganisms.

The efficacy of the system in raw milk from different species and under different ambient conditions was also considered. The Codex guidelines focus on the application of the LP-s to cow and buffalo milk. However, the meeting concluded that the same time-temperature combination as outlined in the Codex guidelines (CAC, 1991b) can also be applied to goat and sheep milk. The LP-s has also been shown to be effective in camel milk although the presence of other antimicrobials in this milk mean that a different pattern in terms of the level of activity at various temperatures may be observed.

An important consideration of the meeting was the impact of the LP-s on pathogenic microorganisms in milk. Based on the available evidence the meeting concluded that the LP-s does not promote the growth of pathogenic microorganisms after completion of the bacteriostatic effect[6] and there is no evidence to show that the long-term use of the LP-s would lead to any such microbiological risks, e.g. development or accumulation of toxin-producing bacteria. Furthermore, the meeting concluded that the application of the LP-s is not likely to stimulate the development of resistance to the LP-s itself or other antimicrobial agents but due to the dynamic nature of microorganisms ongoing monitoring of the situation would be reasonable.

The meeting gave particular consideration to data on the effectiveness of the LP-s at time-temperature combinations outside those outlined in the Codex document. It concluded that the LP-s also has a positive impact on the keeping quality of raw milk at ambient temperatures of 31–35 ^{o}C albeit only for 4 to 7 hours. Nevertheless, this was considered important as it may mean the difference in terms of getting milk to a refrigerated collection point in a good condition particularly in areas of warm or very warm ambient temperatures. The impact of the LP-s at refrigeration temperatures was also considered, especially the ability of the system to minimise the growth of psychrotrophic bacteria. The effectiveness of the LP-s at lower temperatures led the

[6] Under laboratory conditions.

meeting to conclude that the application of the system could be broadened to extend the period of refrigerated storage of raw milk.

The kinetics of thermal inactivation of the lactoperoxidase enzyme are well established and the time and temperature of heat treatment will determine the level of destruction of the lactoperoxidase enzyme. The meeting noted suggestions that residual lactoperoxidase activity plays a role in the keeping quality of pasteurized milk and dairy products generally. With regard to further processing of milk it was noted that while there is the potential for the LP-s to have an impact on the organoleptic quality of milk and the manufacture of products, this has not been observed in practice. Numerous observations from laboratory and field studies indicate that the LP-s does not induce adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. In considering the potential impact of the LP-s on fermented products it was noted that the data on this issue was somewhat inconsistent, which appears to relate to the difference in susceptibility of the various starter culture strain to the LP-s. Where negative effects have been reported they were limited.

The meeting concluded that the LP-s has a role to play as part of an integrated system to improve milk quality and safety. It was strongly emphasised that the LP-s cannot be used to disguise poor quality milk and that the system is most effective when implemented in conjunction with good hygienic practices. While cooling and heat treatment are well recognised as effective means of milk preservation, and numerous other systems are used on a smaller scale or being developed, the expansion of milk production particularly in developing countries where appropriate infrastructure and equipment for cooling, heat treatment or other physical processes are not always possible, means that it is important that cost effective alternatives are available. The application of naturally occurring preservation systems, of which the LP-s is one, is an area that is currently being widely investigated for application in a range of different foods and at different points in the food chain. Their application is not being considered as a replacement of existing well serving technologies, such as cooling and heat treatment, but to provide complimentary alternatives, particularly at the primary production stage when the other approaches are not available, feasible or suitable.

In this context, the meeting considered that the LP-s provides a real alternative in terms of short-term raw milk preservation. The fact that it can be used without any expensive infrastructure or equipment makes it a potentially viable option especially for many small rural milk producers. The ability to extend the shelf-life of raw milk, in a regulated way, is critical to ensuring that safe milk is made available for consumers and there is an economic benefit for the small dairy holder. Extension of the shelf-life of raw milk can ensure that it is still in a good condition when it reaches the processing facility despite long distances or poor transport infrastructure under warm or very warm ambient conditions. Milk losses are reduced again benefiting both producer and consumer.

Noting the increasing regional and international trade in milk and dairy products from countries which were, in the past, major milk importers and the increasing demand and milk production

growth in developing and transitional countries, the meeting emphasised that the implementation of standards that fulfil obligations under the WTO agreements are of growing importance. Such standards are often based on Codex standards as these are considered the benchmark standard under WTO for foods in international trade. However, the provision relating to the use of the LP-s makes this somewhat of an exception and is an important limitation to the adoption of the system because of the potential of being shut out of regional and international trade in these products.

In this context the health and nutritional aspects of milk, particularly milk that had been subjected to the LP-s was considered. In terms of human health and nutrition it was firstly concluded that the advantages of the LP-s mainly result from significantly reduced spoilage losses of milk and thus improved availability of milk as a good nutrient source in the diet and benefiting both milk producers and consumers. Milk improves health and reduces morbidity and mortality from childhood disease. Therefore, the application of the LP-s could be considered as part of a system to improve public health by increasing the availability and safety of milk. The meeting reviewed the available toxicological data on the LP-s and confirmed the evaluation of the 35th JECFA that the LP-s does not present a toxicological hazard when implemented according to established Codex guidelines. The meeting also noted that very few new data have become available since the JECFA evaluation. Nevertheless, the meeting recognised the significance of iodine deficiency and emphasised that where iodine deficiency is common, public health measures to rectify this situation are needed whether or not the LP-s is used.

Overall the meeting concluded that the LP-s has numerous advantages to offer when used as part of an integrated system to improve milk quality and safety, reduce milk losses and enhance its availability. Based on the available data and an evaluation thereof, the technical meeting considered the LP-s to be a safe method of raw milk preservation. When implemented according to established Codex guidelines the meeting concluded that there is currently no scientific basis for continuing the provision related to the limitation on the international trade of LP-s treated milk and dairy products.

Recommendations

In making its recommendations the meeting reiterated the safety of the lactoperoxidase system of raw milk preservation when used according to the existing guidelines (CAC 13/91), recommending its use in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities. Based on its deliberations the following specific recommendations were made.

To Codex

Consider expanding the guideline for the use of this system with regard to temperature of application of the LP-s to also include the temperature range from 31 ºC to 35 ºC for 4–7 hours and down to 4 ºC for 5–6 days.

Develop milk and dairy product standards that can be easily adopted at regional or national level through the encouragement and support of active participation of a representative range of country members in the development of standards.

Remove the current provision regarding the restriction on the use of LP-s in milk or dairy products intended for international trade as the meeting found no scientific or technical basis or economic justification for the provision.

To member countries, FAO, WHO, Codex, NGOs and the dairy industry

Acknowledge the LP-s as an effective and feasible method of raw milk preservation that does not display a negative impact on the further processing of milk.

Owing to its bacteriostatic effect, give consideration to the application of the LP-s as part of a programme to improve milk hygiene and safety along the milk chain.

Consider the application of the LP-s to complement cooling in order to extend the keeping quality of raw milk and halt proliferation of milk spoilage and pathogenic microorganisms.

Use the LP-s to improve the quality of processed products based on its proven bacteriostatic effect from milk collection to final processing and in particular to extend milk collection distances in developing countries, thereby increasing the amount of milk available for marketing. This can have significant direct benefits for both milk producers and consumers.

Recognise that the use of the LP-s is an economically viable option (either standalone or in combination with refrigeration) to significantly reduce milk losses and increase milk availability.

In addition to those recommendations specific to the use of the LP-s a number of other related issues were discussed, based on which the technical meeting made the following recommendations.

Promote the consumption of milk as a valuable source of human nutrition contributing to healthy development and growth.

Promote the contribution of small-scale dairying to household nutrition, food security, and poverty alleviation.

Implement measures to rectify iodine deficiency in recognised IDD areas accompanied by appropriate monitoring of its prevalence. Milk can also be a valuable source of iodine, providing there is adequate iodine in the diet of the milk-producing animals.

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APPENDIX A – Papers submitted in response to the FAO/WHO call for data

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APPENDIX B – Additional background papers made available in the course of the meeting

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APPENDIX C - Summary table comparing LP-s, refrigeration and the combination of LP-s with refrigeration

	Safety	**Microbiological performance**	**Applicability**	**Cost/benefit**
LP-s	No safety concern for public health when used in accordance with the Codex guidelines.	1. Primarily bacteriostatic for many milk-borne and other human pathogenic microorganisms. 2. Maintains initial milk quality for 4–7 hours (at 30 to 35 ºC) and up to 24–26 hours at 15 ºC. 3. Does not improve milk quality. 4. No long-term microbiological resistance expected.	Milk of all species. May interfere with fermentation when milk is not adequately heat treated. No significant adverse effects on the chemical, physical or sensory characteristics of raw milk and dairy products.	1. Low start-up and maintenance costs. 2. No energy requirements. 3. Can be applied in areas where refrigeration is not a viable option. 4. May increase availability of milk and dairy products. 5. Requires appropriate training of personnel for use.
Refrigeration	No safety concern for public health.	1. Primarily bacteriostatic for many milk-borne and other human pathogenic microorganisms. 2. Maintains initial milk quality for several days (depending on temp. of refrigeration and initial microbial quality of milk). 3. Does not improve milk quality	Milk of all species. Limited negative physical and chemical effects.	1. Extends keeping time of milk by several days. 2. Nothing added to milk. 3. Requires electricity. 4. Relative high cost for initial investment and maintenance.
Refrigeration with the LP-s	No safety concern for public health when used in accordance with the Codex guidelines.	1. Primarily bacteriostatic for many milk-borne and other human pathogenic microorganisms. 2. Maintains initial milk quality for 5–6 days at 4 ºC. 3. Does not improve milk quality. 4. No long-term microbiological resistance expected.	Milk of all species.	1. Increases shelf-life of milk and dairy products as compared to refrigeration alone 2. Minimal increase in cost.

APPENDIX D - Thiocyanate exposure based on the GEMS/Food regional diets both with and without lactoperoxidase treated milk

Thiocyanate exposure without LP-s using food supply of GEMS/Food regional diets in milligrams/year

GEMS/Food Consumption Cluster Diets[7]	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**
Brassica vegetables	87.8	1001.5	379.1	1761.9	1267.1	1125.5	1071.2	203.5	492.0	80.4	173.3	2240.2	814.4
Tomato	19.6	370.0	236.0	121.4	63.2	74.4	47.1	63.1	29.9	25.0	71.2	19.9	180.5
Cassava	971.2	0.0	0.1	0.0	0.0	0.0	62.4	96.5	685.3	1128.7	230.9	79.2	2.6
Lima beans, dry	0.0	4.8	4.7	17.4	0.0	0.0	9.5	32.9	0.0	0.0	0.0	15.4	2.9
Milk only	344.2	953.3	396.6	1512.6	898.0	1189.4	330.1	604.0	408.1	511.6	1038.4	285.5	1439.7
Total thiocyanate exposure (milligrams/year)	1422.9	2329.7	1016.5	3413.4	2228.3	2389.3	1520.3	999.9	1615.3	1745.7	1513.9	2640.2	2440.1
Total thiocyanate exposure (milligrams/day)	**4.0**	**6.5**	**2.8**	**9.5**	**6.2**	**6.6**	**4.2**	**2.8**	**4.5**	**4.8**	**4.2**	**7.3**	**6.8**

Thiocyanate exposure adding LP-s using food supply of GEMS/Food regional diets in milligrams/year[8]

Brassica vegetables	87.8	1001.5	379.1	1761.9	1267.1	1125.5	1071.2	203.5	492.0	80.4	173.3	2240.2	814.4
Tomato	19.6	370.0	236.0	121.4	63.2	74.4	47.1	63.1	29.9	25.0	71.2	19.9	180.5
Cassava	971.2	0.0	0.1	0.0	0.0	0.0	62.4	96.5	685.3	1128.7	230.9	79.2	2.6
Lima beans, dry	0.0	4.8	4.7	17.4	0.0	0.0	9.5	32.9	0.0	0.0	0.0	15.4	2.9
Milk only	1307.9	3622.5	1507.2	5747.9	3412.3	4519.6	1254.3	2295.0	1550.7	1944.0	3945.8	1084.9	5470.7
Total thiocyanate exposure* *incl. 100% LP-s treated milk (milligrams/year)	2386.6	4998.9	2127.1	7648.7	4742.6	5719.5	2444.5	2691.0	2757.9	3178.2	4421.4	3439.5	6471.2
Total thiocyanate exposure*	**6.6**	**13.9**	**5.9**	**21.2**	**13.2**	**15.9**	**6.8**	**7.5**	**7.7**	**8.8**	**12.3**	**9.6**	**18.0**

[7] For complete list of country assignment codes (listed A-M above) see http://www.who.int/foodsafety/chem/gems/en/index1.html

[8] Mean exposure of sodium thiocyanate has been estimated by multiplying the mean consumption of the 13 GEMS/Food regional diets with the mean concentration in selected foods.

APPENDIX E - Food supply according to GEMS/Food regional[9] diets in kilograms/year

CODE	GEMS	NOTES	A	B	C	D	E	F	G	H	I	J	K	L	M	Sodium thiocyanate or HCN in milligram/kilogram
VB 40	BRASSICA[10] VEGETABLES	(14)	2.2	25.0	9.5	44.0	31.7	28.1	26.8	5.1	12.3	2.0	4.3	56.0	20.4	40[11]
VO 448	TOMATO[12]	(9)	9.8	185.0	118.0	60.7	31.6	37.2	23.5	31.6	15.0	12.5	35.6	9.9	90.3	2
VR 463	CASSAVA[13]	(1)	242.8	0.0	0.0	0.0	0.0	0.0	15.6	24.1	171.3	282.2	57.7	19.8	0.7	4[14]
VD 534	LIMA BEAN (DRY)[15]		0.0	0.2	0.2	0.7	0.0	0.0	0.4	1.3	0.0	0.0	0.0	0.6	0.1	25[16]
ML 106	MILKS[17]	(1) (2)	68.8	190.7	79.3	302.5	179.6	237.9	66.0	120.8	81.6	102.3	207.7	57.1	287.9	5 (19 with LP-s
AO 31	**TOTAL MILK AND MILK PRODUCTS**		70.5	223.4	87.9	317.4	249.7	301.4	66.6	136.2	85.6	103.5	211.7	63.9	333.2	

[9] For complete list of country assignment codes (listed A-M above) see http://www.who.int/foodsafety/chem/gems/en/index1.html
[10] Food Standards Australia and New Zealand, 2002
[11] Cooked (60% leaking into cooking water).
[12] Tonacchera, *et al*., 2004
[13] WHO, 1993
[14] Cooked (1% of raw)
[15] WHO, 1993
[16] Cooked (1% of raw)
[17] Introduction 1.1 of document CAC/GL 13-1991 (CAC, 1991b)

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