codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD HYGIENE

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REQUEST FOR ADDITIONAL INFORMATION REGARDING THE POTENTIAL RISKS IN RESPECT OF THE LACTOPEROXIDASE SYSTEM

In response to CL 2007/31 – FH, Cuba, Canada, the United States of America and Argentina provided additional information regarding the potential risks in respect of the lactoperoxidase system.

CUBA

Information from Cuba in response to Document CL 2007/31-FH issued by the Secretary of the *Codex Alimentarius* Commission: Request for Additional Information Regarding the Potential Risks with Respect to the Lactoperoxidase System

Background

Cuba has 25 years of experience researching the subject of the lactoperoxidase (LP) system and 15 years of its continuous use under the practical conditions of the dairy industry in the country, covering a third of the annual production of milk. Cuba has also obtained results from more than 30 countries around the world, with emphasis in Latin America and the Caribbean. The activation of the LP system is carried out by means of an activating product, which makes the procedure simpler and safer. Next, we present information obtained in the last couple of years (2006-2007), as well as technical comments on those aspects that have been the subject of discussion and concern for some countries.

Thiocyanate toxicity

A series of studies was conducted on the content of thiocyanate in milk, which allowed defining the basic elements regarding toxicity and safety under American tropic conditions:

There is a wide variation in the thiocyanate concentration in milk of individual cows, with a range of 0.04 mmoles/L up to 0.62 mmoles/L. The mean concentration of the thiocyanate ion in mixed milk was 0.141 mmoles/L, and has a much more narrow variation, with a range between 0.08 mmoles/L and 0.21 mmoles/L. The most common concentration, observed in various countries of the region, was found to be between 0.13 and 0.15 mmoles/L. The factors found to increase the content of thiocyanate in milk were the following: feeding with star grass *(Cynodon nlemfuensis)* fertilized with nitrogen, cows from rustic breeds and with low

milk production, presence of mastitis, the colostrum period and old cows. The time of year had no significant impact.

The exposure to annual intake of thiocyanate in milk was analyzed in various scenarios of consumption per capita. The per capita value is related to international reports, from the lowest consumption profile for developing countries, to the highest one for developed countries.

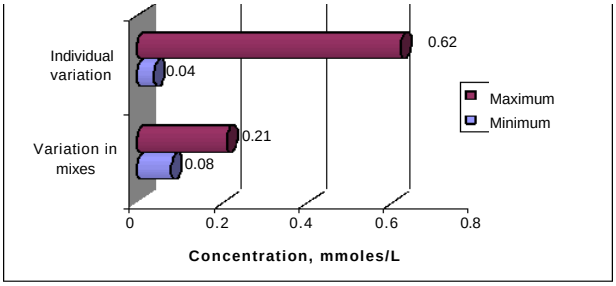
The calculations were based on the following:

- Liquid milk and milk derivatives are considered total intake of milk equivalent.
- Conversion of mmoles/L to mass (weight): by multiplying such value by 58, which is the molecular weight of the thiocyanate ion.
- Natural concentration of thiocyanate in mixed milk: 0.14 mmoles/L (8.12 mg/L).
- Exogenous addition for the activation of the LP system: 0.11 mmoles/L (6.38 mg/L).
- Total amount of thiocyanate in activated milk: 0.25 mmoles/L (14.5 mg/L).

Countries that do not activate the LP system, but that exceed an annual consumption of 150 kg, ingest more thiocyanate in milk by natural means (countries with a developed dairy industry) than those with low consumption. A country with a non-activated milk equivalent of 250 liters per capita consumes 2.7 times more thiocyanate than a country with 75 kg of activated milk per capita.

a) Variation of the concentrations of the thiocyanate ion in milk of individual cows and in mixes and b) Concentration of thiocyanate in different countries

a)



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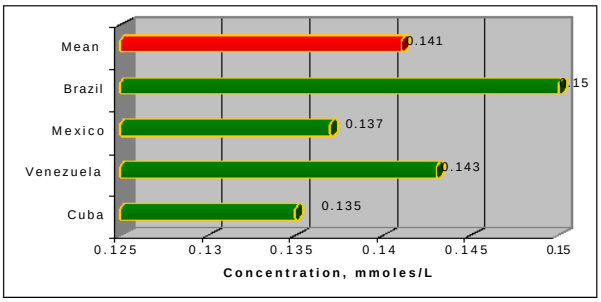


Basis for the establishment of the activation of the LP system with the thiocyanate ion (SCN) under tropic conditions.

Source	Minimum	Maximum
Individual cows	0.04	0.63
Mean value in raw milk mixes	0.08	0.21
Tropic concentration	0.140	
Optimum enzyme concentration	0.25	
Activation concentration according to CAG 13, 1991	0.173 (14 mg NaSCN)	
Proposed activation for the tropic	0.11 (9 mg NaSCN)	
Overdose suspected	0.251 - 0.35	
Overdosage	+0.35	

First value: Concentration of the SCN ion expressed in mmoles/L.

Second value in parenthesis: Sodium thiocyanate salt expressed in mg/L.

Annual intake of thiocyanate according to a per capita level of total milk consumption

Level of annual consumption per capita as milk equivalent (kg/year)	Intake of SCN without activation g/year	Total intake, with LPs activation g/year
25	0.203	0.363
50	0.406	0.725
75	0.609	1.087
100	0.812	1.450
120	0.974	**1.740**
150	1.218	2.175
200	**1.624**	2.90
250	**2.030**	3.625
300	**2.436**	4.35

The following conclusion is derived from this set of results:

1. The total thiocyanate concentration in mixed milk under American tropic conditions is 0.14 mmoles/L. The total concentration established by the Codex Guidelines, CAG/GL 13, 1991, is 2.5 times less than the maximum ion concentration that is found naturally in an individual animal's milk at a point during lactation.

2. The activation of the LP system under American tropic conditions can be achieved with only 9 mg/L of sodium thiocyanate salt, a much lower amount than the 14 mg/L value established in the Codex Alimentarius Guidelines. This criterion increases the level of safety and reduces the risks relative to thiocyanate.

3. The value of overdosage of the thiocyanate ion in raw milk is 0.351 mmoles/L or greater. The greater concentration in mixed milk was taken into consideration, plus one standard deviation. The suspected range was established between 0.251 and 0.35 mmoles/L. This allows for establishing a control for use on a specific quantitative magnitude.

4. Countries that do not activate the LP system, but that exceed an annual consumption of 150 kg of milk equivalent, ingest more thiocyanate in milk by natural means (countries with a developed dairy industry) than those with low consumption, even when consuming all milk in activated form (the system is aimed only at situations where refrigeration is limited and where there is a lack of infrastructure).

5. The daily intake of thiocyanate is influenced by the nature of the total diet components and their proportions, not only by the consumption of milk and milk derivatives. Therefore, the establishment of an ADI would have no practical meaning for the specific use of the LP method nor for its control in milk.

6. The existence of a real concern about the ingestion of thiocyanate, based on scientific elements, turns into a contradiction in terms: It would be a toxicological hazard for those countries with a medium to high level of consumption of milk and milk derivatives, even if they do not activate the LP system (for instance, the United States of America, the European Union, New Zealand, Uruguay), since the content of the natural ion would always be higher than in those countries that need the method to increase their access to milk. Also, milk should not be consumed from only one individual animal, since its natural concentration in a given moment could exceed by far the value of 0.25 mmoles/L. Cuba considers that we should focus on the subject as a risk/benefit matter.

7. The analysis of the international literature on the toxicological subject relative to thiocyanate indicates, almost in its entirety, that the experimental designs are not consistent with those from the CAG/GL 13 Guidelines, 1991, and exceed the concentrations of such ion. The benefit that results from the increase in the consumption of milk could be greater than the theoretical adverse effects created by increasing the consumption of this ion. Common salt iodization and other methods of iodine incorporation into the diet are solutions to the lack of this element in some affected areas, more than limiting the use of the LP system.

8. The results obtained by Cuba could be incorporated into the Codex Guidelines, which would increase the safety in the usage of the lactoperoxidase system.

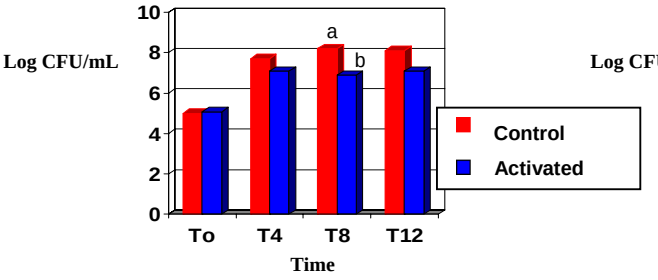
Exacerbation of pathogenic microorganisms

The activation of the lactoperoxidase system, according to the Codex Guidelines, is aimed at preventing the proliferation of saprophytic flora and the deterioration of milk, but not to eliminate pathogenic bacteria. There are numerous reports in the international literature, however, in regards to such effect. The latest studies in Cuba have been aimed at establishing the dynamics of growth-inhibition of the different groups of saprophytic microorganisms and on specific pathogens.

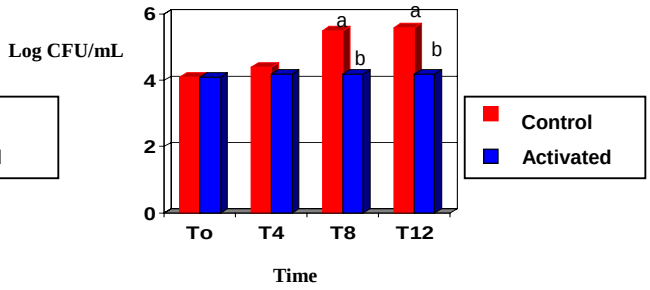
In the case of saprophytic flora, the activation generates an effect practically of bacteriostatic nature. There was a reduction of 27.8 % in the total load in different groups of microorganisms (aerobic mesophilic organisms, coliforms, proteolytic organisms, psychrotrophs, thermoresistant organisms). The effect in warm milk is minimal at the beginning of the activation (two percent); it reaches maximum action between 4 & 8 hours post-activation (35 %), and declines starting at 10-12 hours post-activation (less than 25 %).

Studies on the possible exacerbation of specific pathogenic microorganisms were performed in parallel in two Cuban laboratories (*Centro Nacional de Sanidad Agropecuaria*, CENSA, and *Instituto Nacional de Nutrición e Higiene de los Alimentos*, INHA), and were replicated in the *Istituto Zooprofilattico Sperimentale* (IZS), in Venice, Italy. For the studies, raw milk was contaminated with different strains of reference pathogenic bacteria (*Staphylococcus aureus, Salmonella spp., E. coli 0157:H7, Listeria monocytogenes, Bacillus cereus*), and the evolution of their growth in time was determined while being kept at room temperature.

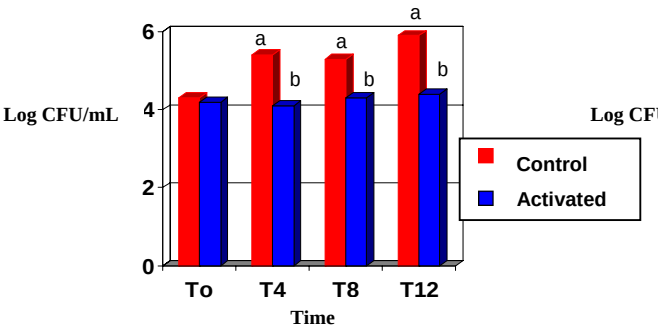
Effect of the activation of the LP system on pathogenic microorganisms in milk contaminated in the laboratory (Results from CENLAC/INHE, Cuba).



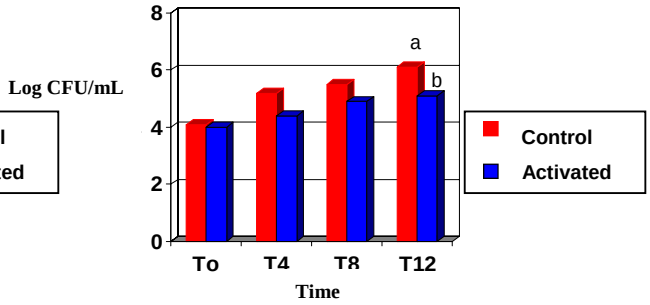
Staphylococcus aureus



Listeria monocitógenes

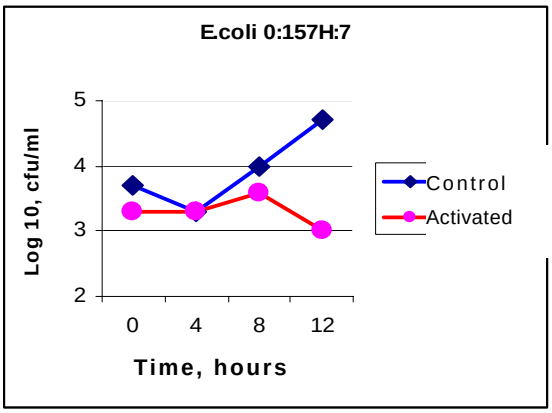


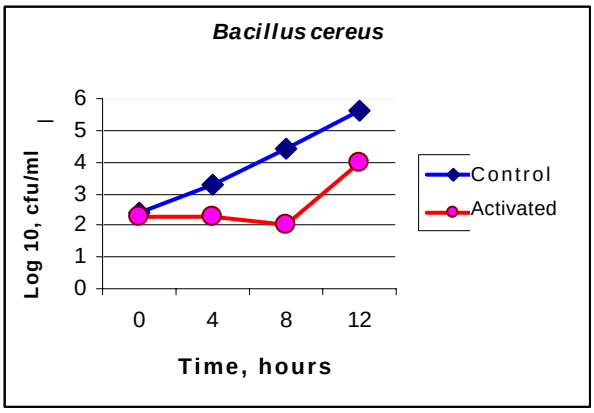
Escherichia coli enterohemorrágica 0157:H7

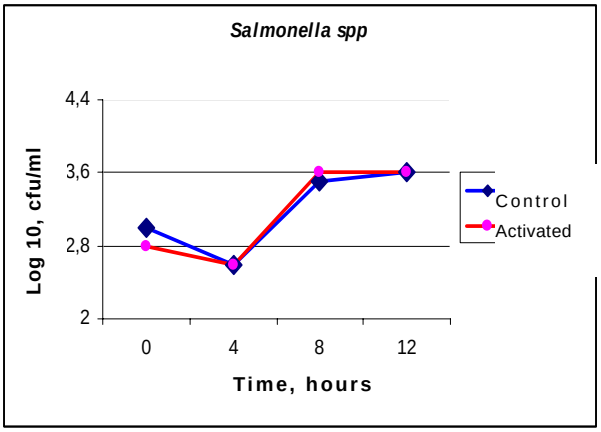


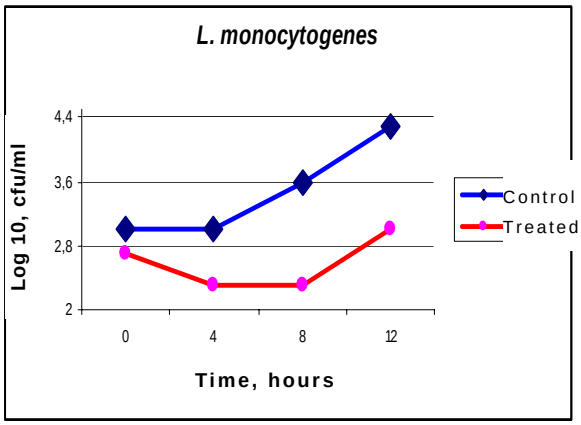
Salmonella typhimurium

The percent reduction at 12 hours post-activation was 8.45, 11.1, 21.4, and 24.2 for *Staphylococcus aureus, Listeria, E. coli, & Salmonella,* respectively. Different letters, $p<0.05$.









Effect of the activation of the LP system on pathogenic microorganisms in milk contaminated in the laboratory (IZS Results, Italia, Dalvit, R. 2006).

Exacerbation was considered to exist when the growth of a specific microorganism in activated milk was significantly greater than the one observed in non-activated contaminated milk. In none of the three assays (with three replicates of each) was exacerbation of any of the pathogenic bacteria observed once the effect of activation either decreased or disappeared (defined as 12 hours). In general a 1 $\log_{10}$ cfu/ml or greater reduction was seen in pathogenic bacteria in activated milk, in comparison to non-activated milk. A comprehensive review of the scientific basis of the LP system action mechanisms was conducted, and there was no scientific element found that would support a possible post-activation exacerbation.

Although theoretically speaking the exacerbation of pathogenic microorganisms can occur, this is not only applicable to the lactoperoxidase system activation, but also to non-activated milk whether refrigerated or warm. There are multiple results that prove that the reduction or disappearance of the inhibition effect of the LP system rapidly increases milk acidity, which constitutes a factor that inhibits the growth of pathogenic bacteria. On the other hand, the activation causes damage at the cellular wall level in bacteria, which increases the efficacy of thermal treatment processes in milk.

During the 15 years of systemic usage of the method, no case of intoxication of a microbiological nature has been reported associated with the consumption of milk or milk products from a source of milk activated with the LP system. The integration of the experimental assays and the practical evidence of the continuous use of the method allow the conclusion to be made that the activation of the LP system does not generate the exacerbation of the growth of pathogenic microorganisms once its activity over the saprophytic flora is weakened, and that the behavior is similar to this one.

Inhibition of lactose-fermenting bacteria

Cuba has been using the activation of the LP system in milk destined for industrial processing for obtaining fermented products and in cheeses matured with the addition of starters. Such practical experiences have also been developed in countries in Latin America and the Caribbean. The results have varied according to the technological characteristics of the process.

Basic results have been obtained in the production of yogurt, with strains of *Lactobacillus bulgaricus* and *Streptococcus termophillus*, with a thermal treatment prior to the activated milk, at a temperature of 85°C during 20 to 25 minutes, before adding the culture. Under these conditions there were no differences observed in the quality indicators of the product in both, activated and non-activated milk. Similar results have also been obtained when strains of *Bifidobacterium bifidus* and *Lactobacillus casei* have been included.

Strains from industrial cultures have also been added to pasteurized milk at 73°C for 15 seconds, and destined for use in the production of Gouda cheese of medium maturation, which was previously activated with the lactoperoxidase system. In the results, both from the physicochemical analysis and from a sensorial

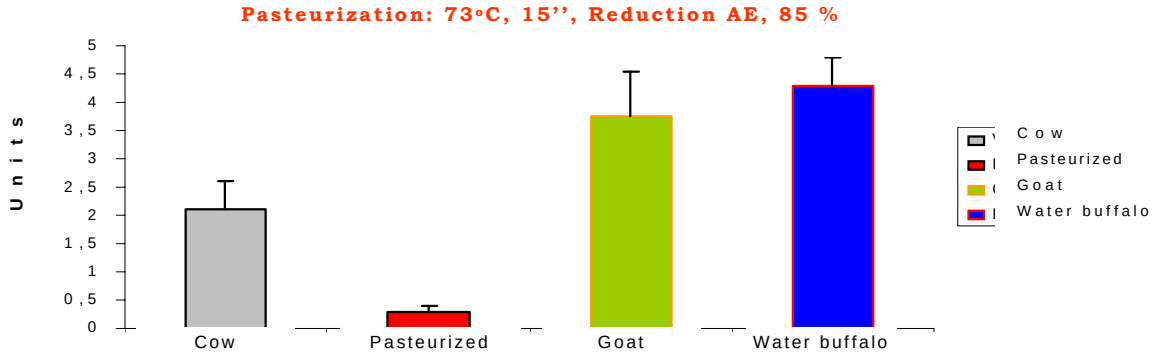
characteristics panel, no differences were found between the quality of the process and that of the final product between the activated and the non-activated source.

The inhibition problems of lactose-fermenting microorganism strains have been observed when the process is conducted directly in raw milk and also at temperatures between 40-60°C, conditions under which the LP system does not uncouple and the enzyme retains its activity. On the other hand, the effect can also be boosted by the presence of other naturally occurring inhibitors in milk. However, the majority of fermented products need the prior application of a strong thermal treatment in milk (with or without the LP system), generally at a temperature of 85°C.

The review of the international reports that encounter the inhibition of the LP system over the starter cultures seems to indicate that this is due to problems of low temperature/time in the treatment of milk. Even the potential generation of hydrogen peroxide by certain strains of lactose-fermenting bacteria, with the capacity to reactivate the LP system, could not be explained at a temperature of 85°C, when the enzymatic activity disappears.

Quality indicators of yogurt at 18 hours of incubation, obtained from raw milk previously activated with the LP system.

Indicator	Control	Activated	Significance
Clotting time, hours	4.0	4.0	NS
Lactic acid, %	0.91	0.89	NS
pH	3.81	3.8	NS
Viscosity index	24.0	23.5	NS



Activity of the lactoperoxidase enzyme in milk from different species

The thermal treatment of the conventional pasteurization of milk (73°C for 15 seconds) reduced the activity of the lactoperoxidase enzyme by 85 %. The literature reports indicate that at 85°C the activity of the enzyme disappears completely.

The integration of the set of experimental results and the experience of the practical use of the LP system, allows the conclusion to be made that the activation does not affect the development of the culture starters, nor the final yogurt quality, when the process of the thermal treatment of milk is conducted at a temperature of 85°C for the specified time. When problems occur in the extension of the clotting time, in the development of acidity or in the characteristics of the clot, other factors associated with fermentation must be examined, including the technological parameters of the process.

CANADA

We have reviewed the document *"Benefits and Potential Risks of the Lactoperoxidase System of Raw Milk Preservation"*, a report of an FAO/WHO technical meeting, which was held at FAO Headquarters, Rome, Italy between November 28 and December 2, 2005.

The main health concern associated with this system of processing milk examined by the technical meeting was the level of thiocyanate, a reaction product of the lactoperoxidase system, in the treated milk. Information on the teratogenicity and reproductive toxicity of thiocyanate were not present in the technical meeting report. This information is important since pregnant women often ingest greater amounts of milk than they would consume when not pregnant, with the consequence that the developing fetus is exposed to a greater potential risk. It was understood that no studies were available at the time the document was prepared but recently a study on the prenatal toxicity study of thiocyanate has been published (1). We would suggest that this study should be evaluated by the FAO/WHO technical committee.

The human observations noted in the FAO/WHO Report were limited to two studies (a third study was cited but did not provide any safety data). One study (2) showed altered thyroid function as a result of people consuming milk containing thiocyanate; the other (3) showed no effect but was of shorter duration than the study with the positive finding. The applicability of these findings with respect to the safety of the proposed use of this system should be clarified.

It is Canada's view that the thiocyanate present in treated milk may not be a concern for persons with adequate iodine in their diet; however, the condition of inadequate iodine is not uncommon. We would suggest that as there is a possibility that persons with iodine deficiency may be at greater risk for thyroid toxicity than persons with a well functioning thyroid, the implications for such individuals on the use of the lactoperoxidase system should be investigated further.

References

1. A..B. de Sousa *et al.*, Reproductive Toxicology, 23, 568-577, 2007.

2. K.K. Banerjee *et al.*, British Journal of Nutrition, 78, 679-681, 1997.

3. P. Dahlberg *et al.*, American Journal of Clinical Nutrition, 39, 416-420, 1984.

UNITED STATES OF AMERICA

The following is the response of the United States to Codex Circular Letter 2007/31 – FH on the *Request for Additional Information Regarding the Potential Risks in Respect of the Lactoperoxidase (LP) System*. We believe that there may be scientific evidence that was not considered by FAO/WHO in developing its report entitled *Benefits and Potential Risks of the Lactoperoxidase System of Raw Milk Preservation.* Although we have additional concerns about potential quality of product and economic issues that may be associated with use of the LP system that were not addressed adequately in the report, we are limiting our comments to our safety concerns with use of the system.

The U.S. believes that there is insufficient information about or attention given to the potential impact of the LP system on the microbiological safety of dairy products that are produced from treated milk. Considering that over 50% of milk production is used to produce fermented dairy products such as cheeses and yogurts, we feel that the following potential risks associated with the use of the LP system must be considered:

> The safety aspects of inhibited acid production by lactic starter cultures are not mentioned within the report. Seifu et al[1] found that starter culture activity in goat milk was found to be sensitive to the LP system (quality/economic issue) and also that activation of the LP system resulted in greater inhibition of acid production than growth of the starter cultures. Rapid acid production during the initial fermentation of fermented dairy products is critical to preventing growth of pathogens that may be present or introduced into the milk during the initial phases of production. For example, *Staphylococcus aureus* outbreaks associated with cheeses and other fermented dairy products have historically been linked to delayed or inhibited acid production by starter cultures. Reduced rates of acid production may also make it easier for acid-resistant strains of pathogens to survive in fermented dairy products. It has been well established that pathogens such as *Escherichia coli*

[1] Seifu et al. Effect of the lactoperoxidase system on the activity of mesophilic cheese starter cultures in goat milk. International Dairy Journal (2003) 13:953-959.

O157:H7 and *Salmonella* have inducible acid resistance systems. The U.S. has concerns that the potential for delayed acid production due to the use of the LP system could provide sufficient time for such system to be to activated and thus decrease the potential inactivation of these pathogens during subsequent maturation of the fermented products.

The FAO/WHO report concluded that use of the LP system is not likely to stimulate resistance to the system in pathogens. However, a study by Sermon et al[2] which was not considered in the report indicates that *E. coli* has a specific genetic response upon exposure to the LP system and analysis of this response reveals a number of pathways which may be involved in antagonizing the toxic effects of the enzyme system and increasing cellular resistance and which may be involved in virulence in pathogenic strains. Given the response by *E. coli*, it seems that there is the possibility that widespread usage of the LP system could result in the development of LP-system-resistant strains of pathogens, which could then pose a public health risk for consumers of products preserved by the LP system.

There are other references[3] supporting the safety concerns described above. The U.S. feels that these areas need to be adequately considered before any change in the current code of practice for dairy products is considered by CCFH. The U.S. plans to introduce a CRD at the 39th Session which more fully describes the scientific literature available related to potential risks associated with the use of the LP system.

ARGENTINA

Argentina appreciates the opportunity to comment on this document.

Argentina wishes to express its dissatisfaction with and opposition to the use of this alternative raw milk preservation system in dairy products intended for international trade.

Like in an earlier consultation, the Committee on Food Hygiene and the Committee on Milk and Milk Products have managed to clearly define and find scientific support for the uses and scope of this system in the dairy production chain. It is apparent that the system per se **has a given "shelf life", so it is not suitable for international trade in milk products, and it would make no sense as a preservation method if used together with cooling.**

In addition, there is evidence that the use of the system **requires specific technical capacity and that such use could facilitate the hiding of inappropriate agricultural practices in farms**. Inappropriate thiocyanate management due to overdosing in this system could cause a safety problem, which needs to be considered in the risk analysis.

Although the use of lactoperoxidase as a raw milk preservation method may be beneficial for some countries or population groups, we believe that given the need **to train specific personnel and and to have sound knowledge of the scope of its use,** the use of the system should be kept within national and/or regional contexts, taking into account the system feasibility over time.

Argentina takes into consideration the *Codex General Standard for the Use of Dairy Terms* (*Codex Stan 206-1999*), which defines milk as "the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for

[2] Sermon et al. Unique stress response to the lactoperoxidase-thiocyanate enzyme system in *Escherichia coli*. Research in Microbiology (2005) 156:225-232.

[3] 1) Seifu et al. Significance of the lactoperoxidase system in the dairy industry and its potential applications: a review. Trends in Food Science and Technology (2005) 16:137-154

2) Nakada et al. Lactoperoxidase suppresses acid production in yoghurt during storage under refrigeration. International Dairy Journal (1996) 6:33-42

3) Sermon et al. CorA affects tolerance of *Escherichia coli* and *Salmonella enterica* Serovar Typhimurium to the lactoperoxidase system but not to other forms of oxidative stress. Applied and Environmental Microbiology (2005) November, 6515-6523

4) Leyer and Johnson. Acid adaptation induces cross-protection against environmental stresses in *Salmonella typhimurium*. Applied and Environmental Microbiology (1993) 1842-1847.

further processing." Argentina does not share the approach of adding an external agent to raw milk marketed as such in international trade.

In addition, as a country fostering high quality dairy production, Argentina has promoted the installation of cooling equipment in primary production units to foster cold milk preservation (as indicated by Codex) with a view to ensuring the safety of the product throughout its shelf life.

In view of the above, Argentina believes that the use of the lactoperoxidase system can be very useful nationally but is inappropriate to promote in international trade.

Specific Comments

In view of the new request for additional information, regarding the potential risks in respect of this system, we wish to point out the following:

Sensitivity to temperature: There is evidence that, according to the temperature curve, lowering the temperature increases the shelf life of this system, alhtough only within a very limited range to consider the possibility of exporting the product, since at 4º C the shelf life period of the system is comparable to traditional cold chain. Therefore, the lactoperoxidase system would make no sense in itself.

Thiocyanate overdosing: as described in the document "Benefits and potential risks of the lactoperoxidase system of raw milk preservation: report of an FAO/WHO technical meeting, FAO Headquarters, Rome, Italy, 28 November - 2 December 2005", although thiocyanate is a safe chemical substance if used at normal levels, **overdosage may occur and become a risk factor for specific populations such as pregnant women, children and people with low blood iodine level.**

Thiocyanate underdosing: for cases of thiocyanate underdosing/normal dosage, consideration should be given to the **potential hazard caused by the presence of bacteria that are more resistant to lactoperoxidase. Furhtermore, pafe 21 of the FAO/WHO document refers to the need to conduct longer-term specific studies to monitor the appearance of resistant microorganisms.**

Training: as stated in the *Guidelines for the Preservation of Raw Milk by Use of the Lactoperoxidase System (CAC/GL 13–1991)* and in page 20, section 4.1 of the FAO/WHO document (according to which thiocyanate is "…recommended for administration by trained personnel only…"), for both of the above cases, **we should take into account the fact that people using this method are, in theory, farmers with no access to electricity, with small-scale production and from developing countries. Thus, it may be inferred that they are unlikely to get access to specific training or primary production education so as to be able to dose chemical products expressed as concentrations in terms of milligrams per liter. In our view, this is a risk factor that needs to be given priority in the management of this alternative system.**

Continuous decline: although the input of this technique is very valuable and important for some population groups or situations in developing countries or peoples with specific electricity supply problems, we consider that it may imply a step backwards in the dairy industry of countries that have been managing milk production continuous improvement programmes. For example, Argentina has been working on colony-forming unit (CFU) control when milk enters dairy plants. As a result of application of these systems involving the addition of bacteriostatic substances to raw milk (lactoperoxidase), it would not be possible to conduct a quality analysis of the milk of primary production units (dairy plants) and of the good hygiene practices of these facilities since this product would be added after milking is finished. Thus, **the system could hide management conditions that are not very hygienic and flaws in GAPs.**