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EVALUATION OF MICROBIOLOGICAL AND CHEMICAL RISKS OF THE LACTOPEROXIDASE SYSTEM ACTIVATION IN RAW MILK

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Abstract

The Lactoperoxidase System is an unspecific natural defense mechanism of the mammary gland in all mammalians including women. It is formed by the lactoperoxidase protein, thiocyanate ions and little concentrations of reactive oxygen. The exogenous activation in milk generates a bacteriostatic/bactericide action, which limits contaminant bacteria growth and consequently its deterioration. That is the reason why it has been extensively studied in the last 40 years, and has been approved by the *Codex Alimentarius* in 1991 as a preservation method of raw milk without refrigeration. Obtaining information about the possible microbiological and chemical risks in the application of the method, specially its effect on pathogen microorganisms, on milk and milk product components and their limitations in the international dairy market has been of great interest either for the *Codex* and for the Global Lactoperoxidase Group, created by FAO in 1998. The study includes an analysis of the scientific literature on the topic, as well as the wide experience of Cuba and other Latinamerican and Caribbean countries. The guidelines established by the *Codex Alimentarius* (CAC/GL-30, 1999) were followed for this study. In the analysis of results, neither microbiological nor chemical risks, associated to the use of the method, were identified since the exacerbation of pathogen microorganisms is not evidenced, but there is a favorable bactericide effect. Alterations were not observed in the diverse experiments and practical experiences about the activation of the Lactoperoxidase System in the dairy components. In a general way, we found beneficial results in the efficacy of industrial processes and in the final quality of products. The Cuban experience has been considered very important since it has been using the method in a practical and stable way during the last 12 years in more than 800 liters of milk, without having any unfavorable criterion by the official organizations of public health, agriculture, industry, science and the National Committee of *Codex*. The evaluation carried out by the group GLP-FAO coincides with the conclusions of this study. We also emphasize the importance of maintaining good hygiene practices in milking and in milk manipulation either in refrigerated or preserved milk by the activation of the LPs.

Key words: Milk and milk products, Lactoperoxidase System, risks
Abbreviations: LPs, Lactoperoxidase System, GLP, Global Lactoperoxidase Group

Introduction

The Lactoperoxidase System is found in its natural way in milk of all mammalians as part of intrinsic mechanisms for the preservation and protection of rearings as well as in other biological fluids such as blood, saliva, gastric juice, lymph and urine. It is composed by three basic elements: the lactoperoxidase enzyme, a protein synthesized in the mammary gland and thiocyanate ions originated by the hepatic metabolism and by reactive oxygen molecules derived from leukocyte and other cell activity. Oxiacids are compounds derived from the transference of reactive oxygen to thiocyanate ions (8,79), which are joint in a specific way with SH groups present in bacterial proteins, causing bacteriostatic/bactericide effects depending on the kind of contaminants in milk (28). Effects of the LPs on sporulated microorganisms, viruses, mycoplasmas, yeasts, fungi, and parasites have also been demonstrated (20, 64,73,91,102,115; 117).

In natural stage, the limiting factors of the Lactoperoxidase System activation are thiocyanate ions and the reactive oxygen. Though they are invariably found in milk, their components depend on many factors like feeding, physiological conditions, management, among others (72, 88).

The use of the lactoperoxidase method in the preservation of raw milk was approved by the *Codex Alimentarius* in 1991 (4,24), and it is based on the exogenous activation of the natural system, using little equimolar concentrations of thiocyanate and hydrogen peroxide for achieving an optimal threshold for the enzyme activity in the order 0.20-0.25 mMoles/L. There are experimental and practical evidences, which demonstrate that the use of this method is innocuous. That is why, the Experts Committee for Food Additives (JECFA) has declared it as acceptable from the toxicological point of view (65). However, more information and evaluation about the microbiological damages associated to pathogen bacteria in milk and by-products and chemical alterations associated to the system components is needed (5,26).

The study was carried out under the principles and guidelines for the application and evaluation of microbiological risks of the *Codex Alimentarius* (25) from the integration and evaluation of three basic information sources: international literature related to the topic, carried out researches and the practical experience of Cuba in using the method for more than 10 years, as well as the evaluations carried out by the Global Lactoperoxidase Group (GLP) from FAO since its creation in 1998.

Identification of Microbiological Risks

The following microorganisms: *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Plesiomonas shigelloides* and *Clostridium* sp. have been recognized as the main pathogens transmitted by milk and by-products (21,37,58,69; 69). They have been identified in different links of the production chain, but with higher frequency at raw milk level.

The principle of use of the LPs is guided towards the bacteria causing acidification and other alterations in the physico-chemical characteristics of milk and not specifically as a way of reducing pathogen microorganisms; due to its antimicrobial nature, it does not exclude activity against them. Guidelines clearly establish the need of maintaining Good Hygiene Practices and also the processing of raw material, so that the LPs does not guarantee by itself milk and by-products inocuity.

The bactericide effect on these pathogen microorganisms: *Salmonella* sp. (32,81), *Escherichia coli* (16,32,59), *Staphylococcus aureus* (16), *Listeria monocytogenes* (34,42,44,67,81,82) and *Yersinia enterocolitica* (39) has been demonstrated. Diverse studies have demonstrated the bactericide/bacteriostatic effect of the LPs activation on the main pathogen microorganisms (Table 1). Due to the existent experimental and practical experiences, other microbiological damages for the use of milk treated with the LPs are not identified (only those recognized for the main classical systems for milk preservation and treatment). An example is that refrigeration inhibits the growth of the main groups of microorganisms. But at low temperatures, psycrotrophic microorganisms producer of undesirable thermo-resistant enzymes (lipases and proteases) are developed. On the other hand, pasteurization presents efficiency between 98-99% and there is a residue of thermo-resistant microorganisms and/or toxins in milk (105).

Table 1. Antimicrobial effect of the LPs application. Results from the international research.

Agent or microbial group	Effect of the System and evaluation conditions	References
Salmonella sp. Salmonella typhimurium Salmonella typhi	Bactericide effect in liquid or frozen culture medium, raw milk, children milk formula and at pH 5 in synthetic medium. Neutralizer of stomach infections and prevention of enteric infections in newborns. Bacteriostatic effect by an incubation period of 16-18 hours with an inoculum of 10^6 ufc/m and in Chihuahua cheese, reduction 3 log ufc/mL during 5 days.	32,43,81,99
Escherichia coli	Bactericide effect at 6 hours in buffer medium, raw milk at 4ºC and in a synthetic medium with activity higher than pH 5. The colonization of the gut decreases by *E. Coli.* Bacteriostatic effect at 30ºC and 37ºC in culture medium, at 37ºC for children formula and in raw milk after 6 hours at 30ºC.	16,30,31,32 39,59,68,99 100
Staphylococcus aureus	Bactericide effect at 4 hours and bacteriostatic at 6 hours at 30ºC in raw milk. Reduces 4 log ufc/mL at 12ºC during 8 hours in milk. Increments elimination by thermal treatment.	16,32,42,66 68,81
Listeria monocytogenes	Bactericide effect in raw milk at 4^0C, 8ºC and 35ºC and at 37ºC during 56 hours. It did not grow from 7 hours to 14 hours at 10ºC. Bacteriostatic effect in milk reducing 3 log ufc/mL at 24 hours at 30ºC. At refrigeration temperatures, the bacteriostatic effect was higher. Elimination is incremented by thermal treatment.	10,17,18,34 42,44,66,81 102,119,120
Yersinia enterocoíitica	Bactericide in culture medium and in raw milk at 4ºC and 30ºC	38
Pseudomonas flourescens	Prevents growth for 5 days at 4ºC or 3 days at 8ºC in milk	120
Viable aerobic mesophylic bacteria	Mainly bacteriostatic but reducing 2 log ufc/mL in raw milk. Reduced 3 log ufc/mL at 8 hours after being activated.	1, 42
Total coliforms	Bactericide in milk at 10 hours	33
Psycrotrophic bacteria	Bactericide in milk at 10 hours	32,33
Fungi and yeasts	Antimicrobial at 30 and 35ºC, when LPs concentration is 30:30 and 30:45 ppm	106,107

Identification of Chemical Risks

The chemical damages of the LPs activation can be associated to the direct action of the system components or the intermediate compounds of the lactoperoxidase enzymatic reaction on the physico-chemical properties of milk and/or its products.

Lactoperoxidase is one of the main properties of milk (15,70). It is found in media concentrations of 30 mg/L, superior to the biological necessities required for an optimal enzymatic reaction (40,109). That is why it is not identified as a chemical damage.

Thiocyanate is an ion widely distributed in all mammalian biological fluids as a result of the hepatic metabolism of sulfurized glucosides and aminoacids. So, a great variability is reported in milk, depending on diet, physiological and racial factors. The wider results have been carried out in Cuba under tropical conditions (84-97), finding variations in individual cows from 0.05 to 0.6 mMoles/L. Variation reduces between 0.1-0.14 mMoles/L in milk mixtures of different cows (Table 2). Concentrations considered as abnormal when controlling the activation of the system can be observed in this table.

Other authors report lower thiocyanate medium concentrations, but very variable individually (0.01-0.18 mMoles/L), changing with species, diet, year season, among other factors (1,11,71,96). This aspect is important from the toxicological point of view since the exogenous activation to a milk mixture, adding either

the thiocyanate added or the natural content in the pool, would be three times lower than the maximal natural values reported for individual animals under determined physiological conditions.

Table 2. Variation in thiocyanate ion concentrations in individual cows and milk mixtures

Source	Minimal	Maximal
Individual cows	2.32 (0.04)	34.8 (0.6)
Mixtures without being activated	6.24 (0.107)	8.35 (0.144)
More common value	8.15 (0.141)	
Final lactation	8.70 (0.15)	
Colostrum	17.40 (0.30)	
Mastitis	20.30 (0.35)	
Optimal concentration	14.5 (0.25)	
Overdose suspicion	17.4 - 23 (0.30 - 0.40)	
Overdosage	+25 (+0.40)	

First value expressed in mg/kg and second value in mMoles/L

From the chemical damage point of view, thiocyanate ion (used as part of the activation of the Lactoperoxidase System and within the levels established by guidelines) does not have any consideration meaning given by a part that does not react with milk components and it is a natural compound. On the other hand, its final concentrations do not surpass the extreme physiological levels reported for mammalian milk, being lower than the concentrations existent in saliva and gastric juice of human being (116).

Chemical damages derived from the possible transformation of thiocyanate ions in cyanide lack of technical support (72,75), because they are very stable and decomposed themselves only at very high temperatures (more than 500^0C), even above sterilization processes and under alkaline conditions. This kind of analysis should be applied to the milk of any species without taking into account if it was activated or not.

Different from oxygenated water (H_2O_2 – Hydrogen peroxide), using milk preservant in concentrations higher than 500 ppm (already excluded), the activation of the LPs is carried out with minimal concentrations in the order 10 ppm, because its purpose is being an enzyme substrate and not for causing reactions of direct oxygenation with bacteria and dairy components, like happens with the Hydrogen peroxide (62,92).

It has been demonstrated that the addition of H_2O_2 till 50 ppm, five times superior to that indicated for activating the LPs, does not affect either the dairy components or nutritional value (70,109). Besides, it is important to take into consideration that the reactive oxygen, added as a substrate for activating the LPs, is quickly consumed in such reaction during more or less five minutes of aggregate (92). This is favored by the action of the catalase enzyme present in milk.

In relation to the effect of oxiacids formed from the reaction, it should be taken into account that dairy proteins have few SH reactive groups, the same as mammalian tissue cells. That is why it is considered that the reaction of this kind of compound has a high specificity against bacteria (57,79).

Studies about the adverse effects of the use of the LPs in milk and by-products

Evaluation studies about the titrable acidity, dairy composition and quality of dairy products were developed in Cuba for 20 years by Ponce (83,86,89,92) and Ponce *et al* (84,85,87,88,90,93,94,95), including more than 100 assays of different nature. It was evidenced that such activation keeps milk initial acidity, within the limits acceptable by the international standards (27) in a time oscillating between 8 hours to 36 hours, depending on storage temperature and milk initial quality (Table 3). These results include practical observations of continues use, either in Cuba or in other countries of the region, showing that when there is a good quality of milk, the beneficial effects are wider in times than those initially established in the guidelines of the *Codex Alimentarius*.

Table 3. Relation between raw milk temperature and LPs preservation capacity

Temperature in ^{0}c	Preservation time in hours
36 - 32	8 -24
32 - 23	8 -36
22 - 16	16 -44
15 - 10	16 -48
8 - 4	48 -120

A clear antimicrobial effect about the main groups of microorganisms (viable mesophylic, coliform, psycrotrophic and thermo-resistant) was demonstrated (92,95) in agreement with the multiple existent experimental evidences (Fig. 1 and 2). When activation was carried out in hot milk of excellent initial quality, the method could maintain such quality for at least 8 hours (Fig. 3). The study about a specific *E. coli* pathogen strain indicated a reduction in at least 2 logs, with a greater effect in time. This demonstrates that there is not an exacerbation effect in the growth of this microorganism (Table 2).

Studies about the dairy components demonstrated that the initial concentrations of protein, lactose or fat were not affected by the activation of the LPs. An effect of proteolysis, lipolysis and lactose fermentation was not evidenced; being different from the milk in which the LPs was not activated (Fig. 4).

The results obtained in pasteurized milk of very bad initial quality (but activated before the thermal process) indicate that the total counting of viable mesophylic bacteria is substantially reduced, and coliform and thermo-resistant bacteria are totally eliminated (Table 5). This phenomenon could be explained by the highest sensitivity to the heat produced by the effect of the system on the cellular wall of such group of microorganisms (66,92).

Studies carried out in cheeses from cow, buffalo, goat and camel (87, 72,98) do not evidence significant differences in the quality of products coming from activated milk in relation with that not activated. This was also observed in three repeated assays in Gouda cheese with activated milk (Table 6).

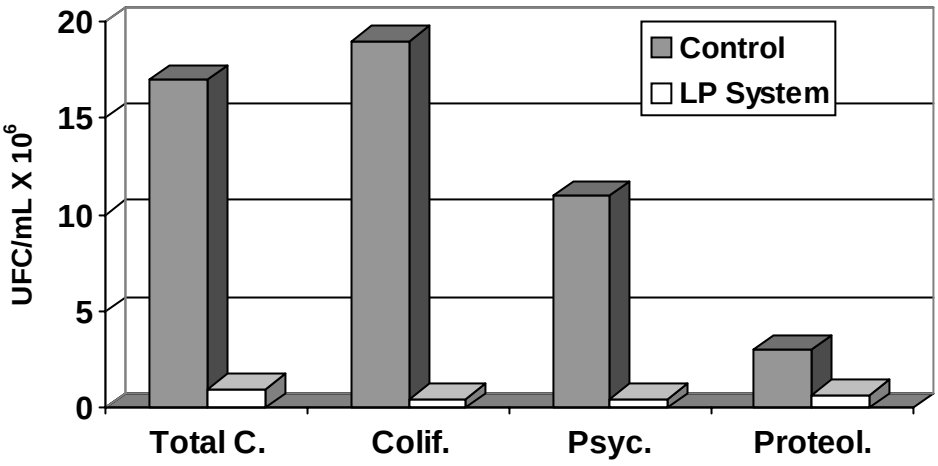

Fig.1. Effect of the LPs activation on contaminant microorganisms in raw milk

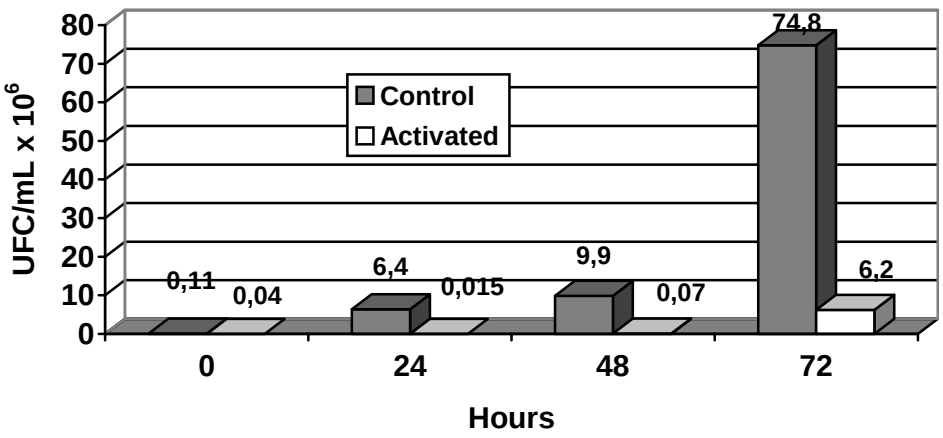

Fig. 2. Effect of the LPs activation on the growth of bacteria in refrigerated milk

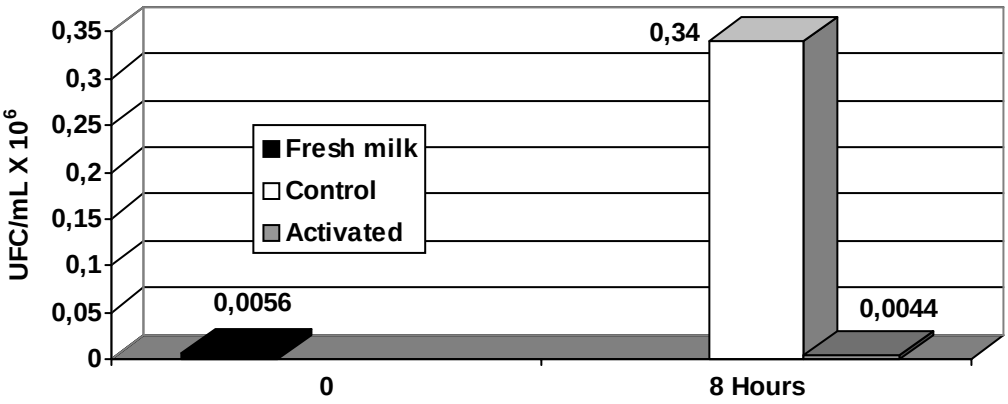

Fig. 3. Effect of the LPs activation on hot raw milk of excellent initial quality

Table 4. Reduction of *E. Coli* inoculated in raw milk by the activation of the LPs

Incubation time in hours	Initial charge, UFC/mL	Medium reduction for the 3 charges
0	10^4,10^6,10^8	2 log
4	10^4,10^6,10^8	4 log
8	10^4,10^6,10^8	4 log
12	10^4,10^6,10^8	6 log

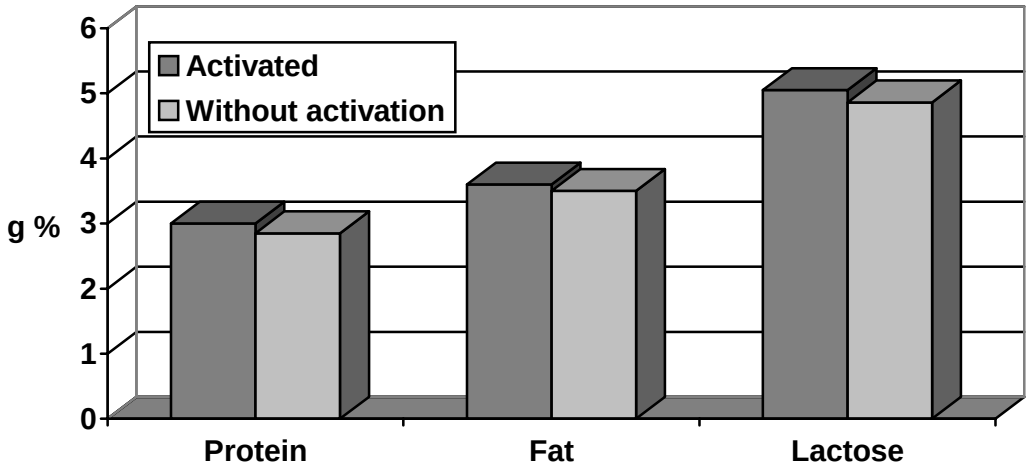

Fig. 4. Effect of the LPs activation on dairy components after 8 hours of activation at room temperature

Table 5. Effect of the LPs activation on the efficiency of the pasteurization process

Treatment	Viable mesophylls ufc/ml	Total coliforms ufc/ml	Thermo-resistant ufc/ml
Raw milk	9×10^6	5×10^5	2.8×10^4
Raw milk LPs	2.5×10^5	1.6×10^4	1.6×10^3
Pasteurized	6×10^4	2×10^3	3.2×10^2
Pasteurized LPs	3×10^3	Negative	Negative

Table 6. Quality indicators and sensorial evaluation in Gouda cheeses

Indicator	No LPs	Treated	Indicator	No LPs	Activated
Fat % (raw milk)	3,53	3,50	Splits	No	No
Protein % (raw milk)	2,90	2,90	Taste (soft-medium)	100	95
Acidity % lactic	0,17	0,17	Smell (soft-medium)	100	93
pH (at cut)	6,39	6,38	Rancidness %	93	0
pH curdled (pre-pressed)	6,29	6,35	Ojos (regular)	41	47
pH final pressed	5,87	5,92	Bark (hard-semi hard)	41	28
% of compressing	100	100	Dry bark	50	33

Reports in the international literature in relation with the effects on milk chemical composition and by-products quality are plentiful. But the existent evidences do not offer elements supporting any alteration about the nutritional quality, chemical composition, organoleptic characteristics or other quality attributes. There are reports, which confirm a beneficial effect on those characteristics as it is shown in Table 7.

Table 7. Evaluation of the LPs activation on milk and by-products composition

Product	Conditions/effects	Results	References
Raw milk	Activation in time on fat, protein and lactose concentrations. Effect on lesser components.	Fat, protein and lactose stable concentrations for a time. Proteolysis and lipolysis inhibition is evidenced. There are not significant changes in fatty acids. Vitamin concentrations are maintained.	2,45,46,70 85,109,111
Pasteurized milk	Activation before pasteurizing/stability/quality.	Increment in useful life. No changes either in the physico-chemical or organoleptic characteristics.	67,92,108
Milk UHT	Activation before sterilization/gelification/precipitations	Lengthening in the time of useful life. Fewer occurrences of alterations in time.	29,52,112
Cheeses	Activation-termization/industrial properties/product quality.	Improvement of non-activated milk quality either in fresh or mature cheeses.	47,77,86,94, 98
Creams	Activation of milk/posterior lipolysis	Diminution of lipolysis Lengthening of shelf life	13,114
Fermented product	Activation-termization /quality indicators	Improvement of the quality parameters. Acidity development is inhibited. Useful life of yogurt is prolonged without altering its biological characteristics.	3,60,72,74 97,118,106 107,118
Sweet, milk, blancmange	Activation/product quality	Improvement of properties.	13

Though there is confusion in relation with the possible inhibitory effect of the LPs in the manufacture of fermented products, it is convenient to point out that the thermal treatment of milk (generally between 80-85^{0}C during at least 20 minutes) is a condition for their industrial productions. It is a process in which the LPs is uncoupled and the biological lactoperoxidase activity disappears (9).

In relation with the dairy products of great importance in the international market, obtained from milk in which the LPs has been previously used such as: cheese, powder milk, dry whey, caseins and butter oil, there is no any technical reason supporting any damage. Thermal treatments for obtaining dehydrated or dry products also uncouple the LPs and affect the enzyme biological activity. In these cases, there is not a medium to propitiate the formation of the reactive oxygen or peroxides.

Evaluation of Exposition

Till 1991, the main results have been offered by researchers from Sweden, England and other countries belonging to the International Dairy Federation (14,15,61,66,100,101). In 1990, the United States stated that due to the hydrogen peroxide and thiocyanate are milk natural compounds, there are no risks recognized by the activation of the system. On the contrary, due to the bactericide and bacteriostatic effect, they give a high security of the inocuity of milk (23). It was recognized that the method is bacteriostatic against streptococcus, lactobacillus and yersinia; bactericide against gram negatives such as: pseudomonas and salmonella. In 2002, the Commission of Standards from Australia and New Zealand (41) concluded that the system is secure and does not constitute any risk from the toxicological point of view, either in milk or in meat. France has developed researches on 18 pathogen microorganisms for the preservation of milk, meat and fish, showing bactericide/bacteriostatic effects (12). The conclusions obtained by the Global Lactoperoxidase Program (GLP-FAO), from two studies guided to

this goal, indicate that there are no risks in its use and there are no technical elements supporting its limitation in the trade of dairy products (53). This group of international experts has been supported by the help from Sweden, Ireland, France, Hungary and the Czech Republic (36), countries that have contributed with great results in the evaluation of the method. It is important to notice that one of the first initiatives of the Project IDASS from ONU-PNUD-OIT for the cooperation between southern countries is the incorporation of the method as a way for stimulating and impelling dairy in such countries (78).

Cuba has used the activation of the LPs since 1992 till now in the 30% of the total volume of milk collected by industry, which comprises 800 million liters of milk. This practical experience has conducted to the evaluation of the possible damages derived from the use of the system. Evaluations on the topic, carried out by different productive and regulatory organizations of the country are reported by Ponce *et al* (95) and appear in Table 8. Such evaluations comprise either microbiological or chemical possible damages, but there are not declarations identifying any of them.

Table 8. Evaluation about the activation of the LPs from Cuba.

Institution, year	Document	Conclusion
Academy of Sciences, 1991. Presidency	Joint Verdict on the Preservation of Raw Milk by the Activation of the LP System.	Approval of the use of the LP System by the present sanitary and control regulations.
Regional Workshop FAO-CENSA, 1995. Committee of the Workshop	Recommendations to governments and organizations of the region.	Adopting the correspondent measures which facilitate the use of the method.
Agricultural Ministry, 2000. Vice-minister MINAG	Evaluative report about the use of the LPs activation as part of the National Program for Milk Quality and Improvement.	It is being used in farms since 1991, covering the 30% of the national production of milk. Excellent criteria of producers. Damages by its use are not reported.
Provincial Center of Hygiene and Epidemiology, Havana Province, 2000.	Report about the possible toxicological damages of the LPs in Havana Province.	Not any toxicological damages or health affectations associated to the method are reported.
Food Industry Ministry, 2001. Vice-minister MINAL	Guarantee of the Vice-ministry about the use of the method in dairy industry.	There is a strict control of its use. There are not any harmful effects on processes and by-products. No reports of damages to consumers.
National Institute of Food Hygiene and Nutrition, 2001.	Declaration about the use of the LPs activation.	Neither intoxications nor damages to health have been reported during 9 years.
Central Scientific Council from CENSA, Higher Education Ministry, 2001.	Study about the security of the use of the LPs activation for Public Health.	No evidences of toxic damages for consumers of milk and by-products during 10 years of using the method.

The results of various researches, observations and field experiences, obtained in some Latinamerican and Caribbean countries (including those carried out by GLP sponsorships) are summarized in Table 9. Diversity of countries, conditions and products, included in the studies, are highlighted.

Table 9. Different reports of the activation of the LP System in Latinamerican and Caribbean countries.

Country, year, origin	Type of evaluation	Results
Bolivia (93)	Field evaluation with producers and industry.	Maintaining quality between 8-16 hours in raw milk at 24-34°C temperature.
Colombia (7,87)	Thesis of grade and some assays for evaluating dairy products and others.	Maintaining initial quality since 8-72 hours depending on temperature and initial quality. Improving UHT milk quality packed in sachet, fresh cheeses and yogurt. Important measure of management and hygiene.
Costa Rica (19)	Thesis of grade: Agricultural Engineer.	Obtained 18 hours of preservation in hot milk at more than 30°C. Did not affect cheeses and yogurt.
Dominican Republic (Author data)	Evaluation in cheese farms and industry.	Maintaining the minimal initial quality of 8 hours at temperatures higher than 32°C. Improving quality of fresh cheeses.
Ecuador (104)	Thesis of grade of VM Controlled experiments.	Positive effect till 9 hours with hot milk. Reduction in the counting of coliforms. Did not affect consumption of milk and fresh cheeses.
Honduras (121)	Thesis of grade: Agricultural Engineer.	Beneficial effect for more than 8 hours in the transport of hot milk. Did not affect quality in cheeses.
Mexico (43,92,103, 114)	Evaluation of the regulatory regulations and others.	Reduction of mesophylic and coliform bacteria. Chemical composition and content of reducer substances are not affected. Inhibitors were not detected. Pay attention to Good Hygiene Practices in milking.
Nicaragua (Author data)	Theoretical-practical course with producers.	Activation effectiveness in the times established by guidelines in shown.
Peru (3,13)	Evaluation in farms and factories.	Effect between 12-30 hours in hot milk and 120 hours in refrigerated milk. Improvement in quality and shelf life of dairy products.
Venezuela. (63,94)	Evaluation of the regulatory organization and CIEPE-Yaracuy.	Beneficial effect in farms and collecting centers. Reduction of mesophylic and coliforms. Improvement of refrigerated milk and mature cheeses.
Colombia, Mexico and Venezuela. (50)	Evaluation in farms and factories. Practical training.	Effects between 8-30 hours depending on initial quality and temperature. Maintenance of refrigerated milk till 72 hours. Quality improvement in UHT milk, cheeses and yogurt. Need of maintaining hygiene measures in milking.

The concern of some people about the possibility that the use of the LPs activation makes some producers to abandon or weaken Good Hygiene Practices could happen. But the analysis is also valid for those using refrigeration in farms and for those processing directly milk in an artisan way or using thermal treatment though not activating the LPs. The practical experience accumulated indicates that the use of the system does not exclude improvement in quality, every time there is a direct relationship in the lengthening of the beneficial effect in milk of better quality. Another interesting aspect is that the activation of the LPs does not neutralize the acidity developed, does not disguise either adulteration by watery or the presence of antibiotics or other kinds of adulterations (76).

Risk Characterization

The experience accumulated on the topic is wide and comprises all continents, conditions and countries. Many of the basic studies and practical applications of the LPs come from the United States, England, France, Sweden, Australia, Spain, Canada and other countries belonging to the European Union. There are also study reports in China, Russia, Estonia, India and Arabian, Asiatic and African countries. They have included cow, buffalo, goat, sheep and camel milk, and also meat, fish and juice preservation (48,61,72,92,94,95,109). The analysis of the results joint to the own natural condition of the LPs and the regulations established for its use confirm it does not constitute a toxicological damage (41,56,107).

In the identification of the microbiological damage developed, we could appreciate that the activation of the LPs has a bactericide/bacteriostatic effect on the main pathogens contaminating milk. That is why; it is a preservation method that besides maintaining stable initial quality of this product, it could also favor the control of pathogen microorganisms. However, we should always insist on the application of Good Hygiene Practices, since any preservation method is able to substitute such actions. The application of the Code of Hygiene Practices for Milk and Dairy Products (6) should contribute to increase quality, security and inocuity of dairy products in all the chain.

In the analysis of the reported experience about the use of the LPs activation, evidences affecting health by the presence of pathogens were not found. Studies carried out in Cuban populations consuming milk treated with the LPs for more than ten years do not report any complain either in industry or in consumers of fluid milk or products originated from activated milk. The study conclusions reached by the Global Lactoperoxidase Group (GLP, 2004) also confirm such elements. A wide evaluative study, published by the Regulatory Organization of Food Standard in Australia and New Zealand (41) in milk and meat, neither brings evidences about any kind of risk in using the activation of the LP System.

Knowledge of the biological and practical bases of the LPs activation already discussed indicates that microbiological and chemical damages do not have proved technical evidence; that is why they are very difficult to be characterized from the qualitative and quantitative point of view.

Conclusions

The exposition evaluation analysis does not offer strong elements about any kind of risk, except about some considerations about the possible inhibitory effect in the making of fermented products occurring frequently in products from milk without being activated, therefore without establishing clearly any relationship dose/reaction. An adequate activation of the system; for example, when adding high concentration (10 times higher than H_2O_2) could produce any oxidative lipolysis effect on fats or when fermented products are produced and milk is not previously thermally treated. Both concentrations are due to mistakes in application and not to limitations of the LPs.

The integration of Cuban results and the international experience indicate there are not any microbiological or chemical risks when using the activation of the Lactoperoxidase System in raw milk.

References

1. **Abd El-Ghany, S., and A. F. Sabed.** 1997. Natural thiocyanate content and optimum conditions for activation of lactoperoxidase system in raw buffalo milk. Egyptian J. Dairy Sci. 25:241-252.
2. **Ahrne L. and Bjork L** 1985. Effect of the lactoperoxidase system on lipoprotein lipase activity and lipolysis in milk. J. Dairy Res. 52: 513-520
3. **Albujar R. Ludeña F. Castillo L.** 2003. Evaluación de la conservación de la leche cruda en distintas regiones del Perú mediante la activación del sistema lactoperoxidasa. IV Taller Internacional sobre calida de la Leche. Sep. 19-23, 2003. CENSA, La Habana.

4. **ALINORM 91/13, 1990.** Proyecto de directrices para la conservación de la leche cruda mediante la aplicación del sistema lactoperoxidasa. Apéndice X, párrafos 82-89.
5. **ALINORM 03/41, 2003.** Twenty sixth session, FAO Headquarters, Rome, ·0 June-7 July.
6. **ALINORM 03/13A.** Anteproyecto de Código de Practicas de Higiene para la Leche y los Productos Lácteos. Apéndice III.
7. **Antolinez V.E. y Tobon R.N.D.** (1994). Conservación de la leche cruda en Colombia mediante la activación del sistema lactoperoxidasa por adición del producto comercial Stabilak. Tesis de grado. Universidad Nacional, Bogotá.
8. **Aune T.M. and Thomas E.L**.1977. Accumulation of hypothiocyanate ion during peroxidase-catalysed oxidation of thiocyanate ion. European J. Biochem.80: 209
9. **Barret N.E. Grandison A.S., Lewis M.J.** 1999. Contribution of the lactoperoxidase system to the keeping quality of pasteurized milk. J. Dairy Res. 66:73-80
10. **Bibi, W., and M. R. Bachmann.** 1990. Antibacterial effect of the lactoperoxidase-thiocyanate-hydrogen peroxide system on the growth of ***Listeria spp***. in skim milk. Milchwiss.45: 26-28
11. **Bibi, W., and M. R. Bachmann.** 1997. The fate of thiocyanate in the lactating bovine. Milchwiss.52:8-10.
12. **Bio Serae, 2001.** Enzymes du Lait et Matrice du Risque Sanitaire des Produits Alimentarires Frais et Crus. Michel Degree, Bio Serae Lab SA. 18 pags
13. **Biovet Peru *S.A.C***. 2001. Resultados de un producto para conservar leche fresca. Efecto sobre la calidad de la crema, dulce de leche y manjar blanco. Ciclo de conferencias Universidad de la Molina, Lima, Nov. 13-17, 2000.
14. **Bjork L.**, 1978. Antibacterial effect of the lactoperoxidase system on psychotrophic bacteria in milk. J. Dairy Res. 45:109-118
15. **Bjork, L.; Rosen, C.; Marshall, V.; Reiter, B.** 1975. Antibacterial activity of the lactoperoxidase system in milk against **Pseudomonas** and others Gram-negative bacteria. Appl. Microbiol.: 30: 199-204.
16. **Bosch, E. H., Van Doormen, and S. De Vries.** 2000. The lactoperoxidase system: the influence of iodide and the chemical and antimicrobial stability over the period of about 18 months. J. Appl. Microbiology 89:2 15-224.
17. **Boussouel, N, F. Mathieu, V. Benoit, M. Linder, A. M. Revel-Junelles, and J. B. Milliere.** 1999. Response surface methodology, an approach to predict the effects of lactoperoxidase system, nisin, alone or in combination, on ***Listeria monocytogenes*** in skim milk. J. Appl. Microbiology 86:642-652.
18. **Boussouel, N., F. Mathieu, A. M. Revel-Junelles, and J. B. Milliere.** 2000. Effects of combinations of lactoperoxidase system and nisin on the behavior of ***Listeria monocytogenes*** in skim milk, International J. Food Microb. 61: 169-175.
19. **Bran J.R.N. y Mora K.V.** 1999. Evaluación del uso de higienizadores en leche cruda sin refrigeración y su efecto en la elaboración de quesos frescos y yogurt. Tesis para opción al grado de ingeniero agrónomo. EARTH, Costa Rica.
20. **Cailliez-Grimal, C., A. M. Revel-Junelles, M. Linder, and J. B. Milliere. 2002.** Antimicrobial activity spectra of the glucose/glucose oxidase and the lactoperoxidase systems (SCN) modified by 1.or 103.anion. Milchwiss.57: 656-660.
21. **Campbell W.** 2001. Supplemental report in favor of grade raw milk. http://home.earthlink.net/˜optimal/3-8.
22. **Capdevila J. Z.** 2000. Activación del sistema lactoperoxidasa para la conservación de la leche cruda: una experiencia en el trópico. Tesis para opción al grado de maestro en ciencias. Universidad Agraria de la Habana, Nov. 2000. 49 pgs.
23. **Comision del Codex Alimentarius, 1990.** Joint FAO/WHO Food Standard Programme. Doc MDS 90/10(a), Rome, Italy, 5-9 Nov. 1990.
24. **Comisión del Codex Alimentarius, Comité del Codex sobre Leche y Productos Lácteos, 1991.** Directrices para la conservación de la leche cruda mediante la aplicación del Sistema de la lactoperoxidasa. CAC/GL 13-1991.

25. **Comision del Codex Alimentarius, 1999.** Principios y Directrices para la Aplicacion de la Evaluacion de Riesgos Microbiologicos.CAC/GL-30, 1999.
26. **Comisión del Codex Alimentarius. Comité del Codex sobre Higiene de los Alimentos, Trigesima Sexta Reunion, 2004.** Documento CDR 32. Washington DC, 29 de Marzo-3 de Abril.
27. **COPANT C 125-2,** 1997. Especificaciones: Leche de vaca, pasteurizada, homogeneizada o no.
28. **Claesson O.** (1993). The use of the lactoperoxidase system in preservation of raw milk at ambient temperatures. Harare, Zimbabwe, 12-16 July 1993.
29. **de Wit, .I. N., and A. C. M. van Hooydonk.** 1996. Structure, functions and applications of lactoperoxidase in natural antimicrobial systems. Neth. Milk and Dairy J. 50:227-244.
30. **Dionysius, D. A., P. A. Grieve, and A. C**. **Vos.** 1992. Studies on the lactoperoxidase system: reaction kinetics and antibacterial activity using two methods for hydrogen peroxide generation. J. Appl. Bacteriology 72: 146-l 53.
31. **Earnshaw, R. G., J. G. Banks, C. Francotte, and D. Defrise.** 1990. Inhibition of ***Salmonella typhimurium*** and ***Escherichia coli*** *in* enfant formula milk by an activated lactoperoxidase system. J. Food Prot. 53: 170-l 72.
32. **El-Agamy, E. I., R. Ruppanner, A. Ismail, C. P. Champagne, and R. Assaf. 1992.** Antibacterial and antiviral activity of camel milk protective proteins. J. Dairy Res. 59:169-175.
33. **El-Agamy, E. I., Y. M. R. Shoukry, and M. S. Elghannam.** 1993. Effect of lactoperoxidase activation system on the shelf-life of raw milk stored at different temperatures. Alex. J. Agricultural Res.38: 35 1-364.
34. **El-Shenawy, M. A., H. S. Garcia, and E. H. Marth.** 1990. Inhibition and inactivation of ***Listeria monocytogenes*** by the lactoperoxidase system in raw milk, buffer or semi-synthetic medium. Milchwiss.45:638-641.
35. **Fajardo-Lira C.E. and Nielsen S.S** 1998. Effect of psychotropic microorganisms on the plasmin system in milk. J. Dairy Sci. 81-901-908.
36. **FAO**. 200l, Conferencia electrónica de FAO sobre Acopio y Procesamiento de Leche en pequeña Escala. El Sistema lactoperoxidasa (s-LP) de conservación de leche. 29 de Mayo-28 de Julio 2000.
37. **FDA/Center for Food Safety & Applied Nutrition** 2004. Foodborne IllnessTen Least Wanted Foodborne Pathogens. Hypertext updated by mow/cjm/kwg/dms/ear/dvd/dav.
38. **Farrag, S. A., F. E. El-Gazzar, and E. H. Marth.** 1992a. Inactivation of ***Yersinia enterocolitica*** by the lactoperoxidase system in a semi-synthetic medium and in raw milk. Milchwiss. 47:95-97.
39. **Farrag, S. A., F. E. El-Gazzar, and E. H. Marth.** 1992b. Use of lactoperoxidase system to inactivate ***Escherichia co/i*** **0157:H7** in a semi-synthetic medium and in raw milk. Milchwiss.47: 15-17.
40. **Fonteh, F. A., A. S. Grandison, and M.** J. **Lewis.** 2002. Variations of lactoperoxidase activity and thiocyanate content in cows' and goats' milk throughout lactation. J. Dairy Res. 69:401-409.
41. **Food Standards, Australia New Zealand, 2002.** Lactoperoxidase System. Final Assessment Report. Application A404, 06/03, 18 Dic, 2002.
42. **Francois, M.D** 1998. Enzymes du lait et maitrise du risque sanitaire des produits alimentaires frais et crus. BIO SERAE LABORATOIRES SA. FRANCE: 1-15
43. **García, H.S.; Pardio, V.T.; Wauszewski, K.N.** 1984. Activación del sistema lactoperoxidasa para preservación de leche cruda. Tecnología Alimentos México: 18:3:13-18.
44. **Gaya, P., M. Medina, and M. Nunez**. 1991. Effect of the lactoperoxidase system on ***Listeria monocytogenes*** behavior in raw milk at refrigeration temperatures. Appl. Environmental Microb.57: 3355-3360.
45. **Ghosh, A., and P. K. Ghatak.** 1994. Influence of LP-system on lipolysis of raw milk. Indian J. Dairy Sci.47: 712-713.
46. **Ghosh, A., P. K. Ghatak, and A. K. Bandyopadhyay.** 1994. Influence of LP-system on proteolysis of raw milk. Ind. J. Dairy Sci.: 863-866.
47. **Girgis, E. S., A. A. Ismail, S. M. El-Dieb, and W. M. Zaky.** 2001. Application of lactoperoxidase system in milk and some dairy products, Egypt. J. Food Sci. 29:233-256.

48. **Global Lactoperoxidase Programme, (FAO-GLP), 1998.** Firth Annual Meeting of the Lactoperoxidase Group of Experts. Uppsala, Sweden
49. **Global Lactoperoxidase Programme (FAO-GLP),** 1999. NEWSLETTER No 1, 2, 3. Dairy Page. FAO.
50. **Global Lactoperoxidase Programme (FAO-GLP),** 2001.Resúmenes de la Tercera Reunión Anual del Grupo de Expertos Lactoperoxidasa. La Habana, Cuba. Marzo 26-29/2001.
51. **Global Lactoperoxidase Programme, (FAO-GLP)**, 2002. Proceedings of the Fourth Annual Meeting of the Lactoperoxidase Group of Experts. 13-15 May 2002. Tongshan, China.
52. **Grupo Global Lactoperoxidasa (FAO-GLP),** 2002. Texto sobre la discusión del procedimiento para la conservación de la leche cruda usando el sistema lactoperoxidasa CAC/GL-13, 1991, referido a ALINORM 13/41.
53. **Global Lactoperoxidase Programme, (FAO-GLP)**, 2004. Proceedings of the Fourth Annual Meeting of the Lactoperoxidase Group of Experts. Cape Town, Africa del Sur.
54. **Global Lactoperoxidase Programme (FAO-GLP).** Evaluación técnica sobre peligros en el uso de la activación del Sistema lactoperoxidasa. Doc. De la presidencia del grupo GLP. Abril 2004.
55. **Guía Práctica Microbiología de la Leche I** 2003. Facultad de Ciencias Veterinarias. Cátedra de Ciencias y Tecnología Leche. Universidad del Zulia. 1-24.
56. **Haddadin, M. S., S. A. Ibrahim, and R. K. Robinson.** 1996, Preservation of raw milk by activation of the natural lactoperoxidase system. Food Cont. 7: 149-l 52.
57. **Haenstroem L, Johansson A y Carlsson J.** 1983. Lactoperoxidase and thiocyanate protect cultured mammalian cells against hydrogen peroxide toxicity. Med. Bilo. 61:268
58. **Headrick ML, Korangy S, Bean NH, Angulo FJ, Altekruse SF, Potter ME, Klontz KC.** 1998**.** The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, through 1973-1992. Am J Public Health. 88(8):1219-1221.
59. **Heuvelink, A. E., B. Bleumink, F. L. A. M. van den Biggelaar, M. C. Te Giffel, R. R. Beumer, and E. de Boer.** 1998. Occurrence and survival of verocytotoxinproducing ***Escherichia coli*** **0157** in raw cow's milk in the Netherlands. J. Food Prot. 61:1597-1601.
60. **Hirano, M.** 1998. Application of lactoperoxidase to yogurt. Milk Science 47: 195-l 99.
61. **IDF** 1991. Significance of the indigenous antimicrobial agents of milk to the dairy industry. IDF-Bulletin No 264:5-8.
62. **IDF.** 1988. Code of practice for the preservation of raw milk by the lactoperoxidase system. IDF-Bulletin No. 234:1-16.
63. **Informe al Ministerio de Salubridad de Venezuela, Comisión de Leche y productos Lácteos** 2002. Conservación de la leche cruda mediante la aplicación del producto Stabilak en Venezuela. Informe presentado al Ministerio de Salubridad, Grupo de Leche y Derivados Lácteos. Caracas
64. **Jacob, B. M., K. Essy Antony, B. Sreekumar, and M. Haridas.** 2000. Thiocyanate: mediated antifungal and antibacterial property of goat milk lactoperoxidase. Life Sci. 66:2433-2439.
65. **JECFA** 1990. 35 Reunión OMS del Comité de Expertos en Aditivos Alimentarios. Informe No 789, 1990
66. **Kamau D.N., Dooresy S. y Pruitt K.M.** 1990. Antibacterial activity of the lactoperoxidase system against ***Listeria monocytogenes*** and ***Staphylococcus aureus*** in milk. J. Food Prot. 53, 1010-1014.
67. **Kamau, D. N., S. Doores, and K. M. Pruitt.** 1991. Activation of the lactoperoxidase system prior to pasteurization for shelf-life extension of milk. Milchwiss.46: 213-214.
68. **Kangumba, J. G. K., E. H. Venter, and J. A. W. Coetzer.** 1997. The effect of the lactoperoxidase system and souring on certain potential human pathogen in cow's milk. J. South African Vet. Ass. 68: 130-136.
69. **Kendall, P.** 2003. Health. Bacterial Food-Borne Illness. Food and nutrition series No.9.300.
70. **Kumar S and Mathur B.N 1989.** Incidence of LP-system on the nutritional quality of milk proteins. Indian J. Dairy Sci. 42: 198-202

71. **Kumar, S., and B. N. Mathur.** 1994. Microflora of raw buffalo milk preserved by LP-system. Indian J. Dairy Sci. 47:770-773.
72. **Mathur, B. N., and R. Chopra.** 1995. Current issues concerning safety of LP-system for preservation of raw milk. Ind. Dairyman 47:4-l 1.
73. **Metwally, M. M. K., and M. M. Nasr.** 1992. Preservation of raw milk by activation of the lactoperoxidase system. Egypt. J. Food Sci. 20: 175-196.
74. **Nakada, M., S. Dosako, R. Hirano, M. Oooka, and I. Nakajima. 1996.** Lactoperoxidase suppresses acid production in yogurt during storage under refrigeration. Int. Dairy J. 6:33-42.
75. **Oae S**. 1977. Organic Chemistry of Sulphur. Plenum Press, New York.
76. **Oliva Y., Escobar A. y Ponce P.** 2004. Efecto del STABILAK sobre la determinación de antibióticos betalactámicos mediante la prueba SNAP en leche cruda. Rev. Salud Animal.
77. **Olkonen A.** 2002. Testing the activation of the LP-s of raw milk and studying the influence on cheese making in Estonia. Fourth Annual Meeting of the Lactoperoxidase Group of Experts. Tongshan, China. 13-15 may, 2002.
78. **ONU-PNUD-OIT, 2003.** Proyecto IDEASS de Cooperación Sur-Sur. Stabilak: Para la conservación natural de la leche. Folleto técnico de presentación de la activación del sistema lactoperoxidasa. Iniciativa para el Desarrollo y la Cooperación Sur-Sur. 12 Pgs.
79. **Oram J.d. and Reiter B.** 1966. The oxidation of thiocyanate and the nature of inhibitory compound. Biochemical J. 100:273-386
80. **Perraudin, J. P.** 1991. A natural preservative. Dairy Industries International 56:21-24.
81. **Pitt W. M., Harden T. J., and H. R. R.** 2000**.** Investigation of the antimicrobial activity of raw milk against several foodborne pathogens. Milchwiss. 55:249- 252.
82. **Pitt, W. M., T. J. Harden, and R. R. Hull.** 1999. Antibacterial activity of raw milk against ***Listeria monocytogenes***. Australian J. Dairy Techn.54:90- 93.
83. **Ponce P.**, 1983. Estudio del sistema lactoperoxidasa y sus posibilidades de uso en la conservación de la leche cruda sin refrigeración. Reporte de Aplicación No 6. Consejo Científico del Centro Nacional de Sanidad Agropecuaria, CENSA. La Habana, Cuba.
84. **Ponce P. López G. Y Martínez E.** 1986. Capacidad preservante del sistema lactoperoxidasa en la calidad higiénico sanitario y composición de la leche entera. III Congreso Cubano de Ciencias Veterinarias. 6-9 de Marzo/La Habana.
85. **Ponce P., López M. G. y Martínez E.** 1987. Conservación de leche cruda mediante la activación del sistema Lactoperoxidasa /tiocianato/peróxido de hidrogeno. Rev. Salud Anim. 9: 120-128.
86. **Ponce P.** 1992. Resultados del uso del producto stabilak en las condiciones de Bolivia. Informe técnico del estudio en las regiones de Santa Cruz, Cochabamba y CENSA, La Habana, Nov. 1992.
87. **Ponce P., Armenteros M, Barrero S, Moran G, Taboada A, Alfonso H.A, Ginorio C, y Alfonso A**. 1993. Propiedades del uso de un conservante de la leche cruda para consumo humano. II Taller Internacional sobre Calidad de la Leche. Ed Universidad Autónoma Metropolitana-Xochimilco, México. Tomo II pag 104-117. Mayo 17-21
88. **Ponce P., Díaz B., Alfonso H. A.** 1995. Factores asociados a la variación de las concentraciones de tiocianato en leche cruda. Rev. Salud Anim., 3:45-49
89. **Ponce P**. 2001. Experiencia nacional e internacional de Cuba en la aplicación del sistema lactoperoxidasa para la conservación de leche cruda. Tercera Reunión del Grupo Global Lactoperoxidasa, 25-30 de Marzo, La Habana, Cuba
90. **Ponce P. Alfonso H. A. Díaz B. Capdevila J. Y Yánez J**. 1995. Factores asociados al contenido de tiocianato en leche cruda. Taller Regional CENSA-FAO sobre manipulación y conservación de leche cruda en condiciones rurales mediante la activación del sistema Lactoperoxidasa. Memorias. La Habana 27-29 de septiembre/95.
91. **Ponce, P.** 1995. Diseño para la evaluación de campo, pruebas de laboratorios y control de calidad del stabilak, un activador del sistema lactoperoxidasa. Taller Regional FAO-CENSA sobre manipulación y conservación de leche cruda en condiciones rurales mediante la activación del sistema Lactoperoxidasa. Memorias. La Habana 27-29 de septiembre/95.

92. **Ponce P. y Clergé L**. 2001. Informe Técnico sobre la misión del Grupo de Expertos GLP a Colombia, México y Venezuela. Tercera Reunión Anual del grupo FAO-GLP, La Habana, 25-30 de Marzo.
93. **Ponce P., Capdevila J., Alfonso H. A., López M.G. León R. y Taboada A**. 1992. Conservation of raw milk through activation of the Lactoperoxidase System in Cuba. World Anim. Review 73:3 l-41.
94. **Ponce P.**, **Capdevila J.Z. Alcalá A,** 2002. Informe de evaluación de la activación del sistema lactoperoxidasa en las condiciones de Venezuela. CENSA, Julio 2002
95. **Ponce P, Clergé L y Capdevila J. Z,** 2004. El programa global lactoperoxidasa para la conservación de leche cruda en el trópico americano y otras experiencias asociadas. (En edición). Articulo por encomienda del grupo de expertos FAO-GLP.
96. **Pramanik, K. K.** 1991. Lactoperoxidase system in milk, its use and utility: a review. Envir. Ecology 9:66-71.
97. **Prasad, V., and M. V. Sukumaran.** 1992. Effect of preservation of milk on lactic count and sensory qualities of yogurt. Journal of Dairying, Foods Home Sci. 11:65-70.
98. **Ramet J.P. and Soukehal A-H. 2003**. Conservation du Lait cru de Chamelle par Reactivation du Systeme Lactoperoxidase et Fabrication de fromages Camelins. GLP-FAO, Tonsghan, China.
99. **Reiter, B.; Marshall. V.; Philipps, S.M**. 1976. Nonspecific bactericidal activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system of milk against ***Escherichia coli*** and some gram-negative pathogens. Infect. Immun: 13 (3): 800-807.
100. **Reiter B**. **1978.** Review of Progress of dairy sciences: antimicrobial systems in milk. J. Dairy Res. 45: 131-147
101. **Reiter,B.** (1985).The Lactoperoxidase system. In: "Developments of Dairy Chemistry", vol.3, pp 294-312, Fox P.F.(eds.) Elsevier Publ.
102. **Revol-Junelles, A. M., N. Boussouel, J. P. Ramet, and J. B. MilliCre.** 2001**.** Antibacterial activities of the lactoperoxidase systems (LPS) modified by 1.and 10, anions. Milchwiss.56: 329-332.
103. **Saldate O.** 1999. Efecto del producto Stabilak adicionado a la leche cruda. Resumen de Informe del estudio realizado por el Laboratorio Nacional de salud Pública de México.
104. **Saltos G. F**. 1996. Uso del Stabilak en la conservación de la leche cruda. Trabajo de opción al grado a Dr. en medicina veterinaria. Universidad Agraria, Fac. Medicina Veterinaria, Quito.
105. **Sánchez, Cecilia,** 2003. La utilización de leche cruda versus pasteurizada en la elaboración de quesos. II. Pasteurización. www.ceniap.gov.ve/publica/divulga/fd57/**leche**.htm - 20k
106. **Sarkar, S., and A. K. Misra.** 1992. Utilization of milk preserved by LP-system for manufacture of cultured milk product. Indian Dairyman 44:536-540.
107. **Sarkar, S., and A. K. Misra.** 1994a. Implication of LP-system on manufacture of fermented milk products. Indian J. Dairy Sci. 47: 133-l 39.
108. **Sarkar, S., and A. K. Misra.** 1994b. Milk preservation by LP-system and its effect on the quality of pasteurized milk. Indian J. Dairy Sci. 47:780-784.
109. **Sharma, V., and D. Raj.** 1999a. Effect of lactoperoxidase system on the quality of mixed raw milk. Indian J.Dairy Sci. 52:58-60.
110. **Sharma, V., and D. Raj.** 1999b. Lactoperoxidase system origin, efficacy and its effect on milk constituent - A review. Indian J. Dairy Bioscience IO: 9 -13.
111. **Siva, C.V., K.G. Upadhyay, and S.S. Sannabhadti.** 1991. Lactoperoxidase/thiocyanate/H_2O_2 system, its uses and implications in manufacture of dairy products. Indian Dairyman 43:240-246.
112. **Solanky, M. J., R. Chopra, and B. N. Mathur.** 1994. Thermal death kinetics of ***Bacillus stearothermophilus*** **subsp.** ***calidolactis*** in buffalo milk preserved by lactoperoxidase system. Microbiologic-Alim.-Nutr. 12:37-42.
113. **Taller Regional FAO/CENSA.** 1995. Manipulación y conservación de leche cruda en condiciones rurales mediante la activación del sistema Lactoperoxidasa. Memorias. La Habana 27-29 de septiembre/95.
114. **Toledo V. M. y García H.S.** 1991. Efecto de la activación del sistema LP sobre la vida de anaquel de cremas dulces y ácidas. IV congreso Panamericano de la Leche. 22-24 de Marzo, Guadalajara, México.

115. **van Hooijdonk, A. C. M., K. D. Kussendrager, and J. M. Steijns.** 2000**.** *In vivo* antimicrobial and antiviral activity of components in bovine milk and colostrums involved in non-specific defense. British J. Nutr. 84: 127-S 134.
116. **Wilson J. and Mattheus D. M. 1966.** Metabolic interrelations between cyanide, thiocyanate and vitamins B_{12} in smokers and non-smokers. Clinical Sci. 31: 1-5.
117. **Wolfson, L. M., and S. S. Sumner.** 1993. Antibacterial activity of the lactoperoxidase system: a review. J. Food Prot. 56:887-892.
118. **Zall R.R. Chen J. H. and Dzurec D. J.** 1983. Effect of thiocyanate and hydrogen peroxide in cultured products. Milcjhwiss.38: 264
119. **Zapico, P., M. Medina, P. Gaya, and M. Núñez.** 1998. Synergistic effect of nisin and lactoperoxidase system on ***Listeria monocytogenes*** in skim milk. International J. Food Microb. 40:35-42.
120. **Zapico, P., P. Gaya, M. Núñez, and M. Medina.** 1995. Activity of goat's milk lactoperoxidase system on ***Pseudomonas fluorescens*** and ***Escherichia coli*** at refrigeration temperature. J. Food Prot. 58: 1136-l 138.
121. **Zúñiga R.A.P**. 2002. Validación del sistema LP en la conservación de la leche durante el transporte sin refrigeración en condiciones tropicales. Tesis para opción al grado de ingeniero agrónomo. Escuela Nacional de Agricultura, Honduras.