

**Artículo en revisión: Journal dairy Science, 2009**

**Lactoperoxidase system under tropical conditions: Use, advantages, limitations and perspectives.**

**Pastor Ponce Ceballo**

Centro de Ensayos para el Control de la Calidad de la Leche y Derivados Lácteos (CENLAC). Centro Nacional de Sanidad Agropecuaria, CENSA. Apartado 10, San José de las Lajas, La Habana, Cuba. Tel 53-7-863145, email [pastor@censa.edu.cu](mailto:pastor@censa.edu.cu)

**Abstract**

The existent knowledge about the use of the Lactoperoxidase system (LPs) on the preservation of raw milk confirms that it is innocuous to human health, but still there are concerns limiting the use of the method in the international dairy marketing. Under tropic conditions, thiocyanate concentration needed for activating the LPs can be reduced to about the half of the established in the *Codex Alimentarius* guidelines, increasing its use security. When the antimicrobial effect of the LPs disappeared, exacerbation of the pathogen microorganisms was not observed, neither an inhibitory effect on the lactofermentant bacteria, always milk was previously pasteurized to 85<sup>0</sup>C during 20 minutes. This method maintains the initial quality of raw milk, classified as excellent, during 8 hours without refrigeration and its use should not be associated to poor hygiene quality conditions; although it is preferable to insert it inside an Integral Milk Quality Control Program. The activation of the LPs before pasteurization increases the efficiency of the thermal treatment, eliminating the contamination with coliforms and thermo-resistant bacteria after treatment. The knowledge bases exist and there are practical needs for an accelerate use of the LPs activation on the food preservation in the next years.

Key Words: Lactoperoxidase system, raw milk, innocuous, exacerbation

## **1. Introduction**

The practical and scientific knowledge obtained on the use of the Lactoperoxidase system (LPs) confirms that it is innocuous to human health [11], but concerns about limiting the method in the international dairy marketing are still maintained [7,11]. The simple name of LPs generates confusion due to the association to hydrogen peroxide or oxygenated water, adding no scientifically sustained criteria on the microbiological, toxicological and technological hazards.

Contrarily, there is a clear tendency about the use of the system for preserving meats, fruits and vegetables, substituting active chloride or other hazardous substances [1, 2, 11]

The most discussed aspects are:

- Toxic potential of the sodium thiocyanate salt used on the exogenous activation of the system due to the possible interference on the iodine metabolism [12, 17]
- Possible risks of the pathogen microorganisms exacerbation, once the systems is inactivated [3, 7,11]
- Possible increase of the microorganisms resistance to the method [7]
- Loss of interest of dairy producers on the hygiene practices [11].

A review on the scientific information available done by a Technical Committee from FAO/WHO in 2005 [11], recommends that its use, in correspondence with the guidelines [6], is a way to stimulate the dairy development in areas in which the adequate infrastructure to apply refrigeration system does not exist. This work integrates several studies carried out in the last 20 years under Cuban and other tropical countries conditions and establishes a current and perspective approach of the subject.

## **2. Materials and Methods:**

The analysis of thiocyanate ion ( $\text{SCN}^-$ ) content in cow bulk and individual raw milk was done in 2 400 samples (895 and 1100 samples respectively), representing a total volume of 4

mmL, using the method described in the guidelines of the *Codex Alimentarius* CAC/GC 13, 1991 [6]. The study on bulk milk included herds from Cuba, Mexico and Venezuela.

The effect of activation upon the contaminant flora of raw milk included cows, goats, buffaloes and sheeps. Twenty three assays were carried out, including the determination of different groups of microorganisms: aerobic viables mesophiles, coliforms, psychrotrophics, thermoresistants and proteolytics, with emphasis in the first two ones, at different activation times: 0, 2, 4, 8 and 12 hours at fluctuating room temperature, between 22-36 °C, accord to the guidelines [6]. The description of the microbiological methods used corresponds with the international norms (24). For the exacerbation studies, raw milk experimentally contaminated with *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* enterohemorrágica O157:H7, *Listeria monocytogenes* ATCC 43256 and *Bacillus cereus* sp was used during the times previously pointed out. For the exacerbation criterion, it was considered that the pathogen microorganisms amounts maintain the same counting (no significant differences), or when a reduction in time occurred, comparing the control milk with the LPs activated sample. An LPs activator product was used [24], containing the quantities of sodium thiocyanate and percarbonate salts established in the guidelines [6]. Activation was done on 2 L milk aliquots (laboratory assays) milk jars of 40-50 L (dairy farm assays) and in tanks of 500-5000 L (collecting assays). In all cases, homogenous mixtures were used to obtain the control and treated samples.

The measure of the activation effect upon the milk components and products includes the of the protein, fat and lactose determination by infrared method in hot raw milk and the final contamination in pasteurized milk. Assays on the final quality of yogurt and matured cheeses were also included. In all cases, the evolution of the quality indicators in activated and not activated milk was studied.

To evaluate the use of the LPs activation within an Integral Program for Milk Quality Improvement, the activation was carried out in 40 L jars or during the collecting process of 500-5000 L, from 700 herds, with a total of 36 000 cows, during seven years.

### **3. Results:**

The variation of thiocyanate ion concentrations in milk from individual cows was very wide, with values from 0,05 mmol.L<sup>-1</sup> to 0,62 mmol.L<sup>-1</sup>, but concentration in bulk milk was much lesser variable, with values maintained in a close range between 0,11-0,18 mmol.L<sup>-1</sup>, with an average of 0,14 mmol.L<sup>-1</sup>, repeated in the major part of observations (Tab. I). Milk from cows consuming star pasture (*Cynodon nlenfluensis*) fertilized with nitrogen (experimental conditions), had the highest values, the concentrations in bulk milk of cows fed in dairy farms using different types of pasture, including star pasture, were lesser; and the average concentrations observed in the different countries were equally similar. The highest concentrations were found in cows with subclinical and clinical mastitis and in animals with more than five lactations (Tab. I).

A tendency to show higher concentrations was also observed in animals from rustic breedings such as Zebu and crossbreedings with Holstein and Brown Swiss. From these results, the general average concentration was established in 0,141 mmol.L<sup>-1</sup> and the concentration needed for activation under the American tropic conditions in 0,11 mmol/L. To account its values, the concentration over 0,35 mmol.L<sup>-1</sup>, can be considered as thiocyanate overdose.

Changes in the mean values of cfu/mL in log<sub>10</sub>, the main groups of milk contaminant bacteria, measured at 4 hours post activation ( Fig. 1), indicate a decrease of the total amount in the order of one log or higher, independently of the microorganism group, the initial contamination and milk temperature. The dynamics of the bactericidal effect in coliforms group ( Tab. 2), shows that such effect is minimal in the first two hours, increases until 8-9

hours and decreases from the 12 hours, occurring concomitant with the bacteriostatic activity , which is the main action of the system [11, 15, 25, 26].

This effect has its expression in decreasing the lactic acid production capacity by lactofermentant bacteria, being the most visible practical base of the activation in hot milk in the tropic, since it avoids the acidification and curdled, either in cow, buffalo, goat and sheep (Fig.2). The use of LPs in hot milk with excellent initial quality (less than 10 000 cfu/mL), indicates that such quality can be maintained stable for at least 8 hours at room temperature between 28-34°C, (Fig. 3), which supposes that the method does not have to be only directed to the conservation of raw milk with high contamination..

The results obtained from the experimental contamination of raw milk with pathogen microorganism strains (Tab. 3), show that no significant differences were observed in any case between the activated and control milk, although the absolute concentration values were always lower in treated milk, at least in one reduction log. More that one reduction log was obtained at 8 hours with *Listeria monocytogenes* and at 8 and 12 hours with *Escherichia coli*. The reduction percent measured at 12 hours post activation was 8,45% in the case of *Staphylococcus aureus* and the highest of 24,19% in *Salmonella typhimurium*. The results are coincident with those obtained in similar conditions in the Zoo-Prophylactic Venice Institute and others [3, 11, 16].

The protein and fat concentrations were stables until 12 hours post activation and until 8 hours in the case of lactose, while activation in highly contaminated milk before the pasteurization process, totally eliminated the presence of coliforms group and thermo resistant bacteria, and did not altered the quality indicators of yogurt, neither the matured cheeses obtained from milk previously activated and pasteurized (Tab. 4), demonstrating that the method does not change the dairy products quality, including those using

lactofermentant bacteria, after an adequate pasteurization of the milk at 85°C during 20 minutes.

The inclusion of the Lactoperoxidase system as a method for maintaining the initial quality of raw milk without refrigeration into a program for improving milk quality, included actions related to the milking hygiene and management, mastitis reduction, improvement of the milk solid content, registers and laboratory technical support [24]. The integration of a hygienic milking and clean packages is aimed to obtain a good initial quality, meanwhile the use of the LPS activator joined with adequate milk manipulation practices, guarantee the non deterioration in time and the arrival to optimal quality to industry. A reduction of 90 percent of acid milk arriving to industry and recolection points was obtained and also a decrease in 2  $\log_{10}$  of milk contamination.

#### **4. Discussion**

The results significantly contribute to a higher security and innocuity in the use of the LPs, since a lesser thiocyanate concentration than that indicated by the Codex Alimentarius Guidelines is used, due that the activation range is much lesser than the maximal natural concentration found in the milk of an individual animal. The obtainment of a limit value to consider an inadequate use of the LPs is also a criteria allowing its control of use.

The nature essentially bacteriostatic of the LPS, avoids the quick multiplication of the milk contaminant saprophytic flora, lowering the deterioration of the initial quality and the losses due to acidification [11, 23, 25, 26], demonstrated in all mammalian species of economical importance.

The growth inhibition effect of pathogen bacteria and the less reduction of bacteria pathogens can be associated to the damage provoked by the action of the Lactoperoxidase system final

products on the bacteria structure and/or function, which restrains its restitution even after the effect has disappeared, and then the exacerbation effect is discarded.

From the practical point of view, the observed increase on the efficacy of pasteurization is very important in those cases in which the processing milk contains a high bacterial amount, and when failures in the itself process exist, since the total elimination of microorganisms is assured with emphasis in the coliform and pathogen groups. The reduction or elimination of the thermoresistant bacteria vegetative forms and potentially of the spores, is an aspect for further research, but it can be a field highly promissory field for long live products and high temperature thermal processes of dairy industry.

The results agree with other reports [4, 16] referring an increase on the efficacy of the thermal process in previously activated milk and the inactivation of the components post activation. The non alteration of cheese and yogurt quality, where live microorganisms are used after the activation and thermal treatment of milk, demonstrates that the LPS is deactivated by the thermal treatment [4, 16, 23, 30]. The majority of reports indicating any inhibitor effect on lactofermentant microorganisms have been obtained in non thermally treated milk or with temperatures lower than those established for such processes (85°C during 20 minutes or more).

The use of the method as part of an integral program for increasing the milk quality and not as an independent measure, assures that the dairy producers use the good practices of milking hygiene and milk manipulation as an essential element of the improvement and the LPs activation as an auxiliary measure to maintain its initial quality [24]. On the other hand, though the method improves the pasteurized milk quality indicators, it does not exclude the need of its thermal treatment of the milk.

The LP system activation effects on the saprophyte flora and some pathogen bacteria are being used for other goals besides the milk preservation. It includes the following potential uses.

- Oxidative stress in relation to intestinal inflammation (9,20)
- Treatment without helicobacter pylori (13, 14, 21, 32)
- Treatment to respiratory diseases (5,8)
- Cosmetics (13)
- Conservation of fish, meats and others (1, 2, 10)
- Conservation of fruits and fruit juices(19, 24, 29)
- Conservation of vegetables from group IV. Substitution of active chloride as disinfectant. (2, 22, 29)
- Use in the development of drugs and dental pastes (14, 18, 28)
- Diagnostic of mastitis in goats (27)

The development of some researches in that field, used before only on milk conservation, shows the high potential of the new uses of the LPs.

## **5. Conclusions**

Concerning the use of the LP system in Cuba and in other tropical countries from America, the determination of normal thiocyanate ion concentrations and overdossages, together with the confirmation that there is not exacerbation of pathogen bacterias, reinforce the criterion of security and innocuity when using the LP system. The calculation of the kinetic of activation as for the combined bacteriostatic and bactericide effects, clarifies the evolution of the activity in time and their scope. The improvement of the efficiency of the pasteurization process in



milk with elevated counting of bacteria and the experience in the activation in milk from four mammal species of economical interest, reinforce the criterion of its use in the practice.

There is a coincidence with the conclusions of FAO/OMS Technical Committee (11), that there are no scientific or practical reasons to maintain the clause that restricts the use of the method in the international market of milk products. The evidences point out to a quick use of the LP system activation for the conservation of raw milk, for other foods and in the development of products for the human health.

## References

- [1]. Agencia Española de Seguridad Alimentaria (AESa). Informe del Comité Científico de AESa sobre la utilización del sistema CATALIC basado en la activación del sistema LP, para el tratamiento de frutas y hortalizas. Nro de Referencia AESa (2005) 2005-012.
- [2]. Agencia Francesa de Seguridad de Alimentos (AFSSA). Avis de l'Agence française de sécurité sanitaire des aliments relatif à l'autorisation d'un système lactoperoxidase comme auxiliaire technologique pour traitement des salades Iveme gamme. Saisine Nro 2003-SA-0015.
- [3]. Armenteros M., Dalvit P., Leyva V., Ponce P., Alfonso P. Risk analysis of the exacerbation of foodborne pathogens in raw milk activated with Lactoperoxidase system. Rev. Salud Anim. 29 (2006) 176-181
- [4]. Barret N.E., Grandison A. S., Lewis J. Contribution of the Lactoperoxidase to the keeping quality pasteurized milk. J. Dairy Res. 66 (1999) 73-80
- [5]. Boots J.W., Floris R. Lactoperoxidase: From catalytic mechanism to practical applications. A Review. Int. Dairy J. 16 (2006), 1271-1276. Us Patent November 21/2000.
- [6] *Codex Alimentarius*. Guidelines for the preservation of raw milk by use of the Lactoperoxidase system. CAC/GL 13-1991. (1991). 5 pp.

- [7]. *Codex Alimentarius*. Request for additional information regarding the potential risk in respect of the lactoperoxidase system. Information from Cuba, Canada, The United States and Canada. CX/FH 07/39/2 Add. 1, October 2007
- [8]. Conner G.E., Wijkstrom-Frei C., Randell S.H., Fernandez V.E., Salathe M. The Lactoperoxidase system links anion transport to host defence in cystic fibrosis. *FEBS Lett* 581 (2007) 271-278.
- [9]. Davies M. J., Hawkins C.L., Pattison D.I., Res M.D. Mammalian home peroxidase: from molecular mechanisms to health implications. *Antioxidants and Redox Signalling* 10 (2008) 1199-1234.
- [10]. Elliot R.M., Mclay J.C., Kennedy M.J., Simmonds R.S. Inhibition of foodborne bacteria by the lactoperoxidase system in a beef cube system. *Int. J. Food Microb.* 91(2005) 73-81.
- [11]. FAO/OMS. Benefits and potential risks of the Lps of raw milk preservation. Inform of the technical meeting FAO/OMS. Available at <http://www.fao.org/ag/dairy.html>. 56 pages, 2006.
- [12]. Fernández O., Marrero E., Capdevila J.Z.. Safety considerations on lactoperoxidase system use for milk preservation. *Rev. Salud Anim.* 27 (2005) 205-209
- [13]. Glanbia Nutrionals Lactoperoxidase: Antimicrobials cosmetics. Available at <http://www.glanbianutritionals.com>. 2007.
- [14]. Haukioja A., Ihalin R., Loimaranta V., Lenander M., Tenuevo J. Sensitivity of *Helicobacter pylori* to an innate defense mechanism, the Lactoperoxidase system, in buffer and human whole saliva. *J. Med. Microbiol.* 53 (2004) 855-860.
- [15]. Jacob, B. M., Essy A., Sreekumar B., Haridas M.. Thiocyanate: mediated antifungal and antibacterial property of goat milk lactoperoxidase. *Life Sci.* 66 (2000) 2433-2439
- [16]. Kamau D.N., Doores S., Pruitt K. Enhanced thermal destruction of *Listeria monocitogenes* and *Staphylococcus aureus* by the lactoperoxidase system. *Appl. and Env. Microb. Sep.* (1990) 2711-2716

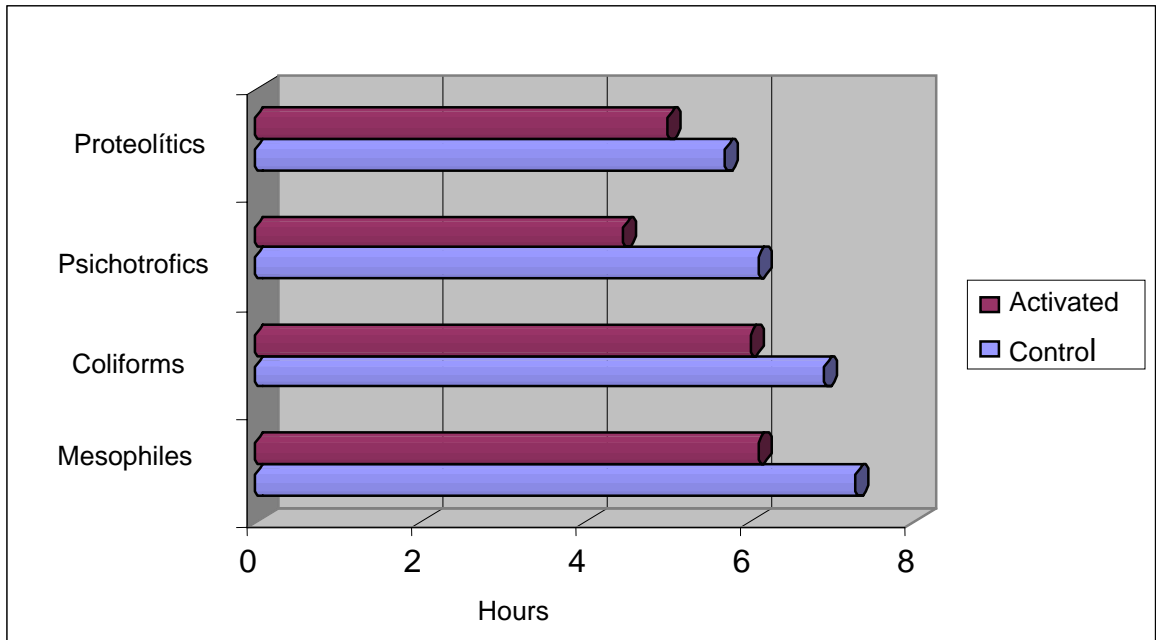
- [17]. Kishore B., Banerjee K., Marimuthu P., Bhattacharyya P., Chatterjee M. Effect of thiocyanate through milk on thyroid hormone homeostasis in women. *Brit. J. Nutrit.* 78 (1997) 679-681
- [18]. Korhonen H., Pihlanto A. Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. *Curr. Pharm. Des.* 13 (2007) 829-843.
- [19]. Le Nguyen D.D., Ducamp M.N., Dornier M., Montet D., Loiseau G. Effect of the lactoperoxidase system against three mayor causal agents of disease in mangoes. *J. Food Prot.* 68 (2005) 1497-1500.
- [20]. Matsushita A., Son D.O., Satsu H., Takano H., Takano., Kawakami H., Totsuda and Shimizu M. Inhibitory effect of lactoperoxidase on the secretion of proinflammatory cytokine interleukin-8 in human epithelial Caco-2 cells. *Int. Dairy J.* 18 (2008) 923-938.
- [21]. Patent Nro US 6149908. Use of lactoperoxidase, a peroxide donor and thiocyanate for the manufacture of a medicament for treating *Helicobacter pylori* infection. Nov 21/ 2000.
- [22]. Patent Nro US 6 447 811 B1. Pesticide against plan-pathogenic microorganisms. Sep 10, 2002.
- [23]. Ponce P., Capdevila J., Armenteros M. Aspectos prácticos y consideraciones sobre peligros microbiológicos y químicos en el uso sistema lactoperoxidasa en el trópico americano. *Rev. Salud Anim.* 25(2004) 163-172
- [24]. Ponce, P. Activación del sistema lactoperoxidasa: un nuevo enfoque para la conservación de leche cruda en el trópico americano. Tesis de Doctor en Ciencias. Ediciones CENSA (2007) 64 pgs, La Habana. Dic/2007.
- [25]. Revol-Jenelles A.M., Milliere, J.B. The Lactoperoxidase system on milk preservation: Its use, antimicrobial activity and effects on milk products. GLP/FAO, 2005.

- [26]. Seifu E., Buys E. M., Donkin E.F. Significance of the lactoperoxidase system in the dairy industry and its potential applications: A Review. Food Sci. and Techn. 16 (2005) 137-154
- [27]. Seifu E., Donkin E.F., Buys M. E. Potential of lactoperoxidase to diagnose subclinical mastitis in goats. Small Rum. Res. 69 (2007) 154-158.
- [28]. Shin K., Yamauchi K., Teraguchi S., Hayasawa H., Imoto I. Susceptibility of *Helicobacter pylori* and its urease activity to the peroxidase-hydrogen peroxide-thiocyanate system. J. Med. Microb. 51 (2002) 231-237
- [29]. Touch V., Hayakawa S., Yamada S., Kaneko S. Effects of a lactoperoxidase-thiocyanate-hydrogen peroxide system on *Salmonella enteritidis* in animal or vegetable foods. Int. J. Food Microb. 93 (2004) 175-183.
- [30]. Trujillo A.J., Pozo P.I., Guamis B. Effect of heat treatment on Lactoperoxidase activity in caprine milk. Small Rum. Res. 67 (2007) 243-246.
- [31]. Uguz M.T. Ozdemir H. Purification of bovine milk Lactoperoxidase and investigation of antibacterial properties at different thiocyanate mediated. Appl. Bioch. and Microb. 41 (2005) 349-353.
- [32]. Zimecki M., Kruzel M.L. Milk-derived proteins and peptides of potential therapeutic and nutritive value. J. Exp. Ther. Oncol. 6 (2007) 89-106.

**Table I. Factors associated to thiocyanate variation in cow raw milk**

<b>Factor</b>	<b>Type of milk</b>	<b>Result</b>
<b>Feeding:</b> Several tropical pastures	Individual	High variation (0,05-0,62 mmol.L <sup>-1</sup> ) Fertilized Star Pasture (0,07-0,64 mmol.L <sup>-1</sup> )
<b>Feeding:</b> Different type of tropical grassy	Farm bulk milk	Low variation (0,12-0,14 mmol.L <sup>-1</sup> ).
<b>Season of year:</b> Dry and rain	Farm bulk milk	Small variations (0,13-0,141 mmol.L <sup>-1</sup> )
<b>Calostral period</b>	Individual cows	Beginning high, normal at 7 days (0,27-0,12 mmol.L <sup>-1</sup> )
<b>Lactation number</b>	Bulks by groups	Slight increasing in consecutive lactations. (0,07, 0,12, 0,14 mmol.L <sup>-1</sup> ) en 1ra, 2da-4ta, +5ta
<b>Mastitis</b>	Individual cow	Normal in healthy cows (0,11 mmol.L <sup>-1</sup> ), Medium in subclinical (0,18 mmol.L <sup>-1</sup> ), High in clinical (0,34 mmol.L <sup>-1</sup> )
<b>Breed</b>	Bulks	Rustic (0,13-0,16 mmol.L <sup>-1</sup> ), Specialized (0,11 mmol.L <sup>-1</sup> )
<b>Country</b>	Bulks Venezuela and Mexico	Low variation, similar to Cuba(0,11-0,15 mmol.L <sup>-1</sup> )
<b>Mean concentration</b>		0,141 mmol.L <sup>-1</sup>
<b>Added Concentration</b>		0,11 mmol.L <sup>-1</sup>
<b>Overdosification criterion</b>		+0,35 mmol.L <sup>-1</sup>

**Figure 1. Effect of the LP system activation on different microorganisms groups ( $\log_{10}$  ufc/ml) at 4 hours post activation**



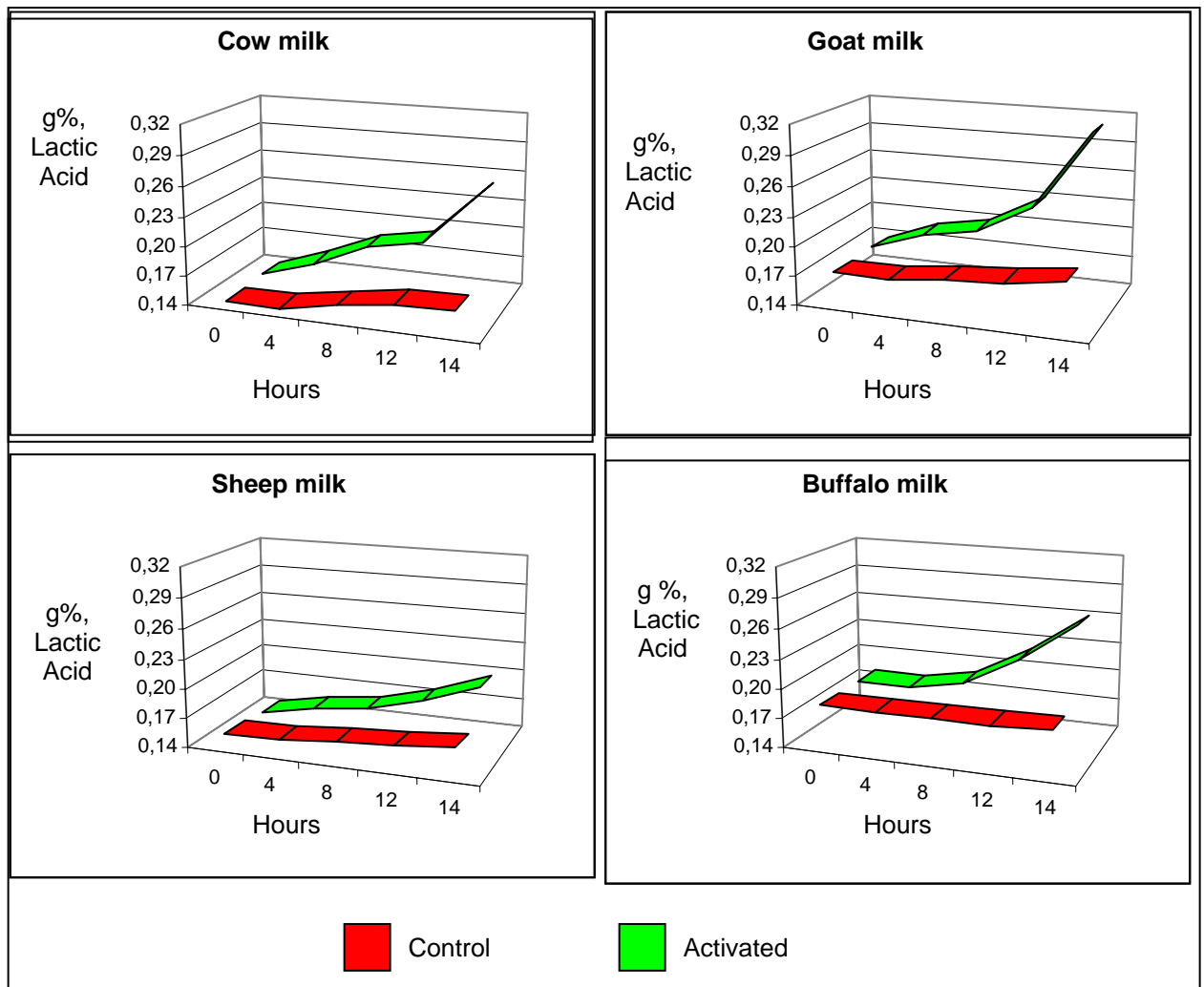
**Conditions:** Means of 27 laboratory tests done during the years 1985-2003. Differences  $p < 0,05$ , in all cases.

**Table II. Dynamic of the LP system activation on the coliform bacteria counting.**

Time, hours	UFC/ml ( $\text{Log}_{10}$ )		% of Reduction	Signification
	Control	Activated		
0	$6,2 \times 10^4$ (4,79)	$5,0 \times 10^4$ (4,70)	1,88	ns
3	$1,85 \times 10^5$ (5,27)	$5,1 \times 10^4$ (4,71)	10,62	$p < 0,05$
6	$2,3 \times 10^6$ (6,36)	$5,0 \times 10^4$ (4,70)	25,10	$p < 0,05$
9	$2,5 \times 10^7$ (7,40)	$5,5 \times 10^4$ (4,74)	35,95	$p < 0,001$
12	$6,2 \times 10^7$ (7,79)	$9,8 \times 10^5$ (5,99)	23,10	$p < 0,01$

**Conditions:** Milk bulks of 5 producers with manual milking, maintained at room temperature between 27.6-34.9°C. Three replicas by origin. First value in ufc/ml, second value in brackets is  $\text{log}_{10}$ .

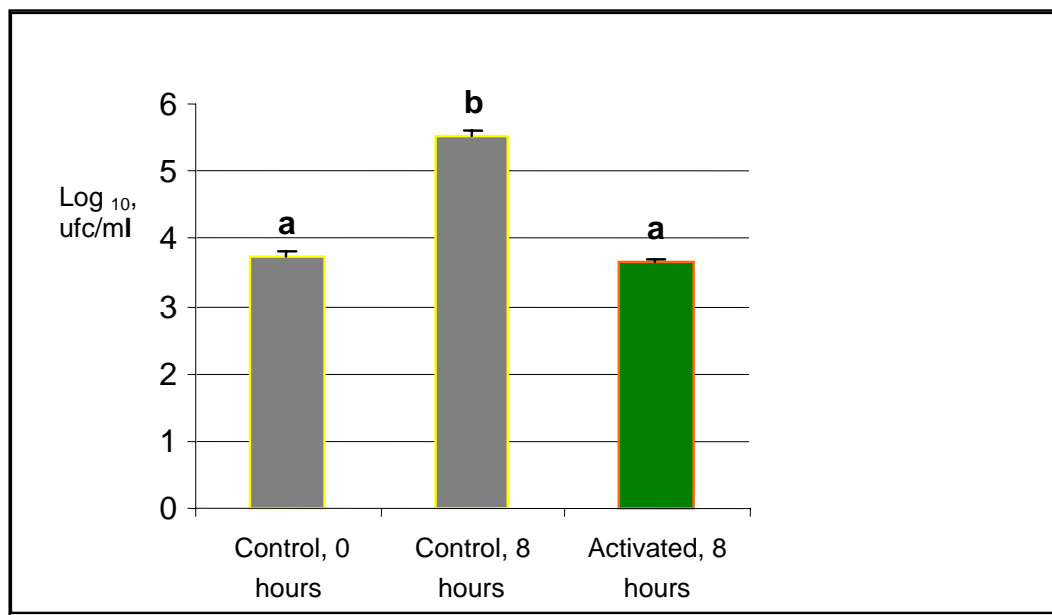
**Figure 2. Effect of the LP system activation on the titlable acidity in cow, goat, sheep and buffalo milk.**



**Conditions:** Mean values of 23 laboratory assays and field observations in different countries during the years 1988-2003. Fluctuating ambient temperature between 22-36°C. Milk of sheep, data of studies only from Albania. Significant differences  $p < 0,05$  starting from 4 horas in cow and goat and from 6 horas in buffalo and sheep.



**Figure 3. Effect of the LP system activation on the mesophile bacteria content in raw milk of excellent initial quality**



**Conditions:** Temperature between 27.7-33.1°C. Volume of 517, 490 and 508 liters,.  
Three replicas in alternate days. Different letters,  $p < 0,001$

**Table III. Reduction Percentage (Activated vs control) in the log<sub>10</sub> of the microorganism amount inoculated in raw milk.**

<i>Microorganism</i>	<i>% Total reduction</i>	<i>% Reduction at 12 hours</i>
<i>Staphylococcus aureos</i> , ATCC 14028	7,74	8,45
<i>Listeria monocitogenes</i> , ATCC 43256	11,13	11,11
<i>E. coli</i> enterohemorrágica, O157:H7,	17,7	21,43
<i>Salmonella typhimurium</i> , ATCC 25923	21,64	24,19
<i>Bacillus cereus</i> , spp	44,0	31,1

Conditions: Contamination with  $10^4$  ufc/ml for each microorganism, except to *Sta aureos* which was  $10^6$  ufc/ml

**Tabla IV. Effect of the LP system activation on the milk components and product quality.**

<b>Product</b>	<b>Characteristics</b>
Raw Milk	Stable concentrations of protein and fat during 12 hours and lactose up to 8 hours. (Three replicas in raw milk maintained at a temperature of 32 <sup>0</sup> C).
Pasteurized Milk	Reduction of 92% of mesophyle viable aerobic and of 100% in coliform and thermo-resistant bacteria. (In all cases raw milk with higher amounts than one million ufc/ml. Pasteurization at 73 <sup>0</sup> C during 20 seconds).
Yogurt	Similar coagulation times, percentage of lactic acid and viscosity index between treated and without treated. (Pasteurization at 85 <sup>0</sup> C during 20 minutes).
Semi hard Gouda Cheese	Non significant differences in time of coagulation, acidity percentage, compactation, flavour, odor, rancidness, eyes, hardness of the bark. (Cheese obtained with 45 days of maturation, 20 lots in alternate days of treated milk /without treated).