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Lactoperoxidase and Lysozyme Activity in Buffalo Milk from Argentine

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Abstract: Lysozyme (LZ) and lactoperoxidase (LP) activity and thiocyanate (SCN^-) content were monitored in the milks of twelve individual Murrah buffalo during mid-lactation. Samples were pasteurized at low (65°C, 30min) and high (72°C, 15s) technique for evaluating heat effect on both enzymes. LP value were 2.49 ± 0.86 $U.mL^{-1}$, thiocyanate level 8.64 ± 2.08 ppm and LZ activity was 424 ± 349 $U.mL^{-1}$. There was difference between heat treatment on both enzymes. LZ was complete inactivated by low and high pasteurization, while LP activity showed 16% of inactivation on low pasteurization and 80% on high pasteurization. SCN^- content was not modified by any heat treatment. This result shows that it is convenient to use low temperatures to preserve buffalo raw milk. LP is the most abundant enzymes in buffalo milk, while LZ has very low activity. Addition of SCN^- or hydrogen peroxide can be carried out when LP is not limitation factor in buffalo milk.

Key words: Buffalo milk, lactoperoxidase, thiocyanate, lysozyme

Introduction

Milk provides the neonate not only with nutrients but also with a host of defence factors such as antibacterial, anti-inflammatory and immuno-modulatory agents (Goldman and Goldblum, 1995). Antimicrobial agents in milk include immunoglobulins, lactoferrin, some lipids and enzymes. Lactoperoxidase and lysozyme are two enzymes present in exocrine secretion of mammals. Both are antimicrobial agents of milk, helping to maintain quality of raw milk. The activity of lactoperoxidase system (thiocyanate, hydrogen peroxide, lactoperoxidase) in ruminant milk and its preservative effect at different temperatures are well known (Björck, 1978; Björck *et al.*, 1975; Oram and Reiter, 1966 and Reikter *et al.*, 1976). Many works has been done to activate LP system in countries where ambient temperatures are higher than 30°C (Björck L., 1979 and Härnulv and Kandasamy, 1982). LP is the most abundant enzyme in bovine milk, constituting about 1 % of whey proteins (Reiter, 1985). Thiocyanate is also present in milk and its derivates from hydrolysis of glucosinolates present in foods (Wood, 1975). The third component of the system, hydrogen peroxide, may be supplied exogenously or produced by lactic acid bacteria present in milk. Lysozyme kills bacteria by cleaving specific bond between N-acetyl muramic acid and N-acetyl glucosamine residues of the peptidoglycan of the bacterial cell wall. Lysozyme content and enzymatic properties vary widely among species. Human and equine milks are very rich in this enzyme (Chandan *et al.*, 1964 and Jauregui, 1975) and content in ruminants is very studied too, because of cause a very important effect on pathogenic and non pathogenic bacteria.

There are few works about those enzymes on buffalo milk (Härnulv and Kandasamy, 1982 and Priyadarshini *et al.*, 2002), an in all cases, the studies were done about Mediterranean or Italian buffalo milk. The aim of this work was study LP and LZ activity in Murrah buffalo milk and the effect of pasteurization on both enzymes, in order to evaluate the feasibility of use one of this enzymes to preserve raw milk quality.

Materials and Methods

Animals: Milk from twelve Murrah buffalo was used in this study. Individual samples were collected every week during 3 weeks. The milk samples were collected at morning and refrigerated to 4°C until study. All animals were 3-4 month lactation (mid-lactation period) Animals fed were consisted in natural pastured *ad libitum*.

Milk treatment: Raw milk was treated to low (65°C, 30 minutes) and high (72°C, 15 s) pasteurization, after that was cooled to 4°C until analyses were carried out.

Lactoperoxidase: LP activity was determined in duplicate following Marshall *et al.*, 1986. The oxidation of substrate 2,2´-azinodi-3-ethyl-benzthiazoline-6-sulphonic acid (ABTS) reactive (Sigma Co) was monitoring at 412 nm using a Beckman spectrophotometer. Unit of enzyme is corresponding to level of LP oxiding 1μmol.min^{-1} of ABTS.

Thiocyanate: Thiocyanate content was determined in duplicate according to IDF Bulletin, 1988. After

deproteinization with 20% (W/V) trichloroacetic acid, as the ferric complex formed with nitrate reagent, by measuring the absorbance at 460 nm. The SCN^- concentration was calculated from a standard curve.

Lysozyme: LZ activity was measure following Tijssen method, 1985, following the absorbance of a solution with *M. lysodeikticus* at 450 nm. The unit of enzyme activity was defined as change in unit absorbance per minute per ml of milk.

Statistical assays: All data were analyzed by ANOVA test. The results are expressed as means ± standard deviations.

Results and Discussion

Lactoperoxidase activity was determined before (Table 1) and after pasteurization (Table 2) of raw milk. LP activity was 2.49 ± 0.86 $U.mL^{-1}$. The range of individual buffalo was 1.40 to 4.11 $U.mL^{-1}$. There were not significantly differences between individual during 3 weeks. Low pasteurization causes an inactivation of 16% of this enzyme. An important decrease of LP activity was observed after high pasteurization process, in this case LP levels decreased as low as 0.62 $U.mL^{-1}$, corresponding to a 75% of inactivation. LP level in our study (2.49 $U.mL^{-1}$) was higher than levels previously found to buffalo milk by Härnluv and Kandasamy, 1982, who reports LP activity of 0.90 $U.mL^{-1}$. LP activity in other ruminant species has been reported, having 0.77 $U.mL^{-1}$ ewe's milk (Medina *et al.*, 1989), 1.55 $U.mL^{-1}$ for goat milk (Zapico *et al.*, 1990) and 1.4 for bovine milk (Stephens *et al.*, 1979). Highest value for LP has been found in guinea pig milk, with a mean content of 22 $U.mL^{-1}$ (Stephens *et al.*, 1979).

High level of LP value for buffalo milk in this work may be due to breed of buffalo. Differences of this kind were found from goat milk, having Murciano Granadina breed more LP activity than Verata breed (Zapico *et al.*, 1990). The levels of thiocyanate anion (SCN^-) were measure (Table 1) and the mean value was 8.64 ± 2.08 ppm. There was a wide variation range between individual samples, varying from 5.47 to 10.56 ppm. For each individual not significantly differences were found during 3 weeks. According to Wood, 1975; Medina *et al.*, 1989, natural SCN^- content of milk varies between 1 and 10 ppm. The observed value of 8.64 ± 2.08 ppm for Murrah buffalo milk is well within these limits. SCN^- level in our study was higher than reported by Takkar and Dave, 1986 for Mediterranean buffalo milk, who found SCN^- content of 5-6 ppm. Moreover, buffalo milk has less content of this anion than sheep according to Medina *et al.*,1989, who reported 10.5 ppm.

Lysozyme activity from raw milk observed in our study was 424 ± 349 $U.mL^{-1}$. There is a wide activity variation between animals (0-950 $U.mL^{-1}$) in each week. Compare LZ activity between related works is very difficult due to different method employed. This enzyme is present in buffalo milk (Priyadarshini *et al.*, 2002) but have low activity. High levels in human and sea animal milks have been showed, arising 11000 and 21100 $U.mL^{-1}$ respectively (Van Nieuwenhove, 1999). Moreover, ruminant animals like goat or sheep has very low LZ activity (300 and 450 $U.mL^{-1}$, respectively) with same method employed. In our study heat treatment of buffalo raw milk produced complete inactivation of this enzyme.

The bacteriological quality of ruminant milk may be improved by activation of LP system at refrigeration temperatures. In buffalo milk addition of SCN^- and H_2O_2 may be an alternative to preserve raw milk, because LP concentration in this animal is not a limiting factor. This results shows that LP is a thermo- resistant enzyme, but

Table 1: LP, LZ and SCN content in Murrah buffalo milk

Milk Component	Content[1]	SD[2]
LP activity ($U.mL^{-1}$)	2.49	0.86
SCN (ppm)	8.64	2.08
LZ activity ($U.mL^{-1}$)	424	349

Resutls and expressed as mean standard devision n = 36

Table 2: Levels of component after heat treatment of raw milk

Milk Component*	Low pasteurization[a]	High pasteurization[b]
LP activity ($U.mL^{-1}$)	1.84 ± 0.72	0.62 ± 0.12
SCN (ppm)	8.59 ± 1.96	8.60 ± 2.11
LZ activity ($U.mL^{-1}$)	0	0

*Results are expressed as mean ± standard deviation

[a]Low pasteurization (65 °C, 30min) [b]High pasteurization (72 °C, 15s) n = 36

its convenient to treat raw milk using low pasteurization technique. SCN^- content was not significantly modified by heat treatment. LP level found for buffalo milk in this study were higher than reported by others author. Differences may be due to animal diet or breed too. LP is major enzyme present in buffalo milk from Murrah breed. These results are according with those reported by Härnulv and Kandasamy, 1982 working with Mediterranean breed. In addition, studies about LP in other ruminant species showed that this enzyme is the principal antimicrobial activity present in milk. LZ is present in buffalo milk too, but have low activity like cow. This study helps to know normal level in buffalo milk. Low pasteurization is recommended to raw milk, due to light effect on LP activity.

The general aim of our work line is to obtain buffalo cheese with high quality from small farms in Argentine. The maintenance of raw milk by activation of LP system and manufacture after low pasteurization are in progress in our laboratory.

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