SUMMARY

Alkaline phosphatase activity in sheep, goat and buffalo milk in relation to the pasteurization heat treatment: experimental study for a compliance limit

Key words: milk, Alkaline phosphatase, sheep, goat, buffalo

Alkaline Phosphatase is a natural enzyme in milk whose activity is used in the dairy industry to determine the pasteurization process' efficiency.
The European Union legislation defines a level of 350 nU/L of alkaline phosphatise activity as safe for the consumption of cow milk (Commission Regulation (EC) No 1664/2006 of 6 November 2006). However no limits have been established for sheep, goat and buffalo milk.
The aim of the study was to evaluate the Alkaline Phosphatase activity (ALP) of raw and pasteurized bulk sheep, goat and buffalo milk by fluorimetric quantitative assay method (ISO 11816-1:2013), during the production season. The fluorimetric determination of ALP activity has been specified as the reference method (Reg CE 1664/2006).
For this purpose, 1179 samples of bulk raw milk from 57 farms located in Lazio region (Italy) were collected. The samples were transported to the laboratory at 4-6°C.
Following parameters were also determined: total bacterial count (cfu·ml^{-1}), fat (%), protein (%), lactose (%), casein (%), solid non fat (%), somatic cells (cell·ml^{-1}), urea (mg/dL), pH.
Total bacterial count was determined by Bactoscan FC; fat, protein, lactose, solid non fat, urea by Milkoscan 7; somatic cells count by Fossomatic FC and pH measurements were performed on an HACH pH meter - HQ411D.
Pasteurized milk samples were obtained in the laboratory by thermal treatment at 63° C±05°C for 30 minutes in a circulation water bath. The milk samples were cooled in an ice bath after heat treatment.
SW MedCalc (version 1412 - © 1993-2014 MedCalc Software bvba) was used for the statistical analysis of the results. The difference between the mean of the raw and that of the pasteurized milk ALP was investigated by one way analysis of variance (ANOVA).
Sheep milk: alkaline phosphatise values ranged from 662,000 mU/L to 6,953,000 mU/L, with an average of 2,686,000 ± 1,099,000 mU/L.
Levels of ALP are low in the early stage of lactation when milk yields are highest.
Significant correlations of ALP are recorded with casein ($r = 0.23$; $p = 0.001$), fat ($r = 0.22$; $p = 0.0001$), lactose ($r = -0.28$; $p < 0.0001$), pH ($r = -0.26$; $p < 0.0001$) and ALP in pasteurized milk ($r = 0.26$; $p < 0.0001$). No correlation was recorded with the total bacterial count.
There were no statistically significant differences between ALP averages in the investigated sheep breeds (Sarda, Comisana, Lacaune and Massese).
The average alkaline phosphatase in pasteurized sheep milk was 272 ± 76 mU/L ranged from 105 mU/L to 526 mU/L. The 86.3% of the milk samples showed ALP activity below 350 mU/L, 8.6% between 351 and 400 mU/L.
Goat milk: alkaline phosphatise values ranged from 7,840 mU/L to 863,800 mU/L, with an average of 135,861 ± 124,826 mU/L.
The monthly average of alkaline phosphatase decreased from january to march and then began to increase gradually until november. Significant correlations of ALP are recorded with proteins ($r = 0.42$; $p < 0.0001$), casein ($r = 0.29$; $p = 0.0003$), urea ($r = 0.23$; $p = 0.0001$), solid non fat ($r = 0.21$; $p = 0.007$).
Pasteurized goat milk: alkaline phosphatise values ranged from 20 mU/L to 325 mU/L, with an average of 105 ± 64 mU/L. The 98.7% of the milk samples showed ALP activity below 300 mU/L, 1.3% between 301 and 350 mU/L. Significant correlations of ALP ($\log_{10}$) are recorded with somatic cells count ($\log_{10}$) ($r = 0.40$; $p = <0.0001$).
Buffalo milk: alkaline phosphatase values ranged from 71,780 mU/L to 3,434,000 mU/L, with an average of 1,184.846 ± 544,833 mU/L.

The monthly average of alkaline phosphatase decreased from december to march and then began to increase gradually until july.

Significant correlations of ALP are recorded with fat ($r = 0.36$; $p < 0.0001$), proteins ($r = 0.26$; $p < 0.0001$), urea ($r = 0.25$; $p < 0.0001$) and negatively with pH ($r = -0.27$; $p < 0.0001$).

Alkaline phosphatase in buffalo pasteurized milk averaged 159 ± 69 mU/L with a range of 45 mU/L - 388 mU/L.

94.7% of pasteurized milk samples showed ALP activity below 300 mU/L, 4.5% between 301 and 350 mU/L. Significant correlations of ALP are recorded with total bacteria count ($r = 0.30$; $p = < 0.0001$) and somatic cells count ($r = 0.15$; $p = 0.0019$).

There was no significant correlation recorded between ALP raw milk and ALP pasteurized milk.

Significant correlations are recorded with the total bacterial count ($r = 0.30$; $p < 0.0001$) and the somatic cells count ($r = 0.15$; $p = 0.0019$).

The results of the present study indicate that the alkaline phosphatase levels in proper pasteurized milk could be quantified as follows: Sheep milk ≤ 530 mU/L; Goat milk ≤ 330 mU/L; Buffalo milk ≤ 380 mU/L.