

ALKALINE PHOSPHATASE ACTIVITY IN SLOVENIAN CHEESE MADE FROM PASTEURIZED, THERMIZED OR RAW MILK

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ABSTRACT

Alkaline phosphatase (ALP) is an enzyme used as an indicator of adequate pasteurization. We measured its concentration following ISO11816-2 in pasteurized cow milk cheeses and in raw and thermized cheeses regardless of the animal species. The amount of ALP in all pasteurized cow milk cheeses (19 samples) was below 10 mU/g of cheese and as such in agreement with the tentative limit set by the European Union Reference Laboratory (EU-RL). The amount of ALP in raw and thermized cow cheese was 4076 and 411 mU/g of cheese, respectively. However, the concentration of ALP in raw sheep milk cheese was 8207 mU/g of cheese, due to the higher fat content of sheep milk. There was one cheese for which the heat treatment was not clearly specified by the producer. Controls of ALP activity should be carried out regularly to ensure that the legislative criteria are followed and in order to verify the correctness of cheese specifications.

Keywords: cheese /pasteurization /thermization / alkaline phosphatase

1 INTRODUCTION

Alkaline phosphatase (ALP) is an enzyme naturally occurring in all mammalian milks. Adequate pasteurization inactivates the enzyme and consequently its activity in milk can be used to demonstrate the efficiency of pasteurization. An ALP test is considered to give a negative result if the measured activity in cow milk is not higher than 350 mU/L (Commission regulation (EC) No 1664/2006). The legal limit of ALP activity in cheese made from pasteurized cow milk has yet to be determined. A tentative limit of < 10 mU/g of cheese has been proposed by the European Union Reference Laboratory (EU-RL).

Milk ALP is a dimer twisted around itself and composed of two equal length 90,000 molecular weight polypeptide chains attached to each other along their length with disulphide bridges. Heat inactivated (un-

folded) ALP is not capable of hydrolyzing any known substrate. Unfolding of ALP is time and temperature dependent so the ALP activity level can be used as a predictor of the time and temperature regime to which milk had been subjected. Measurement of the amount of residual active ALP reveals the amount of ALP that has not yet undergone unfolding and also detects the possible addition of raw milk to pasteurized milk.

Since 1930 several colorimetric techniques have been developed for the measurement of ALP activity (Kay and Graham, 1935; Scharer, 1938; Aschaffenburg and Mullen, 1949, cited in Rocco, 2004). The first fluorometric procedure for the determination of ALP in dairy products was reported in 1990. It was based on a fluorometric substrate Fluorophos (Rocco, 1990a) and is therefore called the Fluorophos method. It is faster and more sensitive compared to the methods developed previously (Payne and Wilbey, 2009). It has been approved by the

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International Organisation for Standardisation (ISO 11816-1) and is required by the Commission regulation (EC) No 1664/2006 as a reference method for determining alkaline phosphatase activity in milk.

ALP activity is measured by a continuous fluorometric direct kinetic assay. A nonfluorescent aromatic monophosphoric ester substrate undergoes hydrolysis of its phosphate radical and becomes a highly fluorescent product. ALP activity is measured in mU/L at 38 °C during 3-min read time. One unit of ALP is amount of enzyme that catalyzes transformation of 1 micromole of substrate/min/L of product. Because of the low levels of ALP in final dairy products, results are reported in milliUnits/L (mU/L) (Rocco, 1990a).

Cheese, whose main raw material is milk, can be roughly classified according to type of milk (cow, sheep, goat), moisture content (hard, semi-hard, soft) and process of heating (raw, thermized, pasteurized), which has the greatest impact on the amount of active ALP in cheese.

Pasteurization is achieved by a procedure involving a high temperature for a short time (at least 72 °C for 15 seconds) and a low temperature for a long time (at least 63 °C for 30 minutes); or any other combination of time-temperature regimes to obtain an equivalent effect such that the products show, where applicable, a negative reaction to an alkaline phosphatase test immediately after such treatment (COMMISSION REGULATION (EC) No 2074/2005). This means that the ALP activity in cow's milk is not higher than 350 mU/L.

Thermization is a process of raw milk heating for at least 15 seconds at a temperature between 57 °C and 68 °C so that after treatment the milk shows a positive reaction to the phosphatase test (Rules on the veterinary-sanitary inspection and control of food production establishments, the veterinary-sanitary checks and the conditions for good health standards for foodstuffs and raw materials of animal origin, 1999).

Concentration of ALP in cheese depends on the milk used (raw, pasteurized or thermized milk) and varies from <10 to 8000 mU/g of cheese. So the ALP activity values can help us evaluate the milk type used for cheese production and verify the classification of the products.

The aim of the EU-RL for Milk and milk products project is to determine the permitted amount of alkaline phosphatase activity in cheese made from pasteurized cow milk. Due to reports from some European NRLs about some types of cheeses made from pasteurized cow milk that may exceed the proposed ALP value (10 mU/g), surveys in member states were conducted. It was presumed that in some special cheese technologies and processes the values of ALP might be exceptionally higher.

The Slovene National Reference Laboratory (NRL) for milk and milk products has the obligation to provide results of ALP activity in cheeses made from pasteurized cow milk produced in Slovenia with the purpose of supporting or refuting the proposed tentative limit of 10 mU/g of ALP in cheese from pasteurized cow milk.

2 MATERIALS, METHODS AND RESULTS

For the purpose of the survey, samples of 24 different cheeses produced in Slovenia were tested for ALP activity. There were 9 hard cheeses produced by 4 different dairy plants, 13 semi-hard cheeses produced by 5 dairy plants and 3 soft cheeses produced by two dairy plants (Table 1).

Initially, the NRL for milk and milk products had asked the producers to provide the cheese samples, but finally a range of cheeses was bought by NRL on the market.

Cheeses were sampled and tested according to ISO 11816-2 (2003).

The experiment was divided into 2 parts. In the first part we measured ALP activity in 19 cheeses made from pasteurized cow milk and in the second part ALP activity was measured in 5 cheeses made from raw or thermized milk regardless of the animal species.

In large wheel-shaped hard cheeses made from raw milk, alkaline phosphatase is not equally distributed. The activity is usually higher between 0 and 0.4 cm immediately below the rind, and lower in the centre. To take the sample, 0.5 cm of the rind of the cheese wheel was removed and then a 1 cm sample was taken 0.5 to 1.5 cm below the rind. For the purpose of this experiment the concentrations in the rind and in the core of the wheel were also determined.

In the semi-hard and soft cheese the concentration of alkaline phosphatase is equally distributed so before sampling, only a thin surface layer of cheese was removed to avoid possible contaminants.

The results of measurements are present in Tables 1 and 2.

3 DISCUSSION

The activity of ALP in all cheeses made from pasteurized cow milk and produced in Slovenia was below 10 mU/g of cheese, which is in accordance with EU-RL and our assumptions.

The activities of ALP in soft and hard raw cheeses and in the thermized cow cheese were 3513, 4076 and 411 mU/g of cheese, respectively. ALP concentration in

Table 1: ALP activity in cheeses made from pasteurized cow milk

No	Type of cheese	Producer	Cheese type	Heat treatment	Sample ² result (mU/g)
1	Hard	1	local 1 ¹	pasteurized	0.79
2	Hard	2	local 2 ¹	pasteurized	1.58
3	Hard	3	local 3 ¹	pasteurized	0.61
4	Hard	3	local 4 ¹	pasteurized	1.02
5	Hard	1	local 5 ¹	pasteurized	0.46
6	Semi-hard	4	gauda type	pasteurized	0.59
7	Semi-hard	1	local 6	pasteurized	0.69
8	Semi-hard	4	local 7	pasteurized	0.60
9	Semi-hard	4	local 8	pasteurized	0.74
10	Semi-hard	4	Trapist type	pasteurized	0.66
11	Semi-hard	1	Edamec type	pasteurized	0.76
12	Semi-hard	1	local 9	pasteurized	3.17
13	Semi-hard	3	local 10	pasteurized	0.86
14	Semi-hard	3	local 11	pasteurized	0.59
15	Semi-hard	4	local 12	pasteurized	0.51
16	Semi-hard	5	local 13	pasteurized	0.96
17	Semi-hard	6	fresh cheese	pasteurized	<1
18	Semi-hard	5	fresh cheese in brine	pasteurized	1.00
19	Soft	5	curd	pasteurized	1.57

¹ Slovenian type of cheese produced according to relevant technological procedure

² Sample – 1 cm of sample 0.5 to 1.5 cm below the rind of the cheese wheel

cheese made from raw sheep milk was 8207 mU/g, which is approximately 50% higher compared to cheeses made from raw cow milk. This difference can be explained by the higher fat content in sheep milk (6 g/100 g) compared to cow milk (3.8 g/100 g). According to Painter and Bradley (1997), 30 to 40% of ALP is located in the fat-globule membrane, which acts as a protective coating against heat treatment. Products with lower fat content have less residual ALP activity than products with higher fat content.

It is assumed that as a consequence of cheese ripening, the concentration of ALP is higher in the rind and lower in the core of cheese wheels. However, our results show that ALP in the tested Slovenian hard cheeses made from raw and thermized milk is equally distributed. Such a distribution could be related to the small dimensions of the cheese wheels. The ALP activities in the rind, core and the sample of raw cow cheese were 2967, 3104 and 4076 mU/g of cheese, respectively. In sheep cheese, the values were 6524, 7297 and 8207 mU/g of cheese in the

Table 2: ALP activity in cheeses made from raw or thermized milk

No	Type of cheese	Producer	Cheese	Heat treatment	Result (mU/g)		
					Sample ²	Rind	Core
20	Hard	3	local 4 ¹	Thermized or raw	4076	2967	3104
21	Hard	7	local 4 ¹	Thermized or raw	1.46	/	/
22	Hard	9	local 14 ³	Raw	8207	6524	7297
23	Hard	8	local 15 ¹	Thermized or raw	411	412	315
24	Soft	2	local 16 ¹	Raw	3513	/	/

¹ Slovenian type of cheese produced according to relevant technological procedure;

² Sample – 1 cm of sample 0.5 to 1.5 cm below the rind of the cheese wheel; ³ Sheep cheese; / not performed

rind, core and sample, respectively. ALP activities in the rind, centre and the sample of thermized cow cheese were 412, 315 and 411 mU/g of cheese, respectively, with a standard deviation of only 56 mU/g.

From our survey we can conclude that producer specification for cheese No 21 (Table 2) regarding milk heat treatment was inconsistent with the relevant technological procedure for such type of cheese. The activity of ALP was 1.46 mU/g of cheese. This cheese type should be produced out of raw or thermized milk and should therefore have a positive reaction to the phosphatase test (Rules on the veterinary-sanitary inspection and control of food production establishments, the veterinary-sanitary checks and the conditions for good health standards for foodstuffs and raw materials of animal origin, 1999).

According to Annex I of Commission regulation (EC) No 2073/2005 there are different microbiological criteria for cheeses made from raw milk and milk that has undergone lower temperature than pasteurization and cheeses made from milk that has undergone pasteurization. Correct specifications and accurate information about the product are therefore crucial, also for the consumer. Measurement of ALP activity can be used as a marker for potential heat treatment of the milk as well as a tool for verifying producer specifications of cheeses.

4 CONCLUSIONS

The tested cheeses made from pasteurized cow milk in Slovenia all had ALP activities of less than 10 mU/g of cheese. We can therefore support the proposed EU-RL tentative limit of 10 mU/g of ALP for this type of cheese. In small cheese wheels, ALP is equally distributed in rind, core and 0.5 to 1.5 cm below the rind. ALP activity could potentially be used not only for testing milk pasteurization but also for verifying producer specifications.

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