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Short communication

Alkaline phosphatase activity in cheese as a tracer for cheese milk pasteurization

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ABSTRACT

Alkaline phosphatase (ALP) activity is used throughout the world as a marker for the proper pasteurization of milk, to guarantee its hygienic safety. The Standard ISO 11816-2/IDF 155-2 describes the analysis of ALP in cheese. However, the method has been questioned in the past because there have sometimes been ambiguous results. The critical operations of the analytical procedure are more precisely defined and a zonal cheese sampling adopted. ALP inactivation is firstly evaluated in the relevant steps of controlled cheese makings of hard (Emmental), semi-hard (Raschera) and soft (Chaource) cheeses. Application of the improved procedure in over 700 samples of typical cheeses from France, Italy and Switzerland proved the applicability of the method. Based on this large study, a limit for ALP activity in cheese from pasteurized milk is proposed at 10 mU/g.

1. Introduction

Alkaline phosphatase (ALP) activity is used throughout the world as a marker for the proper pasteurization of milk, to guarantee its hygienic safety (Rankin, Christiansen, Lee, Banavara, & Lopez-Hernandez, 2010). Milk pasteurization is also adopted in manufacturing of several cheese varieties with the aim of destroying the possible pathogenic microorganisms and reducing those capable of causing defects in the cheese (Kousta, Mataragas, Skandamis, & Drosinos, 2010). Heat-treatments milder than pasteurization are sometimes used to achieve a reduction of undesirable species while preserving the ability of milk to coagulate. At small dairies, milk is often thermally treated using poorly controlled batch processing. In addition, some countries pose restrictions to production or import of cheeses produced from unpasteurized milk (Pellegrino & Donnelly, 2004; Ryser, 2011). Considering all these situations, a simple and reliable method to check proper milk pasteurization in cheese would be of high value for both safety inspection and trading controls. The analysis of ALP in cheese is described by ISO 11816-2/IDF 155-2 (ISO, 2015). In the past, this proposed method was not considered appropriate for reflecting the heat treatment of the cheese milk in some types of cheese (Lechner & Ostertag, 1993). Further studies evidenced that the high variability of different cheese varieties in processing conditions, texture and size affects the residual activity of ALP and its zonal distribution within the cheese (Bisig, Frohlich-Wyder, Jakob, & Wechsler, 2010; Pellegrino, Tirelli, Masotti, & Resmini, 1996). The temperature at which the curd is heated and the size of the cheese wheel are the main parameters influencing residual ALP activity in cheese. Therefore, besides of having a reliable analytical method, there is an urgent need for an appropriate limit for residual ALP activity to characterize cheeses from pasteurized milk. This work performs an in-depth study of the relevant technological parameters for cheese making in order to define the critical analytical conditions and possible limitations on how ALP activity in cheese can be used as a marker for the pasteurization of cheese milk. In addition, a survey of the ALP levels in commercial cheeses has been performed to confirm the feasibility of the proposed approach.

2. Materials and methods

2.1. Cheese production and sampling

Cheeses selected for the study, i.e. Emmental (hard cheese),

Abbreviations: ALP, Alkaline phosphatase.

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Raschera (semi-hard) and Chaource (soft cheese), were manufactured under controlled conditions at respective dairies, and samples were taken at different steps of the process. The usual processing conditions were followed in all cases, and the use of either raw, thermized (63–67 °C for 15–30 s) or pasteurized (73–78 °C for 20–30 s) milk were the only variables adopted in the process. The cheeses were ripened under the usual conditions, and samples were taken at the end of the commercial ripening period, at 4 months, 2 months and 15 days, for Emmental, Raschera and Chaource cheeses, respectively.

Commercial cheese samples from France, Italy and Switzerland were classified into three different groups relating to cheese type (soft, semi-hard and hard cheese) and milk thermal treatment (unheated, thermized, pasteurized), according to the product information. For cheeses produced under controlled conditions, samples for zonal measurements of ALP were taken in the different zones, as shown in the Section 3.2 (Fig. 1C). Commercial cheeses were sampled according to the scheme proposed in Section 3.3. From each cheese, a representative portion of the corresponding gray zone (Fig. 2) was grinded and homogeneously mixed.

2.2. Analysis of alkaline phosphatase with fluorophos

Milk samples were analyzed according to ISO 11816-1/IDF 155-1 (ISO, 2013), while the cheese samples were analyzed based on ISO 11816-2/IDF 155-2 (ISO, 2015), with some optimizations.

Briefly, cheese buffer (Fluorophos Test System, Advanced Instruments Inc., Norwood MA, USA) was used for cheese extraction instead of milk. An aliquot of 0.3–0.5 g of the grinded cheese was weighed in a glass beaker, 5 mL of cheese buffer was added and then this was mixed with a homogenizer (e.g., Ultra Turrax®) to obtain a completely homogenous dispersion. After transferring the sample to a 25 mL volumetric flask, the glass beaker was rinsed with 5 mL of cheese buffer and mixed once again with the homogenizer. The samples were pooled, and the flask was filled to the mark with cheese buffer. The sample was centrifuged at 1000 g at 4 °C for 10 min. The middle transparent phase was collected in a clean tube, and 75 μl were taken for analysis according to ISO 11816-2/IDF 155-2 (ISO, 2015), as indicated above.

2.3. Statistical analysis

At least 150 individual samples were analyzed in duplicate for each cheese type. The data were analyzed for variance by pairwise comparison with the Fisher's 95% Least Significant Difference post hoc test, using least squares means with SYSTAT 13 (Systat Software, Inc., Chicago, IL, USA). Values of $P < 0.001$ were considered to be significant.

3. Results and discussion

3.1. Impact of cheese milk processing on ALP activity

The batches of milk that were used for making the controlled cheeses were analyzed for ALP activity according to the preparation steps. The samples for either raw, thermized or pasteurized milk were taken after milk fat standardization (not done for Raschera cheese), maturation at low temperature (10–16 °C) and heating to the temperature required for coagulation (32–38 °C). Data from the manufacturing process for Emmental cheese are shown in detail, as an example, in Fig. 1. As expected, the ALP activity in the differently heated milk samples decreased with treatment at higher temperatures (Fig. 1A). The subsequent steps did not have any further impact on the ALP activity. Very similar behavior was observed for the other two cheeses (not shown).

3.2. Zonal differences of ALP inactivation in cheese

To study the effects of the heat treatment during cheese production on the resulting zonal differences in heat load in the whole cheese, different types of cheeses were manufactured under controlled conditions with the previously differently heated milk.

It was already demonstrated that in large-size cheeses, such as Grana Padano, Parmigiano-Reggiano and Emmental cheese, ALP is inactivated within the cheese loaf during molding because of the high temperature and the low pH conditions that remain for several hours. Since these cheeses are made with raw milk, residual ALP activity is only present in the peripheral portion of the cheese that cools down faster (Bisig et al., 2010; Pellegrino et al., 1997). In this study, Emmental cheese was chosen as model because of its large-size wheel deriving from the traditional curd pressing process. As for the other cheeses that have been mentioned, the curd is cooked at high temperature (53–56 °C), which has allowed us to study the zonal differences in ALP activity in the ripened cheese depending on preliminary milk heat treatment.

After the cheese had ripened for four months, four different samples were collected from the cheese wheel (Fig. 1 B) and analyzed for ALP activity. One sample was representative of the whole cheese (Fig. 1 B, Whole), while three were zonal samples (Fig. 1 B, C) taken from the outer, intermediate and core zones. As expected, ALP activity was highest for all milk heat treatments in the outer zone and decreased towards the core zone of the cheese loaf (Fig. 1 B). ALP activity in the whole cheese sample was clearly less than the activity of the outer zone. Also, although the differences in the milk heat treatment could be observed in all the samples, they were the greatest in the outer zone. In all three zones, the heat treatment at 73 °C for 20 s, corresponding to milk pasteurization conditions, was found to lead to ALP activities below 10 mU/g (Fig. 1 B). However, in the core zone, the differences in residual ALP activity between treatments at 67 °C/20 s and 73 °C/20 s were very small. This indicates that a distinction between pasteurization and thermization (Ryser, 2011) is not possible in samples from the core zone of the cheese.

In the smaller cheese wheels of both Chaource (soft cheese) and Raschera (semi-hard), the zonal differences were marginal (data not shown) and, therefore, a zonal sampling in those cheeses did not improve the results. In the cheeses produced with pasteurized milk, the ALP activity was below 10 mU/g, irrespective of the sampling procedure.

3.3. Recommended cheese sampling

According to the data that was obtained in this study and reported previously, zonal differences in ALP activity are expected in hard cheeses with big wheels and relatively high curd cooking temperatures. The typical raw milk cheeses from Italy, Switzerland and France, such as Emmental, Grana Padano, Parmigiano-Reggiano, Gruyère, Sbrinz and Bernese Hobel cheese, belong to this group of cheeses. As a result of the zonal differences that were

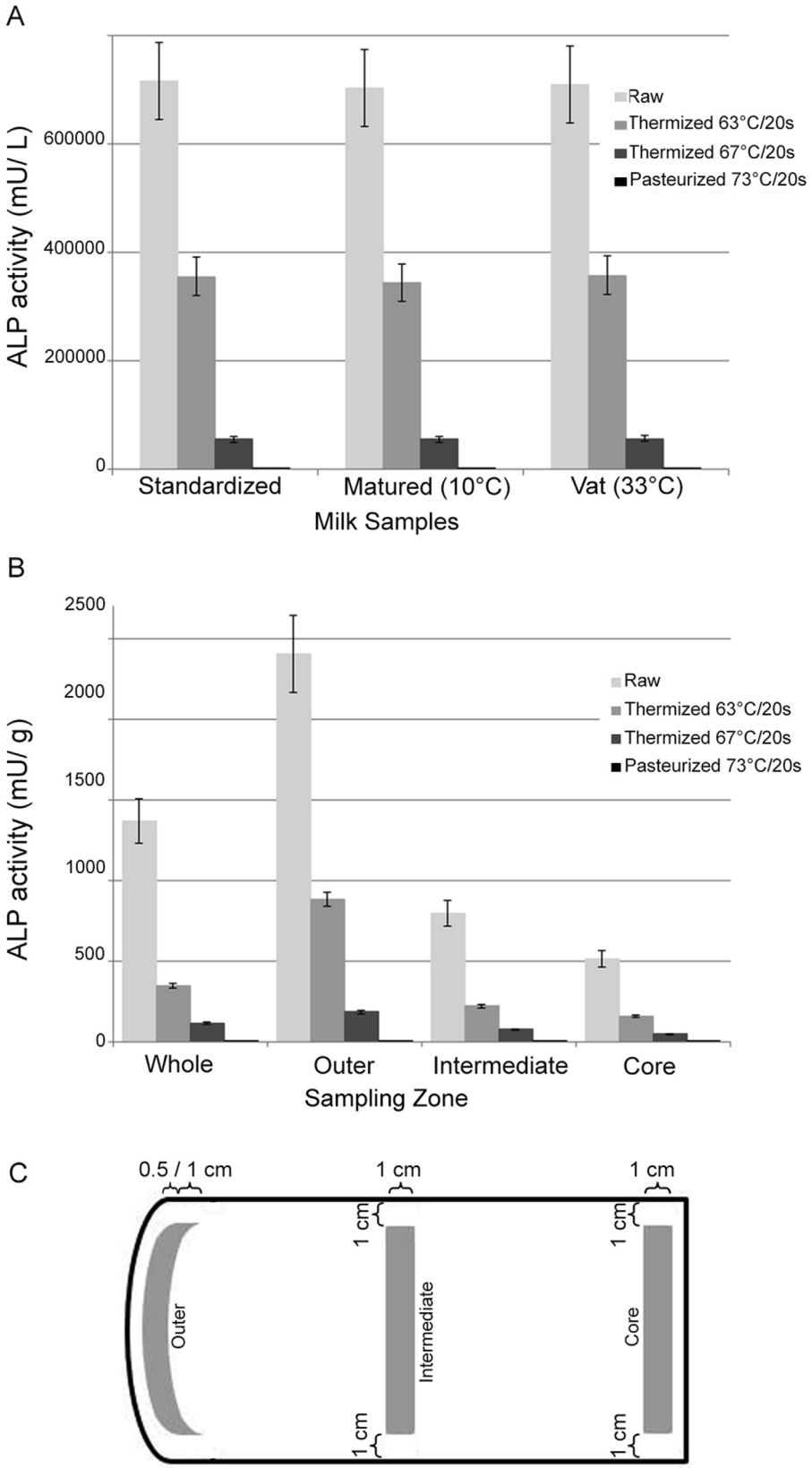

Fig. 1. ALP activities of milk after different heat treatments and of the derived Emmental cheeses. ALP activities of cheese milk following different heat treatments and taken at different steps of the preparation process. Error bars represent standard deviation of duplicated analyses (A). ALP activities measured in different zones of the derived cheeses (B). Sampling zones considered (C).

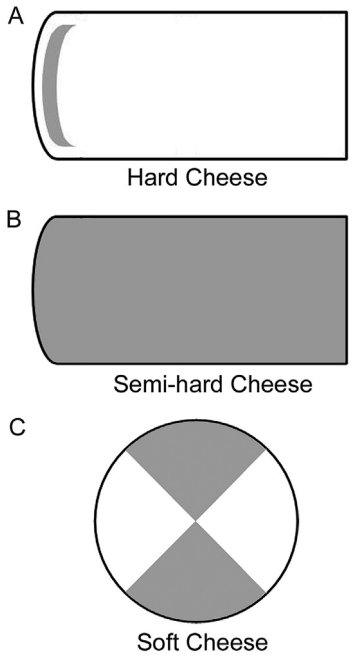

Fig. 2. Cheese Sampling. Gray areas indicate the proposed sampling zones for Hard Cheeses (A), Semi-hard Cheeses (B) and Soft Cheeses (C).

observed in the hard cheeses, a specific sampling scheme was established (Fig. 2). According to this scheme, hard cheeses should only be sampled from the outer zone (A), semi-hard cheeses should be sampled from the entire portion (B) and soft cheeses should be sampled from two opposite quarters of the whole wheel (C). The sampling scheme for semi-hard and soft cheeses corresponds to the ISO 707/IDF 50 Standard (ISO, 2012), while the sampling zone for hard cheeses has not previously been described. In some cases, the assignment of a cheese variety to hard or semi-hard cheese type can be difficult. In Codex Alimentarius (2013) the designation of cheeses according to their firmness is based on the moisture content on a fat-free basis, but the values limiting the two classes partially overlap. Nevertheless, our results demonstrate that sampling a semi-hard cheese using the method described for hard cheeses will not result in different values of ALP activity. Moreover, the sampling for hard cheeses can only be performed on cheese samples where the cheese rind is present and clearly visible, which excludes the analysis of small pieces or grated cheese. Cattaneo et al. (2008) demonstrated that, using a suitable extraction procedure, ALP can be determined in grated Grana Padano cheese. However, grated cheeses only represent a low proportion of commercial cheeses and were not considered in this study.

3.4. ALP activities in pasteurized, thermized and raw milk cheeses

More than 700 samples of 32 different cheese varieties originating from France, Italy and Switzerland were collected, sampled according to the above proposed sampling scheme and analyzed for ALP activity. Cheeses made from pasteurized, thermized and raw milk were considered. All the average values for each cheese variety that was measured, including the standard deviation (SD) and number of analyzed samples (N) are compiled in Table 1. The high SD values for certain types of cheese made from raw milk can be attributed to a number of factors, mainly differences in the cheese-making conditions and in the ripening time. It is also important to consider that many of those cheeses are manufactured at artisanal dairies. Among the raw milk cheeses, the lowest values of ALP activity were found in Grana Padano and Parmigiano-Reggiano cheeses, which are ripened over a period of 12 months at least. In fact, ALP was shown to decrease progressively in these cheese types during the prolonged ripening and to reach approximately 300 mU/g after 34 months (Pellegrino et al., 1996). In contrast, the high values found in the Bernese Hobel cheese could be related to a lower curd heating temperature compared to other hard cheeses and to the smaller cheese mold. However, it is important to note that all the pasteurized cheeses have ALP activities that are clearly below 10 mU/g. This indicates that a clear-cut decision can be made if a specific cheese has been produced from pasteurized milk when the proposed sampling scheme is respected.

Individual statistical analysis of the measured ALP activities for soft (A), semi-hard (B) and hard (C) cheeses showed that, within the three cheese types, three different processing of cheese milk: pasteurization, thermization and no heat treatment (raw milk), were all significantly different from each other ($P < 0.0001$) in their ALP activities (Fig. 3).

3.5. Tentative limit for pasteurized cheese

A clear-cut difference between cheeses from pasteurized milk and cheeses from thermized or raw milk can be drawn at the level of 10 mU/g. To our knowledge, very few data available from the literature was obtained with the same analytical method and is, therefore, comparable to ours. Hodoscek, Rupnik, Ahcin, and Biasizzo (2012) report ALP activity values in the range of 0.5–3.2 mU/g in 19 samples of Slovenian cheeses produced from pasteurized cow milk and including hard, semi-hard and soft varieties. Values of ALP were higher than 400 mU/g in cheeses made from either raw or thermized milk. A recent survey of ALP activity in ten representative cheeses made from pasteurized milk in the United Kingdom indicates levels ranging from 0.1 to 4.6 mU/g (Cassidy, Daly, Early, & Rowe, 2014).

To date, only two cheese types have been found for which the method has not been applicable. Blue cheeses need to be excluded because of the activity of the phosphatases from molds (usually from *Penicillium* spp. and *Aspergillus* spp.) (Rosenthal, Bernstein, & Rosen, 1996). In addition, the temperature over 60 °C reached by the curd for pasta-filata cheeses, while is being stretched with hot water (Kindstedt, Caric, & Milanovic, 2004), inactivate ALP making it impossible to test whether the cheese milk was correctly pasteurized.

4. Conclusions

Alkaline phosphatase activity is a pertinent marker for proper pasteurization of the cheese milk if the proposed cheese sampling, especially for hard cheeses, is respected and with exclusion of blue cheeses and pasta filata cheeses. Based on data obtained from over 700 individual samples from three different countries, a tentative limit for the ALP values of pasteurized cheeses can be set at 10 mU/g. The reliability of this limit is confirmed by literature data. Nevertheless, further work on cheeses from other countries will be performed to support and confirm the applicability of the method as well as the tentative limit of 10 mU/g.

Table 1
Compiled results of ALP activities measured in typical cheeses mainly from France, Italy and Switzerland. SD = Standard deviation, N = number of cheeses analyzed in duplicates. Values written in gray color: analysis of single cheese samples in duplicate (N = 1).

	Cheese Type	Country	Pasteurized			Thermized			Raw		
			Average (mU/g)	SD (mU/g)	N	Average (mU/g)	SD (mU/g)	N	Average (mU/g)	SD (mU/g)	N
Soft Cheeses	Brie	F, CH	3	1	37	60	40	3	1913	565	12
	Camembert	F, CH	3	1	61	648	314	12	3687	928	20
	Acid curd cheese	F	2	1	3						
	Coulommiers	F	2	1	24	920	1109	2	2999	1449	5
	Stracchino	I	2	1	4						
	Limburger	CH	1	2	4						
	Vacherin Mont d'Or	CH				456	500	9			
	Tomme	F, CH	2	3	5	1589	26	2	4306	1869	8
	Chaource	F	5		1	864		1			
	Mascarpone	I	1		1						
Semi-Hard Cheeses	Appenzeller	CH							1284	895	10
	Cream Cheese	F, CH	1	1	8	93		1	3260		1
	Crotto	I	2	1	3						
	Flösser Cheese	CH				325	132	5			
	Mountain Cheese	CH				499	437	9			
	Raclette	F, CH	2	2	16				2643	1679	6
	Raschera	I				562	110	3	3522	1073	6
	St. Paulin	F, CH	2	2	7	77		1	9691		1
	Taleggio, Quartirolo	I	5	2	2				5060		1
	Tête de Moine	CH							3636	631	15
	Tilsiter	CH	0	0	6	1639	1508	6	2274		1
	Vacherin Fribourgeois	CH				102	103	4	3733	518	4
	Valtellina Casera	I	1	1	2						
	Winzer Cheese	CH				326	251	5			
	Caciotta Valle Alpina	I							1977		1
	Fontina	I							1447		1
	Montasio	I							300		1
	Spicy Max	CH				36		1			
Hard Cheeses	Bernese Hobelcheese	CH							3342	350	2
	Comté	F							1846	597	4
	Emmental	F	2	2	12						
	Emmental Switzerland	CH							1117	681	13
	Generic Grana Type	I	2	1	5	318	173	18	1435	745	8
	Grana Padano	I							954	288	134
	Gruyère	CH							1163	785	12
	Parmiggiano Reggiano	I							781	299	114
	Sbrinz	CH							1236	711	6
	Switzerland Swiss	CH	0	0	6						

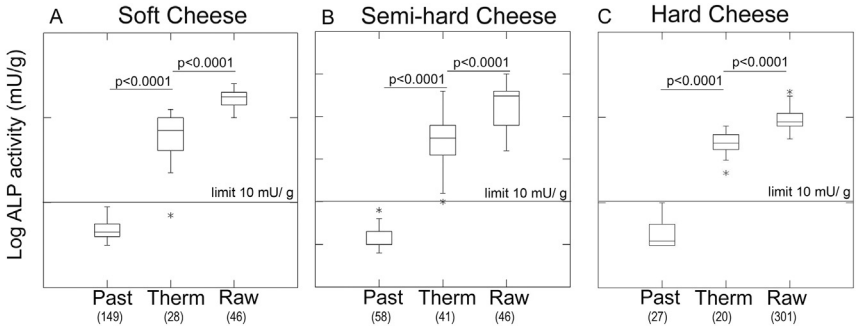

Fig. 3. ALP activities of different cheese types produced with either raw, thermized or pasteurized milk. Whisker plots of ALP activities (log mU/g) measured in Soft, Semi-hard and Hard Cheeses made either from pasteurized (Past), thermized (Therm) and raw milk, respectively. Box plots show median, length of box represents 50% of the central values, and asterisks represent values outside the inner fence (>1.5 times outside the box hinge). Within each box, all three groups are different ($P < 0.0001$). Figures in brackets indicate the number of samples. A tentative limit of 10 mU/g for cheeses made from pasteurized milk is indicated.

Conflict of interest

The authors declare that they have no conflict of interest.

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