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Decision Letter (JDS-14-8906.R1)

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Subject: Journal of Dairy Science - Decision on Manuscript ID JDS-14-8906.R1

Body: 13-Feb-2015

Dear Miss María Vélez:

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Title: A new minicurd model system for hard cooked cheeses

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Sincerely,
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Section Editor, Journal of Dairy Science

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A new minicurd model system for hard cooked cheeses

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Key Words:	minicurd model, hard cheese, cheese making technology

Innovation in cheese models.

Vélez.

Hard cheeses need long ripening periods to reach appropriate quality standards, which means expensive production costs. The view of the present work was to find a new hard cooked cheese model, with cost-effectiveness and simplified experimental manipulation.

SHORT COMMUNICATION: CHEESE MODEL SYSTEM

A new minicurd model system for hard cooked cheeses.

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ABSTRACT

A new minicurd model of hard cheese was proposed and validated. For that, curd particles and whey obtained from conventional cheese-making of Reggianito Argentino were separated and frozen. After that, both fractions were thawed and the mixture whey-curds was reconstituted, from which minicurds were made at laboratory scale. Repeatability, and the effect of freezing on the minicurd composition were investigated by assessing pH, proteins, moisture content, sodium chloride and total thermophilic lactic flora counts. Good repeatability was achieved, and no significant differences were found between minicurds made from fresh and frozen materials. Composition of the minicurd was appropriate to model Reggianito Argentino cheese.

Keywords: cheese making technology, minicurd model, hard cheese

Cheese making experiments aimed at assessing technological innovation or new additives or ingredients are both expensive and time-consuming. That is why a number of cheese models have been developed, in which variations in biochemistry of ripening or cheese ecosystem can be assessed rapidly and without the complexity of real-scale cheese matrix. Scientific research focused on technological changes in food industry needs to be validated at pilot scale, as in vitro assays usually are not enough to inspire confidence and promote changes (Hunter et al., 1997). However, cheese model systems have been quite useful for many applications. Available cheese models include miniature semi-hard or Cheddar cheeses, cheese slurries, Ch-easy (Farkye et al., 1995; Smit et al., 1995; Shakeel-Ur-Rehman et al., 1998; Muehlenkamp-Ulate and Warthesen, 1999; Hynes et al., 2000; Jeanson et al., 2011; Leclercq-Perlat et al., 2004; Milesi et al., 2011). Reggianito Argentino and Parmigiano Reggiano extracts were also developed as a model of the aqueous phase of this type of cheese (Gatti et al., 2008; Bergamini et al., 2013).

Examples of applications of some of these models are: comparison of *Lactobacillus* strains, with or without GDH activity, according to their ability to produce aroma compounds (Kieronczyk et al., 2004); assessing lactic acid bacteria aminopeptidase activities (Gatti et al., 2008), culturing long ripened cheese microflora (Neviani et al., 2009), evaluation of proteolysis induced by different strains (Milesi et al., 2011).

The objective of the present work was to lay out and validate a new cheese model for hard cooked cheeses, consisting of a reconstituted minicurd. We intended that its composition were appropriate to model Reggianito cheese; other requirements for the model were cost-effectiveness and simplified experimental manipulation. For that purpose, we ideated a layout that included one standard cheese-making using 100 L of milk, which we stopped before whey-curds final cooking. Curds and whey were separated at this point and became the raw materials for multiple minicurds elaborations. Freezing of raw materials was also proposed.

Raw materials: Reggianito cheese-making

Raw bulk milk (100 L), pH 6.65 ± 0.05, Dornic acidity 18 ± 1 °D (1D = 100 mg lactic acid L^{-1}) was supplied by a nearby dairy plant (Milkaut Coop. Ltda, Franck, Santa Fe) and standardized to 2.8% fat. Milk was batch pasteurized at 63ºC for 30 min and cooled to 33ºC for cheese making. After that, $CaCl_2$ was added to a final concentration of 0.014% w/v; pH was adjusted to 6.40 with lactic acid (1.5% w/v) as some acidification of cheesemilk is required before coagulation for this type of cheeses. Then, a mixed commercial starter of *Lactobacillus helveticus* and *Lactobacillus bulgaricus* (Chr. Hansen Argentina, Quilmes, Argentina) was added in a concentration of 10^6 UFC mL^{-1} of milk. After 10 min of mechanical stirring, MAXIREN™ 150 (100% chymosin, Gist-Brocades, France) was added at a final concentration of 0.012 g L^{-1} for milk coagulation. After 18–20 min, the curd was cut to the required grain size (approximately half the size of a rice grain) by successive cuts under manual agitation. The mixture of curd particles and whey was continuously stirred, at coagulation temperature (33°C). In that moment, approximately 15 kg of the mixture curd particles-whey were taken from the vat and separated in a sieve (10 ASTM mesh size). Curd particles were quite wet and undergoing syneresis, so the operation was performed as quickly as possible in order to avoid heterogeneity in moisture content, acidification or the formation of a continuous coagulum. Curd particles and whey were packed separately in tight plastic bags (2 L) and frozen at -18ºC in three different freezers for their subsequent use. The proportion of curd and whey in the original mixture was assessed by mass balance: an aliquot of the thoroughly homogeneous mixture and its separate fractions were weighted. The proportion was 1:4 curd-to-whey.

Minicurds preparation

A day before the minicurd preparations, the raw materials – whey and curds - were thawed at 4ºC. Curd particles were disaggregated and mixed with a spatula in order take representative samples from the curd contained in the plastic bags. Curd and whey were tempered at 33ºC during 20 min in a bath to simulate the conditions in the vat, and pH was measured by immersing a pH electrode (Titriskop E516, Suiza). Then, mixtures of ~ 500 g of whey and curds were prepared, in the same proportion found in the vat (1:4). The mixtures were then incubated at 37ºC until they reached a pH of 5.6; this intermediate pH value was chosen in order to approach the standard value commonly found in cheeses (5.4), avoiding the risk of over acidification. After that, cooking step was performed: curds and whey were gently stirred while heating in a bath at 1ºC min^{-1} up to 45ºC. After reaching 45ºC, the mixtures were more rapidly heated (> 1 °C min^{-1}) up to 50 °C. When the mixtures reached the desired curd scalding temperature the stirring was stopped. The curds were separated from the whey by centrifugation (Multi RF ThermoScientific, USA) at 2750 *g* and 37ºC for 20 min in 250 mL-tubes using a swinging bucket rotor to obtain cylindrical shaped minicurds. Minicurds were refrigerated for 5 min and brined in 20% (w/v) brine at 12 °C for 20 min. Four replicates were obtained from each batch of curd particles and whey. In each replicate, two minicurds of approximately 25 g were made in parallel; one was sampled immediately and the other was vacuum packed in plastic film and stored 7 days at 12ºC. A minicurd photograph is shown as an example in Figure 1.

Repeatability

As mentioned above, the repeatability of the model was checked for raw materials coming from different Reggianito cheese-making vats, made on different days and with different milk. Temperature and pH curves were monitored in the curd-whey mixtures during minicurds preparation. Minicurds were analyzed in duplicate at day 1 (preparation) and day 7, as follows: pH, measured by APHA method (Bradley et al., 1992); proteins by the Kjeldahl method (IDF, 1993: 20B), moisture content was determined by oven drying to a constant weight at 102 ±1 ºC according to IDF (IDF, 1982), sodium chloride by atomic absorption spectrophotometry (AOAC, 1990). Thermophilic lactic bacteria were assessed before storage by plating sample dilutions on skim milk agar (SMA) and counting plate colonies after 48 h of incubation at 37°C (Candioti et al. 2002).

Freezing and thawing effect

The effect of freezing and thawing of whey and curd particles on the minicurd composition was also checked. Whey curds mixtures were prepared with fresh materials from different Reggianito cheese vats and employed to obtain 3 minicurds replicates. They were compared with minicurds made with frozen materials. As described above, two minicurds were made in parallel, one for sampling the same day of curd making and the other to store for 7 days at 12 °C.

Evaluation of the model

During minicurd making employing frozen materials, no significant differences were found in the pH and temperature curve (Figures 2 and 3). Comparison was established between two groups of four replicate minicurds, coming from frozen raw materials provided by two cheese vats obtained in different days. Data for pH curves was recorded during the steps of curd maintaining at 37 °C after thawing, and cooking. Temperature monitoring was started when the mixture curd-whey ended the incubation step at 37ºC.

The same samples were analyzed for microb ial counts, and showed that total thermophilic lactic flora exceeded 10^8 CFU at beginning of ripening in all curds (data not shown), which correlated with the similarities found for pH evolution.

For the different cheese makings, also mean values of pH, moisture, sodium chloride and protein content of minicurds did not show significant differences when a t-test was applied for each variable at the two times studied (Table 1). Good repeatability was verified, as the coefficient of variation did not exceed 3%.

In the present work, minicurds were stored during 7 days, a short period aimed at representing the first stage of cheese ripening. It was noticed that pH decrease during storage was not significant probably because of the small curd size and consequent rapid temperature decrease. Moisture content was slightly higher than the one commonly found in mature cooked cheeses (Candioti et al., 2002; Sihufe et al., 2007), but it was inferior to those obtained by Vélez et al. (2011), when the milk was coagulated directly in falcon tubes. As for sodium chloride content, the values obtained were acceptable for young Reggianito cheeses (Shiufe et al., 2007; Wolf et al., 2010; Ceruti et al., 2012).

It is of common knowledge that simplifying cheese matrix brings about the resignation of some aspect: texture, chemical or physical (Shakeel-Ur-Rehman et al., 1998). In recent years there have been several attempts in order to find an ideal cheese model, but the quality of a model is always related to the objectives of each investigation. In general, the composition of model acid curds and slurries greatly differ from a real cheese (Farkye and Fox, 1990; Farkye et al., 1995; Muehlenkamp-Ulate and Warthesen, 1999; Choi et al., 2006; Yu et al., 2012). Choi et al. (2006) obtained curds with 90 % moisture and 3% proteins. Farkye et al. (1995) obtained slurries of Cheddar cheeses with 57.70% moisture and 23.18% proteins; the model proposed by Milesi et al. (2008) contained ~61 % moisture, and 18% proteins. A synthetic model easy to prepare was developed by Salles et al. (1995), made with caseins, low heat milk powder, deodorized milk fat, NaCl and rennet. The model had lower dry matter and higher moisture than a normal hard cheese, but its way of preparation allowed incorporation of not only some lipophilic substances such as aromas but also some water-soluble substances in order to study flavour. Smit et al. prepared a cheese model "Chea-sy" heating a slurry at 80ºC, giving composition and texture similar to natural cheese, but heat treatment changed chemical natural conditions. Boisard et al. (2013) utilized model cheeses with different lipid/protein ratios, made by mixing rennet casein, acid casein, commercial melting salts, anhydrous milk fat, deionized water, NaCl and citric acid; the models allowed the authors to study mobility and release of sodium ions, but they did not reflect the actual environment present in cheeses.

On the other hand, in the present work the experiment aimed at comparing minicurds obtained from either frozen or fresh raw materials showed that no differences existed in the acidification curves. For minicurds obtained from fresh materials, the step of incubation at 37ºC was not performed, as it was not needed to restart acidification. In this curds, draining pH and temperature ramps follow the same temporal steps that Reggianito cheese-making (Zalazar et al. 1999; Vélez et al. 2010). Composition of minicurds made with frozen and fresh raw materials was similar, as pH, moisture, sodium chloride and protein content did not show significant differences. Microbiology counts exceeded 10^8 CFU (Table 2). As far as we know, there is no information reported about the effect of freezing bovine curd grains, but some similar results were found by Picon et al. (2010) and Campos et al. (2011), who studied cheeses made with frozen ewe and goat curds respectively, as follows: curds were pressed, frozen, thawed, cut and mixed with fresh cow milk in order to manufacture cheeses. Authors did not found differences in dry matter, pH and microbiology counts between experimental and control cheeses. Freezing of curd from bovine milk, aimed at producing low moisture Mozzarella is a usual practice in Argenina and other countries in South America, mainly carried out in small dairies and farm factories. Curds are drained, frozen and sold to Mozzarella factories that thaw them, ripen them to get the correct pH and stretch them continuing with the usual cheese-making process (Anuario de la Lechería, 2013).

In this work, we proposed a cheese model based on a minicurd reconstituted from frozen curd grains and whey obtained from standard Reggianito cheesemaking. We were able to conclude that composition and acidification curves during minicurd making were not biased by either minucurds replicate or cheese batch elaboration, and consequently, the model had good repeteability. We also found that composition of minicurds made from curds grains and whey that had been previously frozen did not differ from those made with fresh raw materials.

The model proposed has advantages with respect of previous experimental models; the fact that raw materials were obtained after starter addition and that the curd was cut in the original vat at pilot plant scale, probably improved the simulation of a real cheese curd. Also comparatively with other cheese models, minicurds were easier, quicker to obtain and less expensive.

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Table 1. Moisture, protein content and pH of minicurds made with frozen raw material. (Global mean and standard deviation of 4 replicates are shown)

[a] Values in the same row with different superscripts differ significantly ($p < 0.05$).

Ripening time	1 day			
Cheese making day	1		2	
	Mean ± sd[1]	cv[2]	Mean ± sd[1]	cv[2]
Moisture (%)	37.07[a] ± 0.64	1.54	37.48[a] ± 0.99	1.95
Proteins (%)	30.03[a] ± 0.58	1.74	29.73[a] ± 0.54	2.66
NaCl (S/M)[3]	2.00[a] ± 0.06	3.00	2.07[a] ± 0.04	1.93
pH	5.56[a] ± 0.09	1.93	5.59[a] ± 0.10	1.80
Ripening time	7 days			
Cheese making day	1		2	
Moisture (%)	37.75[a] ±0.64	1.15	38.2[a] ± 1.17	1.58
Proteins (%)	29.96[a] ±0.4	1.68	30.48[a] ±0.70	3.05
NaCl (S/M)[3]	1.95[a] ± 0.04	2.05	2.11[a] ± 0.02	1.42
pH	5.46[a] ±0.06	1.34	5.55[a] ±0.09	2.30

[1]sd: standard deviation
[2]cv: coefficient of variation
[3]Salt in Moisture

Table 2. Moisture, proteins, pH and microbial counts of minicurds made with frozen and fresh materials. (Global mean and standard deviation of 3 replicates are shown.)

Ripening Time	1 day	
	Frozen Materials	No Frozen Materials
Moisture	37.20[a] ± 0.55	38.00[a] ± 1.5
Proteins	30.00[a] ± 1.00	31.5[a] ± 0.70
pH	5.55[a] ± 0.15	5.40[a] ± 0.20
Total thermophilic lactic flora counts (CFU ml^{-1})	$6\ 10^{8}$	$7.5\ 10^{8}$
Ripening Time	**7 days**	
	Frozen Materials	No Frozen Materials
Moisture	37.50[a] ± 0.70	36.20[a] ± 1.0
Proteins	31.50[a] ± 1.30	32.00[a] ± 0.8
pH	5.45[a] ± 0.11	5.50[a] ± 0.15

[a] Values in the same row with different superscripts differ significantly ($p < 0.05$).

Vélez- Figure 1

Figure 1. Minicurd photograph (a) Horizontal view (b) Top view.

Vélez-Figure 2

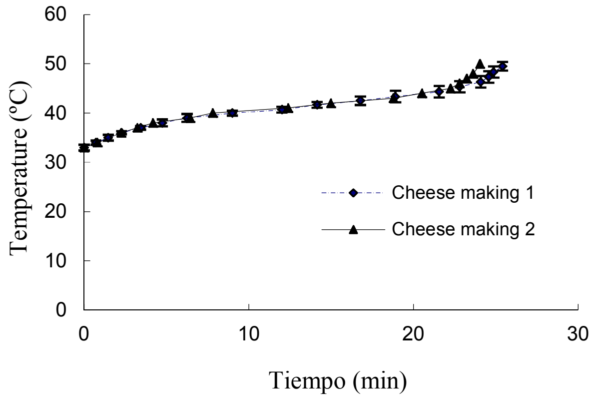

Figure 2 - Temperature curves during elaboration of minicurds. Each curve corresponds to average values of four replicate minicurds by cheese-making day. Recording started when the mixture curd-whey ended the incubation at 37ºC.

Vélez - Figure 3

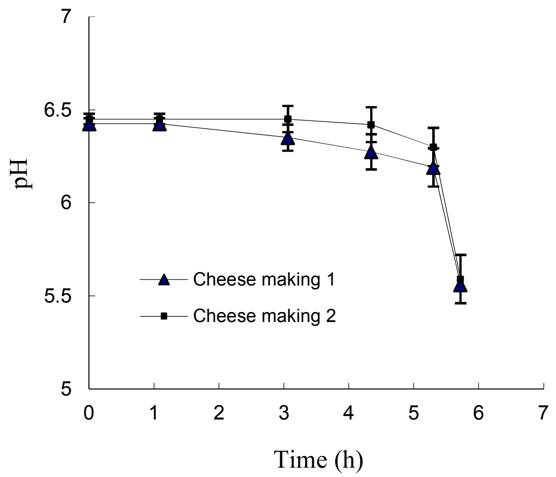

Figure 3. Acidification curves during elaboration process of minicurds made with frozen raw materials. Recording started when the mixture curd-whey attempted the incubation temperature (37°C) up to the end of manufacture.(—▲— —■—) Corresponds to average values of four replicate minicurds for each cheese-making day.

Vélez – Figure 4

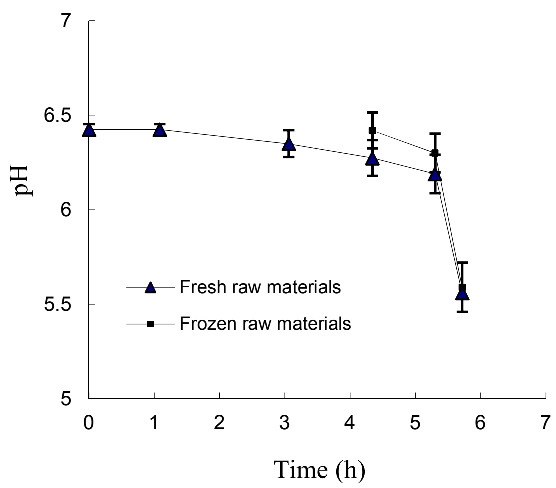

Figure 4. Acidification curves during elaboration process of minicurds made with frozen and fresh materials.