The effect of probiotics (*Lactobacillus rhamnosus* HN001, *Lactobacillus paracasei* LPC-37, and *Lactobacillus acidophilus* NCFM) on the availability of minerals from Dutch-type cheese

Marek Aljewicz,^{*1} Ewa Siemianowska,[†] Grażyna Cichosz,^{*} and Elżbieta Tońska[‡]

^{*}Department of Dairy Science and Quality Management, Faculty of Food Science,

[†]Department of Safety, and

[‡]Department of Science of Commodities and Evaluation of Food Quality, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, 10-726 Olsztyn, Poland

ABSTRACT

The use of probiotic cultures in the production of Dutch-type cheeses did not lead to significant changes in their chemical composition but it lowered their acidity. The availability of calcium and magnesium analyzed by in vitro enzymatic hydrolysis was 19 and 35%, respectively; the availability of phosphorus was significantly higher, at >90%. The use of probiotic cultures significantly increased the availability of calcium (~2.5%), phosphorus (~6%), and magnesium (~18%). The in vitro method supports accurate determination of the effect of the *Lactobacillus* spp. cultures on the availability of mineral compounds ingested with Dutch-type cheese.

Key words: *Lactobacillus*, probiotic, availability, mineral, cheese

INTRODUCTION

Probiotic cultures are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2006). Numerous clinical tests have demonstrated the beneficial effects of probiotic cultures on the gastrointestinal tract (by alleviating symptoms of enteritis and irritable bowel syndrome) and the immune system (Kneifel and Salminen, 2011). Probiotic strains produce metabolites (organic acids, diacetyl, ethanol, hydrogen peroxide, bacteriocins, antibiotics, and carbon dioxide) that stimulate the growth of large intestinal microflora and the immune system. The effectiveness and type of the resulting health benefits are directly determined by the probiotic strain composition, bacterial counts, and the applied carrier.

The effect of probiotic cultures on the bioavailability of mineral compounds is an equally important but

often overlooked factor. Bacteria proliferating in the intestines use nutrients, including mineral compounds in the digesta, for growth. Changes in the composition and abundance of microflora lead to variations in the bioavailability of mineral compounds (Kwong and Kitts, 2003; Ghanem et al., 2004).

Dairy products are the major (~70%) source of calcium in the human diet (Karczmarewicz et al., 2002). Calcium and other mineral compounds are also supplied with foods of plant origin and water, but the calcium content of those sources is significantly lower, at 16 and ~7%, respectively (Guéguen and Pointillart, 2000). Unlike other foods, dairy products do not contain phytates, oxalates, uronic acids, or insoluble dietary fiber fractions, which produce insoluble complexes and decrease the bioavailability of mineral compounds (Wolf et al., 2000).

Ripened cheeses have acceptable sensory characteristics and constitute an abundant source of mineral compounds. Yet the minerals found in ripened cheeses are not always readily available to the human body. The bioavailability of mineral compounds from ripened cheeses is determined by cheese type and the applied production technology, the content of organic acids, the presence of various casein fractions (α_{S1-} , α_{S2-} , and β -CN) and their degradation products, fat content, and FA structure (Guéguen and Pointillart, 2000; Kłobukowski et al., 2009).

Bioavailability is defined as the quantity of mineral compounds and trace elements that can be released (digested), absorbed, and metabolized by the human body. The digestibility of food components is determined with the use of in vivo and in vitro models. The in vitro method relies on enzymatic hydrolysis and an artificial model that simulates the conditions inside the gastrointestinal tract. It is used to determine the degree to which mineral compounds become available (bioavailable) to the human body.

The bioavailability of various mineral compounds and trace elements present in dairy products, including ripened cheeses, is widely discussed in literature.

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¹Corresponding author: marek.aljewicz@uwm.edu.pl

But our knowledge about the bioavailability of calcium, magnesium, and phosphorus from ripened cheeses containing probiotic cultures remains limited. For this reason, the objective of the current study was to determine the effect of 3 probiotic cultures, *Lactobacillus rhamnosus* HN001 (HN001), *Lactobacillus paracasei* LPC-37 (LPC37) and *Lactobacillus acidophilus* NCFM (NCFM), on the availability of calcium, magnesium, and phosphorus from Gouda-type cheese.

MATERIALS AND METHODS

Experimental Design

The experimental material comprised ripened cheese produced in an industrial plant in Gizycko, Poland. Dutch-type cheese (control and experimental) were produced from 10,000 L (each) of premium class milk, which was thermized at 65°C for 15 s and cooled to 4°C. Milk was bacto-fugated, pasteurized at 72.5°C for 15 s, and standardized to 3.0% fat content. It was tempered to 31°C and inoculated with the cheese starter culture and probiotic bacteria. Warmed milk was combined with 3 kg of calcium chloride (Ciech, Warsaw, Poland), 110 mL of coloring agent, 500 mL of lysozyme (Afilact, Chr. Hansen, Czastkow Mazowiecki, Poland), deep-frozen Choozit classic 111 cheese starters (0.06% vol/vol; DuPont, Poznan, Poland), and HN001 (0.03% vol/vol), NCFM (0.03% vol/vol), or LPC37 (0.03% vol/vol; DuPont) probiotic cultures with 430 mL of rennet (Chymax, Chr. Hansen) then added directly to batches of experimental cheese. Every phase of the production process was consistent with industry standards and according to Dutch cheese. After brining, the cheeses were wrapped in Cryovac (Duchnice, Poland) heat shrink, oxygen barrier bags and cold stored under controlled conditions. The cheeses were ripened for 6 wk at a temperature of 12°C and relative humidity of 85%. They were stored at 4°C and relative humidity of 85%. Samples of brined cheeses ripened for 6 wk and stored for 3 mo were collected for analysis. Polish consumers prefer cheeses with a mild taste and a delicate aroma. Because of this, cheeses ripened for 6 wk were analyzed in the study.

Chemical Composition

Grated cheese samples were analyzed in triplicate to determine their salt content by the Volhard method (AOAC International, 2005; method 975.20), fat content by the Van Gulik method (ISO, 2008), and moisture content by oven-drying at 102°C (AOAC International, 2005; method 926.08). The pH of the cheese slurry, prepared by blending 10 g of grated cheese with 10 mL

of H₂O, was measured with a pH meter (Elmetron CP 501, Zabrze, Poland; electrode: Inode, Zabrze, Poland) after calibration with pH 4.0 and 7.0 buffers (Merck, Darmstadt, Germany).

Mineral (Ca, P, Mg) Content of Cheese (Mineralization Stage)

Cheese samples of 1.5 g were weighed (accurate to 0.0001 g), placed in 500-mL Kjeldahl flasks, combined with concentrated HNO₃ (Suprapure, Merck), and HClO₄ (Ultrapure, JT Baker, Deventer, the Netherlands; 3:1) and left to stand for 30 min. The samples were mineralized (Buchi K-439, Flawil, Switzerland) until a colorless solution was obtained. The samples were cooled and transferred to a volumetric flask containing 50 mL of ultrapure deionized water (Merck).

The calcium and magnesium content of cheese samples was determined by atomic absorption spectrometry in an air-acetylene flame using the iCE 3000 Series Atomic Absorption Spectrometer (Thermo-Scientific, Hemel Hempstead, UK), a deuterium lamp for background correction, and cathode lamps suitable for each element. Concentrations of Ca²⁺ were determined by combining the samples with 10% aqueous solution of lanthanum chloride to obtain La³⁺ concentrations of 0.5% in each sample (Whiteside, 1979). Phosphorous content was determined by the molybdenum method with hydroquinone and sulfate using spectrophotometer Helios β (Unicam, Cambridge, UK) at λ = 460 to 480 nm (PKN, 1999).

Availability of Minerals from Cheeses

Mineral availability was determined by enzymatic hydrolysis in vitro in a system that simulates the conditions in the human gastrointestinal tract. Cheese samples of approximately 1.5 g (accurate to 0.0001 g) were mixed with 50 mL of deionized water, pH was adjusted to 2.0 with 1 M HCl (Suprapure, Merck), and 1.6 mL of pepsin solution (16 g of pepsin, P-7000, Sigma-Aldrich, St. Louis, MO, in 100 mL of deionized water) was added. The mixture was incubated in a shaking water bath (Julabo Sw 22, Labortechnik GmbH, Seelbach, Germany) at 37°C for 2 h (shaking frequency adjustable to 100 rpm). The solution was neutralized to pH 6.8 to 7.0 with 6% NaHCO₃ (Merck), and a solution of pancreatin and bile salts [0.4 g of pancreatin (Sigma-Aldrich) in 100 mL of 0.1 M NaHCO₃ and 2.5 g of bile salts (Sigma-Aldrich) in 0.1 M NaHCO₃ (POCH, Gliwice, Poland)] was added at a rate of 15.8 mL per every 50 mL of the reaction solution. The samples were incubated at 37°C for 2 h (shaking frequency adjustable to 100 rpm) and centrifuged at 5,000 × g for 15 min at

37°C (MPW 350R centrifuge, Mechanika precyzyjna, Warsaw, Poland). The supernatant was filtered (0.22 µm, Merck) and mineralized in the K-439 Digestion Unit (Buchi, Flawil, Switzerland). The mineral content of cheese samples was determined in accordance with the method described in the previous section.

Microbiological Analysis

Microbiological examinations were carried out according to the PN-EN ISO 7218 standard (PKN, 2013). A cheese sample of 10 g was combined with 90 mL of sodium citrate solution (20 g/L, POCH) at a temperature of 40°C. The samples were homogenized in a stomacher (BagMixer 400W, Saint Nom, France) to produce a uniform suspension. After homogenization, 10-fold serial dilutions were prepared using peptone water (15 g/L, Merck) as the solvent. The microbiological quality of milk and whey was evaluated by diluting 1 mL of the sample in peptone water.

Total *Lactobacillus* spp. counts were determined in control and experimental (probiotic culture + indigenous *Lactobacillus*) cheeses on Rogosa agar (Merck). The samples were incubated anaerobically at 37°C for 72 h in the AnaeroGen system (Oxoid, Poznan, Poland).

Statistical Analysis

The results were verified for normal distribution and homogeneity of variance. The significance of differences between means was analyzed by the Student's *t*-test. Interactions between 2 factors were determined by ANOVA. The results were processed in Statistica 10.0 PL software (Statsoft 2011, Krakow, Poland) at $P < 0.05$ for $n = 3$ (physicochemical and biochemical

parameters) and $P < 0.05$ at for $n = 3$ (microbiological analysis, in duplicate). All data are presented as mean $\pm$ SEM.

RESULTS

Chemical Composition of Cheeses and Cheese-Like Products

The water content of the analyzed cheeses ranged from 41.82 to 43.18%. Minor differences in the content of protein, fat, and sodium chloride were reported between the examined cheeses. The addition of HN001, LPC37, and NCFM cultures revealed some significant ($P < 0.05$) changes in the chemical composition of the studied Dutch-type cheeses (Table 1).

Cheese Acidity During Ripening and Storage

Immediately after brining, the acidity of control and experimental cheeses (containing HN001 or NCFM) was comparable at 5.44 pH. Cheeses containing LPC37 were characterized by a higher pH of 5.51. After 6 wk of ripening, pH was higher in all experimental cheeses (5.73) than in control cheeses (5.68). During 3 mo of storage, smaller variations in pH were reported in experimental cheeses than in control (Figures 1 and 2).

Changes in *Lactobacillus* spp. Counts

After brining, the average *Lactobacillus* counts in control cheeses were determined at $3.85 \log_{10}$ cfu/g and were significantly ($P < 0.05$) lower than in experimental cheeses ($8.51 \log_{10}$ cfu/g; Table 2). A significant increase ($4.62 \log_{10}$ cfu/g) in *Lactobacillus* spp. counts was reported in control cheeses after 6 wk of ripening.

Table 1. Composition of control and probiotic cheeses¹

Composition (%)	Cheese ²			
	Control	HN001	LPC37	NCFM
Moisture	42.51 $\pm$ 0.08 ^a	41.82 $\pm$ 0.17 ^c	42.88 $\pm$ 0.2 ^{ab}	43.18 $\pm$ 0.13 ^b
Fat	24.84 $\pm$ 0.45 ^c	28.3 $\pm$ 0.16 ^b	27.67 $\pm$ 0.17 ^{ab}	26.84 $\pm$ 0.17 ^a
FDM ³	43.2 $\pm$ 0.79 ^b	48.65 $\pm$ 0.34 ^a	48.44 $\pm$ 0.33 ^a	47.23 $\pm$ 0.24 ^a
Protein	23.64 $\pm$ 0.05 ^{ab}	24.05 $\pm$ 0.11 ^{bc}	24.44 $\pm$ 0.2 ^c	23.29 $\pm$ 0.17 ^a
Salt	1.63 $\pm$ 0.03 ^a	1.62 $\pm$ 0.02 ^a	1.71 $\pm$ 0.02 ^c	1.24 $\pm$ 0.01 ^b
SDM ⁴	2.83 $\pm$ 0.05 ^a	2.78 $\pm$ 0.03 ^a	2.99 $\pm$ 0.02 ^c	2.18 $\pm$ 0.02 ^b

^{a-c}Means in rows with different superscripts differ ($P < 0.05$).

¹Results are expressed as mean $\pm$ SEM; $n = 3$. Analyses were conducted after brining of Dutch-type cheese.

²Control = control cheese only with starter cultures Choozit classic 111 (DuPont, Poznan, Poland); HN001 = cheese with starter cultures Choozit classic and *Lactobacillus rhamnosus* HN001 (DuPont); LPC37 = cheese with starter cultures Choozit classic and *Lactobacillus paracasei* LPC-37 (DuPont); NCFM = cheese with starter cultures Choozit classic and *Lactobacillus acidophilus* NCFM (DuPont).

³FDM = fat in DM.

⁴SDM = salt in DM.

Figure 1. Change in the pH value in Dutch-type cheese after brining (0 wk) and 2, 4, and 6 wk of ripening. Results are significant ($P < 0.05$) and are expressed as mean $\pm$ SD; $n = 3$ sets. Control = control cheese only with starter cultures; HN001 = cheese with starter cultures Choozit classic and *Lactobacillus rhamnosus* HN001 (DuPont, Poznan, Poland); LPC-37 = cheese with starter cultures Choozit classic and *Lactobacillus paracasei* LPC-37 (DuPont); NCFM = cheese with starter cultures Choozit classic and *Lactobacillus acidophilus* NCFM (DuPont).

Lactobacillus spp. counts remained relatively similar in NCFM and LPC37 experimental cheeses. Unlike in LPC37 and NCFM cheeses, a significant increase (1 $\log_{10}$ cfu/g) in *Lactobacillus* spp. counts was observed in the HN001 cheese. After 3 mo of storage, *Lactobacillus* spp. counts increased significantly ($P < 0.05$) in control cheeses and in NCFM (0.2 $\log_{10}$ cfu/g), but decreased to 0.74 and $\sim 1.2 \log_{10}$ cfu/g in LPC37 and HN001, respectively.

Availability of Calcium from Dutch-Type Cheeses

The average calcium content of all cheeses was determined at 938 mg/100 g immediately after brining (Table 3). The availability of calcium from the analyzed products was low ($\sim 19\%$) in the process of in vitro

Figure 2. Change in the pH value in Dutch-type cheese after 1, 2, and 3 mo of storage. Results are significant ($P < 0.05$) and are expressed as mean $\pm$ SD; $n = 3$ sets. Control = control cheese only with starter cultures; HN001 = cheese with starter cultures Choozit classic and *Lactobacillus rhamnosus* HN001 (DuPont, Poznan, Poland); LPC-37 = cheese with starter cultures Choozit classic and *Lactobacillus paracasei* LPC-37 (DuPont); NCFM = cheese with starter cultures Choozit classic and *Lactobacillus acidophilus* NCFM (DuPont).

enzymatic hydrolysis (in a model simulating the conditions inside the human gastrointestinal tract). The addition of probiotic cultures significantly ($P < 0.05$) increased ($\sim 2.5\%$) the availability of calcium relative to the control. A decrease (not statistically significant) in the availability of calcium from both experimental and control cheeses was observed during ripening and storage.

Availability of Magnesium from Dutch-Type Cheeses

After brining, the average content of magnesium in all cheeses was determined at 34 mg/100 g. In the process of in vitro enzymatic hydrolysis, the availability of magnesium was estimated at $\sim 35\%$. The addition of probiotic cultures LPC37, NCFM, and HN001 resulted in a significant ($P < 0.05$) increase in the availability of magnesium relative to control at 24, 20, and 10%, respectively. A similar correlation was noted in cheeses

Table 2. Viability of *Lactobacillus* spp. in Dutch-type cheese after brining (0 d), 6 wk of ripening, and 3 mo of storage¹

Viable count ($\log_{10}$ cfu/g)	Cheese ²			
	Control	HN001	NCFM	LPC37
0 d	3.85 $\pm$ 0.05 ^{a,A}	8.61 $\pm$ 0.01 ^{b,B}	7.59 $\pm$ 0.02 ^{c,A}	9.32 $\pm$ 0.03 ^{d,A}
6 wk	8.47 $\pm$ 0.08 ^{a,B}	9.6 $\pm$ 0.03 ^{b,B}	7.47 $\pm$ 0.02 ^{c,B}	9.35 $\pm$ 0.01 ^{d,A}
3 mo	8.7 $\pm$ 0.09 ^{a,C}	8.39 $\pm$ 0.05 ^{b,C}	7.62 $\pm$ 0.07 ^{c,A}	8.76 $\pm$ 0.1 ^{a,B}

^{a-d}Means in rows with different superscripts differ ($P < 0.05$).

^{A-C}Means in columns with different superscripts differ ($P < 0.05$).

¹Results are expressed as mean $\pm$ SEM; $n = 3$.

²Control = control cheese only with starter cultures Choozit classic 111 (DuPont, Poznan, Poland); HN001 = cheese with starter cultures Choozit classic and *Lactobacillus rhamnosus* HN001 (DuPont); LPC37 = cheese with starter cultures Choozit classic and *Lactobacillus paracasei* LPC-37 (DuPont); NCFM = cheese with starter cultures Choozit classic and *Lactobacillus acidophilus* NCFM (DuPont).

Table 3. Mineral content (mg/100 g) and changes in mineral availability from Dutch-type cheese after brining (0 d), 6 wk of ripening, and 3 mo of storage¹

Cheese ²	Before in vitro hydrolysis	After in vitro hydrolysis			Amount of mineral released (%)		
	0 d	0 d	6 wk	3 mo	0	6 wk	3 mo
Control							
Ca	903.041 ± 12.29 ^{a,A}	127.711 ± 3.16 ^{b,A}	139.336 ± 3.45 ^{b,A}	175.578 ± 5.71 ^{c,A}	14	15	19
P	551.016 ± 3.89 ^{a,A}	553.503 ± 0.89 ^{bc,A}	561.037 ± 0.90 ^{b,A}	535.401 ± 6.88 ^{c,A}	100	102	97
Mg	32.456 ± 0.71 ^{a,A}	6.688 ± 0.66 ^{b,A}	6.715 ± 0.66 ^{b,A}	6.64 ± 0.14 ^{b,A}	21	21	20
HN001							
Ca	914.935 ± 0.29 ^{a,A}	169.943 ± 1.26 ^{b,B}	173.589 ± 2.36 ^{b,B}	199.355 ± 0.75 ^{c,B}	19	19	22
P	563.382 ± 6.19 ^{a,B}	476.552 ± 4.6 ^{b,B}	507.957 ± 4.14 ^{c,B}	524.434 ± 2.37 ^{d,B}	85	90	93
Mg	32.884 ± 0.72 ^{a,A}	22.935 ± 0.8 ^{b,B}	21.642 ± 3.69 ^{b,B}	22.145 ± 0.26 ^{b,B}	70	66	67
LPC37							
Ca	980.549 ± 5.69 ^{a,B}	172.418 ± 5.08 ^{b,B}	124.888 ± 0.52 ^{c,C}	121.643 ± 2.37 ^{c,C}	18	13	12
P	569.914 ± 7.40 ^{a,B}	562.802 ± 5.60 ^{a,C}	569.361 ± 5.13 ^{c,C}	629.481 ± 4.14 ^{b,C}	99	100	110
Mg	27.525 ± 1.06 ^{a,B}	12.828 ± 0.56 ^{b,C}	24.518 ± 0.36 ^{c,B}	39.298 ± 2.34 ^{d,C}	47	89	99
NCFM							
Ca	960.255 ± 4.50 ^{a,C}	206.356 ± 6.08 ^{b,C}	134.862 ± 1.03 ^{c,A}	106.923 ± 6.08 ^{c,D}	21	14	11
P	579.377 ± 4.72 ^{a,B}	606.223 ± 18.85 ^{b,D}	598.607 ± 4.32 ^{c,D}	599.879 ± 4.73 ^{c,D}	105	103	104
Mg	35.771 ± 0.24 ^{a,C}	10.798 ± 0.94 ^{b,D}	22.753 ± 1.44 ^{c,B}	27.258 ± 0.73 ^{d,D}	30	64	110

^{a-d}Means in rows with similar superscripts do not differ ($P < 0.05$).

^{A-D}Means in column with similar superscripts do not differ ($P < 0.05$).

¹Results are expressed as mean ± SEM; n = 3.

²Control = control cheese only with starter cultures Choozit classic 111 (DuPont, Poznan, Poland); HN001 = cheese with starter cultures Choozit classic and *Lactobacillus rhamnosus* HN001 (DuPont); LPC37 = cheese with starter cultures Choozit classic and *Lactobacillus paracasei* LPC-37 (DuPont); NCFM = cheese with starter cultures Choozit classic and *Lactobacillus acidophilus* NCFM (DuPont).

after 3 mo of storage. The availability of magnesium was highest after 3 mo of storage and lowest after brining (Table 3).

Availability of Phosphorus from Dutch-Type Cheeses

The average content of phosphorus in cheeses after brining was determined at 573 mg/100 g. In the process of in vitro enzymatic hydrolysis, the availability of phosphorus reached 89 and ~96% from control and experimental cheeses, respectively. The addition of HN001 led to a minor increase (2%) in phosphorus availability. The availability of phosphorus was similar (~98%) after 3 mo of storage (Table 3).

DISCUSSION

The influence of bacterial cultures, including probiotics, on changes in the availability of mineral compounds from food products has been explored by a growing number of research studies (Henry, 1995; Bronner and Pansu, 1999; Guéguen and Pointillart, 2000; Tsuchita et al., 2001; Scholz-Ahrens et al., 2007). The beneficial or adverse effect of bacterial cultures is not explicit, and it is often determined by the applied strain (Ghanem et al., 2004; Gilman and Cashman, 2006; Klobukowski et al., 2009). Probiotic culture starters (*Lactobacillus* spp.), microflora not originating from sourdough fer-

mentation, and technologically unsuitable microorganisms synthesize metabolites that affect acidity (pH) in early stages of production and during ripening and storage. The ionization yield of mineral compounds, mostly calcium, increases with a decrease in pH.

The rate of lactic acid fermentation determines the chemical composition of ripened cheeses, in particular in early stages of production (curd cutting, drying, supplementary drying). It is practically impossible to produce 2 batches of cheese with identical chemical composition. Automatic cheese-forming systems (Caso-matic, Obram, Olsztyn, Poland) are used to minimize differences in the chemical composition of cheese. In the present study, experimental cheeses containing probiotic cultures and control cheeses differed in their chemical composition, but the observed differences were not statistically significant. Similar results were reported by other authors (Gardiner et al., 1998; El-Tanboly et al., 2010; Burns et al., 2012).

The chemical composition and acidity of fresh cheese determine its microbiological quality (i.e., the abundance of secondary microflora and technologically unsuitable microorganisms). An increase in secondary microflora counts contributes to changes in acidity during ripening. In the current study, a gradual increase in pH was observed in experimental and control cheeses. The addition of probiotic cultures contributed to a higher pH in experimental cheeses than in the control,

both after 6 wk of ripening and 3 mo of storage. The observed differences in acidity resulted from higher *Lactobacillus* spp. counts in experimental cheeses, in particular in early stages of ripening (Table 2).

The rate of changes in the pH of cheese was one of the key determinants of calcium, magnesium, and phosphorus availability. Gradual dissociation of carboxylic acids in the aqueous phase of cheese, accompanied by a decrease in acidity, was observed in the initial stages of ripening. This led to the dissociation of mineral compounds in cheese, which substantially increased their availability. Ionized minerals easily penetrate the mucin layer and intestinal epithelial cells.

The calcium content of control cheese and experimental cheeses containing HN001 and NCFM (945, 918, and 920 mg/100 g, respectively) was lower in comparison with experimental cheese enhanced with LPC37 (967 mg/100 g). Immediately after salting, the cheese containing LPC37 was characterized by higher pH, which lowered calcium retention in whey. Ripened cheeses were more abundant in calcium than in Cheddar (721 mg/100 g; O'Brien and O'Connor, 2004) and Gouda cheese (740–880 mg/100 g; Kusiuk et al., 2008; Reykdal et al., 2011). The content of magnesium in the studied cheeses was similar, whereas the content of phosphorus was significantly lower than that reported by other authors (Gambelli et al., 1999; O'Brien and O'Connor, 2004; Kusiuk et al., 2008). The differences in the mineral content of the analyzed cheeses and the results presented by other authors can be attributed to variations in the chemical composition and starter culture used of the compared products.

The mineral compounds present in ripened cheese are not always readily available to consumers. In vitro enzymatic hydrolysis, which simulates the conditions inside the human gastrointestinal tract, demonstrated that the availability of calcium from ripened cheeses is low. The probiotic cultures used in the production of Dutch-type cheese increased calcium availability by 2.5%, on average, and contributed to a significant increase in magnesium availability (18%).

The low availability of calcium from dairy products has been demonstrated by other studies. The availability of calcium from milk and yogurt was reported at 23 to 27 and 25.4%, respectively (Bacciottini et al. 2004; O'Brien and O'Connor, 2004). In ripened cheeses, calcium availability is determined by fat content, and it is higher in low-fat cheeses (35%; Delisle et al., 1995) than in full-fat cheeses (22.9%). According to (Unal et al., 2005), the availability of calcium from full-fat cheeses is lower (13.85%) than from semi-fat cheeses (24.13%). Van Dokkum et al. (1996) demonstrated that the availability of calcium from cottage cheese is relatively high, in the range of 37 to 42%.

The low availability of calcium and magnesium from the analyzed cheeses can be attributed to the presence of SFA. Bond formation by bivalent metal cations is proportional to the content of SFA, in particular long-chain SFA (C16 and C18), but the process does not occur evenly (Jenkins and Palmquist, 1982; Kies, 1985). In the analyzed cheeses, calcium was probably less available than magnesium due to the higher energy of ionic bonding between the COO^- (carboxyl) group and calcium ions. The resulting quantity of bonded calcium can reach 42 to 57% (Bronner and Pansu, 1999).

The availability of calcium, magnesium, and phosphorus from various ripe cheeses is also determined by proteolysis and lipolysis products. The content of bioactive peptides, including calcium phosphocaseinate (Tsuchita et al., 2001; Narva et al., 2004; Cross et al., 2005; Miquel et al., 2005), low-molecular-weight peptides (<600 Da), and free AA (Cichosz et al., 2006; Aljewicz et al., 2010), increases gradually during cheese ripening due to the activity of bacterial proteinases and peptidases. The availability of calcium and magnesium is conditioned by the chelation (formation of a covalent bond or an ionic bond between the COO^- group and a metal ion or a covalent bond between an amino group and a metal ion) of metal ions by free AA, dipeptides, and tripeptides.

The results of the current study and the findings of other authors indicate that chelated minerals are characterized by higher availability (Henry, 1995). Proliferating probiotic cultures are capable of producing ionophoric peptides (bacteriocins), including rhamnosin A produced by HN001 (Dimitrijević et al., 2009) and lactocin produced by NCFM and LPC37 (Altermann et al., 2005; Uniprot, 2014). Bacteriocins form ion channels in colonocytes and facilitate ion transport. Free AA and low-molecular-weight peptides bind to FFA and metal ions, thus stimulating the availability of mineral compounds.

The stimulating effect of probiotic cultures on the availability of mineral compounds in cheese can be attributed to intensified enzymatic conversion, mainly proteolysis and lipolysis. Peptides, free AA, FFA, and their complexes with calcium, magnesium, and phosphorus ions stimulate gastrointestinal function by increasing the activity of digestive enzymes, boosting colonocyte proliferation and ion transport (Lutz and Scharrer, 1991; Banasaz et al., 2002; Scholz-Ahrens et al., 2007).

CONCLUSIONS

The cheeses containing HN001, LPC37, or NCFM probiotic cultures were characterized by lower acidity than control cheeses (without probiotic cultures). Rip-

ened cheese is a rich source of calcium, magnesium, and phosphorus in the human diet. Cheese has a high content of SFA, and its mineral compounds are less available to consumers than the minerals present in milk and yogurt. Despite this, cheese is a much more abundant source of calcium than milk and yogurt. The use of probiotic cultures in the production of Dutch-type cheeses increased the availability of calcium, magnesium, and phosphorus. The in vitro method supports an accurate determination of the effect of the *Lactobacillus* spp. cultures on the availability of mineral compounds from Dutch-type cheese. The results of this study further our understanding of the interactions that determine nutrient absorption from the human gastrointestinal tract.

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