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ORIGINAL PAPER

FORTIFICATION OF YOGHURTS WITH CALCIUM COMPOUNDS*

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Abstract

The main sources of calcium intake in a diet are milk and dairy products, and dietary calcium absorption ranges from 10% to 40%. Approximately 32% of calcium is absorbed from milk and dairy products. An insufficient intake of calcium by the Polish population has stimulated the marketing of an increasing number of food products fortified with calcium, enriched fruit juices and dairy products in particular. In this study, yoghurts were produced from 2% fat milk, thickened with 3% of milk powder, fortified with calcium compounds 800 Ca (mg kg^{-1} of milk) and pasteurized at the temperature of 72°C for 15 seconds. Fermentation with the yoghurt starter culture YC-X16 (Chr. Hansen) was performed at 45°C for 4.5 h and then the fermented beverages were cooled down to 5°C. The beverages were examined during the incubation for a change in yoghurt acidity directly after the addition of the starter, after 2 h and after 4 h of incubation. After 24 h of cold storage, yoghurts were analyzed for syneresis, pH, texture and colour. The addition of calcium compounds to milk at a dose of 800 Ca (mg kg^{-1} of milk) determined pH and total acidity. Milk was soured the most by calcium D-gluconate, and neutralized the most by calcium bisglycinate. After 4 h of fermentation, the acidity of yoghurt most similar to the control sample was found only in the yoghurt fortified with calcium chloride. However, after 24 h of storage, the acidity and syneresis most similar to the control sample were determined in yoghurt with calcium citrate. Fortification with calcium chloride and calcium bisglycinate darkened the colour of yoghurts. The addition of a dose of 800 Ca (mg kg^{-1} of milk) in the form of lactate, chloride and citrate increased hardness and adhesiveness of yoghurts.

Keywords: fermented milk, minerals, syneresis, colour, fermentation process, texture.

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INTRODUCTION

Calcium is one of the elements that should be consumed regularly to ensure proper development of the body. The recommended daily intake of calcium is generally known, but there is little information on the bioavailability of this element. The bioavailability of calcium depends on many factors and can vary in a range from a few to nearly one hundred per cent (Szeleszczuk, Kuras 2014). Currently, the daily requirement for calcium according to EFSA is 1000 mg per day (URL 2014). Lactose, insulin, some fructooligosaccharides and casein phosphopeptides, lysine and arginine are the factors that enhance the absorption of calcium (Gibney et al. 2005, Heaney 2009). The main sources of calcium intake in a diet are milk and dairy products, and dietary calcium absorption ranges from 10% to 40%. Approximately 32% of calcium is absorbed from milk and dairy products (Kiel 1990, Greenberg et al. 2008, Jarosz Bułhak-Jachymczyk 2008, Weaver 2008). Insufficient intake of calcium by the Polish population has stimulated the marketing of an increasing number of food products fortified with calcium, enriched fruit juices and dairy products in particular (Pirkul et al. 1997, Klahorst 2001, Gerstner 2003). Yoghurt and fermented milk products are among the most popular dairy foodstuffs consumed all over the world. Bioavailability of calcium from yoghurt is even higher than that from milk. The acidic pH of yoghurt ionizes calcium and thus facilitates intestinal calcium absorption (Bronner Pansu 1999, Unal et al. 2005).

Absorption of minerals does not depend entirely on the amount of a given element present in a dairy product, but is also controlled by the solubility of the element's chemical form and composition of a diet (Delisle et al. 1995), and thus the mineral availability of dairy products depends on the type of the complex used.

The aim of the study was to analyze the dynamics of milk souring and properties of yoghurt depending on the calcium compound used for fortification.

MATERIAL AND METHODS

The experiment was composed of two stages. The first stage included production of yoghurts (2% fat milk) thickened with 3% of milk powder and fortified with calcium compounds 800 Ca (mg kg^{-1} of milk): calcium chloride hexahydrate pure p.a. ($CaCl_2 \cdot 6H_2O$) Chempur, Poland; calcium lactate pentahydrate pure p.a. ($C_6H_{10}CaO_6 \cdot 5H_2O$) Chempur, Poland; calcium carbonate pure ($CaCO_3$) Chempur, Poland; calcium D-gluconate monohydrate ($C_{12}H_{22}CaO_{14} \cdot H_2O$) Sigma – Aldrich, China; calcium citrate tetrahydrate ($Ca_3(C_6H_5O_7)_2$) Barentz, Poland; calcium bisglycinate ($C_4H_8CaN_2O_4$) Olimp –

Labs, Poland, and it was pasteurized at the temperature of 72°C for 15 seconds. To be in line with the Commission Regulation (EC) No 1662/2006 of 6 November 2006 amending Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin, the temperature used during research was 72°C. Calcium compounds increase the risk of denaturization of milk proteins at high temperature, and the use of this pasteurization temperature not only minimizes the denaturization process, but also helps to avoid using different pasteurization temperatures for various calcium compounds (ZIARNO et al. 2009). The use of calcium did not cause thermal denaturation of milk proteins during its pasteurization (at the temperature of 72°C for 15 s). Fermentation with the yoghurt starter culture YC-X16 (mixed strain culture containing *Streptococcus thermophilus* and *Lactobacillus delbruckii* subsp. *Bulgaricus*) (Chr. Hansen) was performed at 45°C for 4.5 h and then fermented beverages were cooled down to 5°C. The quality of yogurt was analyzed during incubation for a change in yoghurt acidity directly after the addition of the starter, after 2 h and after 4 h of incubation. In the second stage of the study, after 24 h of cold storage (at 5°C), yoghurts were analyzed for syneresis: a 25 g sample of yoghurt was placed on a paper filter in a funnel. The funnel was placed in a measuring cylinder (of known weight) and left for 120 min at 5°C. Whey leakage (the quantity of whey) was weighed and the percentage was calculated in relation to the weight of the sample (CUEVA ARYANA 2008). The pH was determined with the electrometric method by measuring the activity of hydrogen ions with a pHmeter Mettler Toledo FiveEasy (Switzerland). Determination of potential acidity was performed by titration of 0.25 N samples with the standard solution of sodium hydroxide in the presence of phenolphthalein as an indicator. Texture was assessed with an analyzer Brookfield CT3 (USA) equipped with Brookfield Texture Pro CT software. For determination, the TPA (Cycle Count 2) was selected and a sample of solid state yoghurt was chosen. A container with 100 ml of yoghurt, not mixed, was submitted to the following settings of the analyzer: cylinder 66.00 mm x 33.86 mm, force 0.1 N, head speed 1 mm s^{-1}, table TA-BTKIT, probe TA3/100 (acrylic cylinder 35 mm in length). The colour was analyzed with a Minolta chromameter CR-400 microcolour tristimulus colorimeter (Konica Minolta Sensing Inc., Milton Keynes, UK) for the determination of L*, a*, and b* values.

The experiment was repeated three times, and each parameter was measured five times. The mean and standard deviation values were derived from the results with the help of Statistica 12.0 software (StatSoft, USA). The univariate analysis of variance was used to evaluate the influence of the type of calcium compound used on the properties of fermented milk beverage. Significance of differences between the averages ($p \leq 0.05$) was estimated with the Tukey's test.

RESULTS AND DISCUSSION

In the experiment, lactate, chloride and D-gluconate were the calcium compounds that caused the highest souring of milk directly after the addition of starter (Table 1). Addition of calcium chloride to milk causes a decrease in pH (for example, a dose of 50 mM reduced the pH from 6.6 to 5.8), but coagulation was shown to be induced not only by an acid reaction because it also occurred when the pH was readjusted to 6.6, although the yield of coagulum was reduced (Ramasubramanian et al. 2013). In contrast, calcium bisglycinate changed the reaction of milk to alkaline compared with the control sample. After the addition of calcium citrate and calcium carbonate, no significant effect on pH of milk was observed following the dosing of starter cultures. After two hours of fermentation, the pH was significantly higher than in the control sample only in milk fortified with calcium lactate. After four hours of incubation, the pH was significantly higher than in control in the samples with calcium lactate, chloride, bisglycinate, citrate, and D-gluconate.

While analyzing the effect of calcium compounds directly after the addition of the starter to milk, significantly higher total acidity was found in milk enriched with calcium D-gluconate (Table 2). In contrast, the addition of calcium bisglycinate resulted in a significant reduction in the total acidity of milk immediately after the addition of the starter in comparison with the control sample. After two hours of incubation, significantly lower acidity relative to the control was determined in the milk samples enriched with calcium lactate, carbonate, chloride, bisglycinate and citrate. The total acidity being significantly lower than in the control after 4 h of fermentation was determined in yoghurts with the addition of lactate, bisglycinate and citrate, while the fortification with calcium carbonate, chloride and D-gluconate did not influence significantly the total acidity of yoghurts after 4 h of incubation.

After incubation and cooling of the tested yoghurts, the highest pH was determined in yoghurts enriched with calcium lactate and bisglycinate as compared with the control sample (Table 3). Fortification with calcium chloride, citrate and D-gluconate did not significantly affect pH of yoghurts, especially when compared to the non-fortified samples. It should be added that acidity significantly lower than in the control also distinguished yoghurts fortified with calcium lactate and bisglycinate.

In the research by Ziarno et.al (2004) exploring a possibility of using calcium compounds to enrich cottage cheese with calcium, it was shown that calcium compounds soluble in water caused a change in the acidity of cream consisting of a lower pH and higher titratable acidity, which resulted in a reduced stability and higher susceptibility of proteins to thermal coagulation. In this respect, however, a significant difference was observed between the use of calcium compounds soluble in water (e.g. calcium lactate, gluconate,

Table 1

The effect of the type of a calcium compound on pH of yoghurts during incubation

Symbols		Calcium lactate	Calcium carbonate	Calcium chloride	Calcium bisglycinate	Calcium citrate	Calcium D-gluconate	Control
pH	after addition of the starter	$6.120^{a} \pm 0.030$	$6.440^{b} \pm 0.002$	$6.200^{ba} \pm 0.010$	$6.710^{c} \pm 0.040$	$6.375^{b} \pm 0.020$	$6.115^{a} \pm 0.050$	$6.335^{b}\pm0.040$
	after 2 h of incubation	$5.325^{a} \pm 0.020$	$5.065^{b} \pm 0.050$	$5.035^{b} \pm 0.020$	$5.140^{b} \pm 0.030$	$5.095^{b} \pm 0.110$	$5.015^{b} \pm 0.010$	$5.140^{b}\pm0.010$
	after 4 h of incubation	$4.990^{c} \pm 0.100$	$4.660^{a} \pm 0.001$	$4.550^{b} \pm 0.030$	$4.745^{a} \pm 0.020$	$4.665^{a} \pm 0.010$	$4.665^{a} \pm 0.010$	$4.535^{b}\pm0.010$

Explanatory notes:
The table shows mean values and standard deviation.
a, *b*, *c* – mean values denoted with different letters within rows differ statistically significantly ($p \leq 0.05$)

Table 2

The effect of the type of a calcium compound on titratable acidity of yoghurts during incubation

Symbols		Calcium lactate	Calcium carbonate	Calcium chloride	Calcium bisglycinate	Calcium citrate	Calcium D-gluconate	Control
Titratable acidity (°SH)	after addition of the starter	$12.40^{b} \pm 0.000$	$11.20^{b} \pm 0.570$	$12.00^{b} \pm 0.000$	$8.400^{a} \pm 0.000$	$12.00^{b} \pm 0.000$	$13.80^{c} \pm 0.280$	$12.00^{b} \pm 0.000$
	after 2 h of incubation	$24.40^{a} \pm 0.570$	$31.00^{b} \pm 0.280$	$31.60^{b} \pm 0.570$	$30.60^{b} \pm 0.850$	$30.60^{b} \pm 0.850$	$32.20^{c} \pm 0.280$	$33.00^{c} \pm 1.410$
	after 4 h of incubation	$36.80^{a} \pm 3.960$	$39.40^{ab} \pm 0.280$	$41.40^{b} \pm 0.850$	$36.20^{a} \pm 0.280$	$37.00^{a} \pm 0.850$	$39.20^{ab} \pm 1.130$	$41.00^{b} \pm 1.410$

Explanatory notes:
The table shows mean values and standard deviation.
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Table 3

The effect of the type of a calcium compound on pH, titratable acidity (°SH), syneresis (%) and colour of yoghurts after 24 h of cold storage

Properties		Calcium lactate	Calcium carbonate	Calcium chloride	Calcium bisglycinate	Calcium citrate	Calcium D-gluconate	Control
pH		$4.770^{a} \pm 0.030$	$4.660^{b} \pm 0.000$	$4.510^{c} \pm 0.030$	$4.770^{a} \pm 0.010$	$4.530^{c} \pm 0.030$	$4.590^{bc} \pm 0.040$	$4.530^{c} \pm 0.010$
Titratable acidity (°SH)		$38.80^{b} \pm 0.570$	$40.20^{b} \pm 0.280$	$41.60^{b} \pm 2.260$	$38.00^{b} \pm 0.000$	$41.40^{a} \pm 0.850$	$38.00^{ab} \pm 1.700$	$40.60^{a} \pm 0.850$
Syneresis (%)		$49.99^{b} \pm 0.210$	$45.90^{c} \pm 1.240$	$45.04^{c} \pm 0.540$	$46.11^{cb} \pm 0.690$	$38.35^{a} \pm 2.400$	$46.73^{cb} \pm 0.420$	$37.10^{a} \pm 1.180$
Colour	L*	$98.84^{b} \pm 0.770$	$98.31^{b} \pm 1.520$	$94.95^{a} \pm 0.490$	$96.49^{a} \pm 0.600$	$98.70^{b} \pm 1.003$	$98.19^{b} \pm 0.840$	$98.47^{b} \pm 1.060$
	a*	$-4.03^{b} \pm 0.040$	$-4.010^{b} \pm 0.010$	$-4.030^{b} \pm 0.030$	$-4.070^{b} \pm 0.050$	$-3.710^{a} \pm 0.060$	$-3.820^{a} \pm 0.030$	$-4.150^{b} \pm 0.230$
	b*	$15.20^{a} \pm 0.380$	$14.62^{ab} \pm 0.310$	$14.26^{bc} \pm 0.070$	$14.42^{b} \pm 0.150$	$13.78^{c} \pm 0.210$	$14.39^{b} \pm 0.140$	$14.41^{b} \pm 0.460$

Explanatory notes:
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lactogluconate or chloride) and water-insoluble salts (e.g. calcium citrate or carbonate). Water-soluble salts strongly affected the acidity of cream, resulting in rapid thermal destabilization of proteins. In contrast, water-insoluble salts slightly or hardly affected the acidity of cream samples and did not influence the stability of proteins present in milk.

Velez-Ruiz and Rivas (2001) performed analyses of physicochemical properties such as acidity, pH, density and texture of yoghurt fortified with calcium lactate and calcium chloride, accompanied by sensory evaluations. The researchers found that yoghurt properties were similar between plain and fortified yoghurt, and determined that calcium levels of 50 mg 100 ml^{-1} of yoghurt may be incorporated in the yoghurt samples without consumer rejection.

Fortification of milk with calcium in the production of yoghurts helped to increase syneresis (Table 3). In the carried-out research the highest syneresis was found in the yoghurt enriched with calcium lactate. In this case the size of syneresis surely was affected by water connected with the molecule of calcium lactate (lactate pentahydrate). Also increased values of syneresis in the yoghurts with the addition of calcium chloride and D-gluconate can be explained by the degree of hydration of the added calcium compound. The syneresis closest to the control yoghurt was determined in the yoghurt with the addition of calcium citrate. According to Ziarno et al (2004), calcium citrate or carbonate seem to be preferable sources of calcium and can be successfully used for supplementation of many products. Their disadvantage, however, is their very poor solubility. The data in the literature show that solubility of calcium citrate under standard conditions is about 0.2 g dm^{-3}, but it changes in a sour medium (Kuntz 1998, Klahorst 2001, Kuntz 2003).

The addition to milk, prior to fermentation, of 800 Ca (mg kg^{-1} of milk) of milk in the form of chloride and bisglycinate caused some darkening of the colour of yoghurts relative to the control yoghurts, as evidenced by the values of L* coefficient (Table 3). In contrast, the addition of calcium lactate, carbonate, citrate and D-gluconate did not differentiate significantly the value L*. The value of chromaticity of a* colour, contained in Table 3, shows that the addition of 800 Ca (mg kg^{-1} of milk) to yoghurts causes a colour shift in the space towards green colour. The largest proportion of green colour was in the yoghurts enriched with calcium citrate and D-gluconate in comparison with the control sample. Fortification of yoghurts with different calcium compounds also differentiates the value b* significantly. A significantly higher proportion of yellow colour characterized yoghurt with the addition of calcium lactate as compared to the control sample, while fortification of yoghurts with calcium citrate caused a shift of colours in the space toward the blue colour. The values of colour (L*, a*, b*) closest to the control yoghurt were achieved for yoghurt fortified with calcium carbonate.

In the research by Achanta et al. (2007), mean L* values of yoghurts fortified with chromium, magnesium, manganese and molybdenum were higher than in the control. The minerals may have altered the casein micelle matrix structure of yoghurts in such a way as to have contributed to the white opacity of yoghurts leading to increased L* values.

Milk salts greatly influence the functional properties of milk and dairy products including gelation, protein stability, emulsification, foaming and curd texture, primarily by influencing the structural integrity of casein micelles or the sensitivity to aggregation of caseins. The concentration of milk salts can be varied by processing conditions, including acidification or the addition of minerals and metal chelators/sequestrants. During the acidification of milk, the release of Ca^{2+} arising from the solubilization of colloidal calcium phosphate (CCP). Addition of calcium to milk causes an increase in CCP (colloidal calcium phosphate) cross-linking between casein micelles (Lucey, Horne 2009). It is known that more soluble salts result in more free calcium ions in the solution and are more readily available for reaction than less soluble salts. This is the case when natural milk components such as phosphate and proteins react with available calcium (Münchbach, Gerstne 2010). In the present study, fortification of yoghurts with calcium lactate, chloride and citrate significantly increases hardness and adhesiveness of curd as compared to the control sample (Table 4). In contrast, hardness and adhesiveness of curd is significantly reduced by the addition of calcium D-gluconate. In our experiment, it was shown that only the fortification with calcium chloride significantly reduces elasticity of the curd. In terms of the properties of the texturometric profile, the yoghurt which turned out to be the closest to the control yoghurt was the one enriched with calcium carbonate, which is insoluble in water, as evidenced by the literature data (Rutkowski 1993, Ziarno et al. 2004). Addition of less soluble calcium salts to milk during yoghurt manufacture can cause complications in the form of calcium sediments which can develop with time of storage or when the product is heated during manufacture (Münchbach, Gerstne 2010). Differences in texture parameters of the analyzed yoghurts could be the result of various content of calcium ions in the solution. Ramasubramanian et al. (2008), who investigated the effect of calcium on the viscosity, firmness, and smoothness of stirred probiotic yogurt, reported that addition of Ca^{2+} (as calcium chloride) to stirred yoghurt increased gel firmness and viscosity up to ~2 mM addition and decreased these properties with further addition to the maximum added amount of 13.6 mM. Curiously, a soluble nonionic form of calcium, calcium potassium citrate, behaved quite differently from ionised calcium salts when incorporated into stirred yoghurt. The gel firmness and viscosity of yoghurt showed considerable increases with additions up to 50 mM (Ramasubramanian et al. 2008).

Chelatants (e.g. citrate, bisglycinate) have high affinities for cations and are able to displace them, especially calcium (Gaucheron 2005). In the study of Johnston and Murphy (1992), the addition of Ca-chelating agents to milk has been reported to increase the firmness of acid milk gels made with glucono-lactone (GDL).

Table 4

The effect of the type of a calcium compound on texture of yoghurts after 24 h of cold storage

Properties	Calcium lactate	Calcium carbonate	Calcium chloride	Calcium bisglycinate	Calcium citrate	Calcium D-gluconate	Control
Hardness (N)	$3.167^{a} \pm 0.033$	$1.685^{b} \pm 0.086$	$3.200^{a} \pm 0.123$	$2.279^{b} \pm 0.832$	$3.167^{a} \pm 0.042$	$1.060^{c} \pm 0.047$	$1.743^{b} \pm 0.077$
Adhesiveness (mJ)	$9.167^{a} \pm 0.329$	$3.825^{b} \pm 0.250$	$9.909^{a} \pm 1.046$	$6.329^{a} \pm 2.688$	$9.167^{a} \pm 0.404$	$1.950^{c} \pm 0.238$	$4.475^{b} \pm 0.660$
Springiness (mm)	$13.72^{a} \pm 1.930$	$14.26^{a} \pm 0.332$	$0.960^{b} \pm 0.087$	$14.12^{a} \pm 1.454$	$13.72^{a} \pm 2.364$	$14.24^{a} \pm 0.173$	$14.12^{a} \pm 0.478$

Explanatory notes:
The table shows mean values and standard deviation.
a, *b*, *c* – mean values denoted with different letters within rows differ statistically significantly ($p \leq 0.05$)

CONCLUSIONS

1. The addition of calcium compounds to milk at a dose of 800 Ca (mg kg^{-1} of milk) determined pH and total acidity. Milk was soured the most by calcium D-gluconate, and neutralized most intensively by calcium bisglycinate. After 4 h of fermentation, the acidity most similar to the control sample was determined only in yoghurt fortified with calcium chloride. However, after 24 h of storage, the acidity and syneresis most similar to control sample were determined in yoghurt with calcium citrate.

2. Fortification with calcium chloride and bisglycinate darkened the colour of yoghurts.

3. The addition of a dose of 800 Ca (mg kg^{-1} of milk) in the form of lactate, chloride and citrate increased hardness and adhesiveness of yoghurts.

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