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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 Salminem, 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.

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

The bioavailability of various mineral compounds and trace elements present in dairy products, including ripened cheeses, is widely discussed in literature. 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 bactofugated, 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 H_2O, 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 HNO_3 (Suprapure, Merck), and $HClO_4$ (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 Ca^{2+} were determined by combining the samples with 10% aqueous solution of lanthanum chloride to obtain La^{3+} 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 $\lambda = 460$ 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% $NaHCO_3$ (Merck), and a solution of pancreatin and bile salts [0.4 g of pancreatin (Sigma-Aldrich) in 100 mL of 0.1 *M* $NaHCO_3$ and 2.5 g of bile salts (Sigma-Aldrich) in 0.1 *M* $NaHCO_3$ (POCH, Gliwice, Poland)] was added at a rate of 15.8 mL per