LWT - Food Science and Technology 93 (2018) 287–292

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Sodium reduction and flavor enhancers addition: is there an impact on the availability of minerals from probiotic Prato cheese?

Hugo L.A. Silva[a], Celso F. Balthazar[a], Ramon S. Rocha[b], Jeremias Moraes[b], Erick A. Esmerino[a], Marcia C. Silva[b], Renata S.L. Raices[b], Tatiana C. Pimentel[c], Mônica Q. Freitas[a], Adriano G. Cruz[b,*]

[a] Universidade Federal Fluminense (UFF), Faculdade de Veterinária, 24230-340, Niterói, Brazil
[b] Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Departamento de Alimentos, 20270-021, Rio de Janeiro, Brazil
[c] Instituto Federal do Paraná (IFPR), Campus Paranavaí, 87703-536, Paranavaí, Paraná, Brazil

ARTICLE INFO

Keywords:
Prato cheese
Sodium reduction
Probiotic
Bioaccessibility
Mineral

ABSTRACT

The effect of partial substitution of NaCl with KCl and the flavor enhancers addition (arginine, yeast and oregano extract) on the bioaccessibility of five minerals (Mg, Ca, K, P and Zn) from potentially Probiotic Prato cheese was evaluated. After 1, 30, and 60 d of refrigerated storage (processing, ripening and refrigerated storage), total concentrations of Mg, Ca, K, P and Zn were determined by flame atomic absorption spectrometry (FAAS). In addition, bioaccessibility assay with a simulated (*in vitro*) gastrointestinal digestion model was used. Sodium reduction and the addition of flavor enhancers revealed some significant ($p < 0.05$) changes in the chemical composition and pH of potentially Probiotic Prato cheese. Overall, P was the least-available element (~4.8%). The mean availability of Mg, Ca, K and Zn was significantly ~42.4%, 12.7%, 10.9%, and 9.2%, respectively. Na reduction and addition of flavors enhancers increased the bioaccessibility of Mg (~6.4%), Ca (~2.5%), K (~4.5%), Zn (~6.0%) and P (~2.5%). The *in vitro* method supports accurate determination of the effect of the sodium reduction and addition of flavor enhancers on availability of mineral compounds ingested from potentially probiotic Prato cheese during processing, ripening and refrigerated storage.

1. Introduction

In recent years, the cheese production has increased worldwide, particularly in the European Union (+2.35%), United States (+2.83%), and Australia (+5.45%) (Manuelian, Currò, Penasa, Cassandro, and Marchi, 2017). Cheese supplies essential nutrients for human nutrition, especially proteins, bioactive peptides, lipids, vitamins, and minerals (FAO., 2013), mainly calcium, magnesium and phosphorus (Kira & Maihara, 2007).

Calcium is the basic structural element of bones and teeth. It is also an element with a wide range of physiological functions; it is responsible for normal heart function, hormonal secretion, blood clotting, cell membrane permeability and activation of various enzymes (Kłobukowski, Skibniewska, & Kowalski, 2014). Besides calcium, cheese is also a good source of phosphorus, zinc, and magnesium (Walther, Schmid, Sieber, & Wehrmüller, 2008). Phosphorus is a component of all bodily cells, it is required for maintenance of the acid-base homeostasis, ATP and energy production (Gharibzahedi & Jafari, 2017) while magnesium plays numerous physiological roles in the human body and is implicated in many critical health issues, such as metabolic syndrome and skeletal muscle loss (Oh & Deeth, 2017). Finally, zinc is a trace mineral needed for making protein and genetic material; has a decisive role in sperm production, normal fetal development and immune system health; and not less important, potassium is needed for proper fluid balance, nerve transmission, muscle contraction, suitable maintenance of blood pressure, and waste elimination (Gharibzahedi & Jafari, 2017).

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer health benefits on the host (Hill et al., 2014). Ripened cheese is increasingly regarded as a bio-matrix of probiotics that remain viable over extended periods of time (Cichosz, Aljewicz, & Nalepa, 2014; Cruz, Buriti, de Souza, Faria, & Saad, 2009). In addition to its acceptable sensory characteristics, it constitutes an abundant source of essential minerals. However, the minerals found in ripened cheeses are not always readily available to the human body (Aljewicz, Siemianowska, Cichosz, & Tońska, 2014). Their availability is determined by the cheese type and the applied production technology, the content of organic acids, the presence of several casein fractions (αS1-, αS2-, and β-CN), and their degradation products, fat content, and fatty acid structure (Kłobukowski, Modzelewska-Kapitula,

* Corresponding author.
E-mail addresses: adriano.cruz@ifrj.edu.br, food@globo.com (A.G. Cruz).

https://doi.org/10.1016/j.lwt.2018.03.053
Received 16 January 2018; Received in revised form 19 March 2018; Accepted 19 March 2018
Available online 20 March 2018

& Kornacki, 2009).

In vitro methods were commonly used to evaluate the bioaccessibility of minerals and trace elements from different food products (Khouzam, Pohl, & Lobinski, 2011). A growing number of research studies has explored the influence of bacterial cultures on changes in the availability of minerals from dairy products (Aljewicz & Cichosz, 2015; Aljewicz et al., 2014; Bergillos-Meca et al., 2013, 2015).

Prato cheese corresponds about 20% of all cheese produced in Brazil (Nepomuceno, Junior, & Costa, 2016) and presents high sodium content (Felicio et al., 2013). To promote the reduction of sodium content in cheeses, an interesting alternative is the use of flavor enhancers in addition to the replace of NaCl by KCl (Cruz et al., 2011). Arginine is a conditionally essential amino acid that provides metabolic benefits and improves the sensory profile of products (Felicio et al., 2016). Yeast extract is a natural source of volatile aroma compounds and is a commonly used flavoring agent (Mahadevan & Farmer, 2006). Oregano is widely used in food, yielding a pleasant aroma and taste (Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011). Recent studies reported that the sodium substitution with KCl and the addition of flavor enhancers in Prato cheese did not influenced the survival of probiotic bacteria (Silva et al., 2017, 2018). However, the effects of NaCl reduction and addition of flavor enhancers on the bioaccessibility of minerals from potentially probiotic Prato cheese during manufacturing, ripening and storage are not fully described.

Considering that cheese is a good source of minerals and no previous investigations including simulated gastrointestinal digestion and evaluation of minerals from potentially probiotic Prato cheese have been carried out, it is of interest to determine not only the total element content of these minerals but also their availability. This study aimed to evaluate the effect of NaCl substitution with KCl and flavor enhancers' addition on the bioaccessibility of five essential micronutrients of potentially probiotic Prato cheese during storage at 4 °C for 60 days (immediately after processing, ripening time and refrigerated storage).

2. Material and methods

2.1. Lactic and probiotic strain

Lactococcus lactis ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* R-704 (Chr. Hansen, Valinhos, Brazil) were used as lactic starters for Prato cheese productions and *Lactobacillus casei*-01 (Chr. Hansen, Valinhos, Brazil) was the selected probiotic bacteria. All cultures were freeze dried commercial cultures for direct vat inoculation.

Lactobacillus casei-01 is a known probiotic strain and has been shown several benefits in different clinical trials, either human or animal. This strain is related with the improvement of inflammatory status in patients with rheumatoid arthritis (Vaghef-Mehrabany et al., 2014) and decrease of rheumatoid arthritis symptoms in women (Alipour et al., 2014). It is also related with memory-enhancing effect of LSPC in scopolamine-induced amnesia in mice (Xiao et al., 2014), decrease the blood pressure, improvement of lipid profile and immune system in overweighed women (Sperry, Silva, Silva, Esmerino, & Cruz, 2016), and reduction inflammation in the TNBS model of rat colitis (Ivanovska et al., 2017). In addition, it also presented good adhesion in Caco-2 cells (Balthazar et al., 2018) and viability and survival during gastrointestinal condition in different processed foods, as chocolate (Kemsawasd, Chaikham, & Rattanasena, 2016), fruit juices and yogurt (Chaikham, 2015) and fermented sausages (Vasilev et al., 2016).

2.2. Cheese processing

Prato Cheese was produced by a traditional manufacturing method as described by Silva et al. (2017). The experiment was conducted at the *Núcleo Avançado em Tecnologia de Alimentos plant* (NATA), using 120 L of whole pasteurized milk (65 °C/30 min). Milk was cooled to 32–34 °C, and the frozen lactic/probiotic bacteria cultures were added directly to the milk (1% v/v, approximately 7–8 log CFU/g) and incubated for 40 min. Then, calcium chloride (80 mL/120 L milk), annatto dye (36 mL/120 L milk) and coagulant (Ha La 1175, Chr. Hansen, Valinhos - SP, Brazil) sufficient to coagulate the milk within 35–50 min were added. The curd was cut into 1 cm cubes and submitted to slow continuous mixing for 15 min, which was followed by removal of part of the whey (30%) and further heating to 42 °C by progressively adding hot water (25 L - 80 °C) to increase the temperature by 1 °C every 3 min. This temperature was maintained for 40 min. After that, whey was drained off and 5 portions were separated being the ingredients corresponding to each formulation were added in a dry way by manual homogenization: Probiotic control (100% w/w NaCl) and four probiotic reduced sodium formulations: 1 NaCl:1 KCl (w/w); 1 NaCl:1 KCl (w/w) and 1% (w/w) arginine (Vetec, Rio de Janeiro, Brazil); 1 NaCl:1 KCl (w/w) and 1% (w/w) yeast extract from *Saccharomyces cerevisiae* (Bionis YE GMX 18, Biorigin, Lençóis Paulistas, SP, Brazil); 1 NaCl:1 KCl (w/w) and 1% w/w of oregano extract. Then, the curd was placed in rectangular plastic molds (2 kg) and pressed (0.1 MPa for 15 min; 0.24 MPa for 30 min; and 0.31 MPa for 90 min). Cheeses were fermented for 5 h at room temperature (~25 °C). The cheeses were then dried at 12 °C for 72 h, vacuum-packed into heat-shrinkable plastic bags, and stored at 12 °C for 60 days. Five cheeses were manufactured: CI (NaCl only + *L. casei*), CII (1 NaCl:1 KCl w/w) + *L. casei*), CIII (1 NaCl:1 KCl (w/w); 1% w/w arginine + *L. casei*), CIV (1 NaCl:1 KCl (w/w); 1% w/w yeast extract + *L. casei*), CV (1 NaCl:1KCl (w/w); 1% w/w oregano extract + *L. casei*). The processing was repeated twice, being all analyses were carried out in triplicate after 1, 30 and 60 days.

2.3. Chemical composition

Chemical composition (g/100 g moisture, protein and fat) was determined using traditional methods. Moisture was determined by drying 5 g sample at 100–105 °C for 24 h (Brasil, 2006). Protein was determined by the Kjeldahl method (Brasil, 2006), and fat was quantified by the Gerber method (Brasil, 2006). The pH values were carried out using a digital pH meter (Micronal, B-375, Digimed, Piracicaba, São Paulo, Brasil) by direct insertion of the electrode into the sample (Marshall, 1993). All analyzes were performed in triplicate.

2.4. Mineral (Ca, Mg, P, K, Zn) content of cheese

The mineral content analysis was performed according to Aljewicz et al. (2014). Cheese samples (1.5 g) were weighed, placed in 500 mL Kjeldahl flasks, combined with concentrated HNO_3 and $HClO_4$ (3:1 v/v), and left to stand for 30 min. The samples were mineralized (Buchi K-439, Flawil, Switzerland) until a colorless solution was obtained. Finally, were cooled and transferred to a volumetric flask containing 50 mL of ultra-pure deionized water (Millipore, Darmstadt, Germany).

The Ca, K, Mg and Zn contents of cheese samples were determined by atomic absorption spectrometry in an air-acetylene flame using the iCE 3000 Series Atomic Absorption Spectrometer (Thermo-Scientific, Hemel Hempstead, Hertfordshire, UK), a deuterium lamp (SMI-Labhut Ltd., Churcham, Gloucester, UK) for background correction and cathode lamps (SMI-Labhut Ltd.) suitable for each element. The concentrations of Ca^{+2} were determined by combining the samples with a 10% aqueous solution of lanthanum chloride to obtain La^{+3} concentrations of 0.5% in each sample. The phosphorous content was determined by the molybdenum method with hydroquinone and sulfate using spectrophotometer Helios β at ƛ = 460–480 nm (PN-ISO 13730).

2.5. Bioaccessibility of minerals from cheeses

Minerals *in vitro* availability was determined by enzymatic hydrolysis in a system, according to Aljewicz and Cichosz (2015), which simulated the conditions of the human gastrointestinal tract. The previously-grated cheese samples (1.5 g) were mixed with 50 mL of