Food Research International xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

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

Effect of sodium reduction and flavor enhancer addition on probiotic Prato cheese processing

H.L.A. Silva[a], C.F. Balthazar[a], E.A. Esmerino[a], A.H. Vieira[a], L.P. Cappato[b], R.P.C. Neto[c], S. Verruck[d], R.N. Cavalcanti[e], J.B. Portela[f], M.M. Andrade[f], J. Moraes[f], R.M. Franco[a], M.I.B. Tavares[c], E.S. Prudencio[d], M.Q. Freitas[a], J.S. Nascimento[f], M.C. Silva[f], R.S.L. Raices[f], A.G. Cruz[f,*]

[a] Universidade Federal Fluminense (UFF), Faculdade de Veterinária, 24230-340, Niterói, Brazil
[b] Universidade Federal Rural do Rio de Janeiro (UFRRJ), Instituto de Tecnologia (IT), BR 465, Km 7, 23890-000 Seropédica, RJ, Brazil
[c] Universidade Federal do Rio de Janeiro (UFRJ), Instituto de Macromoléculas Professora Eloisa Mano (IMA), 21941-598 Rio de Janeiro, Brazil
[d] Universidade Federal de Santa Catarina, Departamento de Ciência e Tecnologia de Alimentos, 88034-001 Florianópolis, Brazil
[e] Universidade Estadual de Campinas (UNICAMP), Faculdade de Engenharia de Alimentos (FEA), 13083-862, Campinas, São Paulo, Brazil
[f] Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Departamento de Alimentos, 20270-021 Rio de Janeiro, Brazil

ARTICLE INFO

Keywords:
Prato cheese
Sodium reduction
Flavor enhancers
Probiotic bacteria
Bioactivity
Rheology
Microstructure
Sensory acceptance

ABSTRACT

The effect of partial substitution of NaCl with KCl and the flavor enhancers addition (arginine, yeast extract and oregano extract) on Probiotic Prato cheese processing with (*L. casei* 01, 7 log CFU/mL) was investigated. Microbiological (lactic acid bacteria and probiotic counts), physicochemical (proximate composition, pH, proteolysis), bioactivity (antioxidant and angiotensin I-converting enzyme inhibitory activity), rheological (uniaxial compression and creep tests), water mobility (time domain low field magnetic resonance), microstructure (scanning electron microscopy) and sensory evaluation (consumer test) were performed. Sodium reduction and flavor enhancers addition did not constitute an obstacle to the survival of lactic and probiotic bacteria. Proximate composition, antioxidant and angiotensin I-converting enzyme inhibitory activity, and the rheological parameters were affected by the addition of flavor enhancer. No change in the fatty acid profile of cheeses was observed while good performance in the consumer test was obtained by the addition of yeast extract and oregano extract. Prato cheese can be an adequate carrier of probiotics and the addition of different flavor enhancers can contribute developing this functional product in the cheese industry.

1. Introduction

In today's competitive scenario, the dairy industry has sought to meet the demands of the consumer market, developing products aimed at providing functional components and/or to meet specific dietary needs, as in cases of obesity and hypertension (Ferrão et al., 2016). In addition, the probiotic microorganisms which when administered in adequate amounts, confer a health benefit on the host (Marchesi et al., 2016). The probiotic market is witnessing unprecedented growth, and the application to dairy products has the potential to improve the lives of millions of people worldwide (Reid, 2015; Almada, Nunes de Almada, Martinez, & Sant'Ana A. de S., 2015).

Cheese is a rich source of essential nutrients such as proteins, lipids, vitamins and minerals that perform an integral part of a healthy diet (Ash & Wilbey, 2010). The wide variety of cheese all over the world constitutes a growing market for probiotic cheese with potential advantages and is a valuable alternative to the dairy industry (Cruz, Buriti, de Souza, Faria, & Saad, 2009). The high sodium levels in cheeses is of major concern for public health worldwide (Felicio et al., 2013), as it has been associated with a high risk of hypertension, cardiovascular disease, osteoporosis, and other chronic noncommunicable diseases. Health authorities have recommended that sodium intake should not exceed 2000 mg per day, which corresponds to 5 g of salt (WHO, 2011).

Prato cheese corresponds about 20% of all cheese produced in Brazil and together with Mozzarella and Minas cheese is one of the most consumed in the country (Nepomuceno, Junior, & Costa, 2016). A widespread technological alternative to reduce sodium content in cheese is to replace NaCl by potassium chloride (KCl), which contributes to keep the salty taste by reducing the salt content in food by

* Corresponding author.
E-mail address: food@globo.com (A.G. Cruz).

http://dx.doi.org/10.1016/j.foodres.2017.05.018
Received 24 January 2017; Received in revised form 22 May 2017; Accepted 22 May 2017

25% without loss of palatability (Gomes et al., 2011, Soares, Fernando, Alvarenga, & Martins, 2016). However, higher substitution levels of sodium chloride by potassium chloride results in a higher perception of metallic taste, which is often associated with bitter taste, making necessary the addition of taste masking agents (Felicio et al., 2016).

The use of ingredients that can improve the sensory attributes of KCl is a promising alternative. Flavor enhancers are responsible for the umami, brothy, and savory taste, allowing the production of low-sodium cheese with high saltiness intensity and masking the bitter flavor (Grummer, Bobowski, Karalus, Vickers, & Schoenfuss, 2013). Arginine, in addition to improving the sensory profile of the product, can also provide metabolic benefits to the body (Virarkar, Alappat, Bradford, & Awad, 2013). Yeast extract is composed primarily of amino acids, peptides and nucleotides, with high protein content, being rich in B vitamins, selenium and dietary fiber (Halász & Lásztity, 1991). Oregano is a widely used ingredient in raw and cooked foods, providing distinct aroma and flavor, being known for its antioxidant activity (Karre, Lopez, & Getty, 2013).

Considering the Prato cheese as a cheese marketed and consumed on a large scale in Brazil, the benefits of cheeses as probiotic food carriers and the importance of reducing sodium levels of dairy foods, the present study evaluated the physicochemical, microbiological, rheological, microstructural, and sensory characteristics of different reduced-sodium probiotic Prato cheese containing flavor enhancers.

2. Material and methods

2.1. Cheese processing

The experimental design covered five cheeses: 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:1 KCl (w/w); 1% w/w oregano extract + *L. casei*). Prato Cheese was produced by a traditional manufacturing method as described by Mazal, Vianna, Santos, and Gigante (2007). The experiment was conducted at the Advanced Nucleon in Food Technology (NATA), using 120 L of full-fat pasteurized milk (65 °C/30 min). The milk was cooled 32–34 °C and the lactic culture (*Lactococcus lactis* ssp. *Lactis* and *Lactococcus lactis* ssp. *Cremoris* (Sacco, Brazil) and the probiotic bacteira *Lactobacillus casei-01* (Chr. Hansen, Valinhos, Brazil) cultures were added directly to the milk (1% v/v, approximately 7–8 log CFU/g) and incubated for 40 min. Both cultures were freeze-dried commercial cultures for direct vat inoculation. 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) were added for milk coagulation within 35–50 min.

The curd firmness was evaluated by inserting a sanitized spatula into the coagulum at a 45° angle, gently lifting the spatula upwards and observing the curd when it opened. Then the optimal curd set point was determined and the curd was cut into 1 cm cubes and submitted to slow continuous mixing for 15 min, which was followed by removal of part (30%) of the whey 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 until the mass point was reached. 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); NaCl:1 KCl (w/w) and 1% (w/w) yeast extract from *Saccharomyces cerevisiae* (Bionis YE GMX 18, Biorigin, Lençóis Paulistas, SP, Brazil); NaCl:1 KCl (w/w) and 1% w/w of oregano extract. Then, 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. The cheeses were then dried at 12 °C for 72 h and vacuum-packed into heat-shrinkable plastic bags and stored at 12 °C for 25 days.

2.2. Lactic and probiotic bacteria counts

25 g cheese and 225 mL sterile 0.1% peptone water (w/v) (Oxoid, São Paulo, Brazil) were homogenized, and serial dilutions were made. The microbial counts were carried out in duplicate using the pour plate technique. For enumeration of starter *L. lactis*, the culture medium M17 agar (Oxoid, São Paulo, Brazil) was used, which was performed in duplicate under aerobic conditions, incubated at 37 °C/72 h. The enumeration of *L. casei-01* was performed in duplicate under anaerobic conditions using MRS agar (Oxoid, São Paulo, Brazil) at 37 °C/72 h with Vancomycin (Darukaradhya, Phillips, & Kailasapathy, 2006).

Lactobacillus casei-01 survival after gastrointestinal condition was according previous study (Fernandes et al., 2013), with modifications. *Lactobacillus casei*-01 counts were determined after exposure to simulated gastrointestinal conditions (acidic pH and exposure to bile salts). One gram of cheese was homogenized with 9 mL gastric juice in test tubes. During the gastric phase, the pH was adjusted to 2.0–2.3 using sterile 0.5 N HCl. Sterile pepsin (Sigma-Aldrich, St. Louis, MO, USA) and lipase (Amano lipase F-AP15; Aldrich Chemical Company, Milwaukee, WI, USA) solutions were added to gastric juice to reach concentrations of 3 g/L and 0.9 mg/L, respectively and incubated at 37 °C for 30 min. One mL of this mixture was then transferred to tubes containing 9 mL of intestinal juice. In the enteric phase, the pH was raised to 7.0–8.0 using a sterile alkaline solution containing bile and pancreatin at the concentrations of 10 and 1 g/L, respectively and incubated at 37 °C for 60 min to simulate the conditions in the gastrointestinal tract.

2.3. Physicochemical analyses

Proximate composition (g/100 g moisture, protein and fat) was determined using traditional methods (Brasil, 2006). Moisture was determined by drying 5 g sample at 100–105 °C for 24 h. Protein was determined in duplicate by the Kjeldahl method, and multiplying the nitrogen content by the factor 6.38, and fat was quantified by the Gerber method. The pH values were carried out using a digital pH meter (Micronal, B-375, Digimed, Piracicaba, São Paulo, Brasil).

The mineral content was determined by Inductively Coupled Plasma (ICP) Optical Emission Spectrometry (Spectro Analytical Instruments, Kleve, Germany) according to Felicio et al. (2016).

Proteolytic activity was determined using the OPA method (Gomes et al., 2011). Although OPA method is very simple proteolysis indicator, it has been used in several probiotic cheeses as Minas frescal cheese (Gomes et al., 2011, Felicio et al., 2016) and petit Suisse cheeses (Pereira et al., 2016).

2.4. Bioactivity

The bioactivity of low sodium Prato cheese was evaluated thorough the angiotensin I-converting enzyme inhibitory (ACE) activity and the antioxidant activity. The angiotensin I-converting enzyme inhibitory (ACEI) was determined following the spectrophotometric assay (Torres-Llanez, González-Córdova, Hernandez-Mendoza, Garcia, & Vallejo-Cordoba, 2011). The extent of inhibition was calculated as follows: ACEI activity (%) = $[(B - A)/(B - C)] \times 100$, where the absorbance in the presence of ACE and the ACEI component is A, the absorbance without the ACEI component is B, and C is the absorbance without ACE (Torres-Llanez et al., 2011).

The antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method (Lee, Jeewanthi, Park, & Paik, 2016). The DPPH radical-scavenging activity was calculated using the following formula: DPPH radical-scavenging activity (%) = [1 − (sample absorbance at 517 nm/control absorbance at 517 nm)] × 100.