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Physico-chemical changes during storage and sensory acceptance of low sodium probiotic Minas cheese added with arginine

T.L. Felicio [a], E.A. Esmerino [b], V.A.S. Vidal [b], L.P. Cappato [c], R.K.A. Garcia [b], R.N. Cavalcanti [b], M.Q. Freitas [d], C.A. Conte Junior [d], M.C. Padilha [e], M.C. Silva [a], R.S.L. Raices [a], D.B. Arellano [b], H.M.A. Bollini [b], M.A.R. Pollonio [b], A.G. Cruz [a,*]

[a] Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Mestrado Profissional em Ciência e Tecnologia de Alimentos (PGCTA), Rua Senador Furtado, 171, Maracanã, 20270-021 Rio de Janeiro, Brazil
[b] Universidade Estadual de Campinas (UNICAMP), Faculdade de Engenharia de Alimentos (FEA), 13083-862, Cidade Universitária Zeferino Vaz, Campinas, São Paulo, Brazil
[c] Universidade Federal Rural do Rio de Janeiro, Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, 23890-000, BR 465 km 7 Seropédica, Rio de Janeiro, Brazil
[d] Universidade Federal Fluminense (UFF), Faculdade de Medicina Veterinária, 24230-340, Niterói, Rio de Janeiro, Brazil
[e] Universidade Federal do Rio de Janeiro, Instituto de Química (IQ), Laboratório de apoio ao desenvolvimento tecnológico (LADETEC), 21949-900 Rio de Janeiro, Brazil

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ABSTRACT

The partial substitution of sodium chloride by potassium chloride (0%, 25%, and 50%) and addition of arginine (1% w/w) in probiotic Minas cheese was investigated. Microbiological (*Lactococcus lactis* and *Lactobacillus acidophilus* counts, and functionality of the prebiotics *L. acidophilus*), physicochemical (pH, proteolysis, organic acids, fatty acids, and volatile profiles), rheological (uniaxial compression) and sensory (hedonic test with 100 consumers) characterizations were carried out. The sodium reduction and addition of arginine did not constitute a hurdle to lactic and probiotic bacteria survival, with presented values of about 9 log CFU/g, ranging from 7.11 to 9.21 log CFU/g, respectively. In addition, lower pH values, higher proteolysis, and a decrease in toughness, elasticity and firmness were observed, as well as an increase in lactic, citric, and acetic acid contents. In contrast, no change was observed in the fatty acid profile. With respect to the sensory acceptance, the probiotic low-sodium Minas cheese presented scores above 6.00 (liked slightly) for the attributes flavor and overall acceptance. The addition of arginine can be a potential alternative for the development of probiotic dairy products with reduced sodium content.
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1. Introduction

The excessive consumption of sodium chloride has led to public health problems, with strong correlation with high blood pressure (Felicio et al., 2013). Among dairy foods, cheese has contributed to sodium intake (Mhurchu et al., 2011) since salt improves flavor and prevent microbial growth and enzyme activity, which can deteriorate the product, with harmful effects on public health (Cruz et al., 2011). In addition, cheeses are suitable food matrixes for the addition of probiotic bacteria due to their solid consistence, low acidity, and high protein and fat contents, which allows the probiotics survival during the storage (Cruz, Buriti, Souza, Faria, & Saad, 2009).

Fresh Minas cheese is one of the most popular cheeses in Brazil, being produced on a large scale and consumed both at breakfast and as a dessert (Souza, Cruz, Moura, Vieira, & Sant'Ana, 2008). The sodium reduction and addition of probiotic bacteria to fresh Minas cheese has been studied in an isolated way, focusing on the survival of the probiotics in sufficient amounts to supply health benefits to the consumer (Gomes et al., 2011; Lollo et al., 2012), and on the reduction of sodium chloride up to 25% by substituting with potassium chloride, with good sensory acceptance (Gomes et al., 2011).

Thus, studies on the use of flavor maskers to both minimize the metallic off-flavor caused by the excessive addition of potassium chloride and obtain a greater sodium reduction in the product are required (Cruz et al., 2011). The addition of arginine may be an interesting alternative, once besides improving the sensory profile of the product, it also provides metabolic benefits to the human organism (Virarkar, Alappat, Bradford, & Awad, 2013). Arginine is the precursor of nitric oxide, a vasodilator used in the control of high blood pressure, which also inhibits platelet aggregation by activating cGMP (cyclic guanosine monophosphate) and reducing intra-platelet calcium, as well as impeding adhesion of monocytes and neutrophils to the endothelium (Alvares, Conte-Junior, Silva, & Paschoalin, 2012). No reports on the application of arginine in

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

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dairy products such as cheese have been found in literature, thus evidencing the originality of the present study.

Currently, researchers have focused on functional foods worldwide, aiming to elucidate their properties and beneficial effects to the health and well-being of consumers (Granato, Favalli, Cruz, Faria, & Shah, 2010). In this context, the simultaneous addition of probiotic microorganisms and sodium reduction in processed foods constitutes a potential approach for functional foods, considering the positive impact on both gastrointestinal and cardiovascular health. The objective of the present study was to produce probiotic fresh Minas cheese with reduced sodium content and addition of arginine, and to evaluate the possible changes in the quality parameters during shelf life.

2. Material and methods

2.1. Cheese processing

The cheese was processed according to a recently published technology (Gomes, Braga et al., 2011) with some modifications. Fifty liters of pasteurized milk (3.4 g fat/100 L milk, *Cooperativa Barra Mansa*, Rio de Janeiro, Brazil) was heated to 35 °C and the following additions were made: 0.03 g calcium chloride/100 L milk (Vetec, Rio de Janeiro, Brazil), lactic culture (*Lactococcus lactis* R-704, Chr Hansen, Valinhos, São Paulo, Brazil, DVS, approximately 6–7 log CFU/g, 0.05 g/L) and probiotic culture (*Lactobacillus acidophilus* La5 Chr Hansen, Valinhos, São Paulo, Brazil, 8 log CFU/mL). Liquid rennet (1.0 ml/L, HA LA, Chr Hansen, Valinhos, São Paulo, Brazil) was used to coagulate the milk within 40 min. After coagulation, the curd was cut into approximately 2.0 cm cubes, followed by slow agitation for 20 min. The curd was weighed and divided into four equal portions, which were submitted to salting and drying according to the following experimental design: Qc (NaCl, 100%), QI (NaCl/KCl 75/25%), QII (50/50%, NaCl/KCl), and QIII (50/50%, 1% arginine). The salts and arginine (Vetec, Rio de Janeiro, Brasil) concentrations were expressed as g/arginine or salt mixture in relation to the total weight. Then, the cheeses were packed into polyethylene bags and stored at 5 °C for 14 days. The microbiological, physicochemical, and rheological characterizations were carried out after 1, 7, and 14 days of refrigerated storage.

2.2. Microbiological analyses

The samples were prepared by stomaching. For that, 25 g cheese and 225 mL sterile 0.1% w/v peptone water (Oxoid, São Paulo, Brazil) were homogenized, and further dilutions were made. The microbial counts were carried out in duplicate using the pour plate technique. For enumeration of starter *Lactococci*, the culture medium M17 agar (Oxoid, São Paulo, Brazil) was used, which was incubated at 30 °C for 72 h under aerobic conditions. The enumeration of *L. acidophilus* LA-5 was performed in duplicate under aerobic conditions using 0.15 w/v% bile salts MRS agar (Oxoid, São Paulo, Brazil), incubated at 37 °C for 72 h (Gomes, Braga et al., 2011; Gomes, Cruz et al., 2011). *L. acidophilus* La-5 counts were determined after exposure to simulated gastrointestinal conditions (acidic pH and exposure to bile salts) (Fernandes et al., 2013). One gram of cheese was homogenized with 9 mL gastric juice (Vetec, São Paulo, Brazil, 0.2% NaCl, pH 2.0) in test tubes, and incubated at 37 °C for 30 min. One mL of this mixture was then transferred to tubes containing 9 mL of intestinal juice (0.6% bile salts, pH 7.0) and incubated at 37 °C for 60 min to simulate the conditions in the gastrointestinal tract of a healthy individual who had not been fasting for a long time (Mortazavian et al., 2008).

2.3. Physicochemical analyses

The pH measurements were carried out using a digital pH meter (Micronal, B-375, Digimed, Piracicaba, São Paulo, Brasil), by inserting the electrode directly into the samples (Marshall, 1993). Proteolytic activity was determined after reaction with a reactive solution (OPA) containing sodium dodecyl sulfate, sodium tetraborate decahydrate, dithiothreitol, o-phthalaldehyde and ethanol. The amino acids and peptides released from the lactic acid cultures were quantified by absorbance readings of the OPA derivatives at 340 nm (Gomes, Cruz et al., 2011).

The proximate composition (g/100 g moisture, fat and protein) was determined using traditional methods (Brasil, 2006). Moisture was determined by drying 5 g sample at 100–105 °C for 24 h. Fat was quantified by the Gerber method, and protein was determined in duplicate by the Kjeldahl method, by multiplying the nitrogen content by the factor 6.38 (Brasil, 2006).

The mineral content was determined by Inductively Coupled Plasma (ICP) Optical Emission Spectrometry (Spectro Analytical Instruments, Kleve, Germany) according to Moreno-Rojas, Pozo-Lara, Zurera, and Lopez (1993). Calibration curves were constructed using calcium, sodium, and potassium standards. Ten grams of sample were acid hydrolyzed for approximately 16 h at 120 °C ± 2 °C using 2 mL nitric-perchloric acid solution (2:1). The samples were then heated in a digestion block (Technal, São Paulo, Brazil) in a fume hood at slow boil to100 °C ± 2 °C over 1 h and maintained for an additional 2 h at 170 °C (±2 °C). After cooling to room - temperature, 2 mL of nitric-perchloric acid were added to each tube and heated for further 4 h at 170 °C (±2 °C) in the digestion block.

The organic acids (lactic, acetic and citric) were quantified by high performance liquid chromatography (HPLC, Gomes, Braga et al., 2011; Gomes, Cruz et al., 2011) using an Aminex X-87H column (300 mm × 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) and a guard column containing disposable H^+ cartridges (Biorad-Rad Laboratories) at 65 °C. The mobile phase was sulfuric acid (0.009 mol/L), previously diluted in ultra-pure water (Milli-Q system, Millipore Corporation, Billerica, MA, USA), and subsequently filtered and degassed through a 0.45 mm membrane filter (Millipore). The flow rate was 0.6 mL/min, and UV–VIS detection was performed at 220 nm, with volume injection of 25 μL. Standards of lactic, acetic, and citric acids were prepared, and the chromatographic peaks were integrated using the Millenium software.

The fatty acid (FA) profile was measured according to Florence et al. (2012) with some modifications. The fatty acid methyl esters were quantified by gas chromatography using an Agilent Technologies model 5975C gas chromatograph (Santa Clara, California, USA) equipped with a flame-ionization detector (FID), split injection (1:100), a fused silica capillary column (Supelco SP-tm-2560, Supelco, Bellefonte, PA, USA) and a programmed temperature gradient. The initial column temperature was programmed at 65 °C for 4 min, then rising to 185 °C at 16 °C/min, remaining at this temperature for 12 min followed by a second increase to 235 °C at 20 °C/min, remaining at this temperature for further 14 min, giving a total time of 40 min. The gas (White Martins, São Paulo, Brazil) flow rates were 1.4 mL min^{-1} for the stripping gas (H_2), 30 mL min^{-1} for the auxiliary gas (N_2), and 30 and 300 mL min^{-1} respectively for the H_2 and synthetic gas. The split was 1/80, the injection volume 2 μL, and identification and quantification of the methylated samples were made by comparison of their retention times with those of the respective standards (SIGMA 05632 and SIGMA 189–19). Data were collected using the MSD ChemStation software and the FA were classified as short chain FA (SCFA from C2 to C4), medium chain FA (MCFA from C6 to C12) and long chain FA (LCFA from C14 to C24).

The volatile compounds were extracted by solid phase microextraction (SPME) and analyzed by gas chromatography coupled with

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