



Oxidative stress in probiotic Petit Suisse: Is the jabuticaba skin extract a potential option?



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ABSTRACT

This research evaluated the action of jabuticaba skin extract (JSE) obtained through supercritical extraction compared with the antioxidants ascorbic acid, cysteine chloride, and glucose oxidase along probiotic Petit Suisse cheese processing. Physico-chemical analyses (pH, proteolysis, antioxidant activity, organic acids, and fatty acid profile) and microbiological (starter culture and probiotic culture counts) were carried out over 28 days of refrigerated storage. Addition of JSE on Petit Suisse was variable and dependent of the evaluated parameters with satisfactory findings in consumer test. However, the supplementation of probiotic Petit Suisse with JSE should be evaluated with care.

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1. Introduction

The probiotic microorganisms *Lactobacillus acidophilus* and *Bifidobacterium* spp. are derived from the human gastrointestinal tract and are microaerophile and anaerobic, respectively (Granato, Branco, Cruz, Faria, & Shah, 2010). Unlike aerobic bacteria, which completely reduce oxygen in water, in probiotic bacteria this system is absent or incomplete (Cruz, Buriti, Souza, Faria, & Saad, 2009). Therefore, toxic oxygen metabolites such as superoxide anion (O_2^-), hydroxyl radical (OH^\cdot), and hydrogen peroxide (H_2O_2) accumulate, which may lead to cell death (Vasiljevic & Shah, 2008). Hence, oxygen concentration during storage must be as low as possible in order to avoid toxicity and microorganism death and the consequent loss of product functionality (Cruz et al., 2009).

Alternatives that can lower dissolved oxygen levels in probiotic dairy products and decrease the oxy-reduction potential are also strong allies in maintaining probiotic counts and have been reported in previous studies, among which the use of cysteine with or without ascorbic acid (Dave & Shah, 1997a, 1997b; Rodrigues et al., 2011), glucose oxidase (Batista

et al., 2015; Cruz et al., 2010; Cruz, Castro, Faria, Bogusz, et al., 2012a, Cruz, Castro, Faria, Lollo, et al., 2012b, Cruz et al., 2013), and catechins (Gaudreau, Champagne, Remondetto, Bazinet, & Subirade, 2013).

The food industry has a long tradition of using antioxidant compounds, however, growing consumer concern for natural and healthier products has entailed an increase in the use of natural antioxidants (Van den Ende, Peshev & De Gara, 2011). Anthocyanins, which belong to the natural phenolic antioxidant compounds class, are able to sequester free radicals, absorb ultraviolet light between 100 and 400 nm, and chelate transition metals, thus halting auto-oxidation decay and the production of poor odors and textures (Brewer, 2010).

The jabuticaba (*Myrciaria cauliflora* Berg) fruit, native to Brazil, is a pleasant-tasting, dark-colored fruit and a rich source of a wide variety of phenolic compounds, including flavonoids, anthocyanins, tannins, phenolic acids, as well as less well-known polyphenols like depsides (Wu, Long & Kennelly, 2013). Jabuticaba skin boasts a promising antioxidant potential since it carries the anthocyanins cyanidin-3-glucoside and delphinidin 3-glucoside (Santos, Veggi, & Meireles, 2012), which has recently led to it being used as raw material for the production of powdered pigment with functional activity (Silva et al., 2014) and subjected to microencapsulation (Ibrahim Silva, Stringheta, Teófilo, & Oliveira, 2013). In addition, studies have reported the benefits of ingesting jabuticaba skin (Batista et al., 2013; Batista et al., 2014; Leite-Legatti et al., 2012; Lenquiste, Batista, Marineli, Dragano, & Maróstica, 2012).

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This study assessed the performance of the jabuticaba skin extract, compared with other antioxidants used in previous studies to minimize the oxidative stress in probiotic Petit Suisse cheese. In this sense, several intrinsic parameters of quality were evaluated along refrigerated storage as microbiological counts (lactic and probiotic counts) and physico-chemical analysis (pH, proteolysis, antioxidant activity, acid organic production, fatty acid profile). Finally, a consumer test was also performed.

2. Material and methods

2.1. Ingredients

The following commercial ingredients were employed in the production of the probiotic Petit Suisse: pasteurized whole milk (3.0% fat, Laticínio Atilatte LTDA, Itatiba, Brazil), sucrose (Cosan, Tarumã, Brazil), commercial sterilized milk cream (25% fat, Nestlé Brasil LTDA, Araçatuba, Brazil), and Petit Suisse powder (Hexus Petit Suisse 500®, Hexus Food Ingredients, Portão, Brazil). The cultures used were the ones previously described. As antioxidants, ascorbic acid (Casa da Química, Diadema, Brazil), cysteine (Labsynth LTDA, Diadema, Brazil), glucose oxidase (Glucomax CO, Prozyn, São Paulo, Brazil), and jabuticaba extract obtained through supercritical extraction at 60 °C using 80% carbon dioxide and 20% water, 300 bar pressure (Lasefi, Unicamp, Campinas, Brazil) were used.

The samples were packaged in 50 mL polystyrene flasks with high density polyethylene screw caps (Ânfora, Campinas, Brazil).

2.2. Experimental design

The five experimental treatments were performed according to Table 1. For comparison purposes, technological alternatives previously reported in the literature such as cysteine chloride, ascorbic acid, and glucose oxidase enzyme were used (Dave & Shah, 1997a; 1997b; Cruz et al., 2010; Cruz, Castro, Faria, Bogusz, et al., 2012a; Cruz, Castro, Faria, Lollo, et al., 2012b; Cruz et al., 2013).

All treatments used freeze-dried *Streptococcus thermophilus* (TA 040, Danisco, Dangé, France) as starter culture and the probiotic cultures *L. acidophilus* (LA C4, Danisco, Dangé, France) and *Bifidobacterium animalis* subsp. *lactis* (DN 173-010, Danisco, Dangé, France). The microorganisms employed were freeze-dried commercial cultures for direct vat inoculation and were added with 30 mg·L⁻¹ (3% w/v) of *S. thermophilus* and with 50 mg·L⁻¹ (5% w/v) of *L. acidophilus* and *Bifidobacterium lactis*, in order to achieve 6 log CFU·g⁻¹.

2.3. Petit Suisse manufacture

The Petit Suisse cheese was produced according to the recommendations of the Petit Suisse powder manufacturer. Milk (78.5% w/v), sucrose (10.5% w/v), and Hexus Petit Suisse 500® powder (6.0% w/v) were mixed and kept under stirring for 1 h for the hydration of the system. After this period, the sterilized milk cream was added (5.0% w/v) and the mixture was pasteurized at 90 °C for 5 min. The mixture was then cooled down to 37 °C and all cultures were incubated. After that, the product was fermented at 37 °C until a pH of 4.7 to 4.9 was reached

(approximately 6 h). At this point, the product was separated into five portions and the antioxidants were added, as presented in Table 1. The portions (treatments T1, T2, T3, T4, and T5) were distributed in the flasks and kept under refrigeration (4 ± 1 °C) for up to 28 days.

2.4. Microbiological analysis

L. acidophilus, *B. animalis* subsp. *L. actis*, and *S. thermophilus* counts were performed weekly during the storage period (28 days) for all trials. For microbiological analysis, 25 g portions of duplicate cheese samples were blended with 225 mL of 0.1 g·100 mL⁻¹ peptone water and submitted to serial dilutions with the same diluent. *L. acidophilus* was counted by pour-plating 1 mL of each dilution in DeMan–Rogosa–Sharpe (MRS) agar (Oxoid, São Paulo, Brazil), with 0.15% (w/v) of bile salt (Mortazavian, Ehsani, Sohrabvandi, & Reiheimer, 2007), after 3 days of aerobic incubation at 37 °C. *Bifidobacterium* spp. was counted by pour-plating 1 mL of each dilution in MRS agar (Oxoid, São Paulo, Brazil), to which 0.9% (w/v) sodium propionate and 0.6% (w/v) lithium chloride solutions were added, after 3 days of anaerobic incubation (Anaerobic System Anaerogen, Oxoid, São Paulo, Brazil) at 37 °C (Zacarchenco & Massaguer-Roig, 2004). *S. thermophilus* was counted by pour-plating 1 mL of each dilution in M17 agar (Oxoid, São Paulo, Brazil), followed by 2 days of aerobic incubation at 37 °C. Microbiological analyses were carried out in triplicate.

2.5. pH

The pH changes in the assays were monitored weekly by direct insertion using a pH meter (Digimed DM-20, São Paulo, Brazil).

2.6. Proteolysis

The level of proteolysis of the samples was assessed based on the method described by Donkor, Henriksson, Vasiljevic, and Shah (2007). A 5 g portion of each treatment was mixed with 10 mL of 0.75% (w/v) trichloroacetic acid (TCA – Synth, Diadema, Brazil) and filtered using qualitative filter paper (Whatman, England). The filtrate (150 µL) was then mixed with 3 mL OPA reagent and left at room temperature (≈20 °C) for 2 min. The absorbance of the solution was measured by a spectrophotometer (Beckman, model DU-70) at 340 nm. The relative proteolytic activity of these organisms was expressed as the absorbance of free amino groups, measured using the TCA solution as a blank. The analysis was carried out weekly in triplicate.

The preparation of OPA reagent consisted in weighing 0.9 g sodium tetraborate (Casa da Química, Diadema, Brazil) and 0.5 g sodium dodecyl sulfate (Sigma-Aldrich, São Paulo, Brazil) directly in a 50 mL volumetric flask. Approximately 35 mL deionized water were added and the mixture was solubilized in an ultrasound bath for 30 min. In a small beaker, 40 mg OPA (Sigma-Aldrich, São Paulo, Brazil) were weighed and solubilization was carried out with 1 mL methanol P.A. (Synth, Diadema, Brazil). The mixture was then transferred into the volumetric flask with the sodium tetraborate and sodium dodecyl sulfate solution. 100 µL β-mercaptoethanol (Sigma-Aldrich, São Paulo, Brazil) were added and the volume was completed with deionized water (Church, Swaisgood, Porter, & Catignani, 1983). The whole procedure was carried out protected from light and the solution was stored in an amber glass flask and kept under refrigeration until use.

2.7. Extract chemical composition

2.7.1. Total phenolic content

TPC (total phenolic content) of the jabuticaba skin extract was determined using the Folin–Ciocalteu method described by Singleton, Orthofer, and Lamuela-Raventos (1998). First, a stock solution was prepared in a 100 mL volumetric flask by dissolving 0.5 g gallic acid in 10 mL ethanol, then the volume was completed with distilled water.

Table 1
Experimental design of the probiotic Petit Suisse with antioxidants.

	T1	T2	T3	T4	T5
Antioxidant	-	Ascorbic Acid	Glucose Oxidase + Glucose	Cysteine	Jabuticaba Extract
Concentration (mg·kg ⁻¹)	-	250.00	62.32 + 4.35	280.00	5000.00
References	-	(I)	(II)	(III)	(IV)

(I) Dave & Shah 1997b; (II) Cruz et al. 2010; (III) Dave & Shah 1997a; (IV) Michael, Phebus, & Schmidt 2010.

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