

## Full Length Research Paper

## Development and evaluation of sundae-type “Coalhada” containing *Lactobacillus paracasei* and blueberry (*Vaccinium ashei*) preparation

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“Coalhada” is a type of fermented milk (made with *Lactococcus lactis* spp. *lactis* and *Lactococcus lactis* spp. *cremoris*) very popular in Brazil, which is widely consumed due to its pleasant texture and mild taste and aroma. Its acceptance is possible to increase by adding a fruit preparation. Fermented milk is the most common food matrix used for delivery probiotic bacteria, which are able to survive to the passage through the gastrointestinal tract, reaching the colon in high concentration where they can effectively bind and subsequently, improve the body functions as a whole. In this study, two formulations of “Coalhada” were developed which resemble a sundae (in which, the fruit preparation stays on the bottom of the package and the fermented milk on the upper part) used for a blueberry preparation. The formulation F1 was the control, with no addition of probiotic bacteria and to the formulation F2 the probiotic *Lactobacillus paracasei* was added. The formulations were evaluated over 14 days of storage under refrigeration. The characteristics evaluated in the product were: moisture, pH, acidity, lipids and ash content and all of them were not significantly affected ( $p > 0.05$ ) by the addition of the probiotic bacteria. However, the addition of *Lactobacillus paracasei* significantly decreased ( $p < 0.05$ ) the syneresis of the product during the refrigerated storage. Blueberry preparation had a high antioxidant activity which was  $330.8 \mu\text{mol TEAC } 100 \text{ g}^{-1}$  and the total phenolic compounds content was  $36.65 \text{ mg GAE } 100 \text{ g}^{-1}$ , showing that the addition of blueberry can also improve the nutritional characteristics of the final product. The count of the lactic acid bacteria (including the probiotic one) remained high ( $> 10^9 \text{ CFU g}^{-1}$ ) during the 14 days of storage, but the shelf life of the two formulations was around seven days, since in the second week of storage the counts of yeasts and molds reached  $10^2 \text{ CFU g}^{-1}$  (which is the maximum allowed by Brazilian legislation). It is important to note that there was no addition of preservatives to the blueberry preparation, although the Brazilian legislation allows this and it could extend the shelf life of the final product, because yeasts and molds found in the product, probably comes from the fruits. Concluding that it was feasible to use blueberries in functional fermented milk such as “sundae-type Coalhada” and that it was a good way to innovate and to add value to the final product.

**Key words:** Fermented milk (“coalhada”), *Lactococcus lactis*, *Lactobacillus paracasei*, blueberry (*Vaccinium ashei*), shelf-life, lactic acid bacteria.

## INTRODUCTION

In Brazil, “Coalhada” is a dairy product obtained through the fermentation of pasteurized or sterilized milk, being used with one or more species of mesophilic lactic acid bacteria (*Lactococcus lactis*). The microorganisms used should be viable, active and until the end of the shelf life of the product, the minimum concentration required in the final product should be  $10^6$  CFU  $g^{-1}$ . Its acidity must be between 0.5 and 1.5% of lactic acid according to the Brazilian Legislation (BRASIL, 2007).

The processing procedure of “Coalhada” is very similar to that used for yogurt production, but it differs in terms of the fermentation temperature. The ideal temperature is around 30°C and the microorganisms used for fermentation can be: *Lactococcus lactis*, *Lactococcus cremoris* and/or *Lactococcus diacetylactis* (Lerayer et al., 2009).

According to the Brazilian Legislation (BRASIL, 2007), fermented milks comprised standardized milk and/or reconstituted milk and the specific lactic acid bacteria for which type of fermented milk. Other ingredients, such as milk powder or milk protein concentrated, whey protein, caseinate, among others can be added and the addition of fruit pulp or fruit preparation is also permitted up to a maximum of 30% (w/w).

According to Lerayer et al. (2009), it is possible to divide the lactic acid bacteria (LAB) used to ferment milk into two main categories. The first one are the technological cultures, which are the acidifiers, responsible for the milk fermentation (to obtain the nutrients to multiply themselves) and they also give to the product a palatable characteristic; and the second group are the probiotic cultures, which are beneficial to the health of the host.

Probiotic are living microorganisms, which when ingested in sufficient amounts, will beneficially influence the health of the host by improving the composition of intestinal microbiota. They should be able to survive to the passage through the gastrointestinal tract and to bind to the colon (FAO/WHO, 2006).

A healthy and balanced enteric microbiota results in the normal performance of the physiological functions. With a high amount of probiotics in the colon, it is possible to maintain an environment hostile to the pathogens microorganisms (which could cause infections, besides the fact that they can produce toxins), by inhibiting their multiplication. In addition to the antimicrobial action, due to the production of compounds such as nisin, the probiotics also have hydrolytic activity on bile salts, contribute nutritionally and modulate immune activity. Probiotic bacteria should remain stable and viable in high amount during all the shelf life of the product (Saad et

al., 2013). Lactobacilli are non-spore-forming, rod-shaped, anaerobic Gram-positive bacteria used for fermentation, especially, in the dairy industry. *Lactobacillus paracasei* is a probiotic bacteria used for cheese, yoghurt and other fermented milk production (Schmid et al., 2006). There are several scientific studies on the beneficial effects of *L. paracasei* ssp. *paracasei* in fermented products, which had shown that these bacteria helps in the reduction of hypercholesterolemia, hypertension, allergies, gastric damage, osteoporosis and obesity (Saad et al., 2013).

Many fruits are highly protective for human health, especially against ageing and oxidative-stress related to many diseases, due to their content of healthy phytochemicals. The berries are generally a small fruit that lacks big seeds and often are the richest source of natural antioxidants phytochemicals. Anthocyanin is a pigment that can be red, purple, violet or blue, it is a water soluble polyphenolic pigment widely found in the “berries fruits” which can prevent oxidative stress (Pojer et al., 2013).

Blueberry is a small fruit native from the United States; it is a flowering plant which belongs to the genus *Vaccinium* and to the family Ericaceae. In Brazil the cultivation of the species of *Vaccinium ashei* is still small but growing and it is concentrated in the southern states, where there are several days of cold during the winter, which allows the production of this berry (FACHINELLO, 2008).

Blueberry is one of the “super fruits” due to their potential bioactive compounds and with this fruit is possible to produce many functional foods. Blueberries have a flattened shape with a diameter between 1 and 2.5 cm and weight from 1.5 to 4 g. Its taste varies from sweet acid to acid and it has many seeds inside. It is known that anthocyanins are the nature’s most potent antioxidants and have demonstrated properties that extend well beyond suppressing free radicals (Srivastava et al., 2007). The blueberries may alleviate the cognitive decline in Alzheimer’s disease and other conditions of aging (Krikorian et al., 2010).

The high content of functional compounds makes the blueberry one of the richest fruits in terms of antioxidant compounds, since it contains significant levels of phenolic compounds, including anthocyanins, flavonoids and procyanidins, with a high level of biological activity, providing different health benefits (Koca and Karadeniz, 2009). Continued intake of phenolic compounds is associated with prevention of some degenerative diseases (Silva et al., 2010).

Blueberries are widely consumed *in natura* and often

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they are processed into a fruit preparation that can be used in production of fermented milk, in yoghurt, in ice cream, or in bakery products (Stewart, 2004). An attractive red color dependent on the pigment concentration is one of the most important quality characteristics of berry yoghurts.

The consumption of fermented milks is increasing worldwide and consumers are demanding new products, different formulations, organic and minimally processed fruit. In this research, fermented milk with a fruit preparation, the “Blueberry Coalhada”, which resembles a sundae, with and without the addition of the probiotic *Lactobacillus paracasei* was developed. The “Blueberry Coalhada” sundae type was characterized to evaluate the influence of the probiotic bacteria on the technological characteristics of the final product during the refrigerated storage.

## MATERIALS AND METHODS

Ingredients (UHT milk, skimmed powdered milk and sugar) were purchased from a local store in Florianópolis, Santa Catarina State (SC), in Brazil for the preparation of two formulations of “Blueberry Coalhada”. Blueberries (cultivar of highbush blueberry - *Vaccinium corymbosum* L.) were purchased from a local market, in São José, SC, Brazil. Lyophilized bacteria used for the fermentation were the commercial lactic acid bacteria (DVS), a mixture of *Lactococcus lactis* and *Lactococcus cremoris* (R-704, from Chr Hansen®) and the probiotic *Lactobacillus paracasei* (BGP 93, from Sacco®).

“Coalhada” were produced on a laboratory scale using UHT milk (fat content 32 g L<sup>-1</sup>) and was added 5% of sugar (w/w) and the mixture was heat-treated at 95°C for 5 min. The protein content of the “coalhada” was increased by addition of 5% of non-fat milk powder. This mixture was cooled to 30°C for the inoculation with the lactic acid bacteria. Both formulations (F1 and F2) were inoculated with 1% of a mixture of *Lactococcus lactis* and *Lactococcus cremoris* starter and afterwards, at formulation F2, 1% of the probiotic *Lactobacillus paracasei* was added. F1 was the control formulation, without the addition of *L. paracasei*. The fermentation was carried out at 30°C until a pH decrease at 4.6, when the products were cooled to 4°C to dramatically reduce the fermentation.

The fresh blueberries were washed and sanitized with sodium hypochlorite solution (100 mg L<sup>-1</sup> during 15 min), then they were rinsed in potable water. To the blueberry preparation, it was added 10% of water and 40% of sugar in relation to fruit weight. This mixture was concentrated and also pasteurized by heat treatment (95°C during 10 min), then 1% (w/w) of pectin was added to the mixture and boiled for 5 min. The addition of pectin was in the end of the heating process to avoid its degradation during the heat treatment. The preparation was hot fill packaged into a previously sanitized glass container and it maintained under refrigeration at 4°C until usage. The pectin was added to increase the viscosity of the blueberry preparation, a slight jellification is important to avoid the mixture with the inoculated milk which was added in the upper part of the product.

Blueberry *in nature* and the blueberry preparation were analyzed by the following determined parameters: content of total soluble solids (°Brix) using a digital refractometer (Mark Mettler, Toledo) and pH using a digital potentiometer (from Digimed Brand), according to the recommendation of the manufacturers. Determination of the phenolic compounds content was performed by the Folin-Ciocalteu spectrophotometric method (Singleton and Rossi, 1965) and the antioxidant capacity according to Brand-

Williams et al. (1995).

Physicochemical characterization of final product: content of moisture, protein, lipids, carbohydrates, ash and total acidity was carried out according to the methods described in AOAC (2010). To evaluate the spontaneous syneresis was applying the method described by Amatayakul et al. (2006).

The microbiological analyses of the two formulations were performed every seven days, by means of the counts for the yeasts and molds, lactic acid bacteria and total and thermotolerant coliforms according to APHA (2001, 2004).

The analyses were performed in triplicate and the results were subjected to variance analysis (ANOVA) and Tukey test to identify the significant differences ( $p < 0.05$ ) between the means, using the software Statistica 7.0®.

## RESULTS AND DISCUSSION

After 5 h and 30 min of fermentation at 30°C and the pH reached 4.6, leading to the coagulation of the milk and resulting in the “Coalhada”. There was no difference regarding the final pH of the two formulations (F1 and F2), so the addition of the *L. paracasei* did not affect the fermentation time. According to Arruda (2013), the fermentation of five different formulations of “coalhada” with the addition of passion fruit required 8 to 12 h, much longer than it takes for yoghurt fermentation after probiotic addition, as reported by Gallina et al. (2011).

The pH reduction is attributed to the continuous production of lactic acid by the inoculated lactic acid bacteria that destabilizes the casein micelles and it aggregates therefore, leading to the formation of the casein acid coagulum. It occurs when the pH reaches the isoelectric point of the casein (pH of 4.6), when its solubility is minimal (Tamime and Robinson, 2007).

The pH evaluation during the fermentation is important, since the low acidity (pH > 4.6) can cause separation of the serum from the coagulum once it is not completely formed and on the other hand, a sharp fermentation with a pH < 4.0 promotes the coagulum contraction due to poor proteins hydration, causing a severe syneresis (Tamime and Robison, 2007).

During the 14 days of storage under refrigeration, a slight post acidification in both formulations, with a decrease in the pH from 4.83 to 4.53 was observed; although this pH variation was not significant ( $p > 0.05$ ).

However, since the first week of evaluation, the syneresis was higher in F1 than in F2 (Table 1), and after 14 days of storage, the serum separation was 17.68 and 6.08% for F1 and F2, respectively. These results suggest that the addition of *L. paracasei* reduced substantially the syneresis without affecting significantly the post acidification, thus improving the technological characteristics of the developed product.

Although the blueberries have a low pH, it is still possible yeasts and molds growth, especially the acid-tolerant molds, due to that the blueberry preparation was pasteurized. The heat treatment also has the function to contribute in the dissolution of sugar (Moura et al., 2009). A partial evaporation of water during the processing

**Table 1.** The pH-values and percentage of syneresis of Sundae-type “coalhada” with blueberry (F1) and Sundae-type “coalhada” with blueberry containing *Lactobacillus paracasei* (F2) during storage.

Storage (days)	pH		Syneresis (%)	
	F1	F2	F1	F2
1	4.83 <sup>aA</sup>	4.85 <sup>aA</sup>	0 <sup>xx</sup>	0 <sup>xx</sup>
7	4.64 <sup>aB</sup>	4.54 <sup>bB</sup>	8.56 <sup>xy</sup>	3.16 <sup>yY</sup>
14	4.54 <sup>aC</sup>	4.53 <sup>aC</sup>	17.68 <sup>xZ</sup>	6.08 <sup>yZ</sup>

Values in the same line with different superscript small letters are significantly different ( $p < 0.05$ ). Values in the same column with different superscript capital letters are significantly different ( $p < 0.05$ ) according to the Tukey test.

**Table 2.** Centesimal composition of Sundae-type “coalhada” with blueberry (F1) and Sundae-type “coalhada” with blueberry containing *Lactobacillus paracasei* (F2).

Composition	Mean ( $\pm$ standard deviation)	
	F1	F2
Moisture	73.40 $\pm$ 0.02 <sup>a</sup>	73.35 $\pm$ 0.15 <sup>a</sup>
Protein	4.35 $\pm$ 0.08 <sup>a</sup>	3.91 $\pm$ 0.06 <sup>b</sup>
Lipids	2.70 $\pm$ 0.12 <sup>a</sup>	2.54 $\pm$ 0.15 <sup>a</sup>
Ashes	1.08 $\pm$ 0.01 <sup>a</sup>	1.09 $\pm$ 0.01 <sup>a</sup>
Carbohydrates*	18.49 $\pm$ 0.02 <sup>a</sup>	19.05 $\pm$ 0.12 <sup>b</sup>
Acidity**	0.95 $\pm$ 0.01 <sup>a</sup>	0.91 $\pm$ 0.02 <sup>a</sup>

\*Carbohydrates calculated by difference. \*\*Acid content in % of lactic acid. Values in the same line with different superscript letters are significantly different ( $p < 0.05$ ) according to the Tukey test.

occurs and the combination of this effect with the sugar addition made the soluble solids increased from 17.4°Brix (in the fresh fruit) to 50°Brix at the blueberry preparation. The blueberry preparation showed a 366.59 mg of gallic acid equivalents (GAE) per 100 g of total phenolic compounds, which is consistent with the results found by other researchers. Gesser et al. (2012) observed that, in a blueberry preparation with 30°Brix (which was used for the production of a probiotic fermented milk), the content of phenolic compounds was 336.41. Rocha (2009), Silveira et al. (2007) and Rodrigues et al. (2011) analyzing the blueberry pulp, the blueberry extract and the fresh fruits found 317.6, 277.4 and 436.6 mg GAE 100 g<sup>-1</sup>, respectively.

The antioxidant activity determined in this study was 3308.1  $\mu$ mol of TEAC in 100 g of sample and it was in the range that Sellappan et al. (2002) reported that using the same method, they found values between 811 and 3829  $\mu$ mol of TEAC 100 g<sup>-1</sup> of sample for several varieties of blueberry.

Table 2 shows the chemical composition of the two

formulations (F1 and F2), with the mean and the standard calculated. Regarding to the chemical composition of the two formulations, there was no significant difference ( $p > 0.05$ ) in relation to the moisture, lipids and ash content. Taking into consideration the lipid content on “coalhada”, the final product was partially defatted, since the fat contents ranging from 0.6 to 2.9%. Protein and carbohydrate content were statistically different (Table 2). Protein was lower in F2, and due to that, carbohydrate content was also lower because it was calculated by the difference.

According to Tamime and Robinson (2007), it is better to add skimmed milk powder than whey protein or caseinate to increase the protein content in order to have a firm gel with the minimum syneresis. The addition of 5% of skimmed milk powder to both formulations in this study aimed to raise the protein content of the final product, improving its texture without increasing the fat content.

It was observed that a normal acidity for both formulations, is within the values expected for “coalhada” (0.5 to 1.5), and the difference between the two formulations was not statistically significant. According to Tamime and Robinson (2007), acidity is one of the factors limiting the acceptance of fermented milk.

The counts for the total and thermotolerant coliforms for both formulations were less than 3 MPN g<sup>-1</sup>, indicating that good manufacturing practices (GMP) were applied during the production, once these microorganisms are easily eliminated by washing and sanitizing all the surfaces or by heating the ingredients (Franco and Landgraf, 2008).

The total count for the lactic acid bacteria remained constant at 10<sup>9</sup> CFU g<sup>-1</sup> for both formulations during the 14 days of analysis. Identity and quality standards of “coalhada” provide that the starters used must be viable, active and present in a concentration higher than 10<sup>6</sup> CFU g<sup>-1</sup> throughout the shelf life of the product (Brasil, 2007). And if the product has at least 10<sup>8</sup> CFU g<sup>-1</sup> of *Lactobacillus paracasei*, it can have a probiotic claim. In the first day of the analysis, the counts of the yeasts and molds were less than 10 CFU g<sup>-1</sup> for both formulations, but after seven days of storage, the F1 formulation had reached 5  $\times$  10 CFU g<sup>-1</sup> and after 14 days, both formulations had values above the upper limit established by the Brazilian legislation (10<sup>2</sup> CFU g<sup>-1</sup>). Arruda (2013) observed a shelf life of 28 days for “coalhada” mixed with passion fruit and Gesser (2012) reported a shelf life of 14 days for a probiotic fermented milk containing a blueberry preparation.

The presence of molds and yeasts can also be an indicator of poor sanitary practices in the manufacture and packaging of the final product. However, to these products was added sugar and fruit that are especially susceptible to the development of spoilage yeasts and typically, the shelf life of these products is limited by the multiplication of such microorganisms.

## Conclusion

Based on the results of this study, it was concluded that the *sundae*-type “coalhada” with a blueberry preparation had a shelf life of seven days, which was limited by the yeasts and molds count, although the total lactic acid bacteria remained high throughout the analysis period. It is important to note that there was no addition of conservatives to the blueberry preparation, which is allowed by Brazilian legislation and could extend the shelf life of the product up to three or four weeks. The addition of *L. paracasei* did not interfere with the fermentation, but it had a positive effect, since it significantly decreased the syneresis of the product.

## Conflict of interests

The authors have not declared any conflict of interests.

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