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ORIGINAL ARTICLE

Lactose-free *dulce de leche* with different concentrations of green banana biomass

Doce de leite sem lactose com diferentes concentrações de biomassa de banana verde

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Abstract

The production and consumption of *dulce de leche* is one of the most important sweets in the Mercosul Region. However, recent studies have shown consumers' interest for healthier food and no lactose products, hence creating a demand for this study aims to develop a more nutritional lactose-free *dulce de leche* with less calories and higher yielding by adding green banana biomass. Five lactose-free *dulce de leche* formulas have been elaborated in which four were added 5%, 10%, 15% and 20% of green banana biomass and one of them received a standard 0.5% of commercial starch addition. The samples were evaluated regarding its microbiological, physicochemical and sensorial quality. The different lactose-free *dulce de leche* formulas attended the microbiological standards and demonstrated an increased yield of the green banana biomass concentration. The results indicated that the rise of the green banana biomass concentration has increased moisture and decreased protein, lipids and calories levels. In regards of acceptance, all samples evaluated received grades between 7.23 to 8.72, that is, above 6 which is the minimum value accepted to all the evaluated attributes hence demonstrating the acceptance on behalf of evaluators. All samples presented high ratios of intent to purchase therefore confirming the acceptance results found. It has been observed that the use of green banana biomass aggregated positive characteristics to the product and did not influence the sensorial quality of the sweets, seeing that all of them could improve their yield and thus demonstrating to be a good option to the development of healthier products.

Keywords: *Dulce de leche*; Biomass; Sensorial; Microbiology; Physicochemical; Yield.

Resumo

O doce de leite está entre os mais importantes doces consumidos na região do Mercosul, o que estimula o estudo de sua produção. Estudos recentes têm mostrado o interesse dos consumidores por alimentos mais saudáveis, demandando o presente estudo, que teve como objetivo desenvolver um doce de leite mais nutritivo, menos calórico e com maior rendimento, por meio da adição da biomassa de banana verde. Foram elaboradas cinco formulações de doce de leite, sendo quatro delas com adição de 5%, 10%, 15% e 20% de biomassa de banana verde

e uma formulação padrão adicionada de 0,5% de amido comercial. As amostras foram avaliadas quanto à qualidade microbiológica e aos parâmetros físico-químico e sensorial. As diferentes formulações de doce de leite atenderam aos padrões microbiológicos e apresentaram aumento no rendimento com a adição da biomassa. Os resultados indicaram que o aumento da concentração de biomassa de banana verde aumentou o teor de umidade e diminuiu o teor de proteínas, lipídeos e calorias. Em relação à aceitabilidade, todas as amostras avaliadas apresentaram notas entre 7,23 e 8,72, ou seja, acima do mínimo aceitável, que é 6, para todos os atributos avaliados, indicando aceitação por parte dos julgadores. Todas as amostras apresentaram alto índice de intenção de compra, o que comprova os resultados encontrados de aceitabilidade. Observou-se que a utilização da biomassa de banana verde agregou características positivas ao produto, não influenciando na qualidade sensorial dos doces, e ainda melhorou seu rendimento, sendo uma boa opção para o desenvolvimento de produtos mais saudáveis.

Palavras-chave: Doce de leite; Biomassa; Sensorial; Microbiologia; Físico-química; Rendimento.

1 Introduction

Dulce de leche is a dairy product typical from Argentina being produced and consumed in large scales in Brazil and Argentina (Silva et al., 2015a). This product results basically from milk and sugar cooking until desired concentration (Konkel et al., 2004). However, for Gaze et al. (2015), *dulce de leche* is composed of milk, sucrose, sodium bicarbonate and other additives, it may exhibit distinct characteristics that vary greatly among industries and production areas.

According to Brazilian legislation, it is allowed the addition of 0.5% of starch or modified starch and from 5 to 30% of other isolated or mixed food products in *dulce de leche* composition (Brasil, 1978).

Many consumers have increased interest in products released into the market and that makes food industry dedicate even more in this area so as to attend this demand. An example of this is associated with the zero lactose products.

Lactose intolerance is correlated with the body incapacity to digest lactose, the main carbohydrate from milk, and it is related to the lactase enzyme deficiency or absence, which is produced in the intestines (Koblitz, 2012). This enzyme is responsible for the lactose molecule brake into monosaccharides allowing its absorption by the intestines (Robert, 2017). For Maurício & Trentinalha (2010), in a high part of the world population, after weaning, there was a gradual or total decline in the lactase enzyme activity hence an observance in the increase in the cases where people are intolerant to this sugar.

In this context people are showing a higher level of interest for healthier food which then leads the food industry to develop products that go beyond the delivery of basic nutrients and palate satisfaction of consumers. Among the food from which health allegations have been disclosed by media and scientific papers published, the green banana biomass stands out (Rabbani et al., 2004; Ranieri & Delani, 2014; Silva et al., 2015b). Brigadeiro, cheese bread, and energy juice are among the products currently developed demonstrating that the green banana biomass has a potential to be applied in different formulations, thus enhancing their nutritional characteristics without loss of sensory acceptance (Marques et al., 2017).

The banana Brazilian production is of 8 million tons a year, although 60% of national crop gets lost before even getting to final consumers, for the fruit presents a very short lifetime and needs to be consumed fast in order to avoid a significant waste. For all of these the use of the green fruit as biomass is an excellent alternative to its usage (Lajolo & Menezes, 2008).

The green banana biomass acts as a powerful thickener having as one of its attributes the tasteless flavor and therefore not altering the taste of food in which it is being added. Not only it increases volume as well as it also adds vitamins B and C and minerals such as potassium and calcium. Aside from its thickening effect, the green banana biomass stands out for the presence of resistant starch, phytosterol and phenolic compounds (Mellor, 1984; Alkarkhi et al., 2011, Sarawong et al., 2014).

In regards of the search for no lactose food and studies about green banana biomass effects, the following study aimed to develop a lactose-free *dulce de leche* with green banana biomassa, as well as identifying its acceptability and buying intentions and characterizing it with respect to its physicochemical and microbiological characteristics.

2 Material and methods

2.1 Green banana biomass elaboration

The green bananas of the caturra variety were cleaned in running water and sanitized with 200 ppm chlorinated water for 15 min. After sanitizing, the bananas were cooked under pressure in a pan for 20 minutes with sufficient water to cover them. After cooked the bananas were peeled and grinded in a blender until homogeneous mass consistency. The adding of 1% of water to this could assist in this process. In addtion, they were stored in polypropylene containers and preserved at -18 °C.

2.2 *Dulce de leche* elaboration

To make the sweets it was used: whole fat milk UHT without lactose from the same batch; green banana biomass; demerara sugar; glucose; sodium PA bicarbonate; and corn starch. Five formulations of *dulce de leche* without lactose were made, from which four had addition of 5, 10, 15 and 20% of green banana biomass and one formulation had the standard adding of 0.5% of commercial starch. The other ingredients were added in the same proportion.

Firstly, the milk was poured into a stainless steel double-walled pan, added 2% of bicarbonate to correct acidity from 18° Dornic to 13° Dornic. In addition, the other ingredients were added, i.e., demerara sugar (20%), corn starch (standard formulation) and green banana biomass according to desired formulation. The ingredients were mixed and concentrated using heating and constant stirring until reaching perfect cooking for the *dulce de leche*. The brix varied from 50 to 58° brix, measured in triplicates with a table refractometer. After reaching desired concentration, 2% of glucose was added to the *dulce de leche* and then it was cooled under stirring until 70 °C, envased in glass containers previously sanitized and stored at room temperature for further analyses.

2.3 Chemical analyses

Moisture, total ash, lipid, and crude protein (N x 6.25) were determined through Association of Official Analytical Chemists (AOAC) methods (Association Offficial Analytical Chemistis, 2005). Titratable acidity, pH, refractometric method (Brix) and water activity (Aw) were determined through Instituto Adolfo Lutz methods (Instituto Adolfo Lutz, 2008). Total carbohydrate was calculated by the difference. Energy values (kilocalories) were obtained by applying factors 4, 9, and 4 for each gram of protein, lipid, and carbohydrate, respectively.

2.4 Physical characteristics of *dulce de leche*

The yield of *dulce de leche* was estimated by difference between the weight results of the *dulce de leche* before and after cooking. Color measurements of *dulce de leche* were carried out using a colorimeter Minolta Chroma Meter CR-400 (Konica Minolta) with D65 illuminant and 10° angle of vision. The mean values for L^* (lightness), a^* (redness to greenness), b^* (yellowness to blueness), chroma [($a^*2+ b^*2)1/2$] and hue angle [tan-1 (b^*/a^*)] were determined according to the CIE Laboratory system. The mean values of the top and bottom surface color of each *dulce de leche* were evaluated. Color parameters were determined in triplicate, and 10 measurements were carried out for each sample.

2.5 Microbiological analyses

The microbiological analyses were developed according to Instructive Norm 62/2003 – MAPA (Brasil, 2003) for 45 °C thermotolerant coliforms, coagulase reactive *Staphylococcus*, *Salmonella sp.*, molds and yeasts. The results obtained were compared to limits of current legislation – *Resolução de Diretoria Colegiada* (RDC) n° 331, December 2019 (Brasil, 2019a) and Normative Instruction no° 60, December 2019 (Brasil, 2019b).

2.6 Consumer acceptance and purchase intent of *dulce de leche*

A total of 52 volunteers, habitual *dulce de leche* consumers (men and women, between 17 and 62 years old), were recruited. Consumer acceptance test was performed according to the methods described by Meilgaard et al. (2007) to evaluate the overall acceptability of *dulce de leche* samples that had been stored for 24 hours in glass packaging room temperature (25 ± 2 °C). A nine-point hedonic scale ranking from "dislike it extremely" to "like it extremely" was used, corresponding to the lowest and highest scores of "1" and "9", respectively. The purchase intent was also evaluated on a five-point scale from "definitely would not buy it" to "definitely would buy it" corresponding to the lower and higher scores of "1" and "5", respectively (Meilgaard et al., 2007).

Protocol number 27312/2017 was approved by Human Research Ethics Committee at the Universsidade Comunitária da Região de Chapecó (UNOCHAPECÓ) and subjects signed an informed consent document prior to participation.

2.7 Statistical analysis.

All analytical determinations were carried out in triplicate. Mean ± SD values were calculated, and the data were subjected to Analysis of Variance (ANOVA). Tukey's test for multiple comparisons of average was performed to determine differences (p <0.05) between treatments.

3 Results and discussion

3.1 Yield of *dulce de leche*

The amounts of *dulce de leche* with and without the addition of different green banana biomass concentration may be observed in Figure 1. There was an increase in yield without lactose after adding the green banana biomass. The standard lactose-free *dulce de leche* had the lowest yield of 39%, and the lactose-free *dulce de leche* in which 20% of green banana biomass had been added demonstrated the highest yield of 74%. This yield increase was observed even when using a high moisture green banana biomass, which possesses a lower level of starch when compared to the biomass flour and commercial starch.

With such yield gain from the addition of green banana biomass, raw material rich in resistant starch, the *dulce de leche* increased its resistant starch concentration and proportionally reduced it sugar and other components levels, which comes to demonstrate that the addition is a great nutritional benefit of a sweet commonly so full of sugar.

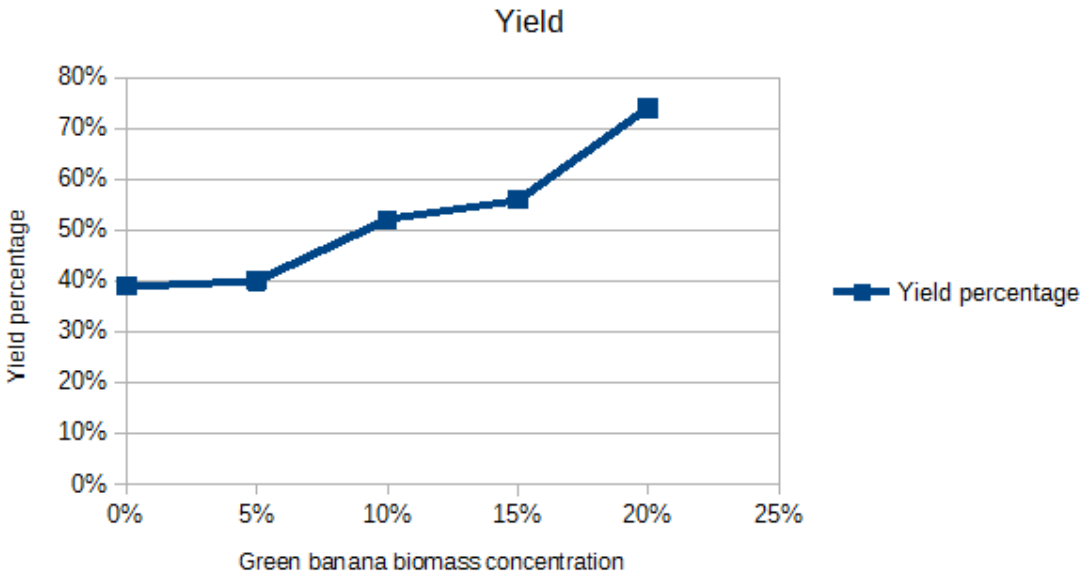

Figure 1. Yield of standard lactose-free *dulce de leche* and of lactose-free *dulce de leche* added of 5%, 10%, 15% and 20% of green banana biomass.

3.2 Physicochemical analysis

Table 1 shows the average obtained to the studied variables. According to Ordinance n° 354 from September 4 of 1997 (Brasil, 1997), creamy *dulce de leche* must contain a 30% of moisture and 2% of ashes (p/p) maximum levels. Minimum level of protein must be of 5% (p/p) and content of fat must be between 6.0% – 9.0% (p/p).

It was observed that all samples showed an elevated moisture level, above the stated by law, indicating that the added green banana biomass rich in resistant starch allowed a more adequate consistency to the *dulce de leche.* This resistant starch capacity to retain water justifies the yield enhancement and higher moisture. Regarding the protein content, lipids and ashes, all samples demonstrated results within legislation standards.

Supplementing the *dulce de leche* with the green banana biomass in rising levels reduced significantly ($p \leq 0.05$) the protein, lipids, carbohydrates and energy content as it can be observed in Table 1. Similar results was obtained by Ferreira et al. (2012) when whey and coffee were added to the *dulce de leche.*

Values of pH and acidity of this study were superior and carbohydrates were similar to the ones found by Gaze et al. (2015), which in its research characterized commercial *dulces de leche* and obtained pH values that varied from 6.14 to 6.37, acidity from 0.23 to 0.50 (lactic acid g/100 g) and carbohydrates values from 43.67 to 61.08 (g/100 g).

Silva et al. (2015a) evaluated the effect of adding five types of modified starch in the creamy *dulce de leche* characteristics and obtained soluble solids values (°Brix), moisture (g/100 g), lipids (g/100 g), protein (g/100 g), ashes (g/100 g) and carbohydrates (g/100 g) that varied respectively from 66.7 to 69.5, 30.12 to 33.74, 6.1 to 7.4, 6.9 to 7.2, 1.74 to 1.80 and from 50.92 to 53.74. The results of this work were similar to these, except the soluble solids, inferring that the green banana biomass had effects similar to the ones from starch in physical-chemical characteristics but can stand out by aggregating higher nutritional and functional value to the product due to the peculiar composition of this ingredient.

Table 1. Soluble solids values (°Brix), pH, acidity, moisture, ashes, lipids, protein, carbohydrates and energy of different samples of standard lactose-free *dulce de leche* and of different added concentrations of green banana biomass lactose-free *dulce de leche.*

	Type of *dulce de leche* (%)				
Analysis	**0**	**5**	**10**	**15**	**20**
°Brix	58 ± 1a	57 ± 1a	55 ± 0b	53 ± 0b	50 ± 0c
pH	8.04 ± 0.03a	7.98 ± 0.01a	7.92 ± 0,04a	7.25 ± 0,01b	7.15 ± 0,01b
Acidity (g/100 g of lactic acid)	6.41 ± 0.0d	7.25 ± 0.0c	7.72 ± 0,04b	7.93 ± 0,01a	8.04 ± 0,03a
Moisture (g/100 g)	21.2 ± 1.25a	23.4 ± 0.60a	27.1 ± 0,35b	31.3 ± 0,57b	35.4 ± 0,25c
Total ash (g/100 g)	1.97 ± 0.01a	1.90 ± 0,02a	1.38 ± 0,01a	1.33 ± 0,01a	1.2 ± 0,01a
Crude protein (g/100 g)	8.5 ± 0.01a	8.0 ± 0,08a	7.5 ± 0,02b	7.2 ± 0,09b	6.8 ± 0,01b
Lipid (g/100 g)	8.0 ± 0.20a	7.7 ± 0,25a	7.0 ± 0,21ab	6.67 ± 0,25b	6.13 ± 0,23b
Carbohydrate[a] (g/100 g)	60.26 ± 1.21a	58.93 ± 0,51a	56,93 ± 0,67a	53,47 ± 0,52b	50,43 ± 0,32b
Energy (kcal/100 g)	347.5 ± 5.3a	337.6 ± 3,65a	321.1 ± 1.39ab	302.7 ± 3.19b	284.1 ± 2b

Mean values ± SD of triplicate determinations; Mean values in the same line followed by different letters are significantly different ($p < 0.05$). [a] By difference.

3.3 Microbiological analyses

Table 2 demonstrates the samples microbiological results. Based in the results, *dulce de leche* samples added of different green banana biomass formulations showed counting < 1,0 × 10^2 CFU/g for coagulase-positive *Staphylococcus*, as well as for mould and yeast, coliforms counting inferior to 3 PWN/g and absence of *Salmonella spp*, attending then food microbiological standards according to RDC n° 12 resolution from *the Agência Nacional de Vigilância Sanitária* (ANVISA) (Brasil, 2001).

Table 2. Microbiological analyses results from standard *dulce de leche* and with different green banana biomass concentration.

Analysis	**Samples with different green banana biomass concentration**				
	0%	**5%**	**10%**	**15%**	**20%**
Coagulase-positive staphylococcus (CFU/g)	< 1x 10^2	< 1x 10^2	< 1x 10^2	< 1x 10^2	< 1x 10^2
Salmonella spp (em 25g)	Absence	Absence	Absence	Absence	Absence
Mold and Yeast (CFU/g)	< 1,0 x 10^2	< 1,0 x 10^2	< 1,0 x 10^2	< 1,0 x 10^2	< 1,0 x 10^2
Total Coliforms (PWN/g)	< 3	< 3	< 3	< 3	< 3

Pieretti et al. (2012) obtained similar results to this study with all samples in accordance with Brazilian legislation (Brasil, 2001). Pereira et al. (2018) have also found *dulce de leche* fit for consumption coming from Argentina and Uruguai, commercialized in border region of Rio Grande do Sul, although the same result does not apply to other analyzed products.

The obtained microbiological results can be explained by the barrier concept. According to Leistner, from Germany, many factors and technics are simultaneously applied to control micro-organisms in food. This practice has been applied in food for over a century (Jay, 2005). Applying the theory to the present study, the combination of the cooking temperature with elevated osmotic pressure by the sugar addition during the *dulce de leche* cooking along with disinfection and the cooking englobing the green banana biomass sterilization, diminishes drastically the possibility for the existence of any live micro-organism in the food.

Besides, recipients boiling process for product storage also reduces the product contamination possibilities by non-sporulating micro-organisms.

The biggest part of pathogens micro-organisms like *Salmonella* sp., positive-coagulase staphylococcus, coliforms and even fungus and yeasts were destroyed by simple food pasteurization (Franco & Landgraf, 2008). According to Timm et al. (2007), mould and yeast provide information on general hygiene conditions of processing, storage and transportation of food working as important indicators of food deterioration.

One of the product contamination possibilities is the environmental, specifically during filling procedures for it is possible to find fungus spores in the air. When such spores are able to carry out secondary contamination for not having local competitors and being the *dulce de leche* a nutritionally rich product, with favorable microbial growth physicochemical characteristics (Table 2), they can then develop and deteriorate the product.

Since the *dulce de leche* is not manipulated by hands, contamination possibility by staphylococcus and coliforms are practically null unless utensils are appropriately sterilized. Hentges et al. (2010) contaminated artificially *dulce de leche* samples with *Salmonella typhimurium*, *listeria monocytogenes*, *Escherichia coli* O157:H7 and *S. aureus* and verified that the pathogens were able to survive in the product for 30 days. Hence the importance of good manipulation practices of food avoiding microbial contamination.

3.4 Sensorial analyses

The results obtained in sensorial analyses of the five lactose-free *dulce de leche* formulation to each sensorial attribute and for the buying intention are presented in Table 3. Regarding acceptance it is observed grades varying from 7.23 to 8.72, that is, above the minimum accepted, six (6).

All attributes evaluated obtained values above the ones found in several similar published works, as the *dulce de leche* added with mascavo sugar (Pieretti et al., 2012), dulce de leche added with commercial starch (Konkel et al., 2004), *dulce de leche* added with whey cheese and coffee (Ferreira et al., 2012).

In attributes of appearance, viscosity, flavor and general impression with the addition of up to 15% of biomass the samples did not show significant difference. When adding 20% of biomass, the *dulce de leche* demonstrated a lower acceptance in regards of other samples in level of 5% significance to all evaluated attributes. To attributes of color and odor, they showed a reduction of its acceptance as the added biomass increased. Thus to mask these two attributes the industry could add natural caramel food coloring.

Nevertheless, it was observed that using green banana biomass not only did not influence in the *dulce de leche* sensorial quality but also enhanced it nutritional quality, then according to Ranieri & Delani (2014), the green banana biomass contains resistant starch quantities that assist in preventing intestine infections and the development of colon inflammatory diseases like diverticulitis and even intestine cancer.

Table 3. Results of acceptance sensorial analyses of lactose-free *dulce de leche* added with different concentrations of green banana biomass and standard lactose-free *dulce de leche.*

Analyzed attributes	Lactose-free *dulce de leche* added with different concentrations of green banana biomass and standard lactose-free *dulce de leche*				
	0%	5%	10%	15%	20%
Appearance*	8,5 ± 0,59^{a}	8,43 ± 0,65^{a}	8,04 ± 1,32^{a}	8,26 ± 0,64^{a}	7,35 ± 1,36^{b}
Color*	8,63 ± 0,51^{a}	8,46 ± 0,72^{ab}	7,96 ± 1,65^{b}	8,22 ± 0,84^{ab}	7,25 ± 1,72^{c}
Odor*	8,48 ± 0,63^{a}	8,35 ± 0,74^{ab}	7,88 ± 1,39^{abc}	7,83 ± 1,07^{bc}	7,52 ± 1,38^{c}
Viscosity*	8,44 ± 0,62^{a}	8,3 ± 0,81^{a}	7,88 ± 1,43^{ab}	8,11 ± 0,90^{a}	7,23 ± 1,62^{b}
Flavor*	8,72 ± 0,42^{a}	8,48 ± 0,67^{a}	8,31 ± 1,19^{a}	8,45 ± 0,77^{a}	7,48 ± 1,53^{b}
General impression*	8,71 ± 0,41^{a}	8,42 ± 0,62^{a}	8,3 ± 0,97^{a}	8,24 ± 0,76^{a}	7,56 ± 1,39^{b}
Buying intention**	4,89 ± 0,31^{a}	4,54 ± 0,81^{ab}	4,29 ± 1,89^{b}	4,66 ± 0,58^{ab}	3,63 ± 1,12^{c}

Average values ± SD of determinations by 52 judges; The average values in the same line, followed by distinct letters are significantly different ($p \leq 0,05$) according to Tukey's test. *Attributes evaluated for acceptability using hedonic scale of 9 points. ** Buying intention evaluated using scale of 5 points.

As it can be observed in Table 3, all *dulce de leche* samples demonstrated high level of buying intention. Except for the 20% addition of green banana biomass, all samples were classified between four (4) ("possibly would buy it") and five (5) ("would definitely buy it") which meets results verified in regards of acceptability.

4 Conclusion

All lactose-free *dulce de leche* samples were accepted by tasters independently of added biomass quantity. Therefore, the following study has proven that green banana biomass can be used to enrich the nutritional value of *dulce de leche* when compared to traditional ones as well as demonstrating a yield and hence showing itself as an alternative for the industry.

It is suggested for future studies the evaluation of these *dulce de leche* stability through the evaluation of its shelf-life as well as the use of higher proportions of green banana biomass.

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