Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2563
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v39i4.29396

# Storage time effect on 'Dulce de leche' characteristics with coffee and whey

**Larissa de Oliveira Ferreira Rocha[1*], Sandra Maria Pinto[2], Luiz Ronaldo de Abreu[2] and Carlos José Pimenta[2]**

*[1]Programa de Pós-Graduação em Ciência dos Alimentos, Universidade Federal de Lavras, Campus Universitário, Cx. Postal 3037, 37200-000, Lavras, Minas Gerais, Brazil, [2]Departamento de Ciência dos Alimentos, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. *Author for correspondence. E-mail: larioliv@hotmail.com*

**ABSTRACT.** The 'Dulce de leche' (milk-based caramel sauce) is a product that has good chemical and microbiological stability, but low physical stability. Physical stability refers to the maintenance of fine texture, without the appearance of 'grittiness' given by large lactose crystals which are slowly formed during storage, causing great inconvenience in the product preservation. This work was carried out with the objective to verify the storage time effect on physical, physicochemical microbiological and sensory characteristics of the 'Dulce de leche' with coffee added whey and modified starch. It was found that the storage time has a significant effect on color variables b* and L* and in parameters texture (hardness, adhesiveness, elasticity, cohesiveness and gumminess). There was no formation of lactose crystals and microbiological quality was maintained throughout 'Dulce de leche' storage time. The time did not influence in the 'Dulce de leche' acceptance in relation to flavor, texture and overall impression, which shows that variations of color and instrumental texture did not affect the product sensory quality, providing an alternative for the use of whey and coffee in 'Dulce de leche' production, thus obtaining a new product.

**Keywords:** microbiological quality, sensory quality, crystallization.

## Efeito do tempo de armazenamento nas características do doce de leite com café e soro

**RESUMO.** O doce de leite é um produto que apresenta boa estabilidade química e microbiológica, porém baixa estabilidade física. A estabilidade física refere-se à manutenção da textura fina, sem aparecimento de 'arenosidade' dada por grandes cristais de lactose que se formam lentamente durante o armazenamento, sendo essa a grande dificuldade para a conservação do produto. Este trabalho foi realizado com o objetivo de verificar o efeito do tempo de armazenamento nas características físicas, físico-químicas, microbiológicas e sensoriais de doce de leite com café com adição de soro e amido modificado. Verificou-se que o tempo de armazenamento exerceu efeito significativo nas variáveis de cor b* e L* e nos parâmetros de textura (dureza, adesividade, elasticidade, coesividade e gomosidade). Não houve formação de cristais de lactose e a qualidade microbiológica foi mantida durante todo o período de estocagem do doce. O tempo não influenciou na aceitação do doce em relação ao sabor, textura e impressão global, o que demonstra que as variações de cor e textura instrumentais não comprometeram a qualidade sensorial do produto, constituindo uma alternativa de utilização do soro de leite e do café na fabricação do doce de leite, obtendo assim um novo produto.

**Palavras-chave:** qualidade microbiológica, qualidade sensorial, cristalização.

## Introduction

The 'Dulce de leche' is a dairy product similar to sweetened condensed milk, which is known in some South American countries such as Argentina and Uruguay (Giménez, Ares, & Gámbaro, 2008). Mainly consumed as a sweet or spread as jelly, it is named 'Dulce de leche' in Argentina and Uruguay, 'arequipe' in Colombia, 'manjar blanco' in Peru, and 'doce de leite' in Brazil. It is prepared boiling whole milk, added sucrose until reaching about 70% of total soluble solids (Ranalli, Andrés, & Califano, 2012). The sodium bicarbonate is added to avoid the casein coagulation, and favor the Maillard reaction, responsible for its typical brown color (Giménez et al., 2008).

The 'Dulce de leche' is a product that has good chemical and microbiological stability but low physical stability due to the crystallization of both lactose as well as added sugars, which may give the product a sandy texture (Silva et al., 2015). The 'Dulce de leche' industries in Brazil have been partially replacing the milk with whey in the manufacturing process of sweet milk. Although the

addition of whey is not forbidden, since the Brazilian legislation classifies it as dairy ingredient, its use should be declared to avoid economic frauds (Machado & Viotto, 2007).

The whey is a by-product mainly obtained by means of the manufacturing of cheese or casein. It comes in liquid form and yellow-green color (Carvalho, Prazeres, & Rivas, 2013). It contains more that the half of solids found in the milk, including whey proteins, about 20% of total protein, and the majority of lactose, minerals and water-soluble vitamins (Baldasso, Barros, & Tessaro, 2011). One of the major problems faced in the dairy sector worldwide is the destination given to the whey from cheese manufacturing, by being an effluent that shows high quality of organic matter. However, new uses of whey are ancient needs of dairy products, because it may work within the requirements of the environmental protection agencies.

In addition, the use of whey in the 'Dulce de leche' manufacturing technology increases the content of lactose in the sweet, what increases the tendency for appearance of lactose crystals perceptible in the taste. However, the control of crystallization is the major problem in the manufacturing process of 'Dulce de leche'. To solve this problem, it has been proposed the reduction of concentration of lactose by the addition of β-galactosidase (lactase) (Klein, Jong, & Révillion, 2010), forced or induced crystallization (Martínez, Hough, & Contarini, 1990) or the increase of product viscosity (Perrone; Costa Júnior, & Magalhães, 2008).

The use of native or modified corn starched in its manufacture began to be allowed by Brazilian law in 1997, but within a limit of 0.5 g 100 $mL^{-1}$. Its use can contribute to the consistency and product yield as a function of water retention provided, and assist in controlling the crystallization of lactose (Silva et al., 2015).

Different coffee constituents have been suggested as potentially chemo-protectors in different chemical and biological systems. Caffeine is the most popular due to its physiological and pharmacological properties. Chlorogenic acids are the main and most abundant phenolic compounds with antioxidant properties in coffee (Monteiro & Trugo, 2005). The trigonelline, compound found in green coffee bean is a precursor to niacin in the roasting process (Nogueira & Trugo, 2003).

Although there are several possibilities of use of whey, only part of the generated whey is used due to the high cost and processing difficulty. Knowing that the concentration of a mixture of milk, whey and sugar makes possible the obtainment of 'Dulce de leche' similar to traditional one, and new products based on coffee have been used, due to their great acceptance and popularization, this work was carried out with the objective to verify the storage time effect on physical, physicochemical, microbiological and sensory characteristics of the 'Dulce de leche' with coffee added whey. In addition, it was sought to manufacture a differentiated product, with coffee flavor, and sustainable by means of whey.

## Material and methods

### Raw material

We used the following ingredients to manufacture sweet milk: pasteurized whole milk, liquid whey from the fresh cheese manufacturing, sugar, sodium bicarbonate, modified maize starch (Amidogen 8500) supplied by Gemacom Tech®, sodium citrate, soluble extra strong coffee, and potassium sorbate.

### Manufacturing of 'Dulce de leche'

The manufacturing of 'Dulce de leche' was performed at Pilot Plant of Products Processing of the Department of Food Sciences of the Federal University of Lavras. Eleven formulations (tests) of the sweet were obtained (Table 1). The equipment used to this purpose consisted of an open pan (Macanuda®, Joinville, Santa Catarina) with rotary vertical shovel and working capacity about 20 liters of milk.

**Table 1.** Formulation of 'Dulce de leche' using the central composite rotational design ($2^2$) with 2 independent variables, 3 replicates at the central point (c), and 4 axial points.

| Tests | Variables coded | | Real variables | |
|---|---|---|---|---|
| | $x_1$[1] | $x_2$[2] | $X_1$ (%) | $X_2$ (%) |
| 1 | -1 | -1 | 15 | 0.5 |
| 2 | +1 | -1 | 45 | 0.5 |
| 3 | -1 | +1 | 15 | 1 |
| 4 | +1 | +1 | 45 | 1 |
| 5 | -1.41 | 0 | 8.85 | 0.75 |
| 6 | +1.41 | 0 | 51.15 | 0.75 |
| 7 | 0 | -1.41 | 30 | 0.397 |
| 8 | 0 | +1.41 | 30 | 1.10 |
| 9 | 0 | 0 | 30 | 0.75 |
| 10 | 0 | 0 | 30 | 0.75 |
| 11 | 0 | 0 | 30 | 0.75 |

[1]$x_1$: % of substitution of milk by whey; [2]$x_2$:% of starch addition.

Milk and whey were firstly placed in the pan in the right proportion. Then, we added bicarbonate to correct the acidity for 1 and 0.8 g of lactic acid $L^{-1}$ of milk and whey, respectively. The mixture (milk + whey) was placed in the equipment, and after heating it, sugar (20%), sodium citrate (0.08%), and starch (Table 1) was added. The different

formulations of 'Dulce de leche' were cooked until they reached a soluble solid level of ±70%. After reaching the end point was added potassium sorbate (0.02%) and the instant coffee, the coffee was previously diluted in hot water, was added at a ratio of 1:1 (coffee:water). Coffee, sugar, sodium citrate, and starch were added according to the desired amount of mixture (milk + whey). After the addition of coffee, the 'Dulce de leche' was bottled in glass jars and stored in cardboard boxes for the analyses.

### Selection of 'Dulce de leche' using sensory analysis

The sample selection for evaluation effect of time on the 'Dulce de leche' characteristics was performed using acceptance test. The sensory analysis was performed by 60 consumers among them students and office staff aged between 18 and 40 years. About 10 g of each formulation was provided in disposable cups coded using three-digit numbers. The 11 samples were presented in the balanced way, according to Wakeling and MacFie (1995) in two sessions, where the same consumers tasted five samples in the first moment and six in the second. At the end of the testing process, water-salt cookies were given to cleansing the flavor between tests of samples. The test was performed in the afternoon, at the Laboratory of Sensory Analysis at Federal University of Lavras, in individual cabins and white light. A nine-point structured hedonic scale (1 = disliked and 9 = liked extremely) was used in the acceptance test of 'Dulce de leche' for the evaluation of color, taste, texture, and global impression attributes according to Stone and Sidel (1993).

### Composition of 'Dulce de leche' selected

'Dulce de leche' composition was evaluated in triplicate, performed immediately after production. The moisture was determined according to the methodology suggested by the Association of Official Analitical Chemistry (AOAC, 2006). The sample was first diluted at a ratio of 20 g until the volume of 100 mL of warm water for lipid determination. Soon after, there was fat extraction using Gerber lactobutyrometer for milk, according to the official methodology for determination of lipids in fluid milk (Brasil, 2006). Proteins were quantified by the Kjeldahl method as described by AOAC (2006). The ash content was determined according to the technique described by AOAC (2006). Carbohydrates were determined by difference, according to Association of Official Analitical Chemistry (AOAC, 2006).

### Evaluation of the effect of storage time on 'Dulce de leche' characteristics

To evaluate the time effect on 'Dulce de leche' characteristics were held water activity analysis ($a_w$), color evaluation, texture, crystallization, microbiological and sensorial analysis, as methodologies described below.

Water activity ($a_w$): the 'Dulce de leche' water activity was determined using AquaLab equipment (Decagon model 3 TE), with readings in controlled temperature at 25 ± 0.3°C.

Color evaluation: the L★, a★ and b★ values were determined by Minolta CR 400 colorimeter, working with $D_{65}$ (daylight) and using the CIElab standards.

Texture evaluation: the texture profile analysis (TPA) was held in texturometer Stable Micro Systems, TAXT2i model. The parameters analyzed were: hardness, adhesiveness, elasticity, cohesiveness and gumminess. The samples were evaluated in triplicate, in the 'Dulce de leche' jar. Pre-test speed: 2.0 mm $s^{-1}$; Test speed: 1.0 mm; Post-test speed: 2.0 mm $s^{-1}$; distance: 10.0 mm; time: 5.0 s; Contact force: 5.0 g; probe: acrylic cylinder 20.0 mm (P20).

Microbiological analysis: for the microbiological quality of the product, we carried out analysis for total coliforms at 35°C, and thermotolerant coliforms at 45°C, fungi, yeasts and positive *Staphylococcus coagulase* and *Salmonella* sp., as outlined in the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant & Splittstoesser, 1992).

Crystallization: the analysis of lactose crystallization was carried out by means of microscopy evaluation using the method proposed by Hough, Martínez, and Contarini (1990). The sample of sweet milk, about 0.003 g, was weighted on a slide. A cover slip was placed on the sample. With the support of other cover slip, a pressure was exerted on the slide containing the sweet, seeking to form a thin and circular layer about 10 mm diameter. The slide was evaluated in polarized light microscopy; model 49901-35 PAL COLOR with the 10x and 40x objectives. To estimate the number of crystals, pictures of 10 microscopic fields were randomly taken using a Meiji brand camera. The number and amount of lactose crystals present in the sweet was assessed for 180 storage days.

Sensory analysis: the sensory analysis was performed by 50 consumers among students and office staff aged between 18 and 40 years. The analysis was performed under the same conditions of the optimization process. Mean scores were recorded throughout the 180 days.

### Experimental design and statistical analysis

For the selection of the 'Dulce de leche' a central composite design was used, $2^2$ full factorial (level ± 1) with the addition of 3 central points (level 0) and axial points (level ± 1.41), according to the Table 1. The data relating to the samples acceptance were evaluated by variance analysis (ANOVA) followed by average tests (Tukey, $p \leq 0.05$).

To estimate the effect of storage time in the characteristics of the 'Dulce de leche' with coffee and whey the randomized block design with one treatment and three blocks was used Each block consisted of one panful of 'Dulce de leche'. The results were submitted to Variance Analysis (ANOVA), when significant, were fitted regression models to assess the time effect.

Data processing was carried out using the Statistic for Windows 5.0 (Statistical Analysis and Data Mining Software, 1995).

This study was accepted by the Committee of Ethics of the President Antônio Carlos University (nr 670/2010) located in Barbacena, State of Minas Gerais, Brazil.

## Results and discussion

### Selection of the 'Dulce de leche' using sensory analysis

The averages for the sensorial attributes assessed are between 7 and 8 (Table 2), which correspond, on the hedonic scale, to scores corresponding to 'moderately liked' and 'liked very much', respectively; and indicate a good product acceptance.

**Table 2.** Average scores assigned by the panelists for color, taste, texture, and global impression.

| Tests | Color | Texture | Taste | Global impression |
|---|---|---|---|---|
| 1 | 7.62 a | 7.10bc | 7.50 a | 7.34bc |
| 2 | 7.64 a | 7.72ab | 7.34 a | 7.40abc |
| 3 | 7.86 a | 7.78ab | 7.72 a | 7.72abc |
| 4 | 7.96 a | 7.9a | 7.96 a | 7.98a |
| 5 | 7.68 a | 7.80ab | 7.82 a | 7.86ab |
| 6 | 8.00 a | 7.78 a | 7.84 a | 7.86ab |
| 7 | 7.84 a | 7.68ab | 7.48 a | 7.72abc |
| 8 | 7.50 a | 6.86c | 7.46 a | 7.18c |
| 9 | 7.93 a | 7.64ab | 7.74 a | 7.73abc |
| CV% | 11.91 | 14.42 | 14.07 | 12.93 |

Means in columns followed by same letter do not differ statistically ($p < 0.05$). CV% = coefficient of variation.

There was no significant difference ($p \geq 0.05$) among samples as to the attributes color and taste. There was a significant difference ($p < 0.05$) among samples in relation to the texture and overall impression, and the sample 8 obtained the lowest average, being considered the least preferred sample in relation to this attribute. Sample 4 had the highest average as the overall impression, which is the preferred sample in relation to this sensory attribute. Once sample 4 has displayed the highest average in relation to the overall impression of the product, this sample was selected for time effect evaluation step on 'Dulce de leche' characteristics.

### Composition of 'Dulce de leche' selected

In Table 3 the composition results of 'Dulce de leche' is shown.

**Table 3.** Centesimal Composition of 'Dulce de leche'.

| Compound | DLCW[1] | Standard[2] |
|---|---|---|
| Moisture (%) | 32 | max. 30% |
| Protein (%) | 6.36 | min. 5% |
| Fat (%) | 4.45 | 6 – 9% |
| Ash (%) | 1.69 | max. 2% |
| Carbohydrates (%) | 87.51 | - |

[1]DLCW: 'Dulce de leche' with coffee and whey; [2]Decree nº 354 - September 4, 1997 (Brasil, 1997).

It was observed that (Table 3) in relation to the fat content, the 'Dulce de leche' showed content below the limit established by Decree nº 354 - September 4, 1997 (Brasil, 1997), this was due to the high whey concentration, since the whey (0.3%) has a low fat content compared to milk (3.5%) used in the study. It was observed that the 'Dulce de leche' showed a reduction of 25% in fat content when compared with the minimum requirements of Decree nº 354 de 1997 (Table 3), which can be called as light or reduced in fat. According to RDC nº 54 - November 12, 2012, a product to be considered light in relation to fat content must have a minimum 25% reduction in the total fat content compared to conventional product (Brasil, 2012).

The 'Dulce de leche' presented moisture content above the maximum requirements established by law (Table 3) consequently this higher moisture content is due to water retention by the starch. Konkel, Oliveira, Simões, and Demiate (2004) stated that in candies made with excess of starch, there was excess moisture.

Ferreira, Pereira, Maria, and Pinto (2012) evaluated the replacing effect of milk by whey and the effect of coffee addition on 'Dulce de leche' composition. According to the authors, the increase of whey concentration increased the moisture content and decreased protein and fat content, which corroborates to the obtained results. The addition of coffee altered only the moisture, being smaller as higher the coffee concentration.

### Storage time effect

In Table 4 is shown the p values for the variables water activity, color (L, $a^\star$, $b^\star$) and texture (hardness, adhesiveness, elasticity, cohesiveness and gumminess) of 'Dulce de leche' during storage.

**Table 4.** p values for the variables water activity, color (L, a★, b★) and texture (hardness, adhesiveness, elasticity, cohesiveness and gumminess) of 'Dulce de leche' during storage.

| Variables | Average | p value |
|---|---|---|
| Water activity | 0.86 | 0.0572 |
| L★ | 30.16 | 0.0162* |
| a★ | 10.38 | 0.0924 |
| b★ | 13.22 | 0.0147* |
| Hardness (g) | 130.8 | 0.0000** |
| Adhesiveness (g $s^{-1}$) | -366.7 | 0.0000** |
| Elasticity | 0.93 | 0.0217* |
| Cohesiveness | 0.64 | 0.0005** |
| Gumminess | 82.30 | 0.0000** |

**Significant at the 1% level for the F test *Significant at the 5% level for the F test.

It is observed in Table 4 that the storage time did not influence water activity ($a_w$) and value a★. Water activity is a factor which directly affects the food characteristics and its stability, since expressed water content lying in the 'free state' causes transformations or growth of microorganisms (Gonçalves et al., 2014). Most microorganisms that cause deterioration have difficulty to develop in products with $a_w$ below 0.90. The growth of yeast and fungi ends in $a_w$ below 0.85 and 0.70, respectively (Correia-Oliveira et al., 2008). The average water activity displayed during the storage time was 0.86, a value which contributes to the product conservation.

The storage time has a significant effect on L★, b★ value, hardness, adhesiveness, elasticity, cohesiveness and gumminess (Table 4). The adjusted model with the respective coefficient of determination ($R^2$) to the results of these variables can be found in Figures 1 and 2.

The storage time only influenced components L★ and b★, which are the components that characterize 'Dulce de leche' color. The L value expresses the luminosity and clarity of the sample, as closer to 100 in most of the samples. Analyzing the average values of L★ (30.16) during storage (Figure 1), it can be said that the 'Dulce de leche' presented dark color.

The component b★ ranges from yellow (+) to blue (-). Like all values of b★ (average value 13.22) were positive, it means that the 'Dulce de leche' had only wavelength reflection associated to yellow color. The positive values found for the color coordinates a★ and b★ indicate that the 'Dulce de leche' is a product with a tendency to red and yellow, with a predominance of the yellow, due to higher b★ values found in all samples.

Ferreira et al. (2012) evaluated the color of different brands of doughy 'Dulce de leche'. According to the authors, the average values obtained for the L parameter (from 53.41 to 59.60), was above the value found in the study (30.16). In relation to the yellow component intensity (b★), the values obtained (17.28 to 28.08) were below to the observed value. Therefore, it is observed that coffee influenced in 'Dulce de leche' color.

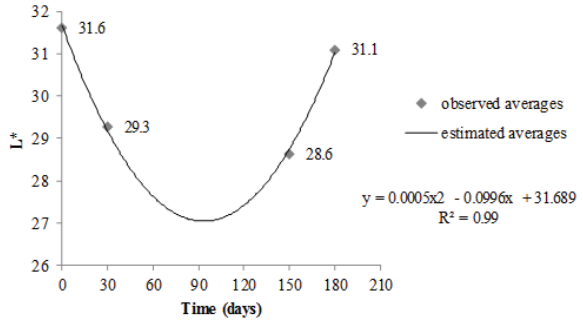


**Figure 1.** Average value of L★ during 'Dulce de leche' storage time.

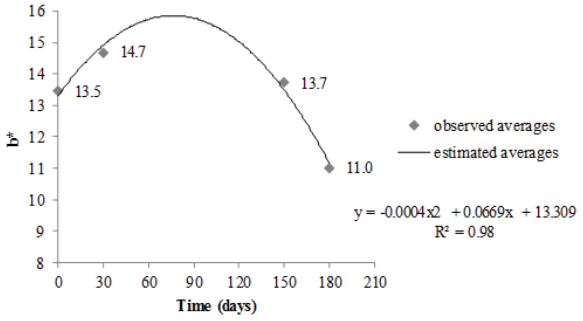


**Figure 2**. Average value of b★ during 'Dulce de leche' storage time.

Gaze et al. (2015) evaluated the color of Brazilian commercial brands of 'Dulce de leche'. According to authors, the average values ranged from 48.59 to 62.11 for L parameter, and from 13.60 to 16.90 for color coordinate a★, and from17.89 to 27.70 for color coordinate b★.

However, even in traditional 'Dulce de leche', there is a marked difference in color. Color represents a regional pattern. Possibly for this reason, differences between the results of the work are noticeable.

It is observed in Figure 3, 4, 5 and 6 the intensity of hardness, adhesiveness, elasticity and gumminess during 'Dulce de leche' storage time.

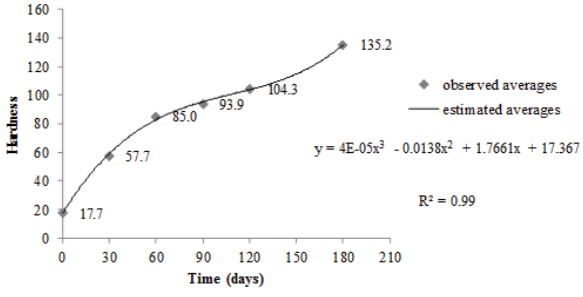


**Figure 3.** Average value of hardness during 'Dulce de leche' storage time.

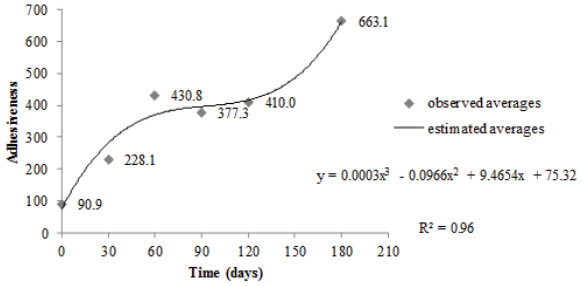


**Figure 4.** Average value of adhesiveness during 'Dulce de leche' storage time.

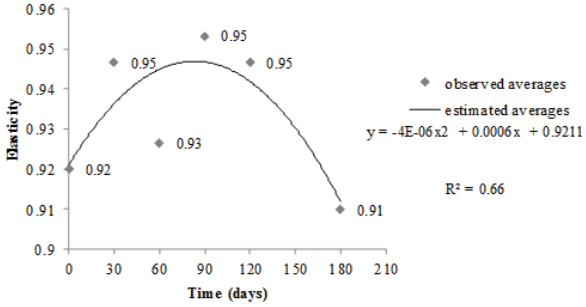


**Figure 5.** Average value of elasticity during 'Dulce de leche' storage time.

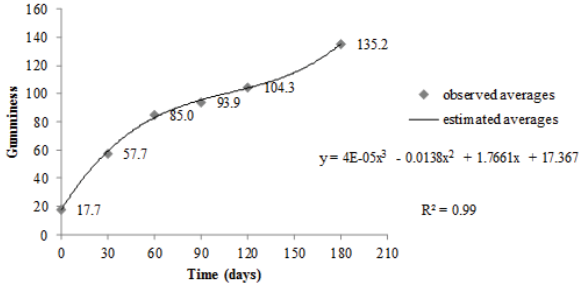


**Figure 6.** Average value of gumminess during 'Dulce de leche' storage time.

The storage time caused a cohesiveness reduction (Figure 7) which passes from 0.72 to 0.67.

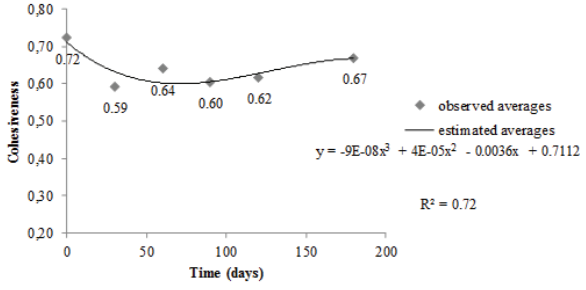


**Figure 7.** Average value of cohesiveness during 'Dulce de leche' storage time.

Ferreira et al. (2012) evaluated the characteristics of quality in commercial 'Dulce de leche'. It differed widely in texture and found values ranging from 66.10 to 206.46 in hardness, -121.99 to 418.34 in adhesiveness, 0.87 to 0.89 in elasticity, 0.58 to 0.69 in cohesiveness and 43.58 to 120.12 in gumminess. According to the authors the hardness and gumminess are important parameters in choosing the product, because the consumers preferred the less gummy and softer samples.

The average values from 130.8 for hardness, -366.7 for adhesiveness, 0.93 for elasticity, 0.64 for cohesiveness and 82.30 for gumminess, these values are different from those found 297.5 – 473.7, 20.54 – 36.28, 18.08 – 19.45, 0.83 – 0.94, 255.9 – 431.1, respectively, commercial samples of 'Dulce de Leche' by Silva et al. (2015).

In Table 5 are presented the criteria and the results obtained for count *Staphylococcus coagulase* positive, fungi and yeasts, total coliforms and *Salmonella sp*.

The results obtained for the positive *Staphylococcus coagulase*, fungi, yeasts, total coliforms and *Salmonella* sp. (Table 5) showed that samples of 'Dulce de leche' were according to what is outlined in the 354 Decree of 4th September 1997 (Brasil, 1997) and in the RDC 12 of 2nd January 2001 (Brasil, 2001), indicating that the whole process, including bottling, was performed in hygienic conditions.

**Table 5.** Microbiological pattern of 'Dulce de leche'during the storage period.

| Standard | Positive *Staphylococcus* coagulase (CFU $g^{-1}$) [1] | Fungi and yeasts (CFU $g^{-1}$)[1] | Total coliforms (MPN $g^{-1}$)[2] | *Salmonella* sp. (CFU $g^{-1}$)[1] |
|---|---|---|---|---|
| | $1 \times 10^2$ | $1 \times 10^2$ | $5 \times 10^1$ | Absence |
| Time (days) | | | | |
| 0 | $<1x10^1$ | $<1x10^1$ | <3,0 | Absence |
| 30 | $<1x10^1$ | $<1x10^1$ | <3,0 | Absence |
| 60 | $<1x10^1$ | $<1x10^1$ | <3,0 | Absence |
| 90 | $<1x10^1$ | $<1x10^1$ | <3,0 | Absence |
| 120 | $<1x10^1$ | $<1x10^1$ | <3,0 | Absence |
| 150 | $<1x10^1$ | $<1x10^1$ | <3,0 | Absence |
| 180 | $<1x10^1$ | $<1x10^1$ | <3,0 | Absence |

[1]CFU: colony forming units. [2]MPN: most probable number.

Madrona, Zotarelli, and Bergamasco (2008) analyzed the effect of the addition of whey in the microbiological quality of 'Dulce de leche' doughy. They did not found the presence of fungi, yeasts and positive *Staphylococcus coagulase* in all samples; which is similar to results obtained in this study.

Timm et al. (2007) studied the microbiological quality of 28 samples of 'Dulce de leche' fractioned into portions to be sold by retail in supermarkets of Pelotas, Brazil, and isolated *Salmonella* from one sample. All analyzed samples showed coagulase-positive *Staphylococcus* count <10 CFU $g^{-1}$. However, only one sample showed mold and yeast count with acceptable values by Brazilian Norms and all the others showed count $>1.0 \times 10^2$ CFU $g^{-1}$.

These authors consider that the common practice of opening the 'Dulce de leche' containers in retail markets to sell it in small amounts increases the hazard of contamination and transmission of undesirable microorganisms to consumers.

The storage time did not affect the acceptance of 'Dulce de leche', i.e., there was no significant difference ($p \geq 0.05$) in relation to taste, texture, and overall impression during all months (Table 6).

The scores obtained in the acceptance test were greater than six and, from this score, there is reference to 'like the product', however, it is not possible to establish its rejection before this period.

**Table 6.** p values for the variables taste, texture and overall impression.

| Variables | Average | p valor |
|---|---|---|
| Taste | 8.13 | 0.1136 |
| Texture | 8.22 | 0.4600 |
| Overall impression | 8.15 | 0.1037 |

The mean score for all parameters was greater than eight, which corresponds to the category 'liked very much' in the hedonic scale. Therefore, regardless the time, the sweet showed a great acceptance.

Microscopy analysis carried out for six storage months did not show presence of lactose crystals. Similar results were observed by Silva et al. (2015). Machado and Viotto (2007) demonstrated that the addition of 0.75% of modified starch and 45% of whey provided the obtainment of sweet with no lactose crystals until the ninth month of storage. According to Perrone et al. (2008) the use of starch in the 'Dulce de leche' manufacturing increases viscosity of the sweet, preventing the crystallization.

## Conclusion

It was found that the storage time has a significant effect on color variables $b^\star$ and $L^\star$ and in parameters texture (hardness, adhesiveness, cohesiveness and gumminess). There was no formation of lactose crystals and microbiological quality was maintained throughout storage time. The time did not influence in the 'Dulce de Leche' acceptance in relation to flavor, texture and overall impression, which shows that variations of color and instrumental texture did not affect the product sensory quality, providing an alternative for the use of whey and coffee in its production, thus obtaining a new product.

## Acknowledgements

The authors acknowledge the fellowship provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and are grateful for the financial support provided by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig).

## References

Association of Official Analitical Chemistry. (2006). *Official methods of analysis of the association of analitical chemists.* Arlington, TX: AOAC International.

Baldasso, C., Barros, T. C., & Tessaro, I. C. (2011). Concentration and purification of whey proteins by ultrafiltration. *Desalination, 278*(1-3), 381-386.

Brasil. Agência Nacional de Vigilância Sanitária. (2012). Resolução RDC n. 54, de 12 de novembro de 2012. Regulamento técnico sobre informação nutricional complementar. *Diário Oficial da União*, Brasília, DF.

Brasil. Agência Nacional de Vigilância Sanitária. (2001). Resolução RDC nº 12, de 2 de janeiro de 2001. Dispõe sobre o regulamento técnico sobre padrões microbiológicos para alimentos. *Diário Oficial da União*, Brasília, DF.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. (2006). Instrução Normativa nº 68. Métodos analíticos oficiais físico-químicos, para controle de leite e produtos lácteos. *Diário Oficial da União*, Brasília, DF.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. (1997). Portaria n. 354, de 4 de setembro de 1997. Regulamento técnico Mercosul para fixação de identidade e qualidade de doce de leite. *Diário Oficial da União*, Brasília, DF.

Carvalho, F., Prazeres, A. R., & Rivas, J. (2013, February). Cheese whey wastewater: Characterization and treatment. *Science of the Total Environment, 445-446*, 385-396.

Correia-Oliveira, M. E., Ferreira, A. F., Poderoso, J. C. M., Lessa, A. C. V., Araújo, E. D., Carnelossi, M. A. G., & Ribeiro, G. T. (2008). Atividade de Água (Aw) em amostras de pólen apícola desidratado e mel do estado de Sergipe. *Revista da Fapese, 4*(2), 27-36.

Ferreira, L. O., Pereira, P. A. P., Maria, J., & Pinto, S. M. (2012). Avaliação das características de qualidade de doces de leite comerciais. *Revista do Instituto de Laticínios Cândido Tostes, 387*(67), 5-11.

Gaze, L. V., Costa, M. P., Monteiro, M. L. G., Lavorato, J. A. A., Conte Júnior, C. A., Raices, R. S. L, ... Freitas, M. Q. (2015, February). Dulce de leche, a typical product of Latin America: Characterisation by physicochemical, optical and instrumental methods. *Food Chemistry, 169*, 471-477.

Giménez, A., Ares, G., & Gámbaro, A. (2008). Consumer reaction to changes in sensory profile of dulce de leche due to lactose hydrolysis. *International Dairy Journal, 18*(9), 951-955.

Gonçalves, G. R. F., Oliveira, J. S., Bonomo, R. C. F., Melo Neto, A., Leite, C. X. S., & Gomes, G. M. S. (2014). Caracterização físico-química do doce de leite em barra com diferentes concentrações de líquor de

cacau. *Revista Brasileira de Produtos Agroindustriais, 16*(1), 27-32.

Hough, G., Martínez, E., & Contarini, A. (1990). Sensory and objective measurement of sandiness in Dulce de Leche, a typical argentine dairy product. *Journal of Dairy Science*, 73(3), 604-611.

Klein, M. P., Jong, E. V., & Révillion, J. P. P. (2010). Utilização da β-galactosidase para prevenção da cristalização em doce de leite. *Ciência e Agrotecnologia, 34*(6), 1530-1535.

Konkel, F. E., Oliveira, S. M. R., Simões, D. R. S., & Demiate, I. M. (2004). Avaliação sensorial de doce de leite pastoso com diferentes concentrações de amido. *Ciência e Tecnologia de Alimentos, 24*(2), 249-254.

Machado, L. M. P., & Viotto, W. H. (2007). Estudo sobre a cristalização da lactose em doce de leite pastoso elaborado com diferentes concentrações de soro de queijo e amido de milho modificado. *Revista Ciência e Cultura, 2*(2), 69-74.

Madrona, G. S., Zotarelli, M. F., & Bergamasco, R. (2008). Estudo do efeito da adição de soro de queijo na qualidade microbiológica do doce de leite pastoso. *Revista Brasileira de Tecnologia Agroindustrial, 2*(1), 81-86.

Martínez, E., Hough, G., & Contarini, A. (1990). Sandiness prevention in dulce de leche by seeding with lactose microcrystals. *Journal of Dairy Science, 73*(3), 612-616.

Monteiro, M. C., & Trugo, L. C. (2005). Determinação de compostos bioativos em amostras de café torrado. *Química Nova, 28*(4), 637-641.

Nogueira, M., & Trugo, L. C. (2003). Distribuição de isômeros de ácido clorogênico e teores de cafeína e trigonelina em cafés solúveis brasileiros. *Ciência e Tecnologia de Alimentos, 23*(2), 296-299.

Perrone, I. T., Costa Júnior, L. C. G, & Magalhães, F. A. R. (2008). Características tecnológicas, de rendimento e cristalização em doce de leite obtido de mistura de leite e soro de fabricação de queijo. *Revista do Instituto de Laticínios Cândido Tostes, 62*(354), 43-49.

Ranalli, N., Andrés, S. C., & Califano, A. N. (2012). Physicochemical and rheological characterization of 'Dulce de leche'. *Journal of Texture Studies, 43*(2), 115-123.

Silva, F. L., Ferreira, H. A. L., Souza, A. B., Almeida, D. F., Stephani, R., Pirozi, M. R., ... Perrone, I. T. (2015). Production of Dulce de leche: the effect of starch addition. *Food Science and Technology, 62*(1), 417-423.

Statistical Analysis and Data Mining Software. (1995). *Statistica for Windows: versão 5.0*. Retrieved from www.statsoft.com

Stone, H. S., & Sidel, J. L. (1993). *Sensory evaluation practices*. San Diego, CA: Academic.

Timm, C. D., Conceição, R. C. S., Coelho, F. J. O., Roos, T. B., Tejada, T. S., Quevedo, P. S., ... Brasil, N. D. A. (2007). Avaliação microbiológica de doce de leite pastoso. *Revista do Instituto Adolfo Lutz, 66*(3), 275-277.

Vanderzant, C., & Splittstoesser, D. F. (1992). *Compendium of methods for microbiological examination of foods* (3rd ed.). Washington, DC: American Public Health Association.

Wakeling, I. N., & MacFie, H. J. H. (1995). Designing consumer trials balanced for first and higher orders of carry-over effect when only a subset of *k* samples from *t* may be tested. *Food Quality and Preference, 6*(4), 299-308.

*Received on October 7, 2015.*
*Accepted on July 6, 2016.*