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Characterization of Lactose-Free Dulce de Leche

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ABSTRACT

The aim of the study is to be determine the effect of lactose hydrolysis and sugar content on physicochemical properties, sensory profile and HMF (5-hydroxymethylfurfural) content in Dulce de Leche (DL). Lactose free (lactose-hydrolysed) dairy products as well as low sugar products have been developed to supply consumer demand. Two different sucrose concentrations, 16% and 20%, were used in milk jam samples produced by the traditional method. For the formation of desired colour and flavour in the Dulce de Leche, Maillard reaction products are mainly responsible. HMF is Maillard reaction indicator analysed in this work. Fat, protein, solid content, ash, lactic acid and pH analysis were carried out. Sucrose, glucose, fructose and lactose concentrations were determined. Lightness, yellowness and redness as colour parameters of DL samples were evaluated and sensory analyses were also performed. It has been revealed that lactose hydrolysis causes significant changes in the color parameters and sensory profile of the samples. In this study, less or no HMF was detected in the lactose hydrolyzed DL samples compared to the control samples.

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Introduction

Dulce de Leche (DL) (or milk caramel or milk jam) is a sweetened concentrated milk product, which is popular in some South American countries. DL is consumed especially as a spread, as a dessert and at breakfast and can be used for confectionary and ice-cream manufacture. In traditional DL production, milk and sugar mixture is concentrated by boiling at atmosphere pressure to a solid content of approximately 70% (Zalazar and Perotti, 2011). Sodium bicarbonate ($NaHCO_3$) is added during production in order to avoid casein coagulation and favor the Maillard reaction, responsible for its typical flavor and brown color (Gimenez et al., 2008).

Maillard browning is the main reaction between sugars and milk proteins during the production of DL. Maillard reaction occurs between the free amino groups of proteins and carbonyl groups of reducing sugars and the reaction is very important because of its effects on food quality (Van Boekel, 1998; Walstra et al., 2006). The reaction also has considerable consequences on the quality of dairy products. Because the brown and flavor compounds, anticarcinogenic, mutagenic, anti-mutagenic substances and antibacterial compounds are formed during the reaction (Van Boekel, 1998).

The most common method used to reduce the lactose content is the method in which lactose is hydrolyzed with the lactase enzyme before DL production (Castelao et al., 1977; Gimenez et al., 2008; Silva, 1980; Silva et al., 2015;). Lactose hydrolysis can cause increasing in rate of Maillard reaction because of the increase in reducing sugar content (Gimenez et al., 2008; Francisquini et al., 2019).

Lactose-free milk products (LFM) can provide the essential nutrients of dairy products to individuals with lactose intolerance. LFM currently has a wide and growing health appeal to consumers. It's reported that LFM is now the fastest growing market in the dairy industry (Dekker et al., 2019).

The aim of the present work was to determine the effect of lactose hydrolysis on physicochemical and sensory characteristics of DL and to evaluate development of Maillard reaction in lactose-free DL through monitor of 5-hydroxymethylfurfural (HMF) content.

Material and Methods

Materials

DL samples were produced from commercial pasteurized whole milk (3.0% fat, 3.0% protein, pH=6.8). Production of DL samples was carried out using the method described by Yüksel (2018). In preparation of control samples, temperature of pasteurized milk was heated to 40°C and keep for 4 hours. Concentrations of 20% and 16% (w/v) of sucrose were used. Sodium bicarbonate (dissolved in water) was added to adjust pH 7.0 of milk. According to traditional production method, the mixture was boiled during approximately 120 minutes, keeping a constant manual agitation. After its cream like consistency was obtained, the DL samples were cooled to about 50°C and packaged in glass containers.

Lactase (ß-galactosidase) (NOLA Fit 5500 Chr Hansen, 5500 BLU/g) was used to hydrolyze lactose in milk. In preparation of lactose-free DL samples, temperature of pasteurized milk was heated to 40°C, then the enzyme (0.25%, v/v) was added, and the sample incubated for 4 hours. After incubation, lactose-free DL samples were produced according to traditional method mentioned above.

Sample codes and their information (Table 1), and production curves (Figure) are given.

Physicochemical Analyses

Moisture content was determined gravimetrically by drying at 105°C until constant weight. Fat content was determined using Gerber method. Protein content was obtained using micro-Kjeldahl method. Ash content was obtained gravimetrically after incineration of 3 g of sample at 550°C. The analyses followed AOAC International official methods (2005).

The lactic acid (%) and pH were determined. The pH was measured in a pH meter (Sartorius, TE214S, Germany). The lactic acid was measured by alkaline titration method (AOAC, 2005).

Sugar Analyses by HPLC

Dry individual sugar standards (fructose, glucose, sucrose, maltose and lactose) were purchased from Merk (Germany). The analyses of sugars were performed by HPLC (high performance liquid chromatography) according to the AOAC International official method (2006). All standards were dissolved in deionized water and serially diluted to concentration 50 µg/ml. They were prepared daily. 10 g of DL was weighted into centrifuge bottle and added 50 ml petroleum ether. Then the sample was centrifuged for 15 minutes at 500 x g. Supernatant was decanted and discarded, and extraction was repeated. Precipitate was pulverized with a glass rod and added 100 g deionized water and weighted and placed in 85°C water bath for 25 minutes. And then it was cooled to room temperature and added deionized water to original weight. It was centrifuged for 10 min at 500 x g until withdrawing portion of clear supernatant. The clear supernatant was filtered through 0.45 µm filter (Sartorius, GmbH, Germany). The HPLC analysis of sugars was performed on an Agilent 1200 series HPLC system consisting of a detector RID 1200 (Agilent Technologies). Filtered samples were injected (20 µl) into HPLC using a Waters µ-Bondapark carbohydrate column (300 mm x 4 mm i.d.). The mobile phase was acetonitrile: water (80:20) (v/v) at flow rate of 1.5 ml/min. Column temperature was 30°C.

HMF Analyses by HPLC

The analysis of HMF was performed by HPLC according to the methodology described by Makawi et al., 2009.

Table 1. Sample codes (C1, C2, H1 and H2) and their information.

Sample codes	Samples' information
C1	Non-hydrolyzed DL with the addition of 20% sucrose and pH adjusted to 7.0 (control sample)
C2	Non-hydrolyzed DL with the addition of 16% sucrose and pH adjusted to 7.0 (control sample)
H1	Lactose hydrolyzed DL with the addition of 20% sucrose and pH adjusted to 7.0
H2	Lactose hydrolyzed DL with the addition of 16% sucrose and pH adjusted to 7.0

(C1: non-hydrolyzed DL with the addition of 20% sucrose, C2: non-hydrolyzed DL with the addition of 16% sucrose, H1: hydrolyzed DL with the addition of 20% sucrose, H2: hydrolyzed DL with the addition of 16% sucrose).

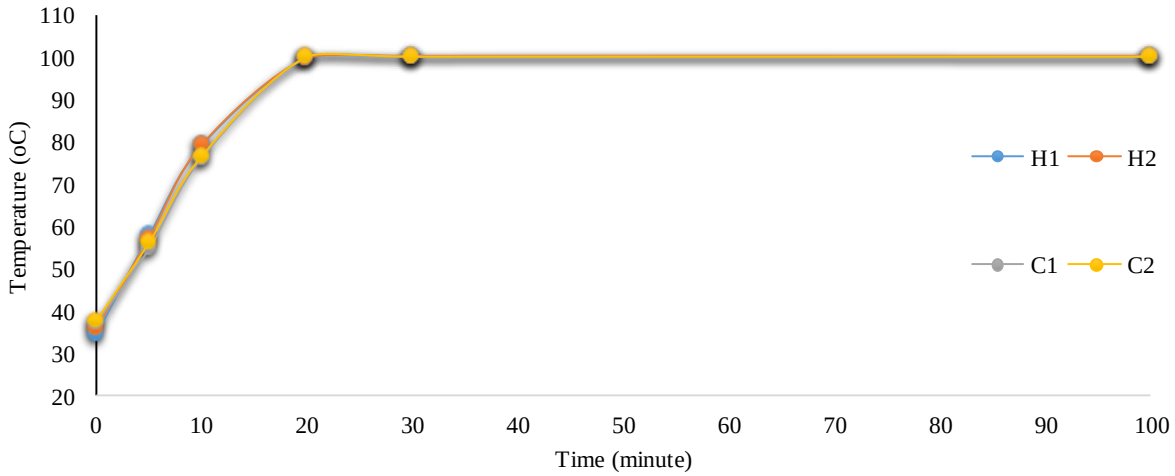

Figure 1. Production curves of DL samples.

HMF standard was purchased from Sigma Aldrich (Germany).

5 g of DL sample were dissolved in 25 ml deionized water and diluted to 50 ml with deionized water, filtered through a 0.45 µm filter before injection into the HPLA column (C18, 150 mm x 4.6 mm i.d. with 5µm particle size). UV-VIS detector (Agilent Technologies) was used. The mobile phase was 5% acetic acid w/v in water: methanol (80:20) (v/v) at flow rate 1 ml/min. Detection wavelength was 285 nm. Injection volume was 20 µl and column temperature was 40°C.

Colour Measurements

The colour parameters were determined by using a spectrophotometer (M-3600d, Minolta Co., Japan) at room temperature. The results were expressed in L* (lightness; 0 = black, 100 = white), a* (+ a = redness, - a = greenness) and b* (+ b = yellowness, - b = blueness) values.

Consumer Acceptance Test

Consumer acceptance testing was conducted on DL samples with participation of university students and stuffs. Fifty consumers, ages ranging between 18 and 65, and 24 of women and 26 of men, evaluated the four samples of DL. Samples were served in closed odorless plastic containers at room temperature and the plastic containers were labelled with three-digit random codes. Each DL sample was evaluated for appearance, flavour and texture on hedonic scale of nine points ranging from 1 (disliked it extremely) to 9 (liked it extremely). Consumers were also asked to rank samples according to their acceptance.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using the ANOVA procedures of the IBM-SPSS statistics software (version 23.0).

Results and Discussion

The chemical compositions and acidity values (lactic acid contents and pH values) of control samples and lactose-free DL samples with two different sucrose concentrations were presented in Table 2 and Table 3, respectively. It has been reported that the moisture content of the DL composition should be at most 30%, at least 24% of non-fat dry matter, at most 2% of ash and at least 5% of protein content (Zalazar and Perotti, 2011). It was seen that the moisture content of the DL samples produced using the traditional method varies between 32.24% and 42.42% when the results of this study are evaluated (Table 2). The fact that the production was carried out using the homemade technique and that some samples were produced using less sucrose (16%) have been among the reasons for the high moisture content of the product.

The ash contents of the samples were found to be between 1.54% and 1.65% on average. It was revealed that the total sugar content of the samples varied between 33.31% and 43.06%. It has been seen that the protein content of the samples varies between 5.52 and 5.87 g/100g on average (Table 2).

In the literature, in different DL samples collected from the market, the lactic acid values were found to be between 0.1% and 0.39% and the pH values were between 6.09 and 6.75 (Yüksel, 2018). In another study, it was observed that lactic acid content varied between 0.2% and 0.5% and pH values varied between 6.14 and 6.37 in seven different DL samples (Gaze et al., 2015).

In this study, the lactic acid contents of the control samples were found to be 0.1%, and the lactic acid contents of the lactose-free DL samples were found to be between 0.15% and 0.16%. It was determined that the pH values for all samples varied between 6.76 and 6.89 (Table 3).

When the lactic acid values of both control and lactose-free DL samples were compared with the findings in the literature, it was determined that the lactic acid values of the samples in our study were lower, albeit at a low level. This has been thought to be since the pH value of the milk was adjusted to 7.0 before the production.

The sugar amounts determined by HPLC analyzes of the samples are given in Table 4.

When the sugar contents of the samples were examined (Table 4), lactose could not be detected in the lactose hydrolyzed DL samples (H1 and H2). In these samples, as expected, the hydrolysis product of glucose, which was released as a result of the enzymatic reaction, was determined. This has indicated that all the lactose was hydrolyzed enzymatically. While lactose was found in the control samples, the hydrolyzed product glucose was not detected. Since there was no hydrolysis in the control samples, an average of 7.68% to 7.95% of lactose was found, while an average of 6.48% to 6.72% glucose was found in the hydrolyzed DL samples.

Color parameters were evaluated as "Lightness" (L^*), "redness" (a^*) and "yellowness" (b^*). The color results were presented in Table 5. Photograph of the samples was also given in Figure 2.

In general, the changes in the color values of the samples can be evaluated as an indicator for the non-enzymatic browning reactions (Maillard reaction and caramelization) that occur during the heat treatment in production. Considering the color measurements given in Table 5, there were differences in the color parameters of all samples in general ($P<0.05$).

Table 2. Chemical composition for the DL samples *

Samples	Protein (g/100g)	Moisture (g/100g)	Total sugar (g/100g)	Total sugar in DM (g/100g)	Protein in DM (g/100g)	Fat (g/100g)	Ash (g/100g)
C1	$5.80^{b}\pm0.19$	$32,24^{a}\pm2.22$	$43.06^{d}\pm6.01$	$63.44^{c}\pm7.00$	$8.56^{a}\pm0.15$	$5.33^{a}\pm0.47$	$1.56^{a}\pm0.09$
C2	$5.87^{c}\pm0.15$	$42.42^{c}\pm3.77$	$37.83^{b}\pm4.46$	$65.74^{c}\pm6.81$	$10.23^{b}\pm0.52$	$5.76^{c}\pm0.47$	$1.58^{a}\pm0.07$
H1	$5.52^{a}\pm0.16$	$35.90^{b}\pm5.50$	$41.14^{c}\pm5.37$	$64.27^{c}\pm7.17$	$8.63^{a}\pm0.16$	$5.66^{b}\pm0.75$	$1.54^{a}\pm0.02$
H2	$5.86^{c}\pm0.30$	$42.09^{c}\pm5.20$	$33.31^{a}\pm1.04$	$57.80^{a}\pm4.64$	$10.14^{b}\pm0.40$	$5.86^{d}\pm0.92$	$1.65^{b}\pm0.05$

* Values are means ± standard deviation; Analyses were performed in triplicate; [a-d] Means with different letters in the same column are significantly different ($P<0.05$) using Tukey's test; (DM represents dry matter); (C1: non-hydrolyzed DL with the addition of 20 % sucrose, C2: non-hydrolyzed DL with the addition of 16 % sucrose, H1: hydrolyzed DL with the addition of 20% sucrose, H2: hydrolyzed DL with the addition of 16% sucrose).

Table 3. Acidity values for DL samples.

Samples	Lactic acid (g/100g)	pH
C1	$0.10^{a}\pm0.00$	$6.79^{a}\pm0.09$
C2	$0.10^{a}\pm0.01$	$6.89^{c}\pm0.05$
H1	$0.16^{b}\pm0.01$	$6.76^{a}\pm0.20$
H2	$0.15^{b}\pm0.00$	$6.81^{a}\pm0.23$

*Values are means ± standard deviation; Analyses were performed in triplicate; [a-c] Means with different letters in the same column are significantly different (P<0.05) using Tukey's test; (C1: non-hydrolyzed DL with the addition of 20 % sucrose, C2: non-hydrolyzed DL with the addition of 16% sucrose, H1: hydrolyzed DL with the addition of 20 % sucrose, H2: hydrolyzed DL with the addition of 16 % sucrose).

Table 4. Sugars (lactose, sucrose and glucose) in DL samples obtained by HPLC

Samples	Lactose (g/100g)	Sucrose (g/100g)	Glucose (g/100g)
C1	$7.68^{a}\pm0.72$	$35.38^{c}\pm5.45$	-
C2	$7.95^{a}\pm0.90$	$29.88^{b}\pm3.60$	-
H1	-	$34.42^{c}\pm4.96$	$6.72^{a}\pm1.12$
H2	-	$26.83^{a}\pm1.78$	$6.48^{a}\pm1.34$

*Values are means ± standard deviation; Analyses were performed in triplicate; [a-c] Means with different letters in the same column are significantly different (P<0.05) using Tukey's test; (C1: non-hydrolyzed DL with the addition of 20% sucrose, C2: non-hydrolyzed DL with the addition of 16% sucrose, H1: hydrolyzed DL with the addition of 20% sucrose, H2: hydrolyzed DL with the addition of 16% sucrose).

Table 5. Colour parameters of the DL samples (L*; lightness, a*; redness, b*; yellowness)

Samples	L*	a*	b*
C1	$38.33^{b}\pm4.47$	$4.94^{b}\pm0.61$	$7.55^{b}\pm2.22$
C2	$42.05^{c}\pm3.57$	$6.49^{d}\pm0.35$	$10.90^{d}\pm1.23$
H1	$35.31^{a}\pm1.82$	$4.56^{a}\pm1.09$	$6.68^{a}\pm1.86$
H2	$37.32^{b}\pm4.22$	$5.67^{c}\pm1.81$	$8.45^{c}\pm3.40$

*Values are means ± standard deviation;Analyses were performed in triplicate; [a-d] Means with different letters in the same column are significantly different (P<0.05) using Tukey's test; (C1: non-hydrolyzed DL with the addition of 20% sucrose, C2: non-hydrolyzed DL with the addition of 16% sucrose, H1: hydrolyzed DL with the addition of 20% sucrose, H2: hydrolyzed DL with the addition of 16% sucrose).

The positive values of a^* and b^* color parameters determined in DL samples indicated that the product was tending to be red and yellow colors (Gaze et al., 2015). Therefore, these two equivalent results could explain the browning of milk jam samples by Maillard and caramelization reactions.

A lower L^* value indicates darker color formation. According to Table 5, it has been seen that the DL sample with the lowest L^* value was the lactose-free DL sample (H1) with 20% sucrose content.

The L*, a* and b* values of C2 and H2 samples with low sucrose content were found to be higher than their control samples (C1 and H1). This led to an increase in the tendency of DL samples to red (a*) and yellow (b*) colors and a lighter color formation (L*) as the amount of sucrose used decreased in both hydrolyzed and non-hydrolyzed samples.

As shown in Table 6 differences were observed in HMF contents between samples (P<0.05). Control sample C2 showed the highest the HMF content while HMF formation was not detected in sample H2. It has been revealed that Maillard reaction levels in DL samples vary depending on lactose hydrolysis and the amount of sucrose used in production. The HMF content of the lactose-free DL sample containing 20% sucrose (H1) was found to be lower than the HMF content of the control samples. It has been determined that lactose hydrolysis prevents or reduces the formation of HMF.

In a study in which the effect of lactose hydrolysis on HMF formation in DL was investigated in the literature, it was shown that lactose hydrolysis resulted in higher HMF formation and, consequently, higher rate of Maillard reaction products (Francisquini et al, 2019). Contrary to the results of the study, the results obtained within the scope of this study have showed that lactose hydrolysis leads to lower HMF formation.

In the Maillard reaction, some degradation products have been formed depending on the temperature and pH of food. In neutral or acidic foods, while if the initial sugar is pentose, furfurals form; if hexose, the formation of HMF takes place. In alkaline foods, it consists of reductones and fission products such as acetol, pruvaldehyde, and diacetyl. All these released Maillard reaction products are very reactive and play an important role in the next steps (Villamiel et al., 2006). Because of the reactivity of HMF, it can easily transform into other compounds. Moreover, this might be due to fact that HMF was not only formed from the Amadori compound but also from sugars (Yuksel, 2014). HMF formation in lactose non-hydrolyzed DL samples (C1 and C2) may be due to lactose isomerization and degradation. The reason why HMF could not be detected in the H2 sample. It may be due to HMF being reactive and converting to other compound or compounds in the next step. In this context, both the hydrolysis of lactose and the lower sugar content in the H2 sample may be among the effects that accelerate the progress of the reaction.

Figure 2. Photograph of produced DL samples (C1, C2, H1 and H2 samples in order from left to right).

Table 6. HMF concentrations of the DL samples

Samples	HMF (ppm)
C1	$2.63^{b}\pm1.01$
C2	$3.34^{c}\pm0.87$
H1	$1.03^{a}\pm0.42$
H2	N/A

*Values are means ± standard deviation; Analyses were performed in triplicate; [a-c] Means with different letters in the same column are significantly different (P<0.05) using Tukey's test; (C1: non-hydrolyzed DL with the addition of 20% sucrose, C2: non-hydrolyzed DL with the addition of 16 % sucrose, H1: hydrolyzed DL with the addition of 20% sucrose, H2: hydrolyzed DL with the addition of 16% sucrose).

Table 7. Consumer acceptance test results of DL samples

	Appearance	Consistency	Flavor
C1	$7.43^{c}\pm0.12$	$7.00^{a}\pm0.36$	$7.02^{b}\pm0.05$
C2	$7.53^{c}\pm0.15$	$7.19^{b}\pm0.29$	$7.13^{c}\pm0.04$
H1	$6.83^{a}\pm0.73$	$6.90^{a}\pm0.36$	$6.71^{a}\pm0.69$
H2	$7.15^{b}\pm0.94$	$7.11^{b}\pm0.24$	$6.93^{b}\pm0.49$

*Values are means ± standard deviation; Analyses were performed in triplicate; [a-c] Means with different letters in the same column are significantly different (P<0.05) using Tukey's test; (C1: non-hydrolyzed DL with the addition of 20% sucrose, C2: non-hydrolyzed DL with the addition of 16 % sucrose, H1: hydrolyzed DL with the addition of 20% sucrose, H2: hydrolyzed DL with the addition of 16% sucrose).

There are clues to the final stage of the Maillard effect, the construction of the nitrogenous brown polymer and copolymers known as melanoidins (Villamiel et al., 2006). Melanoidins are defined as low molecular weight colored substances. It has been suggested that colored melanoidins with high molecular weight can be produced with cross-linked proteins via lysine or arginine. It has also been suggested that these are polymers consisting of repeating units of furan and/or pyrroles formed in the advanced stages of the Maillard reaction (Martins and van Boekel, 2003).

The low or undetectable HMF ratio in lactose hydrolyzed samples can be explained by the possibility of formation of dark-colored melanoidins, which were among the final products of the reaction. The fact that the lactose-free samples were significantly darker may also support this result.

Results of consumer acceptance test of DL samples were given in Table 7.

It was found that there were significant differences between DL samples in terms of appearance, consistency and taste ($P < 0.05$). Lactose hydrolyzed DL samples were generally found to be darker in color and sweeter in taste than non-hydrolyzed samples. When evaluated in terms of consistency, it has been seen that lactose hydrolysis had no effect on consistency, but sugar content had an effect on consistency (Table 7).

In these consumer tests, among the lactose hydrolyzed samples it was found that the sample with 16% sucrose content was more liked in terms of appearance, flavor and consistency compared to hydrolyzed sample with 20% sucrose content.

Conclusion

According to the consumer acceptance test results, since the sweetness level was higher in hydrolyzed samples, this brought about a decrease in flavor appreciation scores. Some information on the sweetness levels of DL samples was also obtained according to the results of the reasons for appreciation, which are optional to be written (the results were not presented). From the test results obtained, DL samples produced with 20% sugar content were found to be too sweet, and lactose hydrolyzed 20% DL sample was evaluated as the sweetest jam. Lactose non-hydrolyzed product with 16% sugar content was the most appreciated in terms of flavor, while the lactose hydrolyzed product with 16% sugar content was evaluated as sweeter. Since the sweetness rate of lactose was low, the release of glucose by lactose hydrolysis caused milk jam to be perceived as sweeter.

Consumer currently try to reduce consumption of products containing lactose as well as containing high sugar products. In conclusion, findings of this study show that sugar levels can be further reduced in lactose-free DL, resulting in the production of less sweet and therefore more health-friendly, low-calorie products. The important advantage of this is that the product is lactose-free, as well as reducing the amount of sucrose to be used in production. Another important finding is that HMF was at the lowest level (in the sample containing 20% sucrose) and not detected at all (in the sample containing 16% sucrose) in lactose-free milk jam samples. Some consumers have a tendency not to prefer processed food due to the harmful compounds such as HMF that may occur during the processing of food. The fact that HMF could not be detected in the lactose-free DL sample produced with low sucrose content (H2) is an important finding in this respect. With this study, it is foreseen that Dulce de Leche can be produced with the traditional method in a way that will be lower in calories and healthier.

In future studies, it is planned to determine the content of melanoidins in addition to these analyzes in DL samples that will also be produced with less sucrose content than 16%.

Conflict of interests

The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

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