5-Hydroxymethylfurfural formation and color change in lactose-hydrolyzed dulce de leche

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Short title: **5-Hydroxymethylfurfural and color in dulce de leche**

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Supplementary File

Introduction

European companies operations on this market allow Occident Europe to be considered the biggest producer of lactose free milk and dairy products. According to Future Market Insights report (APEJ, 2018), North America market for dairy products with lactose free claim was worth US$ 10,582.5 million by the end of 2017 with growing projection to became US$ 17,809.4 million by the end of 2027. Therefore, the partial or total hydrolysis of lactose have been adopted by food industries as consequence of an increased number of consumers with lactose intolerance or that adopted a lactose free diet.

Composition, physical chemistry characteristics, colorimetric analysis and heat treatment indicators are important parameters of characterization, classification and processing monitoring applied during manufacture. And, according to Van Boekel (1998), 5-hydroxymethylfurfural or HMF concentration direct increases with the intensity of heat treatment, so it is consider a good indicator for MR in dairy products. Besides, this indicator can be easily detected by spectrophotometric technics (Francisquini *et al*. 2018), which make it easy the continuous assessment even by less specialized personnel.

Material &methods

Lactose-hydrolysis in milk

Pasteurized whole milk was added of 0·2% $w \cdot w^{-1}$ of lactase (Maxilact-DSM®) and kept at 34 ±1°C for 24 h, according to Fialho *et al.* (2017). The hydrolysis was conducted until ~100% lactose concentration reduction as measured by Lactozym® Pure Lacto Monitor™ (Novozymes/Dinamarca) following manufacturer instructions. Pasteurized whole milk with 50% of lactose reduction was obtained by mixture of equal amounts of regular and lactose free milk.

Food industry adopts the total or partial hydrolysis of lactose on dairy products manufacture (Luthy et al., 2017), hence 50 and 100% reduction were studied on this work. Pasteurized whole milk (0% lactose hydrolysis) was used as control.

Adjustment of milk pH

The pH of whole milk with or without lactose hydrolysis was measured with pH meter (PG 180 - Gehaka) and adjustments were done with sodium bicarbonate (Farmax).

To avoid technological problems as protein precipitation during production, milk initial pH must be around 7 (Perrone *et al*. 2007; Fennema, 2010). For this work, milk pH was adjusted to 6.85, 6.95 or 7.05.

Water activity and colorimetric analysis

The colorimetric analyses were determined through direct reading of system reflectance of coordinates L* (lightness), a* (red/green coordinate) and b* (yellow/blue coordenate), using CIELAB color scale with Illuminant D65 and standard observer function of 10° (Nachtigall *et al*. 2009).

Analysis of moisture, lipids, protein, ashes and carbohydrates

In moisture analysis the sample was weighted on a dish with sand and took to an oven. The samples were periodically removed from the oven, put on a desiccator to cool down and weigh. The process was repeated until constant weigh.

Fat analysis was performed in a milk butyrometer with scale range from 0 to 8% (Original, São Paulo), 11 mL of dulce de leche solution (20 % $m \cdot v^{-1}$) was added replacing milk. After mixing with the other reagents, the butyrometer was centrifuged for 5 min at 200·g (ThermoScientific™ Heraeus™ Biofuge™ Stratos™ ThermoFisherScientific, EUA) and transferred to boiling bath at 65± 2ºC for 10 min before direct reading on the butyrometer scale. The result was obtained after multiplication by dilution factor (5x).

The carbohydrates were obtained by difference from the other determined attributes.

Analysis of Maillard reaction indicators

Free HMF was analyzed without dilution on times 0 and 20 min, while 4 g of dulce were diluted in 20 g of distilled water before analysis on times 40 and 80 min. Total HMF was analyzed after dilution of 0.25 g of dulce de leche in 100 g of water.

For free HMF, 5 mL of sample/solution was transferred to a tube and added 5 mL of oxalic acid 0.3 $mol \cdot L^{-1}$(> 99.5 % PA; Dinâmica) and 5 mL of trichloroacetic acid

40% w·v^{-1} (TCA > 99 % PA; Vetec). After filtration in paper filter (Quanty – J. Prolab, 15 cm, 8 µm), 4 mL of permeated was transferred to a tube, added 1 mL of thiobarbituric acid 0.05 mol·L^{-1} (> 97.5 % PA; Merck) and then heated at 40°C for 30 min. Afterwards, samples were read in UV/Visible spectrophotometer (model Evolution 60S, ThermoScientific®, Madison) at 443 nm. For total HMF, the sample and the oxalic acid were kept in boiling water for 60 min, cooled down and then added the of TCA. From this point, the analysis followed the same protocol of free HMF.

HMF concentration (µmol·kg^{-1}) was obtained from the analytical curve (Figure 1).

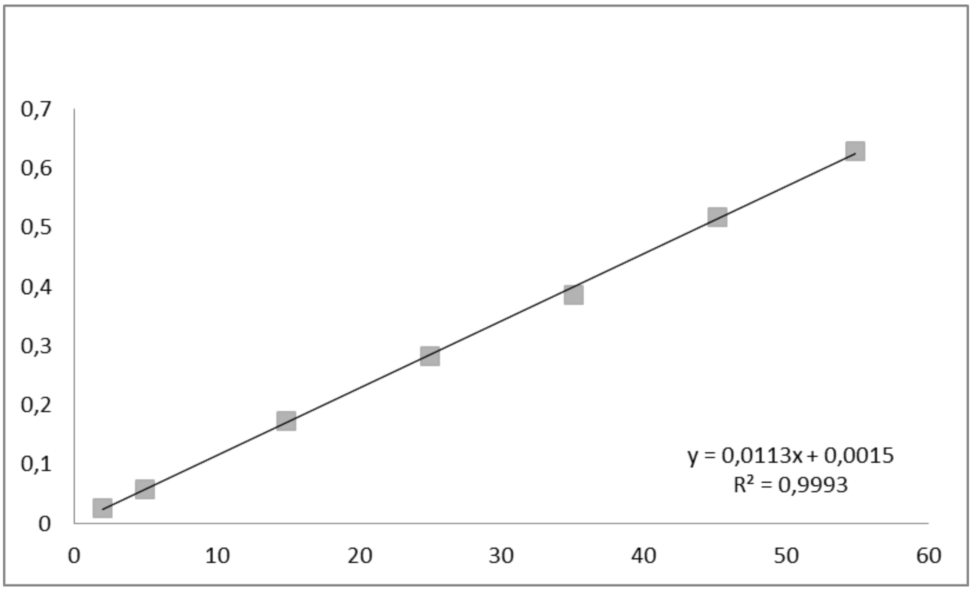

Figure 1: Standard curve for obtaining the concentration of 5-hydroxymethylfurfural (µmol·kg^{-1}). In which Y represents absorvance; X represents HMF content; and R^2 is the coefficient of correlation.

Statistical analysis

MATLAB 7.10 software was used for Principal Components Analysis (PCA). The data were normalized using SNV (Standard Normal Variate) pre-processing, which normalizes the data using the weighted average. In the model constructed by PCA, two main components (CPs) were chosen, with about 98% of the total variance captured. The choice of CPs was based on the graph of eigenvalues versus number of principal components.

Results and discussion

Dulce de leche production with or without lactose hydrolysis on manufacture scale is produced in open pan evaporators with steam jacket. Considering the necessity to precisely control product's temperature to study the development of Maillard reaction, a process simulator was used to better adjust heating rate and agitation.

Hydroxymethylfurfural analysis throughout production time

During dulce de leche production, the ingredients mixture (milk + sucrose) was kept at controlled temperature of 110 ± 15 °C until concentration achieved 62 °Brix. Production time took between 80 and 100 min depending on formulation.

Periodically analyses during the 80 min production length for the different formulations (Figure 1) were used to evaluate the HMF accumulation rate. Free HMF versus processing time curves were built from groups of similar samples with same degree of lactose hydrolysis (Figure 1A), percentage of sucrose (Figure 1 B) or pH (Figure 1C).

The relation between Free HMF concentration and processing time can be adjusted by quadratic equations as shown by Figure 1. Free HMF accumulation rate at 80 minutes increased within the increase of the degree of lactose hydrolysis, with values of 0.32; 0.58 e 0.67 $\mu mol.g^{-1}.min^{-1}$ for 0, 50 e >99% of hydrolysis, respectively (Figure 1A).

Considering samples with the same percentage of sucrose (Figure 1B), HMF accumulation rate in 80 minutes of process was 0.40; 0.57 and 0.53 $\mu mol \cdot g^{-1}.min^{-1}$ for treatments with 15, 20 and 25% of sucrose. Using the same approach for samples at the same pH (Figure 1C), HMF accumulation rate was 0.58; 0.43 and 0.58 $\mu mol \cdot g^{-1}.min^{-1}$ for pH 6.85; 6.95 and 7.05, respectively.

The rate of change in HMF concentration in the samples were influenced to a greater or lesser extent by the % of lactose hydrolysis, % of sucrose and pH of the formulations (Figure 1). The major rate of variation on HMF accumulation (0.32 to 0.67 $\mu mol \cdot (g \cdot min)^{-1}$) was observed for % of lactose hydrolysis, which suggests that the presence of glucose and galactose promote Maillard reaction propagation.

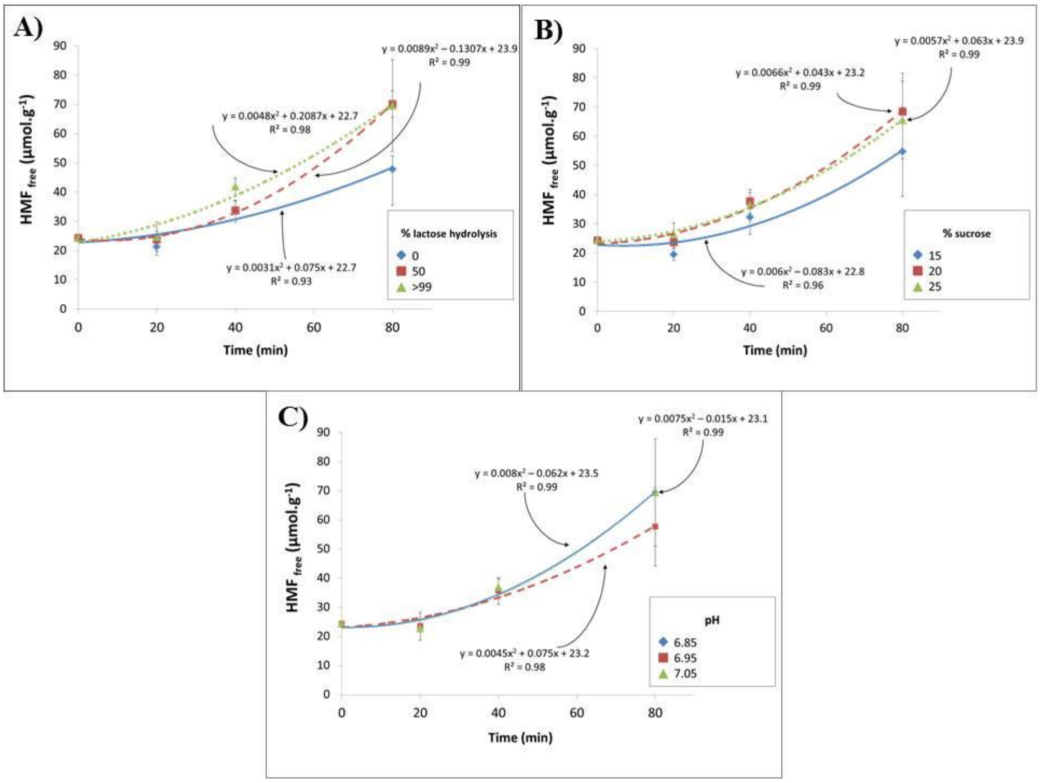

Figure 2: Kinetics of Free HMF accumulation during processing of different dulce de leche formulations. Lactose hydrolysis effect (A); sucrose percentage (B) and pH (C).

Maillard reaction starts with the condensation of a reducing sugar with the amino group of aminoacids, which is more likely to happen at higher pH (pH>9) (Fennema, 2010). Ergo, slightly alkaline dairy products are expected to present a tendency to accumulate more HMF.

Relation colorimetric analysis and HMF

Formulation at pH 6.85 and 7.05 were respectively light and dark, despite the level of hydrolysis or sucrose concentration (Table 2). Products at pH 6.95 presented different colors as function of the level of lactose hydrolysis: 0% lactose hydrolysis (light color); 50 or >99% lactose hydrolysis (dark color) (Table 3). Sucrose concentration did not present any influence on the samples classification as dark or light.

Table 2: Color of formulations at pH 6.85 or 7.05.

Trials	Hydrolysis(%)	Sucrose (%)	pH	Color
2	0	20	7.05	Dark
3		20	6.85	Light

5	50	15	7.05	Dark
6		15	6.85	Light
10		25	7.05	Dark
11		25	6.85	Light
13	>99	20	7.05	Dark
14			6.85	Light

Table 3: Color of formulations at pH 6.95.

Trials	Hydrolysis (%)	Sucrose (%)	pH	Color
1	0	15	6.95	Light
4	0	25		Light
8	50	20		Dark
12	>99	15		Dark
15	>99	25		Dark

Dulces with lower luminosity show a tendency of higher HMF concentration, despite poor correlation of a direct relation between these two factors (Figure 3).

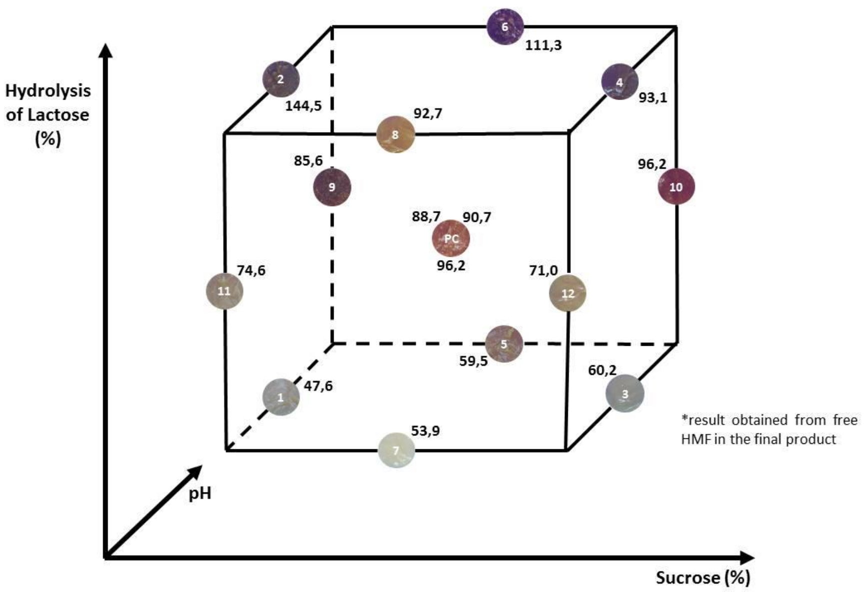

Figure 3: Schematic representation of the three levels used on the Box-Behnken design.

Relation of HMF, physical chemical and compositional attributes

The Box-Behnken desing used on this work is a rotation class or approximately rotation class, corresponding to a second order design and being based on an incomplete factorial design of three levels. These three levels can be graphically represented to help understand the obtained results (Ferreira *et al*. 2007). Figure 3 shows the graph that represents this study.

From Figure 3, it is possible to observe the products arrangement according to the parameters: % of sucrose, % of lactose hydrolysis and pH, in addition to the specific coloration of each product and its respective free HMF index. This graph confirms the previous presented results and shows the clear influence of the different parameters used on this design on coloration and free HMF index.

Figure 4 shows the Principal Component Analysis (PCA), which was elaborated to graphically show the observations, to facilitate the visualization and to evaluate the similarities and differences between the attributes analyzed in the present work.

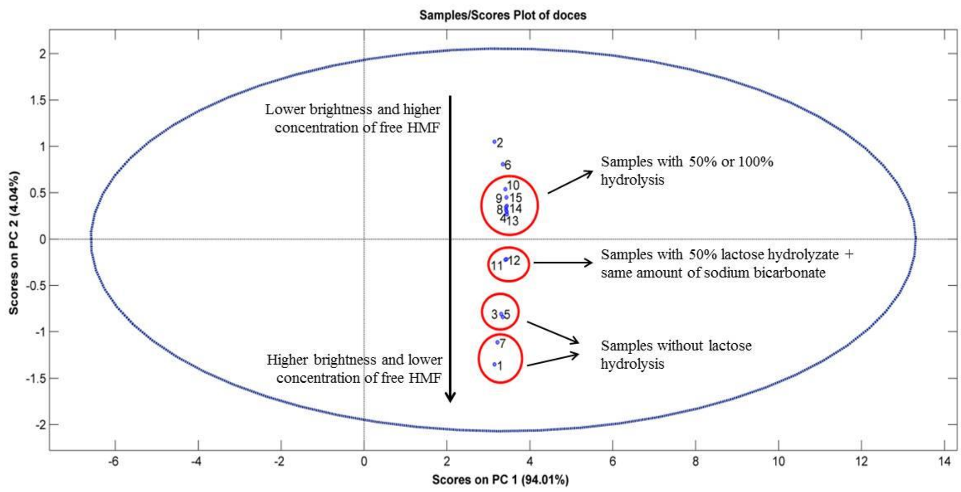

Figure 4: Graphic of scores PC1 versus PC2 of composition characterization data and free HMF formation at the times 0, 20, 40, 60, 80 and final minutes of evaporation (soluble solids content, water activity, moisture content, protein, lipid, ashes, carbohydrate, luminosity, free HMF 0, free HMF 20, free HMF 40, free HMF 80, free HMF final).

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