Influence of sucrose reduction on fouling during the production of dulce de leche

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Short title: **Fouling according to sucrose reduction in dulce de leche**

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Supplementary File

Introduction

Products with low sugar content are justified by recent studies correlating the decrease in dietary sugar (mainly sucrose) with the prevention, combat and recovery of a series of pathologies such as diabetes, hypertension, obesity, cardiovascular diseases, metabolic syndrome, dementia , depression, anxiety, cancer, dental problems, ulcers, hiatal hernia, Crohn's disease, irritable bowel syndrome, dermatitis, liver disease (Chow, 2017; Della Corte *et al*., 2018; Khan & Sievenpiper, 2016; Lenne & Mann, 2020). These products with low content or without sugar meet the demand for healthy foods, however they can present different functional, rheological, sweetness, stability, particle size distribution and texture when compared to traditional products, thus needing to use other compounds that provide body, texture and sweetness to it (Belščak-Cvitanović *et al*., 2015).

In order to avoid the problems associated with excessive sugar intake and to meet the growing demand of consumers for a healthier lifestyle, the dairy industries have made use of these new standards and concerns to manufacture products such as dulce de leche, chocolate, ice cream and yogurt with low sugar content (Castanheira, 2012; McCain *et al.*, 2018; Moore *et al*., 2020). Nutritionally, dulce de leche (DL) has high energy value and high concentration of proteins, minerals, fats and carbohydrates (BRASIL, 1997; Francisquini *et al*., 2018; Stephani *et al*., 2019). Normally, the pans for the production of DL are simple to operate and maintain, being made of stainless steel with a double wall through which the steam circulates and an agitator shaft. This has the purpose of preventing the portion of the liquid in direct contact with the equipment walls from burning (modifying the sensory characteristic of the product) or forming incrustations (fouling) along the concentration in addition to causing the temperature to be homogenous thus facilitating the evaporation of water. Upon reaching the desired point the product must be cooled and packaged (Perrone *et al*., 2019; Stephani *et al*., 2019).

With the heat treatment of milk, two types of inlays can occur in the pan: type A (formed mostly by proteins with a soft, white appearance) and type B (formed mostly from minerals, being more compact and grayer) (Bansal & Chen, 2006). As they differ in composition, it is necessary to use different cleaning processes to efficiently remove each type of deposit (Fickak *et al*., 2011; Goode *et al*., 2013; Jeurnink & Brinkman, 1994; Morison & Thorpe, 2002). When adding sugar to milk, in the manufacture of DL, there is a reduction in the occurrence of these incrustations, since there is an increase in the surface tension of water and in the chemical potential of proteins, favoring the state of lower surface of the proteins (native protein). Moreover, the addition of sugar causes rheological and kinetic changes that interfere with protein-protein interactions (Baier *et al*., 2004; Kendrick *et al*., 1997). Thus, the traditional DL industry does not present considerably many cases of encrustation, but when it comes to dulce de leche with low sugar content, changes in the product occur and they can lead to the formation of deposits on the equipment. These fouling can result in loss of productivity, in loss of the thermal exchange efficiency, in pressure drop in the equipment and in increase of additional costs related to cleaning products and energy (Gandhi *et al*., 2017; Tanguy *et al*., 2019; 2016).

Material & methods

Deposit formation

Pasteurized whole milk used was purchased in the local market (Viçosa, Brazil). The temperature of the inlet product was 4 °C and added with different sucrose contents (0%, 5%, 10%, 15% and 20% ($w \cdot w^{-1}$)). This process ensured the same protein content of all blends since for all the mixtures the same amount of milk was weighed and only then added the sucrose according to the desired percentages. The samples were concentrated in a laboratory scale atmospheric pressure evaporator (process simulator) (Vorwerk

Thermomix TM5) nearly at 125 °C for 90 min with 1 g (centrifugal force) of rotation. The deposits formed were collected after the end of the concentration by scraping the surface of the equipment - mass of deposit - and weighed in analytical balance (BEL engineering® Mark 214). For the characterization of the deposits, was performed dehydration using the freeze-drying method (Labconco - Freezone 2.5 Plus) with temperature -88 °C and pressure 0.014 mbar. This method was used to standardize the samples.

Characterization of deposits

Total protein

Total nitrogen from each deposit formed from the different samples was determined by using the micro Kjeldahl method, according the AOAC 982.38 method, it is an indirect methodology because when considering a conversion factor of 6.38 it is possible to estimate the protein contents (Wang *et al*., 2016), the results were expressed in % dry basis.

Moisture and dry matter

The moisture content was measured by using 1.90 ± 0.05 g of each deposit and a thermogravimetric balance (Sartorius® MA150). The total solids content was obtained by percentage difference of the moisture content found and with this percentage is possible to calculate the dry extract.

Carbon, Hydrogen and Nitrogen

The Elemental Analysis was performed by means of an elemental analyzer (Perkin Elmer 2400 series ii) to determine the percentages of carbon, hydrogen, and nitrogen of the

105 samples of the deposits formed in the evaporator. This analysis is based on the Pregl-
106 Dumas method whose samples are subjected to combustion in an atmosphere of pure
107 oxygen and the gases resulting from this combustion are quantified in a TCD detector
108 (thermal conductivity detector).
109
110 *Calcium and Phosphorus*
111 For the analysis of the trend of calcium and phosphorus content in the deposits it has been
112 used a semi-quantitative analysis by energy dispersive spectrophotometer – EDS
113 (HITACHI® TM3000).
114 And for quantitative analysis, the methods used were carried out according to the
115 methodology of AOAC (2012), as official methods 985.35 using flame atomic absorption
116 spectroscopy (FAAS) and 984.27 using inductively coupled plasma-atomic emission
117 spectrometry (ICP-AES) for the calcium and phosphorus analyzes, respectively.
118
119 *Scanning electron microscopy*
120 Samples of deposits were analyzed by scanning electron microscopy – SEM (HITACHI®
121 TM3000) with increase of 500x.
122
123 *FT-Raman spectroscopy*
124 Fourier-transform Raman spectrometer (Bruker, RFS 100/S) with a germanium detector
125 using liquid nitrogen as coolant and with 1064 nm excitation from a Nd:YAG laser was
126 used to characterize the deposits according to their dispersive ability. A few milligrams
127 of the sample were placed into a small aluminum sample cup, the laser light with a power
128 of 20 mW was introduced and focused on the sample, then the scattered radiation was
129 collected at 180°. For each spectrum an average of 512 scans were performed at a
130 resolution of 4 cm^{-1}, over the 4000–50 cm^{-1} range. The OPUS 6.0 (Bruker Optik,

Ettlingen, Germany) software program was used for Raman data acquisition (Almeida, Oliveira, Stephani & De Oliveira, 2011; Rodrigues Júnior et al., 2016).

Results and discussion

Concentration of Milk

Corroborating with Figure 1, the same is seen after weighing the deposits, which goes from 1.9 g to 21.5 g in the mix with 20% $m \cdot m^{-1}$ of sucrose and without added sucrose, respectively, which represents an increase of more than 11 times in incrustation.

Physical-chemical characterization of deposits

When calculating the water-protein ratio of the deposits, it has been observed that with the decrease in the sucrose content there is a greater water retention by the proteins reaching approximately 4 g $H_2O \cdot g$ proteína^{-1} corroborating with the literature (Walstra, Wouters, & Geurts, 2006).

It is still possible to say that the relationship between the lower content of deposits in the production of traditional DL is not only related to the dilution of milk constituents by the addition of sucrose, since the increase in deposits does not evolve in the same proportion as the decrease of the dilution factor, thus indicating the protective effect of sucrose amidst the concentration of the product (de Jong *et al.*, 1992).

Regarding the low content of calcium and phosphorus in the deposit resulting from the product with greater sucrose addition, it corroborates with the previous results and indicates that sucrose stabilizes milk proteins (Lee & Timasheff, 1981). As the relationship between calcium and phosphorus ($Ca \cdot P^{-1}$) remains around 1.75, with no statistically significant difference by the Tukey test ($p < 0.05$) for different sucrose levels, this indicates that despite the increase in minerals in the deposit, these are precipitated in the same proportion, therefore, the phosphate ester groups of the caseins together with

157 the bound calcium are assumed not to be part of the calcium phosphate matrix, thus being
158 of the tricalcium phosphate type (Gaucheron, 2005).
159 Pearson's correlation was used in order to observe whether the interactions between the
160 analyzes performed and the variables of the experiment (added sucrose content, dilution
161 factors and concentration) were significant. Based on Table S1, it is possible to observe
162 that the relationship between calcium and phosphorus does not present a statistically
163 significant correlation to the analyzed parameters. However, all other variables are
164 statistically significant in relation to the added sucrose content and to the dilution factor.
165 But when analyzing in relation to the concentration factor, it is observed that the
166 percentage of total protein is not significant as it is in relation to the dilution factor,
167 therefore it is possible to conclude that the concentration, despite influencing proteins,
168 the addition of sugar ends up generating a protective effect on them that is removed by
169 completely eliminating sucrose from milk.
170
171 *Scanning Electron Microscopy*
172 The deposit observed in Figure A1 indicates Type A deposits (Burton, 1968), that is, with
173 high protein content, which goes with the other analyzes carried out and that show the
174 relationship between the decrease in sucrose and the increase in proteins (from 12.94 to
175 31.31% ($w \cdot w^{-1}$) of protein on a dry basis for deposits with the addition of 20 and 0% ($w \cdot w^{-1}$)
176 of sucrose, respectively) and also to the increase in moisture present in this type of
177 deposit (going from 18.43 to 54.23% humidity for deposits with addition of 20 and 0%
178 ($w \cdot w^{-1}$) sucrose, respectively), once the rough structure is able to retain more water than
179 the compact structure of the deposit formed in production of traditional DL. A change in
180 the quantity and structure of the deposits with the addition of sucrose is also observed by
181 Zhang *et al*. (2018). Besides, this structure shows that the adopted cleaning procedures
182 must be different, since the protein deposit requires specific cleaning and specific

chemical products in a certain sequence: first the alkaline solution for the removal of the protein layer (top) and then the acidic solution for the removal of the innermost layer - minerals (Jeurnink & Brinkman, 1994).

Raman spectroscopy

In C2 (dulce de leche) and C3 (sucrose) spectra it is possible to perceive some of the main marking bands of sucrose, such as the band observed at 850 cm^{-1} referring to the vibrational deformation mode of CH_2, the bands at 642 and 523 cm^{-1}, referring to the deformation modes of the glucofuran ring, and the 403 cm^{-1} band, referring to a coupled mode to the sum of the deformation modes of the O39-C32-C31 bond and to the deformation of the C37-C31-O27 group of the glucopyran ring (Brizuela et al., 2012).

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Table legends:

Table 1:

Pearson correlations between the analyzed parameters and the sucrose content, dilution factor and evaporation rate in the manufacture of DL.

	Sucrose added (% w·w^{-1})	**Dilution Factor**	**Evaporation Ratio**
Deposit (% w·w^{-1})	-0.942	-0.948	0.997
Moisture (% w·w^{-1})	-0.953	-0.952	0.793
Total protein (% w·w^{-1})	-0.901	-0.897	0.687
Protein on dry basis (% w·w^{-1})	-0.957	-0.956	0.815
Nitrogen (%)	-0.953	-0.945	0.846
Calcium (norm. Wt. %)	-0.920	-0.926	0.997
Phosphorus (norm. Wt. %)	-0.902	-0.906	0.987
Ca·P^{-1}	-0.062	-0.053	-0.157
Calcium (mg·100g^{-1})	-0.984	-0.984	0.855
Phosphorus (mg·100g^{-1})	-0.994	-0.995	0.887
Ca·P^{-1}	-0.691	-0.683	0.386