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**Title: Transient changes of volatile organic compounds (VOCs) during dulce de leche preparation by a direct injection mass spectrometer based on proton transfer reaction (PTR-MS)**

**Running Title:** Property changes in dulce de leche production**.**

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**ABSTRACT**

The capacity of a direct injection mass spectrometer based on proton transfer reaction (PTR-MS) to monitor the transient changes of the volatile organic compounds (VOCs) during *dulce de leche* production was investigated. We found a correlation between the mass fingerprint and some physio-chemical properties of the *dulce de leche* samples. The intensity of the selected mass fragments related to VOCs was positively correlated with the changes of solids content but negatively with that of water activity. Rheological and textural changes were also highly correlated with the intensity observed for several mass fragments. Hence, this relationship could be useful to predict the textural and rheological changes during heating a complex fluid. Although not all the correlations between physical changes and VOCs formation reflected a direct cause-effect relationship, but those results demonstrated the suitability of the PTR-MS technique to monitor the quality changes even in complex food mixture during thermal processing.

**Keywords:** *dulce de leche*; proton transfer reaction spectrometry; aroma profile; physical properties.

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## INTRODUCTION

*Dulce de leche* is a dairy product from Latin America. It is generally obtained after extensive heating of milk and sugar under mild alkaline conditions. During heating, Maillard reaction products are accumulated. They confer typical browning colors and complex aroma profile (Gaze et al. 2015; Francisquini et al. 2018). In addition, the extensive heating and the consequent evaporation of water leads to a thicker texture and a pseudoplastic rheological behavior (Ranalli Andrés, & Califano, 2017).

The heat-induced mechanism of aroma evolution is quite complex. Generally, the control of the Maillard reaction is based mainly on the measurement of the color and the percent of solids (Quintas Brandão, & Silva, 2007; Rodríguez et al. 2017). At the best of our knowledge, the transient changes occurring to the volatile compounds responsible for the aroma of *dulce de leche* have never been reported.

The knowledge of the volatile organic compounds (VOCs) evolved during processing is important because characterizes one of the most important quality attributes of the product. However, VOCs analysis is also important for checking its safety. For instance, 5-hydroxymethyl-furfural (HMF), one of the major intermediate products in the Maillard reaction, may be cytotoxic at high concentrations (Surh & Tannenbaum, 1994). Therefore, the Codex Alimentarius Standard commission has set the maximum limit for HMF, for instance, in honey (40 mg/kg) (Codex alimentarius, 2001). For these reasons, recently, the aroma profile of *dulce de leche* has been characterized by GC-MS (Gaze et al. 2015). Thirty-two key-aroma compounds have been identified, such as: acids (i.e. acetic acid), ketones (i.e. 2-nonanone and 2-undecanone), aldehydes (i.e. furfural), alcohols (i.e. 2-furanmethanol), furans (i.e. 2-methyl furan) and others like dimethyl disulphide. The formation of such compounds derives from the thermal degradation of reducing sugars (i.e. lactose, glucose or fructose) and the amino acids present in the mixture (Giménez Ares, & Gámbaro, 2008). Moreover, the rate at which such products are formed is greatly dependent also to the pH, time, and temperature of the heating treatment (Martins and Van Boekel 2005).

However, the results reported so far show only the situation when the product is finished. Instead, there is a growing need to develop methods for the continuous monitoring of the heating treatment

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(Francisquini et al. 2019). For this reason, the aim of this work is to report on the use of PTR-MS as a sensitive and rapid method for the monitoring of the aroma evolution of *dulce de leche* during its heating treatment. The changes in the mass fingerprint profile will be correlated with the transient chemical and physical changes occurring during heating, including solid content, water activity, pH, color, viscosity and texture.

## MATERIALS AND METHODS

### *Materials*

Sodium bicarbonate and glucose were purchased from Sigma-Aldrich (Hamburg, Germany). Pasteurized whole milk (3.5% fat) was received from Mila Südtirol (Bolzano, Italy).

### *Dulce de leche preparation*

*Dulce de leche* was produced according to the traditional process in a stainless-steel pot (Kenwood cooking chef) under atmospheric pressure (Zalazar and Perotti 2011). Each time a batch of 1.3 kg was prepared inside a stainless-steel bowl, consisting of milk (1005 g), cane sugar (215 g), glucose powder (75 g) and sodium bicarbonate (5 g). The batch was mixed with a blander for 10 min at 30°C. Then the mixture was heated to reach 100°C (3.5°C/min) and, then, boiled for about 70 min. During heating, stirring paddles were spinning at min level (20 rpm). Heating lasted when total soluble solids (TSS) reached 68±1 °Bx. After cooling at room temperature, the final brix value was 70%.

### *Headspace volatile analysis by PTR-MS*

The headspace measurements were performed using a high-sensitivity PTR-MS (Ionicon, Analytik GmbH, Innsbruck, Austria). The instrumental conditions in the drift tube were as follows: drift voltage 600 V, drift pressure 2.2 mbar and drift temperature +70°C, resulting an E/N value of 141 TD (1 TD = $10^{-17}$ V $cm^2$) according to the method described by Asaduzzaman et al 2020. For the analysis, *dulce de leche* samples (1.0 g) were transferred into 40 mL screw-capped glass vials and incubated at 30°C for 30 min. Samples were analysed in a random order to reduce the source of bias. The vial headspace was transferred at a flow rate of 30 mL $min^{-1}$ through a heated (+80°C) capillary inlet system (1/16″, outer diameter, Silcosteel™, Restek GmbH, Germany) directly into the drift tube of the PTR-MS. The headspace was replaced by clean air by passing through an activated charcoal filter (Supelpure™ HC, USA). The instrument was operated in mass scan mode over m/z 21 to 200 with a

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dwell time of 100 ms per m/z. For each sample, first four scans were averaged to obtain raw signal counts. The signal intensity also corrected for transmission factor. The PTR-MS raw data was normalized against primary ion ($H_3O^+$), and water cluster ($H_2O \cdot H_3O^+$) to reduce long-term variability of the instrumental (Beauchamp et al. 2013). To calculate a normalized VOCs count rate (ncps) the following equation was used:

$$ncps = \frac{cps_{[RH^+]}}{500 \cdot cps_{[H_3^{18}O^+]} + cps_{[H_2O \cdot H_3O^+]}} 10^7 \quad (1)$$

Where, $cps_{RH^+}$ is the count per second of the ionized analyte ($RH^+$), $cps_{H_3^{18}O^+}$ is the count rate for the m/z 21 and $cps_{H_2O \cdot H_3O^+}$ is the count rate for the m/z 37.

The PTR-MS data on volatile profile of *dulce de leche* allowed to understand the homogeneity of the production steps. Form the spectra interesting mass having coefficient of variability 4.5% (n=10) were selected which represented the 75% of the initial ion count. The signal of those selected fragments represents the fingerprint of dulce de leche samples and the variation of such fingerprint reflects the evolution of the aroma over time. Afterwards, principle component analysis (PCA) was applied to discriminate *dulce de leche* samples based on different thermal treatments.

*Physicochemical analysis*

In every batch, physicochemical properties were measured for samples collected at each time point according to Ranalli et al 2012. The concentrations of soluble solids were determined by refractometer (VWR, Leuven, Belgium). Water activity was analyzed by water activity meter (Aqua Lab 4TE, USA), pH was measured by pH meter (sensION$^{TM}$ PH3, Vetrotecnica srl, Italy). The color of *dulce de leche* was measured by spectrophotometer (Konica Minolta CM-5, Japan) using illuminant D65, previously calibrated, with specular reflectance included, and CIELAB color system (Gaze et al 2015). The following factors have been assessed: lightness (L*), obtained from a* and b*, expressing light variation at wavelengths from red to green and yellow to blue, respectively. In all cases samples were measured in triplicate. All measurements were averages of two replicates from the same batch of sample.

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*Rheological characteristic*

The rheological characteristics of the samples were measured using a Brookfield RST-CC controlled-stress Rheometer (Ametek Brookfield, Middleboro, MA, USA), equipped with a conical concentric cylinder geometry (CCT-25, according to DIN 53019) and a cup (27.12 mm diameter). For the characterization of the flow curves, samples were mixed gently and transferred into the cup up to the mark of conical swelling. Flow curves were measured according to Ranalli et al 2012 with slight modification as shear rate between 100 to 1000 $s^{-1}$ (60 measuring points) and at a temperature of 30°C. The experimental data were fitted to the Hershel-Bulkley model (Rovedo, Viollaz & Suarez, 1991).

$$\tau = \tau_0 + k \cdot \gamma^n \quad (2)$$

Where K is the consistency index and 'n' is the flow behavior index. For Newtonian fluid, n= 1 and for non-Newtonian fluid n is far from 1. All measurements were averages of two replicates from the same batch of sample.

*Texture analysis*

The texture properties of dulce de leche was measured with a texture analyzer (TA.XT Texture Analyzer, Stable Micro System, EN.CO. s.r.l., Italy. Samples were placed in 80-mm-diameter plastic containers and kept at 25 °C. The system was equipped with a 40-mm flat-end plastic compression cylinder (probe P/40 Stable Micro Systems). Force and time data were recorded with Texture Expert from Stable Micro Systems Ltd. Software. Samples were compressed 5 mm. The speed of the probe was 1.0 mm/s during the pretest, 1.0 mm/s during compression and 10.0 mm/s during post-test. Duplicate measurements for each sample were performed. From the force versus time curve, values for the following textural parameters were calculated: firmness, consistency, cohesiveness, and work of cohesion (da Silva et al 2015). All measurements were averages of two replicates from the same batch of sample.

**RESULTS AND DISCUSSION**

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*Aroma profile of dulce de leche*

**Figure 1** shows the typical mass spectrum obtained from the analysis of the headspace of *dulce de leche* samples by direct injection mass spectrometry based on proton transfer reaction (PTR-MS). The intensity of each nominal mass ions within the range from 21 to 200 m/z was collected in less than 20 seconds (dwell time of 100 milliseconds per m/z). Such scan cycle was repeated at least ten times, among which first four scans were averaged to obtain raw signal counts. For each sample batch, the procedure was repeated four times and the resulting signals averaged. Finally, the signals were normalized against the primary ion (m/z 21) and the water cluster (m/z 37), according to eq. 1 (Beauchamp et al. 2013). **Figure 1** shows the mass spectrum (mass fragments from 21 to 37 were omitted) obtained as average of ten independent batches of *dulce de leche*, each repeated four times.

The sum of all the normalized signals between m/z 39 and 200 gave an overall intensity of (310 ± 14) $\times 10^3$ cps (n = 10). Although each sample was prepared in different days, the resulting overall mass intensity showed a variability below 5%. A small variability of the mass fragment profile among independent samples is desired as it represents a reproducible production method.

The main volatile compounds of *dulce de leche* were previously identified by Gaze and his co-workers (Gaze et al. 2015). Table 1 reports those volatile compounds, whose nominal parent ion were also detectable by PTR-MS. Moreover, the protonated molecular ions of each volatile compound and their main fragmentation pattern are also reported. Overall, from a list of 21 volatile compounds, eighteen unique mass fragments were selected (m/z 39, 41, 43, 45, 55, 57, 59, 61, 69, 71, 75, 83, 87, 89, 93, 95, 97 and 99). The total intensity of such mass fragments contributes to the 75% of the overall signal.

*Evolution of dulce de leche volatiles during processing*

The eighteen mass fragments previously selected provided an overall signal intensity equal to (238 ± 12) $\times 10^3$ cps (n = 10). **Figure 2** shows the evolution of the overall intensity of the selected mass fragments. For each point, the whole experiment was repeated independently ten times. For the analysis, the headspace sampling was identical to **Figure 1**. However, here, samples of *dulce de leche* were taken every 10 min from the boiling pan. The results show that, during the earlier stage of

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heating, the evolution of the selected mass fragments was minimal. However, after 20 min, the signal intensity suddenly increased, reaching a maximum plateau value at about 60 min. Overall, the period between 30 and 70 min was considered the most important for the evolution of the final *dulce de leche* aroma.

This period of heating was next analyzed in detail by principal component analysis (PCA). **Figure 3-A** shows the resulting score plot (A). This first principal component (PC) explained 66% of the total variance. Along this PC, *dulce de leche* samples formed well-defined clusters according to their heating period. Thus, the first PC may be interpreted with the product variability over time. Conversely, the second PC (which explained 12% of the total variance) reflected mainly the VOCs variation within the sample clusters. This second PC may be interpreted with the process variability over time.

Concerning the loading plot (**Figure 3-B**), the most important variables along the first PC (together with the volatiles species that can contribute to that signal intensity, according to Table 1) are the m/z 69 (2-pentanone, 2-nonanone), 97 (furfural and 4-cyclopentene-1,3-dione), 75 (1-hydroxy-2-propanone and heptanoic acid), 57 (4-amino-1-pentanol, heptanoic acid, nonanal), 89 (3-methyl-oxiran-2-yl-methanol, 2-methyl-propanoic acid, and butanoic acid), 71 (propanoic acid, 2-methyl, butanoic acid, hexanoic acid) and 99 (2-furanmethanol) (Gaze et al. 2015; Asaduzzaman et al. 2017; Dursun, Güler, & Şekerli, 2017; Francisquini et al. 2018).

Along the second principal component, the most important variables are the mass ions m/z 39, 55, 59, and 93. Their signal intensity can be explained by the evolution of acetic acid, hexanal, 5-hydroxymethylfurfural (HMF) and 2-nonanone (Gaze et al. 2015; Dursun, Güler, & Şekerli, 2017; Francisquini et al. 2018). These fragments are present in all the sample batches, regardless to the time of heating. Thus, their variability provides an index of the process variability.

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*Transient aroma and physicochemical changes during heating*

During the heating of *dulce de leche*, not only the aroma, but also several chemical and physical properties change over time. **Figure 4** shows the transient changes of pH, soluble solids, water activity, color, rheological and textural properties. Finally, **Figure 5** reports a map that highlights the correlation between the evolutions of selected mass fragments with the changes in the physico-chemical changes. This map expresses the Pearson's correlation coefficients with circles, whose color (red or blue) identifies positive or negative correlations, whereas the area and color intensity are proportional to its values. As it will discussed in the next sections, some correlations describe a direct cause-effect relationship, whereas others should be regarded with caution. However, the purpose of this map is to investigate which of the mass fragments recorded by PTR-MS could be used in future works as biomarkers for controlling the quality of *dulce de leche*.

*pH changes during heating*

Before starting the heating treatment, a small amount of sodium bicarbonate was added. According to **Figure 4-A**, during the first min of heating, the pH of the mixture increased up to pH 8.6 – 9.0. Higher pHs are desired as they increase the formation of Maillard reaction products, resulting in darker foods (Francisquini et al. 2019). However, after 20 min of heating, the pH dropped down constantly, reaching a pH of 6.5 after 70 min. Such acidification can be explained with different mechanisms (Fox, 1981). The most important is the lactose degradation, which forms organic acid like acetic acid (m/z 61 and 43). Consistently, from **Figure 5**, the signal intensity of the mass fragments m/z 61 and 43 are strongly correlated with the pH changes.

*Solid content during heating*

The strong evaporation occurring in the production of *dulce de leche* constantly increased the concentration of solids and, consequently, decreased the water activity value. The solid content in *dulce de leche* is an important indicator to control the endpoint of the cooking process (Moro and Hough, 1985). The observed changes in the solid content increased from 30° Brix of the initial mixture to 70° Brix after 70 min of continuous heating. The increase of solids is consistent with what reported in previous works (Francisquini et al. 2018). The increase of reducing sugar concentration has the effect of decreasing the water activity level, from an initial value of 0.98 ± 0.01 down to 0.84

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± 0.03 (n = 2). Similar values were reported by Francisquini et al. in 2019. From **Figure 5**, the intensity of most of the selected mass fragments are positively correlated with the increase of solids and negatively correlated with the reduction of water activity. Because of the strong evaporation occurring to the *dulce de leche* mixture during the heating treatment, most of the VOCs are also inevitably concentrated. This explains the wide correlation with most of the selected mass fragments.

*Color changes during heating*

The color of *dulce de leche* is one of the most distinctive quality attributes. Concerning its evolutions, color was measured in terms of CIELAB coordinates. Figure 4-B shows the changes of lightness (L) over time (where L = 0% yields black and L = 100% indicates diffuse white). The changes followed a sigmoidal decay. Within the first 10–15 min, the L-value was about 70 – 80%. After about 50 min of continuous heating, the L-value dropped to 30%. Regarding the other two-color coordinates, the initial values of *a* (where negative values indicate green and positive values indicate red) and *b* (where negative values indicate blue and positive values indicate yellow) ranged from -2 to +5 units. During heating, both indices increased, reaching two distinct peaks, respectively, after 35 and 25 min of continuous heating. Afterwards, both indices decreased, returning to values between +5 to +10 units. Overall, such variations reflect the color changes, from the initial white to dark brown.

From **Figure 5**, the highest correlations were observed, among others, with the mass fragments m/z 97 and 99. Among the typical volatiles found in *dulce de leche* products (see **Table 1**), such fragments correspond, respectively, to the molecular ions of furfural and furfuryl alcohol. Both species have pleasant almond-like odor and turn dark brown in the presence of air. Furans like furfuryl alcohol were found in dairy products as a result of Maillard reactions ((Wherry, Jo, & Drake, 2019) and in the extensive browning of coffee during roasting (Yeretzian et al. 2002; Petisca et al. 2013).

*Textural and rheological changes during heating*

Previous works have shown that the heating greatly affects the viscoelastic properties of *dulce de leche* (Ranalli et al. 2012; Barbosa et al. 2013; Ranalli, Andres & Califano, 2017). Accordingly, back extrusion experiments were applied to characterize *dulce de leche* consistency, firmness, cohesiveness and work of cohesion. Concerning consistency and firmness, they were determined, respectively, with

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the max force and area under the curve recorded during the probe penetration. Similarly, cohesiveness and work of cohesion were determined with the max (negative) force and area under the curve recorded during the removal of the probe from the sample. As shown in **Figure 4-C**, such four indices were highly correlated ($R^2 > 0.997$). Also, the rate of change of these parameters was negligible during the first period of heating, then, they greatly increased after 45 – 50 min of constant heating.

Regarding the rheological behavior of *dulce de leche*, the shear stress (τ) could be related to the shear rate (γ) by several constitutive equations. Although previous works proposed the use of the Bingham model (Barbosa et al. 2013) and the power law (Navarro, Ferrero & Zaritzky, 1999), in this work, the more general Hershel-Bulkley model gave the best fit, as in the work of Rovedo et al. 1991.

$$\tau = \tau_0 + k \cdot \gamma^n \quad (3)$$

This equation allowed the description of the rheological behavior of the initial mixture of ingredients and the following slurries obtained during increasing heating times. The results showed that, for heating times < 30 min, the mixture behaves like a Bingham plastic fluid, with flow index ($n$) equal to 1, and yield stress ($\tau_0$) equal to 3 Pa. Afterwards, $\tau_0$ became negligible, whereas $n$ constantly decreased down to 0.5, suggesting a transition of the fluid towards a pseudoplastic behavior. In all cases, the fitting of the model to the experimental data gave coefficients of determination > 0.999.

Rheological and textural changes in *dulce de leche* samples were highly correlated with the changes in the intensity observed for several mass fragments. However, it should be also emphasized that a significant correlation between such attributes does not necessarily imply a causal relationship between the two (Cook et al. 2005). For the purposes of this discussion, it is intriguing to conclude that, according to the correlation map of **Figure 5**, the measurements based on mass fingerprint profile could be used to predict also the textural and rheological changes of a fluid subjected to extensive heating. From **Figure 5**, the higher the intensity of the spot, the closer the coefficient was to 1 or -1. Low correlations were identified by smaller circle sizes and lighter spot colors (i.e. white = 0). The crosses represented statistical absence of significant correlation (p-values > 0.01).

Further studies should be encouraged to use electronic noses devices based on PTR-MS to further understand the impact of texture and viscosity on the aroma evolution. Indeed, there are increasing

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concerns about the impact of VOCs from food production on our environment and health. As shown with the present application, PTR-MS could be a promising emerging technique allowing direct, fast and sensitive monitoring of VOCs. Moreover, the technique could be applied online avoiding the need of sampling procedures to carry out texture and viscosity measurements.

## CONCLUSIONS

PTR-MS was applied for the monitoring of *dulce de leche* production. Its advantages as a non-destructive and online measurement, which in addition can be completely automatized. Furthermore, it was more sensitive to detect the end point of the process, compared to other methods. Thus, the proposed approach is suitable for the online monitoring of the quality changes of *dulce de leche* and other similar processes.

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**Conflict of Interest:** The authors declare that there is no conflict of interest.

**Ethical Review:** This study does not involve any human or animal testing.

**Data Availability Statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

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**Annotated References**

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Gaze et al. (2015): This paper highlights the physiochemical properties and major volatiles found in the commercial *Dulce de Leche*.

Martins and Van Boekel (2005): This paper highlights the effect of pH and concentration of sugar and protein on the formation of Maillard reaction products.

Francisquini et al. (2018): This paper highlights the effect of formula and heating intensity on the formation of 5-hydroxymethylfurfural (HMF), a Maillard reaction markers, during *Dulce de Leche* production

da Silva et al. (2015): This paper highlights the physiochemical and rheological properties of the commercial *Dulce de Leche*

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**Legends to Figures**

**Figure 1.** Mass fingerprint of *dulce de leche* samples obtained by PTR-MS. Number labels indicate selected mass fragments adopted from the GC-MS analysis of Gaze in 2015.

**Figure 2.** Evolution of total volatile compounds in *dulce de leche* samples as a function of different heating times. Error bars for each experimental point are calculated as standard deviations of n = 10 repetitions of the whole experiment. Also shown the sixth order polynomial fitting and its confidence interval (p = 0.95).

**Figure 3.** Score and loading plots of the mass fragment profile of *dulce de leche* samples analyzed by PTR-MS over sequential boiling times (from 30 to 70 min): (A) score plot, (B) loading plot.

**Figure 4.** Physical and chemical changes of *dulce de leche* as a function of the heating time. Variables in the plot are: A) Soluble solids (4%), $a_w$ (0.3%) and pH (2%); B) Color changes in terms of Lab color scale: lightness (5%), chroma a (5%), chroma b (5%); C) Texture parameters: firmness (5%), consistency (5%), cohesiveness (5%) and work of cohesion (5%); D) Rheological parameters derived from Hershel-Bulkley model: yield stress (5%), flow index (5%) and consistency index (5%).

**Figure 5.** Correlation map of the eighteen mass fragments determined by PTR-MS and the physical and chemical properties evolved during the heating of *dulce de leche*. Pearson's correlation coefficient was displayed by the color gradient. The blue and red circles corresponded, respectively, to positive and negative Pearson's correlation coefficients.

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Table 1. Volatile compounds in samples of *dulce de leche* samples identified by CG-MS (Gaze et al. 2015) and detectable by PTR-MS, together with their typical fragmentation profile.

| | **$[M-H]^+$** | **Fragments** | **Ref** |
|---|---|---|---|
| *Acids* | | | |
| Acetic acid | $(C_2H_4O_2)H^+$ | 61, 43 | (Boscaini et al. 2003, Aprea et al. 2007; von Hartungen et al. 2004, Romano et al 2018, Pu et al. 2019 ) |
| Propanoic acid, 2-methyl | $(C_4H_8O_2)H^+$ | 89, 71, 43 | |
| Butanoic acid | $(C_4H_8O_2)H^+$ | 89, 71 | |
| Hexanoic acid | $(C_6H_{12}O_2)H^+$ | 99, 71, 43, 41 | |
| Heptanoic acid | $(C_7H_{14}O_2)H^+$ | 75, 57, 43, 89 | |
| *Ketones* | | | |
| 2-Propanone, 1-hydroxy | $(C_3H_6O_2)H^+$ | 75 | (Buhr et al. 2002; Koss et al. 2018; Malásková et al. 2019) |
| 2-Pentanone | $(C_5H_{10}O)H^+$ | 87, 69, 45 | |
| 2-Nonanone | $(C_9H_{18}O)H^+$ | 83, 69, 55, 41, 39 | |
| *Aldehydes* | | | |
| Furfural | $(C_5H_4O_2)H^+$ | 97 | (Buhr et al. 2002; Kuś & van Ruth 2015) |
| Nonanal | $(C_9H_{18}O)H^+$ | 83, 69, 57, 55, 41 | |
| *Amides* | | | |
| Butanimidamide | $(C_4H_{10}N_2)H^+$ | 87, 41 | (Gaze et al 2015) |
| *Alcohols* | | | |
| 2-Furanmethanol | $(C_5H_6O_2)H^+$ | 99 | (Boscaini et al. 2003, Koss et al. 2018, Mayr et al 2003, Khomenko et al 2017) |
| (3-Methyl-oxiran-2-yl)-methanol | $(C_4H_8O_2)H^+$ | 89 | |
| 1-Propanol, 2-methyl | $(C_4H_{10}O)H^+$ | 41, 57 | |
| 1-Butanol fragment | $(C_4H_9)H^+$ | 57 | |
| *Hydrocarbons* | | | |
| Toluene | $(C_7H_8)H^+$ | 93 | (Majchrzak et al 2018, Gaze et al 2015, Amador-Muñoz et al 2016) |
| 4-Cyclopentene-1,3-dione | $(C_5H_4O_2)H^+$ | 97 | |
| n-Hexane | $(C_6H_{14})H^+$ | 87 | |

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| | | | |
|---|---|---|---|
| *Furan* | | | |
| Furan, 2-methyl | $(C_5H_6O)H^+$ | 83, 69 | (Märk et al. 2006) |
| *Lactones* | | | |
| Butyrolactone | $(C_4H_6O_2)H^+$ | 87, 59 | (Jürschik et al. 2012) |
| *Others* | | | |
| Disulphide, dimethyl | $(C_2H_6S_2)H^+$ | 95 | (Koss et al. 2018) |

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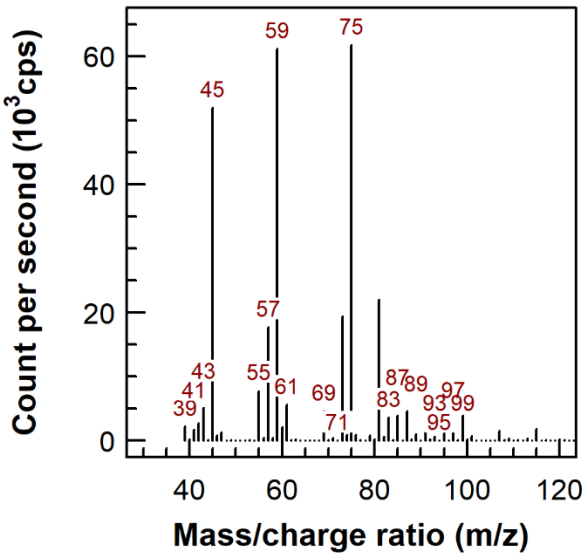


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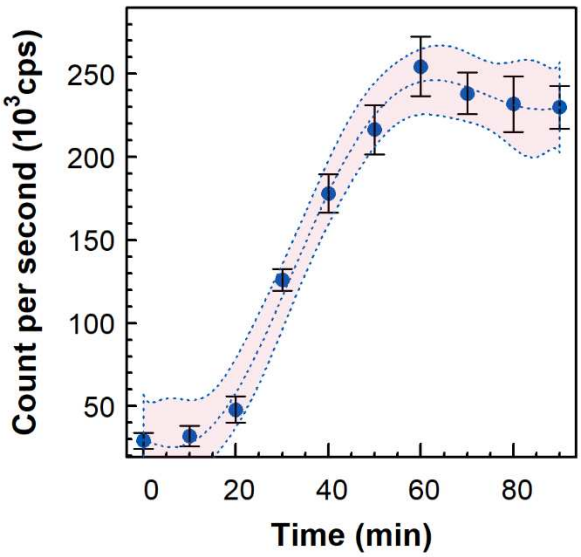


**Figure** 2 Evolution of total volatile compounds in *dulce de leche* samples as a function of different heating times. Error bars for each experimental point are calculated as standard deviations of n = 10 repetitions of the whole experiment. Also shown the sixth order polynomial fitting and its confidence interval (p = 0.95).

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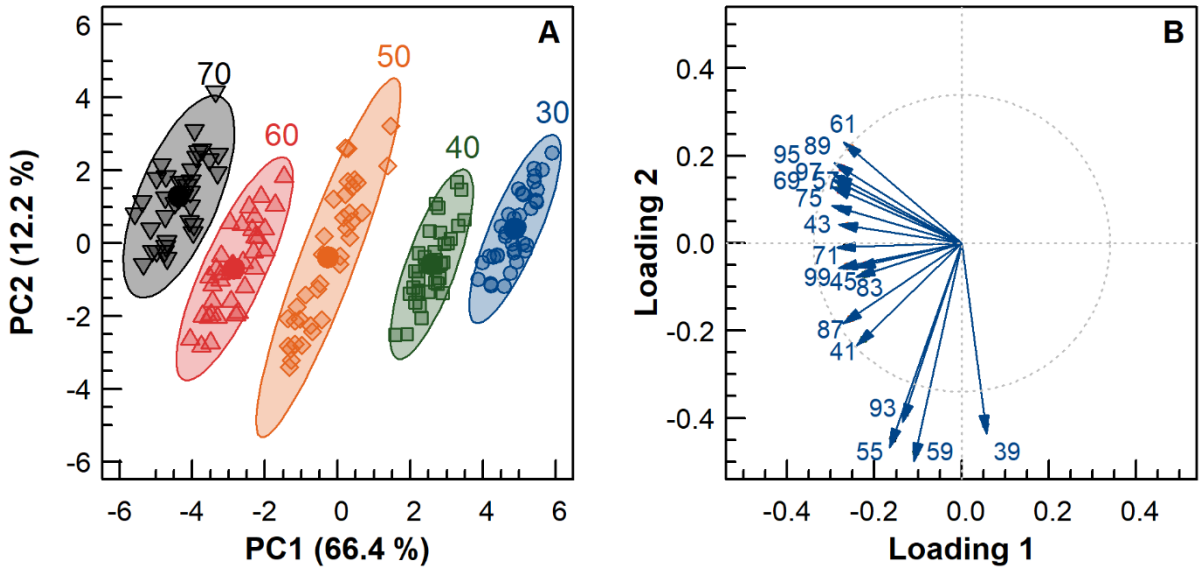


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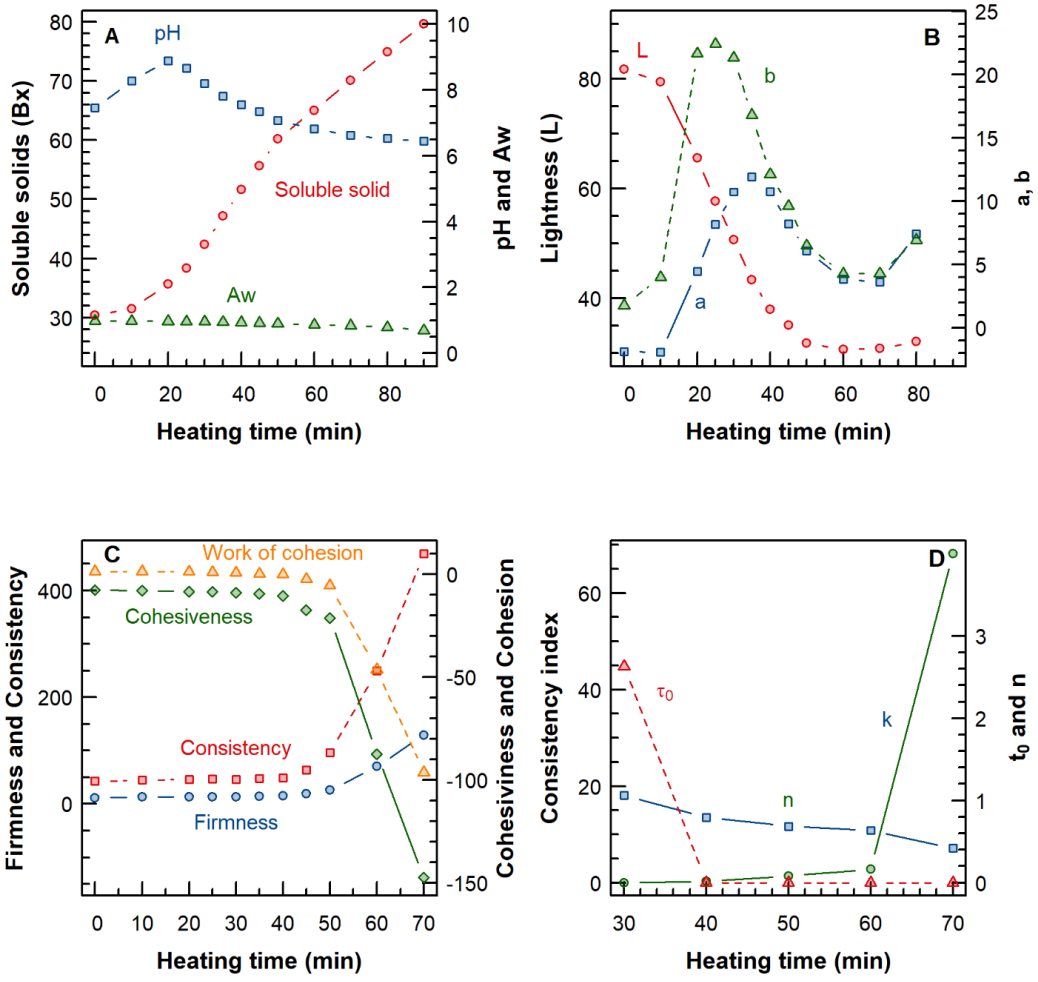


**Figure 4** Physical and chemical changes of *dulce de leche* as a function of the heating time. Variables in the plot are: A) Soluble solids (4%), $a_w$ (0.3%) and pH (2%); B) Color changes in terms of Lab color scale: lightness (5%), chroma a (5%), chroma b (5%); C) Texture parameters: firmness (5%), consistency (5%), cohesiveness (5%) and work of cohesion (5%); D) Rheological parameters derived from Hershel-Bulkley model: yield stress (5%), flow index (5%) and consistency index (5%).

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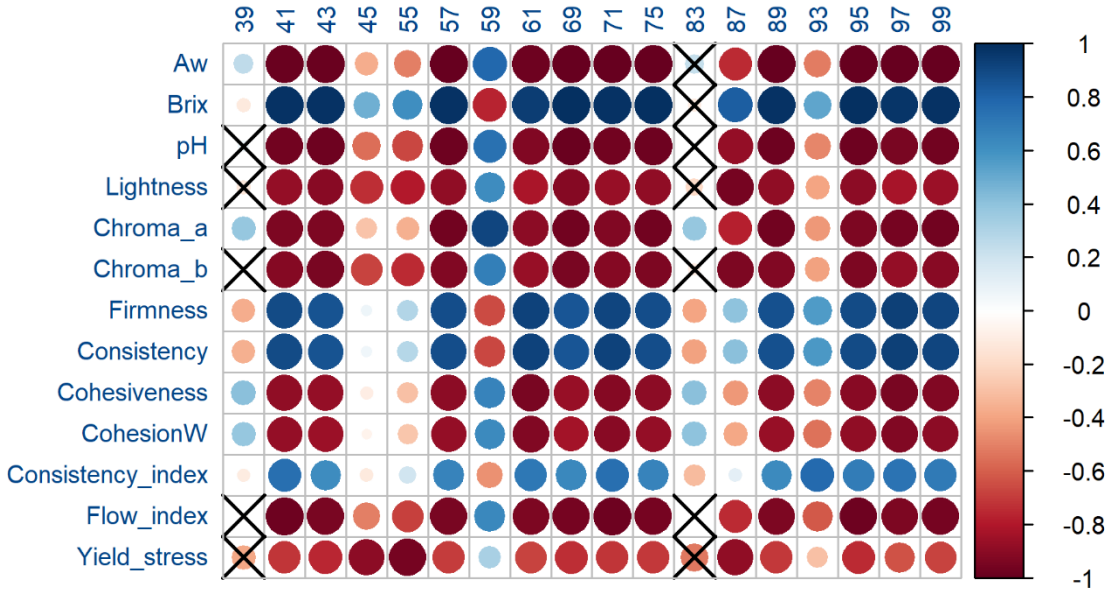


**Figure** 5 Correlation map of the eighteen mass fragments determined by PTR-MS and the physical and chemical properties evolved during the heating of *dulce de leche*. Pearson's correlation coefficient was displayed by the color gradient. The blue and red circles corresponded, respectively, to positive and negative Pearson's correlation coefficients

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