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Manufacture of *Requeijão cremoso* processed cheese with galactooligosaccharide

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ABSTRACT

The addition of galactooligosaccharide, (GOS, 0, 1.5, 3 or 4 g/100 g) on the quality parameters of requeijão cremoso was investigated. Chemical characteristics (pH, moisture, fat and protein), color (L*, a*, b*), water mobility by TD- nuclear magnetic resonance, rheology (flow curve and oscillatory tests), microstructure and sensory acceptance (consumer test) were evaluated. The addition of GOS provided a denser and compact structure and reduced number and size of fat globules. Increased GOS level (3 and 4 g/100 g) improved the softness and spreadability (decrease of G′, G″ and apparent viscosity and an increase of tan δ and melting index) and impacted positively on the aroma and taste of the requeijão cremoso. Overall, the addition of GOS in requeijão cremoso was proved to be a potential and interesting technological option.

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1. Introduction

Prebiotics are non-viable food components that confer benefits upon host health associated with the modulation of the microbiota (FAO/AGNS, 2007). Prebiotic galactooligosaccharides (GOS) are produced by transgalactosylation of lactose catalyzed by β-galactosidase from different sources (Maawia et al., 2016). GOS consumption is related to the improvement in the immune system (Fernandes, do Rosario, Mocellin, Kuntz, & Trindade, 2016), modulation of intestinal microbiota (Monteagudo-Mera et al., 2016), reduction of cancer risk (Bruno-Barcena & Azcarate-Peril, 2015), increased mineral absorption (Maawia et al., 2016) and decreased appetite and food intake (Morel et al., 2015).

GOS are added as functional food ingredients, alone or in combination with inulin-type fructans, into infant formulas to mimic the beneficial effects of human milk oligosaccharides. Other processed foods that can be manufactured with GOS are beverages, bakery and dairy products. GOS can be added in those products because of their functional and technological aspects, that is, high solubility, clean taste, stability and low glycemic index (Vénica, Bergamini, Rebechi, & Perotti, 2015). It is important to note that GOS can improve the texture and mouthfeel of dairy foods (Balthazar et al., 2017).

Requeijão cremoso processed cheese is the product obtained by the fusion of the curd mass, cooked or not, drained and washed, which is obtained by acid and/or enzymatic coagulation of milk and has consistency that can be spread with a knife at room temperature. This characteristic is due to the absence of a rigid protein matrix as a result of the strong agitation and homogenization to which the product is subjected during processing (Ramos, Haddad, Ramos, & Pinto, 2012).

The consumption of requeijão cremoso stands out in the dairy sector. Therefore, studies have been carried out aiming to improve its formulation and functionality but there are no studies evaluating the applicability of GOS in requeijão cremoso. Therefore, the

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objective of this study was to evaluate the effect of the addition of GOS (0, 1.5, 3 or 4 g/100 g) on chemical, rheological and microstructural characteristics and acceptance of requeijão cremoso.

2. Materials and methods

2.1. Processing of requeijão cremoso

The processing of requeijão cremoso was carried out according to Van Dender, Spadoti, Zacarchenco, Galina & Alves (2012). The ingredients used were: 500 g of curd mass (3.5 g/100 g fat, NATA, São Gonçalo, Rio de Janeiro), 185 mL fresh milk cream, 7.5 g Joha S9 fondant salt, 0.5 g potassium sorbate, 7.5 g of NaCl, and 100 mL of water. Four formulations were prepared: (RC) traditional, (RI) requeijão cremoso with 1.5 g/100 g GOS, (RII) requeijão cremoso with 3 g/100 g GOS, and (RIII) requeijão cremoso with 4 g/100 g GOS (Vicinal® GOS Syrup, Friesland Campina Domo®, Borculo, Holland). Preliminary experiments were performed considering the addition of 4.5 g/100 g GOS and it was not noted difference on the several quality parameters *requeijao cremoso* when compared to the product added with 4 g/100 g. Therefore, considering economical purpose, we opted to add only 4 g/100 g GOS at the *requeijao cremoso* formulation.

All the ingredients were weighed, mixed and heated (90 ± 5 °C) until complete melting, whereupon the remainder of the milk cream and water were added. After 5 min of cooking, the heating was interrupted and the curd packed in polypropylene containers. The products were stored under refrigeration at 5 ± 1 °C and the chemical and rheological analyses were performed at seven days of storage and color was assessed on the first day of storage.

2.2. Chemical and color analyses

The pH of the samples was measured using a potentiometer (Digimed DM-20, São Paulo – SP), while moisture, protein and fat were determined according to Brasil (2006). The color was determined using a portable colorimeter (CR-410, Minolta Sensing Konica, Inc., Tokyo, Japan), obtaining the coordinates L * (brightness), a* (green-red) and b* (blue-yellow). Chroma (C *) (Eq. (1)), whiteness index (WI, Eq. (2), Balthazar et al., 2017) and yellow index (Eq. (3), Pathare, Opara, & Al-Said, 2013) were also calculated.

$$C*_{ab} = (a*^2 + b*^2)^{1/2} \quad (1)$$

$$\mathrm{WI} = 100 - [(100 - L*)^2 + a*^2 + b*^2]^{1/2} \quad (2)$$

$$\mathrm{YI} = \mathrm{YI} = 142.86\, b*/L* \quad (3)$$

2.3. Rheology

The rheological measurements were performed using steady-state (flow curves) and dynamic (oscillatory) flow tests on a Paar Physica MCR 300 strain gage (Anton Paar GmbH, Graz, Austria), with cone-plate geometry (5 cm, 2°), 53 μm gap and a temperature control system (Physica TEK 150P). Samples were placed in the dish and allowed to stand at 10 ± 0.1 °C for 10 min to recover the structure.

To obtain the flow curves, shear stress scans were performed: the first scan was performed with increasing strain rate (0–100 s^{-1}), while the second strain rate decreased (100–0 s^{-1}) and the third strain rate increased (0–100 s^{-1}). Data from the third curve were fitted to the power law model (Eq. (4)) using non-linear regression analysis using STATISTICA 5.0 software (Statsoft, Tulsa, OK, USA).

$$\sigma = k\gamma^n \quad (4)$$

where σ represents the shear stress (Pa), k is the consistency index (Pa.sn), $\dot{\gamma}$ is the shear rate (s^{-1}), and n represents the consistency index (dimensionless).

In the oscillatory tests, G′ (elastic modulus) and G″ (viscous modulus) were measured at frequencies between 0.01 and 10 Hz, with a constant voltage (0.3 Pa), within the viscoelasticity range (0.01–10%), with a fixed frequency of 1 Hz (Veiga, Cunha, Viotto, & Petenate, 2000). The tangent of the phase angle was determined according to Eq. (5). The elastic (G′) and viscous (G″) modules were used to calculate the complex modulus (G*) (Eq. (6)).

$$\tan\delta = \frac{G''}{G'} \quad (5)$$

$$G* = \sqrt{G'^2 + G''^2} \quad (6)$$

The Winter and Chambon (1986) critical gel model was used to evaluate changes in the viscoelastic properties of the samples as a function of frequency (Eq. (7)). Data were adjusted by non-linear regression analysis using STATISTICA 5.0 software (Statsoft, Tulsa, OK, USA).

$$G* = A_F \omega^{1/z} \quad (7)$$

where A_F (Pa $s^{1/z}$) represents the gel force, ω is the frequency in Hz and z (dimensionless) corresponds to the interaction factor which is defined as the number of structural units interacting with each other in a three-dimensional network.

2.4. Melting behavior

Fifteen grams of each sample were weighed directly into melting tubes (250-mm long glass cylinders × 30 mm diameter, supplied with rubber corks at both extremities) with 2 reference lines (i.e., longitudinal and transversal) engraved on the glass. The tubes were left upright in an ice bath for 30 min. Then, the rubber cork was adjusted so that the cheese surface was in line with the engraved transversal reference line. The tubes were then placed horizontally in a support and placed in a forced air incubator at 110 °C for 5 min. The support was removed from the oven and inclined at 45 °C to interrupt the cheese flow. The distance flowed as from the reference line to the edge of the melted sample was marked. The support was then returned to the horizontal position and returned to the incubator for 2 min and the marking of the distance flowed repeated. The total distance (cm) covered by the sample during the 5 + 2 min of heating was called the "cheeseflow" and used as the melting index (Cunha, Dias, & Viotto, 2010).

2.5. Scanning electron microscopy

Analogous portions of the requeijao cremoso processed cheese (5 g) were fixed with 2.5 mL/100 mL of glutaraldehyde for 1 h, washed three times with phosphate buffer, placed in 0.2 g/100 mL of OsO4, left overnight, washed three times with (50–70–90–100 mL/100 mL, 20 min per step) and placed in pure ethanol for 24 h. The samples were dried, ground and coated with Au by cathodic spray coating. Micrographs were performed with a QUANTA-200 (FEI) at an acceleration voltage of 10 kV and the electron microscopy was carried out using the JEOL JSM apparatus (JEOL-USA, Inc., Peabody).

2.6. Time domain nuclear magnetic resonance (TD-NMR)

The TD-NMR measurements were performed in a low field spectrometer operating at 23.4 MHz for proton (MARAN Ultra 0.54 T, Oxford Instruments) with 7.5 μs and 90° pulse length. Transversal relaxation time (T2) was measured using Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with 2048 echoes, 200 μs between each

Table 1
pH, gross composition and color parameters (L*, C*,WI,YI) of requeijão cremoso processed cheese added of galactooligosaccharide.*

Parameters	RC	RI	RII	RIII
pH	$6.2^{a} \pm 0.04$	$6.3^{a} \pm 0.08$	$6.3^{a} \pm 0.08$	$6.3^{a} \pm 0.07$
Moisture	$64.5^{a} \pm 1.14$	$64.1^{a} \pm 1.16$	$64.2^{a} \pm 1.12$	$64.2^{a} \pm 1.32$
Dry Matter	$35.5^{a} \pm 1.22$	$35.8^{a} \pm 1.17$	$35.7^{a} \pm 1.88$	$35.7^{a} \pm 1.45$
Protein	$15.7^{a} \pm 1.32$	$15.8^{a} \pm 1.54$	$15.8^{a} \pm 1.45$	$15.4^{a} \pm 1.11$
Fat	$19.9^{a} \pm 1.11$	$19.8^{a} \pm 1.02$	$19.8^{a} \pm 0.34$	$19.7^{a} \pm 1.22$
Fat/Dry Matter	$56.3^{a} \pm 1.84$	$55.5^{a} \pm 1.74$	$55.6^{a} \pm 1.14$	$55.3^{a} \pm 1.64$
Luminosity (L*)	$94.3^{a} \pm 1.31$	$88.7^{c} \pm 0.9$	$91.2^{b} \pm 0.9$	$88.4^{c} \pm 0.2$
Chroma (C*)	$25.6^{b} \pm 1.71$	$26.1^{ab} \pm 0.8$	$26.2^{ab} \pm 0.6$	$26.5^{a} \pm 0.5$
Whiteness Index (WI)	$73.67^{a} \pm 1.80$	$71.4^{c} \pm 1.1$	$72.3^{b} \pm 0.4$	$70.9^{c} \pm 0.4$
Yelowness Index (YI)	$38.8^{c} \pm 1.20$	$42.1^{ab} \pm 0.2$	$41.1^{b} \pm 0.6$	$42.9^{a} \pm 0.3$

* Values are expressed ± standard deviation. Analysis performed in triplicate. pH, L., C*, WI and YI are admensional. Moisture, Dry Matter, Protein, Fat and Fat/Dry matter are expressed in g/100 g. Different letters at the same line indicate statistical difference according the Tukey test ($p < 0.05$). RC, RI, RII, RIII = see text.

echo and 16 scans with a waiting time of 5 s. Only even echoes were considered for exponential fitting to eliminate the errors associated with 90° pulses. The acquired signal decay (M(t)) for all samples was better fitted using simple exponentials that returned a minor chi-square value, according to the function in Eq. (8).

$$M(t) = A1 \exp(-t/T_{2,1}) + A2 \exp(-t/T_{2,2}) \quad (8)$$

Where component 1 refers to protons of fat, structural water and bound water (water linked with protein) (Halle, 2004), whereas component 2 refers to free water and protons provided by moisture (Hills, Takacs & Belton, 1990).

2.7. Sensory analysis

The affective test (appearance, aroma, taste, texture, and overall impression) was performed with 80 consumers (50 women, 30 men, age 19–48 years) using a 9-point hybrid hedonic scale (1 = disliked extremely and 9 = liked extremely). Samples (10 g) were presented monadically at 5 °C in a complete balanced block design (Drake, 2007).

2.8. Statistical analyses

All the processing was repeated three times being the analysis performed in triplicate.Data were analyzed by one-way analysis of variance (ANOVA) and the means were compared using the Tukey test ($p > 0.05\%$) using the software XLSTAT 2017.1 (Adinsof, Paris, France).

3. Results and discussion

3.1. Chemical and color parameters

The results of the chemical and the color parameters are presented in Table 1. Overall, there was no influence of the addition of GOS on the chemical parameters ($p > 0.05$) and results were within the range: pH (6.23–6.25), dry matter (35.50–35.81 g/100 g), fat (19.78–19.99 g/100 g), fat in dry matter (55.3–56.3 g/100 g), and protein (15.45–15.89 g/100 g).

pH is an important factor for cheese identity and quality because it directly affects its structure and rheological properties, altering the chemical interactions between the structural components (proteins, water and minerals). The pH values (≈6.2) remained within the established quality limits (pH 5.4–6.2). Values below 5.4 damage the structure and taste and tend to form a very firm and granular texture, while samples with pH values between 5.5 and 5.7 result in cheeses of creamy and firm consistency. Requeijão cremoso that presents pH values higher than 6.2 may have a decrease in protein–protein interaction and increased protein hydration, leaving the curd less firm, reducing its durability, causing changes in its taste (salty taste, soap flavor and fat separation), and altering the product consistency (Van Dender, 2006).

The moisture of requeijão cremoso is economically important because it increases the yield of the product and directly influences the texture (Van Dender, 2006). A maximum moisture content of 65 g/100 g is recommended by the current official legislation (Brasil, 1997). Therefore, the samples manufactured with GOS had a moisture content within this limit (64.19–64.5 g/100 g). The Brazilian legislation also provides a fat content in the dry extract of less than 55 g/100 g (Brasil, 1997), but the samples manufactured with GOS presented slightly higher levels (55.3–56.3 g/100 g). The fat content can be standardized with slight changes in the formulation of the products.

The minimum daily intake suggested for prebiotics to obtain beneficial health effects on humans is 3 g (Slavin, 2013), suggesting that the consumption of 60 g (2 spoons) of the requeijão cremoso formulations manufactured in this study would provide 30–80% of the suggested consumption. It is important to note that the GOS content was not determined and the calculations were performed based on the added concentrations, considering that losses during the processing are generally inexpressive (Torres et al., 2010).

Requeijão cremoso processed cheese is usually characterized by a slightly yellowish-white color (Cunha et al., 2010). The addition of GOS caused a decrease in the L* and WI parameters and increased the YI parameter ($p \leq 0.05$), regardless of the concentration used (RI, RII, or RIII). The addition of 4 g GOS/100 g (sample RIII) resulted in an increase in the b* and C* parameters ($p \leq 0.05$). Chroma (C *) is a quantitative color attribute directly related to the color intensity of the samples. Whiteness indices (WI) correlate closely with consumers' preferences for white colors. Yellowness index (YI) indicates the degree of yellowness (Pathare, Opara & Al-Said, 2013). The results indicate that requeijão cremoso manufactured with GOS presented a dark and yellow color. In addition, the product with addition of 4 g GOS/100 g (sample RIII) had a more intense color. Such color variation was expected as the GOS syrup used in the experiment had yellowish coloration.

3.2. Rheology

3.2.1. Flow curves

Fig. 1 shows the typical steady-state flow curves (1A) and the apparent viscosity (1B). The samples showed similar behavior; however, the control (RC) presented higher values of shear stress by deformation rate and viscosity (for strain rates <20 s^{-1}), indicating a possible loss of viscosity with the incorporation of GOS.

Table 2 presents the results for the rheological and melting characteristics of the requeijão cremoso samples. The addition of GOS significantly increased the consistency index of samples RII and RIII, whereas the RI sample presented the lowest consistency index ($p \leq 0.05$). The consistency index (k), in processed cheeses, is related to interactions and intermolecular attraction forces between protein particles, suggesting that, due to the water retention capacity of GOS (>3 g/100 g), there was a decrease in the mobility of the protein matrix and an increase in consistency.

All samples showed pseudoplastic behavior ($n < 1$) in which the apparent viscosity decreases with the increase of the applied shear rate (Fig. 1B). Macromolecules (protein and fat) tend to orient in the direction of the fluid, decreasing the flow resistance when the shear rate is increased. The samples RII and RIII had significantly lower n values than the other samples ($p \leq 0.05$) indicating that the addition of fibers provided an increase of the pseudoplastic character, that is, GOS provided a more fluid structure.

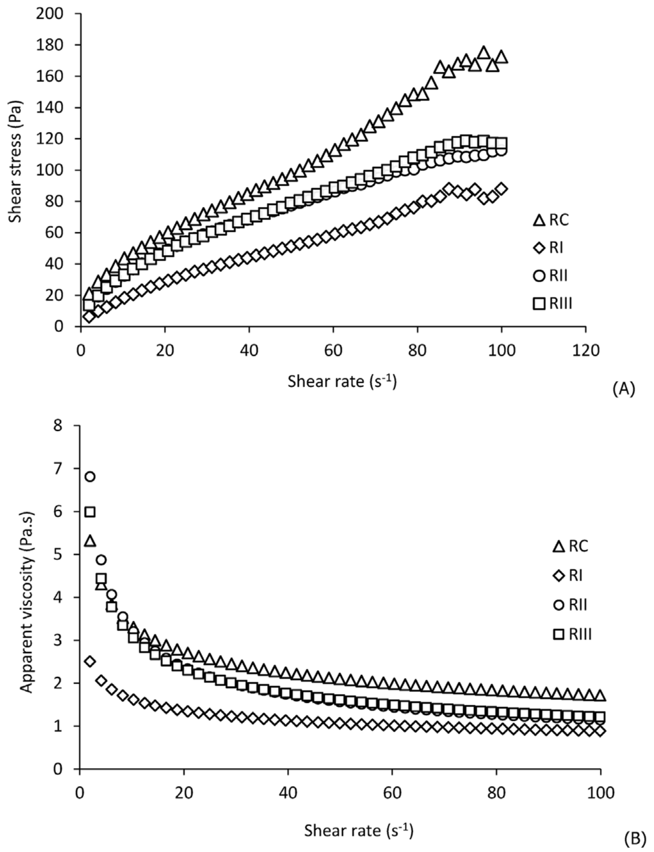

Fig. 1. Flow curves (A) e apparent viscosity (B) of *requeijão cremoso* processed cheese added with galactooligosaccharide RC, RI, RII, RIII = see text.

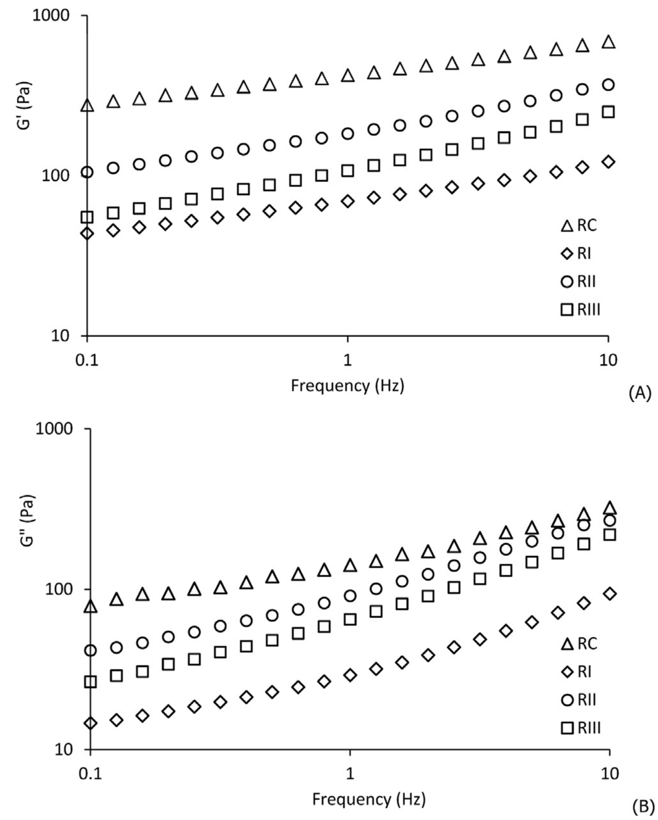

Fig. 2. Elastic (A) and viscous modulus (B) of *requeijão cremoso* processed cheese added with galactooligosaccharide. RC, RI, RII, RIII = see text.

Table 2
Rheological Parameters, spin-spin relaxation times (ms), domain percentage (%) and melting (cm) of *requeijão cremoso* processed cheese added of galactooligosaccharide.*

Parameters	RC	RI	RII	RIII
k (Pa.s^n)	$6.4^b \pm 0.55$	$2.9^c \pm 0.18$	$9.26^a \pm 0.12$	$7.8^a \pm 0.24$
n	$0.7^a \pm 0.02$	$0.7^a \pm 0.02$	$0.5^a \pm 0.03$	$0.5^a \pm 0.07$
R^2	0.9798	0.991	0.999	0.996
A_F (Pa.s$^{1/z}$)	$456.3^a \pm 2.1$	$77.5^c \pm 0.9$	$211.5^b \pm 2.3$	$15.45^c \pm 1.11$
z (−)	$4.7^a \pm 0.01$	$3.6^b \pm 0.03$	$3.0^b \pm 0.02$	$2.5^c \pm 0.02$
R^2	0.996	0.987	0.993	0.994
Melting (cm)	$6.1^b \pm 0.2$	$7.9^a \pm 0.9$	$7.2^a \pm 0.3$	$7.9^a \pm 0.3$
$T_{2,1}$ (ms)	40.6^a	47.3^a	47.5^a	46.1^a
A1 (%)	$84.7^a \pm 0.7$	$83.9^a \pm 0.6$	$82.2^b \pm 0.7$	$80.2^c \pm 0.2$
$T_{2,2}$ (ms)	139^a	137^a	140^a	127.5^b
A_2 (%)	$15.3^c \pm 0.7$	$16.1^c \pm 0.6$	$17.9^b \pm 0.7$	$19.8^a \pm 0.2$

* Values are expressed ± standard deviation. Analysis performed in triplicate. Different letters at the same line indicate statistical difference according the Tukey test ($p < 0.05$). RC, RI, RII, RIII = see text.

3.2.2. Oscillatory tests and melting index

The storage (G′) and loss (G″) moduli represent the relative degrees of elastic and viscous behavior of viscoelastic materials. If G′ > G″, a processed cheese exhibits elastic (gel) properties, and when G′ < G″, a processed cheese has viscous properties (Silva, Ferreira, Bruschi, Britten & Matumoto-Pinto, 2016). As shown in Fig. 2, requeijão cremoso cheeses are viscoelastic systems as G′ > G″, indicating that solid-type structures were present. The increase in frequency leads to an increase in the G′ and G″ values. However, the slope of the line was slightly higher in the samples RII and RIII, indicating that these cheeses have rheological properties more susceptible to the variation of the frequency.

The control sample showed higher G′ and G″ values than the other formulations, followed by samples RII, RIII and RI. These data indicate that the addition of GOS made the cheeses less elastic and viscous, probably because of interactions between the protein aggregates and GOS in the continuous phase (Florencia, 2013).

Fig. 3A shows the tangent of the phase angle (tan δ = G″/G′), which is useful to have a direct view of the solid/liquid behavior of the formulations when they are subjected to oscillatory deformation. When tan δ > 1, a liquid-like behavior is attributed to the sample, whereas tan δ < 1 is associated with a solid-like behavior (Silva et al., 2016). All samples presented low values of tan δ (<0.5), indicating that all cheese formulations maintained a solid-like behavior throughout the frequency range tested. Probably, the intermolecular bonds did not have enough time to break during the period of oscillation and, for this reason, the network behaved as a gel (Cunha et al., 2013). The tan δ values tend to increase when the frequency is increased, indicating that there was not enough time for relaxation between the bonds in the matrix, which indicates typical behavior of a weak gel.

The addition of GOS caused an increase of tan δ, indicating a decrease of the elastic properties in relation to the viscous properties. This behavior indicates that the addition of GOS interfered in the formation of the protein network by decreasing the gel strength, making the product more fluid. This observation can be corroborated by the analysis of the complex modulus (G*) (Fig. 3B), by the model proposed by Winter and Chambon (1986) (Table 2), and the interaction factor (z): the higher the value of these parameters, the greater the firmness of the samples. Therefore, sample RC (negative control) was the strongest sample followed (descending order) by samples RII, RIII and RI. These data indicate that the weakening of the intermolecular interactions with the addition of the fibers leads to a decrease in gel strength and, consequently, impacts on a decrease in the rigidity of cheeses. Although the sample RII pre-

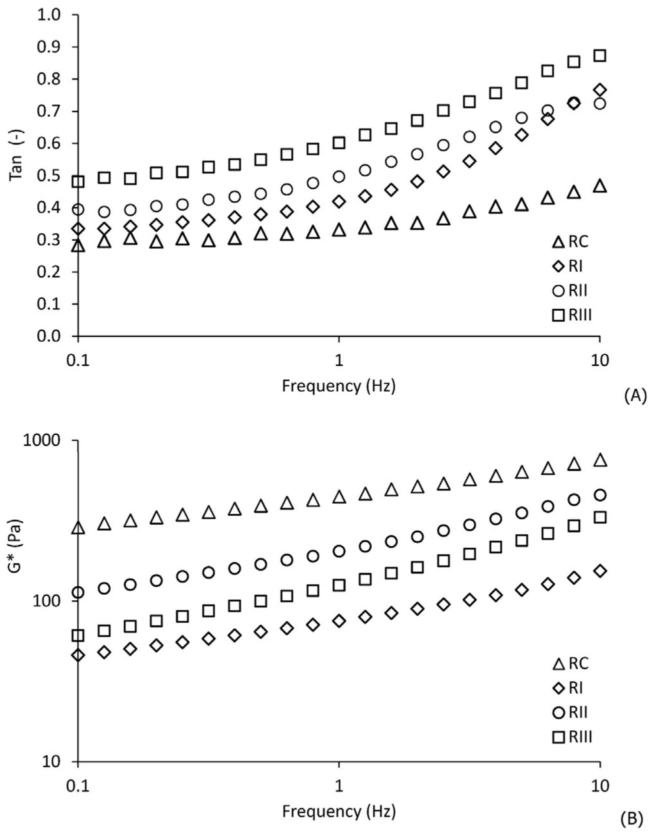

Fig. 3. Tangent of the phase angle (tan δ) (A) and complex modulus (B) of *requeijão cremoso* processed cheese added with galactooligosaccharide. RC, RI, RII, RIII = see text.

sented AF values twice as low as the sample RC, the addition of 3 g GOS/100 g impacted on the most similar behavior that of the control.

The addition of GOS increased the melting index of the products (RI, RII and RIII) when compared to the control (sample RC) ($p \leq 0.05$). When the product is consumed as spreadable processed cheese, the melting index is not an important attribute. When it is used as an ingredient for frozen food and snack fillings, it is important that the melting index is neither too high nor too low, so that cheese maintains part of its structure when submitted to high temperatures in the oven (Cunha et al., 2010). Probably, the casein of the samples manufactured with GOS was more disassociated and the structure was less intact, and the product flowed with less difficulty (Cunha et al., 2010).

3.3. Time domain nuclear magnetic resonance

TD-nuclear magnetic resonance properties can give relevant information about the dynamics of water through the measure of transverse relaxation time (T_2), which correlates with total water content and helps in quantifying the impact of ingredients and processing steps on the properties of the final product (Santos, Pereira-Filho & Colnago, 2016). Thus, the water mobility using TD-NMR is a useful and practical tool in studies of molecular interactions and relaxations existing in dairy products (Balthazar et al., 2017). It is reported that the moisture in processed cheese is mainly bound water combined with the fat globule and hydrated casein (Ferrão et al., 2016). Due to the large amount of hydroxyl groups present in the galactooligosaccharide molecule, its hydrophilic character is expected to lead to an increase in the hygroscopicity of the product. Additionally, as any processed cheese, the structure of requeijão cremoso essentially consists of a fat phase evenly dispersed in the form of fat globules in a partially dispersed casein gel network, in which the total water content (in this case, the moisture) is represented by the bound water associated with the fat globule and hydrated casein (Chen & Liu, 2012).

In the region represented by $T_{2,1}$, there was an increase in the mobility of protons from fat and structural water, and a progressive and significant decrease ($p < 0.05$) of those fractions (A1) in the spreadable cheeses with higher concentrations of GOS (Table 2). Possibly, GOS molecules acted as a kind of “plasticizer”, that is, a physical interaction between the long chains of fatty acids increased the space between these molecules, which favored molecular motility of structural water.

On the other hand, the domain represented by free water showed a tendency to reduce the time ($T_{2,2}$) in relation to the control cheese and impacted on a significant increase ($p < 0.05$) of free water (A_2) in the processed cheeses with higher concentrations of GOS. This fact probably occurred due to the incorporation of moisture contained in the GOS syrup. In addition, the change between calcium and sodium with the simultaneous heating and shearing leads to hydration and dispersion of the caseinate, where the soluble caseinate covers the fat globules, resulting in the emulsification (Chen & Liu, 2012). Thus, during the cooling stage, the partially dispersed caseinate matrix forms “flocks” and the flocks subsequently interact to form a uniform, closely knit, gel network (Zhong & Daubert, 2004).

Overall, the increasing concentration of GOS contributed to higher moisture absorption, explained by the increase of the $T_{2,2}$ value in the requeijão cremoso manufactured with 3 g GOS/100 g. Simultaneously, the water absorption that occurred in the cheeses manufactured with GOS reduced the relaxation time compared to the control sample.

3.4. Scanning electron microscopy

Fig. 4 shows the Scanning Electron Microscopy (SEM) of the *requeijão cremoso* samples. The samples had empty spaces in the protein matrix indicating the initial presence of fat particles, which were extracted during the preparation for analysis. These interruptions of the protein matrix are caused by the fat globules contributing decisively to the desirable creamy texture of processed cheeses (Mistry & Anderson, 1993). There is also a large number of fat globules eventually distributed by the protein matrix with varying sizes and shapes, producing a “sponge” or “honeycomb” structure.

The sample RII (3 g GOS/100 g, 4C) was different from the structural standpoint in relation to the sample RC. Sample RII presented a denser and more compact structure and had a reduced number and size of the fat globules, corroborating the rheological results. As the protein matrix in products manufactured with GOS did not have open structure, the protein molecules showed reduced resistance to flow and presented a low apparent viscosity (Fox, 2000). Additionally, these data show that even the emulsification reduced the dimensions of the fat particles. In turn, RC (negative control – without GOS) presented an open conformation, increased resistance to flow and thus high apparent viscosity, while in sample RIII (4 g GOS/100 g) the protein matrix presented a more open microstructure, with larger fat globules. This observation may be related not only to the high fiber concentration but also to the type of fiber that reduced the number of bonds between the casein chains, which favored the porous and open structure of the protein matrix.

3.5. Sensory analysis

Table 3 presents the sensory data. The requeijao cremoso added with 4 g GOS/100 g (sample RIII) showed the lowest acceptance of

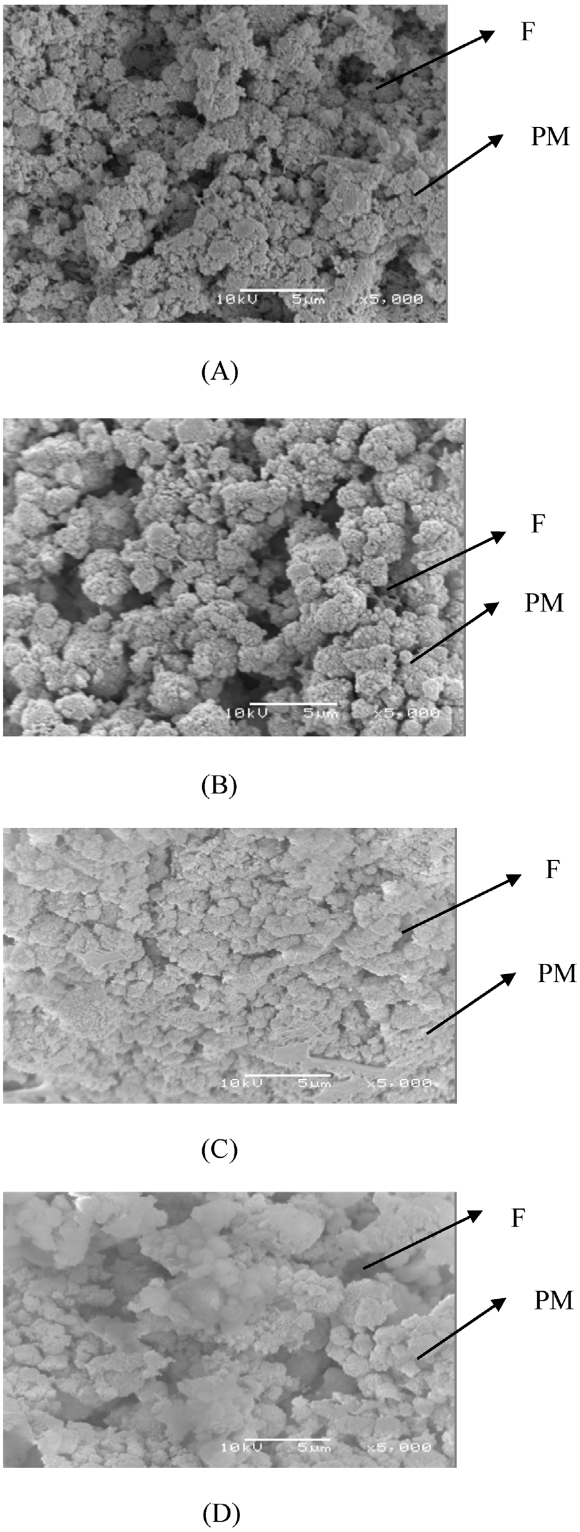

Fig. 4. Scanning electron microscopy (SEM) of *requeijão cremoso* processed cheese (A) RC; (B) RI, (C) RII e (D) RIII. Increase of 5000×, 10 kv. F represents the void space of fat globule, PM represents the protein matrix.

appearance ($p \leq 0.05$), indicating that the more intense yellow color had a negative impact on the sensory attribute.

There was no significant effect of GOS on the acceptance of the requeijão cremoso samples ($p > 0.05$), indicating that the addition of the fibers resulted in changes only in the rheological characteristics of the products. However, the addition of GOS (3 and 4 g/100 g)

Table 3
Sensory acceptance of *requeijão cremoso* processed cheese added of galactooligosaccharide.*

Samples	Appearance	Aroma	Taste	Texture	Overall liking
RC	$7.1^{a} \pm 0.6$	$6.1^{b} \pm 1.2$	$6.8^{b} \pm 0.6$	$6.7^{a} \pm 0.8$	$7.0^{a} \pm 0.8$
RI	$7.2^{a} \pm 1.1$	$6.3^{b} \pm 1.7$	$7.1^{b} \pm 1.4$	$6.5^{a} \pm 0.2$	$7.2^{b} \pm 1.4$
RII	$7.3^{a} \pm 0.8$	$7.7^{a} \pm 1.6$	$8.3^{a} \pm 0.7$	$6.9^{a} \pm 11$	$7.7^{a} \pm 0.3$
RIII	$7.8^{b} \pm 0.4$	$7.6^{a} \pm 1.3$	$8.5^{a} \pm 0.9$	$7.1^{a} \pm 1.2$	$7.8^{a} \pm 1.4$

* Values are expressed ± standard deviation. Different letters at the same column indicate statistical difference according the Tukey test ($p < 0.05$). Mean data from 80 consumers and based on a 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). RC, RI, RII, RIII = see text.

enhanced the aroma and taste of requeijão cremoso samples, when additions. Taste was highly affected by the different concentrations of GOS, in which RIII stood out positively with a mean of 8.5, while the control curd (RC) presented a mean of 6.8. GOS has 0.3–0.6 the sweet taste of sucrose, because it contains oligosaccharides and hydrolyzed sugars (Torres et al., 2010). It is widely accepted that oligosaccharides, such as GOS, have a bland neutral taste without any off-flavor or aftertaste. Therefore, GOS can be combined with other food ingredients without modifying delicate flavors and may enhance the natural flavor of some products (Tárrega & Costell, 2006). Furthermore, the compounds responsible for the aroma of the products manufactured with GOS may be released more quickly in the mouth.

Considering that approximately 80% of the consumers were young (18–30 years old), consumers of *requeijão cremoso* at least 2 times a week, the requeijão cremoso samples manufactured with GOS garnered satisfactory mean values for all evaluated sensory attributes and, therefore, were well accepted by the selected consumers. In this sense, this study presents relevant technological contribution (rheology and sensory acceptance) of GOS as a potential ingredient for the manufacture of requeijão cremoso processed cheese.

4. Conclusion

The GOS addition did not provide significant changes in the proximal composition and the chemical characteristics of requeijão cremoso samples. However, the addition of higher levels (3 and 4 g GOS/100 g) showed more uniform and less porous microstructure, improved softness and spreadability (decrease of G′, G″ and an increase of tan δ and melting index) and improvement in the aroma and taste. Therefore, our findings indicate that GOS may be a potential option as ingredient to be used at *requeijão cremoso* processed cheese formulation for dairy factories, adding a functional value at this product. Future studies should investigate the effect of GOS addition on the sensory characteristics of the products using a trained panel, to confirm its role on the improvement of the mouthfeel and possible enhancement of the natural flavor of the products. Furthermore, a clinical study (considering animal model or humans) should be useful to evaluate the impact of the repeated ingestion of the product at the consumer's health.

References

Balthazar, C. F., Silva, H. L. A., Vieira, A. H., Neto, R. P. C., Cappato, L. P., Coimbra, P. T., et al. (2017). Assessing the effects of different prebiotic dietary oligosaccharides in sheep milk ice cream. *Food Research International*, *91*, 38–46.

Brasil. (1997). Ministério da Agricultura e do Abastecimento. *Regulamento Técnico para Fixação de Identidade e Qualidade do Requeijão Cremoso ou Requesón.* Available in www.agricultura.gov.br.

Brasil. (2006). Ministério da Agricultura, Pecuária e Abastecimento. Oficializa os Métodos Analíticos Oficiais Físico- Químicos para Controle de Leite e Produtos Lácteos. Available in www.agricultura.gov.br.

Bruno-Barcena, J. M., & Azcarate-Peril, M. A. (2015). Galacto-oligosaccharides and colorectal cancer: Feeding our intestinal probiome. *Journal of Functional Foods*, *12*, 92–108.
Chen, L., & Liu, H. (2012). Effect of emulsifying salts on the physicochemical properties of processed cheese made from Mozzarella. *Journal of Dairy Science*, *95*, 4823–4830.
Cunha, C. R., Dias, A. I., & Viotto, W. H. (2010). Microstructure texture, color and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. *Food Research International*, *43*, 723–729.
Cunha, C. R., Grimaldi, R., Alcantara, M. R., & Viotto, W. H. (2013). Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. *International Journal of Dairy Technology*, *66*, 54–62.
Drake, M. (2007). Sensory analysis in dairy foods. *Journal of Dairy Science*, *90*, 4925–4937.
FAO/AGNS. (2007). *FAO technical meeting report on prebiotics*. Available in: http://www.fao.org/ag/agn/agns/files/Prebiotics_Tech_Meeting_Report.pdf.
Fernandes, R., do Rosario, V. A., Mocellin, M. C., Kuntz, M. G. F., & Trindade, E. B. S. M. (2016). Effects of inulin-type fructans, galacto-oligosaccharides and related synbiotics on inflammatory markers in adult patients with overweight or obesity: A systematic review. *Clinical Nutrition* [in press]. https://doi.org/10.1016/j.clnu.2016.10.003
Ferrão, L. L., Silva, E. B., Silva, H. L. A., Silva, R., Silva Mollakhalili, N., Granato, D., et al. (2016). Strategies to develop healthier processed cheeses: Reduction of sodium and fat contents and use of prebiotics. *Food Research International*, *86*, 93–102.
Florencia, F. S. (2013). Rheology of spreadable goat cheese made with autochthonous lactic cultures differing in their ability to produce exopolysaccharides. *Food Science and Technology*, *33*, 233–238.
Halle, B. (2004). Protein hydration dynamics in solution: A critical survey. *Philosophical Transactions of The Royal Society B*, *359*, 1207–1224.
Hills, B. P., Takacs, S. F., & Belton, P. S. (1990). A new interpretation of proton NMR relaxation time measurements of water in food. *Food Chemistry*, *37*, 95–111.
Maawia, K., Iqbal, S., Qamar, T. R., Rafiq, P., Ullah, A., & Ahmad, M. (2016). Production of impure prebiotic galacto-oligosaccharides and their effect on calcium, magnesium, iron and zinc absorption in Sprague-Dawley rats. *PharmaNutrition*, *4*, 154–160.
Mistry, V. V., & Anderson, D. L. (1993). Composition and microstructure of commercial full fat and low fat cheeses. *Food Structure*, *12*, 259–266.
Monteagudo-Mera, A., Arthur, J. C., Jobin, C., Keku, T., Bruno-Barcena, J. M., & Azcarate-Peril, M. A. (2016). High purity galacto-oligosaccharides enhance specific *Bifidobacterium* species and their metabolic activity in the mouse gut microbiome. *Beneficial Microbes*, *7*, 247–264.
Morel, F. B., Dai, Q., Ni, J., Thomas, D., Parnet, P., & Fança-Berthon, P. (2015). α-Galacto-oligosaccharides dose-dependently reduce appetite and decrease inflammation in overweight adults. *Journal of Nutrition*, *145*, 2052–2059.
Pathare, P. B., Opara, U. L., & Al-J. Al Said, F. (2013). Colour measurement and analysis in fresh and processed foods: A review. *Food Bioprocessing Technology*, *6*, 36–60.
Ramos, F. M., Haddad, F. F., Ramos, R. A., & Pinto, S. M. (2012). Adequacy of creamy requeijão cheese to the Standards of identity and quality and the labeling Standards. *Revista do Instituto de Laticínios Cândido Tostes*, *389*, 5–14.
Santos, P. M., Pereira-Filho, E. R., & Colnago, L. A. (2016). Detection and quantification of milk adulteration using time domain nuclear magnetic resonance (TD-NMR). *Microchemical Journal*, *124*, 15–19.
Silva, D. F., Ferreira, S. B. D., Bruschi, M. L., Britten, M., & Matumoto-Pintro, P. T. (2016). Effect of commercial konjac glucomannan and konjac flours on textural, rheological and microstructural properties of low fat processed cheese. *Food Hydrocolloids*, *60*, 308–316.
Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, *22*, 1417–1435.
Tárrega, A., & Costell, E. (2006). Effect of inulin addition on rheological and sensory properties of fat-free starch-based dairy desserts. *International Dairy Journal*, *16*, 1104–1112.
Torres, D. M. P., Gonçalves, M. P. F., Teixeira, J. A., & Rodrigues, L. R. (2010). Galacto-oligosaccharides: production, properties, applications: and significance as prebiotics. *Comprehensive Reviews in Food Science and Food Safety*, *9*, 438–454.
Vénica, C. I., Bergamini, C. V., Rebechi, S. R., & Perotti, M. C. (2015). Galacto-oligosaccharides formation during manufacture of different varieties of yogurt: Stability through storage. *LWT –*, *63*, 198–205.
Van Dender, A. G., Spadoti, L. M., Zacarchenco, P. B., Galina, D. A., & Alves, A. T. S. (2012). Requeijão cremoso: pesquisas e inovações tecnológicas do século XXI. *Leite & Derivados*, *133*, 86–97.
Van Dender, A. G. F. (2006). Requeijão: aspectos gerais. In A. G. F. Van Dender (Ed.), *Requeijão cremoso e outros queijos fundidos: tecnologia de fabricação, controle do processo e aspectos de marcado*. São Paulo: Fonte Comunicações e Editora Ltda.
Veiga, P. G., Cunha, R. L., Viotto, W. H., & Petenate, A. J. (2000). Caracterização química, reológica e aceitação sensorial do queijo petit suisse brasileiro. *Sociedade Brasileira de Ciência e Tecnologia de Alimentos*, *20*, 349–357.
Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *Journal of Rheology*, *30*, 367–382.
Zhong, Q., & Daubert, C. R. (2004). Kinetics of rennet casein gelation at different cooling rates. *Journal of Colloid and Interface Science*, *279*, 88–94.