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Developing a prebiotic yogurt: Rheological, physico-chemical and microbiological aspects and adequacy of survival analysis methodology

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ABSTRACT

The addition of prebiotics such as oligofructose to yogurt can result in a product with consumer benefits, since they stimulate growth of benefic bacteria present in the intestine and also provide a low calorie product, since one can add less sugar to the formulation due to their sweetening power. This work aimed to evaluate the effect of increasing concentrations of oligofructose addition on physicochemical, rheological and microbiological characteristics of non-flavored yogurt. Furthermore, it was investigated the reaction of consumers with the use of the survival analysis methodology. The addition of oligofructose showed no influence on the pH, proteolysis or the viability of *Streptococcus thermophilus* or *Lactobacillus bulgaricus* during 28 days of refrigerated storage ($p > 0.05$). According to rheological measurements the yogurt supplemented with oligofructose was characterized as a weak gel, showing thixotropic and pseudoplastic behavior. Survival analysis was used to investigate consumer responses with respect to different levels of supplementation of plain yogurt with oligofructose (0%, 2%, 4%, 6% and 8% wt. v^{-1}). Using the survival analysis and considering a rejection by 25% of the consumers, the level of oligofructose that can be added to the yogurt was shown to be 2.58% wt. v^{-1} .

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1. Introduction

Oligofructose is a short-chain inulin, a linear non-digestible oligosaccharide composed of linear fructose units connected to each other by β bonds ($2 \rightarrow 1$) with a terminal glucose unit, obtained from the partial enzymatic hydrolysis of native inulin with endoinulinase presenting a polymerization degree oscillating between 2 and 7 (Roberfroid, 2002; Villegas and Costell, 2007). It is a non-toxic and safe human additive (Bolye et al., 2008), whose beneficial effects on human health are not only limited to its use as a dietetic fiber (intestinal traffic control, reduction of cholesterol and increase in calcium absorption) (Waligora-Dupriet et al., 2007; Rao, 2001), but also the benefits derived from its prebiotic nature. Prebiotic ingredients, as oligofructose, promote stimulation of growth and/or activity of colonic bacteria and the regulation of intestinal

flora in the colon, as well as inhibiting the growth of pathogens and harmful microorganisms (Tárrega et al., 2010).

In addition to its beneficial effects on human health as dietetic fiber, prebiotic ingredient and satiety responses (Hess et al., 2011), oligofructose has interesting effects as a low-calorie sweetener. The oligofructose is much more soluble, sweeter and less caloric than native inulin and can be used for partial sucrose replacement (Villegas and Costell, 2007; Wang, 2009). Regarding sensory aspects, oligofructose neither crystallizes nor precipitates and does not leave a dry or sandy sensation in the mouth, showing a viscosity similar to that of sucrose. Furthermore, oligofructose is not degraded by heat treatment, and can be used in processes that use temperatures of up to approximately 140 °C (Wada et al., 2005). Several studies involving the addition of prebiotic ingredients to dairy products have reported a positive effect, both on the growth of probiotic bacteria and on the sensory, rheological and physico-chemical attributes (Cunha et al., 2008; Castro et al., 2009a,b; Oliveira et al., 2009; Arcia et al., 2011; Gonzalez et al., 2011; Debon et al., 2012). However, there is still little information available about the effects of oligofructose addition on the rheological and sensory characteristics of dairy products.

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The understanding of the underlying needs, values and insights of the consumers are the key to new product development, especially functional foods, which it means the consumer perception should be implemented in a consequent way to food products, delivering a health or wellness benefit (Biel, 2010). In this context, survival analysis can be faced with a useful sensory methodology (Hough and Garitta, 2012), as it takes in account the consumer interaction with the food product, resulting in a processed product with optimized formulation, and adequate acceptance by the consumers (Hough, 2010). One differential of this methodology is the presence of censured data, which occurs when the event of interest cannot be observed with exactness, and its occurrence is only known during a certain time interval (Hough and Garitta, 2012).

In this context, the possibility of finding an optimized formulation of yogurt added of oligofructose through survival analysis methodology seems interesting; In addition, it is important the evaluation of the intrinsic quality parameters along the shelf life is also essential. It will proportionate the development of an acceptable product according with potential benefits for the consumers, identifying the changes which occurs along the refrigerated storage. This information can be useful for functional foods processors which added prebiotic ingredients in the products. Therefore, this study aimed to evaluate the effect of the addition of increasing oligofructose concentrations on physicochemical, rheological and microbiological characteristics of plain yogurt. Furthermore, consumers responses were evaluated using the survival analysis methodology.

2. Material and methods

2.1. Materials

Raw milk (3.4% fat w/w) was used for yogurt processing. The oligofructose was obtained from Raftilose, P95-BENEO, Orafiti, Oreye, Belgium. The freeze-dried lactic cultures *Streptococcus thermophilus* TA040 and *Lactobacillus bulgaricus* LB 340 were purchased from Danisco, Sao Paulo, Brazil.

2.2. Yogurt processing

The prebiotic plain yogurt were processed according to Cruz et al. (2010a) with some modifications. Oligofructose (Raftilose P95- BENEO, Orafiti, Oreye, Bélgica) was added in the proportions 0%, 2%, 4%, 6% and 8% wt. v^{-1} (Y_0 , Y_2 , Y_4 , Y_6 and Y_8) to 10 L of raw milk (Atilatti, Itatiba, Brazil) previously standardized with skimmed milk powder at 3.5% wt. v^{-1} (Molico, São Paulo, Brazil). The oligofructose dosage was based in preliminary test and economical considerations. Subsequently, the mixture was submitted to heat treatment at 95 °C for 15 min, cooled to 45 °C and inoculated with 1% wt. v^{-1} ($6-7 \log CFU mL^{-1}$) of freeze-dried lactic cultures of *S. thermophilus* TA 040 and *L. delbrueckii* spp. *bulgaricus* LB 340. These operational parameters have been employed before in previous works (Cruz et al., 2010a,b,c; Cruz et al., 2012a,b). The fermentation of the inoculated milk at 45 °C was monitored and performed until pH value of 4.6 ± 0.05 . Then, the mixture was cooled to 10 °C and the gel was broken manually by stirring for 10 min (Cruz et al., 2012a,b). Finally, the prebiotic yogurts were packaged in 200 mL polypropylene cups (PP permeability of 0.20 cm^3 of O_2 per pot.day, Dixie Toga, São Paulo, Brazil) and stored at 5 °C (± 0.2) during 28 days.

2.3. Physicochemical and microbiological analysis

The yogurts supplemented with different concentration of oligofructose were subjected to the physicochemical and microbio-

logical analysis at 1, 7, 14, 21 and 28 days of refrigerated storage at 5 °C, using methodologies published elsewhere being easily available: pH (Marshall, 1993), proteolytic activity using the OPA method (Church et al., 1983), *S. thermophilus* and *L. bulgaricus* were enumerated using M17 Agar and MRS Agar (pH 4.5; the pH was adjusted using acetic acid) with incubation at 37 °C for 48 and 72 h, respectively (Cruz et al., 2012a,b).

2.4. Rheological analysis

The rheological analyses were carried out on days 1 and 28 of refrigerated storage by a controlled tension rheometer (TA Instruments, model AR 1500ex, USA) aimed to give a overall idea of the effect of the oligofructose in the yogurt matrix. (Fritzen-Freire et al., 2010). The flow curves and the oscillatory assays was performed using a stainless steel cone and plate geometry with 6 cm of diameter and a gap of 1 mm, determining the flow curves and oscillatory trials. The yogurts were loaded on the inset plate and the temperature was maintained at $5 \text{ °C} \pm 0.1$ during the tests. All trials were carried out in triplicate.

The flow curves of the yogurts were determined by scanning between deformation rates of 0 and 300 s^{-1} , submitting them to three flow ramps (up, down and up-cycles) in order to evaluate and eliminate the effect of thixotropy. The first and second up-cycle were denominated as increasing I and II curves, respectively, and they were fitted to the Power Law (Eq. (1)) model using the software Statistica 5.0 (Statsoft, Tulsa, USA). The thixotropic behavior of the samples was evaluated by estimating the hysteresis loop area ($A_{up} - A_{down}$) between the upward (A_{up}) and downward (A_{down}) flow curves.

$$\sigma = K\dot{\gamma}^n \quad (1)$$

where σ is the tension (Pa), K the consistency index (Pa s^n), $\dot{\gamma}$ the deformation rate (s^{-1}), and n the flow behavior index (dimensionless).

The oscillatory trials were carried out with maximum deformation of 1% and the frequency varied between 0.01 and 10 Hz. The parameters assessed in all samples were: storage (G') and loss (G'') moduli, as previous studies covering yogurts (Ramirez-Santiago et al., 2010; Peng et al., 2010; Ozcan et al., 2011; Glibowski and Kowalska, 2012).

2.5. Consumer test

One hundred and twenty consumers (Drake, 2007; Cruz et al., 2011), all habitual yogurt consumers, were recruited at random through notices in various places throughout the campus of the University of Campinas (UNICAMP), Brazil. They were invited to take part in an acceptance test for six yogurts supplemented with different oligofructose concentrations. Each sample contained 30 mL and was presented monadically in coded plastic cups in a random order. The first-order and carry-over effects were balanced using a Randomized Complete Block Design, where each consumer evaluated all the yogurt samples (MacFie et al., 1989). The consumers were asked to reply to the following question in a dichotomous way (yes or no): "Would you normally consume this product?". These answers were used to calculate the oligofructose concentration to be added to the yogurt formulation according to the survival analysis methodology (Libertino et al., 2011). The test was carried out under controlled conditions, with mineral water and cream crackers being made available to the consumers. As our main purpose was to find an optimal concentration of oligofructose at the yogurt formulation applying survival analysis methodology, any hedonic affective question was performed in this consumer test.

2.6. Statistical analysis

The experiment was repeated twice, being the physicochemical, microbiological and rheological data were obtained at least in triplicate. The results were submitted to a one-way analysis of variance (ANOVA) to identify contrasts amongst the yogurt samples, followed by Tukey's test with a significance level of 5.0%. All these analyses were carried out using the XLSTAT 2011 (Addinsoft, New York, NY, USA).

The consumer reactions to the different oligofructose levels in the yogurt were evaluated using the survival analysis, and the theoretical basis of this methodology is widely available (Hough, 2010). The SAS system (version 9.1.3, SAS Institute Inc, Cary, NC, USA) was used and the following parameters were defined:

- ✓ O : random variable, representing the level (percentage) of oligofructose added to the sample which resulted in consumer rejection of the sample.
- ✓ $F(o)$: rejection function, representing the probability of a consumer reject a yogurt with an oligofructose level below o , that is, $F(o) = P(O \leq o)$.
- ✓ $S(o)$: rejection function, representing the probability of a consumer reject a yogurt with an oligofructose level below o , that is, $S(o) = P(O > o)$.

In the present work, the event of interest was rejection by the consumer with respect to purchasing a yogurt with a determined concentration of oligofructose, and this event was achieved when, from the sensory point of view, the shelf life of the products depends on their interaction with the consumer (Hough, 2010). It is also known that $F(o) = 1 - S(o)$. Since the data were discreet, the random variable O can never be observed exactly, due to the presence of censure. The discreet statistical distributions (Weibull, Lognormal, Logistic, Logrank) were fitted to the data obtained in the sensory test, and the best fit (obtained by a visual inspection of the curve) was used to express $F(o)$. The optimum level of oligofructose in the yogurt was obtained after constructing a graph with the level of oligofructose in the yogurt on the abscissa (x), and consumer rejection in percent data on the ordinate (y), subsequently considering the following values: 10%, 25% and 50% consumer rejection (Hough et al., 1999, 2003; Gimenez et al., 2007; Cruz et al., 2010b).

3. Results and discussion

3.1. Physicochemical and microbiological analysis

No effect of the addition of oligofructose on the various parameters analyzed was observed ($p > 0.05$). In general similar post-acidification and proteolysis were observed, with values for pH and proteolysis along 28 days of refrigerated storage varying between 4.1 and 4.2 and 0.789 and 0.802, respectively. Such results suggest a poor post-acidification which is a desirable characteristic in a modern yogurt industry, being related to the choose of the metabolism of latic culture used. With respect to the microbiological analyses, counts of 9.34 and 9.56 and 8.56 and 8.21 log CFU mL⁻¹ were observed for *S. thermophilus* and *L. bulgaricus*, respectively, which are in accordance with the Brazilian legislation which establishes a minimum of 7 log CFU mL⁻¹ for the lactic bacteria count to the fermented milk is considered a yogurt (Brasil, 2007). Similar results were reported in recent works (Isik et al., 2011; Marafon et al., 2011; Elizaquível et al., 2011; Kim et al., 2011).

In addition, these results suggest that the oligofructose was not used by these microorganisms as a nutrient source. This was ex-

pected since only probiotic microorganisms are able to metabolize this ingredient (Heydari et al., 2011; Kemal-Seckin and Yeliz-Ozkilinc, 2011; Gonzalez et al., 2011).

3.2. Rheological properties

3.2.1. Thixotropy and steady-state rheology

Fig. 1(A and B) show the flow curves for the prebiotic yogurts supplemented with different levels of oligofructose along the 28 days of refrigerated storage. It can be seen that all yogurts showed thixotropic and pseudoplastic behavior which it can be observed in others researches covering dairy foods (Debon et al., 2010; Castro et al., 2009b; Hennigsson et al., 2006; Paseephol et al., 2008).

Fig. 1A shows that flow curves of all yogurts added with oligofructose presented similar behavior to each other at 1 day of storage, whereas yogurt without added oligosaccharide showed the lowest value of shear stress as compared to others. It can be seen in Fig. 1B that the curve of the yogurt without added prebiotic presented a pronounced increase in shear stress at low values of shear rate (0–50 s⁻¹) in 28 days of storage. On the other hand, all yogurts supplemented with oligofructose showed a similar smooth increase in shear stress with shear rate. The curves of increasing deformation rate were mathematically modeled using the Power law and the results are shown in Table 1.

Thixotropy is detected in especially fragile structures, which the three-dimensional network formed is completely destroyed as in the case of the yogurts (Steffe, 1996). Since the energy required to break the yogurt structure is proportional to the area of hysteresis (Schramm, 1998), thixotropy can be estimated by the difference between the areas under the curves of shear stress-shear rate (increasing I and II) (Steffe, 1996; Sato and Cunha, 2007). This measurement can be used as a qualitative comparison between the different yogurts evaluated, and the values are presented in Fig. 2. According to this figure, the incorporation of 8% of oligofructose significantly increased ($p < 0.05$) thixotropy in the yogurt at 1 day of storage. However, the thixotropy degree did not vary significantly ($p < 0.05$) among the other prebiotic concentrations and yogurt without oligofructose. Likewise, Debon et al. (2010) and González-Tomás et al. (2009) observed an increase in hysteresis loop area with native inulin and oligofructose incorporation in dairy products. On the other hand, Paseephol et al. (2008) and Castro et al. (2009b) noticed a significant ($p < 0.05$) decrease in hysteresis loop area with oligofructose addition in low-fat yogurts and probiotic lactic beverages, respectively. All samples clearly presented a pseudoplastic behavior, showing flow indices ($0 < n < 1$) in the first up-cycle (increasing I curve). Along the storage no yogurt have shown significant difference ($p < 0.05$) in hysteresis loop area, except yogurt without prebiotic and with 8% of oligofructose which presented a significant increase ($p < 0.05$) in thixotropy. Paseephol et al., 2008) observed a decrease in hysteresis during storage in yogurt with added oligofructose (4% wt. v⁻¹). The same variation was not verified for full-fat yogurts without prebiotic. Such differences of results may be related to food matrix, formulation, mathematical model or packaging used.

According to the mathematical modeling results presented in Table 1, it was noticed that the increasing II was the curve that presented the best approach ($R^2 = 0.95$ – 0.99) to Newtonian behavior since n values tend to 1. Thus, data of increasing II curve were used to estimate the apparent viscosity in the stationary state for different shear rates (10, 50, and 100 s⁻¹) whose values were chosen based on literature data (Shama and Sherman, 1973; Bourne, 2002; Tárrega and Costell, 2007). The estimated values of apparent viscosity have demonstrated that the addition of oligofructose increased the apparent viscosity ($p < 0.05$) as compared to the yogurt without added oligofructose for 1 day of storage (Table 2). With

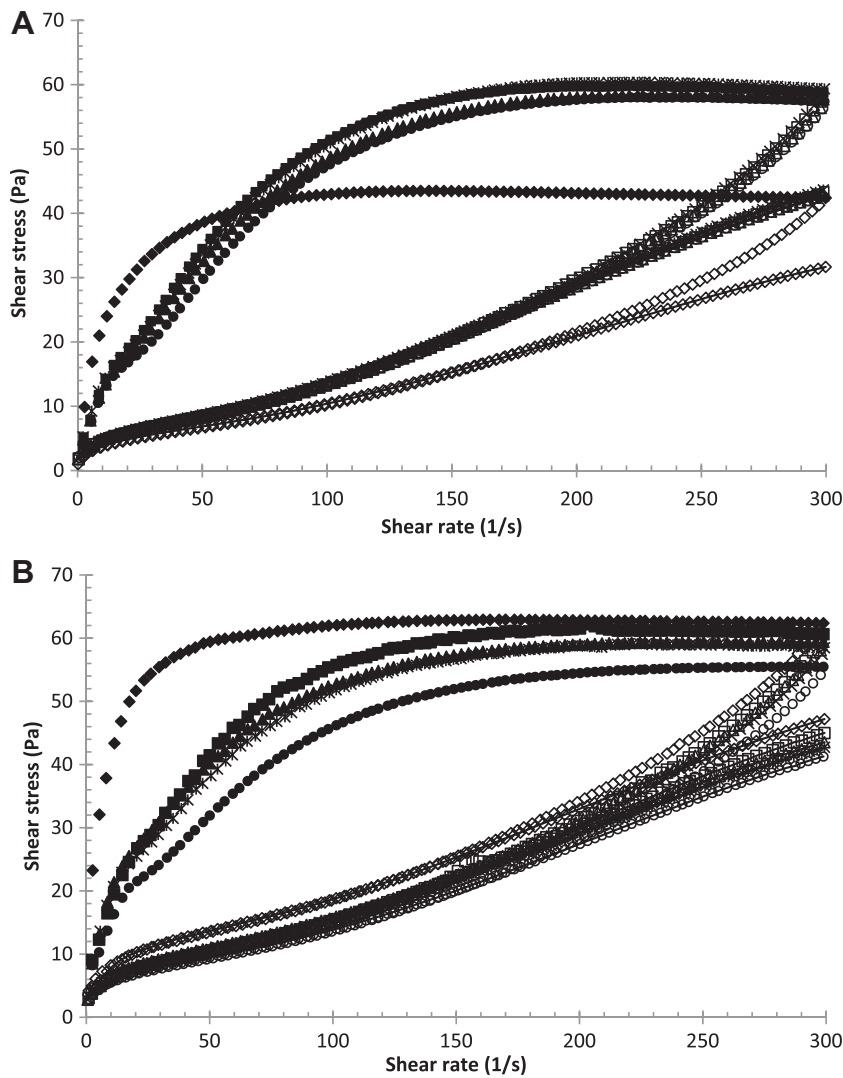


Fig. 1. Flow curves, shear stress versus shear rate for prebiotic yogurt with increasing levels of oligofructose concentration (♦: 0%; ▲: 2%; ●: 4%; *: 6%; ■: 8% wt. v⁻¹, respectively) stored at 5 °C during 1 day (A) and 28 days (B).

Table 1
Rheological parameters obtained using the Power Law model for prebiotic yogurt supplemented with different levels of oligofructose, at 1 day and 28 days refrigerated storage.

Yogurt	Increasing I			Increasing II		
	<i>n</i>	<i>K</i> (Pa s ^{<i>n</i>})	<i>r</i> ²	<i>n</i>	<i>K</i> (Pa s ^{<i>n</i>})	<i>R</i> ²
<i>Day 1</i>						
<i>Y</i> ₀	0.142 ± 0.008 ^a	20.052 ± 0.505 ^a	0.748	0.957 ± 0.009 ^a	0.131 ± 0.009 ^a	0.989
<i>Y</i> ₂	0.346 ± 0.011 ^b	9.237 ± 0.882 ^b	0.909	0.976 ± 0.017 ^a	0.167 ± 0.020 ^a	0.951
<i>Y</i> ₄	0.387 ± 0.011 ^b	7.297 ± 0.259 ^b	0.920	0.985 ± 0.006 ^a	0.157 ± 0.001 ^a	0.990
<i>Y</i> ₆	0.360 ± 0.013 ^b	8.518 ± 0.836 ^b	0.906	1.004 ± 0.064 ^a	0.140 ± 0.008 ^a	0.991
<i>Y</i> ₈	0.349 ± 0.015 ^b	9.087 ± 0.850 ^b	0.900	1.009 ± 0.014 ^a	0.137 ± 0.012 ^a	0.991
<i>Day 28</i>						
<i>Y</i> ₀	0.085 ± 0.013 ^c	40.374 ± 2.734 ^c	0.735	0.756 ± 0.006 ^b	0.610 ± 0.024 ^b	0.979
<i>Y</i> ₂	0.237 ± 0.044 ^d	16.530 ± 3.876 ^d	0.891	0.845 ± 0.002 ^c	0.336 ± 0.008 ^c	0.983
<i>Y</i> ₄	0.311 ± 0.043 ^b	11.010 ± 3.373 ^d	0.936	0.882 ± 0.046 ^a	0.282 ± 0.090 ^c	0.983
<i>Y</i> ₆	0.272 ± 0.017 ^d	14.059 ± 1.837 ^d	0.911	0.850 ± 0.024 ^c	0.340 ± 0.052 ^c	0.983
<i>Y</i> ₈	0.279 ± 0.010 ^d	13.820 ± 0.795 ^d	0.897	0.895 ± 0.010 ^c	0.272 ± 0.015 ^c	0.984

Mean ± standard deviation. *Y*₀, *Y*₂, *Y*₄, *Y*₆, and *Y*₈ represent 0%, 2%, 4%, 6%, and 8% of oligofructose concentration (wt. v⁻¹), respectively. Values followed by different letters (a–d) in the same column indicate significant differences between the formulations on the same storage day, according to the Tukey Test (*p* < 0.05). *k* = consistency index, *n* = flow behavior index, *R*² = coefficient of determination.

respect to yogurts containing added prebiotic, the difference in oligofructose concentration did not influence the apparent viscosity (*p* < 0.05) for the same storage time. This behavior is in accordance

with earlier studies (Kip et al., 2006; Guggisberg et al., 2009; Debon et al., 2010), who reported that the apparent viscosity increased with the addition of inulin and/or oligofructose. This behavior

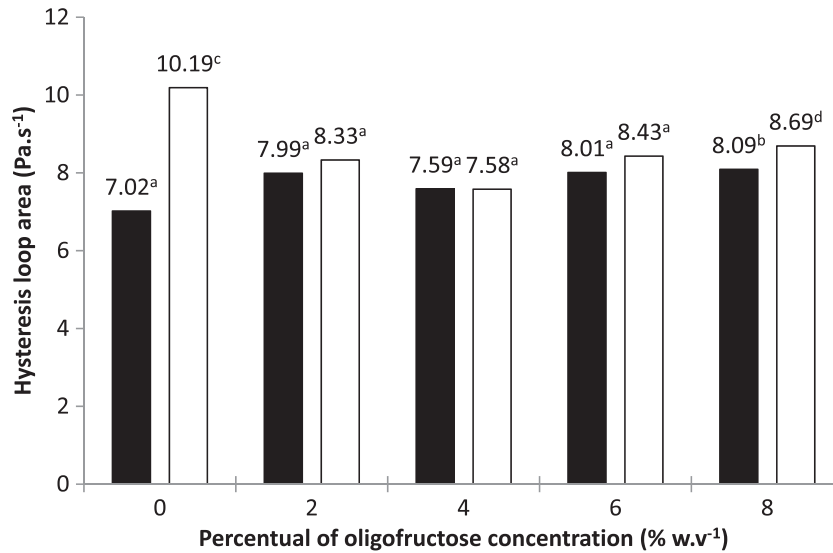


Fig. 2. Hysteresis loop area for prebiotic yogurt supplemented with increasing levels of oligofructose concentration (% wt. v⁻¹) stored at 5 °C during (■) 1 day and (□) 28 days.

Table 2

Apparent viscosity (Pa s) of prebiotic yogurt supplemented with different levels of oligofructose for the steady-state (increasing II).

Shear rate (s ⁻¹)	Day 1				
	Y ₀	Y ₂	Y ₄	Y ₆	Y ₈
10	0.33 ± 0.009 ^{a,A}	0.46 ± 0.029 ^{b,A}	0.46 ± 0.007 ^{b,A}	0.44 ± 0.007 ^{b,A}	0.44 ± 0.015 ^{b,A}
50	0.13 ± 0.004 ^{a,A}	0.18 ± 0.008 ^{b,A}	0.18 ± 0.002 ^{b,A}	0.17 ± 0.005 ^{b,A}	0.17 ± 0.005 ^{b,A}
100	0.10 ± 0.003 ^{a,A}	0.14 ± 0.006 ^{b,A}	0.14 ± 0.003 ^{b,A}	0.13 ± 0.003 ^{b,A}	0.13 ± 0.003 ^{b,A}
	Day 28				
10	0.73 ± 0.016 ^{a,B}	0.59 ± 0.022 ^{b,B}	0.57 ± 0.071 ^{b,B}	0.61 ± 0.033 ^{b,B}	0.59 ± 0.012 ^{b,B}
50	0.27 ± 0.003 ^{a,B}	0.22 ± 0.01 ^{b,B}	0.21 ± 0.023 ^{b,B}	0.22 ± 0.012 ^{b,B}	0.21 ± 0.005 ^{b,B}
100	0.18 ± 0.003 ^{a,B}	0.15 ± 0.004 ^{b,B}	0.15 ± 0.014 ^{b,B}	0.16 ± 0.008 ^{b,B}	0.16 ± 0.002 ^{b,B}

Mean ± standard deviation. Y₀, Y₂, Y₄, Y₆, and Y₈ represent 0%, 2%, 4%, 6%, and 8% of oligofructose concentration (wt. v⁻¹), respectively.

Values followed by different letters (a–d) in the same line indicate significant differences between the formulations on the same storage day, according to the Tukey test ($p < 0.05$).

Values followed by different letters (A and B) in the same column indicate significant differences between the formulations on the same deformation rate, according to the Tukey test ($p < 0.05$).

could be a consequence of the increase in solids content or because the prebiotic takes part in the constitution of the product gel. However, after 28 days of storage the yogurt without prebiotic showed the highest values of apparent viscosity ($p < 0.05$) as compared to the others yogurts. Similar results were achieved by [Castro et al. \(2009b\)](#) which reported a viscosity reduction with the increase in oligofructose concentration. This inconsistent behavior can be related to the influence of several factors during storage such as the water retention capacity ([Soukoulis et al., 2009](#)); interaction with milk proteins ([Schaller-Povolmy and Smith, 2001](#)); formation of small aggregates of microcrystals capable of retaining water ([Gonzalez-Tomás et al., 2008](#)); and by changes in total solids content ([Meyer et al., 2011](#)). These results indicate that the addition of oligofructose had a positive effect in the stability of the apparent viscosity of the yogurts.

3.2.2. Dynamic-shear rheology

[Fig. 3](#) shows the mechanical spectrum of the yogurts. This figure shows that the storage (G') and loss (G'') moduli were little dependent on the frequency, although G' was larger than G'' at all the frequencies studied, a characteristic of weak gels which is typical of yogurts ([Meyer et al., 2011](#)). G' value is a measure of the deformation energy stored in the sample during the shear process, representing the elastic behavior of a sample. On the other hand, G''

value is a measure of the deformation energy used up in the sample during the shear and lost to the sample afterwards, representing the viscous behavior of a sample. If G' is much greater than G'' , the material will behave more like a solid; that is, the deformations will be essentially elastic or recoverable. However, if G'' is much greater than G' , the energy used to deform the material is dissipated viscously and the materials behavior is liquid-like ([Tabilo-Munizaga and Barbosa-Cánovas, 2005](#)).

The rheological behavior of yogurt is a result of the formation of a three-dimensional network composed of casein and denatured whey protein. Mild acidification leads to the formation of aggregates by way of hydrophobic and electrostatic bonds, resulting in the gel structure. The firmness of a yogurt gel is related to the total solids content and to the content and type of protein ([Tamime, 2006](#)).

The addition of oligofructose influenced the rheological behavior of the yogurt ($p < 0.05$). The G' and G'' curves of the yogurt without oligofructose showed greater values than the yogurts added with the oligosaccharide, independent of the storage time. However, the variation in oligofructose concentration ranged 2% to 8% had no significant influence ($p < 0.05$). This behavior suggests that the addition of oligosaccharide interferes with the formation of the protein network, decreasing the gel strength. Previous trials also showed a decrease in gel strength with the addition of 4% inulin and oligofructose ([Paseephol et al., 2008](#)). Inulin has been reported

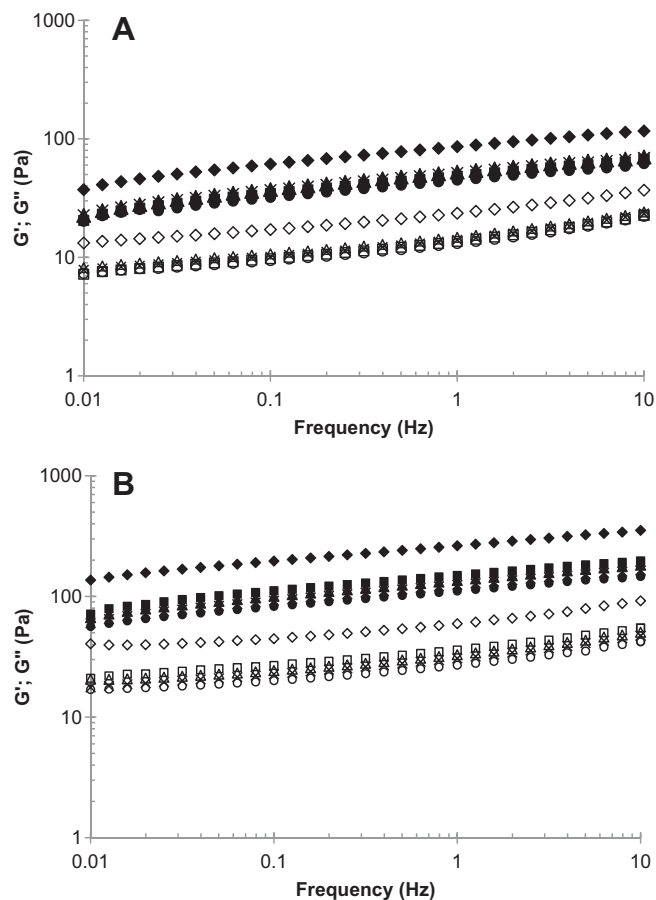


Fig. 3. Mechanical spectrum of prebiotic yogurt supplemented with increasing levels of oligofructose concentration (\diamond : 0%; \triangle : 2%; \square : 4%; $*$: 6%; \blacksquare : 8% wt. v^{-1} , respectively) stored at 5 °C during 1 day (A) and 28 days (B). G' : storage modulus (solid symbol); G'' : loss modulus (open symbol).

as an inert filler or structure breaker, preventing the formation of a protein network (Chiavaro et al., 2007) due to the formation of complexes with protein aggregates in the yogurt by way of hydrogen bonds (Kip et al., 2006). According to the mechanical spectra, it can be seen that the yogurts stored for 28 days (Fig. 3B) showed higher values for G' and G'' than yogurts stored for just one day (Fig. 3A) ($p < 0.05$), which could be associated with the large number of protein interactions and rearrangements (Ross-Murphy, 1990) occurring during storage.

3.3. Survival analysis methodology

There are no statistical tests that can compare the different parametric models used in survival analysis methodology with respect to interval censoring, which is given when the time of interest falls into an observed interval (Hough and Garitta, 2012). Therefore, a visual evaluation of the models in order to choose the most adequate model is very important. However, due to its increased use in the area of studies involving shelf life and food stability (Cruz et al., 2010a), the Weibull distribution showed an adequate fit and was therefore chosen for the subsequent calculations. The estimates of maximum likelihood of the parameters of the Weibull distribution correspond to: Intercept (μ) = 1.8787, and scale factor (σ) = 0.7462. These parameters were used to draw the graph of the percent rejection by the consumers versus the percentage of oligofructose.

The oligofructose level was the only different parameter at the yogurt formulation, which it suggests the different levels of accep-

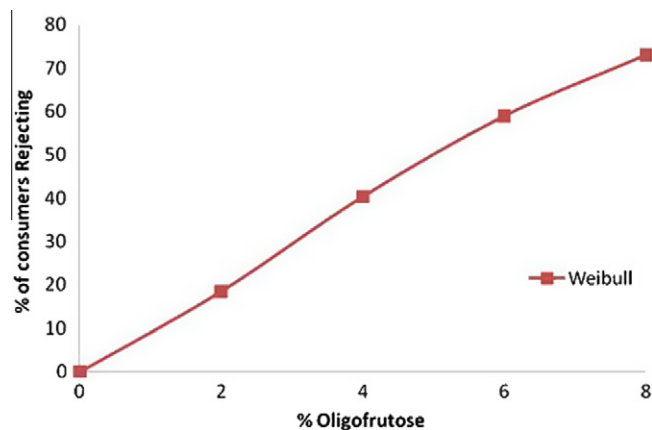


Fig. 4. Percentage of consumers that reject the yogurt supplemented with different concentrations of oligofructose by the Weibull distribution.

tance and rejection are related to this ingredient. As it shown, the percent of oligofructose added increased as consumer rejection with respect to purchase the yogurt also increased (Fig. 4). Despite the fact that the addition of oligofructose to yogurt is safe for human consumption and non-toxic (Bolye et al., 2008), and its ingestion is related to benefic effects on human health, such as the growth of probiotic bacteria (Waligora-Dupriet et al., 2007), the consumers rejected buying a yogurt with an elevated addition of oligofructose due to changes in the yogurt characteristics.

Overall, the inclusion of prebiotics in yogurts has been responsible for increasing their sensory acceptance, making them more preferred in relation to similar probiotic and conventional products (Allgeyer et al., 2010a,b), reinforcing the idea that a functional food, such as that with added prebiotic, should show similar behavior to the original product (Cruz et al., 2010c). However it has been shown that this effect depends on the fiber added, since negative results have also been reported (Ares et al., 2009). Our results emphasize the importance of carrying out sensory tests with regular consumers of the product as well as to use these data for adjust the product formulation.

In studies involving the survival analysis, values of 10%, 25% and 50% as the percentages of consumer rejection have been considered (Gambarro et al., 2006; Hough et al., 2003; Jacobo-Velázquez and Hernández-Brenes, 2010). In the present study, values of 10% and 25% were taken into consideration, since high rejection levels could result in economic losses for the oligofructose supplier, which would be prohibitive. Using this percent rejection, the percent oligofructose to be added to the yogurt would be 1.22% and 2.58% w/v, respectively.

Regulatory aspects indicate that the label of liquid foods with a prebiotic claim should indicate a minimum amount of 1.5 g of the prebiotic ingredient (Brasil, 2008) for a portion of 200 mL of yogurt (Brasil, 2003). Therefore, a daily ingestion of 150 mL of yogurt would be sufficient to establish a prebiotic effect and consequently, lead to benefits for the consumer health without an occurrence of gastrointestinal intolerance such as flatulence, abdominal discomfort or increased purge, observed in adult studies typically with doses above 15 g/day (Saavedra and Tschernia, 2002). As most yogurt packages available worldwide are presented in 200 mL plastic cups, this means that a daily ingestion of 1 cup of yogurt.

4. Conclusion

The supplementation with increasing levels of oligofructose concentration has demonstrated a variable effect on the yogurt quality parameters. From the rheological point of view, the yogurt

containing oligofructose has shown a weak gel behavior. The increase in the oligosaccharide content caused an increase in thixotropy, apparent viscosity, and storage and loss moduli while no interference was observed at the viability of the yogurt cultures and post acidification or proteolysis.

Using the survival analysis, it was shown that the addition of increasing amounts of oligofructose to the yoghurt caused changes in the sensory characteristics of the product, resulting in greater consumer rejection. It was observed that supplementation with 2.58% of oligofructose was adequate, considering 25% of rejection of the consumer. In this context, the development of functional foods such as prebiotic yogurts should obligatorily give priority to the interaction between the consumers and the product to be consumed in order to have a chance of success on a market constantly increasing in its competitiveness and increasingly more monitored by the Regulatory Agencies.

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