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Effect of microbial transglutaminase on functional and rheological properties of ice cream with different fat contents

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This study evaluated the effects of microbial transglutaminase (TG) (*Streptoverticillium mobaraense*) on the properties of ice cream with 4, 6 and 8 g/100 g fat. The TG was added at a concentration of 4 U g^{-1} and the chemical characteristics, capacity to incorporate air (overrun), fat coalescence, melting behavior, rheological properties and texture were evaluated. The TG was effective in controlling the ice cream properties providing greater overrun, greater fat coalescence and melting resistance in relation to samples without TG. These modifications can be attributed to the formation of a more cohesive protein network which increased the stability of the ice cream. Regarding the rheological parameters, it was found that TG caused an increase in the flow behavior index and pseudoplastic properties of the samples. The firmness of the ice cream was decreased by the addition of TG and was inversely proportional to the fat content. Ice cream with fat contents of 4 and 6 g/100 g subjected to enzymatic treatment had similar characteristics to samples formulated with 8 g/100 g fat, demonstrating that TG can be used to partially replace fat in ice cream.

1. Introduction

Ice cream is complex-colloidal systems which in their frozen state is comprised of ice crystals, air bubbles, partially-coalesced fat globules and aggregates, all in discrete phases surrounded by an unfrozen continuous matrix of sugars, proteins, salts, polysaccharides and water (Goff, 2002). Ice cream contains a high concentration of fat (Metwally, 2007), which is considered a multifunctional ingredient and influences the creaminess (Koxholt, Eisenmann, & Hinrichs, 2001), texture, mouthfeel (Adapa, Dingeldein, Schmidt, & Herald, 2000), color and flavor of these products (González-Tomás, Bayarri, Taylor, & Costell, 2008). Fat contributes to the properties of ice cream during freezing and beating, especially through the formation of a three-dimensional network of partially-coalesced fat globules. Some of the fat globules surround air bubbles, stabilizing the air phase and increasing the levels of fat aggregation, thus improving the melting resistance (Granger, Legerb, Barey, Langendorff, & Cansell, 2005) and ice recrystallization (Goff, 2002).

Milk proteins present in ice cream formulations emulsify the fat and contribute to partial coalescence and fat structure formation. They are adsorbed at the air interface, leading to enhanced aeration and foam stability. The proteins not present at interfaces contribute to enhancing the viscosity and textural quality of the ice cream (Vega & Goff, 2005).

The formation of the ice cream structure is hindered when the fat content is reduced and attributes related to quality, such as viscosity, ice crystallization, hardness, melting rate and flavor, are affected (El-Nagar, Clowes, Tudorică, Kuri, & Brennan, 2002). When the fat components are reduced they are often replaced by carbohydrates and proteins which can perform similar functional properties as fats (Benjamins, Vingerhoeds, Zoet, Hoog, & van Aken, 2009). The enzyme transglutaminase (TG), which is Generally Recognized As Safe (GRAS) by the Food and Drug Administration (FDA, 2010), has a high affinity for dairy proteins and modifies their functional properties.

Microbial TG (EC 2.3.2.13) is an enzyme that catalyzes a transfer reaction between the acyl and γ-carboxyamide of peptide-bound glutamine residues and primary amino groups in a variety of amino components. This enzyme acts by modifying the functional properties of food proteins through the incorporation of amine, cross-linking and deamidation (Motoki & Seguro, 1998). The affinity of TG for different types of protein is dependent on the distribution of glutamine residues as well on the secondary and tertiary structures of the proteins. The TG structure is stabilized by strong covalent ε-(γ-glutamyl)lysine cross-links between the peptide chains

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(Ionescu, Aprodu, Darabă & Porneală, 2008). Among the milk proteins present in ice cream formulations, the κ- and β-caseins are most susceptible to TG attack (Rossa, Sá, Burin, & Bordignon-Luiz, 2011). Whey proteins, α-lactalbumin and β-lactoglobulin, which usually require prior treatments such as heating to achieve their denaturation, increasing their interaction with the casein micelles as a consequence, increase the susceptibility of proteins to reaction with TG (Rodriguez-Nogales, 2006; Rossa et al., 2011).

The aim of this study was to evaluate the effects of the addition of the microbial enzyme TG (*Streptoverticillium mobaraense*) on the functional properties (melting rate, fat destabilization and overrun), rheological properties and texture of ice creams made with different fat contents.

2. Materials and methods

2.1. Materials

The following ingredients were used to manufacture the ice cream: skimmed cow's milk (67 g/100 g), sucrose (17 g/100 g), skimmed milk powder (7 g/100 g), Emustab® emulsifier (Duas Rodas, Jaraguá do Sul, SC, Brazil) (0.5 g/100 g), and Super Liga Neutra® stabilizer (Duas Rodas, Jaraguá do Sul, SC, Brazil) (0.5 g/100 g). Cream was added only to the ice cream samples with 6 and 8 g/100 g fat. The microbial transglutaminase (composed of lactose, maltodextrin and transglutaminase) was provided by Ajinomoto® (Ajinomoto, São Paulo, SP, Brazil). The enzymatic activity of the TG was 100 U g^{-1} (manufacturer's data) and it was used in the original form without further purification. All reagents were of analytical grade.

Six different ice cream formulations were prepared. The samples were coded as: ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).

2.2. Methods

2.2.1. Enzymatic cross-linking and ice cream preparation

The milk was subjected to heat treatment at 78 °C for 15 min for denaturation of the whey proteins (Rodriguez-Nogales, 2006). After cooling (25 °C), TG was added to the milk before the addition of the ice cream ingredients and mixing of the sample. The TG concentrations were calculated considering the ice cream protein content, quantified by the Kjeldahl method (AOAC, 2005). The conditions for enzyme activity were: 4 U g^{-1} protein, 40 °C and 90 min. After TG incubation, the enzyme was deactivation using heat treatment at 80 °C for 2 min (Rossa et al., 2011).

The ingredients, with the exception of the emulsifier, were mixed and pasteurized at 85 ± 2 °C for 15 min with constant stirring. After the pasteurization the ice cream mix was rapidly cooled to 50 °C and homogenized for 3 min. For aging, the ice cream mix was then stored at 4 ± 1 °C for 24 h and after this period the emulsifier was added. The samples of ice cream were produced using a processor (Britania, Curitiba, Brazil) with a churning speed of 815 rpm at −8 °C. The samples were cooled in a freezer (Consul, Whirlpool S.A., São Paulo, Brazil) at −20 ± 1 °C and stored under this condition until the analysis was carried out.

The samples IC4, IC6 and IC8 were prepared following the procedure described above, but without addition of the TG enzyme.

2.2.2. Chemical parameters

The chemical parameters evaluated were pH, fat (Soxhlet method), proteins (Kjeldahl method), total sugars (titration), ash and total solids (gravimetric method) (AOAC, 2005).

2.2.3. Overrun

The overrun was evaluated as ((Wt. of mix − Wt. of same vol. of ice cream)/Wt. of same vol. of ice cream) × 100% (Wildmoser, Scheiwiller, & Windhab, 2004).

2.2.4. Fat destabilization

The fat destabilization of the ice cream samples was evaluated according to the methodology proposed by Goff and Jordan (1989). The ice cream was diluted 500 times with distilled and deionized water and then centrifuged for 5 min at 1200 g (Jaetzki K24, Jena, Germany). The absorbance was measured 10 min later at 540 nm (spectrophotometer model Hitachi U2010, U2010, Tokyo, Japan). Distilled and deionized water was used as the blank. Fat destabilization was calculated as $(A_{mix} - A_{frozen})/A_{mix} \times 100$.

2.2.5. Melting rate

The melting rate of the ice cream samples was evaluated using the Lee and White (1991) method. The sample (120 g) was placed on a grid with 2 mm hole diameter in a funnel that drained into a graduated cylinder. The sample was allowed to melt in a controlled-temperature room at 25.0 ± 1.0 °C. The weight of the drainage was determined at 10 min intervals and the percentage of melted ice cream was then calculated as a function of time.

2.2.6. Rheological measurements

The rheological measurements of the samples of melted ice cream were carried out with a Brookfield rotational rheometer with a concentric cylinder (model DV-III Ultra, Brookfield Engineering Laboratories, Stoughton, MA, USA) and a ULA spindle. Data were collected using the software 32 Rheocalc® version 2.5 (Brookfield Engineering Laboratories, Inc, Middleboro, MA, USA). The rheometer was thermostatically controlled by a water circulator (model TE-184, TECNAL, São Paulo, Brazil) at 4.0 ± 0.1 °C, and the samples were left to stand for 15 min to ensure stability. The flow behavior of the samples was measured by the linearity of the shear rate from 19.6 to 67.3 s^{-1} in 20 min and returning to 19.6 s^{-1} over a further 20 min.

The hysteresis of the samples was evaluated from de area between the shear stress/shear rate curves.

The Power Law model (Equation (1)) was applied to describe the flow behavior and the consistency index of the samples treated with TG. The apparent viscosity of ice cream samples as a function of time at a constant shear rate was evaluated under a constant shear rate of 20 s^{-1}.

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

where σ is the shear stress (Pa); K the consistency index (Pa s^n); γ the shear rate (s^{-1}); and n the flow behavior index (adimensional).

The time-dependent rheological data were fitted using the Weltmann Model (Equation (2)) for a shear rate of 18 s^{-1} for 70 min, in order to characterize the thixotropic behavior of the ice cream samples.

$$\sigma = A + B \log t \tag{2}$$

where σ is the shear stress (Pa); A is the initial shear stress (Pa); B is the time coefficient of the thixotropic breakdown (Pa); and t is time (s).

2.2.7. Texture analysis

The texture analysis was conducted using a Texture Analyzer (TA-TX2, Model TA1000, Stevens LFRA, England, UK) and the software Exponent 32 (Stable Systems, version 4.0.13.0, 2007). The samples were kept in 80 mL plastic containers (50 mm diameter) and stored at −20.0 ± 1.0 °C until the analysis. For each sample six measurements were carried out using a Delrin polyacetate cylindrical probe (12 mm diameter; PL 0.5) attached to a 50 kg load cell. The penetration depth at the geometrical center of the samples was 35 mm and the penetration speed was set at 2.0 mm s^{-1}. The hardness was determined as the peak compression force during penetration.

2.2.8. Statistical analysis

Statistical analysis was carried out by analysis of variance (ANOVA) and the Tukey test ($P < 0.05$). This analysis was evaluated using the software Statistica® (version 8.0, StatSoft Inc., Tulsa, OK, USA, 2007). The rheological models were evaluated on OriginLab® software (version 6.0, Microcal Software Inc., Northampton, MA, USA, 2007). All tests were performed in triplicate. The interactions between the parameters (incorporation of air, fat destabilization, melting rate, rheological properties and texture) were evaluated by Principal Component Analysis (PCA).

3. Results and discussion

3.1. Chemical parameters

The data on the composition of the ice cream samples are given in Table 1 and they did not change significantly ($P < 0.05$) with the addition of TG. The average fat concentration for the samples IC4 and IC4-TG was 4.23 g/100 g, for IC6 and IC6-TG it was 6.5 g/100 g and for IC8 and IC8-TG it was 8.51 g/100 g.

3.2. Overrun and fat destabilization

Overrun is a measurement that relates to an increase in the volume of an ice cream product during processing (Cruz, Antunes, Sousa, Faria, & Saad, 2009). It was observed that the amount of overrun for the ice cream samples ranged from 39.13 to 107.15 g/100 g depending on the composition (Table 2). The greatest overrun was observed for the sample IC4-TG, followed by IC6-TG and IC8-TG. The addition of TG increased the overrun of the ice cream samples compared to the controls (without TG). According to Faergemand, Murray, Dickinson, and Qvist (1999), TG polymerizes the caseins through covalent and intermolecular bonds, making them capable of stabilizing emulsions and foams. Thus, the formation of casein polymers involving air bubbles was probably responsible for the increased volume and air bubble stabilization in the samples. Besides the action of the TG, the reduction in fat was also favorable for the incorporation of air (IC4-TG). A significant increase ($P < 0.05$) in overrun was observed with decreased fat concentration. This inverse relationship between fat content and overrun was also observed by Adapa et al. (2000) and Alamprese, Foschino, Rossi, Pompei, and Savani (2002). According to Stanley, Goff, and Smith (1996), the high-viscosity does not favor the formation of foam but rather the stability of foams.

The spectroturbidity method was applied to confirm the differences in the fat destabilization of the ice cream samples. The fat destabilization, related to the process of partial coalescence of fat globules, increased significantly ($P < 0.05$) in the ice cream samples that were submitted to enzymatic treatment with TG (Table 2). Fat coalescence was highest in the sample IC8-TG and lowest in IC4.

Ice cream fat which is coated with a protein/emulsifier layer and partially coalesced influences the ice cream quality, contributing mainly to the texture, body (Adapa et al., 2000) and stabilization of the structure of the air bubbles and foam (Granger et al., 2005). In a study by Metwally (2007), the TG, through polymerization of the whey protein and casein present in the fat globules, increased the cohesive properties of the membranes of the air bubbles and the adherence of the adsorbed film of the fat globules. This action, together with the increased fat concentration, was probably responsible for the increase in the percentage of coalesced fat in the ice cream samples with TG.

3.3. Melting rate

Fig. 1 shows the melting rate of the ice cream samples at 25 °C. It was observed that TG increased the stability of the samples, providing greater resistance to ice cream melting compared to the control (without TG). This result can be attributed to the polymerization of the milk proteins by the action of TG (Rossa et al., 2011) which led to an increase in the stability of the ice cream, especially when the amount of fat in the formulation is reduced. TG thus represents a potential substitute for fat in these products.

The ice creams with higher fat concentrations showed greater resistance to melting (Fig. 1), as also observed by Koxholt, Eisenmnn and Hinrichs (2001) and Karaca, Güven, Yasar, Kaya, and Kahyaoglu (2009). The sample IC8-TG showed the highest resistance followed by IC8 and C6-TG and IC4 melted the fastest. This result is consistent with the behavior observed in the fat destabilization analysis, because the sample that showed the greatest destabilization (IC8-TG) was that which melted the slowest. According to Cruz et al. (2009), the melting time of ice cream is related to its stability after overrun and indicates the extent of the stabilization and partial coalescence of fat. Furthermore, an increase in coalesced fat provides greater resistance to flow of the liquid phase resulting in slower melting (Muse & Hartel, 2004).

3.4. Rheological measurements

The data on the apparent viscosity, consistency index and flow behavior index of the ice cream samples produced with different fat contents and subjected to treatment with TG are shown in Table 3.

Table 1
Chemical parameters of ice cream samples.

	Samples[a]					
	IC4	IC4-TG	IC6	IC6-TG	IC8	IC8-TG
Protein (g/100 g)[b]	3.82a ± 0.14	3.91a ± 0.02	3.83a ± 0.05	3.91a ± 0.08	3.87a ± 0.01	3.84a ± 0.09
Fat (g/100 g)[b]	4.14a ± 0.11	4.31a ± 0.02	6.49b ± 0.01	6.51b ± 0.28	8.55c ± 0.12	8.47c ± 0.08
Total sugar (g/100 g)[b]	22.39a ± 0.49	22.57a ± 0.24	22.48a ± 0.42	21.56a ± 0.32	22.37a ± 0.51	21.65a ± 0.11
Total solids (g/100 g)[b]	33.18a ± 0.08	33.32a ± 0.03	33.17a ± 0.01	33.30a ± 0.04	33.19a ± 0.05	33.27a ± 0.04
Moisture (g/100 g)[b]	66.82a ± 0.08	66.68a ± 0.03	66.83a ± 0.01	66.70a ± 0.04	66.81a ± 0.05	66.73a ± 0.04
Ash (g/100 g)[b]	0.94a ± 0.01	0.96a ± 0.04	0.89a ± 0.02	0.98a ± 0.02	0.90a ± 0.02	0.88a ± 0.01
pH[b]	6.94a ± 0.06	6.96a ± 0.00	7.00a ± 0.01	6.83a ± 0.23	6.89a ± 0.13	7.00a ± 0.01

[a] Ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).

[b] Mean values ± standard deviation. Values with different letters in the same row are significantly different ($P < 0.05$) (Tukey test).

Table 2
Effect of the transglutaminase addition on the parameters overrun and fat destabilization of ice cream samples.

Samples[a]	Overrun[b] (g/100 g)	Fat destabilization[b] (g/100 g)
IC4	67.19a ± 1.77	21.83a ± 0.36
IC4-TG	107.15b ± 0.92	37.94b ± 0.23
IC6	58.06c ± 2.03	48.81c ± 0.55
IC6-TG	63.86d ± 1.56	54.77d ± 0.18
IC8	39.13e ± 1.20	79.45e ± 0.50
IC8-TG	47.90f ± 1.76	91.95f ± 0.57

[a] Ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).
[b] Mean values ± standard deviation. Values with different letters in the same column are significantly different ($P < 0.05$) (Tukey test).

These parameters were obtained by the Power Law model ($R^2 > 0.99$) of upward (increase of shear rate) and downward (decrease of shear rate) curves.

It was observed that the apparent viscosity obtained from both the upward and downward curves, measured under a constant shear rate of 20 s^{-1} at 4 °C, was influenced by the enzymatic treatment with TG and the fat content (Table 3, Fig. 2).

All samples containing TG had a significantly higher apparent viscosity compared to their control samples (without TG), probably due to the ability of TG to form high-molecular-weight polymers from monomers of proteins, conferring greater resistance to flow. The sample IC4-TG showed the highest apparent viscosity, followed by IC6-TG and IC8-TG (Table 3). These results demonstrate that the addition of TG may be an effective method for increasing the ice cream viscosity while maintaining a lower fat content. In Fig. 2 it can be observed that the sample IC8-TG, with the greatest fat content, showed the least difference in viscosity compared with the control sample, probably due to the lower contribution of polymerized proteins to the viscosity of the samples with greater fat content.

On analyzing the samples without enzymatic treatment it was observed that the samples with higher fat content had higher apparent viscosity (Table 3). This result can be explained by the degree of fat crystallization occurring during the ice cream aging process (the higher the fat content the higher the concentration of crystalline fat). These crystals behave like hard spheres providing greater resistance to shear stress, thereby increasing the viscosity of the ice cream (Goh, Ye, & Dale, 2006).

All samples showed non-Newtonian behavior, which decreasing viscosity with increasing shear rate (Fig. 2). This decrease is related to the aggregation of fat globules which decrease in size during shearing and hence influence the viscosity of the ice cream (Nazaruddin, Syaliza, & Rosnani, 2008).

The Power Law model gave a good fit with the data ($R^2 > 0.99$) and was used to calculate the flow behavior index (n) and consistency index (K) of different ice cream samples. As in the case of the apparent viscosity, the addition of TG increased the consistency index, especially in the sample IC4-TG (Table 3) as result of the aggregation of proteins and increased protein polymerization catalyzed by TG, without altering the chemical characteristics of the ice cream (Table 1).

Another parameter obtained from application of the Power Law model was the flow behavior index, which indicates the degree of pseudoplasticity or the dilatant character of a fluid. The flow behavior index (n) ranged from 0.55 to 0.64 ($n = 1$), indicating that all ice cream samples behaved as pseudoplastic fluids (Table 3). According to González-Tomás et al. (2008), the rheological properties of ice cream are described as pseudoplastic. For the ice cream submitted to enzymatic treatment, there was an increase in the pseudoplastic properties as the flow behavior index approached zero. This behavior was also evidenced by Gauche, Vieira, Ogliari, and Bordignon-Luiz (2008) with the cross-linking of whey proteins through the action of TG. The favoring of a pseudoplastic behavior probably occurred due to higher-molecular-weight polymers formed during the cross-linking reaction promoted by the TG. Innocente, Comparin, and Corradini (2002) affirmed that with an increase in the shear rate, large polymer molecules tend to disentangle and possibly align in the flow field, offering less resistance to flow.

The rheological behavior of the samples after the reduction of shear rate (downward curves) can be seen in Table 3. Using the Power Law model it was observed that K varied from 0.08 to 0.15 Pa s^{-1}, values which are lower than those obtained for the upward curve. All samples containing TG had higher K values than the respective samples without TG, demonstrating that the addition of TG gives the ice cream a greater resistance to structural rupture. Moreover, the values of the flow behavior index (n) were greater than those of the upward curve, showing that there was a decrease in the pseudoplastic properties when the shear rate decreased. The decrease in K and increase in n can be attributed to the structural breakdown of the protein network of the ice cream due to shearing, which favors this behavior.

An important feature of the shear stress versus shear rate results, obtained by increasing and then decreasing the shear rate, is the formation of hysteresis. The area formed between the curves indicates that the fluid viscosity is time dependent (Tárrega, Durán, & Costell, 2004). Table 4 shows the hysteresis values for the ice cream samples. It can be observed that the TG addition caused an increase in the degree of hysteresis when compared with the controls (without TG). Samples IC4-TG and IC6-TG (Table 4) showed the greatest degree of hysteresis with no significant differences ($P < 0.05$) between them. This demonstrates that these two samples needed more energy to break the ice cream structure formed from the protein polymerization, providing a firmer product. IC4-TG and IC6-TG were also the samples that showed the highest apparent viscosity and consistency index. According to Tárrega et al. (2004), a high-viscosity thixotropic fluid may show a larger hysteresis area than a lower viscosity one, even if the latter undergoes a more accentuated destruction of the structure. The presence of hysteresis was also observed by González-Thomás et al., (2008) and Karaca et al. (2009) in studies on ice cream.

The time-dependent rheological data were fitted using the Weltman model in order to characterize the thixotropic behavior of the ice cream samples. It was observed that the TG addition resulted in a significant increase in the initial tension required (A) to initiate the breaking of the ice cream structure (Table 5) due to the formation of a more stable network. Similar results have been observed in yogurt (Gauche, Tomazi, Barreto, Ogliari, & Bordignon-Luiz, 2009) and processed cheeses (Sá & Bordignon-Luiz, 2010), both with TG added. The sample IC4-TG had the highest values for initial stress, followed by IC6-TG and IC8-TG, and the latter two did not show significant differences ($P < 0.05$). The coefficient of thixotropic breakdown (B) was lower in samples with TG compared with the controls (without TG). Evaluation of the samples without TG (IC4, IC6 and IC8) and with TG (IC4-TG, IC6-TG and IC8-TG), separately, revealed that the coefficient B showed higher values for samples with higher concentrations of fat, with no significant differences ($P < 0.05$) between samples IC6 and IC8 and between IC4-TG and IC6-TG.

3.5. Texture analysis

The hardness of the ice cream samples was evaluated using the penetration test with the aid of a texturometer. The maximum force (g) required to penetrate the ice cream is shown in Fig. 3. The use of

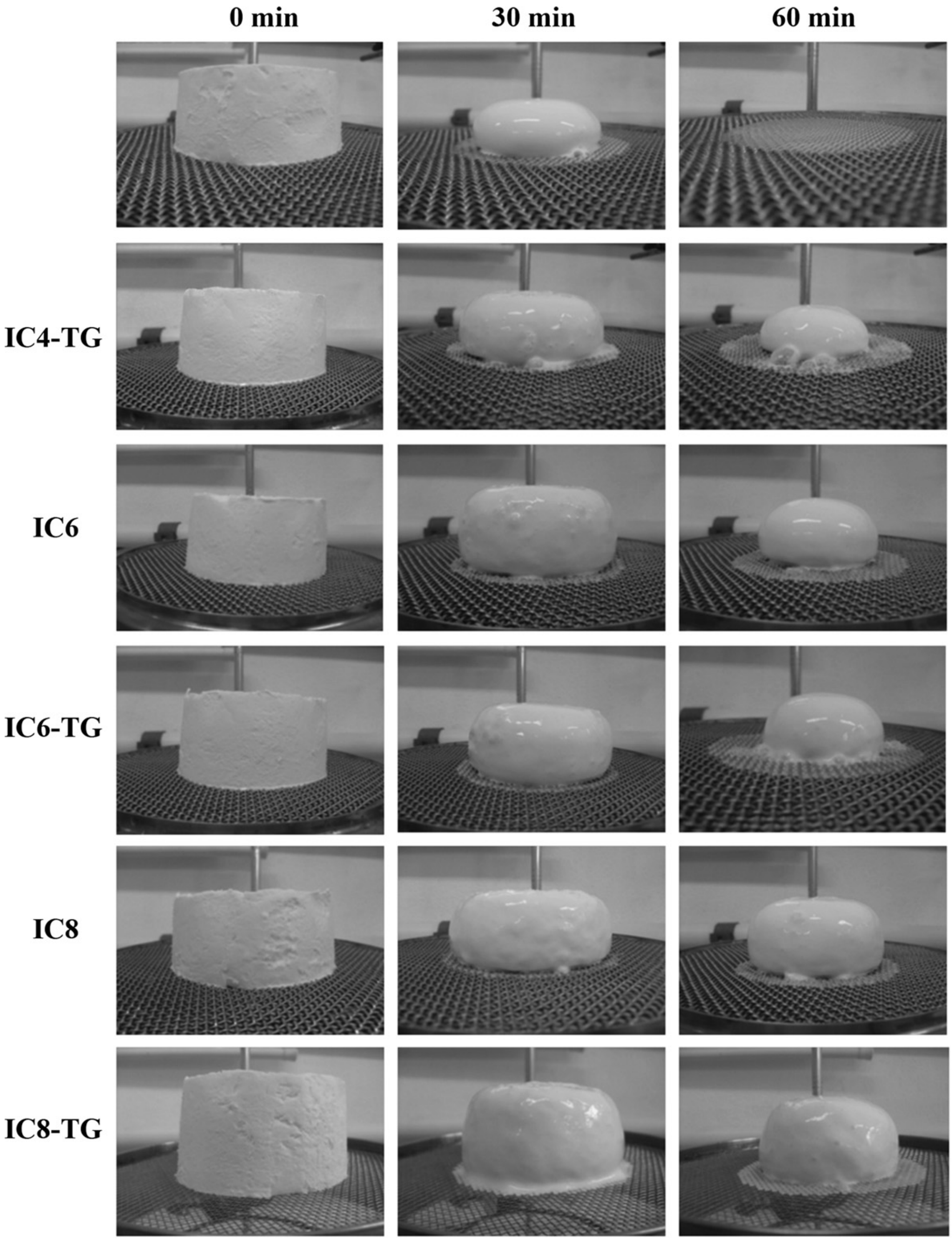

Fig. 1. Melting rate of ice cream samples with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).

a TG concentration of 4 U g^{-1} protein led to an ice cream sample with less firmness in relation to the control sample (without TG). The strengthening of the protein network produces a uniform and stable emulsion and reduces the formation of ice crystals during storage (El-Nagar et al., 2002). The presence of TG results in the formation of a more cohesive protein network through the milk protein polymerization, and this probably leads to a decrease in ice crystallization, reducing the hardness of the ice cream.

Increasing the fat concentration also reduced the hardness of the ice cream samples (Fig. 3). These results are consistent with those observed by Alamprese et al. (2002) and El-Nagar et al. (2002), who demonstrated that the hardness was inversely proportional to the fat content. According to Guinard et al. (1997), an increase in the fat content leads to a decrease in the formation of ice crystals, and subsequently a product of less hardness.

Table 3
Rheological parameters of the ice cream samples obtained using the Power Law model.

Samples[a]	Apparent viscosity (mPa s)[b]	K (Pa s^n)[b]	n[b]	R^{2c}
	Upward curves			
IC4	122.00a ± 2.09	0.28a ± 0.02	0.64a ± 0.01	0.9976
IC4-TG	187.20b ± 1.65	0.71b ± 0.01	0.57b ± 0.01	0.9924
IC6	158.93c ± 0.98	0.48c ± 0.01	0.60c ± 0.00	0.9989
IC6-TG	172.87d ± 1.01	0.67d ± 0.02	0.55d ± 0.02	0.9954
IC8	161.20e ± 1.23	0.52e ± 0.03	0.63a ± 0.02	0.9995
IC8-TG	167.60f ± 0.76	0.57f ± 0.03	0.56bd ± 0.01	0.9998
	Downward curves			
IC4	69.60a ± 1.76	0.08a ± 0.01	0.92a ± 0.02	0.9996
IC4-TG	121.20b ± 2.01	0.15b ± 0.02	0.97b ± 0.01	0.9990
IC6	79.22c ± 1.02	0.03c ± 0.02	0.95a ± 0.03	0.9997
IC6-TG	107.60d ± 1.33	0.10a ± 0.01	0.98b ± 0.03	0.9984
IC8	91.87e ± 2.11	0.11ab ± 0.01	0.93a ± 0.01	0.9989
IC8-TG	100.27f ± 0.89	0.12ab ± 0.01	0.92a ± 0.02	0.9993

[a] K = consistency index; n = flow behavior index; ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).
[b] Mean values ± standard deviation. Values with different letters in the same column are significantly different ($P < 0.05$) (Tukey test).
[c] Coefficient of determination.

Table 4
Hysteresis of ice cream samples with and without transglutaminase.

Samples[a]	Hysteresis (Pa)[b]
IC4	20.50a ± 1.02
IC4-TG	59.31b ± 0.87
IC6	39.58c ± 1.43
IC6-TG	59.49b ± 1.56
IC8	32.34d ± 1.55
IC8-TG	38.90c ± 0.69

[a] Ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).
[b] Mean values ± standard deviation. Values followed by different letters are significantly different ($P < 0.05$) (Tukey test).

Table 5
Thixotropic parameters obtained using the Weltman model for ice cream samples.

Samples[a]	Initial shear stress (A, Pa)[b]	Coefficient of thixotropic breakdown (B, Pa)[b]	(R^2)
IC4	3.81a ± 0.22	1.05a ± 0.05	0.95
IC4-TG	5.55b ± 0.41	1.90b ± 0.11	0.99
IC6	2.56c ± 0.09	0.52c ± 0.01	0.98
IC6-TG	4.33a ± 0.03	1.62b ± 0.04	0.99
IC8	3.21a ± 0.28	0.63c ± 0.21	0.99
IC8-TG	4.41ac ± 0.13	1.16a ± 0.03	0.97

[a] Ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).
[b] Mean values ± standard deviation. Values followed by different letters in the same column are significantly different ($P < 0.05$) (Tukey test).

3.6. Principal component analysis

Principal component analysis (PCA) was performed using the fat content (FAT), overrun (OVE), partial fat coalescence (PFC), melting rate (MR) after exposure of the ice cream to 25 °C for 1 h, as well as the rheological parameters apparent viscosity (VIS), consistency index (K), flow behavior index (n), hysteresis (HYS), initial tension required to initiate the structural breaking of the samples of ice cream (A), coefficient of thixotropic breakdown (B), and hardness (HARD) of the ice cream samples.

Fig. 4 shows that the ice cream samples were clearly separated by two principal functions (Factor 1 × Factor 2), which explain 88.65% of the total data variability. Ice cream samples with and without TG were separated along Factor 1, which explained the greatest variability of the data (49.95%). It was observed that the ice cream samples with TG (IC4-TG, IC6-TG and IC8-TG) were positively correlated with Factor 1, while samples without TG (IC4, IC6 and IC8) were negatively correlated with this factor.

This analysis showed that, of the variables considered, the rheological parameters viscosity (VIS), consistency index (K), hysteresis (HYS) and tension (A) were strongly positively correlated with Factor 1, while the flow behavior index (n), coefficient of thixotropic breakdown (B) and hardness (HARD) were negatively correlated with this factor. It can also be observed that the fat

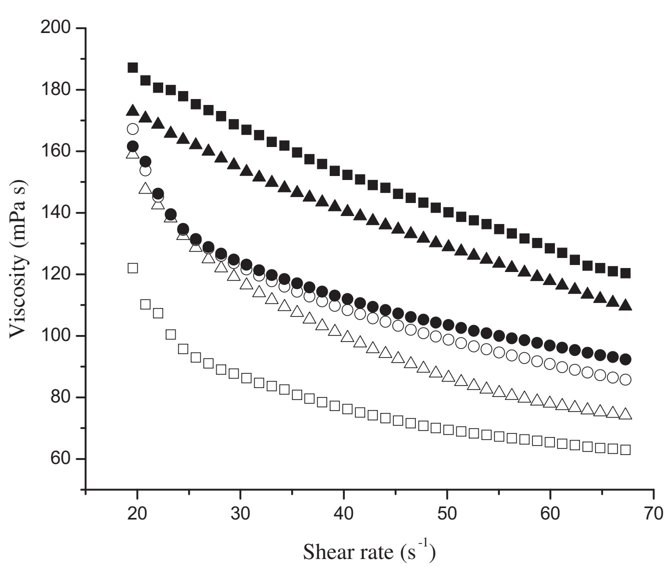

Fig. 2. Effect of shear rate on the apparent viscosity of ice cream samples. (□) ice cream with 4 g/100 g fat without TG (IC4) and (■) with TG (IC4-TG), (△) ice cream with 6 g/100 g fat without TG (IC6) and (▲) with TG (IC6-TG), (○) ice cream with 8 g/100 g fat without TG (IC8) and (●) with TG (IC8-TG).

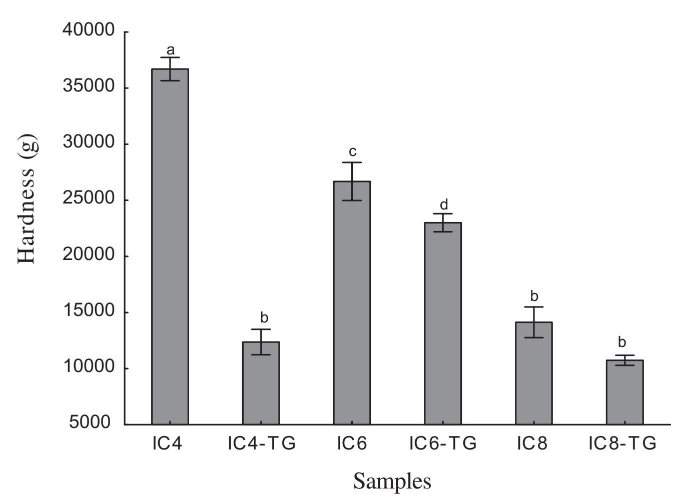

Fig. 3. Hardness of ice cream obtained from the experimental texture analysis expressed in force (g). Ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG). Different letters indicate a significant difference between samples ($P < 0.05$) (Tukey test).

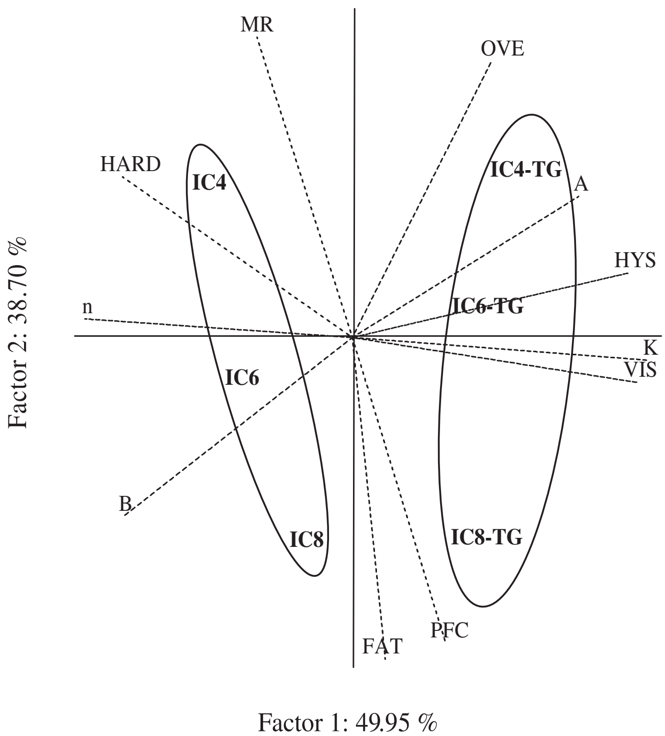

Fig. 4. Principal components analysis of the ice cream with 4 g/100 g fat without TG (IC4) and with TG (IC4-TG); ice cream with 6 g/100 g fat without TG (IC6) and with TG (IC6-TG); ice cream with 8 g/100 g fat without TG (IC8) and with TG (IC8-TG).

content (FAT) and partial fat coalescence (PFC) had a strong negative correlation with Factor 2, while the overrun (OVE) and the melting rate (MR) were positively correlated with this factor (Fig. 4).

4. Conclusions

The enzymatic treatment with TG of the ice cream samples with 4, 6 and 8 g/100 g fat led to an increase in the overrun, partial coalescence of fat globules, melting resistance and hardness compared with the samples without enzyme treatment. Regarding the rheological parameters, protein polymerization induced by TG favored the pseudoplastic properties of the ice cream and gave higher values for the apparent viscosity, consistency index, hysteresis and initial tension required to initiate the structural break of the samples. The addition of transglutaminase led to the ice cream samples with 4 g/100 g and 8 g/100 g fat having similar characteristics, indicating that the enzyme can be used as a partial replacement for fat in ice cream, without affecting the functional and rheological properties of these products.

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