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Influence of hydrogenated oil as cocoa butter replacers in the development of sugar-free compound chocolates: Use of inulin as stabilizing agent

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ABSTRACT

The effect of the addition of inulin as a surfactant or stability agent on white compound chocolate sweetened with sucralose and Stevia was studied. Samples were stored at 7, 15 and 30 °C during 100 days and the influence of inulin on rheological properties, sensorial attributes, shelf-life, physical properties such as melting, crystallization and blooming were analyzed. The shelf-life of the compound chocolate with the incorporation of inulin was higher than the control sample without replacement. Compound chocolate with inulin at 10% w/w showed a dense matrix structure, reducing the size and number of fat crystals formed during storage; furthermore they presented higher values of brightness and *WI*. This chocolate also showed less fracturability and improved thermal properties. DSC studies revealed increased values of onset and peak temperatures and enthalpy of melting of the polymorphic form V, at higher storage temperatures, achieving greater stability against degradation processes.

1. Introduction

Chocolate is a high energy product with a unique taste and texture, containing many carbohydrates and fats. True chocolate contains cocoa butter, which is extracted from cacao beans. Cocoa butter is an expensive ingredient that requires going through a tempering process during melting, which re-establishes the cocoa butter crystals, giving the chocolate the proper sheen, snap and taste. Tempering prevents bloom, where the cocoa butter separates from the cocoa solids and comes to the surface, turning the chocolate whitish or grayish in colour. Compound chocolate is a product made from a combination of cocoa, vegetable fat, and sweeteners. It is used as a lower-cost alternative to true chocolate; it utilizes less-expensive hard vegetable fats instead of the more expensive cocoa butter (Geron & Charaderian, 2013).

Sucrose is the most commonly used sugar in the confectionery industry and constitutes 30–60% of the chocolate, depending on type (Aidoo, Depypere, OheneAfoakwa, & Dewettinck, 2013). It is a multi-functional ingredient due to the structural and sweetening characteristics that sugar offer to these types of products (Aidoo, Afoakwa, & Dewettinck, 2015; Aidoo et al., 2013). However, there is a large market of consumers who demand sugar-free chocolates because diabetes is one of the fastest-growing chronic diseases. Low calorie sweeteners are an important alternative for the production of no- and low-sugar products. The full replacement of sugar represents a challenge because it affects physical quality characteristics like rheological properties and texture, melting behaviors, bloom formation and other characteristics that influence the final stability of chocolate, requiring strategy for their formulation. Combination of sweeteners with bulking and stabilizing agents is needed to provide an integral solution for sugar replacement. A technological resource for this problem may be the addition of fiber or fiber-like ingredients known as low-digestible carbohydrate polymers. Regarding this, the oligosaccharide inulin can be a good alternative as a stabilizing for the manufacture of sugar-free chocolates, trying to keep all the characteristic sensory properties. The incorporation of inulin in foods presents different technology advantages, such as texturizing, humectant, water holding agent, thickener, emulsifier, gelling agent, sugar and fat substitute, among others (Rosell, Santos, & Collar, 2009; Shourideh, Taslimi, Azizi, & Mohammadifar, 2012).

The use of surfactants and polymers as stabilizing agent in emulsions and suspensions has attracted much attention in recent years. Surfactants are important ingredients in the manufacture of chocolate; their function is to coat the surfaces of the sugar and cocoa particles dispersed in fat, generally cocoa butter, to maintain or improve the fluidity of the melted chocolate. Coating the

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surfaces of the dispersed particles with a surfactant reduces interparticle interactions responsible of particle aggregation (Do, Mitchell, & Vieira, 2010). The flow behavior of molten chocolate is an important characteristic directly related with an optimal mouthfeel. Polymeric surfactants of high molecular weight contribute to the stability of the sample, improving the dispersion of the product matrix in time; they are very efficient in terms of steric stabilization due to their molecular size and the formation of multiple binding sites at the interface (Do et al., 2010).

Previous studies reported the influence of fibers in chocolate formulations. The polysaccharide inulin was previously employed in sugar-free chocolate sweetened with Stevia and thaumatin by Aidoo et al. (2015). Tadros, Vandamme, Levecke, Booten, and Stevens (2004), found that inulin, a sugar-based polymeric surfactant is effective in long-term stabilization of emulsions. Inulin and polydextrose were used as bulking agent in the production of free sucrose chocolates (Shah, Jones, & Vasiljevic, 2010). Shourideh et al. (2012) studied the effect of d-tagatose and inulin on some physicochemical, sensory and rheological properties of black chocolate. Farzanmehr and Abbasi (2009) evaluated the effects of inulin, polydextrose and maltodextrin as bulking agents on the rheological properties of chocolate formulations and concluded that inulin and polydextrose can be used to improve the properties of chocolate.

Hydrogenated fat used in compound chocolate have a different triglyceride structure with respect to cocoa butter and can only support a small proportion of this ingredient (Lipp & Anklam, 1998). Cocoa butter has a unique triglyceride composition responsible for its various polymorphic crystallized forms that determines its chemical and physical properties, like melting and crystallization behavior. Moreover, the fatty acid composition results in the form that liquid fat converts into a solid that influences the final texture and microstructure properties (Jahurul et al., 2014).

Moreover, the triglyceride compositions of cocoa butter are responsible for its various polymorphic crystallization forms, whereas liquid fat converts into a solid as a result of fatty acid compositions

The aim of this work was to investigate the influence of inulin as a surfactant on the stability and physicochemical properties of sugar-free white compound chocolate using Stevia and sucralose as sweeteners. Cocoa butter was replaced partially with hydrogenated oil (20% w/w) to obtain compound chocolate. Kinetic studies on the formation of non-enzymatic browning products, evaluation of the changes in surface colour, free fat, rheological behavior of the melted product, textural properties and sensory analysis were carried out. Microstructure was analyzed by scanning electron microscopy and differential scanning calorimetry was applied to characterize the effect of inulin addition, on the crystallinity and melting profiles of the products.

2. Materials and methods

2.1. Raw materials

The raw materials used for production of white compound chocolate were: Cocoa Butter (Arcor SAIC, San Luis, Argentine), whole milk powder (Ylolay, Argentine), skim milk powder (La Serenisima, Argentine), Stevia powder (Tanki SA, Argentine), sucralose (Sucaryl Sucralosa, Merisant, Argentine), vanilla (Alicante, Argentine), soy lecithin, inulin (Orafti Chile S. A.) as surfactant or stabilizer agent (anti-bloom agent) and hydrogenated oil (Danica, Argentine) as cocoa butter replacer.

2.2. Chocolate formulations

Low sugar white chocolate was obtained using Cocoa butter 50%, w/w; Stevia 2.1% w/w; sucralose 1.4% w/w; whole milk powder 26% w/w; skim milk powder 19.7% w/w; soya lecithin 0.7% w/w; vanilla 0.1% w/w. Cocoa butter was partially replaced in a 20% (w/w) with hydrogenated oils (Sample 20%R) to obtain compound chocolate. Inulin at 5% and 10% (w/w) of the total weight of chocolate (20%R + 5%I and 20%R + 10%I) was added as surfactant agent.

2.3. Manufacture process of white chocolate

The low sugar white chocolate was produced through the following stages: sugar was milled together with milk powder using a grain mill (Corn-Grain-Cereal-Mill, Chinese) and a grinder. Then, cocoa butter or/and dehydrated oils were melted in a water bath (T < 45 °C). Sweeteners, milk powder and cocoa butter were mixed in a planetary mixer, (Santini, model MP8, Italian), for 5 min. Preparation was refined using a multi-hole screw extruder for 1 h at 35 °C. The conched was carried out under constant stirring at 200 rpm at 45 °C for 7 h. Lecithin and vanilla were added in the last 30 min of conching. Subsequently tempered by cooling to 23–24 °C and then heating to 28–29 °C was performed. All samples were tempered because cocoa butter in compound chocolate was not completely replaced. Samples were molded and cooled for 2 h at 7 °C. After cooling the product was packaged with a flexible material (Al-PET, water vapor transmission rate (WVTR) < 1 g m^{-2} day^{-1}) to avoid the effect of the ambient humidity.

2.4. Determination of white chocolate shelf life

For the kinetics study the samples were stored in chambers at a constant temperature of 30 °C and refrigerated at 7 ± 2 °C or 15 ± 2 °C over a period of 100 days. Non-enzymatic browning compounds and surface colour were periodically tested in triplicate during storage.

2.4.1. Non-enzymatic browning reactions

Four grams of grated chocolate in centrifuge tubes were weighted, and defatted with 25 ml of a mixture of chloroform/methanol (95:5) the sample was vigorously stirred and centrifuged at 3000 rpm for 30 min. The solvent fraction was decanted and solvent was evaporated in a bath under constant air flow, obtaining fatty extract. The fatty extract was weighted to obtain the percentage of fat in the sample. Then, the defatted pellet was suspended in deionized water at 50 °C in a 50-ml volumetric flask and vigorously stirred for 1 min and clarified with 0.5 ml each of Carrez I (potassium ferrocyanide, 15% w/v) and Carrez II (zinc acetate 30% w/v) solutions. The solution was left to rest for 10 min and the volume was adjusted to 50 ml with distilled water. The solution was filtered and the filtrate was used for PNE measurements by reading the absorbance at 280 nm using a spectrophotometer UV–Visible, double beam – (Shimadzu, USA), (Vercet, 2003).

2.4.2. Surface colour determination

The surface colour of the chocolate samples were measured in three different zones with a spectrophotometer MiniScan EZ, using the CIELAB colour parameters (L^*, a^* and b^*). "L^*" value defines luminance of the samples between 0 and 100 scale in which 0 defines black and 100 defines white colour, "a^*" value describes colour categorizing from green (−) to red (+), while "b^*" value describes colour categorizing form yellow (+) to blue (−). The measurement was performed at 7, 15 and 30 °C. Whiteness Index (WI)

for each sample stored at 15 °C was calculated according to the Eq. (1), (Erdem et al., 2014):

$$WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{0.5} \quad (1)$$

2.5. Kinetic model for nonenzymatic browning in white chocolate

The reaction rate of browning product formation can be described by the following differential equation (Dattatreya, Etzel, & Rankin, 2007):

$$\frac{d[Q]}{dt} = k[Q]^n \quad (2)$$

where *[Q]* = quality factor concentration; *k* = degradation rate constant; *n* = reaction order; t = storage time. Integration of Eq. (2), for a second order kinetics, *n* = 2, leads to:

$$\frac{1}{[Q_t]} = \frac{1}{[Q_0]} + kt \quad (3)$$

where the subscripts 0 and *t* were the initial time and sample time (t), after the degradation reaction, respectively. The quality factor selected was the loss of white colour during storage, this is produced mainly by Maillard reaction and was represented by the relative content of non-enzymatic browning compounds and the *WI* parameter obtained from experimental data of absorbance and surface colour respectively.

The Arrhenius equation was used to describe the temperature dependence of the reaction rates constants (Sothornvit & Kiatchanapaibul, 2009):

$$k = Ae^{\left(-\frac{Ea}{RT}\right)} \quad (4)$$

where E_a activation energy (J/mol), *R* gas constant (8.314472 J/K mol), *T* temperature (K), *A* pre-exponential coefficient or frequency factor ($dm^3\ mol^{-1}\ s^{-1}$), indicates the frequency of collisions.

The reference temperature selected for this study was 20 °C. The physical properties (non-enzymatic browning compounds and colour, a^*) of sugar free white chocolate were measured at 7, 15 and 30 °C. From the plot of the experimental data according to Eq. (3), the kinetic constants at different temperatures were obtained; the activation energy *Ea* was determined by applying Eq. (4).

Shelf life is defined as the time during which the product remain safe with desirable sensory, chemical and physical properties, where the consumption of a processed food is desirable. Therefore, it can be considered as the time taken to reach levels of food quality considered unacceptable for consumption.

The shelf-life for a given temperature was calculated from the Eq. (3), defined the concentration of the initial quality factor and the final quality, establishing a final acceptable value for free-sugar white chocolate sweetened with Stevia and sucralose. The values of *k* were obtained from the Arrhenius equation using the experimental values of *Ea* and *A*.

2.6. Scanning electron microscopy

The microstructure of white compound chocolate was analyzed by scanning electron microscopy (SEM, LEO1450VP, Zeiss, Germany). The samples were mounted on double-sided adhesive carbon on aluminum sample holders. The micrographs were determined under VP mode (variable pressure), using 300× and 700× magnifications. The low vacuum mode of SEM is a special type, in which the chamber where the samples are placed can be maintained under low vacuum (Sammons & Marquis, 1997).

2.7. Determination of the free fat in white chocolate

Four grams of white compound chocolate was weighting and melted at 50 °C for 20 min and then centrifuged at 3000 rpm for 30 min. The supernatant was collected, weighted and the amount of free fat percentage was calculated as g/100 g mobile fat in the chocolate (Ziegleder, Amanitis, & Hornik, 2004).

2.8. Rheological properties of white chocolate

Rheological properties of the chocolate samples were measured using a Brookfield DV-III (Brookfield, USA) viscometer. The chocolate was incubated at 50 °C for 75 min and transferred to the viscometer cub, and sheared at 5 s^{-1} for 10 min at 40 °C before the measurement cycles started. The shear stress was measured at 40 °C with a shear rate ramp up and down. The shear rate increased from 0.5 to 17.5 s^{-1} in 90 s and then decreased from 17.5 to 5 s^{-1} in 90 s; 10 measurements for each ramp was performed (Do et al., 2010; Sokmen & Gunes, 2006). Rheological data, shear stress (τ) and shear rate (γ), were analyzed by means of the Herschel-Bulkley model (Eq. (5)), to describe flow behavior and to determine rheological parameters of chocolate. The effectiveness of this model was checked by statistical analysis, through residual plots and normally test using a statistical software Graph Pad In Stat (Sokmen & Gunes, 2006).

$$\tau = \tau_0 + K\gamma^n \quad (5)$$

where τ_0 is the yield stress, *K* is the consistency index, *n* is the flow behavior index.

2.9. Texture measurements

The mechanical properties of compound white chocolate samples were measured using a TMS-TOUCH texture analyzer (Food Technology Corporation, USA) with a penetration probe attached to an extension bar and a 50 N load cell and a platform. Maximum penetration and fracturability forces through a sample (30 × 30 mm, depth 10 mm) were determined with 10 replications at a pre-speed of 30 mm/min, test speed of 90 mm/min, post-speed of 600 mm/min, penetrating 4 mm at 20 °C (Afoakwa, Paterson, Fowler, & Ryan, 2008; Afoakwa, Paterson, Fowler, & Vieira, 2009).

The texture properties determined in the samples were hardness (N), the point of maximum force during penetration; fracturability (brittleness, N), the point of the first peak or fracture.

2.10. Determination of melting and crystallization properties

Melting and crystallization properties were determined by differential scanning calorimetry (DSC, Q100DTA Instrument). Samples (≅5 mg) were loaded into 40 µl capacity pans and sealed with a sample press. Pans were tempered at 5 °C and heated at 3 °C/min from 5 to 55 °C and then cooled at 20 °C/min from 55 to −50 °C in a N_2 stream. Onset temperature (T_{onset}), peak temperature (T_{peak}), peak width at half height (T_{width}) of the melting and crystallization process, enthalpy of melting (ΔH_m) and crystallization (ΔH_c) and crystallization temperature (T_c) were calculated with the TA Universal Analysis software (Afoakwa et al., 2008). In order to analyze stability, samples were stored at three different temperatures 7, 15 and 30 °C for 100 days and were subsequently subjected to a DSC test.

2.11. Sensory analysis

Samples were assigned a random three-digit code and randomly ordered. Water was provided for cleaning the palate between samples. The sample was tested at room temperature

by 50 untrained panelists who judged the samples on a five-point hedonic scale (5 = extremely like, 3 = neither like nor dislike, 1 = extremely dislike). Flavour, aroma, colour, shape melting (how it melts in the mouth) and smoothness (sensation on tongue and roof of mouth while product is melting) were evaluated (El-kalyoubi, Khallaf, Abdelrashid, & Mostafa, 2011).

2.12. Statistical analysis

The Tukey's test and analysis of one way variance was used for establishing the significance of $P < 0.05$ between the means of the analyzed values. The statistical analysis was performed by the statistical GraphPad InStat software (1998).

3. Results and discussion

3.1. Shelf-life of free-sugar white chocolate and compound free-sugar white chocolate

The shelf-life of chocolate, depends on several parameters including: storage temperature and humidity, addition of different ingredients such as fats, among others (Giménez, Ares, & Ares, 2012; Mexis, Badeka, Riganakos, & Kontominas, 2010; Rodriguez Furlán, Pérez Padilla, & Campderrós, 2010). The samples were stored at different temperatures and were analyzed at beginning (t = 0) and after of 60 and 105 days of storage. The experimental data obtained of nonenzymatic browning from the samples stored at 7, 15 and 30 °C, were fitted to a second-order equation (Eq. (3)), obtaining the kinetic rate constants (k) with a coefficients of determination (R^2) between 8.3 and 1.0 (Table 1). The temperature dependence of the quality loss was modeled by Arrhenius equation (Eq. (4)) and E_a values were obtained in each case (Table 1).

Table 1 shows, that replacing cocoa butter with hydrogenated oils did not modify statistically the E_a of the nonenzymatic browning reaction, maintaining a similar stability than the control sample. Furthermore, with the increase of inulin concentration a statistically significant increase in E_a ($P < 0.001$) was observed, denoting a stabilizing effect.

In previous studies, inulin was used as a fat replacer in water-fat suspensions; the fat substituting property of inulin was based on its ability to stabilize the structure of the aqueous phase (Ibrahim, Mehanna, & Gad El-Rab, 2004; Karaca, Güven, Yasar, Kaya, & Kahyaoglu, 2009; Karimi, Hossein Azizi, Ghasemlouc, & Vaziri, 2015; Meyer, Bayarri, Tárrega, & Costell, 2011). However, these properties are unlikely to take place in fat-based suspensions such as chocolate. Hence the formation of a thick viscoelastic film or a gel that enhances the stabilization of emulsions against coalescence is not a probable mechanism for fat-based suspensions like chocolate (Do et al., 2010). The process of stabilization may be attributed to the fact that inulin coats solid particles extending into the lipid continuous phase producing a steric stabilization (Afoakwa, Paterson, & Fowler, 2007). Thus, the higher stability provided by inulin may be due to its effect as an effective surfactant agent, allowing the stabilization of the particles and dispersions of droplets (Berghofer, Cramer, & Schiesser, 1993; Do et al., 2010).

The *WI* was used as a quality factor and changes were measured at different storage temperatures (7, 15 and 30 °C) over a period of time. The rate constant (k) at each temperature was calculated (Table 1), and from the representation of ln k versus reciprocal of temperature ($1/T$) (Arrhenius model, Eq. (4)), *Ea* was obtained with a R^2 between 0.90 and 1.00 (Table 1).

Based on the results of preliminary experiments in which browning and sensory testing were compared, $WI = 55$ was selected as the final acceptable value for free-sugar white chocolate sweetened with Stevia and sucralose. This limit is reached when the chocolate becomes dark yellow or brownish, with these characteristics unacceptable. *WI* values were fitted to a second-order kinetic equation.

The replacement of cocoa butter by hydrogenated oils generated a significant reduction in *Ea* and therefore on the lifetime. The samples with replacement of coca butter by hydrogenated oils and inulin at 5% (w/w) (20%R + 5%I) showed a decrease in *Ea* with respect to the sample 20%R. However the frequency of collisions in the sample 20%R + 5%I were much lower than the control. Therefore, the collisions between reacting molecules were much lower and consequently the shelf-life increased. Besides, the inulin incorporation at low concentrations could act by covering particle surface decreasing particle-particle interaction (Middendorf, Juadjur, Bindrich, & Mischnick, 2015). Furthermore, the significant decrease of *Ea* value ($P < 0.001$), may be due to lower interaction between the different components of the chocolate matrix. However, at higher inulin concentrations (10% w/w), *Ea* value increased ($P < 0.001$) without statistically significant difference with respect the control sample (75%St + 25%Su).

3.2. Colour analysis

Colour is one of the key attribute for consumer acceptance. Surface colour analysis of white chocolate stored at 15 °C during

Table 1
Quality parameters in free-sugar white chocolate: Kinetic rate constants (k), activation energies (E_a), coefficients of determination of k and E_a (R^2) and shelf-life time of browning compounds and *WI* at the tested temperatures (7, 15 and 30 °C). St (Stevia), Su (Sucralose), I (Inulin), R (cocoa butter replacer).

Quality factor	Factor quality: browning compounds					Factor quality: *WI*					
Sample	Temperature (°C)	k x10^7 (dm^3 mol^{-1} s^{-1})	R^2	E_a (kJ/mol)	R^2	k (dm^3 mol^{-1} s^{-1})	R^2	E_a (kJ/mol)	R^2	A (dm^3 mol^{-1} s^{-1})	Shelf-life (years) T = 20 °C
75%St + 25%Su	7	1.90	1.00			3.23×10^{-11}	0.90	96.6 ± 5.3[a]	0.92	1.1×10^7	1.97
	15	2.65	0.87	13.5 ± 0.8[a]	0.85	6.42×10^{-11}	0.87				
	30	3.03	0.83			1.25×10^{-10}	0.91				
20%R	7	1.67	1.00			6.20×10^{-11}	0.81	76.7 ± 4.2[b]	0.90	5.1×10^3	1.18
	15	2.38	0.93	13.6 ± 0.7[a]	0.82	1.07×10^{-10}	0.87				
	30	2.68	0.89			1.82×10^{-10}	0.98				
20%R + 5%I	7	1.23	1.00			6.87×10^{-11}	0.74	53.8 ± 3.4[c]	1.00	3.9×10^{-1}	1.25
	15	2.12	0.90	17 ± 1.0[a]	0.70	1.01×10^{-10}	0.92				
	30	2.27	0.90			1.46×10^{-10}	0.97				
20%R + 10%I	7	1.06	1.00			5.02×10^{-11}	0.95	107.7 ± 7.3[a]	1.00	1.7×10^9	1.20
	15	2.17	0.92	23.5 ± 1.3[b]	0.74	1.08×10^{-10}	0.91				
	30	2.45	0.92			2.27×10^{-10}	0.77				

*Means with equal superscripts for the same column are not significantly different ($P > 0.05$) by the Tukey's test.

a period of 100 days was performed to identify L^*, a^*, b^* values and *WI* values (Table 2). The samples of white compound chocolate showed that L^* increased at low concentration (5% w/w) and then remains constant at higher concentrations. Previous studies performed by Shourideh et al. (2012) in dark chocolate demonstrated that the increase in concentration of inulin increased the L^* value.

The incorporation of inulin at 5%, delayed the increase of a^* and b^* values during of storage, this could be related with a reduction in the Maillard reaction and therefore a decrease in the rate of chocolate darkening.

Whiteness in colour is a desired feature in white chocolate, and a decrease of this factor is an indicator of the deterioration. The incorporation of inulin increased the WI value where differences with respect to the control sample (75%St + 25%Su), ($P > 0.05$) were not statistically significant. After 100 days of storage a decrease of sample whiteness (*WI*) was observed. However, samples with the addition of inulin showed higher values of whiteness, than those in which cocoa butter has been replaced with hydrogenated oil (20%R). No significant differences between the sample with the incorporation of inulin at 5% (w/w) and the control sample (75% St + 25%Su) were observed.

3.3. Rheological behavior of white chocolate and oil release

Shear stress versus shear rate values of the different tested white chocolate samples with and without inulin were fitted to the *Herschel-Bulkley* equation. The rheological parameters obtained are presented in Supplementary Table 1. The diagnostic analysis of the proposed model presented residual plots with no systematic patterns and normally distributed with $P > 0.1$ for all tested samples showing a Gaussian distribution ($R^2 \approx 1$).

The replacement of cocoa butter by hydrogenated oils did not affect the yield stress ($\tau_0 \cong 0$). Besides, the incorporation of inulin at low concentration (20%R + 5%I) did not modify the yield stress with respect to the control (20%R). This may be because inulin could reduce particle-particle interactions obtaining a matrix less structured and therefore a lower stress should be applied to induce flow, so, the yield value decreased. Similar behavior was previously found for PGPR (Polyglycerol polyricinoleate) in samples of black chocolate (Middendorf et al., 2015). However the incorporation of higher amounts of inulin (10%, w/w) increased the yield stress ($\tau_0 = 0.20 \pm 0.01$ mPa). This is, in agreement with the results observed for soy lecithin, for which at a concentration of 0.2 and 0.3% (w/w) the viscosity was reduced, but at a higher concentrations than 0.5% w/w yield value increases. This result is important, because the yield stress maintains small solid particles in suspension, giving greater stability to the chocolate (Sokmen & Gunes, 2006).

The samples showed a pseudoplastic behavior ($0.7 < n < 0.9$), similar to studies performed on samples of chocolate (Sokmen & Gunes, 2006). Replacing of cocoa butter by hydrogenated oils reduces the consistency index K from 2.20 ± 0.10 mPa s to 1.79 ± 0.08 mPa s and the addition of inulin at low and high concentrations further reduces the K value, 0.72 ± 0.03 mPa s and 1.09 ± 0.09 mPa s, respectively.

Samples showed thixotropic behavior with a hysteresis area measured between 5 s^{-1} and 60 s^{-1}; this is the range of rheological measurements for chocolate established by the International Office of Cocoa, Chocolate and Sugar Confectionery (IOCCC), National Confectioners Association (NCA) and Manufacturing Confectioners Association (CMA). Apparent viscosities were measured at a shear rate of 5 s^{-1}. The replacing of cocoa butter with hydrogenated oils decreased hysteresis area (9.6 ± 0.3 J m^{-3}) with respect to the control sample 75%St + 25%Su (7.7 ± 0.2 J m^{-3}, $P < 0.01$), with the decrease higher for inulin at 5% (w/w) (2.7 ± 0.1 J m^{-3}, $P < 0.001$). This may be due to the thixotropic phenomenon that is influenced by the concentration or combination of polymers present in the sample, probably due to the modification of the inter-particle interactions (Lee, Moturi, & Lee, 2009). However, hysteresis area increased with increased of inulin concentration (20%R + 10% I = 5.1 ± 0.1 J m^{-3}).

Flow properties of the suspensions and the particle-particle interactions, are influenced by the amount of fat immobilized on the particle surface (Middendorf et al., 2015). The replacement of cocoa butter by hydrogenated oils did not modify the sample apparent viscosity at a shear rate of 5 s^{-1} (75%St + 25% Su = 1167 ± 58 mPa s and 20%R = 1064 ± 49 mPa s, $P > 0.05$). The incorporation of small amounts of inulin (5%, w/w) in the chocolate matrix further reduced the apparent viscosity (595 ± 29 mPa s, $P < 0.001$). These results suggested that inulin at this concentration would coat solid particles reducing particle-particle interaction, displacing additional cocoa butter to the bulk phase, thus increasing the free fat from 38.30 ± 0.50% (20%R) to 39.53 0.23% ($P < 0.05$). In this way inulin acted like a lubricant. Similar results were found for other surfactants like soy lecithin, commonly used in chocolate (Middendorf et al., 2015).

Table 2
Colour analysis of surface of samples (T = 15 °C). L^*, a^*, b^* values and WI values during storage calculated afterward.

Parameter	Sample	Time (days)			
		0	20	35	100
L^*	75% St + 25% Su	81.28 ± 0.13^a	82.25 ± 0.15^a	80.19 ± 0.07^a	81.66 ± 0.77^a
	20% R	81.13 ± 0.21^a	82.23 ± 0.40^a	80.04 ± 0.44^a	75.00 ± 4.92^a
	20% R + 5% I	82.60 ± 0.39^b	84.00 ± 0.27^b	81.15 ± 0.61^a	78.85 ± 1.28^a
	20% R + 10% I	82.47 ± 0.35^b	83.57 ± 0.53^b	80.93 ± 0.98^a	79.60 ± 0.88^a
a^*	75% St + 25% Su	-0.93 ± 0.02^a	-0.50 ± 0.03^a	-0.41 ± 0.11^a	3.09 ± 0.13^a
	20% R	-0.17 ± 0.09^b	0.18 ± 0.07^b	0.02 ± 0.04^b	6.11 ± 0.34^b
	20% R + 5% I	-0.63 ± 0.03^c	-0.27 ± 0.03^c	-0.31 ± 0.08^a	-0.11 ± 0.10^c
	20% R + 10% I	-0.86 ± 0.07^a	-0.55 ± 0.04^a	-0.69 ± 0.04^c	6.63 ± 0.13^b
b^*	75% St + 25% Su	26.82 ± 0.10^a	27.41 ± 0.05^a	26.13 ± 0.10^a	29.01 ± 0.24^a
	20% R	27.43 ± 0.31^b	28.22 ± 0.25^b	27.50 ± 0.18^b	34.50 ± 0.85^b
	20% R + 5% I	26.16 ± 0.02^c	25.86 ± 0.08^c	25.34 ± 0.05^c	25.85 ± 1.18^c
	20% R + 10% I	26.80 ± 0.34^a	27.46 ± 0.27^a	26.63 ± 0.04^d	34.11 ± 0.33^b
WI	75% St + 25% Su	67.3	67.3	67.2	65.5
	20% R	66.7	66.6	66.0	57.0
	20% R + 5% I	68.6	68.6	68.4	66.6
	20% R + 10% I	68.0	68.0	67.2	60.0

*Means with equal superscripts in each group for the same column are not significantly different ($P > 0.05$) by the Tukey's test.
**St (Stevia), Su (Sucralose), I (Inulin), R (cocoa butter replacer).

Furthermore the addition of inulin at the concentration of 10% (w/w) decreased the percentage of oil released (Supplementary Table 1), from 39.53 ± 0.23–33.88 ± 0.22% ($P < 0.05$), and statistically increased the apparent viscosity of the suspension from 595 ± 29 to 928 ± 51 mPa s. This behavior is similar to that of other surfactants, such as PGPR which interact with lipids from the bulk phase and attract cocoa butter from the surface of the surrounding particles increasing the viscosity of the suspension (Middendorf et al., 2015).

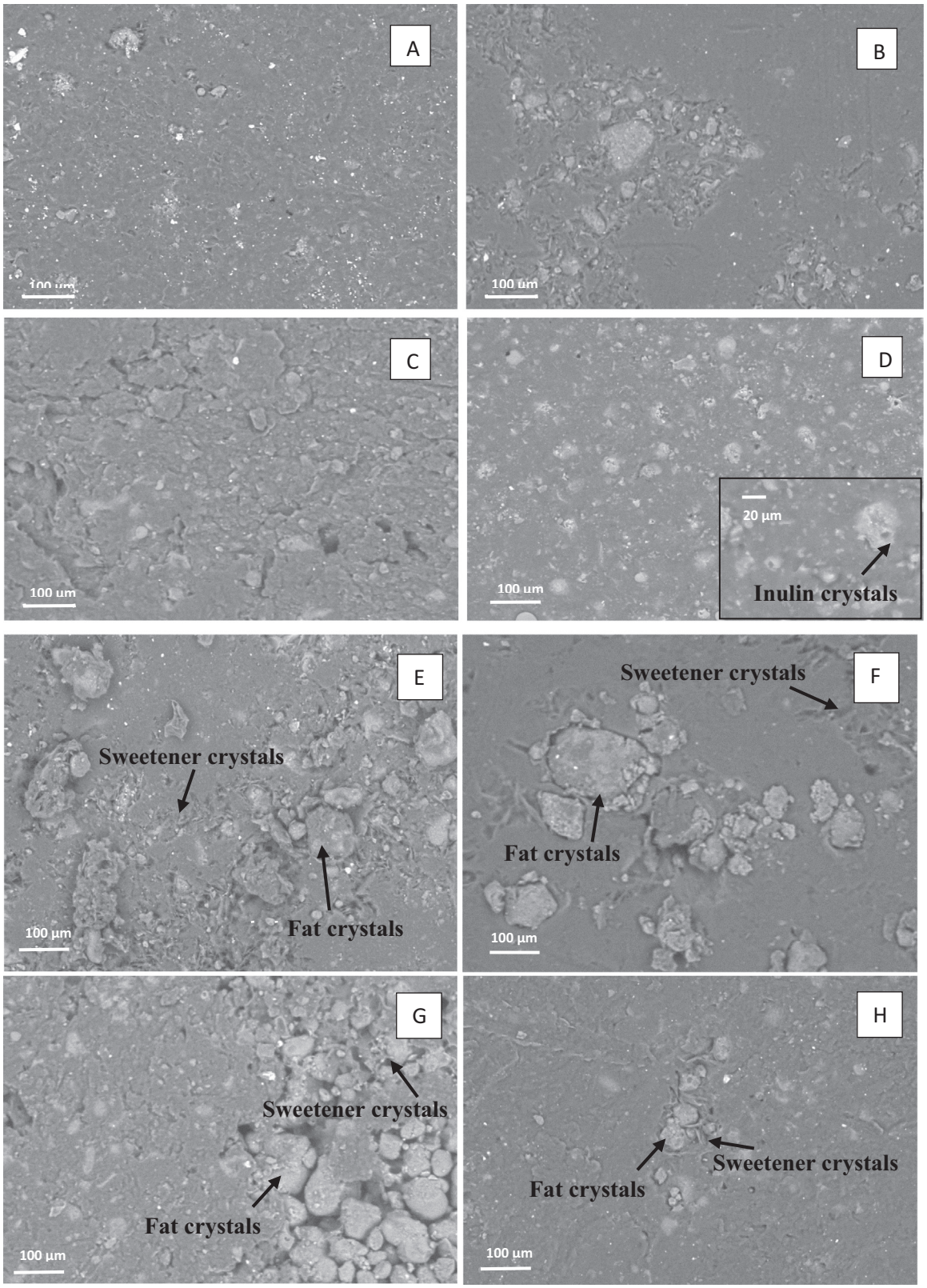

Fig. 1. Scanning electron micrographs showing crystalline network microstructures at magnifications of ×300 (A, B, C, D) and for boomed surface at magnifications of ×300 (A, C, E, F) for the free-sugar white chocolate samples studied: A-E: 75%St + 25%Su; B-F: 20%R; C-G: 20%R + 5%I; D-H: 20%R + 10%I.

3.4. Scanning electron microscopy

Microstructural examination using scanning electron microscopy of white chocolate with and without inulin showed clear variations in the crystalline network structure, inter-crystal connections and spatial distributions of the network (Fig. 1). Micrographs of the 75%St + 25%Su sample showed an even spatial distribution of dense matrix (Fig. 1A). The replacement of cocoa butter with hydrogenated oils generated phase separation with large crystals of fat and sugar (Fig. 1B). The incorporation of inulin at 5% (w/w) generated particles that are dispersed in the matrix surrounded by empty spaces or pores (Fig. 1C), leading to a less inter-particle interaction. This effect results in a less structured matrix which probably generated the decrease in viscosity and yield stress value, previously described. Besides, these pores can continue as channels inside the chocolate, where the fat can more easily spread to the surface, which could influence the fat bloom development (Dahlenborg, Brandner, Fureby, Johansson, & Kalnin, 2011). In contrast the micrographs of compound chocolate with 10% inulin showed a spatial distribution corresponding to a dense mass without empty spaces (Fig. 1D) and dispersed inulin crystals.

Fat bloom in chocolate is a major quality defect depriving it from its smooth appearance, bright colour and gloss. Structure of bloomed chocolate samples stored for 100 days at 15 ± 2 °C was studied and the results are presented in Fig. 1. Comparing the micrographs of the samples 75%St + 25%Su and 20%R (Fig. 1E and F) it can be observed that the replacement of cocoa butter by hydrogenated oils, led to the formation of larger fat crystals. This may be due to a destabilization of the internal structure of chocolate, creating higher aggregates. Furthermore, in both samples, a large amount of saccharides crystallization was observed. The sample 20%R + 5%I (Fig. 1G) showed re-crystallization of the numerous large fat crystals (Ostwald ripening) overlaid with sweetener crystals. The diffusion of the fats from the internal structure resulted in the nucleation and growth of new large crystals on the compound chocolate surface, inducing formation of weak and less inter-crystal connections inside of crystalline structures (Fig. 1G).

Fig. 2A shows the average particle size distribution of the chocolate sample studied. The incorporation of inulin at 10% (w/w) into the matrix (Fig. 1H) decreased the number and size of the sweetener and fat crystals on the chocolate surface when compared to other samples and the control sample. These results demonstrated that, the addition of inulin (10%) as a surfactant agent stabilized the suspension prevented particle separation and recrystallization (sweeteners, etc.) on the chocolate surface; additionally it probably has influence on the mechanical, rheological, melting, sensory properties and the shelf-life of chocolate. This may be because the incorporation of inulin at 10% (w/w) reduced the amounts of cocoa butter available to spread through the matrix toward the surface of the white compound chocolate and to recrystallize forming large aggregates (Ostwald ripening). This can be clearly seen in Fig. 1H, in which the sample 20%R + 10%I shows the formation of smaller fat crystals than in the control sample (75%St + 25%Su).

3.5. Texture

Changes in the composition, processing conditions, storage, etc., of chocolate generates changes in the product quality like texture and its durability. The chocolate hardness must be between a certain range, because if this parameter is small the chocolate is sticky and if is high the chocolate is hard to chew (Alvis, Pérez, & Arrazola, 2011). Hardness of the white free-sugar chocolate (75%St + 25%Su) decreased with the replacement of cocoa butter by hydrogenated oils (20%R) from 30.60 ± 2.80 N to 14.80 ± 1.75 N ($P < 0.001$), while inulin incorporation at 5% (12.48 ± 2.30 N) and 10% (14.50 ± 2.61 N) (w/w), did not modify statistically the sample hardness ($P > 0.05$). Previous studies performed by Shah et al. (2010), who employed Stevia as a sweetening agent and inulin and polydextrose as bulking agents, reported that the incorporation of inulin and polydextrose had no effect on chocolate hardness. In addition, Konar, Özhan, Artık, Dalabasmaz, and Poyrazoglu (2014) found that the incorporation of inulin to milk chocolate without sugar replacement, in a range of 6–12% (w/w), had no significant effect on chocolate hardness. Furthermore, fracturability of the samples was studied. The addition of hydrogenated oils to the control sample reduced the fracturability from 7.52 ± 0.52 N to 5.38 ± 0.66 N ($P < 0.01$). The addition of low concentrations of inulin (5%, w/w) did not modify the sample fracturability (5.34 ± 0.67 N), ($P > 0.05$). However at higher inulin concentrations (20%R + 10%I), an increase in the sample fracturability was observed (7.89 ± 1.38 N), ($P < 0.01$). This may be correlated with the results of the SEM, in which a greater matrix integration with a denser structure was obtained.

3.6. Melting and crystallization properties

Cocoa butter presents polymorphism and can crystallize into six polymorphic forms (I–VI); form I (16–18 °C) and II (22–24 °C) are

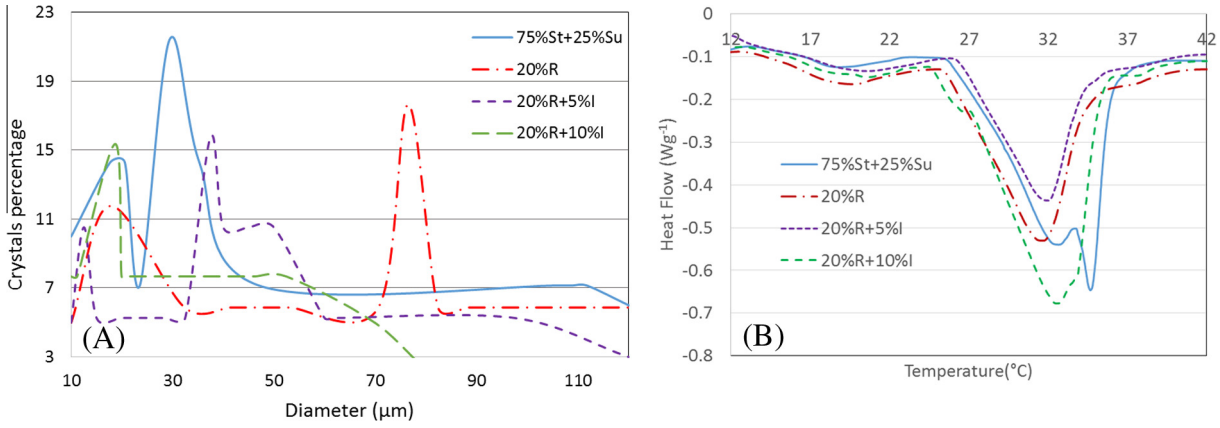

Fig. 2. A: Size distribution of the fat crystals of the samples with development of bloom on the surface. B: Melting properties of sugar-free compound white chocolate with inulin as stabilizing agent compared to the reference chocolate without fat replacer (75%St + 25%Su). A and B: Storage temperature = 15 °C during a period of 100 days.

the least stable and transform slowly into III (24–26 °C) and IV (26–28 °C). Polymorphic V (32–34 °C) is the most desirable form and melts just below body temperature. Lest stable forms of crystalline chocolate (IV, V) can be transformed during prolonged storage to form VI (34–36 °C), the most stable form (Afoakwa et al., 2008; Bui & Coad, 2014). Chocolates with an optimal tempering have the polymorph V, which confer the desired glossy appearance, good snap, contraction and resistance to bloom enhancing the shelf life of the final product (Afoakwa et al., 2008). Fat migration and recrystallization during fat bloom generation (form VI) can be attributed to the insufficient formation of the stable polymorph (form V) in cocoa butter during tempering that causes the formation of large crystals on the surface chocolate (Afoakwa et al., 2009; Bui & Coad, 2014). The fat bloom formation depends on the relative stabilities of crystal forms and temperature (Afoakwa et al., 2009).

Fig. 2B shows DSC thermograms of all the compound chocolate formulations at a storage time of 100 days. The thermograms show the presence of three main polymorphic forms in the control sample (75%St + 25%Su): form I melts at 18.6 °C, the dominant form V at 32.62 °C and a shoulder peak of form VI melting at 34.66 °C. Form V was the dominant polymorph in this sample. Data from the DSC (Fig. 2B) showed that incorporation of hydrogenated oils produced changes in crystallinity and melting properties, observed from the differences in their peak widths and height. Besides, thermograms showed the presence of two main polymorphic forms instead of three: one melting at 19.49 °C and the V polymorph at 31.59 °C. Fig. 2B showed that incorporation of inulin produced changes in crystallinity and melting properties, obtaining optimal values for inulin at 10% (w/w) with three main polymorphic forms similar to the control sample, with melting temperatures of 20.61 °C, 32.57 °C (polymorph V) and 33.92 °C (polymorph VI); the sample consisted mainly of form V, that confers improved quality for the compound chocolate.

Table 3 shows values of T_{peak} and ΔH_m for polymorphic form V for the different samples studied. ΔH_m values of the peaks of the polymorphic form V (the desired for a better chocolate quality), were higher or predominant in the sample 75%St + 25%Su. However, this sample shows a peak quite pronounced of the polymorphic form VI which was related to product quality deterioration or bloom surface formation. The replacement of cocoa butter with hydrogenated oils caused a decrease of the total melting enthalpy (ΔH_{mT}); however, the form V remained predominant. The sample with 5% (w/w) inulin did not produce a statistically significant change in ΔH_{mT} with respect to the sample 20%R, but generated a reduction in ΔH_m of polymorphic form V from 45.99 J/g (20% R) to 34.86 J/g (20% R + 5%I). The addition of inulin 10% (w/w) caused an statistically significant increase in ΔH_m of the polymorphic form V from 45.99 J/g (20%R) to 53.52 J/g (20% R + 10% I), which was statistically superior to the control sample (41.31 J/g). The dominant form V suggest that 20%R + 10%I may have better demoulding characteristics, texture and a more desirable appearance, as well a good resistance to blooming or more stability, compared to the other samples (75%St + 25%Su, 20%R and 20%R + 5%I) Furthermore, the total energy required to melt the sample (ΔH_{mT}) of 20%R + 10% I (80.22 J/g) statistically increased with respect to 20%R (57.92 J/g) and the control sample (64.83 J/g). This can be correlated with a higher melting resistance at higher temperatures. Therefore, at elevated temperatures lower melting triglycerides can be spread on the compound chocolate surface and recrystallize, probably, delaying the appearance of bloom on the surface (Bui & Coad, 2014).

The onset temperatures of polymorphic form V (Table 3) for the samples with replacement of cocoa butter decreased from 26.85 °C (control sample) to 25.68 °C (20%R), ($P < 0.05$). However, the addition of inulin at 5% (w/w) and 10% (w/w) led to higher temperatures of 25.87 °C and 26.10 °C, respectively; non-significant differences between control sample and 20%R + 10%I ($P > 0.05$) were observed. Previous studies performed by Shah et al. (2010) revealed that the replacement of sugar by incorporation of inulin in sugar-free chocolate increases the melting point, being similar to the control sample without sugar replacement. Comparing the peak width at half height (T_{width}) for all the tested samples (Table 3), it was observed that the control sample requires a larger temperature interval to melt (6.25 °C) than the samples with replacement of cocoa butter (5 °C) and with inulin at 5% (w/w), (4.5 °C). However, the incorporation of inulin at 10% (w/w) increased these values to an average value of 5.4 °C. These trends can be associated with the microstructural behavior of the bulk ingredients, where the SEM micrographs showed that control sample (75%St + 25% Su) has minimum inter-particle spaces in comparison to formulations which the replacement of cocoa butter (20%R) and with 5% (w/w) of inulin. The sample 20%R + 5%I revealed large crystals with more void spaces between the crystals indicating limited particle–particle interaction strength. However, the sample with 10% (w/w) inulin resulted in a compound chocolate having dense structure with filling void spaces, high inter-particle interaction, and high solids packing. This was in agreement with the highest values of T_{width}, T_{onset}, ΔH_m and $\Delta H_{m\text{-}total}$ with respect to the sample without addition of inulin (20%R). Glicerina, Balestra, Dalla Rosa, and Romani (2013) also found that higher values of ΔH_m can be related to the existence of very consistent structures, with higher energy requirements for completing fat melting.

All the samples were stored at three different temperatures 7, 15 and 30 °C during 100 days, to evaluate the stability of the different chocolate formulations. DSC thermographs of theses samples are shown in the Fig. 3A–D. The stability test revealed that in all samples $\Delta H_{m\text{-}total}$ values decreased with increasing temperature in the storage period studied. Thus in sample 75%Su + 25%St enthalpy values decreased from 78.37 J/g to 64.38 J/g; in sample 20%R, from 58.37 J/g to 29.42 J/g; in 20%R + 5%I, from 56.20 J/g to 35.14 J/g and in sample 20%R + 10%I, from 87.10 J/g to 51.35 J/g. Therefore, samples with replacement of cocoa butter were more unstable at higher temperatures than the control sample. However, the increase of inulin content increased $\Delta H_{m\text{-}total}$. These results demonstrate that the addition of inulin 10% w/w in the final product gives a positive effect on its thermal properties, as it enables higher storage temperatures, achieving a greater stability against degradation processes.

Table 3
Effect of replacement of cocoa butter with hydrogenated oils and incorporation of inulin at different concentration on onset temperature (T_{onset}), peak maximum (T_{peak}), peak width at half height (T_{width}) of melting of form V; enthalpy of melting of polymorphic form V (ΔH_m); total enthalpy of melting ($\Delta H_{m\text{-}total}$) and temperature ($T_c$) and enthalpy of crystallization (ΔH_c) of free-sugar chocolate storage at 15 °C during 100 days.

Sample	T_{onset} (°C)	T_{width} (°C)	T_{peak} (°C)	ΔH_m (J/g)	$\Delta H_{m\text{-}total}$ (J/g)	T_c (°C)	ΔH_c (J/g)
75%St + 25%Su	26.85 ± 0.34^{a}	6.25 ± 0.36^{a}	32.62 ± 0.36^{a}	41.31 ± 1.23^{a}	64.83 ± 2.12^{a}	8.49 ± 0.13^{a}	45.34 ± 2.86^{a}
20%R	25.68 ± 0.26^{b}	5.00 ± 0.23^{b,c}	31.59 ± 0.29^{b}	45.99 ± 1.10^{b}	57.92 ± 1.95^{b}	5.64 ± 0.18^{b}	34.02 ± 2.41^{b}
20%R + 5%I	25.87 ± 0.36^{b}	4.50 ± 0.31^{b}	31.96 ± 0.34^{a,b}	34.86 ± 0.98^{c}	51.00 ± 1.47^{c}	6.51 ± 0.14^{c}	36.87 ± 3.14^{b}
20%R + 10%I	26.10 ± 0.28^{a,b}	5.40 ± 0.25^{c}	32.57 ± 0.25^{a}	53.52 ± 1.92^{d}	80.22 ± 2.85^{d}	6.99 ± 0.21^{d}	53.33 ± 3.87^{a}

*Means with equal superscripts in each group for the same column are not significantly different ($P > 0.05$) by the Tukey's test.
**St (Stevia), Su (Sucralose), I (Inulin), R (cocoa butter replacer).

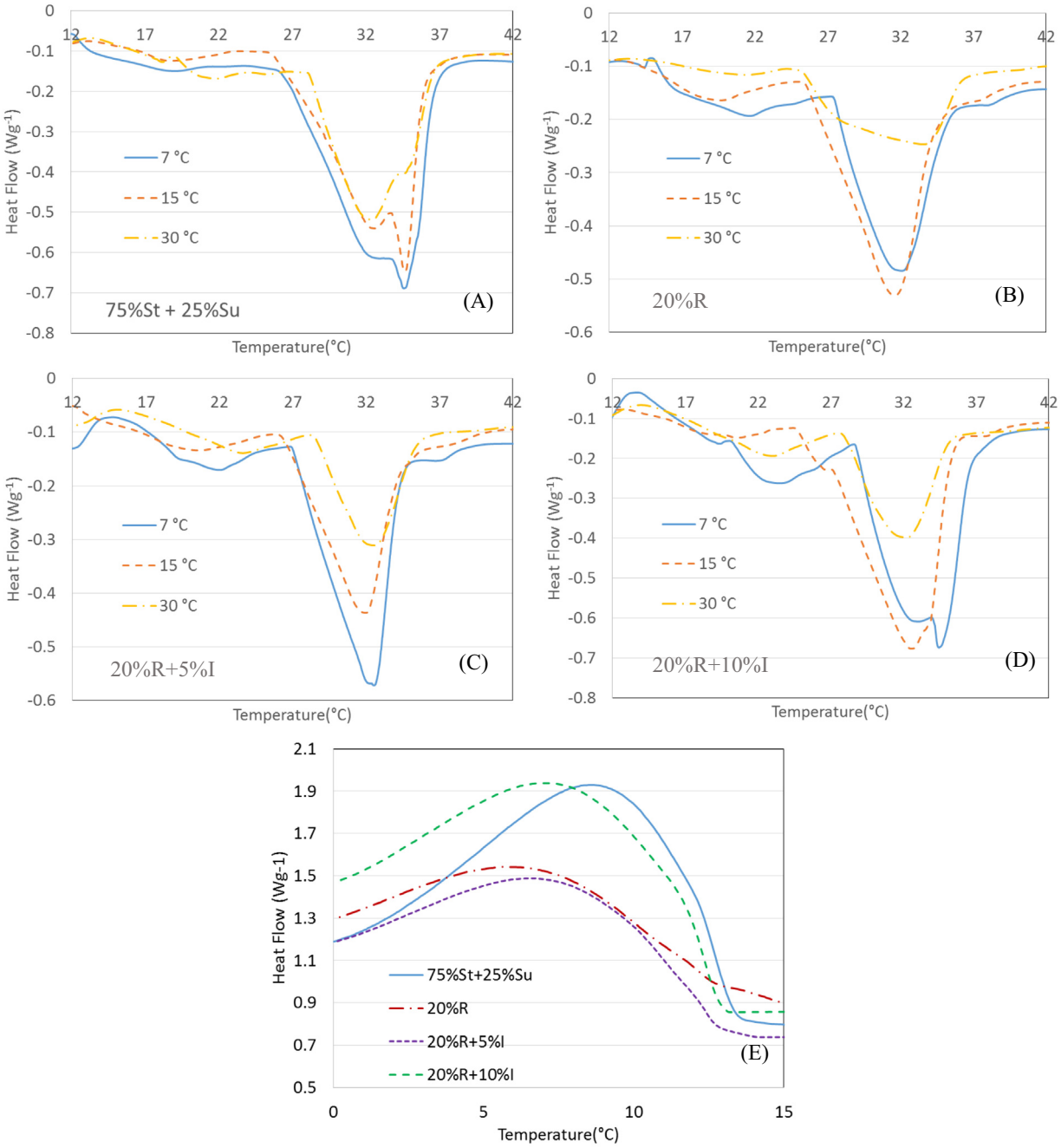

Fig. 3. A-D: DSC thermograms of the melting process and E: Crystallization process of white chocolate and compound chocolate with and without inulin at 5 and 10% (w/w). Samples stored at different temperatures during a period of 100 days.

The crystallization process is presented in Fig. 3E. Results show that by replacing the cocoa butter in the control sample (75%St + 25%Su) with a 20% replacement (20%R) T_c and ΔH_c significantly decreased (T_c from 8.49 °C to 5.64 °C and ΔH_c from 45.34 J/g to 34.02 J/g). However, incorporating inulin, a statistically significant increase ($P < 0.05$) occurs, reaching values similar to the control sample in the case of 10% (w/w) inulin (20%R + 10%I), ($P > 0.05$) (T_c = 7.00 °C and ΔH_c = 53.33 J/g).

3.7. Sensory analysis

Free-sugar white compound chocolate formulations with and without inulin were sensory acceptable. The sensory parameters flavour, aroma, colour, shape melting and smoothness were evaluated (Supplementary Table 2), obtaining higher values than 4.5 between a range of 5–6.5. These samples presented sensory properties similar to the control 75%St + 25%Su ($P > 0.05$). Similar results were found by Golob, Mičović, Bertoncelj, and Jamnik (2004), who studied the influence of inulin and fructose on chocolate sensory characteristics by an evaluation panel. No statistically significant difference was observed between the parameters flavour, aroma, colour and smoothness ($P > 0.05$). However, a decrease was observed for the shape melting parameter between 20%R (5.00 ± 0.38) and control sample (5.84 ± 0.31), ($P < 0.05$). The melting shape is an important parameter because influences the final quality of chocolate, because the melting must be fast

and continuous, with no trace of coarseness. Furthermore, the sample 20%R + 10%I (6.08 ± 0.36) showed a better melting in the mouth than the 20%R + 5%I (5.27 ± 0.41) formulation being similar to the control sample.

4. Conclusions

The effect of replacing cocoa butter with hydrogenated oils in the formulation of sugar free white compound chocolate sweetened with sucralose and Stevia was studied. The analyzed parameters related to product quality were: the formation of non-enzymatic browning compounds and the coloration of chocolate surface during a storage time of 3 months at different temperatures (7, 15 and 30 °C). The results showed that the white compound chocolate with 5% (w/w) of inulin showed the longest lifetime. This behavior could be explained considering that inulin exerts an insulating inter-particle effect, reducing the reactivity and thus the reaction rate of degradation with respect to the selected quality factor, especially at low concentration (5% w/w).

Simultaneously comparing the rheological and free fat studies it can be observed that the addition of inulin at 10% (w/w) resulted in a reduction in the free fat content of the sample generating an increase in viscosity and yield stress with respect to the sample containing less inulin (5% w/w).

Studies of the thermal properties performed by DSC revealed that replacing cocoa butter by reducing hydrogenated oils values decreased the values of ΔH_{mT}, T_{onset}, T_{peak}, ΔH_m in polymorph V. However, the addition of inulin at 10% (w/w) increased these values being higher than the control sample. Texture studies revealed that replacing cocoa butter by hydrogenated oils decreased fracturability. Nevertheless, the addition of 10% inulin improved textural properties of the sample because it produced a statistically significant increase in fracturability with respect to 20% R and no statistically significant difference with the control sample (75%St + 25%Su). Therefore, from these studies formulation of compound chocolate free of sugar with improved properties and stability was developed and characterized, being additionally improved by the addition of a prebiotic such as inulin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.09.054.

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