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RESEARCH ARTICLE

INFLUENCE OF SUCRALOSE AND STEVIA ON THE PHYSICOCHEMICAL PROPERTIES OF FREE SUGAR CHOCOLATE

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ABSTRACT

The purpose of this work was study the influence of the replacement of sucrose by the combination of sucralose (Su) and Stevia (St) for development of formulations of white chocolate suitable for diabetics. The use of a mixture experimental design allowed the modeling of system from kinetic studies of thermal degradation, which showed that binary combinations of Stevia with sucrose had synergistic effects since the sample, presented a high shelf-life time (75%St+25%Su) than the samples formulated from the individual components. From the studies of rheological behavior, a synergy effect between sucralose and Stevia was found. The sample 75%St+25%Su showed a higher viscosity than the samples containing the individual components. Our study provides a chocolate suitable for diabetics, with an appropriate combination of sweeteners (75%St+25%Su) with longer lifetime and higher stability than control sample

INTRODUCTION

Chocolate is a dense suspension consisting of sugar particles between 40-50%, coca solids, and milk power dispersed in cocoa butter as a continuous phase (Sokme and Gunes, 2006). The development of sugar-free chocolates is a challenge for food technology, since sugar is a multifunctional ingredient with properties of a sweetener, a bulking and texturizing agent, hindering its replacement. Besides, chocolate with higher sugar content than 40% had a greater acceptability, because a higher sucrose content decreases the bitter taste present in chocolate with low sucrose content (Aidoo *et al.*, 2013). A high glycemic index of sucrose is dangerous for diabetic people, and therefore, they cannot consume large quantities of such food products. Hence, the products suitable for diabetics should be formulated with sucrose substitutes. However, these substitutes should also simulate the functional properties of sucrose including the chemical stability provided to foodstuffs (Porto Cardoso and Bolini, 2007). During the last decade in many parts of the world, there is a growing interest for different food and beverages that improve or benefit health. The functional food plays an important role, providing a new type of promising tool with beneficial health effects related to particular components present in the food. Therefore, the addition of sweeteners such as Stevia, a natural sweetener with a low calorific value, and a sweetening power 200-300 times higher than sucrose, represents a good alternative as a sucrose substitute. Besides, regular consumption of these extracts of *S. rebaudiana* promotes various beneficial effects on certain physiological systems such as cardiovascular and renal, decreases the content of sugar, radionuclides, and cholesterol in blood, improves cell regeneration and blood coagulation, suppresses neoplastic growth, strengthens blood vessels, and had a significant effect as antioxidant. The extracts can be consumed by healthy persons as well as by diabetics (Erkucuk *et al.*, 2009). Another interesting sweetener and sugar substitute is sucralose, an artificial sweetener with a sweetening power 600 times higher than sucrose. Stevia and sucralose are safe for consumption by diabetics because they do not increase blood glucose levels or insulin resistance (Tadhani *et al.*, 2007). One of the main problems of confectioneries manufactured with chocolate or substitutes that limit their shelf life is the yellowing (loss of white color,

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darkening or browning) during storage. White chocolate is expected to have a white-yellow color, so the presence of a dark yellow color, or even light brown color makes it undesirable by consumers; therefore, the result is a shorter shelf life time. Among the processes that generate the darkening of chocolate, there are the non-enzymatic browning reactions (NEB) that, through Maillard reactions cause the formation of undesirable intermediate compounds, such as furfural and 5-hydroxymethylfurfural (HMF). Therefore, an adequate description of the kinetic reactions in the foods is necessary for the purposes of processing design and storage. Maillard reactions may occur in white chocolate due its composition, i.e. a low water activity, a high fat content, a high relative concentration of reducing sugars (mainly lactose) and proteins (Ibarz *et al.*, 2000; Koca *et al.,* 2003; Vercet, 2003). The occurrence of these reactions depends basically on the temperature (Jardim *et al.,* 2011).

Chocolate blooming induced by exposure to high ambient temperatures involves a gradual change in color and loss of gloss, giving a whitish appearance to the chocolate surface. Temperature fluctuations and improper tempering conditions promote fat migration through the matrix of chocolate and, consequently, recrystallization on the surface (Briones and Aguilera, 2005). Quality evaluation of products is quite subjective, with attributes such as appearance, color, texture and flavor, reviewed by human inspectors. Human perception can easily lead to errors; furthermore, this type of analysis has high labor costs, inconsistency, and variability, requiring an objective measurement system. Recently, automatic inspection system like computer vision has been investigated (Brosnanand Sun, 2004). Computer vision is a technique for color evaluation and quantification which has been applied to chocolate in previous studies performed by Briones *et al.* (2005), and Briones *et al.* (2006), with the objective of detecting blooming. It induces a non-uniform pattern of color on the surface of chocolate. Some recent studies have used Stevia or sucralose as chocolate sugar-free sweetener (Aidoo*et al.,* 2015; Martins Medeiros de Melo *et al.,* 2007, 2009; Shah *et al.,* 2010; Palazzo *et al.,* 2011). However, the effect of replacing sucrose by combinations of Stevia and sucralose sweeteners, and the possible synergy effect resulting from this combination have been slightly investigated. In this context, the development of white chocolate formulations with a total or partial replacement of sucrose by sweeteners as sucralose and Stevia was proposed. Furthermore, we studied the single or combined effect of these sweeteners on stability, bloom development, rheological analysis and shelf life time of the final product, through the use of a mixture experimental design. Stability of different samples was analyzed by: i) physical studies as bloom formation in chocolate surface through image texture analysis and ii)chemical studies as nonenzymatic browning reactions. Besides, the acceptability of the developed product through a sensory analysis was investigated.

MATERIALS AND METHODS

Raw Materials

The materials used in the manufacture of white chocolate were: Cocoa Butter (Arcor SAIC, San Luis), whole milk powder (Ylolay, Argentina), skim milk powder (La Serenisima, Argentina), Sucrose (Ledesma SA, Argentina) , Sucralose (Sucaryl Sucralose, Argentina), Stevia powder (Tanki SA, Argentina), vanilla (Alicante, Argentina), Soy Lecithin (Yeruti S.R.L., Argentina).

Samples preparation

The white chocolate was produced following the next steps: sugar was milled together with milk powder using a grain mill (Corn-Grain-Cereal-Mill, China) and a grinder. Then, the cocoa butter was melted in a water bath. Sugar, milk powder and cocoa butter were mixed in a planetary mixer (Santini, Italy) during 5 min. Preparation was refined using a multihoyo screw extruder for 1h at 35±1 °C. The conchedwas performed under constant stirring at 200 rpm at 45±1 °C for 7 h. Lecithin and vanilla were added in the last 30 min of conching. Subsequently tempered by cooling to 23±1°C and then heated to 28±1°C. Samples were molded and cooled for 2h at 7±1°C. After cooling were packaged with a flexible material (Al-PET). The formulation tested was: Cocoa butter 28%, w/w; sucrose 47% w/w; whole milk powder 14.5% w/w; skim milk powder 11% w/w; soya lecithin 0.4% w/w; vanilla 0.1% w/w.

Experimental design

Response surface methodology (RSM) consists in modeling phenomena, where a several variables are tested simultaneously with a minimum number of trials, which elucidates interactions between variables with a less time-consuming than the classical methods (Carbonell Capella *et al.,* 2013). The influence of the combination of different saccharides as sweetening agents in sensory properties and quality of the products was studied using a mix design of three components (SAS, 1989). White chocolate formulations were made replacing partially or completely the sucrose content by zero-calorie sweeteners. The independent variables of the mixture experimental design were: sucrose (x_1, *S*), sucralose (x_2, *Su*) and Stevia (x_3, *St*). The proportion of the variables in the mix was calculated as percentage where the total amount of the saccharide in the mixture ranged from 0 to 100%. Sweeteners concentration was selected based on its equivalent with sucrose sweetness (1g of Stevia ≡ 15g of sucrose; 1g of Sucralose ≡ 7.5g of Sucrose). The polynomial equation that fitted the experimental data and describes the mixture (linear) model for three components may be represented by:

$$y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 \quad (1)$$

wherey_i is the dependent variable and represents the value of the property of interest, $b_i$are the parameters estimated by the model and x_1=sucrose, x_2=Stevia and x_3=sucralose are the independent variables and represent the equivalent concentrations with respect to sucrose sweetness (Ferreira *et al.*, 2007). The three variables (x_i) representing the proportion of the saccharides in the mixture, with $\Sigma x_i = 1$. The graphs of triangular response surface were constructed from the response of the samples obtained from the regression equations based on the variable of interest, using the Statistical 8 software. Besides to the formulations proposed in the experimental design another formulation was tested with 75% sucralose and 25% Stevia, since the concentration and integration of these components in the chocolate matrix had a great influence on the final product properties.

Shelf life time study

The chocolate samples were packaged in a flexible container (Al-PET) closed by thermal heat sealing that prevents the entry of humidity and oxygen, avoiding the influence of the environmental conditions on the product quality. This packaging avoids the permeation of oxygen and the interfering with the study results. The color products were selected as attributes for determining the quality loss of chocolate, considered the main reaction which limits the shelf-life, is caused by loss of white color during storage (Jardim *et al.,* 2011). The samples were stored in stove at constant temperatures of 30 °C and refrigerated at 7±2 °C and 15±2 °C during 100 days. The quality factors were periodically tested by triplicate, using the methods for determination of surface color.

Color determination: For the color determination of the chocolate surface, the samples were measured in three different zones with a spectrophotometer MiniScan EZ. Then, variation of the surface color (L^*, a^* and b^*) of each sample was monitored over time at different temperatures. The values of the three areas assessed were averaged (Jardim *et al.,* 2011). L^* is the lightness variable from 100 (white) to zero (black), whilst a^* and b^* are chromaticity, +redness/-greenness and +yellowness/-blueness, respectively (Skendi *et al.*, 2010).Whiteness Index (WI) for each sample was calculated according to the Equation (2) (Erdem *et al.,* 2014).

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

Kinetic degradation model

The analytical prediction of the deteriorating of the food quality involves a kinetic/ mathematical model (Dattatreya*et al.*, 2007). Kinetic parameters are sensitive to factors such as food composition and process characteristics. Degradation kinetic model described by Labuza and Riboh (1982) is useful for predicting the behavior of a particular reaction in a food product, and therefore is suitable for modeling the shelf-life of a product. The best known models referred to shelf-life studies are zero-order reactions that represent a linear evolution (retention or degradation) of the parameter, and first-order reactions that represent an exponential evolution of the parameter (Rodriguez Furlán *et al.,* 2014). The reaction rate of browning product formation can be described by the following differential equation:

$$v = \frac{d[P]}{dt} \quad (3)$$

Moreover, the reaction rate can also be expressed according to the following kinetic equation:

$$v = k[P]^n \quad (4)$$

where$n$ is the reaction order, k is the degradation rate constant, *[P]* is the quality factor concentration.

Equating equations (3) and (4) we obtain the general equation of the kinetic degradation model employed to predict the production the nonenzymatic browning (Labuza *et al.,* 1982):

$$-\frac{d[P]}{dt} = k[P]^n \quad (5)$$

where$t$ is the storage time.

Integrating Eq. (5), for a first order kinetics, n= 1:

$$\ln[P] = \ln[P]_0 - kt \quad (6)$$

where the subscripts *0* and *t* were at initial time and at time (t), after the degradation reaction, respectively. The relationship between the reaction constant and the temperature is quantified by the Arrhenius equation (Labuza *et al.,* 1982; Robertson, 1993):

$$k = Ae^{\left(-\frac{Ea}{RT}\right)} \quad (7)$$

whereE_a is the activity energy of the reaction (J/mol), R is the gases universal constant (8.314472 J/K mol), T absolute temperature (K), A pre-exponential constant or frequency factor (1/min), indicates the frequency of collisions.

Rheological properties

Rheological properties of the chocolate samples were measured using a viscometer Brookfield DV-III (Brookfield, USA). The chocolate was incubated at 50°C for 75 min and transferred to the viscometer cub, where was sheared at 5 s^{-1} for 10 min at 40°C before the measurement cycles started. The shear stress was measured at 40°C ranging the shear rate in a ramp up and down. The shear rate was increased 0.5 to 17.5 s^{-1} in 90 s and then was decreased from 17.5 to 5 s^{-1} in 90 s, 10 measurements for each ramp was performed (Do *et al.,* 2010; Sokmenand Gunes, 2006). This test allowed determine if a sample presents a thixotropic behavior, since a sample can be considered thixotropic if when the shear rate is increased, the viscosity decreases to equilibrium value (Do *et al.,* 2010; Fernandes and Sandoval, 2013). Rheological data, shear stress (τ) and shear rate (γ), were analyzed by means of the Herschel-Bulkley model (Equation 10), to describe flow behavior and 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 *et al*., 2006).

$$\tau = \tau_0 + K\gamma^n \quad (8)$$

where τ_0 is the yield stress (initial stress), K is the consistency index, n is the flow behavior index (< 1 for pseudoplastic or shear-thinning fluids).

Statistical analysis

The test de Turkey 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 GraphPadInStat software (1998).

RESULTS AND DISCUSSION

Shelf life time of white chocolate

The shelf life time of a food can be defined as the period in which the level of sensorial, functional and nutritional quality is preserved (Rodriguez Furlán *et al.,* 2010). The main reaction that produces quality loss of white chocolate, and limits its shelf-life is caused by the loss of white color during storage. This quality loss is produced mainly by Maillard reaction and, its consequence is the appearance of browning colors (Jardim *et al.,* 2011). The white chocolate samples were tested by measuring the color production on the chocolate surface. These quality factors as a function of time and temperature were analyzed to determine the reaction kinetics, and to predict the shelf-life time of the product. From the experimental data of color production the Whiteness Index *WI* (defined as a quality factor) as a function of time at different storage temperatures (7 °C, 15 °C and 30 °C), the reaction kinetics and then, the shelf life time were determined on the tested samples. The results obtained for the quality factor are presented in Fig. 1. From the representation of the ln k (rate constants) versus reciprocal of temperature ($1/T$), calculated at 7°C, 15°C and 30°C from the experimental data, *Ea* can be obtained (Fig. 2). Table 1 shows *Ea* data, obtained from the kinetic data of *WI*.

Table 1. Kinetic constants (*k*), activation energies (*Ea)* and pre-exponential factors *(A)* from the experimental data of color production (Whiteness Index)for all samples tested at different storage temperatures (7 , 15 and 30 °C). Shelf life time (T= 20 °C). Rheological parameters obtained from Herschel- Bulkley model and viscosity of white chocolate samples (T= 40 °C).[a]

Samples	E_a (KJ/mol)	A (s^{-1})	k (s^{-1}) T = 20 ºC	$t_{1/2}$ (years) T = 20 ºC	Herschel- Bulkley parameters			Apparent Viscosity at a shear rate of 5 s^{-1} (mPa s)
					τ_0 (Pa)	K (Pa s)	n	
100%S	91.2 ± 5.2[a]	1.1 x 10^6	5.74 x 10^{-11}	2.2	3.4	2.4	0.9	7,860 ± 341[1]
100%St	104.0 ± 6.1[a]	3.3 x 10^8	9.41 x 10^{-11}	1.3	0.0	0.9	0.8	516 ± 26[2]
100%Su	68.6 ± 3.6[b]	1.8 x 10^2	1.06 x 10^{-10}	1.2	0.0	1.1	0.8	783 ± 45[3]
50%S+50%St	72.5 ± 4.0[b]	7.0 x 10^2	8.43 x 10^{-11}	1.5	1.5	2.1	0.8	5,324 ± 280[4]
50%S+50%Su	46.0 ± 2.8[c]	1.1 x 10^{-2}	6.76 x 10^{-11}	1.9	1.2	1.5	1.0	2,832 ± 152[5]
50%Su+50%St	106.2 ± 5.9[a]	1.4 x 10^9	1.55 x 10^{-10}	0.8	0.0	1.0	0.9	890 ± 54[3]
75%St+25%Su	96.6 ± 5.3[a]	1.1 x 10^7	6.42 x 10^{-11}	2.0	0.0	1.7	0.6	1,167 ± 78[5]

The shelf life time ($t_{1/2}$) was defined as the storage time until to get a value of *WI* equal to 55 (final quality factor). This limit was reached when the chocolate became dark yellow or brownish, considered as sensorially unacceptable. The shelf life time of the product stored at different temperatures was calculated by Equations 6 and 7, and using the calculated *Ea* value obtained from the experimental model for each system tested. Model fit was confirmed from the magnitude of the coefficients of R^2 ($0.92<R^2<0.99$). According to Arrhenius in the theory of collisions, the reactants are transformed into products when first reach a minimum amount of energy, called *Ea*. The factor *A* provides information of the number or frequency of collisions. After each individual collision at a molecular level, a successful reaction can be produced. Collision number can be related to product stability, since the molecules must diffuse through the product matrix in order to react. Therefore, while the higher the energy between the molecules absorbed and the molecules within the system matrix, the lower will be the diffusion of the molecules. Table 1 shows the shelf life time data calculated from the values of *Ea* and *A* for each system tested at 20 °C. When comparing the formulations of sucralose with Stevia at different concentrations (50%St+50%Su and 75%St+25%Su), an antagonistic effect by combining 50%Su with 50%St can be observed, since despite the decrease of the matrix sensitivity to the temperature (>*Ea*), the number of collisions increases (>*A*), suggesting that a change in microstructure is leading to a higher value of *k* (Table 3), resulting in lower lifetime (0.81) than the samples 100%Su (1.2) and 100% St (1.3). However a synergistic effect combining 75%St with 25%Su ($P<0.001$) was observed, with high value of *Ea* (higher than the control sample, 100% S) with minor rate constant (Table 1) and so a lifetime improved (2.0 years). Hence, the sample 75%St+25%Su achieve a similar stability than the control sample 100% S (2.2 years). This behavior confirmed the results obtained in a previous stability study, measuring the formation of compounds of non-enzymatic browning.

From E_a(T=20°C) values attained from *WI* values, Scheffe canonical equation was obtained:

Ea= 21,808 S + 24,797 St + 16,364 Su – 23925 S St – 32,340 S Su + 18,676 St Su (9)

The model adequately fits the data with $P<0.05$, a 95% confidence limit and $R^2 = 0.9997$.

By comparing the experimental with theoretical values, an average relative error of 5% was obtained. Only the terms statistically significant ($P<0.05$) were incorporated in the Equation 9.

The binary combination of sucralose with sucrose, contribute more to the model than other factors. A significant antagonistic effect (negative sign in the expression) for the samples: (50%S+50%Su) and Stevia (50%S+50%St) were observed. The model for the system represented by Equation 9was used to generate the response surface graph shown in Fig. 3. The highest values are observed in the right-hand side of the graph which correspond to the binary combination of the sweetener sucralose and Stevia (50%St+50%Su and 75%St+25%Su), being greater than the control sample value (100%S). The *Ea* value decreases to the left-hand side of the graph in the left direction, reaching a minimum for samples containing combinations of sucralose and sucrose (50%S+50%Su).

Rheological behavior

The *Herschel- Bulkley* model was proposed for modeling the rheological behavior of each sample of white chocolate. The diagnostic analysis of the proposed model presented residual plots with no systematic patterns, and normal distribution with $P>0.1$ for all the samples tested. Thus, this model passed the normality test where the data presented a Gaussian distribution ($R^2 \approx 1$).

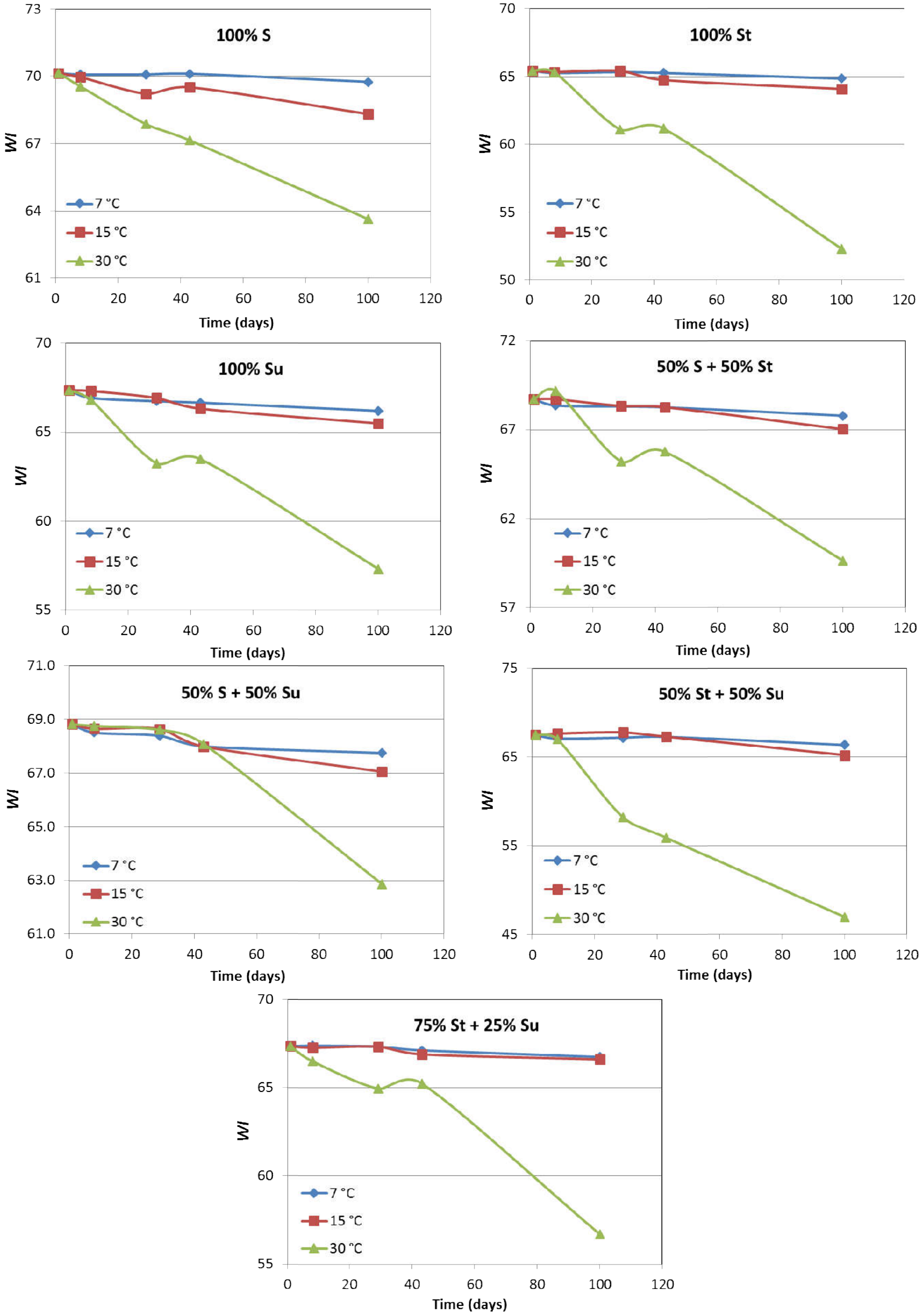

Figure 1. Experimental data for the quality factor color production (Whiteness Index = WI) for chocolate samples with Stevia and sucralose as sugar replacements

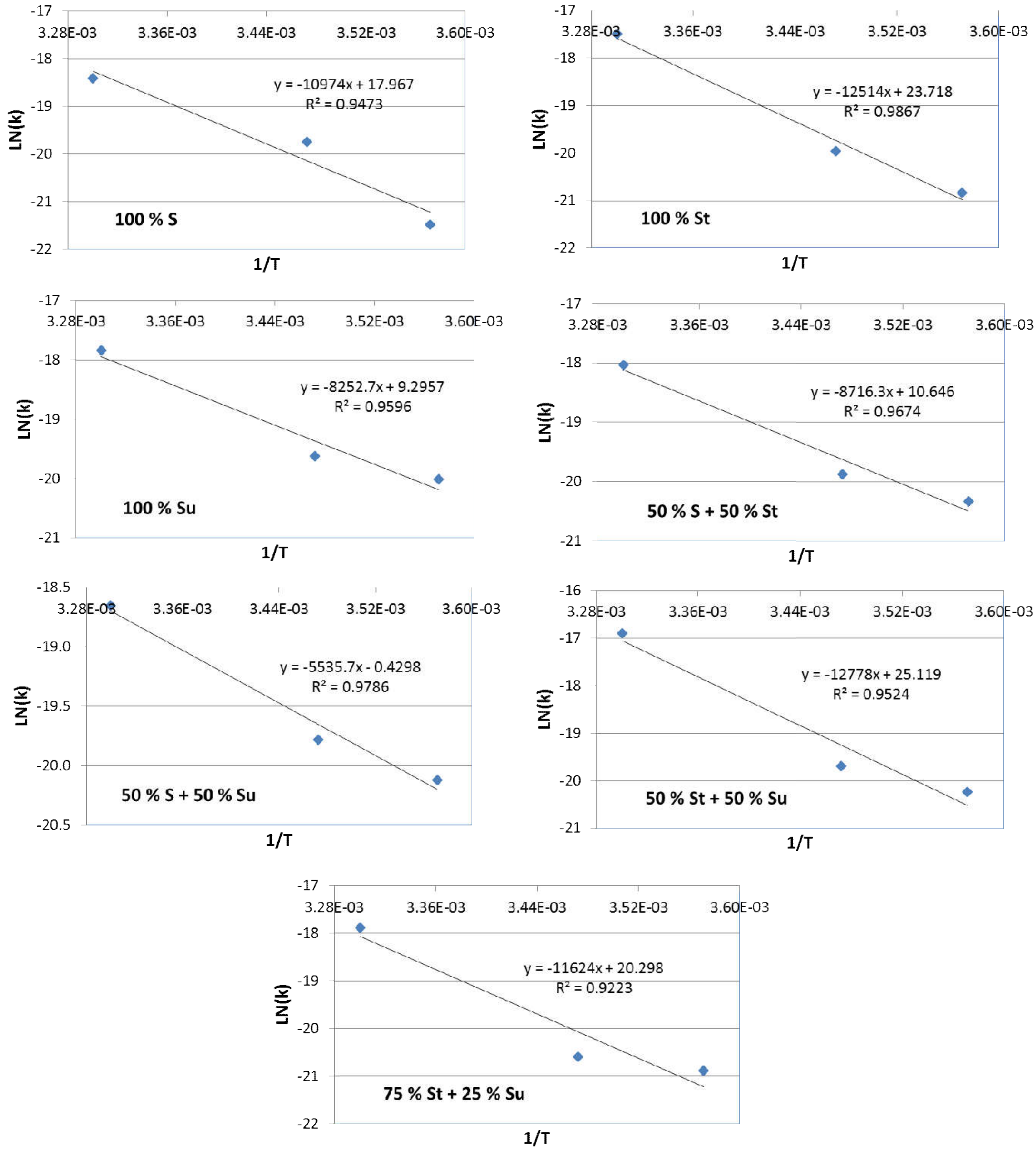

Figure 2. Determination of *Ea* for the quality parameter of surface color in white chocolate formulated with sugar replacement

Sokmen *et al.* (2006) also found that the *Herschel-Bulkley* model described correctly the rheological behavior of chocolate with bulk sweeteners. The parameters of this model (τ_0, K, n) are presented in Table 1. The initial yield stress (τ_0) was zero in all samples except those containing sucrose 100% S, 50%S+50%St and 50%S+50%Su. This result may be due to the greater concentration of solids generating a generated a higher concentration of suspended solids with a greater creep resistance than samples with sweeteners. This behavior is important, because the yield stress keeps small solid particles in suspension, giving greater stability to the chocolate (Sokmen *et al.,* 2006). The flow behavior index obtained from the *Herschel- Bulkley* model is between 0.6 and 1 (Table 1), like pseudoplastic fluids. Previous studies demonstrated a similar behavior in chocolate samples (Sokmen *et al.,* 2006). Samples with sucrose presented a higher consistency index (*K)* than those without sucrose; this may be due to their higher percentage of solids in suspension. International Office of Cocoa, Chocolate and Sugar Confectionery (IOCCC), National Confectioners Association (NCA), and Manufacturing Confectioners Association (CMA) established as acceptable range of rheological measurements for chocolate, a shear rate between 5 s^{1} and 60 s^{-1}. Therefore, a shear rate of 5 s^{-1} was selected; obtained data are shown in Table 4. Sweetener samples (100%St, 100%Su, 50%St+50%Su and 75%St+25%Su) had lower viscosity than the samples 100% S, 50%S+50%St and 50%S+50%Su. This may be due to the high content of solids, since the interactions between particles in suspension and interfacial properties affect the rheological properties of chocolate. Similar results were reported by Belščak-Cvitanović, *et al.* (2012) who worked with chocolate enriched with polyphenol from *Rubusidaeus L* leaves. Furthermore, the flow properties depend on the fat content and on the free fat of the sample because the fat either separates the particles or fills the inter-particle spaces (Ziegleder *et al.,* 2004).

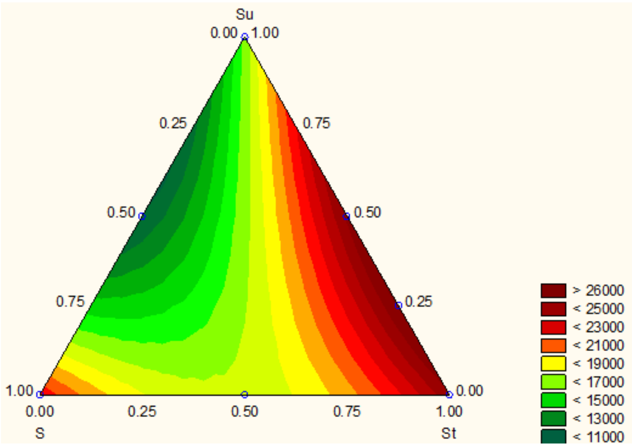

Figure 3. Contour plot of *Ea*in white chocolate containing different blends of sweeteners: Sucrose (S), Sucralose (Su) and Stevia (St)

In this regard, samples without sucrose had a higher fat content, with τ_0 = 0 and hence, a lower viscosity. It is important to highlight that the moisture content of all the studied samples were similar —between 2% and 3.5%— and therefore, it cannot be associated with the different apparent viscosities found. This was also exhibited by Sokmen *et al.* (2006) who study the rheological properties of chocolates with maltitol and isomalt as replacers of sucrose. The viscosity of the samples with single sweeteners 100% St and 100%Su was lower than that of the samples which combined these two sweeteners in different proportions (50%St+50%Su and 75%St+25%Su) (P<0.01). This suggests a synergy effect of sucralose with Stevia. The sample 75%St+25%Su showed a higher viscosity than the samples containing sucralose and Stevia (50%St+50%Su; 100%St and 100%Su); this behavior can be attributed to the adequate interaction between the components at this concentration and with the different components of the chocolate matrix.

Conclusions

Kinetic studies of shelf life time were performed using as indicators of product quality the formation of nonenzymatic browning compounds and the coloration of chocolate surface during a storage time of 3 months, at different temperatures (7 °C, 15 °C and 30 °C). The results showed that white chocolate with a combination of sweeteners 75% Stevia (St) and 25% Sucralose (Su) improved shelf life time (1.3 years), being this value higher than the control sample (100% sucrose, 100% S). The activation energy values obtained from the kinetic study of nonenzymatic chocolate browning by measuring surface color (a*) was modeled by Scheffe canonical equation. This model indicated that binary combinations of sucrose with sucralose, and sucralose with Stevia contributed more to the model than other factors, showing a significant antagonistic and synergistic effect, respectively. From the studies of rheological behavior a synergy effect between sucralose and Stevia was found. The sample 75%St+25%Su showed a higher viscosity than the samples containing sucralose and Stevia (50%St+50%Su; 100%St and 100%Su); this behavior can be attributed to the adequate interaction between the components at this concentration and with the different components of the chocolate matrix. Finally, by this study a formulation of white chocolate (75%St+25%Su) physically acceptable was obtained, being suitable for diabetics, with similar physical properties to the control sample, and with a longer shelf life time.

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