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Development of free sugar white chocolate, suitable for diabetics, using Stevia and sucralose as sweeteners: study of the thermal degradation kinetic

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Abstract-The purpose of this study was the development of formulations of white chocolate for diabetics with replacement of sucrose by sucralose (Su) and Stevia (St) using a mixture experimental design. The kinetic studies of thermodegradation which showed that binary combinations of Stevia with sucrose had synergistic effects since the matrix presented a lower thermal sensitivity to the non-enzymatic browning reaction than the samples formulated from the individual components. The phenomena of blooming during storage were studied by a computer vision system and image analysis. The results of sensory analysis revealed that the sample 100%St was not acceptable; however, combining Stevia with sucrose and sucralose, acceptable sensory chocolates were obtained with no statistically significant differences, compared to control 100%S (P> 0.05). Our study provides a chocolate suitable for diabetics, with an appropriate combination of sensorially acceptable sweeteners with higher stability than control sample.

Index Terms-White chocolate,sucralose, Stevia, Image analysis.

1. 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 [1]. 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 [2]. 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 [3].

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[4].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 [5].

One of the main problems of confectioneries manufactured with chocolate or substitutes that limit their shelf life is the yellowing (loss of white color, darkening or browning) during storage. 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 [6,7,8]. The occurrence of these reactions depends basically on the temperature [9].

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 [10]. 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 [11]. Computer vision is a technique for color evaluation and quantification which has been applied to chocolate in previous studies performed by [10] and [12], 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 [13,14,15,16,17]. However, the effect of replacing sucrose by combinations of Stevia and sucralose sweeteners, and the possible synergy effect resulting from this combination have not 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 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.

2. MATERIALS AND METHODS

2.1. 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).

2.2. 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.

2.3. Experimental design

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 [18]. 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:

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

where y_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 [19]. 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.

2.4. Thermal degradation 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. Since, the formation of lipid oxidation intermediates or lipid peroxidation that can react with intermediates of the Maillard reaction increasing the reaction rate are prevented [20]. The the concentration of non-enzymatic browning products were selected as attributes for determining the quality loss of chocolate[9]. For the kinetics study 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 non-enzymatic browning.

Non-enzymatic browning reactions (NEB): 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 3,000 rpm during 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 forNEB measurements by reading absorbance at 280 nm using a spectrophotometer UV-Visible, double beam - (Shimadzu, USA) [8].

2.5. Kinetic degradation model

Kinetic parameters are sensitive to factors such as food composition and process characteristics. Degradation kinetic model used to predict the production of non-enzymatic browning compounds of the chocolate samples was described by [21]. The best known models referred to thermal degradation studies are first-order reactions that represent an exponential evolution of the parameter [22].

The reaction rate of browning product formation can be described by the following differential equation:

$$v = \frac{d[P]}{dt} \qquad (2)$$

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

$$v = k[P]^n \qquad (3)$$

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

Equating equations (2) and (3) we obtain the general equation of the kinetic degradation model employed to predict the production the nonenzymatic browning [21]:

$$-\frac{d[P]}{dt} = k[P]^n \qquad (4)$$

where t is the storage time.

Integrating Eq. (4), for a first order kinetics,n= 1:

$$\ln[P] = \ln[P]_0 - kt \qquad (5)$$

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 [21,23]:

$$k = Ae^{\left(-\frac{Ea}{RT}\right)} \qquad (6)$$

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.

2.6. Image texture analysis: Bloom development during storage

Computer vision is a technique for color evaluation and quantification, composed basically of a correct illuminant for reduce reflection and shadows, a digital camera of high-resolution, an image capture board, and computer hardware and software to process the images. Image texture represents the spatial variability of pixel gray levels over the whole image and, thus, it provides useful information about color patterns [10,12]. A method of image texture analysis widely used in food industry is the gray level co-occurrence matrix (GLCM). The intensity variation of pixels may be correlated to the sample texture [24]. Different image features can be calculated from these matrices: contrast, homogeneity, entropy and energy [12]. The method of texture analysis is based on human perception of the food surface. These types of analyses include the Fourier Transform, which analyzes the intensity variation in the pixels across the image. Consequently, the texture can be related to sensory properties of food products. These image features have been applied to chocolate in previous studies by [10,12].

The image texture analysis was performed by taking images using a computer vision system. This system comprised a standard gray box (L^*= 50 inCIELab scale) of the following internal dimensions: 30.5 cm wide, 43.3 cm long and 23.2 cm high. The inner box had a pattern of illumination (Illuminant D65, standard light that mimics daylight) consisting of four lamps D65 placed above the sample at a 45° angle to maximize the diffuse reflection responsible for color, and a digital camera (Sony cybershop, USA). The angle between the camera lens axis and the sample was around 90° to reduce gloss. The images were taken without zoom or flash, and were kept in JPG format. After that, the photographs were digitized at 24 bits/pixel formed by levels of primary colors: red, green and blue (RGB), and then, transformed to grayscale [10,12,25]. The images were analyzed using a program designed in Matlab where different parameters related to the characteristics of texture were analyzed: homogeneity, contrast, energy, correlation, entropy, and standard deviation. As contrast and entropy were the only two that detected correctly the appearance of Bloom or roughness during storage, they were the texture parameters selected. Contrast, also called as the sum of variance squares, can be defined as the difference between the values of the highest and lowest set of contiguous pixels. Mathematically, the contrast is represented as:

$$\sum_{i,j} |i-j|^2 P(i,j) \qquad (7)$$

The feature of entropy refers to the amount of energy lost as heat, the "chaos" that appears when a reaction or physical transformation occurs. This term in non-technical language is used as chaos or irremediable disorder. The mathematical formula is given by:

$$-\sum_{i,j} P(i,j)\log[P(i,j)] \qquad (8)$$

A Matlab program was development in this work for analyzing the texture parameters selected (contrast, entropy and deviation). The standard deviation was calculated from the grayscale image with this program. The images were subsequently analyzed with the Image- Pro Plus program to determine the percentage of area occupied by the Bloom, expressed in terms of total area of the different formulations studied.

2.7. Determination of the fat release in white chocolate

Four grams of white chocolate was weighting and melted at 50 °C for 20 min and then centrifuged at 3000 rpm for 30 min. The fat layer on top was separated from the sediment, weighed and calculated the percentage of fat release as g/100 g mobile fat in the chocolate [26].

2.8. Sensory analysis

Each sample of white chocolate was identified with a random three-digit code and randomly ordered. Samples were server in white plastic cups; water and bread were provided for cleaning the palate between samples. The sample was tested at room temperature by 25 semi-untrained panelists who judged the samples through a five-point hedonic scale (5 = extremely like, 3 = neither like nor dislike, 1 = extremely dislike). Flavor, aroma, color, shape melting (how it melts in the mouth) and smoothness (sensation on tongue and roof of mouth while product is melting) were evaluated [27].

2.9. Statistical analysis

The test de Tukey 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).

3. RESULTS AND DISCUSSION

3.1. Thermal degradation kinetic of white chocolate

The white chocolate samples with or without addition of different sweetener concentrations were tested by measuring the concentration of non-enzymatic browning compounds. These quality factor as a function of time and temperature were analyzed to determine the reaction kinetics. The results obtained for the quality factor, defined by the relative content of nonenzymatic browning compounds, are presented in Fig. 1. Experimental data were examined with rate equations of different orders; the first-order equation was found to provide the best fit to describe the production of nonenzymatic browning compounds ($0.83<R^2<1.00$). The linear regressions were calculated from experimental data of the sample absorbance during storage at 7 °C, 15 °C, and 30 °C. According to Arrhenius model (Eq.6), ln k versus the reciprocal of temperature (1/T) was represented, and a straight line with a slope representing $-Ea/R$ was obtained. The model fit was assessed by the regression value ($0.81<R^2<0.94$). Ea values are recorded in Table 1.

Table 1. Activation energyvalues obtained from the Arrhenius equation from the nonenzymatic browning studies (Ea) for all samples tested at different storage temperatures (7 , 15 and 30 °C)[a].

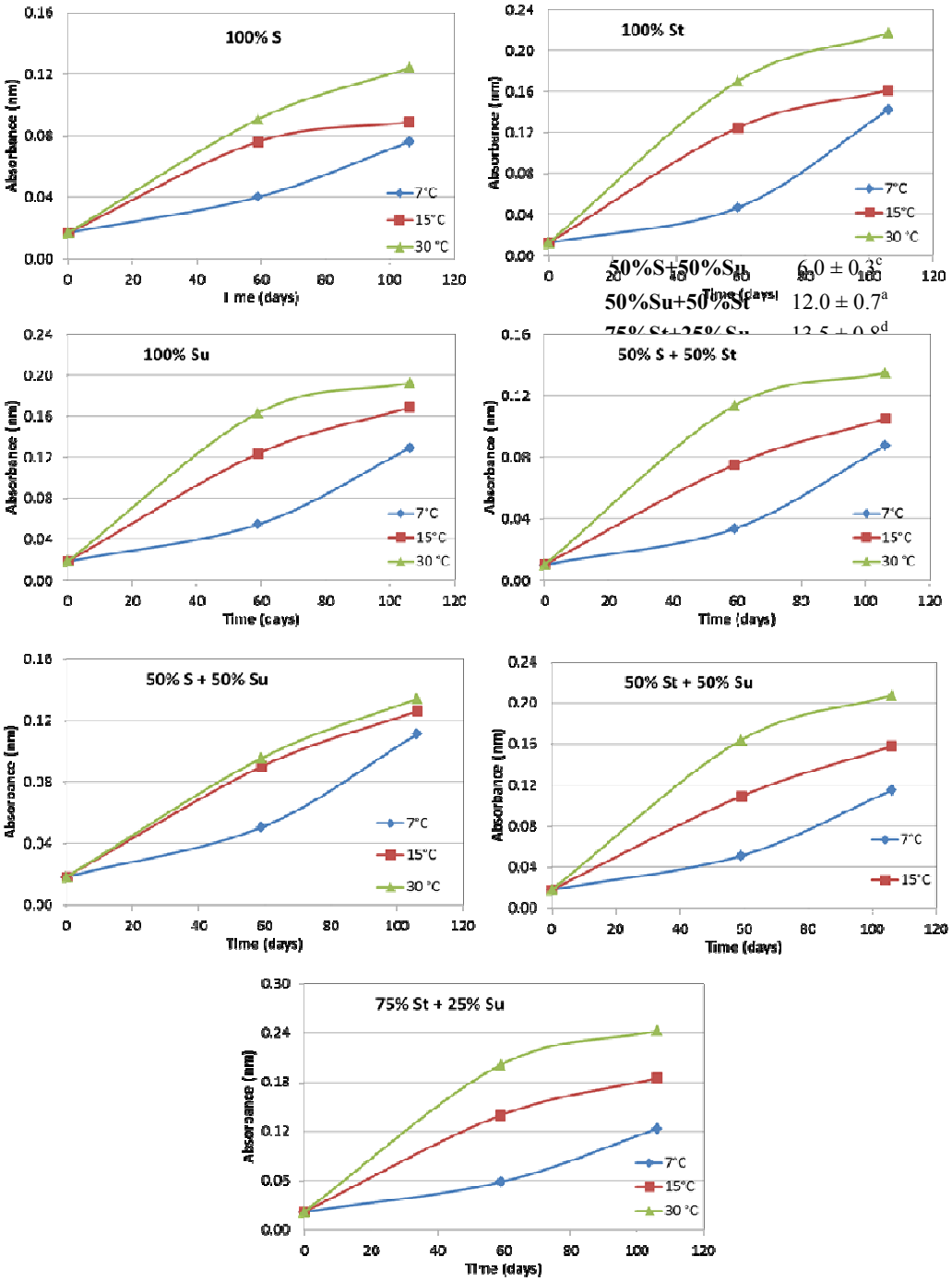

Figure 1.Production of nonenzymatic browning compounds as a function of time for the chocolate samples tested under different storage temperatures (T = 7 °C, 15 °C and 30 °C).

50%S+50%Su 6.0 ± 0.3^{c}

50%Su+50%St 12.0 ± 0.7^{a}

[a]Means with equal superscripts are not significantly different (P>0.05) by the Tukey's test.

These results confirm the close relationship between temperature and the production of non-enzymatic browning in white chocolate. Non statistically significant difference between *Ea* values of samples 100%St, 100%Su and 50%S+50%St was found. The

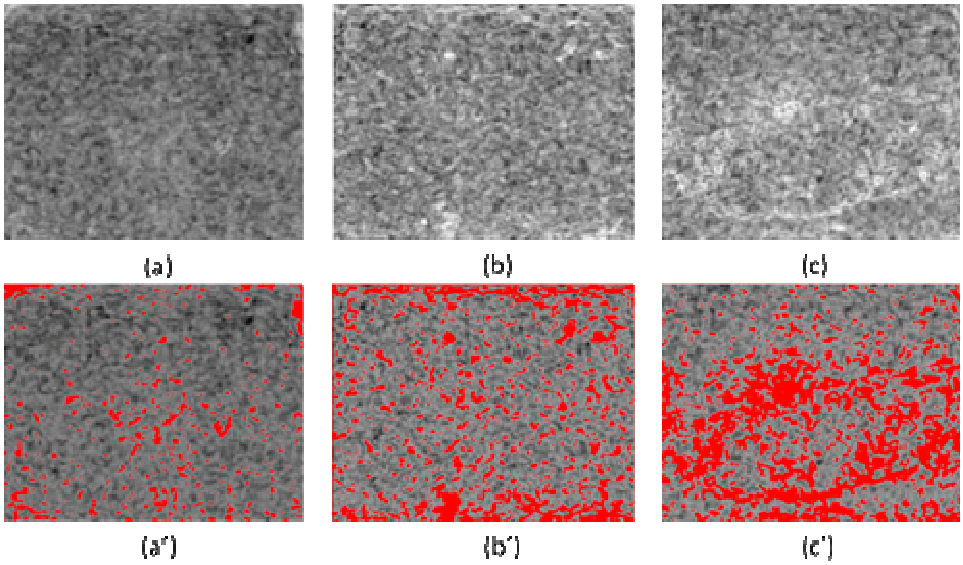

Figure 2. (a) Captured images of a chocolate sample at the beginning of storage, (b) image of chocolate after 10 days of storage, (c) image of chocolate at 78 days of storage. (a '), (b') and (c ') identifying the occurrence of Bloom in chocolate samples (a), (b) and (c) respectively.

samples 100%St and 100%Su presented lower*Ea* values than the control sample (100%S), (P<0.05), showing their lower stability. However, no statistically significant difference between the control (100%S) and the sample 50%S+50%St (P>0.05) was obtained. The formulation with a combination of sucralose and sucrose (50%S+50%Su) presented an important antagonistic effect with respect to the thermal sensitivity of the matrix (<*Ea*) with a lower stability than the control sample (P<0.001). However, the combination of sucralose and Stevia in different concentrations (50%St+50%Su and 75%St+25%Su) improved the stability, especially for the concentration 75%St and 25%Su showing a similar stability to the control sample.

3.2. Image analysis

The evolution of changes in the color distribution pattern ―white background, yellowish background and white specks― can be represented quantitatively as the percentage of the total area [10].

At the beginning of storage, the white specks reached a value of 6.3% (Fig. 2a´) of the total area, increasing rapidly up to 11.9% at the 10th day(Fig. 2b`) and then, the white background began to increase graduallyrising to 30.9% of the total area of the sample (Fig. 2c´). This behavior was also observed in the contrast parameter obtained from texture image analysis (Eq.7), (Fig. 3). Fig. 3 shows that at day 10 of storage, a decrease in the contrast values of the samples 100%S, 100%St, 100%Su and 50%S+50%St (P<0.05) was observed. This may be due to the appearance of round white specks (Fig. 2b).Subsequently, the contrast values tended to increase approaching day 78 of storage. This may be because the white background begins to replace gradually the yellowish background (Fig. 2c).The samples 50%S+50%Su and 50%St+50%Su show a gradual increase in the contrast values along the studied storage period, mainly due to the replacement of the yellowish background by a whitish one. The contrast value of the sample 75% St+25% Su remained approximately constant during this period (P>0.05), indicating a high stability against the Bloom formation on the chocolate surface.Therefore, the different chocolate samples developed dissimilar color patterns during blooming. This behavior was also observed by [10]who studied the Bloom formation on the surface of black chocolate tablets.

3.3. Prediction of fat release in white chocolate

Fig. 4 shows the relationship between the percentage of fat release or fat percentage available to spread rapidly in the chocolate and the percentage of fat present in the sample. This relationship revealed that the percentage of fat release depends on the percentage of fat in the sample.

Release fat propagation in the chocolate was modeled (Fig. 4a). Experimental data was described by the equation of a straight line (R^2> 0.955), [28]:

$$O = d \cdot L - e \qquad (9)$$

where$O$ is fat release (%), L is lipid content (%), d and e are parameters obtained by regression analysis of experimental data.

The values of d and e from the experimental data were obtained (d = 1.5; e = 37.3). The parameter d and e

were used to compute the intersection of the straight line with the x axis. The intercept (*e/d*, % w/w) known as fatspread index represents the critical concentration above which fat can be released from the spread [28].

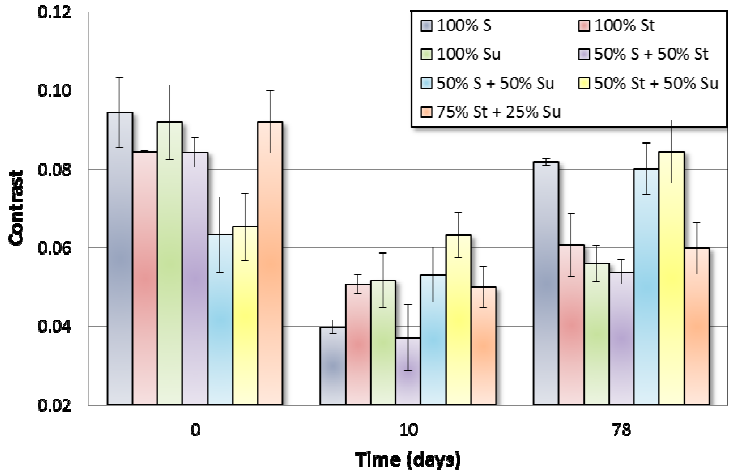

Figure 3. Changes in the contrast of the chocolate surface during storage (average values for all samples).

Therefore, for the samples tested the chocolate spread could be considered physically stable when fat separation by centrifugation is lower than 25% (critical fat spread index, *e/d*). In this sense, the samples which 0% sucrose, have a high propagation rate and therefore, considering this parameter, these samples would be more susceptible to the formation of Bloom in chocolate surface during storage. According to the data shown in Fig. 4b, the tendency for the formation of surface Bloom was 50%St+50%Su < 100%St < 75%St+25%Su < 100%Su, being less propended to fat spread the samples 50%S+50%Su, 50%S+50%St and 100%S. From the data previously obtained in Bloom formation study, the sample 75%St+25%Su was the most stable, contradicting the study of fat propagation. Therefore, the addition of the 75%St+25%Su in chocolate matrix provides a stabilizing effect on the Bloom surface formation delaying this process.

3.4. Sensory analysis

Chocolate formulations of 100%Su, 50%S+50%St, 50%S+50%S, 50%Su+50%St and 75%St+25%Su were sensory acceptable, obtaining a higher value than 4.5 for the tested sensory parameters (flavor, aroma, color,
shape melting and smoothness), among a range of 5 to 6.5. No significant differences between these samples and the control 100%S were found. The taste parameter of the sample 100% St was not sensory acceptable with a value of 2.92±0.65, being less accepted than the control sample 100% S (P<0.05). This result may be because the sweetness profile depends on the food matrix in which the sweetener is incorporated [2]. The results reported by [14] also

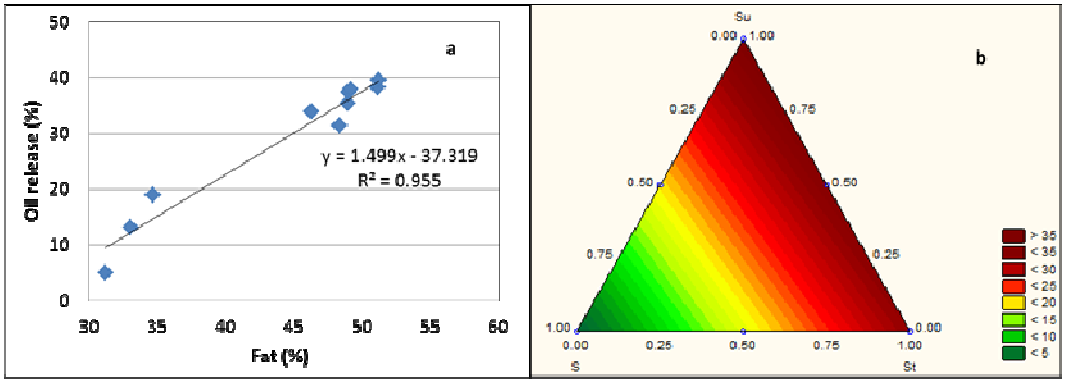

Fig. 4. a) Relationship between oil release (%) and fat content (%) of white chocolate. b) Contour plot of fat releasein white chocolate containing different blends of sweeteners: Sucrose (S), Sucralose (Su) and Stevia (St).

showed a loweracceptability of chocolates sweetened with Stevia. However, the chocolate formulations developed with combinations of sucralose with sucrose and Stevia in different ratios allowed to achieve sensoryacceptable chocolates.

4. CONCLUSIONS

Kinetic of thermal degradation was performed using as indicator of product quality the formation of nonenzymatic browning compounds 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 the stability of the product (>E_a), being this value higher than the control sample (100% S).

Bloom is a complex phenomenon of color change that must be analyzed properly, since it modifies the surface of chocolate tablets. Image analysis represents an appropriate technique to capture the spatial changes, and to measure and analyze the changes in color during the development of fat bloom. Furthermore, this technique is relatively simple, versatile and can be implemented at low cost. The present study showed that sample 75%St+25%Su showed a greater stability than the other sugar-free samples, and that it was similar to control (100%S). Samples with a total sugar replacement presented a high index of fat spread rate. Therefore, considering this parameter, these samples would be more susceptible to the formation of bloom. However, the combination of sweeteners 75%St+25%Su, resulting in a matrix stabilizing effect, reduces the bloom formation on chocolate surface, being comparable to sample 100%S.

The sensory parameters of sample 100%St were not acceptable. However, with the combination of Stevia with sucrose or sucralose, an improvement of the sensory acceptability of this sample was possible. No statistically significant difference was found with the control sample 100% S (P>0.05). Finally, by this study a formulation of white chocolate (75%St+25%Su) sensorially and physically acceptable was obtained, being suitable for diabetics, with similar physical properties to the control sample.

REFERENCES

[1] Sokmen A.; Gunes G. (2006): Influence of some bulk sweeteners on rheological properties of chocolates. LWT- Food Science and Technology,**39**, pp.1053-1058.

[2] Aidoo, R. P.;Depypere, F.;Afoakwa, E. O.;Dewettinck; K. (2013): Industrial manufacture of sugar free chocolates - Applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development. Trends in Food Science & Technology,**32**(2), pp. 84-96.

[3] Porto Cardoso, J. M.; Bolini, H. M. A. (2007): Different sweeteners in peach nectar: Ideal and equivalent sweetness. Food Research International, **40**, pp. 1249-1253.

[4] Erkucuk, A.; Akgun, I. H.;Celiktas-Yesil, O. (2009): Supercritical CO_2 extraction of glycosides from *Stevia rebaudiana* leaves: Identification and optimization. Journal of Supercritical Fluids, **51**, pp. 29-35.

[5] Tadhani, M. B.; Patel, V. H.;Subhash, R. (2007): In vitro antioxidant activities of Stevia rebaudiana leaves and callus. Journal of Food Composition andAnalysis,**20**, pp. 323-329.

[6] Ibarz, A.; Pagan, J.; Garza, S. (2000): Kinetic models of non-enzymatic browning in apple puree. Journal of the Science of Food and Agriculture, **80**, pp. 1162-1168.

[7] Koca, N.;Burlurlu, H. S.;Karadenüz, F. (2003): Kinetics of Nonenzymatic Browning Reaction in Citrus Juice Concentrates during Storage. Turkish Journal of Agriculture and Forestry,**27**, pp. 353-360.

[8] Vercet, A. (2003): Browning of white chocolate during storage. Food Chemistry, **81**, pp.371–377.

[9] Jardim, D. C. P.;Orseb, A. G.;Efraima, P.; de Moura, S. C. S. R. (2011): Procedia Kinetic of white chocolate color loss. Food Science, **1**; 1026-1030.

[10] Briones, V.; Aguilera, J.M. (2005): Image analysis of changes in surfaces color of chocolate. Food Research International,**38**, 87-94.

[11] Brosnan, T.; Sun, D. (2004): Improving quality of food products by computer vision- a review. Journal of Food Engineering,**61**, pp. 3-16.

[12] Briones, V.; Aguilera J. M.; Brown, C. (2006): Effect of surface topography on color and gloss of chocolate samples. Journal and Food Engineering, **77**, pp. 776-783.

[13] Aidoo, R. P.;Afoakwa, E. O.;Dewettinck, K. (2015): Rheological properties, melting behaviours and physical quality characteristics of sugar-free chocolates processed using inulin/ polydextrose bulking mixtures sweetened with stevia and thaumatin extracts. LWT - Food Science and Technology, **62**(1), pp. 592-597.

[14] Martins Medeiros de Melo, L. L.; Bolini, H. M. A.; Efraim, P. (2007): Equisweet milk chocolates with intense sweeteners using time-intensity methods. Journal of Food Quality, **30**, pp. 1056-1067.

[15] Martins Medeiros de Melo, L. L.; André Bolini, H. M.; Efraim, P. (2009): Sensory profile, acceptability, and their relationship for diabetic/reduced calorie chocolates. Food Quality and Preference,**20**, pp. 138–143.

[16] Shah, A. B.; Jones, G. P.; Vasiljevic, T. (2010): Sucrose-free chocolate sweetened with Stevia rebaudiana extract and containing different

bulking agents e effects on physicochemical and sensory properties. International Journal of Food Science and Technology, **45**, pp. 1426-1435.

[17] Palazzo, A. B.; Carvalho, M. A. R.; Efraim, P.; Bolini, H. M. A. (2011): Determination of isosweetness concentration of sucralose, rebaudioside and neotame as sucrose substitutes in new diet chocolate formulations using the time-intensity analysis. Journal of Sensory Studies, **26**, pp. 291-297.

[18] SAS userguide: Statistic. versión (1989). Cary, NC, USA: SAS Inst. Inc.

[19] Ferreira, S. L. C.; Bruns, R. E.; Paranhos da Silva, E. G.; dos Santos, W. N. L.; Quintella, C. M.; David, J. M.; de Andrade, J. B.; Breitkreitz, M. C.; Jardim, I. C. S. F.; Neto, B. B. (2007): Statistical designs and response surface techniques for the optimization of chromatographic systems. Journal of Chromatography A, **1158**, pp. 2-14.

[20] Hidalgo, F. J.; Zamora, R. (2000): The role of lipids in nonenzymatic browning. Grasas y Aceites, **35**(51), pp. 35-49.

[21] Labuza, T. P.; Riboh, D. (1982): Theory and application of Arrhenius kinetics to the prediction of nutrient losses in foods. Food Technology, **36**(10), pp. 66-74.

[22] Rodriguez Furlán, L. T.; Aldrete Herrera, P.; Pérez Padilla, A.; Ortiz Basurto, R. I.; Campderrós, M. E. (2014): Assessment of agave fructans as lyoprotectants of bovine plasma proteins concentrated by ultrafiltration. Food Research International**, 56**, pp. 146-158.

[23] Robertson, G. L. (1993): Food packaging. Principles and practice. New York: Marcel Dekker, pp. 343–351.

[24] Zheng, C., Sun, D.; Zheng, L. (2006): Recent developments and applications of image features for food quality evaluation and inspection- a review. Trends in Food Science & Technology,**17**, pp. 642-655.

[25] Afoakwa, E. O.; Paterson, A.; Fowler, M.; Ryan, A. (2008): Flavor formation and character in cocoa and chocolate: a critical review. Critical Reviews in Food Science and Nutrition, **48**, pp. 1-18.

[26] Ziegleder, G.; Amanitis, A.; Hornik, H. (2004): Thickening of molten white chocolates during storage. Lebensm.-Wiss. u.-Technology, **37**, pp. 771-778.

[27] El-kalyoubi, M.;Khallaf, M. F.;Abdelrashid, A.; Mostafa, E. M. (2011): Quality characteristics of chocolate – Containing some fat replacer. Annals of Agricultural Science,**56**(2), pp. 89-96.

[28] Manzocco, L.;Calligaris, S.;Camerin, M.;Pizzale, L.;Nicoli, M. C. (2014): Prediction of firmness and physical stability of low-fat chocolate spreads. Journal of Food Engineering, **126**, pp. 120-125.