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STEVIA REBAUDIANA, OLIGOFRUCTOSE AND ISOMALTULOSE AS SUGAR REPLACERS IN MARSHMALLOWS - STABILITY AND ANTIOXIDANT PROPERTIES

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ABSTRACT

Consumers are increasingly demanding products with natural ingredients and functional properties. The replacement of conventional sugars with recently available sugars/sweeteners could result in the perception of candies as healthier products. Therefore, the objective of this work was to evaluate the influence of isomaltulose, oligofructose and stevia extracts on the physico-chemical, mechanical, optical and antioxidant properties as well as the shelf-life of marshmallows. A sensory test was carried out in order to evaluate the influence of these ingredients on the acceptance of this product. The instrumental and sensorial textural results indicate that the sucrose and glucose syrup in commercial marshmallows could be replaced by a mixture of isomaltulose, oligofructose and stevia. Adults found the new and the traditional marshmallows to be very similar. However, children only found similarities in terms of the texture. These new marshmallows, besides being more microbiologically stable, have added value due to their antioxidant properties.

PRACTICAL APPLICATIONS

Society is becoming increasingly aware of the importance of nutrition in health, and this has a decisive impact on the proposals of the candy sector in terms of innovation and new product development. The main trends of the market are focused on eliminating the unhealthy ingredients in the formulations, such as sugars, and even incorporate active ingredients with functional properties, but without forgetting customer satisfaction.

At present, the industry is using both intense and volume artificial sweeteners as conventional sugar substitutes. However, the food industry now has the possibility of using alternative natural sweeteners such as stevia, oligofructose and isomaltulose, with the added value of providing certain healthy benefits. The results of the present study could provide pertinent information to the confectionary industry that wishes to take on the challenge of developing candies with functional ingredients.

Keywords: marshmallow, isomaltulose, oligofructose, stevia, antioxidant, texture.

INTRODUCTION

The confectionery industry is a sector which is continually innovating in order to satisfy consumers and develop new sugar free products. The consumption of sweets and candies, due to their high sugar content, has always been linked to the development of tooth decay, an increase in the glycaemic index and obesity. These problems can be reduced by replacing the conventional sugars with alternative healthy sweeteners, which have recently become available on the market as isomaltulose, oligofructose and stevia. In fact, isomaltulose supplies the same amount of energy as sucrose (although it breaks down more slowly), is totally digestible and has positive effects on blood sugar and insulin levels (Hawai et al., 1989; Lina et al., 2002).

Marshmallow is a sweet, soft solid foam made primarily of aerated sugar and gelatine (Kaletunc et al., 1992). The typical structure of marshmallows is formed by the addition of air into the protein-sugar combination through fast stirring. The presence of air not only adds volume to the mixture but also contributes greatly to a soft, light texture, change in rheology, modification of appearance, flavour intensity and mouthfeel, alteration of digestibility and shelf-life due to increased porosity (Groves, 1995, Campbell and Mougeot, 1999). It is well-known that marshmallows produced with conventional sugars have a limited shelf-life due to changes in texture which occur with time, mainly hardening and the loss of elasticity of the foam. Moisture loss, sugar crystallization, foam collapse, gel network formation and the ingredients, could contribute to the hardening process (Lees, 1991; Groves, 1995; Tan and Lim, 2008). In this line, Lim et al. (2006) reported an inhibitory effect of the combination of glucose syrup and high dextrose equivalent on sugar crystallization.

Marshmallows could be produced with alternative sugars/sweeteners, with the aim of not having the negative effects on health caused by conventional sugars (Periche et al., 2015). Among the possible sugar substitutes oligofructose and isomaltulose are interesting as they have similar properties to sugar and glucose syrup, although both have a third of the sweetening power of sucrose (Vasiljevic and Varzakas, 2012; Varzakas and Labropoulos, 2012). Frank (2002) showed that oligofructose had good stability during the usual food processes (e.g. heat treatment), contributed to improved mouthfeel and humectant properties, furthermore contributed to microbiological stability reducing the water activity.

Stevia is a perennial plant from Brazil and Paraguay which is extremely sweet due to the presence of diterpenes (250-300 times more than sucrose), specifically steviol glycosides (Lemus-Moncada et al., 2012). The steviol glycosides are natural sweeteners that show a sufficient thermal stability, making them suitable sucrose replacers in sweet bakery products (Struck et al., 2014). Furthermore, they are apt for foodstuffs with low pH such as candies

(Gong and Bell, 2013). Thus, the use of stevia could compensate the lack of sweetness that the aforementioned sugars have and provide many other functional properties to the products. In fact, the European Food Safety Authority recognized the safety of stevia leaf extracts for alimentary use in November 2011 (EFSA, 2011). The food industry has shown increased interest in plant extracts from stevia as it is another alternative to sucrose, due to its content in non-caloric sweeteners (Carbonell-Capella et al., 2013). Moreover, stevia leaves are increasingly consumed as infusions due to their antioxidant properties (Barba et al., 2015; Periche et al. 2014; Shukla et al., 2012; Muanda et al., 2011) and its beneficial effects on human health as anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory effects (Chatsudthipong and Muanprasat, 2009). However, it is not well known how the replacement of conventional sugars with alternative sweeteners affects the shelf life, texture or colour of this kind of product. Therefore, the aims of the present study were to investigate the influence of isomaltulose, oligofructose and stevia extracts on physico-chemical, mechanical, optical and antioxidant properties as well as shelf-life of marshmallows during storage. Additionally, a consumer test was carried out in order to evaluate the influence of these ingredients on the sensory acceptance of this product.

MATERIAL AND METHODS

Materials

The ingredients used in the formulation of marshmallows were: oligofructose (Frutalose OFP, Sensus, Netherlands), isomaltulose (Beneo-Palatinit; Germany), sucrose (Azucarera, Spain), glucose syrup 43 DE (Emilio Peña, S.A., Spain), gelatin A 220 Bloom (Junca Gelatines S.L.; Spain), corn starch (Roquette, France), natural red liquid colour (Roha Europe S.L.; Spain), strawberry flavouring (Flavorix Aromáticos S.A.; Spain) and sunflower oil (Koipesol, Spain). In addition, *Stevia Rebaudiana* leaves (Raab, Vitalfood, Rohrbach, Germany) were used in order to prepare the aqueous stevia extract incorporated in the

formulations. The leaves were previously treated with UV radiation in order to minimize the microbial load.

Experimental Methodology

The marshmallows prepared in this study consisted of 36% water, 59% sugars (isomaltulose and oligofructose in a ratio 2:3) and 5% gelatine (Edwards, 2002). In the formulations with stevia the water was replaced by an aqueous extract of stevia. This extract was prepared with 1 g of dried leaves in 100 mL of water at 90ºC for 5 minutes. Four different formulations were obtained depending on the amount of water replaced by stevia aqueous extract (%): A (0%), B (33%), C (66%) and D (100%). In addition, a "control sample", prepared in the same way as the commercial ones with only glucose syrup and sucrose (in a ratio 4:6), was used in this study. Furthermore, 0.5 ppm of strawberry flavouring and 0.2 ppm of red colouring were added.

Each formulation was made in a thermal blender (Thermomix, TM31, Vorwerk, Germany) by blending the sugars and water until they reached boiling point at 300 rpm for 10 minutes. This mixture was stirred until it reached 60ºC when pH and ºBrix were measured. The gelatine was then dissolved in water at a ratio of 1:2 (w/w) to obtain a homogeneous mix and subsequently added to the syrup with the flavouring and colouring agents. All the ingredients were blended for 5 minutes at 60 ºC and 300 rpm. Then, the syrup was stirred for 10 minutes at 1860 rpm to add air to the mixture, which is what mainly accounts for the texture of the marshmallows. For moulding purposes, the final mixture was poured into silicone moulds with a thin coating of sunflower oil. The silicone moulds are cylindrical in shape with a diameter of 28 mm and a height of 20 mm. Finally, the moulds were placed in a chamber at 20°C for 24 hours. The samples were then removed from their mould and covered with starch to prevent the samples from sticking together. Samples were stored in plastic bags

(multilayer polyethylene) at room temperature for 45 days. This material was selected as it is normally used in the commercialisation of this type of products.

Physicochemical Analysis

Moisture content and water activity analyses were carried out on the final products. Moisture content was determined gravimetrically by drying to a constant weight in a vacuum oven at 60 ºC (method 20.103 AOAC, 2000). Water activity (a_w) was determined with a dew point hygrometer (LabFerrer, Spain). Soluble solid content (ºBrix) was measured with a refractometer at 20 ºC (ATAGO 3 T) and pH was determined with a pH-meter (SevenEasy, Mettler Toledo) in the initial syrup. All measurements were carried out in triplicate.

Colour

Instrumental measurements of colour were conducted at room temperature with a Minolta spectrophotometer (model CM-3600d) by placing the marshmallow on the diaphragm aperture (8 mm). CIE$L^*a^*b^*$ coordinates were obtained using illuminant D65 and standard observer (10° visual field) as references. Registered parameters were: L^* (brightness), a^* (red component), b^* (yellow component), chroma ($C^*=[(a^*)^2+(b^*)^2]^{1/2}$) and hue ($h^*=arctg(b^*/a^*)$). Global colour differences, $\Delta E^*_{ab,}$ were calculated using the standard formula: $\Delta E^*_{ab}=((\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2)^{1/2}$. These differences were calculated with respect to the control and the sample A. It is assumed that when ΔE^*ab is higher than 3 units, a colour variation can be perceived by an average observer (Francis & Clydesdale, 1975).

Texture

The samples (cylinders with a diameter of 28 mm and a height of 20 mm) were examined with Texture Profile Analysis test (TPA) using a TA.XT plus Texture Analyzer (Stable Micro Systems, U.K.). Instruments were equipped with a load cell of 50 kg and a 45 mm diameter cylindrical probe. The test conditions involved two consecutive cycles of 50% compression with 15 s between cycles. The test speed was 1 mm/s. From the resulting force-

148 time curve the following parameters were quantified: hardness (N) (maximum peak force
149 during the first compression cycle), springiness (the height that the sample recovers during the
150 time that elapses between the end of the first cycle and the beginning of the second cycle),
151 cohesiveness (the ratio of the positive force area during the second compression and the first
152 compression), gumminess (N) (hardness x cohesiveness) (Bourne, 1978).

153 **Determination of total antioxidant capacity**

154 The antioxidant activity (AA) of the extract was analyzed on the basis of the scavenging
155 activities of the stable 2,2-diphenyl-1-picrylhydrazyl free radical as described by Shahidi et al.
156 (2006), with some modifications. Marshmallows were freeze-drying at a vacuum pressure of
157 $9.5x10^{-1}$ mm Hg for 24 hours. Then, they were ground in a grinding mill (A11 Basic, IKA,
158 Germany) and diluted in methanol:water (80:20). 0.1 mL of sample was mixed with 3.9 mL
159 of a methanolic solution of DPPH (0.025mg/mL, prepared in methanol:water (80:20)). The
160 solution was shaken and after 30 min the absorbance of the sample was measured at 515 nm
161 using methanol as a blank. The quantification was made considering a standard curve of
162 Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were
163 expressed as mg of Trolox equivalent per gram of marshmallow.

164 **Microbiological analysis**

165 Serial dilutions were prepared by homogenising 5 g of marshmallow with 45 mL of 1%
166 sterile peptone water in a stomacher bag, using sterile techniques. Mesophilic aerobic
167 populations were analysed in Plate Count Agar (Scharlau, Barcelona, Spain) incubating
168 samples for 72 h at 31 ºC. Yeast and molds were determined on Sabouraud Chloramphenicol
169 Agar (Scharlau, Barcelona, Spain) plates for 5 days at 31 ºC. Samples for analysis were taken
170 on days 1, 7, 14, 21, 28, 35 and 45. Plates were inoculated, in duplicate, with 1 mL of the
171 corresponding dilutions. After the incubation time, Petri dishes with a number of colonies

between 30 and 300 for mesophilic aerobic and between 15 and 150 for molds and yeasts, were considered. Microbial counts were expressed as CFU/g.

Sensorial analysis

An acceptance test using a 9-point hedonic scale (1=dislike extremely, 9=like extremely) was used to evaluate the following attributes: appearance, colour, aroma, sweetness, texture, hardness, gumminess (Sanz et al., 2009; ISO 5492, 2008). Global preference and intention of buying was also evaluated. This study was performance by 50 children aged 11-12 from the State School "Nou d'Octubre" (Tavernes Blanques, Valencia, Spain) and 50 adults (employees and students of the Institute of Food Engineering for Development, Spain). The consumers evaluated 3 formulations (control, A and D); each of the different formulations was presented independently. The consumers were informed about the advantages of the new ingredients (isomaltulose, oligofructose and stevia). The test was conducted in a sensory evaluation laboratory built according to the international standards for tasting rooms (ISO 8589, 2007).

Statistical Analyses

Statgraphics Centurion was used to perform the statistical analyses. Analyses of variance (multifactor ANOVA) were carried out to discern whether the effect of the process variables (formulation and storage time) on the final product was significant. The interactions between factors were considered.

RESULTS AND DISCUSSION

Compositional characteristics, texture and colour

Table 1 shows the mean and standard deviation of ºBrix and pH of the initial syrup as well as the moisture content and water activity of the marshmallows during storage. The significant effect (F-ratio and level of significance from ANOVA) of the factors "formulation" and "storage time" on the physicochemical parameters studied is also shown in

Table 1. As expected, ºBrix were very similar in all cases ranging from 68.65 to 71.6; whereas samples formulated with the healthier sugars (A, B, C and D) exhibited lower pH than the control. A slight increase in moisture content and water activity was found in the samples A, B and C during storage. Water activity increased for samples A, B and C until day 7 and then remained constant. Sample D had a greater initial moisture content and water activity than the other formulations, but no evolution was found with storage time. For the control sample, the values of moisture content and water activity were higher than those values in samples A, B, C and D due to the additional quantity of water provided by the glucose syrup used in the formulation of the control.

Fig. 1 shows the instrumental TPA parameters (springiness, hardness, cohesiveness and gumminess) of the formulated marshmallows. The replacement of glucose syrup and sucrose by isomaltulose and oligofructose resulted in a significant decrease in hardness and gumminess. As expected, the addition of stevia did not affect the mechanical properties. With respect to storage time, a slight increase in hardness and gumminess was found from 0 to 21 days, and a reduction in springiness and cohesiveness, but in no case was a significant effect observed.

Colour planes L*-a* and b*-a* of the samples are shown in Fig. 2. The factor "formulation" had more influence than "time" on the colour of the marshmallows. Samples made with sucrose and glucose syrup showed higher a* and lower L* than the samples formulated with isomaltulose and oligofructose. The higher the replacement of water by stevia extract and the longer the storage time, the lower the luminosity but the higher the a* and b* coordinates of the samples. Table 2 shows the global colour differences (ΔE^*_{ab},) between samples: due to storage time ($\Delta E=S_{t42}-S_{t0}$); at the initial time with respect to sample A ($\Delta E= S_{t0}-A_{t0}$) and the control sample ($\Delta E= S_{t0}-Control_{t0}$); and at the end of storage ($\Delta E= S_{t42}-A_{t42}$ and $\Delta E= S_{t42}-Control_{t42}$).

The colour differences in each sample during storage time ($\Delta E=S_{t42}-S_{t0}$) were lower than 3, except for the control samples. These differences cannot be perceived by the human eye (Francis and Clydesdale, 1975). The colour difference of A, B, C and D with respect to the control samples ($\Delta E= S_{t0}-Control_{t0}$) was higher than 3, mainly due to the different sugars (sucrose and glucose syrup) used in the formulation of the control samples, and also due to the incorporation of stevia aqueous extract in the other samples (B, C and D). As expected, the colour difference of B, C, D with respect to sample A ($\Delta E= S_{t0}-A_{t0}$) was lower because the only change was the incorporation of stevia aqueous extract since oligofructose was present in all these samples.

Total antioxidant capacity

In order to evaluate the contribution of stevia to marshmallows in terms of antioxidant properties, the antioxidant activity (mg trolox/ 100 g of marshmallow) of the formulations (B, C, D) and its evolution with storage time is shown in Fig. 3. The Control and A samples are not shown in the figure as they do not exhibit antioxidant activity, which is logical as they were not made with stevia extract. Taking into account the antioxidant activity of stevia aqueous extract (117 mg trolox/100g stevia aqueous extract) added to the formulations and the values of antioxidant activity at the beginning of storage (1^{st} day of storage) (Fig. 3), it can be affirmed that the processing of marshmallows did not affect the antioxidant activity of the stevia extract. A sharp decrease in antioxidant properties, however, occurred during the first 21 days of storage. From 21^{st} day, antioxidant activity tended to stabilize. Specifically, an average loss of antioxidant activity of 77, 62 and 71 % was registered at the end of storage for the formulations B, C and D, respectively.

Microbiological analysis

Microbial counts of mesophilic aerobics, yeasts and moulds were not found in any of the marshmallows during storage, except the control at the end of storage, although the count

was low ($3*10^1$ CFU/g mesophilic aerobics and $2*10^1$ CFU /g yeasts and moulds). These results make it clear that the product is microbiologically stable during the analysed period of time. The microbiological stability of the samples with stevia could be attributed to the antimicrobial properties of the leaves (Debnath, 2008; Seema, 2010; Sivaram and Mukundan, 2003; Tadhani et al., 2006). Barba et al. (2014) incorporated stevia infusions in other products like fruit extracts with good results in terms of stability. Belda et al. (2014) showed that an infusion of stevia leaves had the highest antimicrobial capability against *L. innocua* in comparison to a crude extract of this leaf, and a purified extract of steviol glycosides.

Sensorial analysis

Fig. 4 shows a radial chart of the average scores for each attribute evaluated (appearance, colour, strawberry flavour, sweetness, texture, hardness, gumminess) besides the global preference and intention to buy. In addition, the F-ratio (of the ANOVA considering "formulation" as a factor) is shown next to each attribute. Two groups of consumers (children and adults) evaluated three samples: the control simple (sucrose and glucose syrup) made with traditional sugars; "A" (isomaltulose and oligofructose) made with natural sugars which substitute the traditional ones and "D" (isomaltulose, oligofructose and stevia) made with natural sugars which substitute the traditional ones and has the highest antioxidant level. Neither of the groups of consumers found significant differences between the samples for the texture parameters (global texture, hardness, gumminess). In relation to the aroma only children found significant differences between samples. Both groups found significant differences between samples for the remaining attributes (appearance, colour, sweetness) and global preference and intention of buying.

Considering that the higher the F-ratio, the greater the effect that a factor has on a variable, sweetness, global preference and intention of buying were most affected by the type of formulation for both types of consumer. As expected, both groups gave higher scores to the

control sample (formulated with sucrose and glucose syrup) than samples A and D (formulated with isomaltulose, oligofructose and stevia). However, adults scored sample D similarly to the control sample, especially for sweetness, gumminess and global preference. The better evaluation by adults in comparison to children could be because they know the benefits of stevia and are more aware of the advantages of the product.

CONCLUSIONS

The instrumental and sensorial textural results, in particular, indicate that traditional sugars in commercial marshmallows could be totally replaced by a mixture of isomaltulose, oligofructose and stevia. The sensory evaluation of these marshmallows by adults, in comparison to children, was very similar to those confected with sucrose and glucose syrup, which could be because they know the benefits of stevia and are more aware of the advantages of the product. These new products, besides being more microbiologically stable, have added value due to their antioxidant properties. However, as the results show that there is a drastic decrease in antioxidants during the first 21 days. It is important to continue studying this topic in greater depth in order to maintain the functional properties of these new marshmallows.

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FIGURE CAPTIONS

FIG. 1 MEAN AND STANDARD DEVIATION OF TEXTURE PARAMETERS (N=3): a) HARDNESS, b) GUMMINESS, c) SPRINGINESS, d) COHESIVENESS. Samples codification: control, A (0%), B (33%) C (66%) and D (100%) (% stevia aqueous extract replacement).

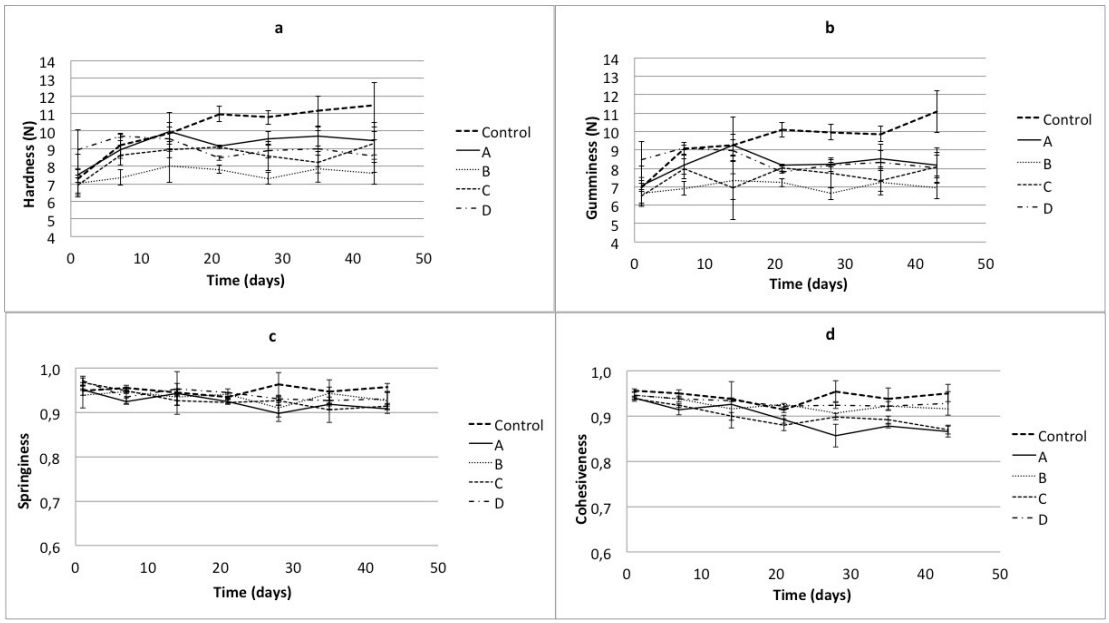

FIG. 2 COLOUR PLANES L*-A* AND B*-A* OF CONTROL SAMPLES AND CONFECTED MARSHMALLOWS WITH STEVIA AQUEOUS EXTRACT DURING STORAGE. Samples codification: control, A (0%), B (33%) C (66%) and D (100%) (% stevia aqueous extract replacement).

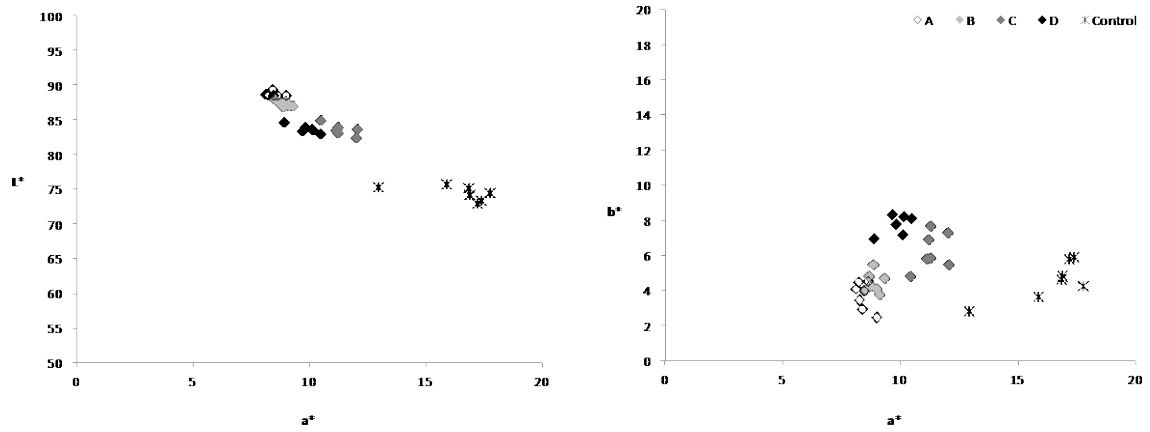

FIG. 3 MEAN AND STANDARD DEVIATION OF THE ANTIOXIDANT ACTIVITY OF MARSHMALLOWS DURING STORAGE TIME (N=3). Samples codification: B (33%) C (66%) and D (100%) (% stevia aqueous extract replacement).

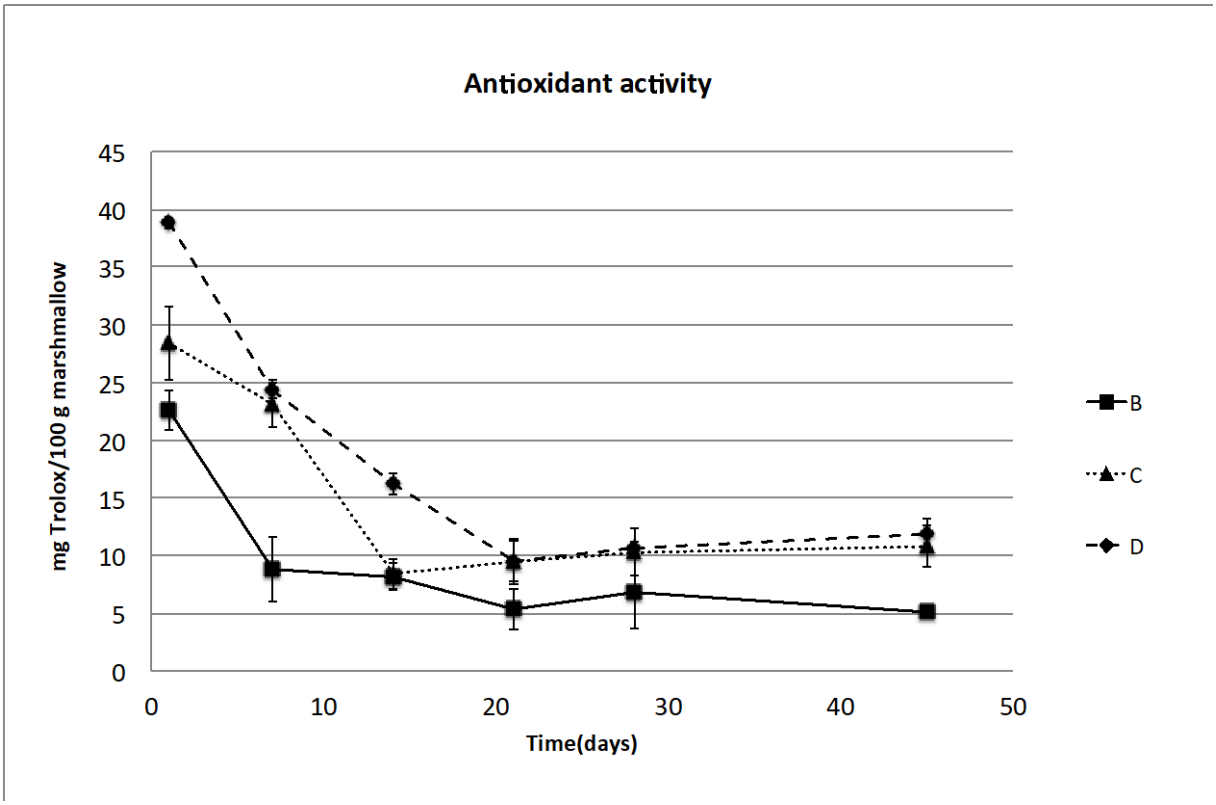

FIG. 4 RADIAL CHART OF THE AVERAGE SCORES FOR EACH ATTRIBUTE. A) CHILDREN, B) ADULTS. Samples codification: control, A (0% stevia aqueous extract) and D (100% stevia aqueous extract). Numbers in brackets refer to the F-ratio of the ANOVA considering "formulation" as a factor. ns=not significant **statistical significance ≥ 99% (p-value ≤ 0.01).

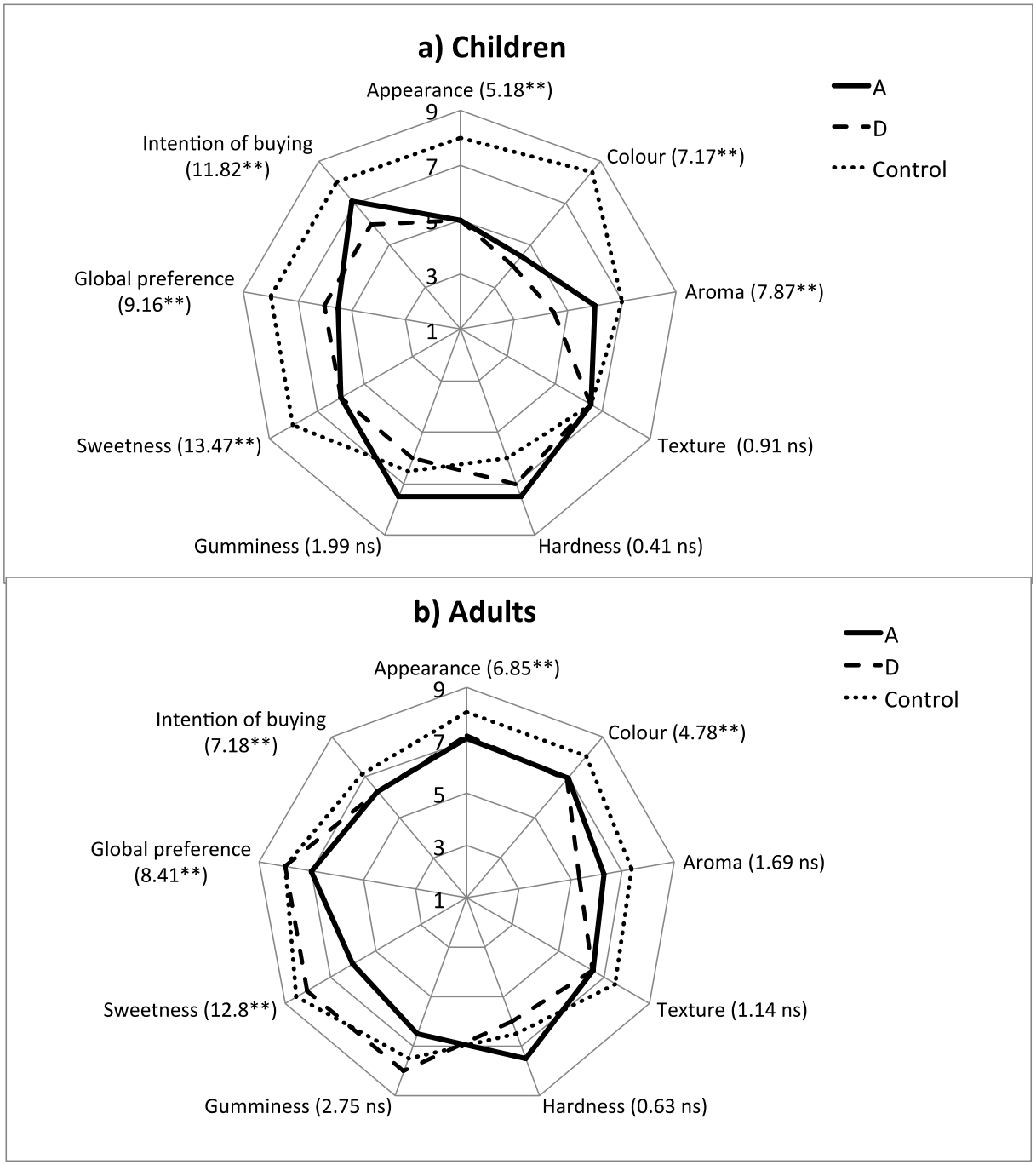

TABLE 1. MEAN AND STANDARD DEVIATION OF °BRIX AND PH OF THE INITIAL SYRUP, MOISTURE CONTENT AND WATER ACTIVITY OF THE MARSHMALLOWS (N=3). SAMPLES CODIFICATION: CONTROL, A (0%), B (33%) C (66%) AND D (100%) (% STEVIA AQUEOUS EXTRACT REPLACEMENT).

		Initial syrup		**Product: marshmallow**	
Formulation	Time (days)	°Brix	pH	Moisture content (g/100g)	a_w
Control	1	70.31(0.04)^{a}	6.92(0.07)^{c}	23.1(0.7)^{d}	0.821(0.002)^{c}
	7			21.9(0.2)^{c}	0.810(0.008)^{c}
	14			19.3(0.3)^{c}	0.815(0.005)^{c}
	21			18.2(0.2)^{b}	0.801(0.004)^{c}
	28			19.9(0.3)^{c}	0.796(0.003)^{b}
	35			19.6(0.7)^{c}	0.759(0.002)^{a}
	42			20.1(0.5)^{d}	0.745(0.007)^{a}
A	1	70.45(0.07)^{a}	6.26(0.06)^{b}	17.4(0.9)^{a}	0.749(0.002)^{a}
	7			17.6(0.3)^{a}	0.785(0.003)^{b}
	14			18.2(0.3)^{b}	0.780(0.005)^{b}
	21			17.5(0.7)^{a}	0.778(0.005)^{b}
	28			18.7(0.3)^{b}	0.780(0.002)^{b}
	35			18.4(0.3)^{b}	0.794(0.003)^{b}
	42			20.3(0.7)^{d}	0.778(0.004)^{b}
B	1	71.60(0.09)^{d}	6.13(0.08)^{c}	17.4(1.7)^{a}	0.757(0.002)^{a}
	7			19.6(1.1)^{bc}	0.820(0.003)^{c}
	14			21.3(0.3)^{d}	0.815(0.005)^{c}
	21			21.0(0.5)^{d}	0.818(0.005)^{c}
	28			22.6(0.3)^{e}	0.809(0.002)^{c}
	35			21.9(0.9)^{c}	0.820(0.003)^{c}
	42			22.1(1.2)^{c}	0.814(0.004)^{c}
C	1	68.85(0.07)^{c}	6.20(0.12)^{bc}	18.3(0.3)^{b}	0.786(0.002)^{b}
	7			16.7(0.6)^{a}	0.791(0.003)^{b}
	14			19.2(0.2)^{c}	0.781(0.005)^{b}
	21			18.1(0.6)^{b}	0.792(0.005)^{b}
	28			18.6(1.3)^{b}	0.803(0.002)^{c}
	35			18.9(0.2)^{b}	0.800(0.003)^{c}
	42			22.1(0.4)^{c}	0.796(0.004)^{b}
D	1	68.65(0.07)^{a}	6.11(0.12)^{bc}	21.6(0.6)^{c}	0.817(0.002)^{c}
	7			20.3(0.5)^{d}	0.826(0.003)^{c}
	14			22.4(0.3)^{c}	0.831(0.005)^{c}
	21			20.9(0.5)^{d}	0.822(0.005)^{c}
	28			21.7(1.2)^{c}	0.817(0.002)^{c}
	35			21.8(0.7)^{c}	0.826(0.003)^{c}
	42			22.9(1.0)^{c}	0.820(0.004)^{c}

ANOVA F-ratio				
	°Brix	**pH**	**Moisture content (g/100g)**	**a_w**
Formulation (F)	104*	3.0*	34.4*	22.9*
Time (T)	-	-	119.5*	107.9*
F x T	-	-	7.2*	8.1*

*Statical significance ≥99% (p-value≤0.01) (The ANOVA homogenous groups are indicated by letters.)

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TABLE 2. GLOBAL COLOUR DIFFERENCES (ΔE*AB,) BETWEEN SAMPLES (N=3): DUE TO STORAGE TIME ($\Delta E=S_{T42}-S_{T0}$); AT THE INITIAL TIME WITH RESPECT TO SAMPLE A ($\Delta E= S_{T0}-A_{T0}$) AND THE CONTROL SAMPLE ($\Delta E= S_{T0}-CONTROL_{T0}$); AND AT THE END OF STORAGE ($\Delta E= S_{T42}-A_{T42}$ AND $\Delta E= S_{T42}-CONTROL_{T42}$).

Sample	$\Delta E=S_{t42}-S_{t0}$	$\Delta E= S_{t0}-Control_{t0}$	$\Delta E= S_{t42}-Control_{t42}$	$\Delta E= S_{t0}-A_{t0}$	$\Delta E= S_{t42}-A_{t42}$
Control	$5.2(0.2)^{c}$				
A	$2.1(0.3)^{ab}$	$13.8(0.3)^{b}$	$17.7(0.7)^{b}$		
B	$1.9(0.2)^{ab}$	$12.4(0.5)^{b}$	$16.0(0.5)^{b}$	$2.0(0.3)^{a}$	$2.0(0.7)^{a}$
C	$2.7(0.9)^{b}$	$10.1(0.9)^{a}$	$12.1(1.7)^{a}$	$4.6(1.3)^{b}$	$5.0(0.2)^{b}$
D	$1.7(0.3)^{a}$	$9.8(0.8)^{a}$	$13.5(1.2)^{a}$	$6.9(0.9)^{c}$	$6.1(1.3)^{b}$
Anova F-ratio	20.58^{***}	25.57^{***}	18.49^{***}	18.66^{**}	16.85^{**}

*p<0.05, **p<0.01, ***p<0.001 (The ANOVA homogeneous group are indicated by letters)