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Effect of incorporation of polyols, fructooligosaccharides and antihypertensive peptides in a deposited marshmallow

Efecto de la incorporación de polialcoholes, fructooligosacáridos y péptidos antihipertensivos en un masmelo depositado

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Abstract Traditional marshmallows are made with sucrose and glucose syrup, an option to make sucrose-free marshmallows, healthy and with functional components, is the use of polyols, fructooligosaccharides (FOS) and antihypertensive peptides. The objective of this research was to evaluate the effect of the inclusion of Nutraflora® (FOS), maltitol (M), sorbitol syrup (SS) and antihypertensive peptides in a marshmallow, on the elastic modulus (EM) of the prototypes elaborated. For this,1 a factorial arrangement (AF) $3x2^2$ was proposed over the EM of the prototypes, also, a completely random design (CRD) to compare a marshmallow control and one of extreme vertex mixtures (DMVE) to optimize the formulation. Finally, the texture profile (TPA) of the developed marshmallow was determined. The proportions of polyols in AF and DCA caused effect on ME (p < 0,05). The DMVE determined the corresponding proportions of 8,45 % FOS; 46,55 % M and 25 % SS. The regression model obtained an r^2 = 93,60 r^2 (adjusted) = 90,40 and an r^2 (prediction) = 80,03.

Keywords: Polyalcohols, fructooligosaccharides, peptides, maltitol, sorbitol, sucralose.

Resumen Los masmelos tradicionales se elaboran con sacarosa y jarabe de glucosa, una opción para elaborar masmelos libres de sacarosa, saludables y con componentes funcionales, es el uso de polialcoholes, fructooligosacáridos (FOS) y péptidos antihipertensivos. El objetivo de esta investigación fue evaluar el efecto de la inclusión de Nutraflora® (FOS), maltitol (M), jarabe de sorbitol (JS) y péptidos antihipertensivos en un masmelo, sobre el módulo elástico (ME) de los prototipos elaborados. Para esto, se planteó un arreglo factorial (AF) $3x2^2$ sobre el ME de los prototipos, también, un diseño completamente al azar (DCA) para comparar un masmelo control y uno de mezclas de vértices extremos (DMVE) para optimizar la formulación. Finalmente, se determinó el perfil de textura (TPA) del masmelo desarrollado. Las proporciones de polialcoholes en el AF y el DCA causaron efecto sobre el ME (p < 0,05). El DMVE determinó las proporciones correspondientes de 8,45 % de FOS; 46,55 % de M y 25 % de JS. El modelo de regresión obtuvo un r^2 = 93,60 r^2 (ajustado) = 90,40 y un r^2 (predicción) = 80,03.

Palabras clave: Polialcoholes, fructooligosacáridos, péptidos, maltitol, sorbitol, sucralosa.

Introduction

Marshmallows are defined as stable foams, with a fluffy texture, with or without the addition of thickener or gelling agent of vegetable or animal origin, with the possibility of being filled or coated with components allowed by the current health legislation of each country or by the Codex Alimentarius (Instituto Colombiano de Normas Técnicas y Certificación [ICONTEC], 2008). A marshmallow includes levels of sucrose and glucose between 30 % and 40 % (Hartel *et al.*, 2018), its sweet taste, textures and diversity of colors are attractive for children and adults; eating sweet in moderate amounts is pleasant. However, they are not commodities in a household basket and their disproportionate consumption is related to increased glycemic index, decay, and obesity (Periche *et al.*, 2015). Colombia has a sucrose-based confectionery production of 615,3 billion pesos by 2022 and a production of 700 billion pesos is projected for 2026, this indicates a growing consumption of sucrose in the Colombian population (Passport Euromonitor International, 2022).

On the other hand, the consumption of sugary drinks is associated with high levels of blood pressure, increasing the incidence of hypertension, suggesting restrictions in the consumption of this type of products (Malik et al., 2014). In American adolescents, the consumption of sugary drinks was associated with high systolic blood pressure levels (Nguyen *et al.*, 2009), 19 % of children aged 11 to 17 years who attended schools in Houston suffered from high blood pressure, 15,7 % had prehypertension and 3,2 % hypertension; high blood pressure was more common in overweight children (Ingelfinger, 2014). Comparisons in several countries showed high blood pressure levels in children and adolescents, 17,3 % in Brazil, 13,8 % in the United States and 12,3 % to 15,1 % in Greece.

The availability of data suggests that if high blood pressure is defined as blood pressure above 120/80 mm Hg, then up to 15% of children and adolescents in the United States of America would have this condition (Feber & Ahmed, 2010). In 2004, seventy-two million adults in the United States (35 %) had hypertension, defined as systolic blood pressure 140 mm Hg and/or diastolic blood pressure 90 mm Hg and another fifty-nine million (29 %) had pre-hypertension defined between 120 to 140 mm Hg (systolic pressure) or 80 to 90 mm Hg (diastolic pressure).

High blood pressure is a risk factor for stroke, cardiovascular disease, kidney disease, all-cause mortality, and short life expectancy (Chen *et al.*, 2013). Results of the 2007 National Health Survey show that in Colombia 22,8 % of the population between 18 and 69 years of age is hypertensive, increasing to 58,9 % in people over 60 years of age (Ministerio de la Protección Social *et al.*, 2015). Therefore, high sugar consumption is a risk factor associated with high blood pressure.

A wide variety of antihypertensive compounds have been characterized from various food sources such as fruits, vegetables, grains, nuts, seeds, legumes, dairy products, meats, and poultry. Most of these inhibit, in vitro, the angiotensin converting enzyme (ACE) and prevent the generation of angiotensin II, a vasoconstrictor compound that increases blood pressure. The main compounds that have this inhibitory effect are flavonoids, flavonols, catechins, anthocyanins, polyphenols, tannins, and phenolic acids of plant origin. In addition, some polysaccharides, saponins, sterols and pigments (Shobako, 2021).

ACE inhibitory peptides, resulting from hydrolysis of protein-containing rice sediments, caused a significant antihypertensive effect in rats (Guan-Hong *et al.*, 2007). On the other hand, cereal proteins such as wheat, rye, barley, and oats possess amino acid sequences homologous to those of ACE inhibitory peptides. These cereals also contain gamma-amino butyric acid (GABA), non-protein, with antihypertensive activity demonstrated in rats and hypertensive human (Ramos-Ruiz *et al.*, 2018).

Milk is a source of bioactive components that prevent cardiovascular disorders, metabolic disorders, promote intestinal health and possess chemo preventive properties (Hsieh *et al.*, 2015). A series of epidemiological studies suggest that the intake of milk and dairy products are related to lower risks of hypertension (Engberink *et al.*, 2009). Proteins and their protein hydrolysates have had an antihypertensive effect, inhibiting blood pressure regulating ACE. In the last 20 years, the antihypertensive effects of some peptides, peptide sequences, doses, and reduction of systolic pressure in rats as well as in hypertensive humans have been evaluated (Anderson & Moore, 2004). Another source of antihypertensive peptides is egg, ovokinin, ovalbumin protein hydrolyzed by pepsin, has demonstrated vasodilator effect (Martínez-Maqueda *et al.*, 2012).

The effects of antihypertensive peptides and the risk factor represented by sugars on the increase in blood pressure were considered, sucrose and glucose syrup were replaced in the formulation of marshmallows, by maltitol (M), sorbitol syrup (SS) and fructoligosaccharic (FOS) and to adjust the levels of sweetness sucralose was included (LOWPEPT®). Finally, the effect that causes the inclusion of polyols, FOS, and antihypertensive peptides in the formulation of deposited marshmallows was evaluated.

Materials and methods

Materials

The prototype marshmallows developed are composed of: fructooligosaccharides (FOS-Ingredion), maltitol (Ingredion), sorbitol syrup (Ingredion), 270 Bloom gelatin (Cimpa Colombia), sodium citrate (Cimpa), vanilla flavor (Silesia), antihypertensive peptides of dairy origin (LOWPEPT®, Innaves Biotech).

The prototypes were developed in the pilot confectionery plant of the Agricultural Center of Buga, SENA Regional Valle del Cauca - Colombia. The mechanical properties were determined in the laboratories of the School of Food Engineering of the Universidad del Valle - Colombia.

Experimental methodology

Development of a prototype marshmallow with incorporation of polyols, fructooligosaccharides and antihypertensive peptides in its formulation. It consists of preliminary prototypes that included fixed proportions of water, sodium citrate, vanilla flavouring, 3 levels of sweeteners; fructooligosaccharides (FOS), maltitol (M) and sorbitol syrup (SS), in the following percentage proportions: FOS-M-SS (15-30-35) % w/w; FOS-M-SS (10-40-30) % w/w and FOS-M-SS (5-50-25) % w/w; two levels of gelatin type B of 270 Bloom at 3,5 and 4 % w/w and two levels of peptides at 1,5 and 3% w/w. Sucralose was included in the prototypes to adjust the sweetness due to the low contribution of the polyols.

For the preparation of marshmallows, a stage of solubilization of fructoligosaccharides, maltitol, sorbitol syrup and sucralose at 110 °C in water was used. In parallel, gelatin plus peptides were hydrated in water, 1:2 ratio, at 70

°C. Once the temperature of the polyol solution reached 80 °C, the hydrated gelatin was added together with the peptides, the system was cooled to 70 °C and air was incorporated until a density of 0,35 to 0,4 g/mL was reached, and then, the marshmallow mass was deposited with the help of funnels on dry starch molds, with a moisture content of 6 % w/w, Figure 1, illustrates the process steps.

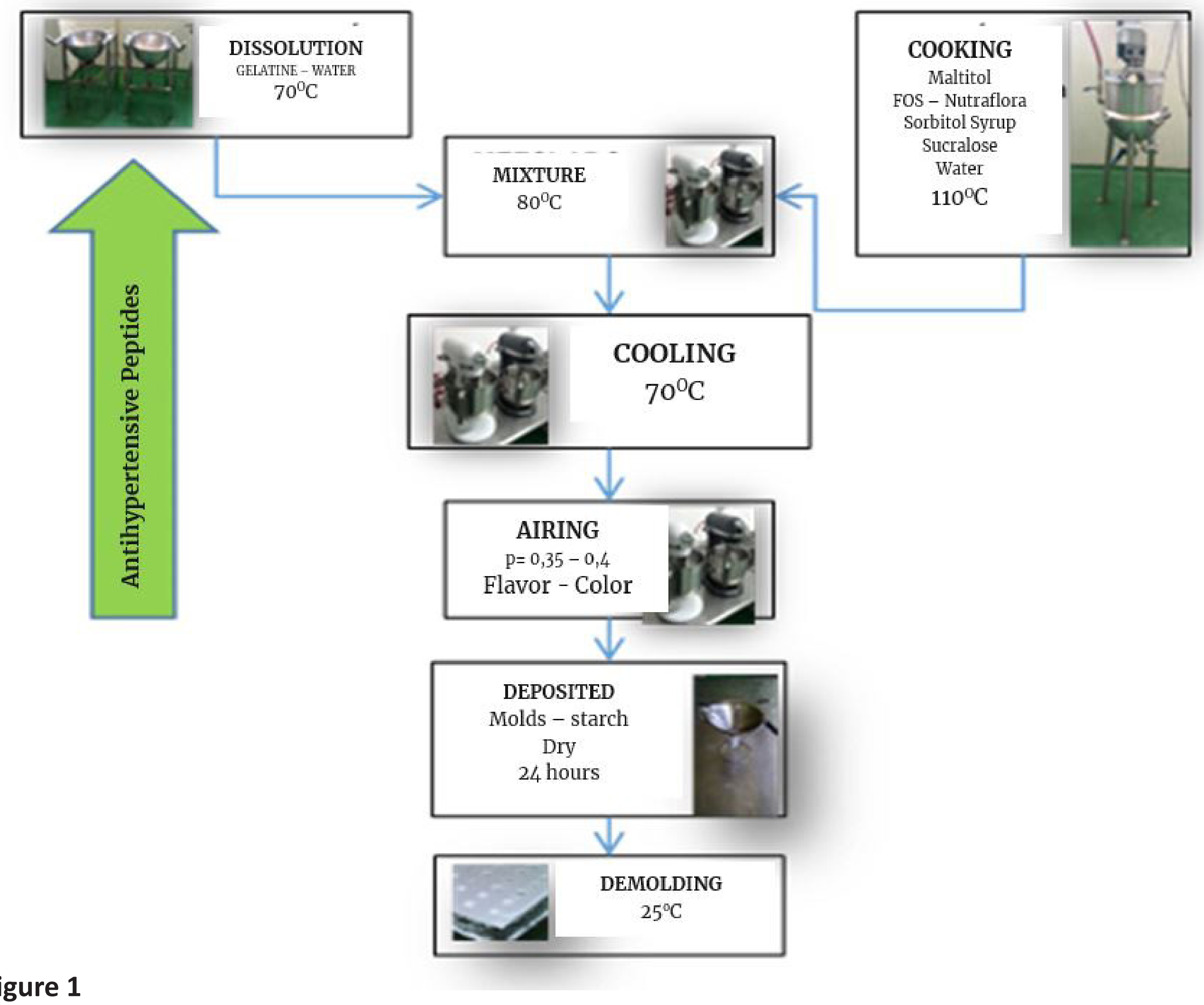

Figure 1

Block diagram for the elaboration of prototype marshmallows

Note. Own elaboration.

A $3x2^2$ mixed factorial experimental design was run, with three replicates (Table 1), to evaluate the effects of the proportions of sweeteners, gelatin, and peptides, on the modulus of elasticity (ME) of the developed marshmallow prototypes. This mechanical property was determined by uniaxial compression tests, on a Shimadzu EZ Test texture meter, with a 50 mm diameter plunger attachment, a travel speed of 20 mm/min and a deformation of 50 %. The marshmallows have a height of 20 mm ± 1,05 mm and a radius of 12,5 mm ± 0,5mm.

The ME is obtained from the slope of the linear zone before the yield point of the true stress curve Ơ(T) Vs strain deformation ЄHF (t), each variable is then defined, in Equation 1:

Equation 1 $$\sigma(T) = \frac{\mathrm{F}\ (\mathrm{t})}{A\ (t)} = \frac{\mathrm{F}\ (\mathrm{t})}{\pi 1_0{}^2 1_0} \mathrm{x} \left(1_0 - d(t)\right) \varepsilon_{HF}\ (t) = Ln\left[\frac{l_0}{(l_0 - d(t))}\right]$$

Where:

$\sigma(T)$ fracture stress (true stress) (Pa)

F: compressive force (N)

A: cross-sectional contact area (m^2)

ε_{HF}: Hencky deformation

l0: initial length (m)

d(t): l_0-l sample distance at time t of the compression (m)

Then ME, defined as the slope, has the following units expressed in Equation 2:

Equation 2 $$\mathrm{ME} = \frac{\frac{N}{m^2}}{\frac{m}{m}} = \frac{N}{m^2} = Pa$$

Where:

$$\mathrm{ME} = \frac{\frac{N}{m^2}}{\frac{m}{m}} = \frac{N}{m^2} = Pa$$

Table 1

Mixed factorial experimental design 3x2²

Proportion of polyols in the initial formulation (%)	Gelatin Ratio (%)	Peptides (%)	Response Variables
FOS-M-SS (15-30-35)	3,5	1,5	ME
FOS-M-SS (10-40-30)	4	3	
FOS-M-SS (5-50-25)			

Note. Own elaboration.

Once the effects of the components on the modulus of elasticity on the modulus of elasticity (ME), the effect of the formulations developed and the witness (control) on the same modulus of elasticity, using a completely randomized design (CRD), (Table 2).

Table 2

DCA for formulations including polyols and marshmallow control

Formulaciones	Módulo de elasticidad
FOS-M-JS (15-30-35)	ME
FOS-M-JS (10-40-30)	ME
FOS-M-JS (5-50-25)	ME
Control marshmallow	ME

Next, the prototype was identified with the modulus of elasticity (ME) close to the control (commercial) marshmallow and optimized with a design of mixtures with extreme vertices (Table 3).

Estimation of the rheological and textural properties of the developed prototype marshmallow. A Shimadzu ETZ texturometer was used, whose necessary operating conditions and accessories to determine the modulus of elasticity (ME) and texture profile (TPA). The parameters corresponding to the texture profile analysis were run with a 50 mm diameter plunger attachment, at a speed of 50 mm/min and a deformation of 50%. The marshmallows have a height of 20mm ± 1.0 mm and a radius of 12,5mm ± 0,5mm. The hardness, elasticity, cohesiveness, adhesiveness, gumminess and chewability were estimated and ten were selected in preliminary testing for (10) replicates for the uniaxial compression and texture profile tests.

Lastly, a five-point hedonic test was used to assess the senses; the witness marshmallow, two trademarks and the control marshmallow; in which 100 judges with basic training in sensory evaluation (apprentices of technology in food quality control from the Agricultural Center of Buga and the Agricultural Center of Popayán), aged between 17 and 56, participated.

Table 3

Design of mixtures with extreme vertices for marshmallows including fructooligosaccharides and polyols

Components	Design Points	
Process Variables	Design Grade	
Total of the mixture		
Limits of the mixture components (in quantity units)		
Component	**Lower**	**Superior**
FOS	0,05	0,15
M	0,30	0,50
JS	0,25	0,35

Note. Own elaboration.

Results and discussion

Development of a prototype deposited marshmallow that incorporates polyols, fructooligosaccharides and antihypertensive peptides in its formulation

The proportions of polyols in the factorial arrangement caused a significant statistical effect ($p < 0,05$) on the MEs of the developed prototypes in which the levels of gelatin and peptide did not cause an effect on the ME, the double interactions of the factors did not present a statistical difference.

In Figure 2, the 1main effects of the evaluated factors are observed, the proportion of polyols is the only main factor that presents a statistical difference, the literature does not report similar experiments to make comparisons and discussions that evaluate the inclusion of polyols in sucrose-free marshmallows. Table 4 records the ANOVA for the $13x2^2$ factorial arrangement Figure 3 lists the intervals for the means of the ME of the developed prototypes and the control marshmallow. The ratio factor of polyols in the DCA, caused a significant statistical effect on the ME ($p < 0,05$), on the contrary, the Tukey test showed that the proportions of polyols FOS-M-SS (15 – 30 – 35) and FOS-M-SS (5 – 50 – 25) did not present a significant statistical difference with the control marshmallow and that of polyols FOS-M-SS (10 – 40 – 30) showed a significant statistical difference with the control marshmallow ME ($p < 0,05$).

Table 5 and 6 record the ANOVA and Tukey test respectively for the DCA.

Once the treatments with the ME close to the control marshmallow were identified, the formulation was optimized by means of a design of mixtures with extreme vertices.

The mixture design determined the proportions of polyalcohols, to reach the ME of the control marshmallow (15,7 Kpa), equivalent to 8,45% of FOS; 46,55% of M and 25% of SS; the regression model obtained an r^2 = 93,60, r^2 (adjusted) = 90,4. Figures 4 and 5 relate the contour plot and the response surface, respectively, and Table 7 shows the ANOVA for the mix design of the prototypes developed.

The optimization of the proportions of polyalcohols in the formulation of the developed marshmallow to achieve the ME of the control marshmallow (15,7 Kpa), is detailed in Figure 6.

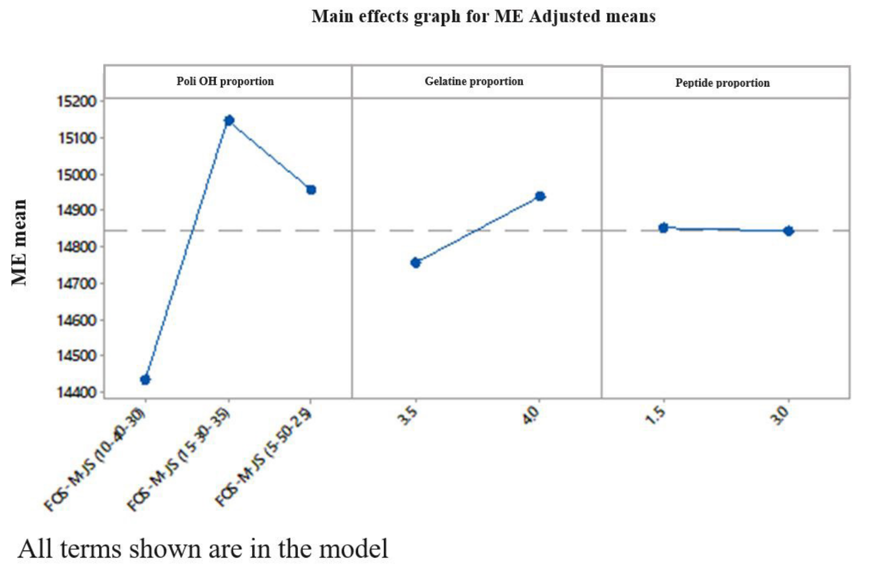

Figure 2

Main effects of the evaluated factors on ME of the developed prototypes
Note. Own elaboration.

Table 4

ANOVA factorial arrangement $3x2^2$

Source	FD	Adj SS	Adj MS	F-Value	P-value
Model	11	5224405	474946	1,25	0,308
Lineal	4	3570962	892741	2,36	0,082
Poly OH Ratio	2	3276414	1638207	4.32	0,025
Gelatin ratio	1	293764	293764	0,78	0,387
Peptide ratio	1	784	784	0,00	0,964
2-Way Interactions	5	1046064	209213	0,55	0,735
Poly Ratio	2	342904	171452	0,45	0,641
OH*Gelatin ratio					
Poly Ratio	2	342904	171452	0,45	0,641
OH*Peptide ratio					
Gelatin ratio * Peptide ratio	1	362404	362404	0,96	0,338
3-Way Interactions	2	607378	303689	0,80	0,460
Poly Ratio					
OH*Gelatin ratio * Peptide ratio	2	607378	303689	0,80	0,460

Source	FD	Adj SS	Adj MS	F-Value	P-value
Error	24	9094303	378929		
Total	35	14318708			

Note. Own elaboration.

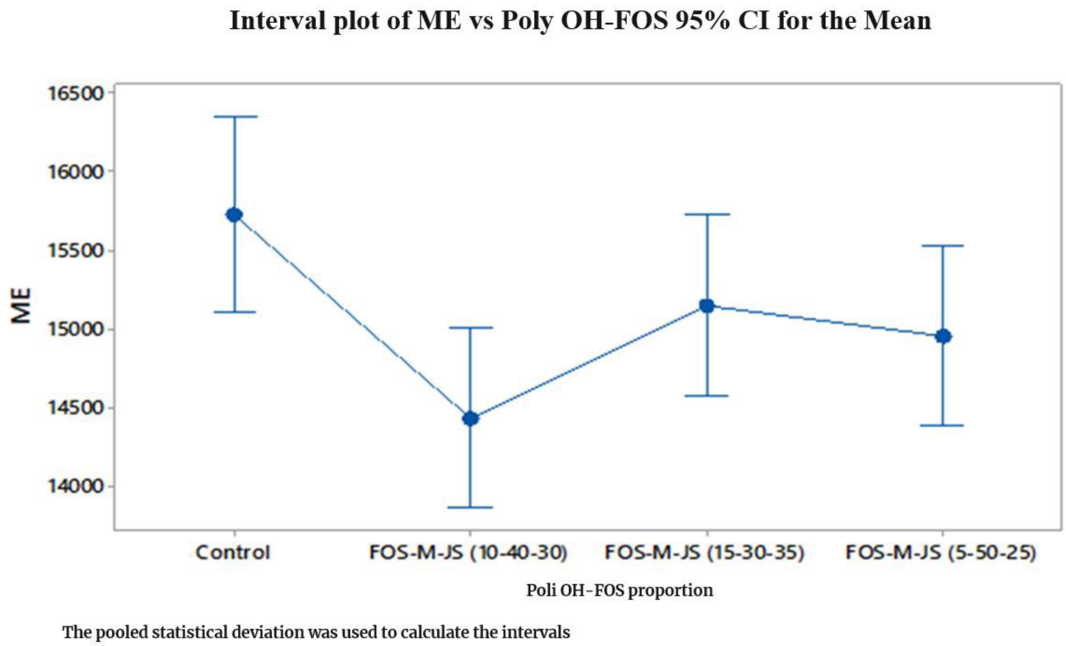

Figure 3

Means ranges of the ME of the developed prototypes, proportion of polyols and control marshmallow

Note. Own elaboration.

Table 5

ANOVA for DCA

Analysis of Variance Source	FD	Adj SS	Adj MS	F-Value	P-value
Poly OH Ratio	3	9329592	3109864	3,25	0,031
Error	42	40245401	958224		
Total	45	49574993			

Note. Own elaboration.

Table 6

Tukey test for DCA

Grouping Information Using the Tukey Method and 95% Confidence				
Poly OH Ratio	N	Mean	Grouping	
Control	10	15724,8	A	
FOS-M-SS (15-30-35)	12	15147,7	A	B
FOS-M-SS (5-50-25)	12	14954,8	A	B
FOS-M-SS (10-40-30)	12	14433,5		B
Means that do not share a letter are significantly different				

Note. Own elaboration.

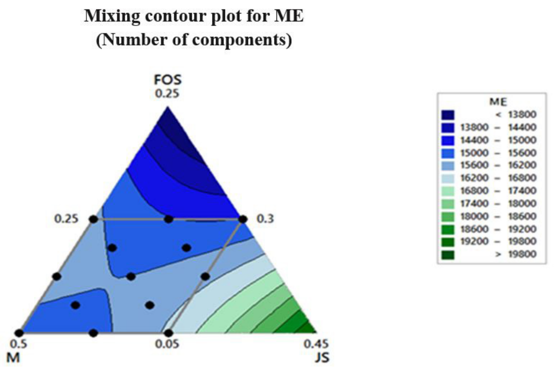

Figure 4

Chart of contour and extreme vertices distributions
Note. Own elaboration.

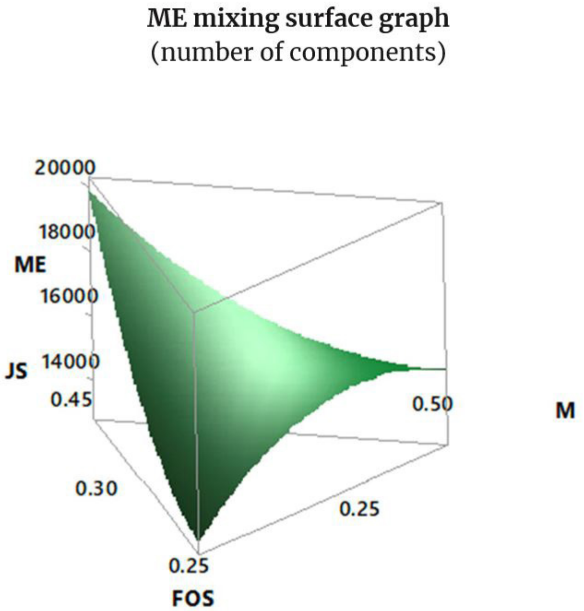

Figure 5

Surface response, design of mixtures with extreme vertices
Note. Own elaboration.

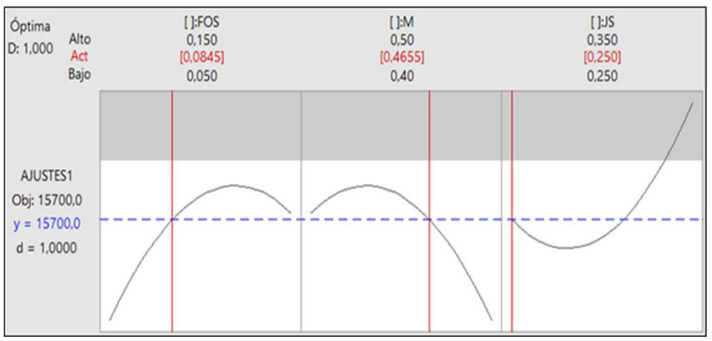

Figure 6

Overall optimal for the marshmallow formulation including polyols and antihypertensive peptides
Note. Own elaboration.

Once the proportions of polyols were determined, the marshmallow was made and the measurements of the ME (15,67 ± 0,67 Kpa) were made, it was compared with the ME of the control marshmallow (15,73 ± 0,58 Kpa) and it was determined that the formulation factor did not cause a significant statistical effect on the ME ($p > 0,05$)

Figure 7, lists the ME means for control and developed marshmallow and Figure 8 depicts characteristic uniaxial compression assays for them, developed including polyols and antihypertensive peptides.

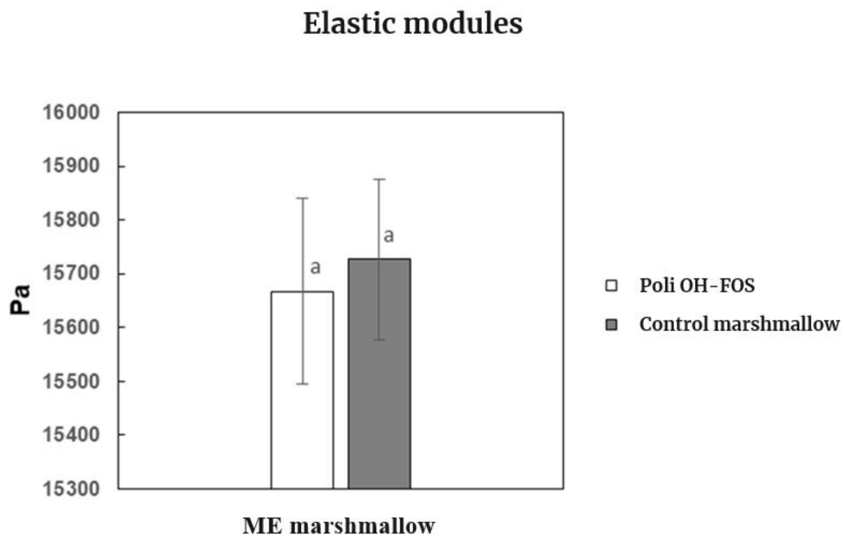

Figure 7

Graph of EM elasticity modules for control and developed marshmallow
Note. Own elaboration.

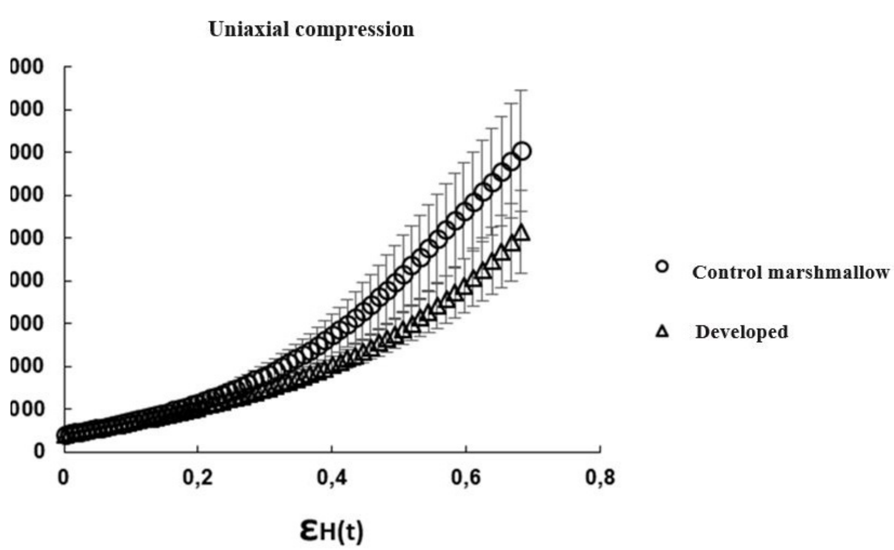

Figure 8

Uniaxial compression for control and developed marshmallow, where σ (Pa) is the true stress in pascals and εH (t) Hencky deformation
Note. Own elaboration.

Estimation of the rheological and textural properties of the developed prototype marshmallow

The parameters of the texture profile analysis were estimated for the developed and control marshmallows, it was performed a one-way analysis of variance between the two and it was estimated that the formulations did not cause significant statistical effect on the parameters of elasticity, cohesiveness, adhesiveness, hardness, gumminess and chewability (p > 0,05). The averages of the estimated parameters are listed in Table 7.

Table 7

TPA parameters for control and developed marshmallow

Marshmallows	Elasticity (mm/mm)		Cohesiveness(mm^2/mm^2)			Adhesiveness (Nxmm)	
TPA POLY OH	1,0023	± 0,0020	0,8760	± 0,008	− 0,284	− 0,284	± 0,208
TPA CONTROL	1,0028	± 0,0016	0,8787	± 0,009	− 0,216	− 0,216	± 0,107
	Hardness (N)		Gumminess (N)			Chewability (Nxmm)	
TPA POLY OH	9,0833	± 1,7730	7,9622		± 1,592	7,9804	± 1,595
TPA CONTROL	9,9125	± 1,0968	8,7136		± 1,008	8,7384	± 1,013

Note. Own elaboration.

Estimated hardness determinations by double compression on an Instron assay machine, reported values from 2,05 N at a zero time, to 35,23 N at week twenty-five of storage.

In the same sense, Peleg (2007) determined values, from 2,8 N at time zero, to 27,33 N at week twenty-two of storage and mastics made with the inclusion of isomaltulose, glucose syrup and fructose syrup in different combinations, as a sucrose substitute, for the same property, recorded from 4,97 N to 27,1 N, for levels with 4 % gelatin of 220 Bloom (Periche *et al.*, 2015). The developed marshmallow has 9,08 N hardness, so it is within the ranges estimated by Tan and Lim (2007), and Periche *et al.* (2015).

The literature reviewed did not report adhesiveness and chewability data in marshmallows and the2 hardness records reported by Peleg (2007) and Periche *et al.* (2015) were taken from the graphs reported using the GetData Graph Digitizer software.

The gumminess estimated presents a range from 5,8 N to 16,09 N. This author also reports the cohesion figures, established from 0,25 for the control marshmallow and 0,73 to 0,88 for those that have inclusion of isomaltulose, fructose syrup and glucose syrup. Therefore, the developed marshmallow is within the estimated ranges, because it has a rubberiness of 7,96 N and a cohesiveness of 0,87.

The elasticity starts from 0,82 for the control marshmallow and 0,8 to 0,88 for the marshmallow that included isomaltulose, fructose syrup and reported glucose syrup (Periche *et al.*, 2015). The estimated value for the developed product is higher (1,0023); possibly due to the inclusion of gelatin with a higher Bloom degree (270), which provides greater elasticity and stability of the foam in the formulation.

Figure 9 shows the TPA curves for control and developed marshmallow including polyols and antihypertensive peptides.

The results show that the developed marshmallow has comparable and acceptable properties, such as hardness, cohesiveness, and gumminess; found within the characteristic ranges for a deposited marshmallow. The values for the elastic modulus, adhesiveness and elasticity were estimated instrumentally.

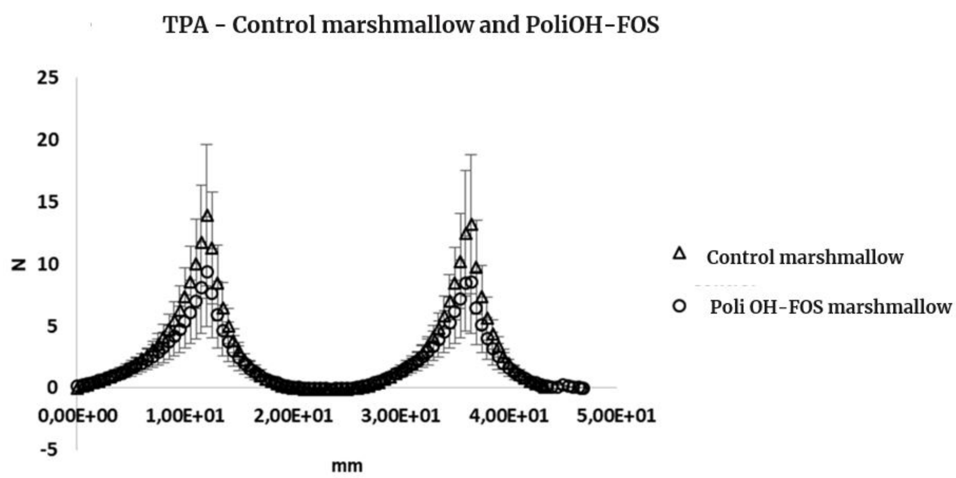

Figure 9

TPA graphic for the control and developed prototype marschmallows

Note. Own elaboration.

Finally, the analysis of variance of the five-point hedonic sensory test determined the formulations that caused significant statistical effect on sensory evaluation *(p* < 0,05). In Figure 10, comparisons between the averages of the sensory evaluation issued by the judges are established.

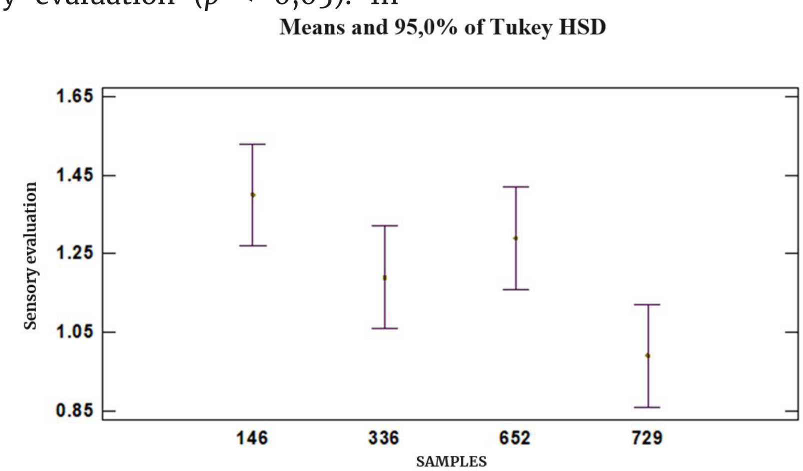

Figure 10

Comparison graph between the averages of the sensory evaluation issued by the judges

Note. Own elaboration.

The control marshmallow was coded with the number 336, the one that has inclusion of polyols, fructooligosaccharides and antihypertensive peptides was catalogued with the number 652, those with codes 146 and 729 are two trademarks. Among these, those who presented a significant statistical difference were: 146 - 729 and 652 - 729 and those who did not present it (p > 0,05) were 336 and 652, they did not show a significant statistical difference.

Marshmallows made by Periche *et al.* (2015) including isomaltulose, glucose syrup and fructose syrup, were evaluated by 20 trained panelists with an acceptance test containing a hedonic scale of 9 points; out of a total of 18 developed marshmallows, 5 (including one commercial) were selected using the technique of main components. The analysis of variance did not report significant differences between the 5 evaluated marshmallows (p > 0,05) and the sensory characteristics assessed were: appearance, color, aroma, texture, elasticity, hardness, gumminess, cohesiveness, sweetness and overall preference (Periche *et al.*, 2015).

Conclusions

The developed marshmallow meets the standards of a commercially deposited marshmallow, the elastic modulus and the parameters of the texture profile are within the specifications of the literature.

The inclusion of polyalcohols and antihypertensive peptides of dairy origin, is possible in a formulation of deposited marshmallow, without causing effect on the textural properties of the product and its elastic modulus. Therefore, it is possible to incorporate them in the levels evaluated in this work, because they maintain the sensory characteristics of a commercial marshmallow.

In the formulations of the developed prototypes marshmallows, the elastic modulus and texture profile of the control marshmallow is achieved. In these, the proportions of maltitol (46,55 %), sorbitol syrup (25 %) and fructoligosaccharides (8,45 %) are responsible for the elasticity and the amounts of gelatin were at the inclusion levels.

The hedonic sensory test determined that the developed marshmallow and the control marshmallow obtained a rating in the "like" category without presenting a statistical difference.

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