POTENTIAL USE OF ISOMALTULOSE TO PRODUCE HEALTHIER MARSHMALLOWS

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ABSTRACT

Isomaltulose is a non-cariogenic sugar with a lower glycemic index but with the same caloric value and visual appearance as sucrose. Therefore, isomaltulose could potentially be used to produce healthier candies. In this regard, the aim of this research was to evaluate isomaltulose as a traditional sugar replacer in soft marshmallow type candies, in order to provide added value to these widely consumed products, making it possible to capture a new market niche. 18 formulations were studied combining different sugars (sucrose, glucose syrup, fructose and isomaltulose) and different percentages of gelatine (4, 5 and 6). Analyses of composition (ºBrix and moisture content), pH and water activity (aw), instrumental colour and texture as well as a sensorial analysis were performed. Marshmallows with isomaltulose combined with fructose exhibited the lowest values of pH (4.99-5.14). Moreover, formulations with similar amount of isomaltulose and fructose presented lower instrumental hardness, higher cohesiveness and springiness, and the best sensory acceptance. A PLS multivariate analysis showed a good correlation between instrumental and sensory-mechanical parameters. Therefore, instrumental measures of texture could be suitable for discerning an overall preference for marshmallows without using trained panellists.
Keywords: marshmallows, isomaltulose, fructose, non-cariogenic, glycemic index and insulinemic index.

1. INTRODUCTION

Sweets are attractive due to their intense colours, shapes and characteristic aroma and taste, both for children and adults. Eating sweets in moderate amounts is pleasing and has a positive effect on well-being attributable to the sugar and flavourings present. However, these food products are not basic necessities and they are actually considered to be quite unhealthy since they are related to different diseases such as tooth decay, a sharp increase in the glycemic index and obesity. Despite these effects, they account for a significant volume of sales, candy consumption in Europe being valued at €710,291,000 in 2011 (Martínez, 2012).

The candy industry is a sector which is continually innovating in order to please consumers and develop new sugar free products meeting the demand for low calorie goods. The artificial sweeteners used as sugar substitutes show different disadvantages. For instance, polyalcohols have a laxative effect (Franz et al., 1994; Edwards, 2002) and intensive sweeteners such as aspartame have been related to the development of cancer and other health issues (AFSSA, 2002; Weihrauch and Diehl, 2004; Soffritti et al., 2007; Renwick and Nordmann, 2007).

In confectionery products, it is usual to find the combination of different sugars such as sucrose, fructose, glucose with inverted sugar or glucose syrups which are meant to increase their solubility, decreasing the water activity of the final product and hence improving their stability. It is important to point out that gummy confectionery products have a particular structure, which is related to the combination of sugars with proteins, resulting in the typical gel texture of jelly babies or others gums. Similarly, marshmallows have a foam-structure formed by the addition of air into the protein-sugar combination through hard stirring. Consequently, the structural role of sugar-substitutes must be studied to assess whether the required mechanical properties of
the traditional product are reached, or on the contrary, whether the end-products might
be considered acceptable by consumers.

An interesting natural sugar that can be used to revise the formula of candies is
isomaltulose. It is a reducing disaccharide composed of glucose and fructose, just like
sugar, but joined by a stronger glycoside bond type α-(1-6) (Weidenhangen & Lorenz,
1957), which is why it cannot be attacked by bacteria responsible for tooth decay
(Matsuyama et al., 1997). Therefore it is noncariogenic, and it is also slowly released in
the bloodstream (Beneo-Palatinit, 2010; Bebyal, 2012; Bucke & Cheetham, 1986). It is
found in small amounts in honey and sugarcane juice (Bárez et al., 2000) but can be
obtained massively from sucrose by means of an enzymatic process (Schiweck et al.,
1990). It has only a third of the sweetening power of sucrose. It supplies the same
amount of energy as table sugar but this energy lasts significantly longer. Furthermore,
it has only a slight effect on sugar and insulin levels in human being and thus, it is
totally digestible (Hawai et al., 1989; Lina et al., 2002).

Fructose is another natural sugar commonly found in fruits, which has been used for
diabetics for many years because it has also a low glycemic index. However there is
increasingly more controversy regarding the hazards of the fructose in high-fructose
corn syrup (HFCS) when used in high amounts. There are some studies which
conclude that high intakes of this syrup increase the risk of obesity since hepatic
metabolism of fructose favours the novo lipogenesis (Bray et al., 2003; Elliot et al.,
2002). Additionally, unlike glucose, fructose does not stimulate insulin secretion or
enhance leptin production. Because insulin and leptin act as key afferent signals in the
regulation of food intake and body weight, it could be suggested that dietary fructose
contributes to an increased energy intake and weight gain. However, fructose could be
safe if consumed in moderate quantities in healthy individuals.

In consideration of all the above, the aim of this study was to evaluate the use of
isomaltulose as a traditional sugar substitute in soft marshmallow type candies, in order
to obtain healthier products. Specifically, the influence of the formulation (type of sugar
and level of gelatine) on compositional parameters, colour, texture and sensory acceptance of the marshmallows was analysed. In addition, a correlation between instrumental and sensory variables was performed.

2. MATERIALS AND METHODS

2.1 Materials

The ingredients used in the formulation of marshmallows were: sucrose (Azucarera Ebro S.L., Burgos, Spain), fructose (Gabot Biochemical Industries, Haifa, Israel), isomaltulose (Beneo-Palatinit, Mannheim, Germany), glucose syrup 43 DE (Emilio Peña, S.A., Valencia, Spain), gelatin A 220 Bloom (Juncà Gelatines S.L., Girona, Spain), corn starch (Roquette Laisa S.A., Valencia, Spain), natural red liquid colour (Roha Europe S.L.U., Valencia, Spain), strawberry flavouring (Flavorix Aromáticos S.A., Madrid, Spain) and sunflower oil (Koipesol, Jaén, Spain).

2.2 Experimental Methodology

The marshmallows were confectioned with 36 g of water/100 g, 58-60 g of sugars/100 g and 4-6 g of gelatine/100 g. The percentage of sugars depended on the amount of gelatine used. Furthermore, 0.5 mg/kg of strawberry flavouring and 0.2 mg/kg of red colouring were added. Six different mixtures of sugars were studied. In the case of the control samples, the total sugar content was composed of 40 and 60 g of sucrose and glucose syrup per 100 g of total sugars respectively, and the code was (S). The new samples were obtained by replacing the sugars with different combinations of isomaltulose, glucose syrup or fructose. In order to simplify the description of each sample, the percentage of the total amount of sugars replaced is shown between brackets along with the code used: isomaltulose:glucose syrup (I: 40 g of isomaltulose and 60 g of glucose syrup per 100 g of total sugars), fructose:glucose syrup (F: 40 g of
fructose and 60 g of glucose syrup per 100 g of total sugars), isomaltulose:fructose
(I30: 30 g of isomaltulose and 70 g of fructose per 100 g of total sugars),
isomaltulose:fructose (I50: 50 g of isomaltulose and 50 g of fructose per 100 g of total
sugar), isomaltulose:fructose (I70: 70 g of isomaltulose and 30 g of fructose per 100 g
of total sugars). Besides, the level of gelatin (4, 5 or 6 g/100 g) was subsequently noted
by including the percentage of gelatine used (S4, S5, S6, I4, I5, I6, etc.) after the code,
a total of 17 different formulations being studied plus the control. Commercial
marshmallows were also analysed to compare them to the new ones only in terms of
composition, water activity and mechanical properties, but not colour since they did not
have similar optical properties.

Each formulation was made in a thermal blender (Thermomix, TM31, Vorwerk,
Wuppertal, Germany) by blending the sugars and water until they reached boiling
temperature at 300 rpm for 10 minutes. This mixture was shaken until reaching 60ºC
and pH and °Brix were measured. The gelatine was then dissolved in water in a ratio of
1 g of gelatine per 2 g of water 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 60ºC and 6.04 G-force. Then, the syrup was shaken for 10 minutes at
231.82 G-force to add air to the mixture, which is what mainly accounts for the texture
of the marshmallows. For molding purposes, the final mixture was poured into silicone
molds with a thin layer of sunflower oil. Finally, the molds were placed in a chamber at
20 ºC for 24 hours. The samples were then removed from their mould and covered with
starch to prevent the samples from sticking together. After an additional 24 hours,
analyses of texture, colour, water activity and moisture performed. Each formulation
was performed by triplicate.

2.3 Analytical determinations
2.3.1 Physicochemical Analyses

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 °C (method 20.103 AOAC, 2000). Water activity ($a_w$) was determined with a dew point hygrometer (FA-st lab, GBX, Valence, France). Soluble solid content (°Brix) was measured with a refractometer at 20 °C (ATAGO 3 T, Tokyo, Japan) and pH was determined with a pH-meter (SevenEasy, Mettler Toledo, Greifensee, Switzerland) in the initial syrup. All measurements were carried out in triplicate.

2.3.2 Colour

Instrumental measurements of colour were conducted at room temperature in a Konica-Minolta spectrophotometer (model CM-3600d, Singapore, Republic of Singapore) by placing the marshmallow on the diaphragm aperture (8 mm). CIEL*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 ($h^*=	ext{arctg}(b^*/a^*)$).

2.3.3 Texture

The samples were examined with Texture Profile Analysis test (TPA) using a TA.XT plus Texture Analyzer (Stable Micro Systems, Godalming, 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-time curve the following parameters were quantified, and are defined by Bourne (1978) as: hardness (N) (maximum peak force during the first compression cycle), springiness (the height that the sample recovers during the time that elapses between the end of the first cycle and the beginning of the second cycle), cohesiveness (the ratio of the positive force area
during the second compression and the first compression), gumminess (N) (hardness x cohesiveness).

2.3.4 Sensory Evaluation

An acceptance test using a 9-point hedonic scale (ISO 4121:2003, Jiménez et al. 2013) was used to evaluate the following attributes: appearance, colour, strawberry flavour, sweetness, texture, hardness, gumminess, springiness, cohesiveness, global preference and intention of buying (ISO 5492:2008). The panel consisted of 20 trained panellists (13 women and 7 males aged from 25 to 45) who are regular consumers of this kind of sweet. The sensory analysis took place in 2 sessions on separate days. The panellists evaluated 3 formulations (S4, I504, I505) on the first day and 2 formulations (I506 and S4-Ar [control which had double the added aroma]) on second day; each of the different formulations (3 units) was presented independently. Testing was conducted in a sensory evaluation laboratory built according to the international standards for test rooms.

2.4 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 (kind of sugar and percentage of gelatine) on the final product was significant. The interactions between factors were considered. Furthermore, Principal Component Analysis (PCA) and Partial Least Square regression (PLS2) were applied to describe the relationships between the sensory and the instrumental texture measurements. These analyses were performed using the Unscrambler version.10X (CAMO Process AS, Oslo, Norway).

3. RESULTS AND DISCUSSION

3.1 Compositional characteristics, pH and water activity
Table 1 shows the resulting °Brix and pH of syrup for each formulation in addition to moisture content (g of water/100 g), water activity and the sweetness of the marshmallows. As can be observed, samples confected with isomaltulose and glucose syrup had the lowest content of soluble solids, which could mean that these products will have a shorter shelf life in comparison with the control sample. However, marshmallows prepared with different proportions of isomaltulose and fructose in granulated form showed values of °Brix which were very similar to those of the control samples.

It is noteworthy that the use of glucose syrup in the preparation of marshmallows led to greater values of pH in the syrup, in comparison to samples prepared directly with mixture of sugars in granulated form. These results could affect the shelf-life of the final product since fructose and other reducing sugars are affected by pH differently. They are stable in modestly acidic environments but become unstable as the pH approaches neutral, and enters the alkaline range. As the pH of the system rises, the sugars become more chemically active and reactive, breaking down into colour bodies and flavour compounds, and reacting with proteins. This pH instability is marked by accelerated colour degradation, going from colourless to yellow to brown (Helstad, 2006). In this respect, given the decrease by almost two points of pH in samples confected with isomaltulose-fructose as compared to the pH in other cases, these products might have an increased shelf life.

On the whole, values of moisture content were in the recommended range for this kind of products (15-22 g of water/100g) (Edwards, 2002). In the case of commercial marshmallows, moisture content was 14.784(0.110) g of water/100g. Moreover, the statistical analysis evidences the significant effect of the interaction between the formulation used and the percentage of gelatine considered. More specifically, each formulation behaved differently depending on the percentage of gelatine used. Thus, control samples (60 g of glucose syrup and 40 g of sucrose per 100 g of total content of sugars) showed the lowest values of moisture content since the percentage of gelatine
increased in proportion to the value of soluble solids observed in the syrup. In contrast, samples with 60 g of glucose syrup and 40 g of isomaltulose per 100 g of total content of sugars, showed the highest values of moisture content when 4 g of gelatine/100 g was used. In samples where other levels of gelatine were used the behaviour was opposite that of the control samples. On the other hand, samples confected with isomaltulose-fructose mixtures had the highest moisture values, which were similar to the values recorded for control samples with 4 and 5 g of gelatine/100 g. Only samples I70 with 4 and 5 g of gelatine/100 g and control samples with 6 g of gelatine/100 g reached lower values of humidity than in the case of commercial marshmallows. Based on these results, it can be concluded that the replacement of sucrose by fructose and especially by isomaltulose in samples with glucose syrup led to an increase in the moisture content of the final product to levels even higher than the recommendable values when low percentages of gelatine were used. However, the combination of fructose-isomaltulose generally decreased the percentage of water in samples regardless of the amount of gelatine used.

In terms of water activity, control samples and marshmallows prepared with isomaltulose and glucose syrup, and with only the lowest level of gelatine, showed the highest water activity, meaning that there was more water susceptible to microbiology spoilage reactions and consequently potential health risks. Moreover, it is noteworthy that among the samples prepared with syrup, those containing isomaltulose showed the greatest water activity, especially for the lowest level of gelatine. In the case of isomaltulose-fructose samples, water activity of I30 was the closest to the water activity in commercial marshmallows (0.6516), the amount of gelatine used having no effect on this value. Besides, water activity rose in proportion to the percentage of isomaltulose.

With respect to the influence of gelatine, higher water activity was only observed in samples I70 with 4 g of gelatine/100 g.

In line with the inherent sweetness of the sugars studied, the higher the proportion of isomaltulose the lower the sweetness of the samples.
To sum up, mixtures of isomaltulose-fructose would be able to reach values of moisture content, soluble solids and water activity similar to the commercial ones. Furthermore, pH was lower than in control samples which could improve their stability.

3.2 Instrumental mechanical and optical properties

Figure 1 shows the instrumental TPA attributes (springiness, hardness, cohesiveness and gumminess) of the marshmallows obtained using the different formulations. Results indicated springiness values for all samples which were higher than 0.9, similar to the values shown for the control samples, regardless of the type of sugar and the percentage of gelatine in their formulation. The inclusion of gelatine in the formulation provided a visco-elastic texture and stable foam that led to the high springiness desirable for this kind of products (PB Gelatines, 2012; Hamann et al.; 2006). With respect to hardness, the higher the percentage of gelatine, the higher the hardness of the samples, except for marshmallows confected with the maximum quantity of isomaltulose (I70). The effect of gelatine on hardness also depended on the blend of sugars used (figure 1), this interaction being more notable in samples confected with the syrup of glucose (S, F and I). The samples confected with the same type of sugars as those used in the control had a higher hardness than that of the other formulations for a given percentage of gelatine, with the exception of samples with the maximum percentage of isomaltulose (I70). The highest value of hardness observed in the samples I70 with 4 and 5 g of gelatine/100 g, could be related to the crystallization of isomaltulose during the cooling step due to the low solubility of this sugar at room temperature (Mitchell, 2006). An increase in the level of jellification to 6 g of gelatine/100 g seemed to limit this phenomenon (PB Gelatins, 2012; Pérez, 2004). This result was also the consequence of the low structural cohesiveness exhibited by these samples (figure 1) which allows isomaltulose molecules to achieve enough mobility to form crystals. In turn, the intermolecular interaction or cohesiveness of any of the
samples I, F, I30 and I50, and therefore their structural integrity, was higher than in control samples (S).

Finally, gumminess, which comes from the interaction between the hardness and the cohesive forces taking place at structural level, was close to 1 in all samples.

Table 2 shows the values of luminosity and coordinates a* and b*, and the chroma and hue of marshmallows depending on the type of sugar and the percentage of gelatine. Table 3 shows the values of the F-ratio for each mechanical and optical parameter obtained in the ANOVA according to the factor studied (formulation and percentage of gelatine) and their interaction.

As can be observed, in all cases the percentage of gelatine was the factor with the greatest influence on luminosity and a* and b* coordinates. Specifically, the lower the level of gelatine the lower the luminosity, especially in samples obtained with glucose syrup-fructose and with glucose syrup-isomaltulose. In control samples, this increase was observed only in the leap between 4 and 5 g of gelatine/100 g, samples with 5 and 6 g of gelatine/100 g having similar values of L*. In this regard, the use of 5 g of gelatine/100 g would be enough to obtain luminosity very close to in the case of the control samples. Besides, for this particular level of gelatine, values of luminosity were very similar in all the formulations considered.

With regard to coordinate a*, the percentage of gelatine again had a greater significant effect than the formulation used or their interaction. Except for in the case of the control samples, marshmallows with 4 g of gelatine/100 g, showed the highest values of coordinate a*, especially in formulation F and I. This behaviour would indicate that samples with a lower content of gelatine would tend to have reddish colours. Nevertheless, in samples with 5 and 6 g of gelatine/100 g, there was no defined tendency with respect to the values of coordinate a* as in the case of luminosity.

In coordinate b*, differences arising due to the percentage of gelatine were less evident than in L* and a*. A significant decrease of coordinate b* was only observed in samples I30, I70 and especially in I, when 4 g of gelatine/100 g was used. On this occasion, the
formulation and percentage of gelatine had a similar effect. In any case, the values of the b* coordinate were very low so the differences found would not lead to important deviations in colour when they are placed in the chromatic diagram b*a*.

In accordance with the previous results, chrome values showed a tendency which was similar to that of the values of coordinate a* due to its higher numerical value in comparison with coordinate b*. With the exception of samples I, hue was kept at an average angle of approximately 8°. Samples I were in the fourth quadrant of the chromatic diagram b*a*, with an angle of 350° which was very close to the other samples.

In general, the colour of the samples prepared with glucose syrup-isomaltulose differed most from the colour of the control samples. However, the differences in colour were minimal, not being visually perceptible. On the other hand, it would be advisable to use a percentage of gelatine of between 4 and 5 g of gelatine/100 g because despite the fact that were changes when 4 g of gelatine/100 g was used, they were almost unnoticeable, and no improvements were observed when using the highest percentage of gelatine, which would also lead to a higher cost.

3.3 Sensory Evaluation

As was described previously, the instrumental texture measurements were made for the 18 possible marshmallows formulations (6 combinations of sugars and 3 levels of gelatine). However, due to the complexity of the sensory studies, only a few of these formulations were selected. To this end, the information given by a principal component analysis (PCA) of the instrumental parameters (hardness, gumminess, cohesiveness and elasticity) obtained from the 18 formulations as well as a commercial sample (C), was taken into account. The latter was also included to facilitate selection of the formulations. The first two components of this PCA explained 83 % of the total variance (PC1, 63 % and PC2, 20 %). The formulations were selected due to their proximity to the commercial sample, which means they had a similar texture profile. S4 (with 4 g of
gelatine/100 g) was selected from those exclusively made with sucrose (sugar used in commercial sweets). The differences between the formulations made with a mixture of isomaltulose and fructose, with a 30:70 ratio of these sugars and a 50:50 ratio, were practically non-existent. Therefore, samples which had the highest levels of isomaltulose (I504, I505, I506) were chosen because of the advantages of this sugar to consumer health.

The result of the ANOVA (using “formulation” as a factor), carried out for the different attributes evaluated by the panellists, is shown in a radial chart (figure 2). This figure shows the average score for each attribute evaluated by the panellists, and the F-ratio of each attribute in brackets. There are no significant differences between the samples evaluated by the panellists for any of the attributes. However, considering the average values, some differences between the samples can be seen. Sample S4 scored lowest on all the attributes except hardness. This low score was reflected in global appreciation and intention of buying.

In relation to global appearance and colour, sample I505 was the most appreciated. Regarding texture attributes (hardness, gumminess, cohesiveness, springiness), samples I504 and I506 had similar scores; on the contrary, sample I505 obtained slightly lower scores. As regards the aroma attribute, sample S4-Ar was the best. As this formulation had double the added aroma, it is clear that the panellists liked a more intense aroma in this kind of product. Finally, sample I504 had the best score for global preference and intention of buying.

In order to ascertain the possible linear dependence between the sensory attributes, and especially to know which attribute has more influence on global preference and intention of buying, Pearson correlation coefficients (95.0% confidence level) were calculated for each pair of variables. Table 4 shows the correlation matrix obtained. The best positive correlations were shown for intention of buying-global preference (0.959) and for intention of buying-texture (0.942). Moreover, a positive correlation between colour-overall appearance (0.908) and cohesiveness-gumminess (0.878)
were found. Therefore, it is texture that defines acceptability and intention of buying the
product.

A PCA analysis was conducted to better understand the relationship between the
samples and the evaluated attributes from a descriptive point of view. Figure 3 shows
the biplot of the sample scores and the attribute loadings obtained by means of this
analysis. The first two dimensions explained 77 % of the total variance (PC1, 50% and
PC2, 27%). Samples with isomaltulose (I504, I505, I506) are placed at the right side
next to the highest values of the sensory variables analysed and hence the most
preferred, especially for I504 (with 4 g of gelatine/100 g). On the other hand S4 and S4-4A
are situated on the opposite side, which implies the lowest values of these variables
for these two last samples, especially for S4.

3.4 **Correlation between sensory and instrumental variables**

As explained before, texture is the characteristic that decisively influences the
consumer when buying this type of product. For this reason, it was decided to assess
whether texture sensory variables, as well as global preference, are correlated with the
instrumental variables. With this aim, figure 4 shows the results from PLS2 regression
analysis, which describes the relationship between the instrumental variables (X-
matrix) and the acceptability score for the sensory attributes (Y-matrix). The sensory
parameters placed in the outer ellipse are correlated with the instrumental variables,
with the exception of sensorial hardness, which being placed in the inner ellipse is not
correlated ($r^2=50\%$). In summary, it could be asserted that the instrumental texture
analyses are suitable and can be used to discern the overall preference for
marshmallows without using trained panellists.

4. **CONCLUSIONS**
According to the mechanical properties, the total sugar content of marshmallows could be replaced by a mixture of isomaltulose and fructose in a 1:1 ratio. Marshmallows prepared under these conditions obtained a better sensory evaluation than those confected with sucrose and glucose syrup. Therefore, isomaltulose could be a good substitute for traditional sugars in marshmallows. Additionally, a good correlation was found between the instrumental parameters and the acceptance sensory attributes, and global preference, indicating that texture measurements can be used for quality assessment purposes. To sum up, isomaltulose could be considered by the confectionery industry to obtain healthier candies.

5. ACKNOWLEDGMENTS

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6. REFERENCES


Beneo-palatinit (2010). Palatinose™. The only toothfriendly sugar for optimized energy supply.


### Table captions

#### Table 1. Mean and standard deviation of °Brix and pH of the initial syrup and moisture content, water activity and sweetness of the marshmallows (n=3)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Gelatin</th>
<th>°Brix</th>
<th>pH</th>
<th>Moisture content (g/100g)</th>
<th>$a_w$</th>
<th>Sweetness. $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>4</td>
<td>75.4(1.3)$^a$</td>
<td>6.57(0.09)$^a$</td>
<td>18.8(0.3)$^{ad}$</td>
<td>0.816(0.002)$^b$</td>
<td>0.42</td>
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<td></td>
<td>5</td>
<td>16.0(1.4)$^b$</td>
<td>12.8(0.8)$^c$</td>
<td>0.736(0.003)$^c$</td>
<td>0.41</td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>20.8(0.3)$^{ad}$</td>
<td>18.1(0.8)$^{ab}$</td>
<td>0.687(0.005)$^d$</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>71(4)$^b$</td>
<td>6.68(0.18)$^a$</td>
<td>19.1(0.5)$^{ad}$</td>
<td>0.721(0.002)$^c$</td>
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<td></td>
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<td>23.9(1.9)$^c$</td>
<td>19.0(0.5)$^{ad}$</td>
<td>0.785(0.004)$^b$</td>
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<tr>
<td></td>
<td>6</td>
<td>18.1(1.7)$^{ab}$</td>
<td>18.4(1.7)$^{ad}$</td>
<td>0.671(0.007)$^d$</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>65.0(0.6)$^c$</td>
<td>6.69(0.13)$^a$</td>
<td>17.2(1.3)$^{ab}$</td>
<td>0.683(0.002)$^d$</td>
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<td>72.9(0.4)$^d$</td>
<td>5.13(0.09)$^b$</td>
<td>17.31(1.06)$^{ab}$</td>
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<td>18.4(1.7)$^{ad}$</td>
<td>18.4(1.7)$^{ad}$</td>
<td>0.671(0.007)$^d$</td>
<td>0.25</td>
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<tr>
<td>I30</td>
<td>4</td>
<td>73.7(1.2)$^d$</td>
<td>5.14(0.15)$^b$</td>
<td>18.3(0.97)$^{ab}$</td>
<td>0.716(0.004)$^c$</td>
<td>0.53</td>
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<tr>
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<td>17.2(1.15)$^{ab}$</td>
<td>17.2(1.15)$^{ab}$</td>
<td>0.678(0.003)$^d$</td>
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<tr>
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<tr>
<td>I50</td>
<td>4</td>
<td>73.4(1.2)$^d$</td>
<td>4.992(0.108)$^c$</td>
<td>17.5(1.3)$^{ab}$</td>
<td>0.709(0.004)$^c$</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10.3(0.3)</td>
<td>10.3(0.3)</td>
<td>10.3(0.3)</td>
<td>10.6(0.3)$^d$</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9.5(0.6)</td>
<td>9.5(0.6)</td>
<td>9.5(0.6)</td>
<td>9.5(0.6)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

S: control (glucose syrup: 60% and sucrose: 40%). F: fructose (glucose syrup: 60% and fructose: 40%). I: isomaltulose (glucose syrup: 60% and Isomaltulose: 40%). I30 (Isomaltulose: 30% and fructose: 70%). I50 (Isomaltulose: 50% and fructose: 50%). I70 (Isomaltulose: 70% and fructose: 30%). The percentages of sugars in the formulations are expressed respect the total amount of sugars in the product.

*Theoretical Sweetness Power (SP) of the marshmallows: SP=∑m, SP/$\sum m_i$ (m: grams of each compound; SP; Sweetness Power of each component (individual sugar)) (González et al., 1989).

Similar letters indicate statistically homogenous groups.

#### Table 2. Mean and standard deviation of Luminosity, coordinates a*, b*, chroma and hue (n=3)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>$C^*$</th>
<th>$h^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S4</td>
<td>85.5(0.7)$^d$</td>
<td>10.9(1.2)$^{bc}$</td>
<td>1.5(0.4)$^c$</td>
<td>11.03(1.07)$^c$</td>
<td>8(3)$^{bc}$</td>
</tr>
<tr>
<td>S5</td>
<td>86.4(0.3)$^{ef}$</td>
<td>10.7(0.3)$^{bc}$</td>
<td>2.009(0.005)$^d$</td>
<td>10.8(0.2)$^c$</td>
<td>10.6(0.3)$^d$</td>
</tr>
<tr>
<td>S6</td>
<td>86.9(0.1)$^{ef}$</td>
<td>10.25(0.12)$^{bc}$</td>
<td>1.70(0.04)$^{ed}$</td>
<td>10.3(0.2)$^{bc}$</td>
<td>9.4(0.3)$^{cd}$</td>
</tr>
<tr>
<td>F4</td>
<td>82.8(0.1)$^b$</td>
<td>14.3(0.3)$^c$</td>
<td>0.4(0.4)$^b$</td>
<td>14.4(0.2)$^f$</td>
<td>1.6(1.6)$^a$</td>
</tr>
<tr>
<td>F5</td>
<td>87.7(0.6)$^{ef}$</td>
<td>9.4(0.6)$^{ab}$</td>
<td>1.4(0.2)$^f$</td>
<td>9.5(0.6)$^{ab}$</td>
<td>8.9(0.8)$^c$</td>
</tr>
<tr>
<td>F6</td>
<td>85.5(0.8)$^{ef}$</td>
<td>11.7(0.8)$^c$</td>
<td>0.26(0.09)$^b$</td>
<td>11.7(0.8)$^{yd}$</td>
<td>1.2(0.4)$^a$</td>
</tr>
<tr>
<td>I4</td>
<td>81.7(0.4)$^a$</td>
<td>16.2(0.6)$^f$</td>
<td>-0.98(0.05)$^a$</td>
<td>16.2(0.6)$^f$</td>
<td>356.51(0.12)$^f$</td>
</tr>
<tr>
<td>I5</td>
<td>86.6(0.4)$^{ef}$</td>
<td>10.1(0.3)$^b$</td>
<td>2.3(0.2)$^f$</td>
<td>10.3(0.3)$^{bc}$</td>
<td>12.9(0.7)$^c$</td>
</tr>
<tr>
<td>I6</td>
<td>84.2(0.9)$^c$</td>
<td>13.08(1.02)$^d$</td>
<td>0.6(0.6)$^b$</td>
<td>13.10(1.02)$^c$</td>
<td>2.8(0.7)$^a$</td>
</tr>
<tr>
<td>I304</td>
<td>84.1(0.2)$^c$</td>
<td>12.7(0.3)$^d$</td>
<td>1.52(0.13)$^f$</td>
<td>12.8(0.3)$^{yd}$</td>
<td>6.8(0.8)$^b$</td>
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<tr>
<td>I305</td>
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<td>10.15(1.17)$^b$</td>
<td>2.1(0.2)$^{dke}$</td>
<td>10.37(1.12)$^{be}$</td>
<td>11.9(0.8)$^c$</td>
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Table 3. F-ratio and interaction of the texture and optical parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor</th>
<th>Formulation</th>
<th>% Gelatin</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td></td>
<td>262.08*</td>
<td>78.66*</td>
<td>74.07*</td>
</tr>
<tr>
<td>Springiness</td>
<td></td>
<td>17.81*</td>
<td>0.61</td>
<td>5.15*</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td></td>
<td>139.82*</td>
<td>8.88*</td>
<td>21.81*</td>
</tr>
<tr>
<td>Gumminess</td>
<td></td>
<td>552.63*</td>
<td>548.23*</td>
<td>197.29*</td>
</tr>
<tr>
<td>L*</td>
<td></td>
<td>52.14*</td>
<td>194.11*</td>
<td>12.74*</td>
</tr>
<tr>
<td>a*</td>
<td></td>
<td>32.92*</td>
<td>119.17*</td>
<td>13.59*</td>
</tr>
<tr>
<td>b*</td>
<td></td>
<td>95.69*</td>
<td>134.90*</td>
<td>36.00*</td>
</tr>
<tr>
<td>C*</td>
<td></td>
<td>31.81*</td>
<td>113.91*</td>
<td>13.02*</td>
</tr>
<tr>
<td>h*</td>
<td></td>
<td>18732.07*</td>
<td>17191.07*</td>
<td>19369.03*</td>
</tr>
</tbody>
</table>

* Statistical significance ≥ 99% (p-value ≤ 0.01)

Table 4. Pearson correlation of different attributes

<table>
<thead>
<tr>
<th></th>
<th>Appearance</th>
<th>Colour</th>
<th>Aroma</th>
<th>Texture</th>
<th>Springiness</th>
<th>Hardness</th>
<th>Gumminess</th>
<th>Cohesiveness</th>
<th>Sweetness</th>
<th>Overall preference</th>
<th>Intention of buying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>1.000</td>
<td></td>
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<tr>
<td>Colour</td>
<td>0.908**</td>
<td>1.000</td>
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<tr>
<td>Aroma</td>
<td>0.116</td>
<td>0.258</td>
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</tr>
<tr>
<td>Texture</td>
<td>0.828</td>
<td>0.617</td>
<td>0.393</td>
<td>1.000</td>
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<td></td>
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</tr>
<tr>
<td>Springiness</td>
<td>0.085</td>
<td>-0.197</td>
<td>0.073</td>
<td>0.497</td>
<td>1.000</td>
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<tr>
<td>Hardness</td>
<td>-0.360</td>
<td>-0.620</td>
<td>0.086</td>
<td>0.063</td>
<td>0.248</td>
<td>1.000</td>
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<tr>
<td>Gumminess</td>
<td>0.052</td>
<td>-0.222</td>
<td>0.475</td>
<td>0.578</td>
<td>0.600</td>
<td>0.790</td>
<td>1.000</td>
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<tr>
<td>Cohesiveness</td>
<td>0.268</td>
<td>-0.096</td>
<td>0.184</td>
<td>0.719</td>
<td>0.845</td>
<td>0.576</td>
<td>0.878**</td>
<td>1.000</td>
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<tr>
<td>Sweetness</td>
<td>0.094</td>
<td>-0.026</td>
<td>0.318</td>
<td>0.268</td>
<td>-0.259</td>
<td>0.724</td>
<td>0.593</td>
<td>0.277</td>
<td>1.000</td>
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<tr>
<td>Overall preference</td>
<td>0.579</td>
<td>0.210</td>
<td>0.052</td>
<td>0.832</td>
<td>0.538</td>
<td>0.497</td>
<td>0.739</td>
<td>0.871</td>
<td>0.493</td>
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<tr>
<td>Intention of buying</td>
<td>0.777</td>
<td>0.469</td>
<td>0.141</td>
<td>0.942*</td>
<td>0.486</td>
<td>0.253</td>
<td>0.617</td>
<td>0.786</td>
<td>0.389</td>
<td>0.959**</td>
<td>1.000</td>
</tr>
</tbody>
</table>

** Statistical significance ≥ 99% (p-value ≤ 0.01) * Statistical significance ≥ 95% (p-value ≤ 0.05)
Figure captions

Figure 1. Values of Hardness (N), Springiness, Cohesiveness and Gumminess (N) of the marshmallows. Codes of formulations were referred to the amount of each kind of sugar used per 100 g of sugars in marshmallows: S (60 g of glucose syrup and 40 g of sucrose), F (60 g of glucose syrup and 40 g of fructose), I (60 g of glucose syrup and 40 g of isomaltulose), I30 (30 g of Isomaltulose and 70 g of fructose), I50 (50 g of isomaltulose and 50 g of fructose) and I70 (70 g of isomaltulose and 30 g of fructose). Dark grey, medium grey and light grey bars correspond to 4, 5 and 6 g of gelatine/100 g of product respectively.

Figure 2. Radial chart of the average scores (1: Dislike extremely, 9: Like extremely) for each attribute and the F-ratio of each attribute in brackets for the evaluated marshmallows. Dotted line refers to control samples (S) formulated with 60 g of glucose syrup and 40 g of sucrose per 100 g of sugars with 4 g of gelatine per 100 g of product. Dashed line refers to control samples with extra aroma (S-Ar). Solid lines refer to formulation I50 which had 50 g of isomaltulose and 50 g of fructose per 100 g of sugars. Black, dark grey and light grey lines indicate 4, 5 and 6 g of gelatine per 100 g of product, respectively.
Figure 3. Bi-plot Principal Components Analysis for the samples and the attributes. Black rhombus (♦) attributes and white rhombus (◊) samples. Codes of formulations were referred to the amount of each kind of sugar used per 100 g of sugars in marshmallows: S and S-Ar (60 g of glucose syrup and 40 g of sucrose), and I50 (50 g of isomaltulose and 50 g of fructose) and I70 (70 g of isomaltulose and 30 g of fructose). S-Ar refers to control with extra aroma. Numbers 4, 5 and 6 after the code correspond to the amount of gelatine used expressed as g of gelatine/100 g of product.
Figure 4. Correlation loadings (X and Y) between instrumental and sensory texture variables. Black rhombus (♦) instrumental values and white rhombus (◊) sensorial values.