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Additional Information

1 **POTENTIAL USE OF ISOMALTULOSE TO PRODUCE HEALTHIER**
2 **MARSHMALLOWS**

3
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10 **ABSTRACT**

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

28 Keywords: marshmallows, isomaltulose, fructose, non-cariogenic, glycemic index and
29 insulinemic index.

30 **1. INTRODUCTION**

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

39 The candy industry is a sector which is continually innovating in order to please
40 consumers and develop new sugar free products meeting the demand for low calorie
41 goods. The artificial sweeteners used as sugar substitutes show different
42 disadvantages. For instance, polyalcohols have a laxative effect (Franz et al., 1994;
43 Edwards, 2002) and intensive sweeteners such as aspartame have been related to the
44 development of cancer and other health issues (AFSSA, 2002; Weihrauch and Diehl,
45 2004; Soffritti et al., 2007; Renwick and Nordmann, 2007).

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

55 the traditional product are reached, or on the contrary, whether the end-products might
56 be considered acceptable by consumers.

57 An interesting natural sugar that can be used to revise the formula of candies is
58 isomaltulose. It is a reducing disaccharide composed of glucose and fructose, just like
59 sugar, but joined by a stronger glycoside bond type α -(1-6) (Weidenhagen & Lorenz,
60 1957), which is why it cannot be attacked by bacteria responsible for tooth decay
61 (Matsuyama et al., 1997). Therefore it is noncariogenic, and it is also slowly released in
62 the bloodstream (Beneo-Palatinit, 2010; Bebyal, 2012; Bucke & Cheetham, 1986). It is
63 found in small amounts in honey and sugarcane juice (Bárez et al., 2000) but can be
64 obtained massively from sucrose by means of an enzymatic process (Schiweck et al.,
65 1990). It has only a third of the sweetening power of sucrose. It supplies the same
66 amount of energy as table sugar but this energy lasts significantly longer. Furthermore,
67 it has only a slight effect on sugar and insulin levels in human being and thus, it is
68 totally digestible (Hawai et al., 1989; Lina et al., 2002).

69 Fructose is another natural sugar commonly found in fruits, which has been used for
70 diabetics for many years because it has also a low glycemic index. However there is
71 increasingly more controversy regarding the hazards of the fructose in high-fructose
72 corn syrup (HFCS) when used in high amounts. There are some studies which
73 conclude that high intakes of this syrup increase the risk of obesity since hepatic
74 metabolism of fructose favours the novo lipogenesis (Bray et al., 2003; Elliot et al.,
75 2002). Additionally, unlike glucose, fructose does not stimulate insulin secretion or
76 enhance leptin production. Because insulin and leptin act as key afferent signals in the
77 regulation of food intake and body weight, it could be suggested that dietary fructose
78 contributes to an increased energy intake and weight gain. However, fructose could be
79 safe if consumed in moderate quantities in healthy individuals.

80 In consideration of all the above, the aim of this study was to evaluate the use of
81 isomaltulose as a traditional sugar substitute in soft marshmallow type candies, in order
82 to obtain healthier products. Specifically, the influence of the formulation (type of sugar

83 and level of gelatine) on compositional parameters, colour, texture and sensory
84 acceptance of the marshmallows was analysed. In addition, a correlation between
85 instrumental and sensory variables was performed.

86

87

88 **2. MATERIALS AND METHODS**

89

90 **2.1 Materials**

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

98

99 **2.2 Experimental Methodology**

100 The marshmallows were confected with 36 g of water/100 g, 58-60 g of sugars/100 g
101 and 4-6 g of gelatine/100 g. The percentage of sugars depended on the amount of
102 gelatine used. Furthermore, 0.5 mg/kg of strawberry flavouring and 0.2 mg/kg of red
103 colouring were added. Six different mixtures of sugars were studied. In the case of the
104 control samples, the total sugar content was composed of 40 and 60 g of sucrose and
105 glucose syrup per 100 g of total sugars respectively, and the code was (S). The new
106 samples were obtained by replacing the sugars with different combinations of
107 isomaltulose, glucose syrup or fructose. In order to simplify the description of each
108 sample, the percentage of the total amount of sugars replaced is shown between
109 brackets along with the code used: isomaltulose:glucose syrup (I: 40 g of isomaltulose
110 and 60 g of glucose syrup per 100 g of total sugars), fructose:glucose syrup (F: 40 g of

111 fructose and 60 g of glucose syrup per 100 g of total sugars), isomaltulose:fructose
112 (I30: 30 g of isomaltulose and 70 g of fructose per 100 g of total sugars),
113 isomaltulose:fructose (I50: 50 g of isomaltulose and 50 g of fructose per 100 g of total
114 sugars), isomaltulose:fructose (I70: 70 g of isomaltulose and 30 g of fructose per 100 g
115 of total sugars). Besides, the level of gelatin (4, 5 or 6 g/100 g) was subsequently noted
116 by including the percentage of gelatine used (S4, S5, S6, I4, I5, I6, etc.) after the code,
117 a total of 17 different formulations being studied plus the control. Commercial
118 marshmallows were also analysed to compare them to the new ones only in terms of
119 composition, water activity and mechanical properties, but not colour since they did not
120 have similar optical properties.

121 Each formulation was made in a thermal blender (Thermomix, TM31, Vorwerk,
122 Wuppertal, Germany) by blending the sugars and water until they reached boiling
123 temperature at 300 rpm for 10 minutes. This mixture was shaken until reaching 60°C
124 and pH and °Brix were measured. The gelatine was then dissolved in water in a ratio of
125 1 g of gelatine per 2 g of water to obtain a homogeneous mix and subsequently added
126 to the syrup with the flavouring and colouring agents. All the ingredients were blended
127 for 5 minutes at 60°C and 6.04 G-force. Then, the syrup was shaken for 10 minutes at
128 231.82 G-force to add air to the mixture, which is what mainly accounts for the texture
129 of the marshmallows. For molding purposes, the final mixture was poured into silicone
130 molds with a thin layer of sunflower oil. Finally, the molds were placed in a chamber at
131 20 °C for 24 hours. The samples were then removed from their mould and covered with
132 starch to prevent the samples from sticking together. After an additional 24 hours,
133 analyses of texture, colour, water activity and moisture performed. Each formulation
134 was performed by triplicate.

135

136 **2.3 Analytical determinations**

137

138 *2.3.1 Physicochemical Analyses*

139 Moisture content and water activity analyses were carried out on the final products.
140 Moisture content was determined gravimetrically by drying to a constant weight in a
141 vacuum oven at 60 °C (method 20.103 AOAC, 2000). Water activity (a_w) was
142 determined with a dew point hygrometer (FA-st lab, GBX, Valence, France). Soluble
143 solid content (°Brix) was measured with a refractometer at 20 °C (ATAGO 3 T, Tokyo,
144 Japan) and pH was determined with a pH-meter (SevenEasy, Mettler Toledo,
145 Greifensee, Switzerland) in the initial syrup. All measurements were carried out in
146 triplicate.

147

148 *2.3.2 Colour*

149 Instrumental measurements of colour were conducted at room temperature in a
150 Konica-Minolta spectrophotometer (model CM-3600d, Singapore, Republic of
151 Singapore) by placing the marshmallow on the diaphragm aperture (8 mm). CIEL*a*b*
152 coordinates were obtained using illuminant D65 and standard observer (10° visual
153 field) as references. Registered parameters were: L* (brightness), a* (red component),
154 b* (yellow component), chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue ($h^* = \arctg(b^*/a^*)$).

155 *2.3.3 Texture*

156 The samples were examined with Texture Profile Analysis test (TPA) using a TA.XT
157 plus Texture Analyzer (Stable Micro Systems, Godalming, U.K.). Instruments were
158 equipped with a load cell of 50 kg and a 45 mm diameter cylindrical probe. The test
159 conditions involved two consecutive cycles of 50% compression with 15 s between
160 cycles. The test speed was 1 mm/s. From the resulting force-time curve the following
161 parameters were quantified, and are defined by Bourne (1978) as: hardness (N)
162 (maximum peak force during the first compression cycle), springiness (the height that
163 the sample recovers during the time that elapses between the end of the first cycle and
164 the beginning of the second cycle), cohesiveness (the ratio of the positive force area

165 during the second compression and the first compression), gumminess (N) (hardness x
166 cohesiveness).

167 *2.3.4 Sensory Evaluation*

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

179

180 **2.4 Statistical Analyses**

181 Statgraphics Centurion was used to perform the statistical analyses. Analyses of
182 variance (multifactor ANOVA) were carried out to discern whether the effect of the
183 process variables (kind of sugar and percentage of gelatine) on the final product was
184 significant. The interactions between factors were considered. Furthermore, Principal
185 Component Analysis (PCA) and Partial Least Square regression (PLS2) were applied
186 to describe the relationships between the sensory and the instrumental texture
187 measurements. These analyses were performed using the Unscrambler version.10X
188 (CAMO Process AS, Oslo, Norway).

189

190 **3. RESULTS AND DISCUSSION**

191 **3.1 Compositional characteristics, pH and water activity**

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

200 It is noteworthy that the use of glucose syrup in the preparation of marshmallows led to
201 greater values of pH in the syrup, in comparison to samples prepared directly with
202 mixture of sugars in granulated form. These results could affect the shelf-life of the final
203 product since fructose and other reducing sugars are affected by pH differently. They
204 are stable in modestly acidic environments but become unstable as the pH approaches
205 neutral, and enters the alkaline range. As the pH of the system rises, the sugars
206 become more chemically active and reactive, breaking down into colour bodies and
207 flavour compounds, and reacting with proteins. This pH instability is marked by
208 accelerated colour degradation, going from colourless to yellow to brown (Helstad,
209 2006). In this respect, given the decrease by almost two points of pH in samples
210 confected with isomaltulose-fructose as compared to the pH in other cases, these
211 products might have an increased shelf life.

212 On the whole, values of moisture content were in the recommended range for this kind
213 of products (15-22 g of water/100g) (Edwards, 2002). In the case of commercial
214 marshmallows, moisture content was 14.784(0.110) g of water/100g. Moreover, the
215 statistical analysis evidences the significant effect of the interaction between the
216 formulation used and the percentage of gelatine considered. More specifically, each
217 formulation behaved differently depending on the percentage of gelatine used. Thus,
218 control samples (60 g of glucose syrup and 40 g of sucrose per 100 g of total content of
219 sugars) showed the lowest values of moisture content since the percentage of gelatine

220 increased in proportion to the value of soluble solids observed in the syrup. In contrast,
221 samples with 60 g of glucose syrup and 40 g of isomaltulose per 100 g of total content
222 of sugars, showed the highest values of moisture content when 4 g of gelatine/100 g
223 was used. In samples where other levels of gelatine were used the behaviour was
224 opposite that of the control samples. On the other hand, samples confectioned with
225 isomaltulose-fructose mixtures had the highest moisture values, which were similar to
226 the values recorded for control samples with 4 and 5 g of gelatine/100 g. Only samples
227 I70 with 4 and 5 g of gelatine/100 g and control samples with 6 g of gelatine/100 g
228 reached lower values of humidity than in the case of commercial marshmallows. Based
229 on these results, it can be concluded that the replacement of sucrose by fructose and
230 especially by isomaltulose in samples with glucose syrup led to an increase in the
231 moisture content of the final product to levels even higher than the recommendable
232 values when low percentages of gelatine were used. However, the combination of
233 fructose-isomaltulose generally decreased the percentage of water in samples
234 regardless of the amount of gelatine used.

235 In terms of water activity, control samples and marshmallows prepared with
236 isomaltulose and glucose syrup, and with only the lowest level of gelatine, showed the
237 highest water activity, meaning that there was more water susceptible to microbiology
238 spoilage reactions and consequently potential health risks. Moreover, it is noteworthy
239 that among the samples prepared with syrup, those containing isomaltulose showed
240 the greatest water activity, especially for the lowest level of gelatine. In the case of
241 isomaltulose-fructose samples, water activity of I30 was the closest to the water activity
242 in commercial marshmallows (0.6516), the amount of gelatine used having no effect on
243 this value. Besides, water activity rose in proportion to the percentage of isomaltulose.
244 With respect to the influence of gelatine, higher water activity was only observed in
245 samples I70 with 4 g of gelatine/100 g.

246 In line with the inherent sweetness of the sugars studied, the higher the proportion of
247 isomaltulose the lower the sweetness of the samples.

248 To sum up, mixtures of isomaltulose-fructose would be able to reach values of moisture
249 content, soluble solids and water activity similar to the commercial ones. Furthermore,
250 pH was lower than in control samples which could improve their stability.

251

252 **3.2 Instrumental mechanical and optical properties**

253 Figure 1 shows the instrumental TPA attributes (springiness, hardness, cohesiveness
254 and gumminess) of the marshmallows obtained using the different formulations.

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

275 samples I, F, I30 and I50, and therefore their structural integrity, was higher than in
276 control samples (S).

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

279 Table 2 shows the values of luminosity and coordinates a^* and b^* , and the chrome and
280 hue of marshmallows depending on the type of sugar and the percentage of gelatine.

281 Table 3 shows the values of the F-ratio for each mechanical and optical parameter
282 obtained in the ANOVA according to the factor studied (formulation and percentage of
283 gelatine) and their interaction.

284 As can be observed, in all cases the percentage of gelatine was the factor with the
285 greatest influence on luminosity and a^* and b^* coordinates. Specifically, the lower the
286 level of gelatine the lower the luminosity, especially in samples obtained with glucose
287 syrup-fructose and with glucose syrup-isomaltulose. In control samples, this increase
288 was observed only in the leap between 4 and 5 g of gelatine/100 g, samples with 5 and
289 6 g of gelatine/100 g having similar values of L^* . In this regard, the use of 5 g of
290 gelatine/100 g would be enough to obtain luminosity very close to in the case of the
291 control samples. Besides, for this particular level of gelatine, values of luminosity were
292 very similar in all the formulations considered.

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

300 In coordinate b^* , differences arising due to the percentage of gelatine were less evident
301 than in L^* and a^* . A significant decrease of coordinate b^* was only observed in samples
302 I30, I70 and especially in I, when 4 g of gelatine/100 g was used. On this occasion, the

303 formulation and percentage of gelatine had a similar effect. In any case, the values of
304 the b^* coordinate were very low so the differences found would not lead to important
305 deviations in colour when they are placed in the chromatic diagram b^*a^* .

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

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

319

320 **3.3 Sensory Evaluation**

321 As was described previously, the instrumental texture measurements were made for
322 the 18 possible marshmallows formulations (6 combinations of sugars and 3 levels of
323 gelatine). However, due to the complexity of the sensory studies, only a few of these
324 formulations were selected. To this end, the information given by a principal component
325 analysis (PCA) of the instrumental parameters (hardness, gumminess, cohesiveness
326 and elasticity) obtained from the 18 formulations as well as a commercial sample (C),
327 was taken into account. The latter was also included to facilitate selection of the
328 formulations. The first two components of this PCA explained 83 % of the total variance
329 (PC1, 63 % and PC2, 20 %). The formulations were selected due to their proximity to
330 the commercial sample, which means they had a similar texture profile. S4 (with 4 g of

331 gelatine/100 g) was selected from those exclusively made with sucrose (sugar used in
332 commercial sweets). The differences between the formulations made with a mixture of
333 isomaltulose and fructose, with a 30:70 ratio of these sugars and a 50:50 ratio, were
334 practically non-existent. Therefore, samples which had the highest levels of
335 isomaltulose (I504, I505, I506) were chosen because of the advantages of this sugar to
336 consumer health.

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

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

352 In order to ascertain the possible linear dependence between the sensory attributes,
353 and especially to know which attribute has more influence on global preference and
354 intention of buying, Pearson correlation coefficients (95.0% confidence level) were
355 calculated for each pair of variables. Table 4 shows the correlation matrix obtained.
356 The best positive correlations were shown for intention of buying-global preference
357 (0.959) and for intention of buying-texture (0.942). Moreover, a positive correlation
358 between colour-overall appearance (0.908) and cohesiveness-gumminess (0.878)

359 were found. Therefore, it is texture that defines acceptability and intention of buying the
360 product.

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

370

371 **3.4 Correlation between sensory and instrumental variables**

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

383

384 **4. CONCLUSIONS**

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

394

395

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399

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471 **Table captions**

472 **Table 1.** Mean and standard deviation of °Brix and pH of the initial syrup and moisture content,
 473 water activity and sweetness of the marshmallows (n=3)
 474

Formulation	Initial syrup			Product: marshmallow		
	% Gelatin	°Brix	pH	Moisture content (g/100g)	a _w	Sweetness.*
S	4			18.8(0.3) ^{ad}	0.816(0.002) ^b	0.42
	5	75.4(1.3) ^a	6.57(0.09) ^a	16.0(1.4) ^b	0.736(0.003) ^c	0.41
	6			12.8(0.8) ^c	0.687(0.005) ^d	0.41
F	4			20.8(0.3) ^{ad}	0.797(0.005) ^b	0.54
	5	71(4) ^b	6.68(0.18) ^a	19.1(0.5) ^{ad}	0.721(0.002) ^c	0.53
	6			18.1(0.8) ^{ab}	0.739(0.003) ^c	0.52
I	4			23.9(1.9) ^e	0.873(0.004) ^a	0.25
	5	65.0(0.6) ^c	6.69(0.13) ^a	19.0(0.5) ^{ad}	0.785(0.004) ^b	0.25
	6			22(5) ^{de}	0.786(0.005) ^b	0.25
I30	4			17.2(1.3) ^{ab}	0.683(0.002) ^d	0.68
	5	72.9(0.4) ^d	5.13(0.09) ^b	17.31(1.06) ^{ab}	0.653(0.004) ^d	0.67
	6			18.4(1.7) ^{ad}	0.671(0.007) ^d	0.66
I50	4			16.2(0.8) ^b	0.715(0.006) ^c	0.54
	5	73.7(1.2) ^d	5.14(0.15) ^b	18.32(0.97) ^{abd}	0.716(0.004) ^c	0.53
	6			17.27(1.15) ^{ab}	0.678(0.003) ^d	0.52
I70	4			13.9(1.9) ^{bc}	0.762(0.003) ^b	0.40
	5	73.4(1.2) ^d	4.992(0.108) ^c	14.0(0.7) ^{bc}	0.679(0.002) ^d	0.39
	6			17.5(1.3) ^{ab}	0.709(0.004) ^c	0.38

475 S: control (glucose syrup: 60% and sucrose: 40%). F: fructose (glucose syrup: 60% and fructose: 40%). I:
 476 isomaltulose (glucose syrup: 60% and Isomaltulose: 40%). I30 (Isomaltulose: 30% and fructose: 70%).
 477 I50 (Isomaltulose: 50% and fructose: 50%). I70 (Isomaltulose: 70% and fructose: 30%). The percentages
 478 of sugars in the formulations are expressed respect the total amount of sugars in the product.
 479 *Theoretical Sweetness Power (SP) of the marshmallows: $SP = \sum m_i \cdot SP_i / \sum m_i$ (m_i : grams of each
 480 compound; SP_i : Sweetness Power of each component (individual sugar)) (González *et al.*, 1989).
 481 Similar letters indicate statistically homogenous groups.
 482

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

Formulation	L*	a*	b*	C*	h*
S4	85.5(0.7) ^d	10.9(1.2) ^{bc}	1.5(0.4) ^c	11.03(1.07) ^c	8(3) ^{bc}
S5	86.4(0.3) ^{ef}	10.7(0.3) ^{bc}	2.009(0.005) ^d	10.8(0.2) ^c	10.6(0.3) ^d
S6	86.9(0.1) ^{ef}	10.25(0.12) ^{bc}	1.70(0.04) ^{cd}	10.3(0.2) ^{bc}	9.4(0.3) ^{cd}
F4	82.8(0.1) ^b	14.3(0.3) ^e	0.4(0.4) ^b	14.4(0.2) ^f	1.6(1.6) ^a
F5	87.7(0.6) ^{fg}	9.4(0.6) ^{ab}	1.4(0.2) ^c	9.5(0.6) ^{ab}	8.9(0.8) ^c
F6	85.5(0.8) ^{de}	11.7(0.8) ^c	0.26(0.09) ^b	11.7(0.8) ^{cd}	1.2(0.4) ^a
I4	81.7(0.4) ^a	16.2(0.6) ^f	-0.98(0.05) ^a	16.2(0.6) ^g	356.51(0.12) ^l
I5	86.6(0.4) ^{ef}	10.1(0.3) ^b	2.3(0.2) ^c	10.3(0.3) ^{bc}	12.9(0.7) ^c
I6	84.2(0.9) ^c	13.08(1.02) ^d	0.6(0.6) ^b	13.10(1.02) ^c	2.8(0.7) ^a
I304	84.1(0.2) ^c	12.7(0.3) ^d	1.52(0.13) ^c	12.8(0.3) ^{de}	6.8(0.8) ^b
I305	87.4(0.9) ^{fg}	10.15(1.17) ^b	2.1(0.2) ^{de}	10.37(1.12) ^{bc}	11.9(0.8) ^c

I306	87.78(0.09) ^g	9.65(0.08) ^{ab}	2.21(0.08) ^{de}	9.90(0.08) ^{ab}	12.9(0.5) ^c
I504	84.8(0.3) ^{cd}	11.95(0.14) ^c	1.786(0.105) ^d	12.09(0.15) ^d	8.5(0.4) ^c
I505	88.2(0.3) ^g	9.3(0.2) ^{ab}	1.93(0.04) ^d	9.5(0.2) ^a	11.7(0.3) ^{de}
I506	87.7(0.6) ^{fg}	10.1(0.7) ^b	1.91(0.03) ^d	10.2(0.6) ^{bc}	10.7(0.5) ^{de}
I704	86.6(0.5) ^{ef}	11.1(0.2) ^c	1.41(0.09) ^c	11.1(0.2) ^c	7.2(0.5) ^{bc}
I705	87.33(0.05) ^f	10.4(0.3) ^{bc}	1.79(0.15) ^d	10.5(0.3) ^{bc}	9.7(0.8) ^{cd}
I706	88.7(0.2) ^g	9.1(0.2) ^a	1.93(0.09) ^d	9.21(0.17) ^a	12.1(0.8) ^{de}

484 S: control (glucose syrup: 60% and sucrose: 40%). F: fructose (glucose syrup: 60% and fructose: 40%). I:
485 isomaltulose (glucose syrup: 60% and Isomaltulose: 40%). I30 (Isomaltulose: 30% and fructose: 70%).
486 I50 (Isomaltulose: 50% and fructose: 50%). I70 (Isomaltulose: 70% and fructose: 30%). The percentages
487 of sugars in the formulations are expressed respect the total amount of sugars in the product. The level of
488 gelatin (4, 5 or 6%) was subsequently noted by including the percentage of gelatine used after the code.
489 [†]ANOVA analysis was performed omitting this case in order to discern the statistical differences among
490 h* values with regard to formulations. Similar letters indicate statistically homogenous groups.
491

492 **Table 3.** F-ratio and interaction of the texture and optical parameters
493

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

* Statistical significance $\geq 99\%$ (p-value ≤ 0.01)

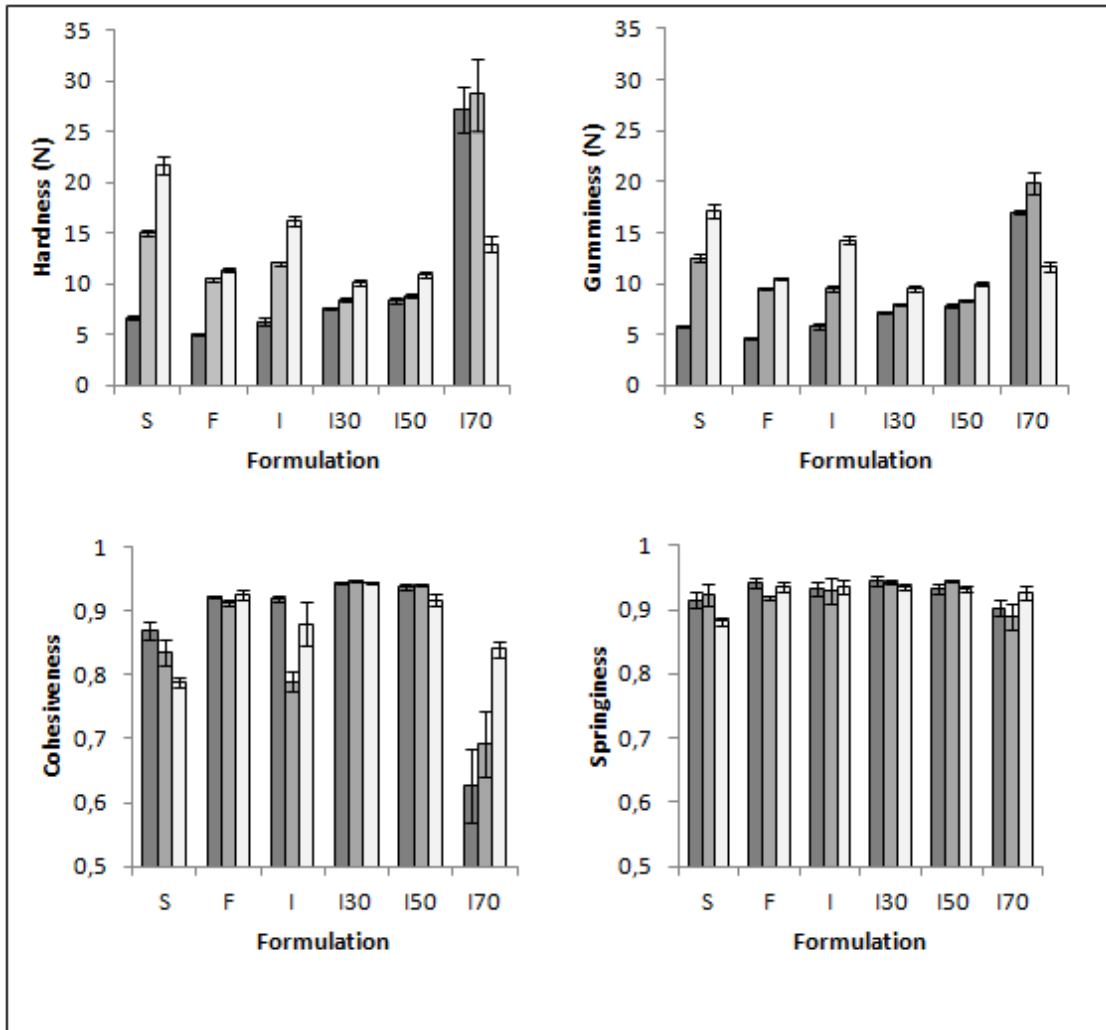
494 **Table 4.** Pearson correlation of different attributes
495
496

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

497 ** Statistical significance $\geq 99\%$ (p-value ≤ 0.01) * Statistical significance $\geq 95\%$ (p-value ≤ 0.05)

498 **Figure captions**

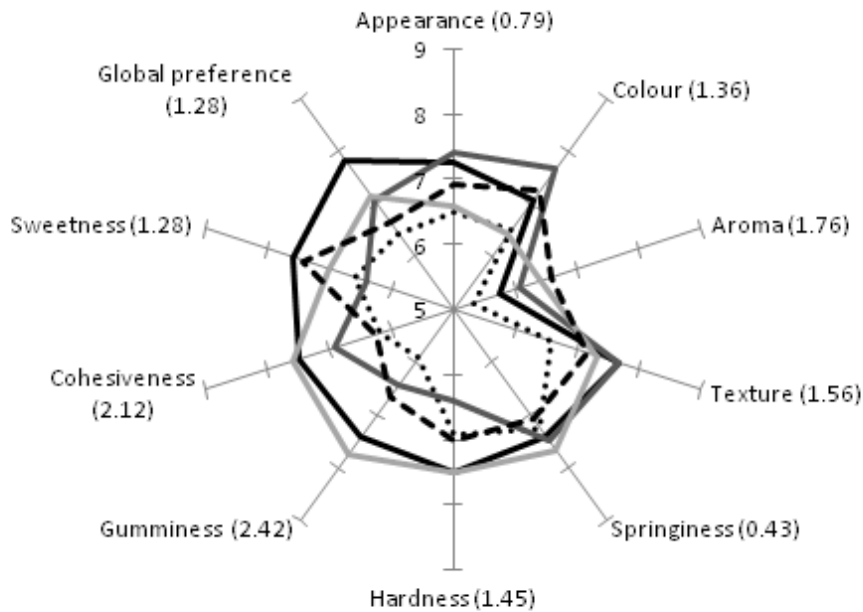
499 **Figure 1.** Values of Hardness (N), Springiness, Cohesiveness and Gumminess (N) of the
 500 marshmallows. Codes of formulations were referred to the amount of each kind of sugar used
 501 per 100 g of sugars in marshmallows: S (60 g of glucose syrup and 40 g of sucrose), F (60 g of
 502 glucose syrup and 40 g of fructose), I (60 g of glucose syrup and 40 g of isomaltulose), I30 (30
 503 g of Isomaltulose and 70 g of fructose), I50 (50 g of isomaltulose and 50 g of fructose) and I70
 504 (70 g of isomaltulose and 30 g of fructose). Dark grey, medium grey and light grey bars
 505 correspond to 4, 5 and 6 g of gelatine/100 g of product respectively.



506

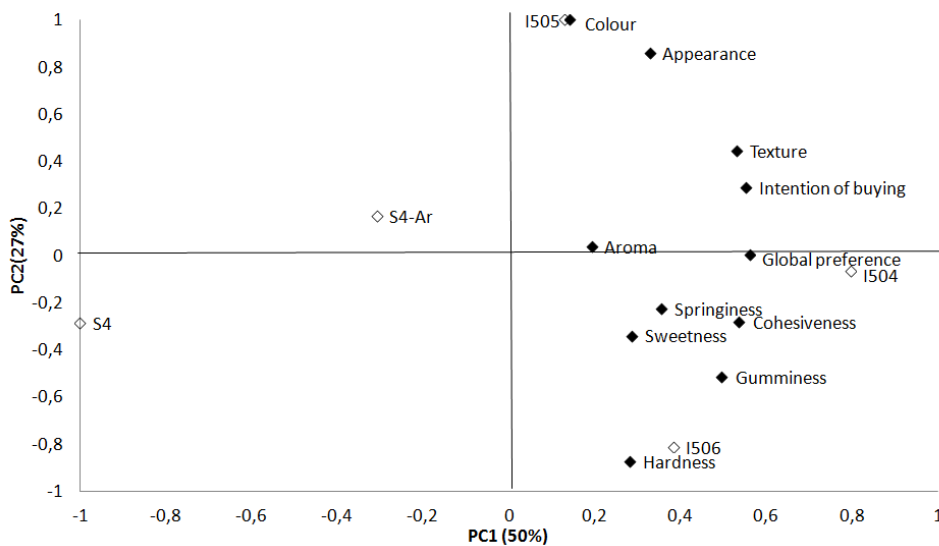
507

508 **Figure 2.** Radial chart of the average scores (1: Dislike extremely, 9: Like extremely) for each
 509 attribute and the F-ratio of each attribute in brackets for the evaluated marshmallows. Dotted
 510 line refers to control samples (S) formulated with 60 g of glucose syrup and 40 g of sucrose per
 511 100 g of sugars with 4 g of gelatine per 100 g of product. Dashed line refers to control samples
 512 with extra aroma (S-Ar). Solid lines refer to formulation I50 which had 50 g of isomaltulose and
 513 50 g of fructose per 100 g of sugars. Black, dark grey and light grey lines indicate 4, 5 and 6 g
 514 of gelatine per 100 of product, respectively.



515

516 **Figure 3.** Bi-plot Principal Components Analysis for the samples and the attributes. Black
 517 rhombus (◆) attributes and white rhombus (◇) samples. Codes of formulations were referred to
 518 the amount of each kind of sugar used per 100 g of sugars in marshmallows: S and S-Ar (60 g
 519 of glucose syrup and 40 g of sucrose), and I50 (50 g of isomaltulose and 50 g of fructose) and
 520 I70 (70 g of isomaltulose and 30 g of fructose). S-Ar refers to control with extra aroma. Numbers
 521 4, 5 and 6 after the code correspond to the amount of gelatine used expressed as g of
 522 gelatine/100 g of product.

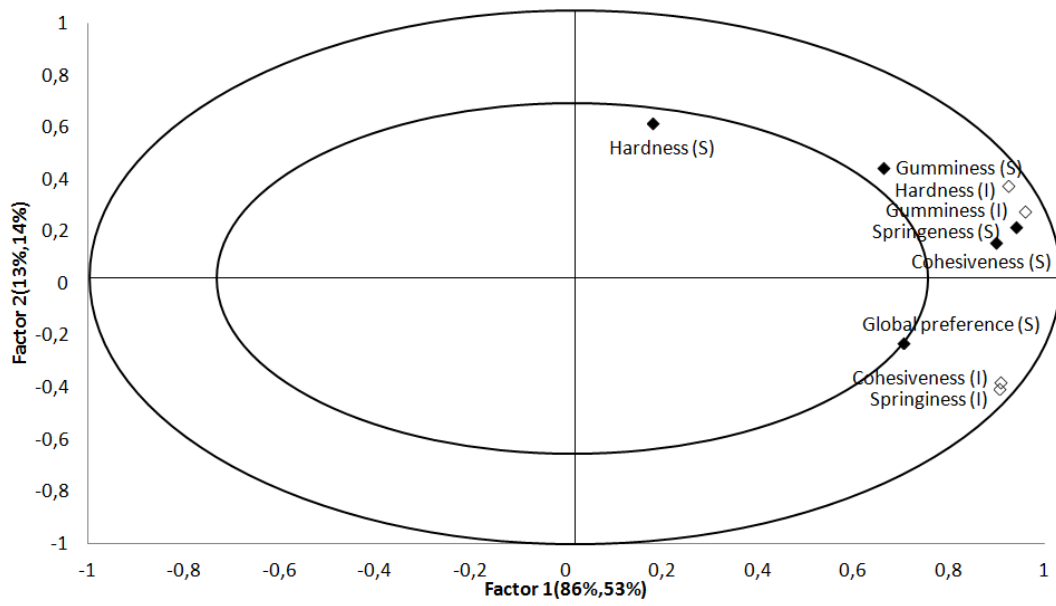


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525 **Figure 4.** Correlation loadings (X and Y) between instrumental and sensory texture variables.

526 Black rhombus (◆) instrumental values and white rhombus (◇) sensorial values.



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