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DEVELOPMENT OF LEMON MARMALADE FORMULATED WITH NEW SWEETENERS (ISOMALTULOSE AND TAGATOSE). EFFECT ON ANTIOXIDANT, RHEOLOGICAL AND OPTICAL PROPERTIES.

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ABSTRACT

The aim of this study was to make lemon marmalades in which sucrose is replaced by sweeteners such as tagatose and isomaltulose. Analyses of ºBrix, pH, moisture, water activity, antioxidant capacity, optical and rheological properties were carried out on marmalades on their first day of storage, and after 60 days of storage. Microbiological analyses were also performed. Moreover, a sensory evaluation was performed to assess its consumer acceptance as compared to marmalade made with sucrose. The results showed that the antioxidant capacity of the new formulations was lower than in marmalade with sucrose. Moreover, marmalades made with healthy sweeteners showed lower consistency than those made with sucrose. Lemon marmalades formulated with a higher proportion of isomaltulose initially had high luminosity compared to the other samples, but browned over time. All marmalades were microbiologically stable, and the marmalades made with healthy sweeteners were scored better than those made with sucrose.

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PRACTICAL APPLICATIONS

The development of new healthier marmalades offer new alternatives to the conventional ones not only to prevent caries and obesity but also to provide functional features associated with the use of tagatose and isomaltulose as sweeteners. However, not always it is possible to replace traditional components by others and that is why is so important to assess their technological influence. In this study, the viability of the use of both sweeteners to prepare lemon marmalades has been checked.

INTRODUCTION

Fruits have a short shelf life but high nutritional value. Marmalades are an alternative to fresh fruit, and they also provide an outlet for surplus fruit production, offering a very stable product. Sucrose has traditionally been used as the main sugar in marmalades. Sucrose provides a high energy input for daily activities due to its high glycemic index, but it is high in calories. Excessive consumption of sucrose can cause several diseases such as tooth decay, obesity and diabetes (Edwards 2002; O'Donnell and Kearsley 2012). However, the food industry offers other natural sweeteners which do not lead to such problems. Tagatose and isomaltulose are two of those non-cariogenic sweeteners that are slowly released into blood. In fact, D-Tagatose (D-tag) it is considered a functional food because it is partially metabolized and the part that is not absorbed (80% of the intake) ferments in the colon, where it performs functions as soluble fiber (Taylor *et al*. 2008) favoring *lactic acid* and *Lactobacillus* bacteria (Petersen-Skytte 2006). Moreover, it is a stereoisomer of D-fructose, it is found naturally in cheese and yoghurt, and it can also be produced from D-galactose (Oh 2007; Lu *et al*. 2008). Furthermore, it is very suitable for confectionary products, ice creams, soft drinks and breakfast cereals (Vastenavond *et al*. 2011), since it is almost as sweet as sucrose and its

texture is very similar to sucrose (Oh 2007; Taylor *et al.* 2008; Calzada-León *et al.* 2013). Additionally, tagatose has only 1.5 kcal/g and it does not cause dental caries (Levin 2002). Tagatose received Generally Recognized as Safe status by the Food and Drug Administration in 2001 and entered the US market as a sweetener in 2003 (Donner *et al.* 2010).

On the other hand, isomaltulose has a third of the sweetening power of sucrose and the physicochemical properties of isomaltulose enable it to be used as a substitute for sucrose in most sweet foods (Lina *et al.* 2002; De Oliva-Neto and Menão 2009; Peinado *et al.* 2013). Furthermore, isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice, its taste and viscosities of aqueous solutions are similar to those of sucrose and it has the same caloric power (Schiweck *et al*. 1990; Periche *et al.* 2014).

Given the characteristics of these two sweeteners, they could be used to reformulate traditional foods to make them healthier for society. Thus, the aim of this study was to evaluate the potential use of healthy sweeteners (isomaltulose and tagatose) as an alternative to sucrose in lemon marmalades, by analyzing their colour, rheological properties, antioxidant capacity, microbiological stability and sensorial acceptance.

MATERIALS AND METHODS

Lemon Marmalade Formulations

The ingredients used in formulation of lemon marmalades were: lemon pulp (*Citrus limon eureka* also known as *Four Seasons*), sucrose (Azucarera Española, Burgos, Spain), agar-agar (Roko Agar, Llanera, Asturias, Spain). Furthermore, we used as healthy sweeteners: isomaltulose (Beneo, Mannheim, Germany) and commercial tagatose (Tagatesse®, Heusden-Zolder, Belgium) which was composed by 39.9% of

tagatose, 39.9% of isomalt, 0.02% of sucralose and 20% of dietary fiber (inulin and oligosaccharides). All the sweeteners were used in podwer format. The following notation was used depending on the combination of sweeteners used: Control marmalade: 100 % sucrose, Marmalade A: 60% isomaltulose and 40% of commercial tagatose, Marmalade B: 50% isomaltulose and 50% of commercial tagatose, and Marmalade C: 30% isomaltulose and 70% of commercial tagatose. A commercial lemon marmalade was also characterized (*Ora et Labora*, Lemon Marmalade, Monasterio Santa Paula, Sevilla, Spain).

Manufacturing Processes

Lemons were selected and picked fresh. Subsequently, they were peeled and mixed with the corresponding combination of healthy sweeteners/sucrose in a ratio 50:50 (w/w) and 1% (w/w) agar-agar in powder form in a thermal blender (Thermomix, TM31, Vorwerk, Germany) for 3 min. Afterwards the mixture was cooked at 100 ºC for 20 min at 350 rpm. The glass jars, which had previously been sterilized in an autoclave at 121ºC for 15 min, were then filled with the marmalade and turned over to ensure proper sealing for 1 hour. Finally, the marmalade was allowed to cool for 24 hours and became jellified. Three batches of lemons were used to prepare the marmalades. They were stored at room temperature (25ºC) and in the dark. Analyses were triplicated on the first day of storage and after 60 days of storage.

Physicochemical analyses

Water activity (a_w) was determined with a dew point water activity meter made by Decagon Devices, Inc. (Aqua Lab 4TE, Pullman, Washington, USA), at 25ºC. The pH was registered with a pH-meter (Seven Easy, Mettler Toledo, Barcelona, Spain). Moisture content (x_w) was determined gravimetrically by drying approximately 1 g of

marmalade until a constant weight, in a vacuum oven at 60ºC (method 934.06, AOAC 2000). The soluble solids content (Brix) was determined in a refractometer at 20ºC (Atago 3T, Tokyo, Japan).

Determination of Antioxidant Capacity

The antioxidant activity of marmalades was analyzed on the basis of the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl free radical (Shahidi *et al*. 2006). 1 g of marmalade was mixed with 6 mL of pure methanol for 5 min in a vortex, keeping the supernatant. This mixture was centrifuged at 13000 rpm for 10 min. The absorbance was read at 515 nm in a spectrocolorimeter manufactured by Thermo Fisher Scientific, Inc. (Helios Zeta UV-VIS, Waltham, Massachusetts, USA). Quantification was performed considering a standard curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as mg of Trolox equivalent per 100 g of marmalade (Rubio-Arraez *et al.* 2015).

Optical Properties

The optical properties of lemon marmalades were measured using a spectrocolorimeter manufactured by Konica Minolta, Inc. (CM-3600d, Tokyo, Japan). CIE-L*a*b* coordinates were obtained using D65 illuminant and 10º observer as the reference system. All analytical determinations were performed on sweet lemon marmalades in 20 mm-wide cuvettes.

Rheological Analysis

The rheological properties of the lemon marmalades studied were measured using a controlled stress rheometer manufactured by Thermo Fisher Scientific, Inc. (Haake RheoStress 1, Waltham, Massachusetts, USA), at 25ºC. Measurements were carried out

in triplicate with plate–plate geometry and a 2.0 mm gap for steady state and oscillatory tests (Sato and Cunha 2009), by means of steady state essays or oscillatory essays to study the pseudoplastic or viscoelastic behavior of marmalades, respectively. The protocol is described in previous studies (Peinado *et al*. 2012; Rubio-Arraez *et al.* 2015). For the steady state measurements the Herschel–Bulkley model (Eq.1) was used.

$$\tau = \tau_0 + \kappa \cdot \gamma^n \quad (1)$$

This model describes Newtonian and a large group of time independent non-Newtonian fluids. There are three parameters: τ is the shear stress (Pa), τ_0 is the yield stress above which the fluid starts flowing (Pa), γ is the shear rate (s^{-1}), k is the index of consistency ($Pa \cdot s^n$) and n is the index of fluidity.

In the case of the oscillatory essays were carried out based on the power-law describing the mechanical spectrum within the linear viscoelastic region in terms of storage (*G'*) and loss (*G"*) modulus as a function of frequency between 0.1-10 Hz (Eqs. 2 and 3):

$$G' = a \cdot \omega^b \quad (2)$$

$$G'' = c \cdot \omega^d \quad (3)$$

Where, ω is the angular speed ($rad \cdot s^{-1}$), a is the low frequency storage modulus (Pa^b); b is the power-law index for the storage modulus (dimensionless); c is the low frequency loss modulus (Pa^d); and, d is the power-law index for the loss modulus (dimensionless).

Microbiological Analysis

Serial dilutions were prepared by homogenizing 10 g of marmalade with 90 mL of 1% sterile peptone water in a stomacher bag, using sterile techniques. Mesophilic aerobic populations were analyzed in a Plate Count Agar (Scharlau Chemie, 1-329, Barcelona, Spain), by incubating samples for 72 hours at 31ºC. Yeast and molds were determined

144 in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1-166, Barcelona, Spain) plates
145 kept at for 5 days. Samples were analyzed initially and after 60 days of storage.

146 **Sensorial Analysis**

147 An acceptance test using a 9-point hedonic scale (ISO 4121) was used to evaluate the
148 following attributes: color, aroma, texture, consistency, spreadable capacity,
149 palatability, flavor, sweetness, bitterness, and global preference (ISO 5492) in the three
150 formulations made with different combinations of healthy sugars (A, B and C), as well
151 as the control marmalade. Additionally, the possible appearance of sineresis and
152 intention of buying were assessed. For this purpose, a panel was formed consisting of
153 30 trained panelists ranging in age from 20 to 50 years old, who are regular consumers
154 of this kind of marmalades. Testing sessions were conducted in a sensory evaluation
155 laboratory built according to the international standards for test rooms (ISO 8589).

156 **Statistical Analysis**

157 Statgraphics plus (Centurion, Statpoint Technologies, Inc. Warrenton, Virginia, USA)
158 software was used to perform the statistical analyses. Analyses of variance (multifactor
159 ANOVA) were performed to study the interactions between the formulation and time of
160 storage on the marmalades.

161 **RESULTS AND DISCUSSION**

162 **Compositional Characterization of Marmalades**

163 Table 1 shows the results reflecting the composition (Brix and moisture content (x_w)),
164 pH, water activity (a_w) and antioxidant capacity of the lemon marmalades studied. It is
165 noteworthy that none of the marmalades prepared with the new sweeteners reached the
166 Brix of the commercial (≈65 Brix) or the control (≈59 Brix) samples. However, the new

167 marmalades do meet the standards of Council Directive 2001/113/EC of 20 December
168 2001 relating to fruit jams, jellies and marmalades and sweetened chestnut purée
169 intended for human consumption, since this Directive allows soluble content lower than
170 60 Brix when sweeteners are used in the formulation for these products, rather than
171 sugars. Over time the values of Brix remained constant. In coherence with the Brix
172 results, marmalades formulated with the new sweeteners showed the highest values for
173 moisture content (x_w), whereas the commercial marmalades showed the lowest value,
174 followed by the control sample. However, in this case, moisture content significantly
175 increased after storage, probably due to the condensation of water vapor in the space
176 located in the inner part of the lids. Accordingly, the water activity of the commercial
177 lemon marmalades was significantly lower than in the other cases. Among the new
178 marmalades, the lowest water activity was registered for marmalade C, which had the
179 highest amount of tagatose. Therefore, tagatose would make the water molecules more
180 compact than isomaltulose. After storage, few changes were observed in a_w. It should
181 also be highlighted that all samples prepared in this study showed lower values of pH
182 than the commercial marmalades. Initially the marmalades B and C showed higher pH
183 values, but all samples reached similar values after storage. Besides, all pH values were
184 lower than 3.5, which would ensure a proper microbiological stability of these products,
185 as was observed in other fruit jams made with strawberry, peach, plum or apricot
186 (Carbonell *et al.* 1991; García-Martínez *et al*. 2002). Also noteworthy was that the
187 lemon marmalade formulations A, B and C showed a lower antioxidant content than the
188 control marmalade, although time significantly reduced the antioxidant content in all
189 cases. Furthermore, the commercial sample showed only 0.11 ± 0.04 mg Trolox eq/ 100
190 g which would be consistent with the rapid deterioration of antioxidant compounds in
191 which occurs in lemon marmalade. The degradation of health related compounds, such

as ascorbic acid and polyphenols, initiated during processing, will continue during storage and the losses observed in stored products can often be more severe than those observed during processing as was also observed by Mazur et al., 2014 who worked with strawberry low-sugar jams. The role of new sweeteners could slow down this degradation as was observed in our previous study (Rubio-Arraez *et al.* 2015) where the highest proportion of oligofructose contributed to improve the initial antioxidant capacity of orange marmalades. However, in the present study no improvement in the antioxidant capacity was registered by using different combinations of isomaltulose-tagatose.

Rheological Properties

The rheological properties of marmalades studied were determined using two tests, both steady and oscillatory, to obtain the parameters of the models considered in each case.

The results obtained for the stationary test of lemon marmalades, based on the combination of sweeteners used and the storage time, is presented in Fig.1. The rheograms of lemon marmalades fluctuated, possibly due to the increased presence of lumpy parts. In any case, over the storage time, the curves remained similar. The rheograms of commercial lemon marmalade showed no clear trend due to the high presence of cells and lemon peel. Therefore, the curve is not shown. The parameters of the Herschel-Bulkley model for lemon marmalades studied at the beginning and the end of the period considered are shown in Table 2. As can be seen, the use of the new sweeteners initially led to lower values of shear stress (τ) than in marmalades prepared with sucrose. However, the consistency index (k) was initially significantly higher in marmalade with the highest amount of tagatose (formulation C). In studies carried out by other authors, (Peinado *et al*. 2012) where sugars were also replaced by other sweeteners (isomaltulose), a decrease in consistency and cohesiveness of strawberry

jams was observed. On the other hand, the storage time caused a compaction in lemon marmalades since the values of shear stress significantly increased. The index of fluidity (*n*) also increased over time but this increase was only significant in formulation A, which might be due to the fact that the isomaltulose content was higher. However, the index of consistency (*k*) significantly decreased in formulation C, dropping to half the initial index, despite registering the highest initial values, as previously mentioned. In our previous study (Rubio-Arraez *et al.* 2015), the orange marmalade with the same proportions of oligofructose and tagatose was more consistent.

As for the results of the oscillatory test, Fig. 2 shows the evolution of the storage (G') and loss (G") moduli *versus* frequency for the lemon marmalades studied. Furthermore, the results of varying both G' and G'' were adjusted for the angular velocity (ω) ($rad \cdot s^{-1}$) to the model of the Power Law. The resulting values of the parameters of this model are presented in Table 2. This type of test determines the ratio between the elastic and viscous component of a material and quantifies to which the material behaves as a solid or liquid. Specifically, the storage modulus (G') is associated with the elastic component of the material, while the loss modulus (G"), is associated with its viscous component. Since in all cases G' was greater than G" moduli the lemon marmalades showed a semi-solid behavior (Peinado *et al.* 2012). This characteristic is typical of a gel, since it is more elastic than viscous. The parameters *a* and *c* decreased significantly with new sweeteners, but there were no differences between the combinations studied. The storage time increased these parameters in all cases, but the increase was only significant in the control marmalade. These results are consistent with the curves presented in Fig. 2, where the marmalade control curves of G' and G" as a function of frequency are placed above the others, especially at the end of storage. Besides, Peinado *et al.* (2012) observed the same when sucrose was replaced by isomaltulose in the

reformulation of different strawberry spreadable products. This decrease was associated with how the type of sugar influences the availability of water in the mixture of pectin-sugar-acid, and therefore in the formation of hydrogen bonds and the possible association of water in the pectin polymer chain (Peinado *et al.* 2012). In the present study, the gelling agent used was agar-agar instead of pectin and it could have homogenized the rheological properties of the marmalade regardless of the type of sugar used. However, the parameters *b* and *d* were similar in all marmalades. Besides, in orange marmalade formulated with oligofructose and tagatose as a substitutes of sucrose and agar-agar there was an increase in the elastic component (G') after 45 days of storage (Rubio-Arraez *et al.* 2015).

Optical Properties

As shown in Fig. 3, the interaction charts for the colorimetric coordinates L*, a* and b*, chroma (C*) and hue (h*) of the different lemon marmalades were studied, both initially and at the end of storage. On the other hand, the values for these coordinates of the commercial lemon marmalade were L*=35.03±0.11, a*= 1.57±0.05, b*= 9.7±0.1, chroma=9.81 ± 0.08 and hue=80.80±0.24. It should be pointed out that the storage time for this commercial marmalade was unknown. As can be seen, the *L** of control marmalade and the samples formulated with combination B and C were very similar, while sample A, which had the highest percentage of isomaltulose, initially had a higher luminosity, although luminosity decreased at the end of storage as in the other cases. In our previously studies (Rubio-Arraez *et al.* 2015) with orange marmalades formulated with oligofructose and tagatose had a similar appearance, but oligofructose reduced L* and the highest content of tagatose also decreased L*, a* and b* after 45 days of storage.

Coordinate a* for the marmalade with formulation B initially showed the highest value. However, after 60 days of storage no significant differences were observed between the new marmalades, although their coordinate a* was higher than in the control marmalade. In contrast, coordinate b* decreased after storage. Consequently, the C* and h* decreased during storage, leading to browning in the marmalades. This browning could also be related to a reduction in polyphenols (antioxidant capacity) over time, which would be also responsible for the previously mentioned increase in pH after storage which occurred in the samples. These results are also consistent with those found by Peinado *et al*. (2015) who reported that strawberry jams formulated with the healthy sweetener isomaltulose and different concentrations of citric acid and pectin darkened during storage. Additionally, the colorimetric coordinates of the products containing the sucrose-isomaltulose mixture seemed to be influenced by the percentages of pectin and citric acid, while the color of the samples containing the fructose-isomaltulose mixture did not seem to be affected by the different variables. Therefore the influence of the different ingredients on the food system does not only depend on their concentration or distribution within the different system phases but also on the different component interactions during the period studied (Dervisi *et al.* 2001; Peinado *et al*. 2015).

Microbiological Analysis

There were no colonies of molds and yeast or aerobic mesophillic found in any of the marmalades in this study over the storage period considered. Therefore, in all cases the products were stable from a microbiological point of view.

Sensory Analysis

289 Fig. 4 shows a radial chart of the average scores for each attribute evaluated. No
290 significant differences in color, aroma, texture, spreadability and consistency were
291 detected. However, new formulations improved palatability with respect to the control
292 marmalade. Moreover, although the bitterness level for the marmalades evaluated in this
293 study was expected to be very high, it seems that the combination of sweeteners reduced
294 this bitterness, due more to the effect of tagatose than to the effect of isomaltulose.
295 Thus, the control marmalade had the highest bitterness followed by formulation A, C
296 and B. Besides, the lemon marmalades with the highest amounts of tagatose (B and C)
297 were the sweetest, showing that there is a sweetness threshold for a concentration of
298 tagatose of 50% in the proportion of sweeteners used in the formulations. Although
299 tagatose should have a sweetening power similar to sucrose (Oh 2007; Taylor *et al.*
300 2008; Calzada-León *et al.* 2013), the sweetening powers were not similar in this case.
301 This behavior could be due to the fact that the commercial tagatose used in this study
302 was composed also by oligosaccharides, isomalt and sucralose, which increased the
303 sweetening power for their due a synergic effect. In fact, according to the Patent
304 EP0946112 B1 (Dörr and Jager 2002), oligosaccharides increase the sweetness and
305 improve the taste of an acesulfame-k/aspartame mixture. Consequently, the formulation
306 B showed the highest values of acceptance and intention of buying, followed by
307 formulation C, without significant differences between the two marmalades.
308 Additionally, the new lemon marmalades were evaluated as being better than the
309 control. According to our studies in orange marmalades (Rubio-Arraez *et al.* 2015)
310 those prepared with new healthy sweeteners (tagatose and oligofructose) had better
311 scored than marmalade prepared with sucrose.

312 **CONCLUSIONS**

The reformulation of lemon marmalade with non-cariogenic sweeteners such as tagatose and isomaltulose is possible, since although the new marmalades did not reach the same concentration of soluble solids as marmalades made with sucrose, they were microbiologically stable over the storage period considered. More specifically, isomaltulose increased their luminosity and hue. Furthermore, the combination of the new sweeteners did not influence viscoelasticity, although it was lower than in marmalade with sucrose. Finally, tagatose led to the best scores for lemon marmalades, mainly due to its high sweetening power.

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FIGURE CAPTIONS

FIG.1. MEAN FLOW CURVES (RHEOGRAMS) OBTAINED FROM THE STEADY ASSAY OF LEMON MARMALADES AT THE BEGINNING AND AT THE END OF STORAGE. SAMPLES WERE CODED BASED ON THE AMOUNT OF SUGARS AS: CONTROL (100% SUCROSE), A (60% ISOMALTULOSE AND 40% TAGATOSE), B (50% ISOMALTULOSE AND 50% TAGATOSE), AND C (30% ISOMALTULOSE AND 70% TAGATOSE).

FIG. 2. AVERAGE FREQUENCY CURVES OBTAINED IN THE OSCILLATORY TEST OF LEMON MARMALADES AT THE BEGINNING AND END OF

STORAGE. SAMPLES WERE CODED BASED ON THE AMOUNT OF SUGARS AS: CONTROL (100% SUCROSE) A (60% ISOMALTULOSE AND 40% TAGATOSE), B (50% ISOMALTULOSE AND 50% TAGATOSE), AND C (30% ISOMALTULOSE AND 70% TAGATOSE).

UNSHADED SYMBOLS REFER TO VALUES OF G' AND SHADED SYMBOLS REFER TO VALUES OF G''.

FIG. 3. INTERACTION GRAPHICS (SIGNIFICANT LEVEL OF 95%) OF COLOR PARAMETERS: L*, a*, b* COORDINATES, CHROMA (C*) AND HUE (H*) OF THE LEMON MARMALADE AS A FUNCTION OF THE FORMULATION AND STORAGE TIME. SAMPLES WERE CODED BASED ON THE AMOUNT OF SUGARS AS: CONTROL (100% SUCROSE); A (60% ISOMALTULOSE AND 40% TAGATOSE); B (50% ISOMALTULOSE AND 50% TAGATOSE), AND C (30% ISOMALTULOSE AND 70% TAGATOSE).

FIG. 4. RESULTS OF THE SENSORY ANALYSIS IN THE EVALUATION OF THE SAMPLES CODED BASED ON THE AMOUNT OF SUGARS AS: CONTROL (100% SUCROSE), A (60% ISOMALTULOSE AND 40% TAGATOSE), B (50% ISOMALTULOSE AND 50% TAGATOSE), AND C (30% ISOMALTULOSE AND 70% TAGATOSE).* p-value <0.05, ** p-value <0.01

TABLE 1. VALUES FOR MOISTURE CONTENT (x_W), BRIX, WATER ACTIVITY (a_W) AND pH OF LEMON MARMALADES INITIALLY AND AFTER 60 DAYS OF STORAGE. VALUES FOR COMMERCIAL LEMON MARMALADE ARE ALSO INCLUDED. EQUAL LETTERS INDICATE HOMOGENEOUS GROUPS (α= 95%).

TABLE 2. RHEOLOGICAL PARAMETERS OF THE HERSCHEL-BULKLEY MODEL AND PARAMETERS OF THE POWER-LAW MODEL FOR LEMON MARMALADES INITIALLY AND AT THE END OF STORAGE. EQUAL LETTERS INDICATE HOMOGENEOUS GROUPS (α= 95%).