# PHYSICOCHEMICAL CHARACTERISTICS OF CITRUS JELLY WITH NON CARIOGENIC AND FUNCTIONAL SWEETENERS


## ABSTRACT

In this study the effect of sweeteners with low glycemic index and non-cariogenic characteristics (isomaltulose, oligofructose and tagatose) in jelly prepared with citrus juice has been evaluated considering as reference a citrus jelly formulated with sucrose. For that, analyses of soluble solids, moisture content, pH, water activity, antioxidant capacity, optical and mechanical properties of different blenders of these new sweeteners have been carried out, initially and after 15, 30 and 45 days of storage. Besides, mesophilic aerobics and moulds and yeasts have been also counted to determine their stability over time. A sensory evaluation of the citrus jelly has also been done. The results showed the antioxidant activity decreased over storage time in all formulations. Throughout time tagatose increased luminosity whereas coordinates a*, b* and chrome of all the new formulations were lower than in jellies with sucrose. Moreover, the formulations of citrus jelly with only oligofructose or tagatose or with the mixture of isomaltulose and tagatose were most closely resembled to the control jelly respect to mechanical properties. Finally, the jelly prepared with the combination of isomaltulose and tagatose in equal proportions obtained the best scored in the sensorial analysis.



**Keywords**: isomaltulose, oligofructose, tagatose, antioxidants, mechanical properties, sensory evaluation.

## Introduction

Traditionally, jelly desserts are mainly produced with edible gelatine, water, sugar and flavors. Although jelly desserts have low content of gelatine this type of protein contains 18 different amino acids, including 8 essential amino acids (GME 2015) being particularly rich in glycine, proline and hydroxyproline. Furthemore, gelatine is a natural colloide with properties of gelling and a stabilizing effect. Therefore, gelatine has a quite high nutritional value but with a low caloric power (17 kJ/ kg or 4 kcal/ g). Other important components of jelly desserts are sugars. It is widely known that their excessive consumption is related to tooth decay, diabetes and obesity (Edwards 2002; O'Donnell and Kearsley 2012), among other illnesses. Concretely, white sugar, which contains high percentage of sucrose, is one of the most usual sweetening agent in confectionary products but it requires calcium and potassium to be digested in detriment for vital organs (Shukla and Kandra 2015).

Despite the fact that this type of dessert is not considered with a high nutritional value, it is important to point out that this situation might change if natural vitamins and antioxidants provided from fruit juice were included in its formulation instead of the water.

Citrus fruits such as orange, lemon and mandarin orange have many beneficial properties due to their high content of fibre, vitamins, minerals, ascorbic acid and specially high content in antioxidant compounds, such as carotenoids, flavonoids and phenolic compounds (Álvarez et al. 2014). As far as we know, a jelly dessert prepared with a mixture of different citrus juices does not exist in the market and it could expand the possibilities of commercialization.

Currently awareness of health-related issues in society has increased the demand of new functional foods and consequently food industry must be constanly innovating to offer consumers new alternative products (Shukla and Kandra 2015). In the confectionary and beverage sectors this concern is mainly focused on the achievement of an adequate sweetness while improving health and appearance, and as a result the use of artificial sweeteners has increased. However artificial sweeteners, such as aspartame, acesulfame-k, saccharin and sodium cyclamate, or polyalcohols have negative connotations due to their possible risk to health and they must be subject to a rigorous assessment before their use in food products and beverages (de Queiroz Pane et al. 2015). Bearing this in mind, the reformulation of jelly desserts with new non-cariogenic sweeteners available in the market could be a good chance to achieve this goal.

To cope with these issues nowadays there are natural sweeteners such as tagatose, isomaltulose and oligofructose (FDA 2005; FDA 2010; FDA 2011), which need to be studied in order to check their capacity to replace sucrose and other sugars in traditional foods as jelly desserts. In this sense, previous studies had been carried out to reformulate confectionary products with isomaltulose such as strawberry jam (Peinado et al. 2012; Peinado et al. 2013), gummy confections (Periche et al. 2014) or marshmallows (Periche et al. 2015a). Tagatose and oligofructose have been also studied in orange marmalade (Rubio-Arraez et al. 2015) and the combination of isomaltulose, stevia and oligofructose in marshmallows (Periche et al. 2015b).

Oligofructose is an oligosaccharide derived from sucrose, which acts as dietary fibre regulating intestinal transit. It presents a prebiotic effect because it favours the selective growth of bifidus bacteria (Ledur et al. 2013). Besides, it reduces cholesterol and blood sugar levels (Chacón-Villalobos 2006) and improves calcium absorption (Van Den Heuvel et al. 1999). Nevertheless, it is highly soluble and possesses technological

properties (sweet taste, stability…) analogous to sucrose (Pimentel et al. 2015). In 2011, oligofructose was recognized as safe (GRAS) (FDA 2011).

D-Tagatose (D-tag) is a ketohexose, a stereoisomer of D-fructose and it is found naturally in several foods, including cheese and yoghurt. Its texture is very similar to sucrose and almost as sweet as sucrose, with only 1.5 kcal/g and it does not provoke dental caries (Levin 2002; Oh 2007; Taylor et al. 2008; Calzada-Leon et al. 2013). Tagatose is very suitable for confectionary products, ice creams, soft drinks and breakfast cereals (Vastenavond et al. 2012). Tagatose is minimally absorbed by the upper gastrointestinal tract. The unabsorbed tagatose is fermented in the intestines, causing a change in the proportions of various short chain fatty acids (Taylor et al. 2008). Thus, it is considered a functional food and besides it performs functions as soluble fibre favouring lactic acid bacteria and Lactobacillus specie bacteria (Petersen-Skytte 2006). D-tagatose received GRAS status by the Food and Drug Administration in 2001 (Levin 2002; FDA 2010).

Isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice, and its appearance, taste and viscosities of aqueous solutions are comparable to sucrose (Periche et al. 2014). Based on its chemical definition compared to sucrose or glucose, it is less insulinemic, less glycemic and is non-cariogenic (Lina et al. 2002). However, it has a third of the sweetening power of sucrose (Lina et al. 2002; De Oliva-Neto and Menão 2009; Peinado et al. 2012). In 2005, isomaltulose was recognized as safe (GRAS) (FDA 2005).

In accordance with the properties of these three sweeteners (oligofructose, isomaltulose and tagatose), the aim of this paper was to evaluate their potential use as an alternative to sucrose in the development of jelly dessert along with the addition of fresh citrus juice on

composition, antioxidant capacity, mechanical and optical properties, and sensory analysis.

## Materials and methods

### *Materials of citrus jelly*

Jelly was manufactured with citrus fruits juice (*Citrus reticulata clementina, Citrus limon eureka, Citrus sinensis navelate*), sugar/sweeteners and gelatine (Junca Gelatines S.L., Girona, Spain). In control jelly sucrose (Azucarera Iberia S.L., Madrid, Spain) whereas in the new jellies the amount of sucrose was replaced by different mixtures of oligofructose obtained from Sensus (Frutalose, Roosendaal, Netherlands), isomaltulose obtained from Beneo (Palatinose, Mannheim, Germany) and tagatose obtained from Damhert Nutrition (Tagatesse, Heusden-Holder, Belgium). The jelly dessert was prepared using the same proportions of ingredients as in a commercial orange flavoured jelly powder (Royal, Kraft Foods, Madrid,Spain) which were: 85.2% of sugars and 9.5% of gelatine. It is important to point out that commercial jelly also contained vitamin C, acidity regulators (fumaric acid, sodium citrate), flavourings and colourants (E100: curcumine and E120: carminic acid) but these components were not included in the jelly of this study. Following the manufacturer's instructions, the content of the powder was diluted with 500 g of water, leading to a final composition of 12.6% of sugars and 1.6% of gelatine. In the jelly prepared with citrus juice, the amount of sugars contained in the juice were taken into account when adding sweeteners in order to maintain the same proportion of sugars and gelatine as in the commercial formula. Furthermore, 50% of the amount of water was replaced by citrus juice. The citrus juice was prepared with the following proportionos of each fruit: lemon juice 14%, orange juice 43% and mandarin orange juice 43%.

Depending on the combination of sucrose/sweeteners used in jelly, the following notation was used: Control: 100% sucrose; I50T50: 50% isomaltulose and 50% tagatose; T: 100% tagatose; I: 100% isomaltulose; I50O50: 50% isomaltulose and 50% oligofructose, and O jelly: 100% oligofructose.

*Jelly preparation*

Figure 1 shows the flow chart of the stages required to prepare jelly for this study. The amounts of each component were weight in an analytical scale (Precisa Gravimetrics AG, model BJ 6100D, Dietikon, Switzerland). Juice was extracted using a liquidiser (Molinex, model vitapress, Mayenne, France). For the stages of mixing and blending, a thermal blender (Thermomix, model TM31, Vorwerk, Wuppertal, Germany) was used. Once the mixture was obtained, containers were filled with it and stored at refrigeration at 4ºC.

*Analytical determinations*

Analysis of moisture content, Brix, pH, water activity, antioxidant capacity, optical and mechanical properties and microbiological analysis were performed for each formulation of citrus jelly at 1, 15, 30 and after 45 days of storage at 4 ºC by triplicate. Next the methodologies followed for each case are described.

*Moisture and soluble solids content, pH and water activity.*

Moisture content ($x_w$: g water/g citrus jelly) was analysed gravimetrically following an adaptation of the AOAC method (2000). Soluble solids content of samples were measured by a refractometer at 20ºC (Atago3T, Tokyo, Japan), the results being obtained in Brix. pH was registered using a pH-meter (Mettler Toledo, model SevenEasy, Barcelona, Spain), previously calibrated with buffered solutions of pH 7.0 and 4.0. Water activity

($a_w$) was determined using a hygrometer (Decagon Devices, Inc., model 4TE, Pullman, Washington, USA), at 25ºC.

*Determination of antioxidant capacity*

The antioxidant activity of citrus jelly was analysed following the method described by Shahidi et al. 2006, based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and measuring the absorbance change of samples at 515 nm in a spectrocolorimeter Thermo Fisher Scientific, Inc. (Helios Zeta UV-VIS, Waltham, Massachusetts, USA). The results were expressed as mg of Trolox equivalent per 100 g of citrus jelly.

*Optical Properties*

The optical properties of citrus jelly placed in 20 mm-wide cuvettes was measured using a spectrocolorimeter UV (Konica Minolta Inc., CM-3600d model, Tokyo, Japan). CIEL*a*b* coordinates were obtained using D65 illuminant and a 10º observer as reference system. Registered parameters were: L* (brightness), a* (red component), b* (yellow component), Chroma ($C^*=(a^{*2}+b^{*2})^{1/2}$) and hue ($h^*=arctg(b^*/a^*)$).

*Mechanical Properties*

The samples were examined with Texture Profile Analysis test (TPA) using a TA.XT plus Texture Analyser (Stable Micro Systems, Godalming, U.K.). For this purpose, a load cell of 50 kg and a 45 mm diameter cylindrical probe were used. The test conditions involved two consecutive cycles of 50% compression with 15 seconds between cycles. The test speed was 1 mm/s. Based on the resulting force-time curve it was possible to measure the following parameters: hardness, cohesiveness, adhesiveness and springiness.

*Microbiological analysis*

Mesophilic aerobic populations and yeast and molds colonies were determined following the procedure described by Rubio-Arraez et al., 2015. Microbial counts were expressed as CFU/g.

*Sensorial Analysis*

An preliminary acceptance test using a 9-point hedonic scale (ISO 4121 2003; ISO 5492 2008) was used to evaluate the following attributes in the samples: color, flavor, texture, sweetness, global preference and intention of buying. The panel consisted of 30 trained panellists (aged from 20 to 50) who are regular consumers of this kind of dessert. Testing was conducted in a sensory evaluation laboratory built according to the international standards for test rooms. In this analysis the citrus jelly formulated using sweeteners containing only isomaltulose (I) and combination isomaltulose-oligofructose (I50O50), were not considered because the other samples of jelly were of a better quality.

*Statistical analysis*

Analyses of variance (multifactor ANOVA) were carried out by Statgraphics plus software (Statpoint Technologies, Inc., Centurion, Warrenton, Virginia, USA) to discern whether the effect of formulation or storage was significant on the citrus jelly studied with a significance level of 95%. Interactions between factors were also considered.

## Results and discussion

*Compositional characterisation of citrus jelly*

Table 1 shows the results of solids soluble content (Brix), moisture content ($x_w$), and water activity ($a_w$), pH, and antioxidant capacity of the jelly formulations with sucrose or new sweeteners (tagatose, oligofructose and isomaltulose). Initially, all jelly desserts reached a concentration of soluble solids around 22 ºBrix, but formulation that contained only

189 oligofructose (O) had the highest values of ºBrix (≈23 ºBrix ) unlike formulations
190 contaning only isomaltulose (I) or tagatose (T) that showed the lowest values of ºBrix
191 (≈21 ºBrix). The storage decreased significantly ºBrix of formulation I50T50 but they
192 increased in formulation T, being control and I50O50 the most stable formulations. Even
193 though, values of soluble content were quite similar in all cases. In terms of moisture
194 content, there were no significant differences due to formulation and only after 30 days
195 of storage there was a significant increased but moisture content was the same as initially
196 after 45 days in all cases. This fact could be due to the permeability to water vapour of
197 the package and also because the relative humidity was not controlled to simulate the
198 conditions of commercialization. Besides, values of water activity were always 0.98,
199 although formulation T showed the highest $a_w$ initially. Again, the pH was very similar
200 in all formulations of jelly, but it was initially lower in formulation T and control jelly,
201 althougth all jellies presented similar values after 45 days of storage.

202 As can be seen in table 1 initially all samples of jelly prepared with citrus juice showed
203 the same antioxidant capacity except for I50T50 and T jellies which had the highest
204 values due to their content in tagatose, which would be responsible for this behaviour.
205 Other authors (Zeng et al. 2012) also detected an improvement in the radical scavenging
206 activity and oxidation reduction potential of the hydrolysates of tune backbone with rare
207 sugars (especially D-tag). However, in all cases there was a significant reduction of the
208 antioxidant capacity over the storage period considered, reaching similar values after 45
209 days for all formulations as was also observed in previous studies of orange marmalade
210 (Rababah et al. 2011) as a consequence of the oxidation of the components responsible
211 of this capacity.

212 *Optical properties*

213 The interaction charts of the colorimetric coordinates L*, a* and b*, chroma (C*) and hue
214 (h*) of the citrus jellies considering as factors the formulation of sucrose/sweeteners used
215 and the storage time are shown in Figure 2. Initially control jelly desserts had more
216 similarities in terms of luminosity with samples containing tagatose, but for coordinates
217 a* and b* and for chroma formulation I50O50 was closed to control jelly. It was also
218 observed that the citrus jelly formulated with tagatose (T and I50T50) showed an increase
219 of their luminosity after 45 days of storage time in contrast with the decrease observed in
220 formulations with isomaltulose and the combination of isomaltulose with oligofructose (I
221 and I50O50) at the end of storage. This behaviour could be related with the low solubility
222 of isomaltulose as was reported (Peinado et al. 2012). Coordinate a* in jellies containing
223 only oligofructose or isomaltulose was the most stable in time but coordinate b* increased
224 over time in formulation I whereas it decreased in formulation O. At the end of storage
225 a*, b* and C* of the new formulations of jellies were lower than in control jellies, except
226 for a* of formulation I50T50 which was equal to the control jelly. In terms of h*, it was
227 noteworthy that all formulations showed values around the results of the control jelly,
228 being formulation I above control jelly in the whole period of storage and formulation O
229 the most similar to control jelly. Thus, the effect of the different ingredients on the food
230 system depends not only on their concentration or distribution but also on the interactions
231 of the components (Peinado et al. 2013)

232 *Mechanical properties*

233 Figure 3 shows the average curves of the TPA analysis carried out on the samples of jelly
234 used in this study. Moreover, Figure 4 shows the interaction charts (with a significant
235 level of 95%) of the mechanical parameters. As can be seen, initially the curves obtained
236 for O jelly (formulated only with oligofructose), showed more pronounced peaks than the
237 other samples and consequently they had the highest values of hardness without statistical

differences respect to citrus jelly formulated with tagatose (T and I50T50), whereas samples prepared with isomaltulose showed the lowest hardness in coherence with the results obtained by Peinado et al. (2012) in strawberry jams formulated with isomaltulose compared with those prepared with sucrose and also by Periche et al. (2014) in gummy confections in which sucrose and glucose syrup were replaced by isomaltulose and/or fructose. This behaviour gives evidence of the lowest capacity of isomaltulose to form gel structure. Besides, the second peak of control jelly was placed on the right of the others formulations. After 15 days of storage, the O jelly curve was overcome by the control jelly curve and its second peak was shifted to the right. However at 30 days of storage the second peaks of all formulation were placed together. Additionally, formulations with only tagatose (T) and oligofructose (O) showed highest peaks at the end of the storage (45 days). Even though, factor time did not have a significant effect on most of the mechanical parameters analyzed in these jellies. However, the formulation composed by isomaltulose and oligofructose (I50O50) showed the highest values of adhesiveness. Furthermore, cohesiveness and springiness were also higher in that formulation and when there was only isomaltulose in the sweetener content of jelly (I). In contrast, gumminess was very low in formulation I50O50. Therefore, the most similar jellies to control samples were those prepared with the mixture of isomaltulose and tagatose (I50T50) followed by those prepared with only oligofructose (O) or tagatose (T).

*Microbiological analysis*

Microbial counts of mesophilic aerobics, yeasts and moulds were not found in any of the citrus jelly at 1, 15, 30 days of storage. However, at the end of storage (45 days) there were presence of mesophilic aerobics, yeasts and moulds, except for the formulation that only contained oligofructose. This protective effect of oligofructose could be due to its selective preference for the growth of bifidus bacteria (Ledur et al. 2013), which were not

enhanced in the agars used for this analysis, specific for mesophilic aerobics, yeasts and moulds. According to Pascual and Calderón (2000), the microbial counts for jelly desserts must not exceed $5 \cdot 10^2$ CFU/g mesophilic aerobics and $5 \cdot 10^1$ CFU /g yeasts and moulds. Even though, the microbial count was bellow those limits ($3 \cdot 10^1$ CFU/g mesophilic aerobics and $2 \cdot 10^1$ CFU /g yeasts and moulds) after 45 days in all cases. These results give evidence that the product was microbiologically stable for the studied period. The microbiological stability of the samples could be attributed to the acidity of citrus juice which gave place to a low pH (≈3.5) in citrus jellies.

*Sensory analysis*

The results of sensory analysis of citrus jelly, depending on their formulation (control, T, I50T50, O), are presented in Figure 5. As can be seen, T and I50T50 formulations showed the highest sweetness, due to their higher content of tagatose. This would coherent with the recommendations given by the manufacturer of the commercial tagatose (two tablespoons of sucrose provides the same sweetness as one tablespoon of tagatose), though as was mentioned in the introduction, tagatose should have similar sweetening power to sucrose (Oh 2007; Taylor et al. 2008; Calzada-León et al. 2013). It is noteworthy that the global preference and intention of buying of jelly formulated with equal proportion of tagatose and isomaltulose (I50T50) presented the better score. Therefore, the replacement of sucrose by a mixture of isomaltulose and tagatose in equal proportion would be feasible from a sensory point of view.

## Conclusions

The reformulation of citrus jelly with non-cariogenic and low glycemic index sweeteners used in this research is viable. Besides, tagatose favoured the antioxidant capacity of citrus jelly initially, but not differences among all formulations were found after storage.

In general, at the end of storage coordinates a*, b* and chrome of the new formulations of jellies were lower than in jellies with sucrose. From the mechanical point of view the recommended formulation would be oligofructose (O) or tagatose (T) or the mixture of isomaltulose and tagatose (I50T50). However the use of isomaltulose (I) or its combination with oligofructose (I50O50) reduced the capacity of gel formation. In citrus jellies with only oligofructose there was no microbial presence in the considered storage period. According to sensorial analysis, I50T50 was the best scored jelly.

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**Figure captions**

**Fig.1** Flow chart of the manufacturing process of citrus jelly

**Fig.2** Interaction graphics (95% of significant level) of colour parameters: L*, a*, b* coordinates, chroma (C*) and hue (h*) of the citrus jelly as a function of the formulation and storage time.

**Fig. 3** Representative curves of TPA test for citrus jelly studied as a function of sweeteners used in its formulation initially (A), at 15 days (B), at 30 days (C) and after 45 days of storage (D).

**Fig. 4** Interaction graphics (95% of significant level) of hardness, adhesiveness, cohesiveness, gumminess and springiness of citrus jelly as a function of the formulation and storage time.

**Fig. 5** Sensory assessment of citrus jelly as a function of the formulation. Level of significance (ns) of the ANOVA considering all jelly: *level of significance: 95%, **level of significance: 99%.

**Table 1** Values for moisture content ($x_w$), Brix, Water activity ($a_w$) and pH of citrus jelly formulated with sucrose (control) or with new sweeteners and their combinations (isomaltulose, oligofructose and tagatose) inicially, 15 days, 30 days and 45 days of storage. Equal letters indicate homogeneous groups.