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EFFECT OF REPLACING SUCROSE WITH TAGATOSE AND ISOMALTULOSE IN MANDARIN ORANGE MARMALADE ON RHEOLOGY, COLOUR, ANTIOXIDANT ACTIVITY, AND SENSORY PROPERTIES

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The aim of this study was to make mandarin orange marmalades, in which sucrose is replaced by sweeteners, such as tagatose and isomaltulose, which are non-cariogenic and have a low glycemic index. Analyses of rheology, colour, antioxidant activity, microbiology, and sensory properties were carried out on marmalades on their first day, and after 90, 180, and 360 days of storage. The results showed that marmalades made with healthy sweeteners had a less elastic character and were thinner in consistency than those made with sucrose. Lightness was shown to be highest in mandarin orange marmalades made with tagatose, although colour was stable for 180 days to one year of storage. Tagatose also enhanced the antioxidant activity of these marmalades. All marmalades were microbiologically stable. Finally, marmalades made with tagatose alone scored the highest for global acceptance and intention of buying by consumers.

Keywords: marmalade, tagatose, isomaltulose, rheology, colour, sensory analysis

Mandarin orange fruit (*Citrus reticulata*) have a high nutritional composition (high content of phenolics, ascorbic acid, dietary fibre, etc.), and their consumption prevents diseases mainly due to this fruit's antioxidant activity (Rosa et al., 2015). Mandarin oranges are usually consumed as fresh fruit, but also as marmalades; most of them are prepared with sucrose. However, this sugar has a high glycemic index, is also high in calories and can cause several diseases (Riedel et al., 2015). Nowadays, there are other alternative natural sweeteners available in the market, such as tagatose and isomaltulose. These sweeteners are non-cariogenic and less glycemic. D-Tagatose is a stereoisomer of D-fructose, it has only 1.5 kcal g^{-1} (Lu et al., 2008). Interest in tagatose results from studies suggesting this monosaccharide has prebiotic properties (Bell, 2015). Furthermore, tagatose can be used to make numerous products, since its texture and sweetness are very similar to sucrose (Calzada-León et al., 2013). Based on its laxative effect, it is advisable to limit intake of D-tagatose to less than 30 g per eating occasion (Buemann et al., 1999). However, around 20 g of marmalades are used per serving, in which approximately 50% are sugars, therefore, this limit is not overpassed by eating one portion of this product. On the other hand, isomaltulose is a reducing disaccharide, which is naturally present in honey and sugar cane juice (Lina et al., 2002). Its caloric power, appearance, taste, and viscosities of aqueous solutions are similar to those of sucrose (Periche et al., 2014). Moreover, given the physicochemical properties of isomaltulose, it can be used as a substitute for sucrose in most sweet foods and it has a third of the sweetening power of sucrose (Peinado et al., 2012). Given the properties of these two sweeteners (isomaltulose and tagatose), the aim of this

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work was to evaluate their potential use as an alternative to sucrose in mandarin orange marmalades. For this purpose, their antioxidant activity, rheology, colour, and sensorial acceptance were analysed.

1. Materials and methods

1.1. Mandarin orange marmalade formulations and manufacturing processes

Marmalades were produced using 50% mandarin orange pulp (*Citrus reticulata Clementina*), 50% sucrose (Azucarera Española, Burgos, Spain) or sweeteners (tagatose (Tagatesse, Heusden-Zolder, Belgium) or isomaltulose (Beneo, Mannheim, Germany)) containing 1% agar-agar (Roko Agar, Asturias, Spain). The following notation was used depending on the combination of sweeteners applied: Control marmalade: 100% sucrose, Marmalade A: 75% tagatose and 25% isomaltulose, Marmalade B: 50% tagatose and 50% isomaltulose, Marmalade C: 25% tagatose and 75% isomaltulose, and Marmalade D: 100% tagatose. Three batches of mandarin oranges were selected and picked fresh. Subsequently, they were peeled and mixed with the corresponding combination of healthy sweeteners/sucrose and the agar-agar in a thermal blender (Thermomix, TM31, Vorwerk, Wuppertal, Germany) for 3 min. Afterwards, the mixture was cooked at 100 ºC for 20 min at 350 r.p.m. Finally, the marmalade was allowed to cool for 24 hours and became jellified in the glass jars. All measurements were carried out in triplicate for each batch on the first day of storage and after 90, 180, and 360 days.

1.2. Rheological analysis

The rheological properties of the mandarin orange marmalades studied were analysed using a controlled stress rheometer manufactured by Thermo Fisher Scientific, Inc. (Haake RheoStress 1, Waltham, Massachusetts, USA), at 25 ºC, using the protocol described in previous studies (Peinado et al., 2012; Rubio-Arraez et al., 2015). Oscillatory or steady-state tests were carried out to study the pseudoplastic or viscoelastic behaviour of marmalades, respectively. In the case of the oscillatory essays the equations 1 and 2 were used.

$$G'=a\cdot\omega^{b} \tag{1}$$

$$G''=c\cdot\omega^{d} \tag{2}$$

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).

For the steady-state measurements the Herschel–Bulkley model (equation 3) was used.

$$\tau = \tau_0+\kappa\cdot\gamma^{n} \tag{3}$$

where: *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.

1.3. Optical properties

The optical properties of mandarin orange marmalades were measured using a spectrocolorimeter manufactured by Konica Minolta, Inc. (CM-3600d, Tokyo, Japan). CIE-L*a*b* coordinates were obtained following the procedure described by Rubio-Arraez and co-workers (2015).

1.4. Antioxidant activity

The antioxidant activity of marmalades was analysed on the basis of the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl free radical following the protocol described in previous studies (Shahidi et al., 2006; Rubio-Arraez et al., 2015).

1.5. Microbiological analysis

Yeasts, moulds, and mesophilic aerobic bacteria were determined following the protocol described in previous paper (Rubio-Arraez et al., 2015). Samples were taken for analysis on days 1, 90, 180, and 360.

1.6. Sensorial analysis

An acceptance test using a 9-point hedonic scale (Rubio-Arraez et al., 2015) was used to evaluate the following attributes: colour, aroma, texture, consistency, spreadable capacity, palatability, flavour, sweetness, bitterness, global preference, and intention of buying in the three formulations made with different combinations of healthy sugars (A, C, and D), as well as the control marmalade. Marmalade B, formulated with the same proportion of isomaltulose and tagatose was not considered in the sensorial analysis, since the aim of this test was to determine the consumers' preference for tagatose or isomaltulose and the other marmalades had a higher amount of each of these sweeteners.

1.7. Statistical analysis

Multifactor ANOVAs were performed using a multiple comparison test and a LSD test (α=95%), with Statgraphics Centurion software (Statpoint Technologies, Inc. Warrenton, Virginia, USA).

2. Results and discussion

2.1. Rheological properties

The rheological results of the oscillatory assay, which were based on the evolution of the storage (G') and loss (G'') moduli versus frequency for the marmalade studied are shown in Figure 1.

As can be observed, the formulation D showed the lowest values of G' and G'', consequently the lowest elastic and viscous behaviours were found. In contrast, the results for marmalade C were most similar to the results for the control marmalade, most likely due to the analogous chemical structure of the sucrose and isomaltulose molecules. However, in our previous work (Rubio-Arraez et al., 2015) carried out on orange marmalade formulated with oligofructose and tagatose as a substitutes for sucrose, there was an increase in the elastic component (G') after 45 days of storage. Consequently, it can be concluded that depending on the nature of the chemical structure of the sweetener used, the rheological behaviour will be different. The parameters of the power-law model are shown in Table 1. According to the results obtained in Figure 1, marmalade D showed the lowest values for the low frequency storage modulus (parameter *a*). Consistent with the evolution of G' curves over time, the *a* parameter significantly decreased over time only in formulation B, i.e. after 360 days of storage, whereas there was an increase in the control marmalade after 180 days of storage. In terms of parameter *b* of the power-law of G', marmalade D again differed from

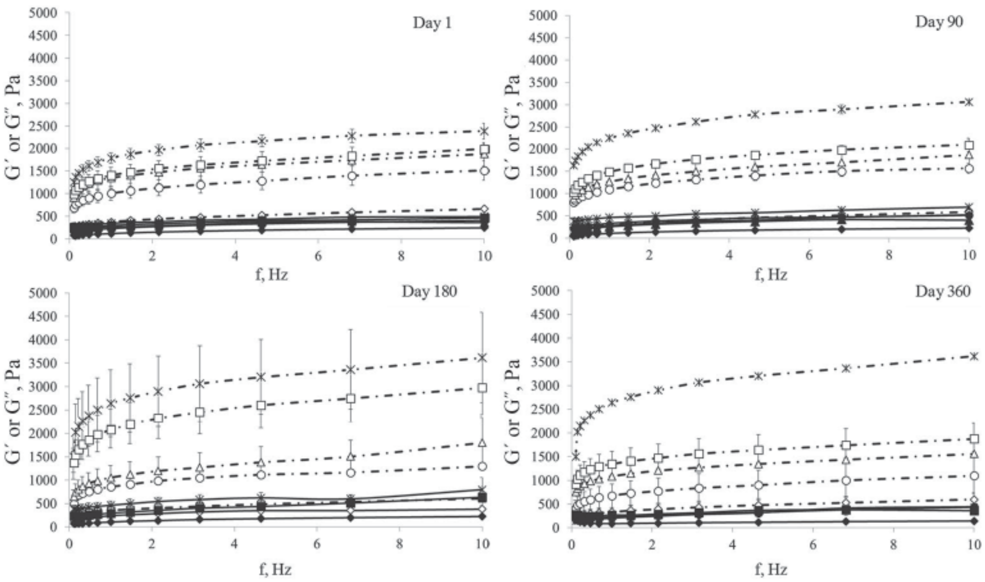

Fig. 1. Average frequency curves obtained in the oscillatory test of mandarin orange marmalades. Unshaded symbols refer to values of G´ and shaded symbols refer to values of G″.
—×—: Control; —○—: A; —△—: B; —□—: C; —◇—: D

the other formulation, but in this case, it showed a higher value. However, there was a significant decrease in formulations A and C after 360 days of storage, with an abrupt fall in the curves shown in Figure 1. In the case of the *c* and *d* parameters of G″ modulus for power-law, the trends shown were similar to those of *a* and *b* parameters but with less marked differences. PEINADO and co-workers (2012) observed that replacing sucrose with isomaltulose in the formulation of different strawberry spreadable products resulted in a decrease in parameters *a* and *c* of the power-law model, but parameters *b* and *d* were similar in all marmalades. Based on these results, the combination of these non-cariogenic sweeteners leads to formulations with less elastic character in comparison to the control marmalade. On the other hand, the results obtained for the steady-state test of mandarin orange marmalades based on the combination of sweeteners used and the storage time, are presented in Figure 2. The rheograms of mandarin orange marmalades for samples formulated with the new sweeteners were below the control samples, but there were only slight differences between them. The parameters of the Herschel–Bulkley model for the mandarin orange marmalades studied over the period considered are shown in Table 1. Nevertheless, the yield stress above which the fluid starts flowing (τ_0) and the index of consistency (k) were the lowest in formulation C, but the index of fluidity (n) was the highest in this formulation. Since formulation C had the greatest amount of isomaltulose, it can be concluded that this sweetener was responsible for the above described behaviour. In the other cases, marmalades also showed values of τ_0 and *k* that were lower than in the control sample but to a lesser extent than in formulation C. These results were consistent with those obtained by PEINADO and co-workers (2012) in jam prepared with osmodehydrated strawberry using isomaltulose as an osmotic agent, who observed a decrease in the consistency and cohesiveness of these jams with respect to those prepared with sucrose. When considering other combinations of sweeteners in orange marmalade (Rubio-Arraez et al., 2015), a blend of oligofructose and tagatose in the same proportions increased the consistency of marmalades during storage. Therefore, it can be concluded that sweeteners with a high amount of fibre (long chain of monosaccharides) would modify the rheological behaviour of marmalades (in the steady-state test) over time, whereas mixtures of sweeteners with short molecules (isomaltulose and tagatose) would give rise to more stable marmalades from a rheological point of view.

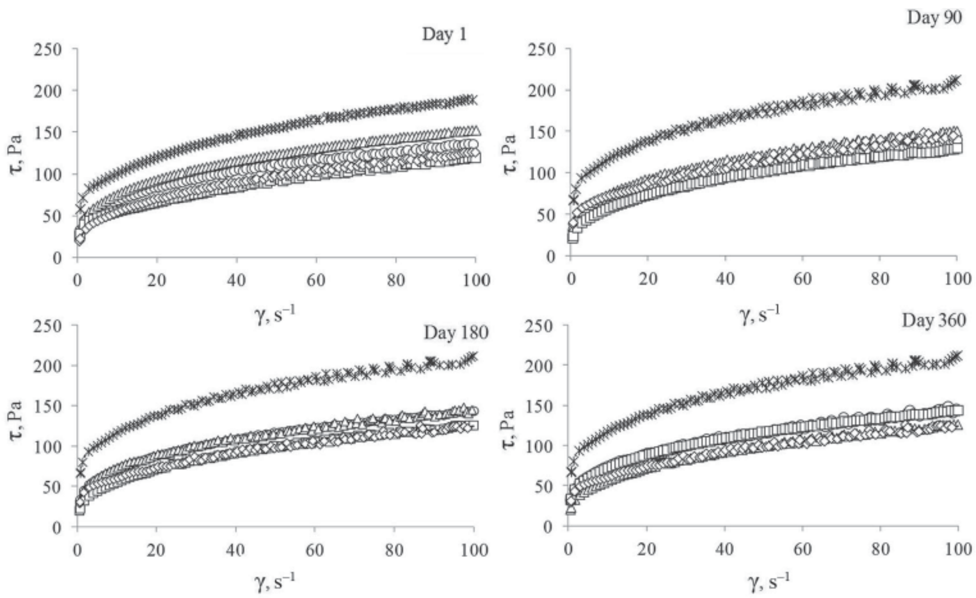

Fig. 2. Mean flow curves (rheograms) obtained from the steady assay of mandarin orange marmalades.
—×—: Control; —○—: A; —△—: B; —□—: C; —◇—: D

2.2. Optical properties

Interaction charts of the colorimetric coordinates *L**, *a** and *b**, chroma *(C*)*, and hue *(h*)* of the different mandarin orange marmalades studied over one year of storage are shown in Figure 3. According to these results, marmalade D had the highest lightness throughout the entire storage time. This formulation was followed by marmalade B, whereas marmalades A, C, and control were initially very similar. During storage, lightness decreased for all combinations studied. The control marmalade initially showed the highest value for coordinate *a*,* but this value decreased after 90 days of storage. Marmalade D had the highest values for coordinate *b** and the *C** throughout the whole storage time, whereas these values decreased in the control marmalade after 90 days of storage, as in coordinate *a**. In contrast, marmalades C showed constant values for *b** and *C** over storage. Therefore, it can be concluded that tagatose was responsible for the changes registered in both parameters. As a consequence of the changes in *a** and *b*,* the initial *h** of the new mandarin orange marmalades was higher than in marmalades prepared with sucrose. Besides, the *h** decreased during the first 180 days of storage in the marmalades formulated with the new sweeteners, especially for formulation A, unlike in the case of the control marmalade. It is noteworthy

Table 1. Rheological parameters of the parameters of the power-law model and Herschel–Bulkley model for marmalades both initially and over the storage period

Formulation	Time (days)	Oscillatory test-power law G´ a^1	G´ b^1	G´´ c^2	G´´ d^2	Steady test Herschel–Bulkley model[3] τ (pa)	k	n	η (–pa·s) (γ=50 s^{-1})
Control	1	1079±34^d	0.14±0.01^b	245±22^c	0.14±0.03^b	243±7^c	59.78±1,01^c	0.245±0.004^a	2.34±0.07^c
	90	1120±27^d	0.15±0.01^b	214±19^c	0.2±0.1^b	224.1±20.3^c	71±7^c	0.231±0.001^a	2.24±0.21^c
	180	1551±268^e	0.16±0.01^b	377±80^d	0.14±0.01^b	236.3±21.2^c	72±7^c	0.231±0.001^a	2.363±0.211^c
	360	1172±347^d	0.13±0.01^b	214±20^c	0.19±0.01^b	234.91±21.01^c	71±7^c	0.230±0.001^a	2.35±0.21^c
A	1	387±30^{ab}	0.16±0.01^{bc}	90±5^{ab}	0.17±0.02^b	181.5±12.4^{bc}	39.1±3.3^b	0.294±0.004^b	1.815±0.124^a
	90	234±18^{ab}	0.20±0.02^{bc}	52±8^{ab}	0.36±0.04^b	169.62±18.73^b	36.2±4.1^b	0.3±0.1^b	1.7±0.2^a
	180	328±30^{ab}	0.2±0.1^c	114±28^{ab}	0.19±0.04^b	203±4^{bc}	38.1±1.1^b	0.286±0.001^b	2.03±0.04^b
	360	308±32^{ab}	0.108±0.002^a	74±6^{ab}	0.011±0.001^a	197±5^{bc}	38.2±1.1^b	0.28±0.01^b	1.96±0.05^b
B	1	755±117^c	0.161±0.002^{bc}	168±29^b	0.20±0.02^b	163±20^{ab}	37±34^b	0.28±0.01^b	1.7±0.2^a
	90	844±33^c	0.151±0.012^b	155±20^b	0.26±0.04^{bc}	209±6^c	40±3^b	0.29±0.01^b	2.1±0.1^b
	180	944±2^c	0.19±0.01^c	216±51^b	0.22±0.01^{bc}	161±7^{ab}	39.1±1.4^b	0.281±0.002^b	1.6±0.1^a
	360	578±6^b	0.19±0.02^c	138±52^b	0.26±0.03^{bc}	161±7^{ab}	39.2±1.3^b	0.281±0.002^b	1.6±0.1^a
C	1	1036±194^{cd}	0.14±0.01^b	241.4±30.3^{bc}	0.09±0.04^{ab}	142.69±13.02^{ab}	26±3^a	0.343±0.002^c	1.43±0.13^a
	90	1088±187^{cd}	0.11±0.01^a	238±46^{bc}	0.015±0.001^a	156.4±3.4^{ab}	27.3±0.3^a	0.336±0.002^c	1.564±0.034^a
	180	943±2^c	0.19±0.01^c	216±51^{bc}	0.21±0.01^b	155.8±6.2^{ab}	26.7±1.5^a	0.336±0,004^c	1.558±0.062^a
	360	935±24^c	0.100±0.002^a	200±10^{bc}	0.015±0.002^a	153.91±5.53^{ab}	26.6±1.4^a	0.333±0.003^c	1.5±0.1^a
D	1	265±26^a	0.21±0.01^c	75±7^a	0.278±0.002^b	135.99±11.64^a	29.2±2.7^a	0.296±0.004^b	1.35±0.12^a
	90	234±18^a	0.20±0.02^c	52±8^a	0.36±0.04^c	168.56±60.34^b	41.3±9.6^b	0.251±0.032^a	1.686±0.603^a
	180	200±1^a	0.22±0.01^c	40±43^a	0.4±0.2^c	145±25^a	33.93±2.54^b	0.271±0.031^b	1.45±0.25^a
	360	190±1^a	0.22±0.01^c	40±43^a	0.4±0.2^c	143±13^a	33.91±2.52^b	0.27±0.03^b	1.430±0.132^a

Equal letters indicate homogeneous groups; [1]: see eg. 1.; [2]: see eg. 2.; [3]: see eg. 3.

that after 180 days, all marmalades showed similar values of h^* except for formulation A. After 180 days of storage, the colour of these marmalades was quite similar regardless of the differences registered before that period. In our previous work carried out on orange marmalade formulated with different combinations of tagatose and oligofructose (Rubio-Arraez et al., 2015), it was observed that marmalades with the highest content of tagatose showed a decrease in L^* and an increase in a^* and b^* coordinates after 45 days of storage. Peinado and co-workers (2012) showed that strawberry jams formulated with isomaltulose and different concentrations of citric acid and pectin jams darkened during storage.

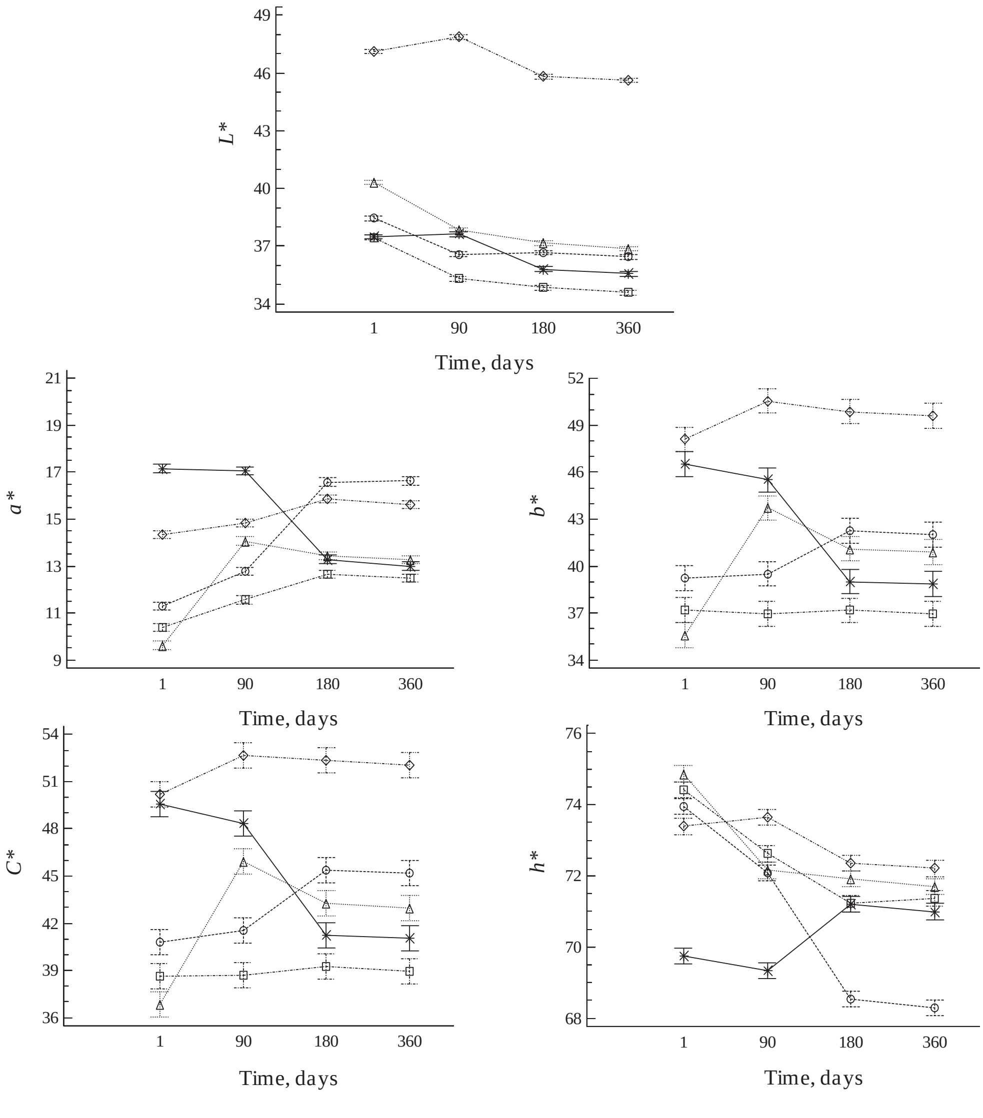

Fig. 3. Interaction graphics (α=95%) of colour parameters: L^*, a^*, b^* coordinates, chroma *(C^*)*, and hue *(h^*)* of the different formulations of mandarin orange marmalade.
—×—: Control; —○—: A; —△—: B; —□—: C; —◇—: D

2.3. Antioxidant activity

The results relating to the antioxidant activity of the mandarin orange marmalades studied are shown in Figure 4. Initially all marmalades showed the same antioxidant activity, but after 90 days of storage, antioxidant activity increased in formulations with the highest content of tagatose. After 90 days of storage, the antioxidant activity of all marmalades slightly reduced, but after one year, values were similar to those initially obtained, except in formulations with more tagatose, which again showed the highest antioxidant activity. Consequently, this new sweetener could enhance the ability of the antioxidants of mandarin orange fruit to scavenger free radicals. Besides, in orange marmalade (Rubio-Arraez et al., 2015) with sucrose or new sweeteners (tagatose and oligofructose) there was also an increase in the antioxidant activity after 45 days of storage, showing possible combinations of components that would lead to the appearance of new antioxidants. In any case, the results of the present study are supported by those obtained by Rosa and co-workers (2015), which qualify all the tested Mediterranean fruit preserves as a good source of biologically active components with considerable antioxidant activity.

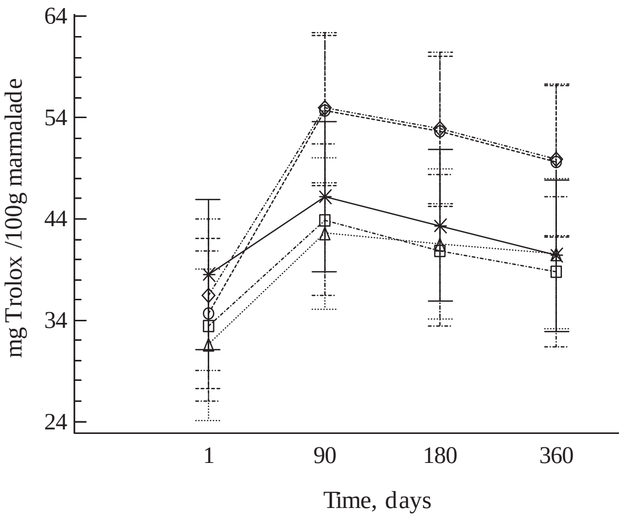

Fig. 4. Interaction graphic (α=95%) of antioxidant activity of the different formulations of mandarin orange marmalade. —×—: Control; —○—: A; —△—: B; —□—: C; —◇—: D

2.4. Microbiological analysis

All mandarin orange marmalades were safe from a microbiological point of view, since no colonies of moulds and yeast or mesophilic aerobic bacteria were found in any of the marmalades studied over the storage period considered.

2.5. Sensory analysis

The results of the sensory analysis of the mandarin orange marmalades formulated with sucrose or new sweeteners are shown in Figure 5. No significant differences were detected in the attributes of colour, aroma, texture, consistency, and spreadable capacity. However, the marmalade D obtained the best scores for palatability, flavour, global preference, and intention of buying. Besides, no significant differences in sweetness were found in formulation D as compared to the control marmalade. Conversely, marmalade C obtained the lowest scores for sweetness, palatability, and flavour, probably due to the low sweetening power of isomaltulose. In the case of orange marmalades (Rubio-Arraez et al., 2015), those prepared with tagatose and oligofructose scored better than marmalade prepared with sucrose. Therefore, the combination of tagatose and isomaltulose leads to greater differences in the sensorial analysis of marmalades than the combination of tagatose and oligofructose.

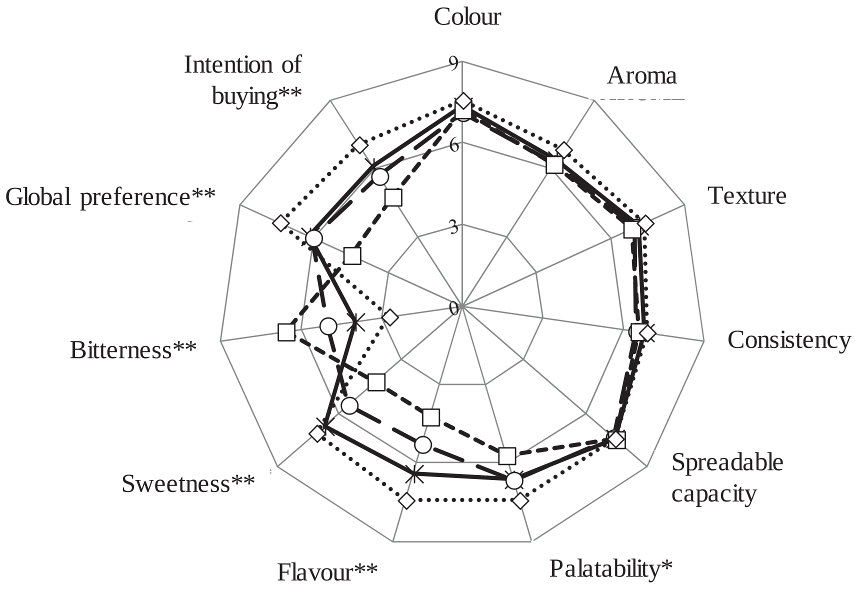

Fig. 5. Results of the sensory analysis.*: P-value<0.05, **: P-value<0.01
—×—: Control; —○—: A; —△—: B; —□—: C; —◇—: D

3. Conclusions

According to these results, it is possible to reformulate mandarin orange marmalade with non-cariogenic sweeteners, such as tagatose and isomaltulose. However, the complete replacement of sucrose with tagatose leads to a significant difference in the rheological behaviour of this type of marmalade, giving rise to a less elastic character. Additionally, tagatose increases the lightness of marmalades, but it improves their antioxidant activity. In all cases, the colour parameters remained constant after 180 days of storage. Finally, the flavour of tagatose scored better than isomaltulose due to the low sweetening power of the latter.

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