Effect of replacing sucrose with tagatose and isomaltulose in mandarin orange marmalade on rheology, colour, antioxidant capacity, 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-carcinogenic and have a low glycemic index. Analyses of rheology, colour, antioxidant capacity, microbiology and sensory properties were carried out on marmalades on their first day of storage, 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. Luminosity was shown to be highest in mandarin orange marmalades made with tagatose, although colour was stable for 6 months 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 fruits (*Citrus reticulata*) have a high nutritional composition (high content of phenolics, ascorbic acid, dietary fiber, etc.) and their consumption prevents diseases mainly due to this fruit's antioxidant activity (BALASUNDRAM *et al.*, 2006). Mandarin oranges are usually consumed as fresh fruit but also as juice. Moreover, fruit preserves, such as marmalades, can also be considered a good source of biologically

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active phenols with considerable antioxidant potential (ROSA *et al.*, 2015). Most marmalades are prepared with sucrose. However, this sugar has a high glycemic index and is also high in calories. Consequently, excessive consumption of sucrose can cause several diseases such as obesity, diabetes and tooth decay (RIEDEL *et al.*, 2015). Nowadays, there are other alternative natural sweeteners available in the market such as tagatose and isomaltulose, whose properties are healthier. These sweeteners are non-cariogenic and are released slowly into blood. D-Tagatose is a stereoisomer of D-fructose used in cheese and yoghurt. It has only 1.5 kcal/g (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 products such as ice creams, soft drinks and breakfast cereals (VASTENAVOND *et al.*, 2012) since its texture is very similar to sucrose and it is almost as sweet as sucrose (CALZADA-LEÓN *et al.*, 2013). On the other hand, isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice. 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 work was to evaluate their potential use as an alternative to sucrose in mandarin orange marmalades. For this purpose, their antioxidant capacity, rheological properties, colour, and sensorial acceptance were analyzed.

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 or isomaltulose) containing 1% agar-agar (Roko Agar, Llanera, Asturias, Spain). Isomaltulose (Beneo, Mannheim, Germany), Tagatose (Tagatesse, Heusden-Zolder, Belgium). The following notation was used depending on the combination of sweeteners used: 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. 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 rpm. Finally, the marmalade was allowed to cool for 24 hours and became jellified into the glass jars. Three batches of mandarins were used to prepare the marmalades. Analyses were triplicated on the first day of storage and after 90, 180 and 360 days of storage.

1.2. Rheological analysis

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

$$G' = a \cdot \omega^b \quad (1)$$

$$G" = c \cdot \omega^d \quad (2)$$

Where: ω is the angular speed (rad·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·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). All analytical determinations were performed on mandarin orange marmalades in 20 mm-wide cuvettes. CIE-L*a*b* coordinates were obtained using D65 illuminant and 10^{o} observer as the reference system.

1.4. 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 following the protocol described in previous studies (SHAHIDI *et al.*, 2006; RUBIO-ARRAEZ et al., 2015).

1.5. Microbiological analysis

Yeast and moulds and mesophilic aerobic were determined following the protocol described in a 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 (ISO 4121, 2003) was used to evaluate the following attributes: colour, arome, 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. A panel was formed consisting of 30-trained panelists (20-50 years old), who are regular consumers of this kind of marmalades. The 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 marmalades prepared by totally replacing sucrose with tagatose (marmalade D) showed the lowest values of G' and G". Since the storage modulus measures the stored energy, which represents elasticity, and the loss modulus measures the energy dissipated as heat, which represents viscosity, marmalades D were found to have the lowest elastic and viscous behavior. In contrast, the results for marmalade prepared with the highest amount of isomaltulose (formulation 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 studies (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 behavior will be different. The parameters of the power-law model are shown in Table 1. According to the results obtained in Figure 1, marmalades formulated with tagatose alone (formulation 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 marmalades after 180 days of storage. In terms of parameter *b* of the power-law of G', marmalade D again differed from 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 the *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 the new sweeteners leads to formulations with less elastic character in comparison to the control marmalade. On the other hand, the results obtained for the stationary 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 behavior. In the other cases, marmalades also showed values of τ_0 and k which were lower than in the control samples 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 our studies on 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 fiber (long chain of monosaccharides) would modify the rheological behavior of marmalades (in the stationary 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.

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 (with the highest content of tagatose) had the highest luminosity throughout the entire storage time. This formulation was followed by sample B (equal proportions of isomaltulose and tagatose), whereas marmalades A, C and control were initially very similar. During storage, luminosity 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, which had the highest amount of isomaltulose, 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 hue 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 marmalades. It is noteworthy that after 180 days, all marmalades showed similar values of hue except for formulation A. After 6 months of storage the colour of these marmalades was quite similar regardless of the differences registered before that period. In our previous studies 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 (2015) showed that strawberry jams formulated with isomaltulose and different concentrations of citric acid and pectin jams darkened during storage.

2.3. Antioxidant capacity

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 3 months of storage, antioxidant activity increases in formulations with the highest content of tagatose. After 3 months 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 capacity 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.

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 aerobics 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, arome, texture, consistency and spreadable capacity. However, the marmalade prepared by replacing sucrose with tagatose alone (formulation 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 with more isomaltulose (formulation C) obtained the lowest scores for sweetness, palatability and flavour, probably due to the low sweetening power of this sugar. In the case of orange marmalades (RUBIO-ARRAEZ *et al.*, 2015), those prepared with the new healthy sweeteners (tagatose and oligofructose) scored better than marmalade prepared with sucrose. Therefore, mandarin orange seems to be more sensitive to the different combinations of sweeteners than orange or otherwise, the combination of tagatose and isomaltulose leads to greater differences in the sensorial analysis of marmalades than the combination of tagatose and oligofructose.

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 behavior of this type of marmalade, giving rise to a less elastic character. Additionally, tagatose increases the luminosity of marmalades but it improves their antioxidant capacity. In all cases, the colour parameters remained constant after 6 months of storage. Finally, the flavour of tagatose scored better than isomaltulose due to the low sweetening power of the latter.

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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. Equal letters indicate homogeneous groups.

		OSCILLATORY TEST-POWER LAW				STEADY TEST HERSCHEL-BULKLEY MODEL			
		G´		G´´					
FORMULATION	**TIME** (days)	***a***	***b***	***c***	***d***	**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.

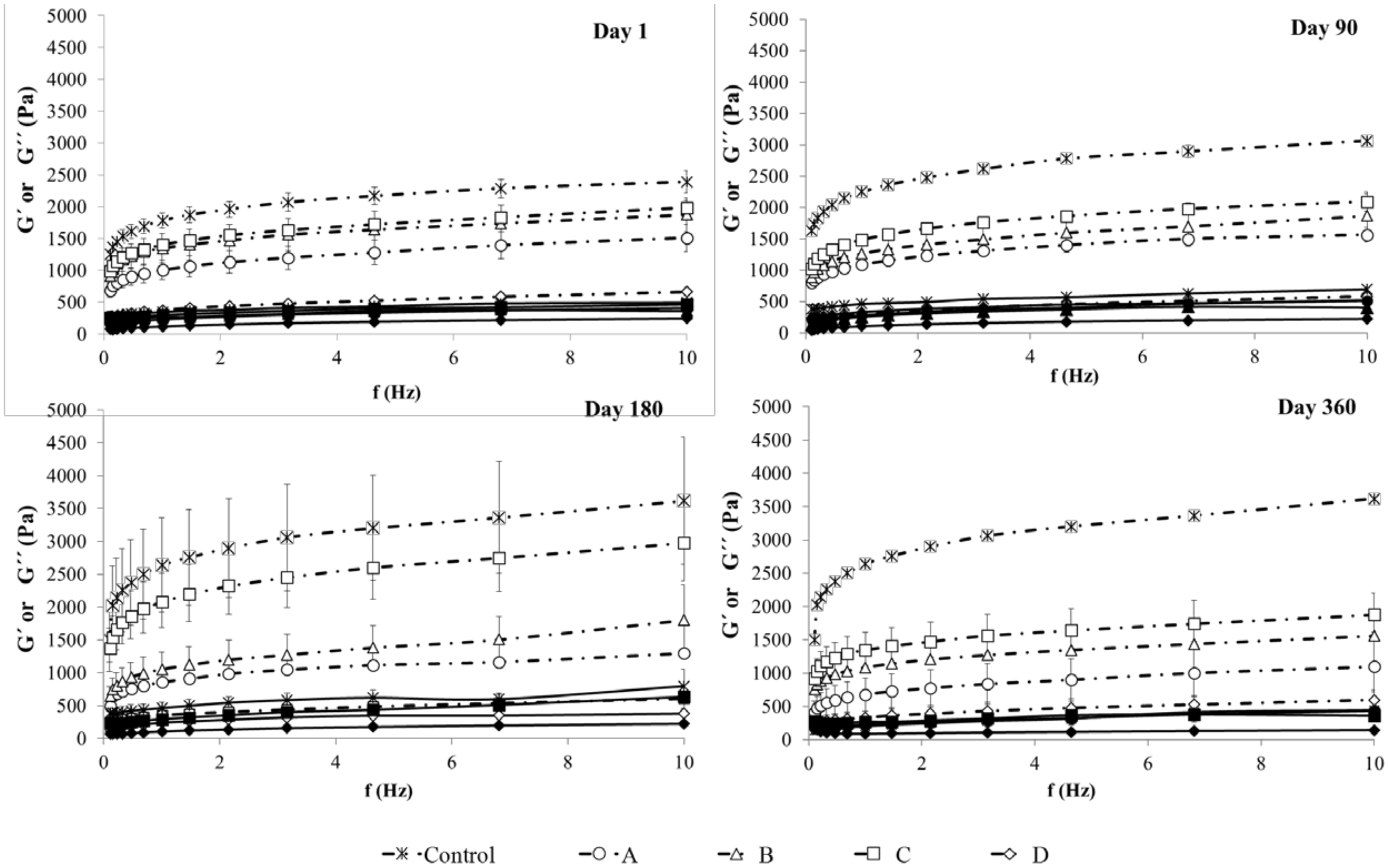

Figure 1. Average frequency curves obtained in the oscillatory test of mandarin orange marmalades. Unshaded symbols refer to values of G´ and shaded symbols refer to values of G´´.

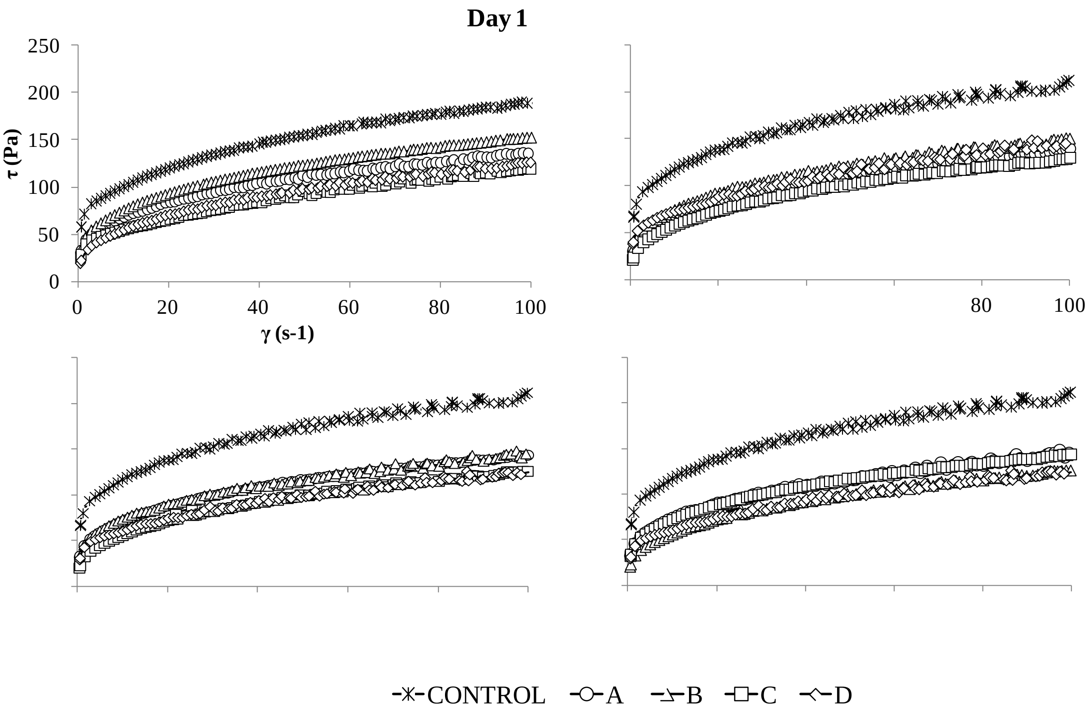

Figure 2. Mean flow curves (rheograms) obtained from the steady assay of mandarin orange marmalades.

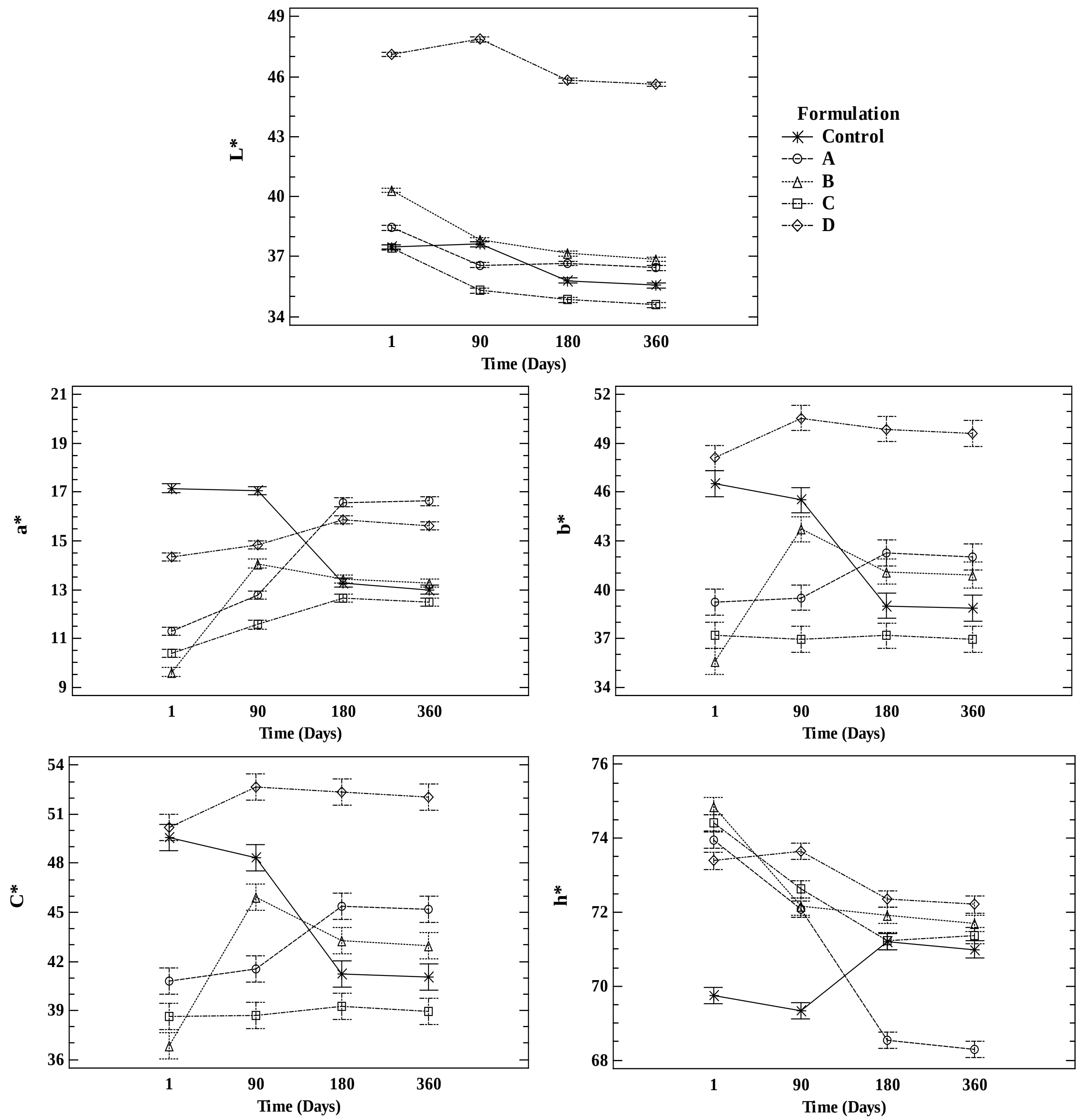

Figure 3. Interaction graphics (α=95%) of colour parameters: L*, a*, b* coordinates, chroma (C*) and hue (h*) of the different formulations of mandarin orange marmalade.

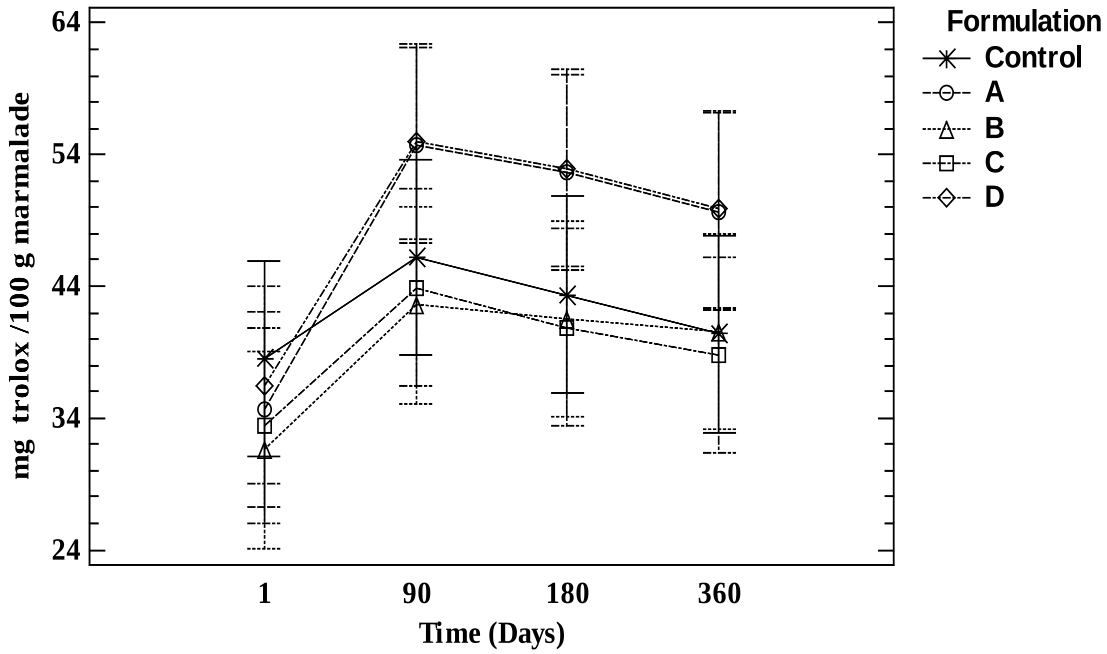

Figure 4. Interaction graphic (α=95%) of antioxidant activity of the different formulations of mandarin orange marmalade.

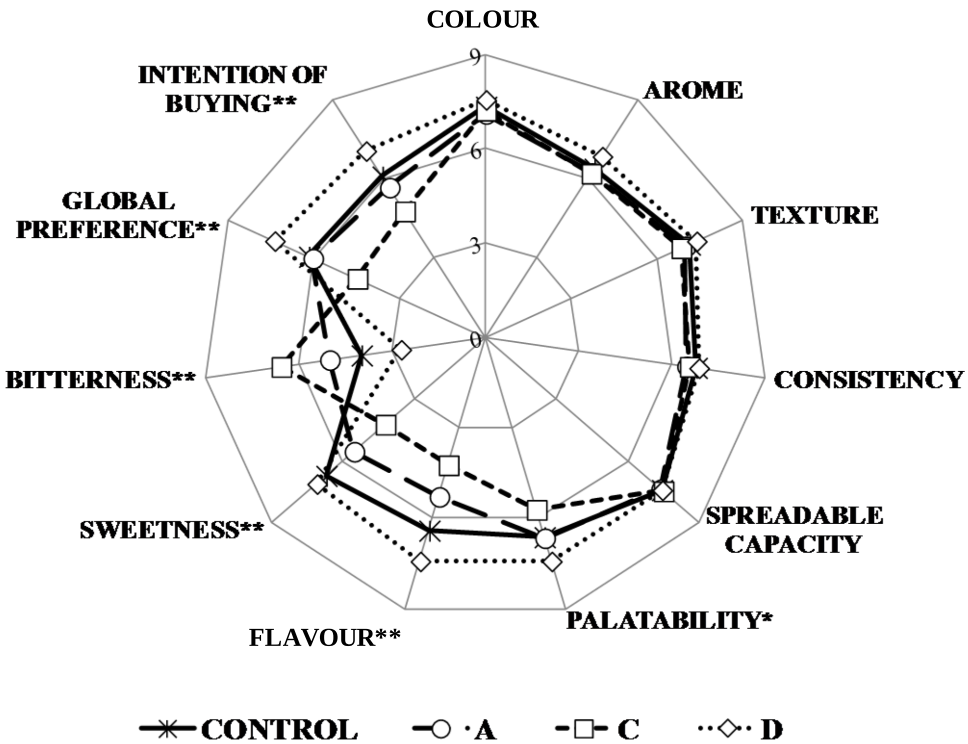

Figure 5. Results of the sensory analysis. *p-value <0.05, ** p-value <0.01