Effects of Cyclodextrin Type on Vitamin C, Antioxidant Activity, and Sensory Attributes of a Mandarin Juice Enriched with Pomegranate and Goji Berries

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Abstract: The effects of the addition of cyclodextrins (CDs), β-CD, or HP-β-CD (1%), on the protection of antioxidant compounds of mandarin juices enriched with pomegranate extract and goji berries juice, was studied. Juices were prepared and after their thermal treatment (98 °C, 30 s) they were stored at 4 °C during 75 d. Vitamin C content, CIE $L^*a^*b^*$ color, antioxidant capacity, retinol equivalents, and sensory properties were studied. Losses on vitamin C were higher (6%) for juices with β-CD than juices with HP-β-CD. Retinol equivalents degradation was lower (3.4%) in juices with HP-β-cyclodextrins than in those treated with β-CD. Lower losses were observed for the instrumental and sensory color intensity in juices with HP-β-CD addition. Finally, the antioxidant capacity was also higher in juices treated with HP-β-CD. Finally, the overall sensory quality of juices with HP-β-CD was the best one after 30 d of cold storage. Even though β-CD addition did not cause any improvement compared with control juice (without CD addition), the benefits of adding HP-β-CD to this particular juice were shown in almost all parameters under study.

Keywords: antioxidant activity, *Citrus reticulata*, color, mandarin juice

Practical Application: The present study deals with the practical aspects of the utilization of CDs and CD-complexes in the food industry. The molecular encapsulation of lipophilic food ingredients with CD are supposed to improve the stability of flavors, vitamins, colorants, and antioxidant activity, leading to extended product shelf life. The HP-β-CD treated mandarin juice enriched with pomegranate extract and juice of goji berries will have a more intense color, higher vitamin C content, retinol equivalents, and antioxidant activity during storage and shelf life than control juices, with no CD addition.

Introduction

Oxidative stress has been involved in the development of numerous chronic diseases and in the aging process (Halliwell and Whiteman 2004). Human diet provides different natural antioxidants, which can be considered as a supplement for the natural organism defenses. In this way, the beneficial effects of the consumption of a diet rich in vegetables have been mainly attributed to vitamins, carotenoids, and phenolic compounds (Prior 2003).

Functional products are designed with the objective of supplying a high amount of antioxidants and to reduce the risk of diseases associated with the oxidative stress (Bello 2006). The term "functional food" is related to an ingredient, which is able to improve the health and/or reduce the risk of diseases (Rafter 2002). The best option is to consume functional foods in a normal diet to obtain the beneficial effects of both the traditional diet and the functional foods (Roberfroid 2002). The product under study here, mandarin juice enriched with pomegranate extract and goji berries juice, will easily fit in this option.

Bioactive compounds, such as vitamin C and polyphenols, have been proved to possess anti-inflamatory, antiatherogenic, antimicrobial, and antioxidant activities (Kris-Etherton and others 2002). These bioactive compounds may or may not remain active in heat-treated products, such as juices, marmalades, jellies, and so on (Zafrilla and others 2001).

Commercial juices are preserved by high temperature pasteurization. The main aim of this heat treatment is to inactive toxic microorganisms; however, changes in desirable components such as nutrients, color, aroma, and texture, also take place (Lee and Coates 2003), and are often related to quality losses. To solve this problem and to improve the bioavailability of different compounds, natural molecules called cyclodextrins (CDs) are being used (Szejtli 1998). The applications of CDs are numerous in the food industry and the main benefits are: (1) prevention of undesirable aroma production, (2) prevention of color degradation, (3) masking of undesirable flavor compounds.

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β-Cyclodextrin and its derivates are widely used in pharmacology and food industry, particularly 2-hydroxypropil-β–CD (HP-β–CD). This compound presents higher water solubility, lower toxicity, and higher hydrophobic cavity than the original product, β-CD (Boudad and others 2001).

The main objective of this study was to evaluate the efficiency of 2 types of CDs (β-CD and HP-β-CD) in encapsulating antioxidant compounds from a functional food, mandarin juice enriched with pomegranate extract and goji berries juice. To reach this objective, vitamin C, retinol equivalents (RE), antioxidant capacity, CIE $L^*a^*b^*$ coordinates and sensory quality were analyzed in samples with and without CD, before and after pasteurization (at 98 °C during 20 s) and during cold storage (up to 75 d at 4 °C).

Materials and Methods

Product description

A product rich in bioactive compounds and with high antioxidant activity was prepared using fresh mandarin juice (96%), goji berries juice (2%), pomegranate extract (1%), and CD (1%). The ingredients selection was based on the high consumers' acceptance of mandarins and goji berries and the high availability of mandarin juice in Spain. Both pomegranate extract and goji berries were added due to their high antioxidant activity, according to the scientific literature (Gil and others 2000; Seeram 2008). The final enriched product must satisfy consumers' requirements and do not decrease the high acceptability of the original product. Our hypothesis was that the addition of pomegranate extract and goji berries juice, at the concentrations used, would not affect consumers' liking but will increase the antioxidant activity and shelf life of the mandarin juice.

Materials

Mandarins used for this study, *Citrus reticulata* cultivar Ortanique, were selected basing on their diameter, pH, total soluble solids content (SSC), titratable acidity (TA), and maturity index (SSC/TA). Goji berries were added as juice, supplied by "Ransom" company, made from concentrated juice, ascorbic acid and potassium sorbate (Ransom & Son Pic, Bradford, U.K.). Pomegranate, in powder extract, was supplied by Probelte Pharma (Murcia, Spain). HP-β-Cyclodextrin and β-CD were from TCI Europe (Zwijndrecht, Belgium). All other chemicals used were of analytical grade and from Panreac S.A. (Barcelona, Spain).

Sample preparation

Total of 3 types of juice were prepared: (1) juice without CD (control juice), (2) juice with β-CD (1%), and (3) juice with HP-β-CD (1%). Freshly squeezed mandarin juice, with the pulp content optimized, was mixed with the rest of ingredients, adding 1% of every type of CD to the corresponding container and shaking the juices for 1 h for a correct and full solubilization of the solid ingredients. All samples were subjected to a high pasteurization step (98 °C for 30 s) and later samples were kept under refrigeration temperature (4 °C) during the storage period (75 d). Samples were analyzed before the thermal treatment (BTT), after the thermal treatment (ATT), and after 7, 30, 46, 60, and 75 d of storage. In each sampling, total soluble solids, titratable acidity, pH, vitamin C, retinol equivalents, antioxidant capacity, and sensory attributes were analyzed. Total of 5 batches of 2 L were prepared for each juice, making a total of 10 L per juice.

Physico-chemical analyses

Total soluble solids content, SSC (°Brix), was determined using a portable refractometer Comecta, S.A., model C3 (Barcelona, Spain). Titratable acidity, TA (% citric acid), was determined in 10 mL of juice by titration to pH 8.2 ± 0.1 with a 0.1 N NaOH solution. The maturity index, MI, was calculated for each juice and expressed as the ratio between the SSC and TA. Five replications were carried out for all physico-chemical analyses.

Vitamin C

Reduced ascorbic acid was measured following the AOAC Official Method 985.33 (Horwitz 2000). Ascorbic acid was estimated by titration with colored oxidation-reduction indicator, 2,6-dichloroindophenol. EDTA was added as chelating agent to remove Fe and Cu interferences. Total of 5 replications were carried out for the vitamin C analysis.

Instrumental measurement of color

Color determinations were made, at 25 ± 1 °C, using a Hunterlab Colorflex® (Hunterlab, Reston, Va., U.S.A.). This spectrophotometer uses an illuminant D65 and a 10° observer as references. A sample cup for reflectance measurements was used (5.9 cm internal diameter × 3.8 cm height) with a path length of light of 10 mm. Blank measurements were made with the cup filled with distilled water against a reference white background (Pérez-López and others 2006). Color data are provided as CIE $L^*a^*b^*$ coordinates. The chroma or color purity ($C_{ab}{}^*$) and the color differences (ΔE^*) were calculated using the following formulas: $C_{ab}{}^* = (a^2 + b^2)^{1/2}$ and $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. The color analyses were run in 5 replicates.

Provitamin A content, expressed as retinol equivalents (RE), was mathematically estimated using CIE $L^*a^*b^*$ color coordinates, as described by Meléndez-Martínez (2005):

$$\mathrm{RE} = 0.308382 \times L^* + 0.108054 \times a^* + 0.337946 \times b^* + 0.004563$$

Antioxidant activity

The Oxygen Radical Absorbance Capacity (ORAC) assay was carried out on a Synergy HT multidetection microplate reader, from Bio-Tek Instruments Inc. (Winooski, Vt., U.S.A.), using 96-well polystyrene microplates with black sides and clear bottom, purchased from Nalge Nunc Intl. (Hereford, U.K.). Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The ORAC was determined as described by Dávalos and others (2004) with slight modifications (Lucas-Abellán and others 2008). All reaction mixtures were prepared in triplicate, and at least 3 independent assays were performed for each sample.

The results were expressed as relative fluorescence with respect to the initial reading. The area under the fluorescence decay curve (AUC) was calculated by the equation:

$$AUC = 1 + \sum_{i=1.14}^{i=120} f_i/f_0$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. The results of antioxidant capacity were defined as mM of Trolox C per 100 mL of juice.

Sensory evaluation with trained panel

Sensory evaluation by a trained panel was used to study the quality of the juices under study. A panel of 10 panelists, ages 20 to 50 y (8 female and 2 male, all members of the Catholic Univ. San Antonio of Murcia), with sensory evaluation experience, was trained in descriptive evaluation of citrus juices (Serrano-Megías and others 2005). The panel was selected and trained following the ISO standard 8586–1 (AENOR 1997; Pérez-López and others 2006).

Measurements were performed in individual booths with controlled illumination (750 to 1000 lux) and temperature (23 ± 2 °C).

The individual products were scored for the intensities of color, sweetness, fresh mandarin flavor, off-flavor and overall quality using a scale of 0 to 10, where: 0 = extremely slight or no intensity and 10 = extremely high intensity.

Samples were presented in 50 mL plastic cups with lids. The entire experiment was repeated 3 times (all judges scored 3 juice samples on each) and the sensory scores were presented as the overall means.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and the Tukey's least significant difference multi-comparison test to determine significant differences among treatments. Significance of differences was represented at $P \leq 0.05$. The statistical analyses were conducted using SPSS 14.0 (SPSS Science, Chicago, Ill., U.S.A.) and figures were prepared using Sigma Plot 9.0 (SPSS Science).

Results and Discussion

Physico-chemical analyses

The pH, SSC, TA, and maturity index values of all juices under study were 3.4 ± 0.2, 12.3 ± 0.4 °Brix, 0.98% ± 0.03% citric acid, and 12.6 ± 0.2, respectively. These values remained constant throughout the experiment and there were not significant differences ($P > 0.05$) among juice samples due to CD addition or storage time.

Vitamin C

Changes in vitamin C have been considered as an indicator of the enzymatic and non-enzymatic degradative reactions, which take place during processing and/or storage (Skrede 1996). For instance, vitamin C maintenance has been used as indicator of shelf life in chilled orange juices. It has been considered that juices with 50% of the initial vitamin C are at the end of their shelf life (Shaw 1992).

Figure 1 shows the degradation of ascorbic acid after the heat treatment and during the cold storage at 4 °C. Initially, the ascorbic acid levels were of 255 ± 7 mg L^{-1} in all samples. After the pasteurization heat treatment (98 °C for 30 s), the levels were of 250 ± 5 mg L^{-1} for the control juice and 245 ± 6 and 255 ± 6 mg L^{-1} for juices treated with β-CD or HP-β-CD, respectively. Vitamin C losses were basically affected by storage time and only slightly by the pasteurization treatment; the losses due to the storage time were about 33% to 35%. The highest final content of vitamin C was found in juices treated with HP-β–CD, 185 ± 5 mg L^{-1}. Nuñez-Delicado and others (1997) added HP-β-CD to a high antioxidant activity matrix and concluded that HP-β-CD acted as secondary antioxidant, preserving the antioxidant capacity of the matrix due to the extra-protector effect of HP-β-CD against the ascorbic acid oxidation.

CIE $L^*a^*b^*$ color

The initial color coordinates before the thermal treatment were $L^* = 39.91 \pm 1.31$, $a^* = 28.28 \pm 2.01$, $b^* = 44.16 \pm 1.56$ for all the samples, corresponding to an intensive orange color (Table 1).

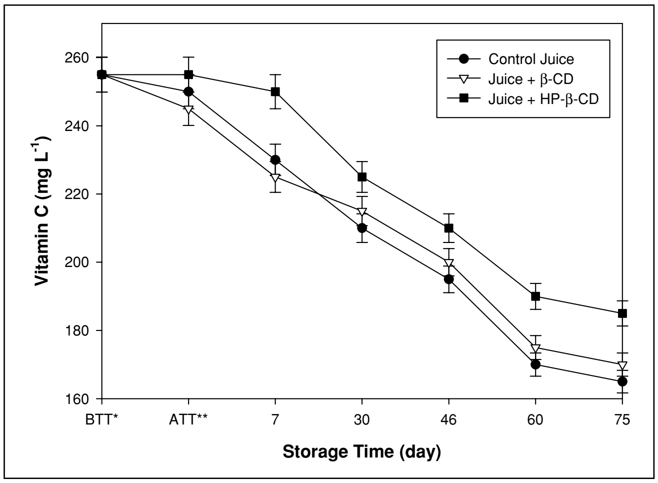

Figure 1–Changes with storage time (75 d) of the vitamin C content (mg L^{-1}) of juices under study. BTT* = before thermal treatment (pasteurization 98 °C for 30 s); ATT** = after thermal treatment.

Table 1–Changes with time of color coordinates (L^*, a^*, b^*, and C_{ab}^*) in mandarin juices without or with cyclodextrin addition, before and after thermal treatment, and after 7, 30, 46, 60, and 75 d of cold storage at 4 °C.

	Color coordinates											
	L^*			a^*			b^*			C_{ab}^*		
Storage time (d)	Control juice	Juice + β-CD	Juice + HP-β-CD	Control juice	Juice + β-CD	Juice + HP-β-CD	Control juice	Juice + β-CD	Juice + HP-β-CD	Control juice	Juice + β-CD	Juice + HP-β-CD
BTT*	39.9 a A[†]	39.9 a A	39.9 a A	28.3 a A	28.3 a A	28.3 a A	44.2 a A	44.2 a A	44.3 a A	52.4 a A	52.4 a A	52.4 a A
ATT*	39.5 a A	39.7 a A	39.0 a A	26.3 ab AB	25.8 bc B	27.9 ab A	43.2 a A	43.4 a A	44.1 a A	50.6 bc B	50.5 b B	52.2 a A
7	39.5 a A	39.8 a A	38.8 a B	24.9 b B	24.8 c B	26.4 b A	43.0 a AB	40.9 b B	44.1 a A	49.7 bc AB	47.9 cd B	51.4 ab A
30	39.6 a A	40.0 a A	38.7 a B	23.9 b B	24.4 c B	26.4 b A	41.7 b B	39.3 c B	44.2 a A	48.1 c B	46.3 d B	51.4 ab A
46	39.6 a A	40.0 a A	38.4 a B	24.0 b B	24.5 c B	26.3 b A	41.1 b B	39.3 c B	43.6 a A	47.6 c B	46.3 d B	50.9 b A
60	39.6 a A	39.9 a A	38.4 a B	24.0 b B	24.3 c B	26.4 b A	41.0 b B	39.3 c B	43.2 a A	47.5 c B	46.2 d B	50.6 b A
75	39.3 a A	39.9 a A	38.4 a B	24.0 b B	24.1 c B	26.4 b A	41.2 b B	39.3 c B	43.1 a A	47.7 c B	46.2 d B	50.5 b A

Values followed by the same "small" letter, in the same column (effect of thermal treatment and storage time), were not significant different ($P < 0.05$), Tukey's multiple-range test. Values followed by the same "capital" letter, in the same row (effect of CD addition) and within the same color coordinate, were not significant different ($P < 0.05$).

Lightness (L^*) was constant in all the juices, with only a degradation of 2% to 3% at the end of the storage time (Table 1). Progressive degradations, with thermal treatment and storage time, of a^* (green-red) and b^* (blue-yellow) were found in all the samples. The losses during the thermal treatment were of approximately 2 units for control juices and those treated with β-CD, and of only 1 unit for juices treated with HP-β-CD. However, at the end of the storage period, losses of a^* and b^* reached up to 4 units.

Changes occurred in coordinates a^* and b^* in all the analyzed samples causing also changes in the chroma coordinate ($C_{ab}{}^*$), which represents the color purity. The chroma values decreased from 52.4 ± 0.65 (before thermal treatment) to 47.7 ± 0.15, 46.2 ± 0.58, and 50.5 ± 0.86 for control samples and juices treated with β-CD and HP-β-CD, respectively (Table 1). The experimental results presented here showed that the color of the juices was mainly affected by the storage time. Some researchers found similar results on fruits and vegetables after thermal treatments and storage (Vikram and others 2005).

After pasteurization, the ΔE^* values were 2.3, 3.3, and 0.97 for control juices and samples treated with β-CD and HP-β-CD, respectively (Figure 2). The biggest changes on ΔE^* were due to the storage time, with values increasing up to 5.36 ± 0.58, 6.08 ± 0.58, and 2.66 ± 0.58, for control juices and samples treated with β-CD and HP-β-CD, respectively (increments of ΔE^* higher than 2 units will cause noticeable visual differences). It can be easily observed in Figure 2 that ΔE^* were lower ($P < 0.001$) in samples treated with HP-β-CD than those from samples treated with β-CD. Others researchers (López-Nicolás and others 2007) obtained similar results in peach juices; these researchers found nonsignificant effects on juice quality after the addition of β-CD but reported positive effects in juices treated with other types of CD, such as maltosil-β-CD or α-CD. On the other hand, some studies (Lee and Coates 2003) showed significant effects on ΔE^* due to thermal treatment of juices.

Provitamin A content

Initially, the provitamin A content reached a value of 30.29 ± 0.15 ER L^{-1} (Figure 3). After the thermal treatment, ER losses were minimum (about 1% to 3%) in all the samples. The effects of the storage time accounted for decreases of about 5% to 6% in control juices and those treated with β-CD and only of 3.4% in juices treated with HP-β-CD. It can be concluded that both the heat treatment and the storage time negatively affected the total carotenoids and therefore the provitamin A, although reductions of ER were lower than 5%. Meléndez-Martínez and others (2007) obtained similar values for different types of oranges. Finally, Mercader-Ros and others (2010b) reported better protection of bioactive compounds in HP-β-CD than in β-CD, similarly to what is reported in the current study.

Antioxidant activity

Figure 4 shows the total antioxidant activity of the fruit juices and its changes during the storage at 4 °C. The ORAC method has been used to evaluate the antioxidant capacity of many types of fruits and vegetables, such as red fruits (Wada and Ou 2002), tomato (Zapata and others 2007), and orange and apple (Wang and others 1996). The antioxidant capacity of the freshly squeezed juices was 25.9 ± 0.4 mMT per 100 mL (Figure 4). The analysis revealed that losses in the juices after the heat treatment ranged

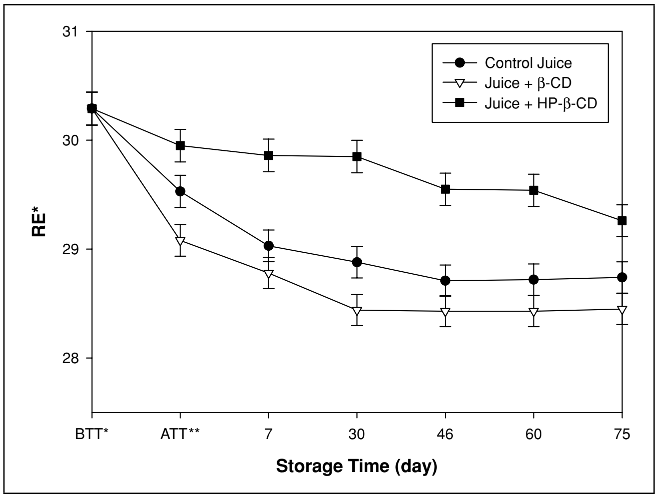

Figure 3–Changes with storage time (75 d) of the retinol equivalents (RE*) of juices under study. BTT* = before thermal treatment (pasteurization 98 °C for 30 s); ATT** = after thermal treatment.

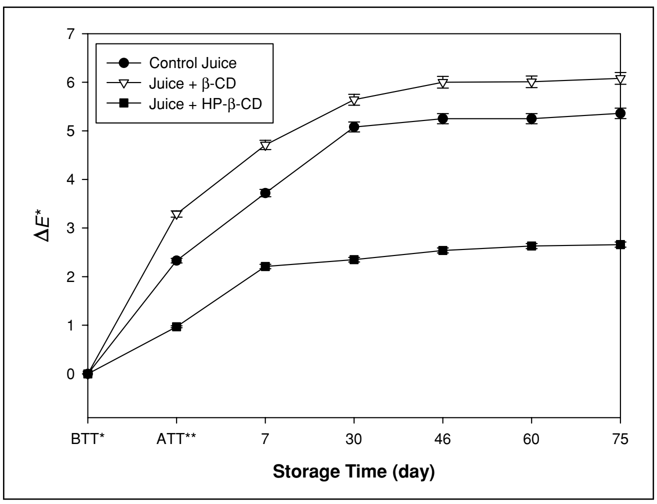

Figure 2–Changes with storage time (75 d) of the total difference color (ΔE*) of juices under study. BTT* = before thermal treatment (pasteurization 98 °C for 30 s); ATT** = after thermal treatment.

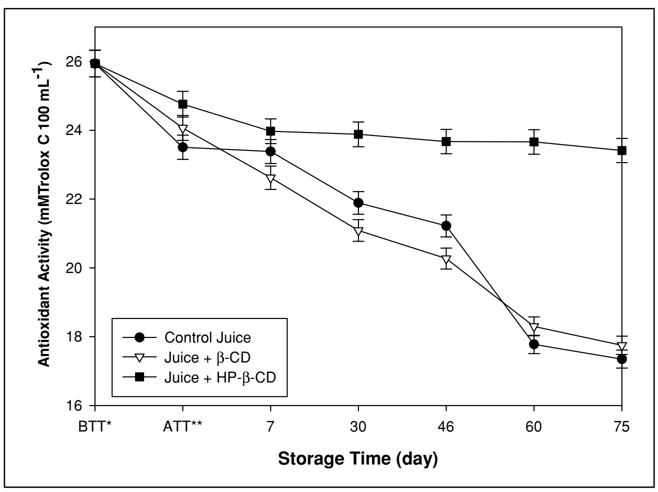

Figure 4–Changes with storage time (75 d) of the antioxidant capacity (mM of Trolox C 100 mL^{-1} juice) of juices under study. BTT* = before thermal treatment (pasteurization 98 °C for 30 s); ATT** = after thermal treatment.

Table 2–Sensory analysis of mandarin juices, without or with cyclodextrin addition, before and after thermal treatment, and after 7, 30, 46, 60, and 75 d of cold storage at 4 °C.

	Sensory properties											
	Color			Fresh mandarin flavor			Off-flavor			Overall quality		
Storage time (d)	Control juice	Juice + β-CD	Juice + HP-β-CD	Control juice	Juice + β-CD	Juice + HP-β-CD	Control juice	Juice + β-CD	Juice + HP-β-CD	Control juice	Juice + β-CD	Juice + HP-β-CD
BTT	8.6 a A†	8.4 a A	8.8 a A	9.0 a A	8.0 a A	7.9 a A	0.5 c A	0.5 b A	0.7 b A	8.8 a A	8.6 a A	8.8 a A
ATT	8.0 a A	7.8 b A	7.7 b A	8.5 b A	8.1 a A	7.3 a B	0.7 b A	0.5 b A	0.6 b A	8.3 a A	8.1 ab A	8.5 a A
7	8.1 a A	7.7 b A	7.8 b A	8.5 b A	7.7 b AB	7.3 a B	1.0 a A	1.0 a A	1.0 a A	8.3 ab A	7.9 bc A	8.5 a A
30	6.5 b B	6.5 c B	7.8 b A	8.4 b A	7.8 b AB	7.2 a B	1.0 a A	1.1 a A	1.2 a A	7.5 b AB	7.1 c B	7.8 b A
46	6.5 b B	6.4 c B	7.8 b A	8.4 b A	7.5 c B	7.2 a B	1.0 a A	1.0 a A	1.2 a A	7.5 b AB	6.9 c B	7.9 b A
60	6.4 b B	6.3 c B	7.6 b A	8.3 b A	7.5 c B	7.1 b B	1.1 a A	1.1 a A	1.1 a A	7.4 b AB	6.9 c B	7.7 b A
75	6.4 b B	6.3 c B	7.6 b A	8.2 b A	7.4 c B	7.0 b B	1.1 a A	1.1 a A	1.1 a A	7.4 b AB	6.7 c B	7.6 b A

Values followed by the same "small" letter, in the same column (effect of thermal treatment and storage time), were not significant different ($P < 0.05$), Tukey's multiple-range test. Values followed by the same "capital" letter, in the same row (effect of CD addition) and within the same color coordinate, were not significant different ($P < 0.05$).

from 8.6% in control juices to 4.5% in juices treated with HP-β-CD. After the storage period (75 d) the antioxidant capacity ranged from 17.8 ± 0.4 mMT 100 mL^{-1} in control juices and juices treated with β-CD and 23.4 ± 0.5 mMT 100 mL^{-1} in juices treated with HP-β-CD, with total reductions (pasteurization and storage) being approximately 31.5% and 9.75%, respectively. There is a clear difference ($P < 0.001$) between the group of 2 samples (control juices and samples treated with β-CD) and juices treated with HP-β-CD. This could be explained considering that HP-β-CD presented a structure much more favorable for the complexion of antioxidant compounds than β-CD. Therefore a higher effectiveness, encapsulating higher contents of bioactive compounds and the juice being more stable, was observed for HP-β-CD than for β-CD (Mercader-Ros and others 2010a).

Sensory evaluation

There were no significant effects ($P > 0.05$) of the 3 factors under study: (1) the CD type, (2) the heat treatment, and (3) the storage time, on the intensity of sweetness (mean value of 7.5 ± 0.1). A similar situation ($P > 0.05$) was observed for the off-flavors intensity (Table 2); however, during the first 7 d of cold storage the intensity of the off-flavors slightly increased.

Before the pasteurization step, the addition of β-CD or HP-β-CD to the juice samples did not cause any significant variation ($P > 0.05$) on the sensory parameters under study. However, significant changes ($P < 0.01$) in the color intensity were detected after 30 d of cold storage; juices treated with HP-β-CD had the highest intensity of orange color (Table 2). This finding agreed with the instrumental data (a^*, b^*, and C_{ab}^*) previously reported (Table 1). López-Nicolás and others (2009) showed that the higher CD concentration, the better the color of pear juices due to the reduction of browning; however, the addition of β-CD had no significant effects on the sensory color intensity.

The juice with the highest intensity ($P < 0.01$) of fresh mandarin flavor was the control one (with no CD addition), followed by juice with β-CD and, finally juice with HP-β-CD (Table 2). The binding between CD and the aroma compounds depends on both hydrophobicity of the guest molecules and their geometric accommodation into the CD cavity. Some researchers showed that β-CD is the most versatile CD for the stabilization of aroma compounds (Reineccius and others 2006). Finally, the overall quality of juices was evaluated by the trained panel after integration of color, taste and flavor attributes. The quality of the 3 juices under study was statistically ($P > 0.05$) similar until day 30, at which the quality of the juice treated with HP-β-CD was quantified being slightly higher ($P < 0.05$) than that of the control and β-CD-treated juices; this same trend was kept until the end of the storage period (75 d).

Conclusions

Cyclodextrin addition caused both positive and negative effects on the composition and quality of the juice under study (mandarin juice enriched with pomegranate extract and goji berries juice). Juices treated with β-CD presented higher intensities of typical aroma (fresh mandarin) than those treated with HP-β-CD and especially control juices. However, juices treated with HP-β-CD presented the highest values of color intensity, antioxidant activity, vitamin C content, retinol equivalents, and overall quality, especially at the end of the cold storage period (75 d). The final conclusion is that HP-β-CD was more efficient than β-CD in improving the quality of mandarin juice enriched with pomegranate extract and goji berries juice.

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