Article

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Protective effect of cyclodextrins on the quality parameters of roast preserved pepper

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Abstract

Total phenolics (TP), vitamin C, antioxidant activity and colour of preserved peppers were evaluated at 4, 25 and 50 °C storage during 30-day intervals. Except for 4 °C, TP decreased during storage at 25 °C and 50 °C, being softer for fortified samples with β-CDs. The protective effect was evident, since 50 °C samples containing β-CDs exhibited lower TP loss (19%) than control samples (38%) for 5 months storage. A decrease in the vitamin C content was observed for both samples as time and temperature progressed. In samples stored at 50 °C the protective effect of β-CD only was evident at the first month, since fortified samples showed lower vitamin C loss (10%) than control samples. The fortified samples with β-CDs exhibited lowest antioxidant activity loss (40%) during 90-day storage at 50 °C, than control samples (64%). The colour changes were in line with those observed for total phenolics and at the end of study, the presence of 1% β-CDs delayed the darkening of samples at both (25 and 50 °C) storage conditions.

Keywords

Total phenolic, antioxidant activity, vitamin C, roast preserved peppers, cyclodextrins

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INTRODUCTION

Fruits and vegetables are rich in minerals such as K^{+}, Mg^{+2}, Ca^{+2} and P and contain vitamins C, E, and phenolic compounds which have antioxidant properties (Sánchez-Moreno et al., 2003).

Peppers (*Capsicum annuum* L.) have long been recognized as an excellent source of vitamin C (Chuah et al., 2008), essential for normal physiological functions such as the metabolism of tyrosine, folic acid and tryptophan. It helps to lower blood cholesterol (Mcrae, 2006) and contributes to the synthesis of carnitine and catecholamine, which regulates nervous system. Being an antioxidant, it protects the body from the harmful effects of free radicals and pollutants, in which it prevents common degenerative processes (Davey et al., 2000). It is known that vitamin C chelates heavy metal ions, reacts with singlet oxygen and other free radicals and suppresses peroxidation, reducing the risk of arteriosclerosis, cardiovascular diseases and some types of cancer (Álvarez-Parrilla et al., 2011). However, as food sage there is a gradual decline in the amount of vitamin C (Platt et al., 1963). Vitamin C is thermo-labile and therefore in fruit and vegetables it provides an indication of the loss of other vitamins, and acts as a valid criterion for other organoleptic or nutritional components degradation such as natural pigments and aromatic substances. Its concentration decreases during thermal treatment and storage conditions such as temperature, oxygen content and light

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(Blasco et al., 2004). Therefore, retention studies of this vitamin are of great importance to food technologist and consumers.

In addition, peppers are rich in polyphenols, particularly the flavonoids, quercetin and luteolin (Lee and Kader, 2000). Polyphenols are a group of phytochemicals responsible of brightly colored pigments and their presence at reasonable levels in foods might be important because they can exert different protective effects.

The contribution of phytochemicals to the antioxidant activity of fresh and processed peppers has been clearly demonstrated (Álvarez-Parrilla et al., 2011). Zepka and Mercadante, (2009) observed that peppers at intermediate ripening stages presented a higher (29%) antioxidant activity than peppers at other ripening stages. Besides ripening, heat processing can also alter the phytochemical composition and bioactivity of peppers since some compounds are highly prone to isomerization, oxidation and degradation under heat conditions (Zepka and Mercadante, 2009). However, these changes depend on many factors; including the heat-processing style/intensity, pepper genotype and ripening stage.

Peppers are commonly consumed raw in salads or blended into juice with others fruits and vegetables. However, vegetables have always been considered to have lower nutritional value after cooking due to the loss of nutritional or phytochemicals compounds (Fennema, 1997). This aspect is of great importance considering that only a small amount of fruit and vegetables are consumed in their raw state, whilst most of them need to be processed for safety, quality and economic reasons.

In fact, seasonality and perishability of foods explain the necessity of applying preservation technologies in order to extend their shelf life. To prolong shelf life, thermal processing is the commonest method in order to inactivate enzymes and destroy microorganisms. However, loss of representative flavour, color and vitamins has been evidenced after different heat processing (Blasco et al., 2004 and Lee and Kader, 2000).

On the basis of these supposed negative effects, in the past few years the main approach for minimizing eventual processing damage was the 'reconstitution', achieved by the addition or the enrichment of the product with natural antioxidants. Various attempts have been made to search for new classes of natural antioxidants to be added to foods (Sandström, 1998).

It is well known that food processing can have many effects, not all of which result in a loss of quality. For instance, it has been recently found that the bioavailability of β-carotene increases as consequence of moderate heating or the enzymatic disruption of the vegetable's cell wall structure (West and Castenmiller, 1998). Blanching also represents a useful strategy in preventing enzymatic oxidations, which are the main cause of loss in naturally occurring antioxidants in raw material of plant origin. In fact, fruit and vegetables subjected to blanching retain most of their original antioxidant properties.

Thus, the evaluation of the influence of food processing and storage on naturally occurring antioxidants is a key factor in finding those necessary technological conditions, to preserve or improve their original activity and bioavailability.

Among the different approaches, the employ of cyclodextrins (CDs) could be a viable short-term solution, as evidenced by success reported in the literature. In fact, the employ of CDs has been proposed for the control of enzymatic browning in apple products by several authors (Gacche et al., 2003, 2004). Also, have been used as protective flavonoid agents (Lucas-Abellán et al., 2007, 2008a, 2008b; Mercader-Ros et al., 2010) leading to an increase in their antioxidant activity due to the protection toward free radical attack (Lucas-Abellán et al., 2008a).

In the basis of a deep bibliographic survey, there have been many studies conducted on fresh peppers, mainly focused to quantify the antioxidant activity of various crops and the influence of maturity or fertilization type on their bioactive compounds (Marín et al., 2004). However, very little information is available on the effect of processing and/or prolonged storage times on the antioxidant activity of peppers. Therefore, the purpose of this study was to investigate the effect of β-CDs on different quality and nutritional parameters (colour, vitamin C, total phenolics, antioxidant activity) of roast preserved peppers under different storage conditions.

MATERIALS AND METHODS

Sample preparation

Fresh peppers (*Capsicum annuum*, Lamuyo, *Herminio* variety, B_1 type) were obtained in the local market. Only fruits free of blemishes, defects and with the same ripening stage (selected taking into account the CIELAB parameters), were included in the study. Peppers were cut into strips and roasted on a hot plate at 210 °C in the pilot plant of Technological Centre for the Canning and the Food Industry (Murcia, Spain). The position of peppers on the hot plate was changed continuously during roasting. After removing the seeds and peel, they were packaged in trays containing 320 g of pepper and covered liquid (pH of 2.5) at 95 °C until a final weight of 427 g. The trays were vacuum sealed (- 0.55 bars) and sterilized (98 °C and 0.8 bars) in a static retort with superheated

water spraying. A probe was employed to monitor the temperature inside the trays over sterilization and cooling -carried out by a cool water spraying system- steps.

To evaluate the effect of CDs on the stabilization of the pepper quality parameters, two types of samples were elaborated: 1) 5% citric acid in the covering liquid and, 2) 5% citric acid plus 1% β-CDs in the covering liquid. For each determination, three aliquots of each sample were used.

Samples containing or not β-CDs were stored at different temperatures (4, 25 and 50 °C). TP, vitamin C, antioxidant activity and colour of preserved peppers were evaluated (by triplicate) 30-day intervals, (150 days total).

For each analysis, the content of one tray (pepper + covering liquid) was homogenized for 1 min in an Ultra Turrax (Ika, Staufen, Germany). The homogenate was centrifuged at 10.000*xg* for 15 min at 4 °C. The pellet was discarded and the supernatant was filtered through a 0.45 μm membrane filter.

As CDs were added to cover liquid, the filtrate was considered the sample and was used to analyze quality parameters.

Reagents

Standard of vitamin C (ascorbic acid) with a purity of 95%, fluorescein (FL), 2,2'-Azobis (2-methylpropionamide) dihydrocloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox C), were supplied by Sigma–Aldrich (Sigma–Aldrich Chemie GmbH, Germany). Gallic acid was from (Fluka, Steinheim, Germany). β-CDs were from TCI Europe N.V. (Zwijndrecht, Belgium). Double-deionized water was obtained with a Milli-Q system from Millipore (Bedford, MA, USA). All other chemicals used were of analytical grade.

Total phenolic content

Total phenolic content of samples were determined according to a previous method (Sun et al., 2011). In brief, phenolic content was estimated by mixing 200 μL of distilled water, 50 μL of diluted extracts and 50 μL of Folin-Ciocalteu reagent. After 6 min, 500 μL of 7.5% sodium carbonate solution were added to the mixture, which was adjusted to 1.3 mL with distilled water and allowed to stand at room temperature for 60 min. Then, the absorbance of each sample was read at 765 nm in a Shimadzu UV-2401PC spectrophotometer (Kyoto, Japan), and comparing it with a standard curve of gallic acid with concentrations between 10 and 300 mg L^{-1}. The results were expressed as mg of gallic acid equivalents/100 g fresh-weight (FW).

2.4. HPLC analysis of ascorbic acid (AA)

AA concentrations were measured according to Asami et al. (2003) with some modifications . Briefly, after homogenization step (see sample preparation) pellet was discarded and the supernatant filtered through a 0.45 μm poly (tetrafluoroethylene) membrane filter. Samples were diluted 1:1 (v/v) with 4.5% metaphosphoric acid, vortexed and filtered through a 0.45 μm filter prior to injection.

Analyses were performed using a Merck HPLC (Darmstad, Germany) pump equipped with a Shimadzu SPD-M6A UV diode array detector (Shimadzu, Kyoto, Japan). Reverse-phase separation was attained using a Merck (Darmstad, Germany) Lichrospher 100 RP-C18 (25 × 0.4 cm, 5 μm particle size). The mobile phase was nanopure water brought to pH 2.2 with sulphuric acid. The flow rate was 0.5 mL min^{-1}, and the detection wavelength was 245 nm.

All samples were run in triplicate and the linearity range was determined from 0.005 to 0.1 mg mL^{-1} ($R^2 = 0.9995$), with a 20 μL injection volume.

ORAC-FL assay

The oxygen radical absorbance capacity (ORAC) analyses were carried out on a Synergy HT multi-detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, Vt, USA), using 96-well polystyrene microplates with black sides and clear bottoms, purchased from Nalge Nunc International. Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5, version 2.01, software. The ORAC was determined as described by Dávalvos et al. (2004) with slight modifications. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL. 100 μL FL (3 nM, final concentration) and 70 μL of the pepper extract were placed in the wells of the microplate. The mixture was pre-incubated for 30 min at 37 °C, before rapidly adding the AAPH [(2,2′-Azobis (2-methylpropionamide) dihydrocloride, 98%)] solution (30 μL; 19 mM, final concentration) using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence recorded every 1.14 min for 120 min. The microplate was automatically shaken prior to each reading. A blank with FL and AAPH using sodium phosphate buffer instead of the antioxidant solution, and eight calibration solutions using Trolox C (6.25, 12.5, 15, 18.75, 21.25, 25, 27.5 and 31.25 μM) as antioxidant were also used in each assay. All reaction mixtures were prepared in triplicate and at least three

independent assays were performed for each sample. In order to avoid a temperature effect, only the inner 60 wells were used for experimental purposes, while the outer wells were filled with 200 µL of distilled water.

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. Data processing was performed using Sigmaplot software package (Jandel Scientific, Germany).

Colour measurement. After homogenization step (see sample preparation), triplicate samples of homogenate (pepper + covering liquid), were immediately evaluated at 25 ± 1 °C for colour, using a Hunterlab Colorflex® (Reston, Virginia, U.S.A.) on the basis of the CIELAB colour system (L*, a*, and b*). This spectrophotometer uses both illuminant D_{65} and a 10° observer angle, as references.

Colour data are provided as CIEL*a*b* coordinates (Bower & Baxter, 2000) which define the colour in a three-dimensional space: L^* indicates lightness and a^* and b^* are the chromaticity coordinates, green-red and blue-yellow coordinates, respectively. L^* is an approximate measurement of luminosity, which is the property according to which each colour can be considered as equivalent to a member of the grey scale, between black and white, taking values within the range 0-100: a^* takes positives values for reddish colours and negative values for the greenish ones, whereas b^* takes positive values for yellowish colours and negative values for the bluish ones.

C^* is chroma $[C^* = \sqrt{(a^{*2}) + (b^{*2})}]$, with 0 at the centre of a colour sphere and increasing according to the distance from the centre. The total colour difference (ΔE^*) was calculated as:

$$\Delta E^* = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

Statistical analysis. All analyses were performed in triplicate and the results were expressed as mean ± standard deviation. The results were analysed using SPSS 17 statistical software package (IBM Corporation, Armonk, New York, United States) and means were accepted as significantly different at 95% confidence interval ($p < 0.05$). Analysis of variance (ANOVA) was performed to determine the effect of storage time at 4, 25 or 50 °C on the CIEL*a*b* colour coordinates, in the absence and in the presence of 1% β-CDs.

RESULTS AND DISCUSSION

TP content, vitamin C, antioxidant activity and colour evolution were studied in samples of roast preserved peppers, packaged in the absence and in the presence of β-CDs in the covering liquid. The effect of storage temperature and time on those parameters was also evaluated.

Total phenolic content has a great importance for the organoleptical properties of vegetables, and therefore in food quality because they are highly sensitive to external agents such us oxygen, light and temperature (Lucas-Abellán et al., 2007; Tomás-Barberan and Espín, 2001). Recently, CDs have been used as protective agents of antioxidant compounds of mandarin juices enriched with pomegranate extract and goji berries (Navarro et al., 2011), in order to increase their stability.

Total phenolic content of roast preserved peppers varied with temperature and storage time. In samples stored at 4 °C, no significant differences were observed in TP during the five months of the experiment both in the absence and presence of β-CDs (Figure 1, squares).

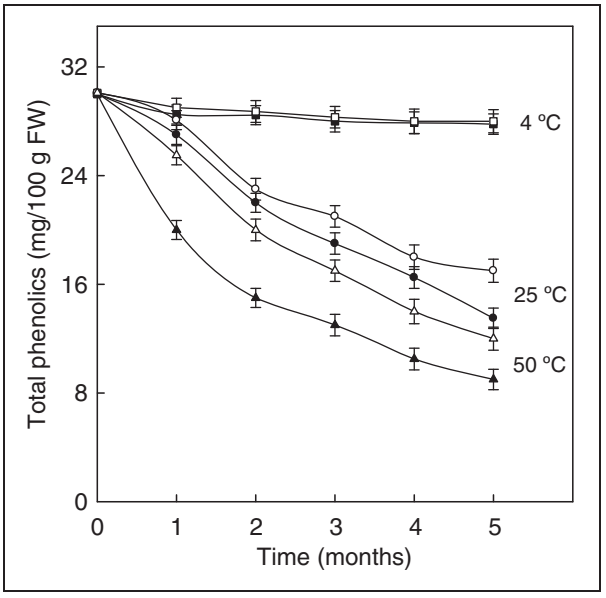

Figure 1. Effect of temperature and storage time on the total phenolic content of roast preserved peppers. [(■) 4 °C, in absence of 1% β-CDs; (□) 4 °C, in the presence of 1% β-CDs; (●) 25 °C, in absence of 1% β-CDs; (○) 25 °C, in the presence of 1% β-CDs, (▲) 50 °C, in absence of 1% β-CDs (Δ) 50 °C, in the presence of 1% β-CDs].

However, in samples stored at 25 °C, an important decrease was observed in absence (Figure 1, filled circles) or presence of 1% β-CDs (Figure 1, open circles). In this case, a reduction of 55% in total phenolic content in the absence of β-CDs and 45% in the presence of 1% β-CDs was observed.

This decrease in total phenolic content was more acute in samples stored at 50 °C. In this case, the presence of 1% β-CDs in the covering liquid had a clear protective effect on total phenolic content, minimising their reduction with storage time from the first month onwards (Figure 1, triangles). After 5 months study, the reduction in total phenolic content was 60% in the presence of β-CDs (Figure 1, open triangles) and 70% in the absence of β-CDs (Figure 1, filled triangles).

As have been described in the literature, significant losses of phenolic compounds by industrial heat processing and storage have been reported in peppers (green, yellow, and red), squash, leek, and other vegetables (Chuah et al., 2008; Turkmen et al., 2005). As we have evidenced, reduction of total phenolic content increases with storage temperature and time, achieving CDs a 10% reduction of total phenolic losses in the most adverse storage conditions (50 °C, five months). This fact could be explained considering that temperature improve the migration and dissolution of phenolics compounds into the covering liquid, thus being more exposed to the effect of certain residual enzymatic activity, oxygen or light in packs that do not contain CDs. In resume, these results confirm that β-CDs have a protective effect in phenolic compound degradation, as previously have been described by our group (Núñez-Delicado et al., 2005), due to their entrapment in the internal hydrophobic cavity of CDs.

Vitamin C level in vegetables depends on several factors (Lee and Kader, 2000; Pérez-López et al., 2008), being peppers the vegetables with the highest content. In addition, the atmospheric oxygen is responsible for much of vitamin C losses during storage and many polymeric packaging allow oxygen flow.

In this study, the changes in the vitamin C content with storage time and temperature were compared in roast preserved peppers packaged in absence and in the presence of β-CDs in the covering liquid.

As described before for total phenolic content, no significant changes were observed at 4 °C during the 5 months, whether in the absence or in the presence of β-CDs (Figure 2, squares).

During storage at 25 °C, a considerable decrease in the vitamin C content was observed for both samples (fortified or not wit β-CDs) as time progressed. However, the decay rate of vitamin C was much lower in the presence of 1% β-CDs (Figure 2, open circles) than in their absence (Figure 2, filled circles).

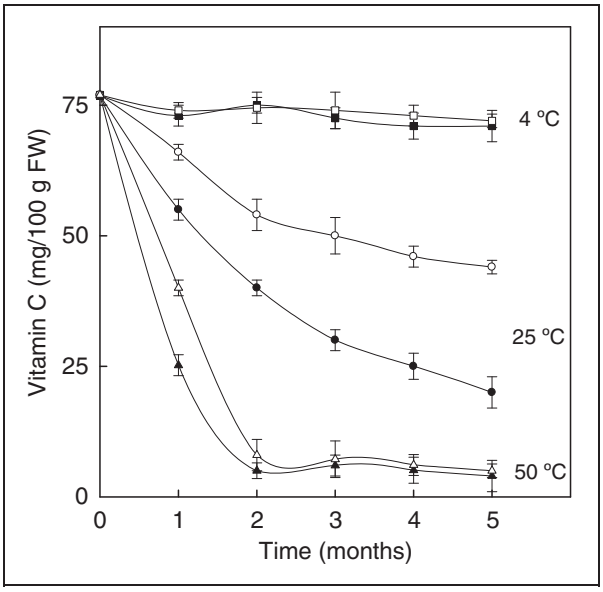

Figure 2. Effect of temperature and storage time on vitamin C content of roast preserved peppers. [(■) 4 °C, in absence of 1% β-CDs; (□) 4 °C, in the presence of 1% β-CDs; (●) 25 °C, in absence of 1% β-CDs; (○) 25 °C, in the presence of 1% β-CDs, (▲) 50 °C, in absence of 1% β-CDs (△) 50 °C, in the presence of 1% β-CDs].

After 5 months of storage, the reduction of vitamin C content was 43% in the presence of 1% β-CDs (Figure 2, open circles) and 74% in the absence of β-CDs (Figure 2, filled circles), pointing to a protective effect of CDs on vitamin C due to its secondary antioxidant role, previously described in the literature (Núñez-Delicado et al., 1997). β-CDs acts by complexing phenolic compounds, inhibiting their oxidation (Figure 1) as well as preventing vitamin C degradation (Figure 2).

The reduction of vitamin C content in samples stored at 50 °C was more pronounced than that observed at 25 °C (Figure 2, triangles). After 2 months of storage, the vitamin C content close to zero, both in the absence and presence of β-CDs. In these adverse conditions, the protective effect of CDs was negligible (Figure 2, triangles). Only after 1 month of storage, the presence of 1% β-CDs show a protective effect on vitamin C, which decreased up to 80% in the absence of CDs and close to 70% in their presence.

These data demonstrate that β-CDs exert a protective effect on vitamin C, similar to that described for phenolic compounds. Our data are in consonance with the results obtained for Romo-Hualde et al. (2012), who propose the encapsulation as an excellent method to increase the stability overtime of vitamins A and E in red pepper extract.

The antioxidant capacity is an interesting parameter for evaluating the nutritional quality of foods

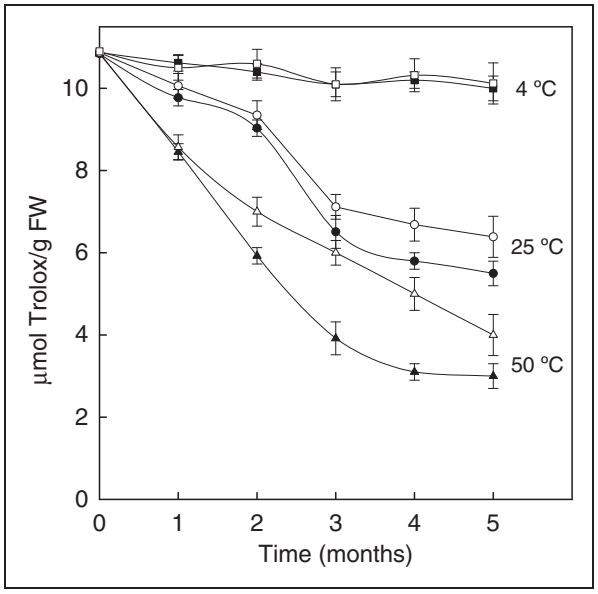

Figure 3. Effect of temperature and storage time on antioxidant activity of roast preserved peppers. [(■) 4 °C, in absence of 1% β-CDs; (□) 4 °C, in the presence of 1% β-CDs; (●) 25 °C, in absence of 1% β-CDs; (○) 25 °C, in the presence of 1% β-CDs, (▲) 50 °C, in absence of 1% β-CDs (△) 50 °C, in the presence of 1% β-CDs].

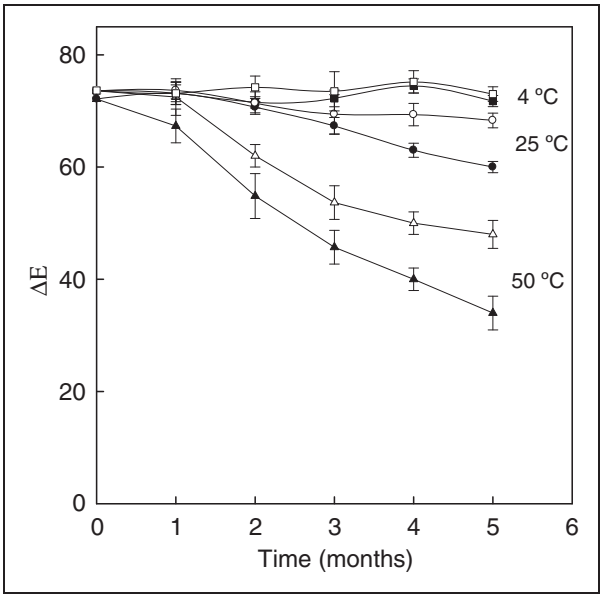

Figure 4. Effect of temperature and storage time on ΔE value of roast preserved peppers. [(■) 4 °C, in absence of 1% β-CDs; (□) 4 °C, in the presence of 1% β-CDs; (●) 25 °C, in absence of 1% β-CDs; (○) 25 °C, in the presence of 1% β-CDs, (▲) 50 °C, in absence of 1% β-CDs (△) 50 °C, in the presence of 1% β-CDs].

(Arnao et al., 1998). The antioxidant activity of fruits and vegetables comes from vitamins C and E, *β*-carotene and polyphenols (flavonols, flavanols, anthocyanidins) (Rice-Evans, 2001). While thermal processing of fruits and vegetables at high temperatures removes the possibility of microbiological damage and reduces the enzymatic activity, it also affects product quality and its antioxidant activity (Siddiqa et al., 2013).

When the antioxidant activity of samples stored at 4 °C was measured, no clear decrease was observed with the storage time whether or not the covering liquid contained CDs (Figure 3, squares), a result that was similar to that described for the evolution of total phenolic content (Figure 1) and vitamin C (Figure 2).

When samples were stored a 25 °C, while the antioxidant activity decreased with the storage time, both in the absence (Figure 3, filled circles) and presence of β-CDs (Figure 3, open circles), the decrease was higher in the absence of β-CDs. Whereas in the absence of CDs the antioxidant activity after a storage period of 5 months had decreased by 50%, in the presence of 1% β-CDs the decrease was only 39%.

The decrease in antioxidant activity was much pronounced in the case of samples stored at 50 °C. After 3 months of storage, the reduction in the antioxidant activity was 64% in the absence (Figure 3, filled triangles) and 40% in the presence of 1% β-CDs (Figure 3, open triangles).

In others words (Heghes et al., 2015), the antioxidant activity observed behaved in the same way as total phenolic content and vitamin C, again confirming the protective effect of 1% β-CDs in the covering liquid over all compounds involved in the antioxidant activity of peppers (Figure 4).

As previously mentioned, colour is an important issue in peppers, for which reason the colour parameters L^*, a^* and b^*, were measured in samples stored at different temperatures (Table 1). In the case of samples stored at 4 °C, no significant differences were observed ($p > 0.05$) during the five months of storage with and without 1% β-CDs (Table 1), since the colour remaining constant in both cases.

When L^*, a^* and b^* values were studied in samples stored at 25 °C (Table 1), the values recorded after 5 months, in the presence of 1% β-CDs were significantly different from the control (L^* 31.16 ± 1 *vs* 29.28 ± 0.7; a^* 47.21 ± 1.3*vs* 43 ± 1.2; b^* 45.80 ± 0.9 *vs* 42.38 ± 0.8). There were significant differences between samples stored at 25 °C with or without 1% β-CDs ($p < 0.001$, for L^* and a^* and $p < 0.05$ for b^*). Nevertheless, after 5 months, the C^* value was higher in the presence (60.37 ± 0.8) than in absence of 1% β-CDs (52.38 ± 1), with a significant difference between them ($p < 0.001$), the presence of 1% β-CDs leading to a more intense red colour due to the minor browning.

As described by Casquero et al. (2010), who carried out a similar experiment at storage temperatures of 8 °C

Table 1. Effect of storage times at 4, 25 and 50 °C on the CIE*L*a*b** colour coordinates, in absence and in the presence of 1% β-CDs

CIEL*a*b*	4 °C				25 °C				50 °C			
Parameters	0 months	p	5 months	p	0 months	p	5 months	p	0 months	p	5 months	p
L*	31.01 ± 1.1	NS	29.62 ± 1.2	NS	31.01 ± 1.1	NS	25.00 ± 1.3	***	31.01 ± 1.1	NS	16.00 ± 1.3	***
L* **(1% β-CDs)**	31.50 ± 1.3		29.30 ± 2.3		31.50 ± 1.3		29.28 ± 0.7		31.50 ± 1.3		24.00 ± 1.2	
a*	46.48 ± 0.7	NS	46.90 ± 0.7	NS	46.48 ± 0.7	NS	38.59 ± 1.1	***	46.48 ± 0.7	NS	18.82 ± 1.3	***
a* **(1% β-CDs)**	46.92 ± 1.1		47.30 ± 1.3		46.92 ± 1.1		43.00 ± 1.2		46.92 ± 1.1		28.00 ± 1.0	
b*	47.15 ± 2.0	NS	45.50 ± 0.8	NS	47.15 ± 2.0	NS	40.48 ± 1.0	*	47.15 ± 2.0	NS	22.70 ± 1.1	***
b* **(1% β-CDs)**	47.65 ± 2.0		45.28 ± 0.9		47.65 ± 2.0		42.38 ± 0.8		47.65 ± 2.0		30.00 ± 1.3	
C*	66.72 ± 1.5	NS	65.34 ± 1.2	NS	66.72 ± 1.5	NS	52.38 ± 1.0	***	66.72 ± 1.5	NS	29.48 ± 0.7	***
C* **(1% β-CDs)**	67.02 ± 1.3		65.48 ± 1.0		67.02 ± 1.3		60.37 ± 0.8		67.02 ± 1.3		41.03 ± 1.0	

Level of significance (p): NS (not significant = $p > 0.05$); Statistically significant: *($p \leq 0.05$); ***($p \leq 0.01$); All samples were run in triplicate.

and 18 °C for 18 days, the colour of roasted pepper was affected positively (first month) by storage conditions (4 °C and 25 °C in our study, filled and empty squares and circles, respectively of Figure 4). However, when the time storage was prolonged the effect was the contrary, diminishing the colour proportionally to storage time. In fact, taking into account storage temperature of 25 °C in absence of 1% β-CDs (Figure 4 filled circles), the value of ΔE parameter decreased as follow: 72,12; 73,12; 70, 67; 67,34; 65,42 and 64,66 for 0; one month; two months; three months; four months and five months, respectively (see Figure 4), demonstrating that after third month, the colour in preserved peppers was negatively affected.

However, in the same conditions as described above (25 °C as storage temperature), the addition of 1% β-CDs (Figure 4 empty circles), showed a soft decrease of ΔE parameter (73.5; 73.6; 71.4; 69.4; 69.3; 68.3; respectively).

In addition, although only one type of treatment was used in our study, also roasting techniques employed should be taking into account since could negatively affect the colour of preserved pepper, as have been previously described by Guerra et al. (2011).

As can be seen in Table 1, in the case of samples stored at 50 °C in the absence of 1% β-CDs, *L** fell by 50% (from 31.65 ± 0.9 to 16.00 ± 1.3) after 5 months storage, while in the presence of 1% β-CDs, the decrease was only 24% compared with the control (from 31.65 ± 0.9 to 24.00 ± 1.2), the difference between samples stored with or without 1% β-CDs being statistically significant ($p < 0.001$). In addition, the colour of the samples was darker in the absence (*L** 16.00 ± 1.3) than in the presence of 1% β-CDs (*L** 24.00 ± 1.2).

As regards the green-red coordinate *a** (−a green, +a red), the lowest value was obtained after 5 months storage in the absence of 1% β-CDs (18.82 ± 1.3). The reduction of 59.5% reflected the browning process that took place during storage. In the presence of 1% β-CDs, the decrease in *a** value after 5 months was 40.5% (compared with 59.5 in the absence of 1% β-CDs), indicating the protective effect that β-CDs have on the browning process of pepper; the difference between samples stored at 50 °C with or without 1% β-CDs was significant ($p < 0.001$). The protective effect that β-CDs play on phenolics compounds, reduce the free radical generation and so avoid the browning process, acting thus CDs as secondary antioxidant agents. The greatest browning was observed in absence of 1% β-CDs and high temperatures.

Similar trends to those described for *L** and *a** coordinates were also found for the blue-yellow coordinate b*. In the case of samples stored a 50 °C, *b** decreased with time, both in the absence and in the presence of 1% β-CDs, although the decrease was greater in the absence (52%) than in the presence of 1% β-CDs (36.4%) (see Table 1), a difference that was statistically significant ($p < 0.001$). This decrease also pointed to the browning process that took place in samples stored at 50 °C, both in absence and in the presence of 1% β-CDs, because the b* values were near the centre of the colour sphere.

In general, samples with the most attractive colour have the highest *C** values. As can be seen in Table 1, when the evolution with time of *C** value was studied in samples stored at 50 °C, a noticeable decrease was observed, both in the absence and in the presence of 1% β-CDs. The *C** value was significantly lower ($p < 0.001$) in samples stored for 5 months at 50 °C in the absence (29.48 ± 0.7) than in the presence (41.03 ± 1.0) of 1% β-CDs. After 5 months, the reduction in *C** value was 55.8% in the absence and 38.5% in the presence of 1% β-CDs, indicating that the browning process was more intense in the absence of 1% β-CDs.

The ΔE value integrates a^*, b^* and L^* in a unique value. As can be seen in Figure 4, in samples stored at 4 °C no significant differences in the ΔE value were observed during the 5 months of storage, whether or not 1% β-CDs was used (Figure 4, squares).

When the evolution with time of ΔE values (see colour measurement) were studied in samples stored a 25 °C, a slight decrease was observed, both in absence and in the presence of 1% β-CDs (Figure 4, circles), the decrease being more acute in the absence of CDs (Figure 4, filled circles).

This decrease in ΔE value was even more pronounced in samples stored at 50 °C, both in the absence and in the presence of 1% β-CDs (Figure 4, triangles), which reflects the darkening of samples. After 5 months, the reduction in ΔE was greats in the absence (Figure 4, filled triangles) than in the presence of CDs (Figure 4, open triangles). Therefore, the presence of 1% β-CDs delayed the darkening of samples. This protective effect of β-CDs on the colour of samples has been previously described (Núñez-Delicado et al., 1997). In summary, complexation of the compounds involved delayed the browning process.

CONCLUSIONS

The content of TP, vitamin C, antioxidant activity and colour of roast preserved peppers decreased with storage time and temperature.

The addition of 1% β-CDs to the covering liquid of roast preserved peppers had a significant protective effect on the phenolic compounds and vitamin C content, the antioxidant activity and colour of the product.

The evaluation of the instrumental colour parameters demonstrated higher stability of the colour indices when β-CDs were included into the covering liquid. The colour changes were in line with those observed for total phenolics and confirmed a higher suitability to storage at room temperature of roast preserved peppers with 1% β-CDs.

Complexation of the compounds involved delayed the browning process. Therefore, the addition of β-CDs may be useful for the application as natural additives in foods.

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