



Quality stability of clear pomegranate juice treated with cyclodextrin

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Abstract Protective effect of cyclodextrin (CD) on some quality characteristics of clear pomegranate juice (CPJ) was investigated. CPJ with no CD addition (Control) and CPJ samples, subjected to different type (β -CD and Hydroxy propyl (HP- β -CD) and concentration (0.5, 1% and 2%) combinations of CD, were stored at 25 °C for 3 months. The changes in color characteristics (polymeric color and total color difference – ΔE), total phenolic content (TPC) and antioxidant activity (AA) of all PJ samples monitored at 1 month intervals. Although overall color change of the PJ samples was hardly perceived visually, none of the treatments were successful to protect the initial color of clear PJ technically ($P > 0.05$). Among the CD treatments, HP- β -CD with a concentration of 0.5% was found effective to prevent TPC and AA losses of PJ ($P < 0.05$). On the other hand, β -CD addition did not cause any improvement in protecting color, TPC and AA of clear PJ during storage, compared to control juice.

Keywords Antioxidant activity · Color · Cyclodextrin · Clear pomegranate juice · Storage

Introduction

Pomegranate (*Punica granatum* L) fruit has been accepted as a functional food due to its high phenolic content (Poyrazoğlu et al. 2002) and antioxidant activity (Gil et al. 2000). The whole fruit, as well as its juice and other extracts have been shown anti-atherogenic (Aviram et al. 2008), anti-oxidative (Rosenblat and Aviram 2006) and anti-inflammatory (Shukla et al. 2008) effects in different in vivo and in vitro studies. Therefore, compositional changes and nutritional losses of this fruit and its other products during maturation (Al-Maiman and Ahmad 2002), processing (Turfan et al. 2011) and storage (Öziyci et al. 2013) have been of interest among researchers.

To reduce food quality loss during processing and storage; natural molecules called cyclodextrins (CD) are being used in food industry (Szejtli 1998). CDs have different preserving effects on food quality such as controlling enzymes responsible for browning in foods such as polyphenol oxidase (PPO) (Billaud et al. 1995; López-Nicolás et al. 2007a, b), stabilizing color of pear and chokeberry juices by keeping anthocyanins from degradation or polymerization (Howard et al. 2013; López-Nicolás and García-Carmona 2007) and protecting nutritional value of fruit juices by preventing Vitamin C and antioxidant activity losses (Navarro et al. 2011a). On the other hand, to our knowledge, no published data exists about investigating the protective effect of cyclodextrins on a dark colored juice that is produced from a single variety.

The main objective of this study was to investigate the protective effect of CD on some quality characteristics of clear pomegranate juice. To this end; two different CD types (β - and 2-Hydroxypropyl- β -CD) at three different concentrations (0.5, 1 and 2%) were used. Also in the study, to be able to depict losses on an industrial scale,

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clear pomegranate juice was produced and processed according to the procedure steps used in an industrial fruit juice factory. Furthermore, the temperature of 25 °C at which the processed fruit juices are most stored at the points of sale, was chosen as the temperature parameter that the juice quality would be observed in the storage period. Polymeric color, total color difference (ΔE), total phenolic content and free radical scavenging activity were monitored as juice quality parameters in production and storage periods (3 months) of clear PJ.

Materials and methods

Chemicals and reagents

Gallic acid standard, 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical, β - and 2-Hydroxypropyl (HP)- β -cyclodextrins were purchased from Sigma-Aldrich (Steinheim, Germany). Folin–Ciocalteu reagent, sodium carbonate, sodium hydroxide and methanol were purchased from Merck (Darmstadt, Germany).

Fruit juice production and processing

Raw pomegranate juice (RPC), clear PJ that was used as control (C) and other CD-treated PJ samples (0.5B, 1B, 2B, 0.5H, 1H and 2H) were produced in a fruit juice factory of Dimes Gıda Sanayi ve Ticaret A.Ş. company located in Tokat, Turkey. Seeds of pomegranate fruit (*Punica granatum* L., Hicaz variety) was used for the juice production. After performing peel removal and seed granulation steps with the system designed by the same company, RPJ was extracted from granulated pomegranate seeds by using a horizontal press (Bucher-Guyer, AG, Switzerland). Hot clarification treatment (50 °C, 2 h) was accomplished with adding pre-determined volumes of clarifying agents [bentonite (1.2 g/L), gelatin (0.4 g/L), and kieselsol (1 g/L) solutions] to the RPJ. At the end of the clarification process; the juice was filtered through cellulose filter, then divided into different parts for CD treatments. One part of the juice was pasteurized directly without any type of cyclodextrin (CD) addition [Control (C) group]. Remaining juice parts were treated with two different CD types [β - and Hydroxypropyl (HP)- β] in different concentrations (0.5, 1 and 2%; w/v), and shaken 1 h for fully solubilization at room temperature. Then the samples were filled to the poly-carbon glass bottles and subjected to the pasteurization process (85 °C, 15 min).

Because pomegranate juice is highly acidic in nature (also not included in this study, pH of all PJ samples was around 3.30), the heating temperature used to prevent microbial growth during the storage period was enough,

therefore no extra preservative was added to all PJ samples before pasteurization step. After cooling to the room temperature, pasteurized juice samples were kept at 25 °C, during the 3-month storage period (Fig. 1). For the monthly analyses, the pasteurized and bottled fruit juices kept in the incubator were taken out as needed and these bottles were opened for the first time during the analyses and the remaining parts were discarded after the analyzes.

Color characteristics

Total color difference (ΔE)

Total color difference (ΔE) value was one of the color characteristics measured in this study to indicate the magnitude of color change during storage of all PJ samples. For this purpose; L, a, b values [L (+): lightness, L (−): darkness; a (+): redness, a (−): greenness; b (+): yellowness, b (−): blueness] were recorded in the CIE-L*, a*, b' uniform color space system by using a CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan) with a D65 illuminant and a 2° observer angle at 25 °C.

Obtained L, a, b values were put into the following Eq. (1) to calculate ΔE values (Karangwa et al. 2012):

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2 \right]^{1/2} \quad (1)$$

Polymeric color (PC)

Polymeric color (PC) was another color characteristic in this study to interpret polymerization of PJ color pigments during storage. Giusti and Wrolstad's (2001) analytical method was used to determine PC. All samples were diluted with distilled water in order to get an absorbance reading between 0.5 and 1.0 during spectrophotometric (UV–Vis 160A Shimadzu, Japan) measurements. For the analysis; 2.8 ml of each diluted sample was transferred to the two spectrophotometric cuvettes, then 0.2 ml of bisulfite solution (1 g $K_2S_2O_5$ /5 ml distilled water) was added to the one cuvette (bisulfite-bleached sample) and 0.2 ml of distilled water was added to the other cuvette (non-bleached control sample). After equilibrating for 15 min, absorbance values were measured at $\lambda = 700$ (to correct for haze), 523 ($A_{\lambda \text{ vis-max}}$) and 420 nm (index for browning). PC values were calculated according to following Eq. (2):

$$\text{Polymeric Color (PC)} = [(A_{420 \text{ nm}} - A_{700 \text{ nm}}) + (A_{\lambda \text{ vis-max}} - A_{700 \text{ nm}})] \times DF \quad (2)$$

where DF is the dilution factor

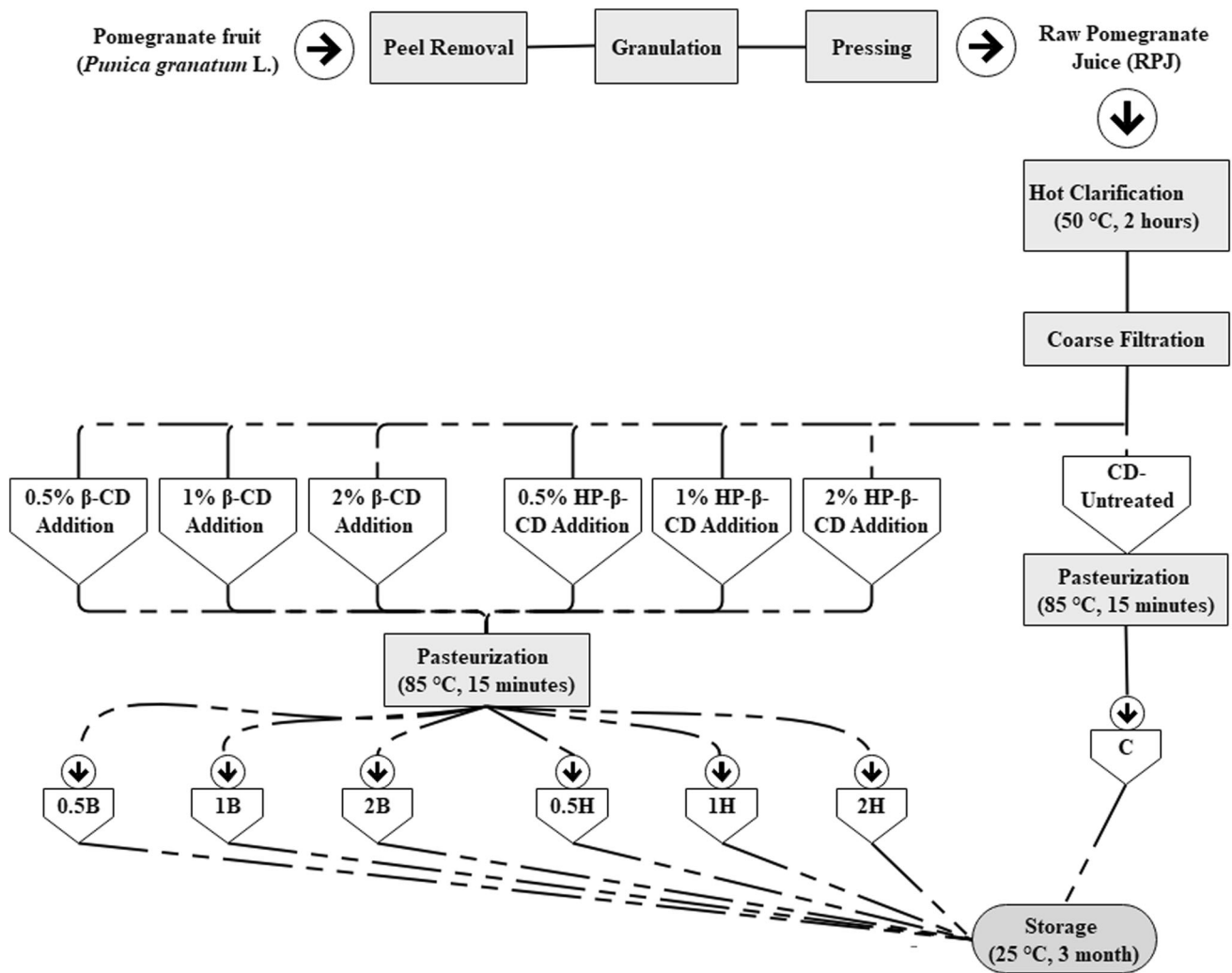


Fig. 1 Production of all PJ samples

Total phenolic content (TPC)

Total phenolic contents (TPC) of all samples were determined with the Folin–Ciocalteu’s reagent method (Javanmardi et al. 2003; Spanos and Wrolstad 1990). Accordingly, 2.5 ml of 1/10 dilution of Folin–Ciocalteu’s reagent and 2 ml of Na₂CO₃ (7.5%, w/v) were added to 50 µl of each sample. Total mixture (5 ml) was mixed well by using a vortex then incubated at 45 °C for 15 min. After cooling to the room temperature, absorbance values were measured at 765 nm with a spectrophotometer (Shimadzu UV-160A, Japan).

TPC values were calculated by using the calibration curve equation obtained by plotting the absorbance values of different concentrations (1–100 ppm) of gallic acid standard. Results were expressed as mg gallic acid equivalents (GAE) per liter fruit juice (mg GAE/L).

Free radical scavenging activity

Free radical scavenging activity, namely antioxidant activity of all PJ samples was determined and calculated according to DPPH radical scavenging method used in the studies published by Dinçer et al. (2013) and Sharma et al. (2015). For preparing a series of five different concentrations; first, each juice sample was diluted in an appropriate ratio with distilled water (at a reduction rate of 10–90% in absorbance). Then, 0.1 ml of each diluted sample was mixed with 4 ml of DPPH methanolic solution (6×10^{-5} M) and after shaking vigorously with a vortex, left to stand in a dark place during 30 min.

The absorbance values of each diluted juice sample with DPPH (A_s) and only DPPH solution without sample (Control—A_c) were recorded during spectrophotometric measurements at 516 nm (Shimadzu UV-160A, Japan). The percentage of DPPH radical scavenging activity was calculated with the following Eq. (3):

$$\text{DPPH radical scavenging activity (\%)} = [(Ac-As)/Ac] \times 100 \quad (3)$$

To determine the sample amount necessary to scavenge 50% of DPPH free radicals (called IC₅₀), the percentage of DPPH radical-scavenging activity was plotted against the sample concentration. Lower IC₅₀ value indicates higher antioxidant activity. IC₅₀ was expressed as µL sample per mg DPPH.

Statistical analysis

All statistical analyses were performed by GraphPad Prism software. The results (three times; two repetitions per each) were expressed as Mean ± S.D. Non-normally distributed data were analyzed by means of non-parametric Kruskal–Wallis ANOVA on ranks with Dunn's post hoc test (including a correction for multiple comparisons). A *P* value less than 0.05 was considered as significant.

Results and discussion

Compositional change: from raw to clear

Processes such as peel removal, granulation, pressing and any heating process such as clarification or pasteurization are known to decrease/increase the initial values of quality characteristics in fruit juices. Also in this study, mainly these processing steps caused to the compositional changes in the pomegranate juice (Table 1).

In this study, both hot clarification and pasteurization processes increased polymeric color of RPJ (approximately 8%) when compared to “C” sample (*P* < 0.05; Table 1). Heating generally causes anthocyanin loss and polymeric color increase (Howard et al. 2013).

Also, even statistically not important (*P* > 0.05), there was a slight decrease (approximately 3%) in TPC concentration when RPJ subjected to different processes to produce clear PJ (Table 1). Juice production steps such as pressing, clarification (due to the gelatin-polyphenol flocculation) and thermal treatment can reduce phenolic compounds (Alper et al. 2005), (Lee et al. 2002) and antioxidant activity (Guiné and Barroca 2014) in fruit

juices. IC₅₀ value of the pasteurized, clear PJ (C) was higher than the value of RPJ means that the antioxidant activity of RPJ decreased significantly when the juice was subjected to hot clarification and pasteurization processes in order to produce clear PJ ready to be sold. This was due to the TPC loss during clarification and pasteurization (*P* < 0.05; Table 1).

Change in chemical composition of all pomegranate juice samples during storage

Polymeric color

Anthocyanins are the major color pigments responsible for red color of PJ (Gil et al. 1995). During storage, total concentration of these pigments can decrease due to the factors such as storage temperature (Alighourchi and Barzegar 2009), ascorbic acid (Choi et al. 2002) etc. This decrease is mainly due to not their loss but polymerization (Ochoa et al. 1999). The parameter of polymeric color (PC) can be used to assess this color loss. Because it is a measure of the pigment resistant to bleaching and indicates anthocyanin polymerization, to some degree.

When the change in the PC values of the samples during the storage period of 3 months is evaluated; the highest increase in the polymeric color occurred in the 0.5% HP-β-CD treated PJ (Fig. 2; *P* < 0.05). PC values of other CD treatments showed the same increase at the same level as control except this treatment, there was no difference among other treatments and control (Fig. 2; *P* > 0.05). As understood from these results; CD treatment (both β and HP-β), with the concentrations (0.5–2%) used in this study was not effective to prevent color loss, namely anthocyanin polymerization in PJ during storage. These results were similar with the findings that Howard et al. (2013) studied. They studied the effects of β-CD and pH on total anthocyanin content and % PC of pasteurized chokeberry juice stored at 25 °C and 4 °C over 8 months. According to their results; although the 0.5 and 1% of β-CD treatments did not help to antocyanins retention in chokeberry juice and thus increase in % PC was higher at these CD concentrations, a 3% of β-CD treatment was found as effective in protection of antocyanins from degradation. They also reported greater anthocyanin degradation at ambient storage temperature, as found the same in this study. Higher storage temperatures (more than 4 °C) are known to cause faster PC changes (Aşkın and Atik 2016).

Processed fruit juices are usually stored at room temperature (25 °C) at points of sale, however, as may be seen with the findings of this study, this high storage temperature will decrease the nutritional quality of the juice therefore, more attention should be paid while storing the fruit juices at cooler temperatures until consumption.

Table 1 Compositional change of raw and clear pomegranate juices

Sample	PC* (%)	TPC* (g GAE/L)	IC ₅₀ (µl PJ/mg DPPH)
RPJ	2.05 ^a ± 0.01	7.02 ^a ± 0.13	14.23 ^a ± 0.02
C	2.15 ^b ± 0.05	6.85 ^a ± 0.05	15.82 ^b ± 0.56

*Means with the same letter in a column, are not significantly different from each other (*P* > 0.05)

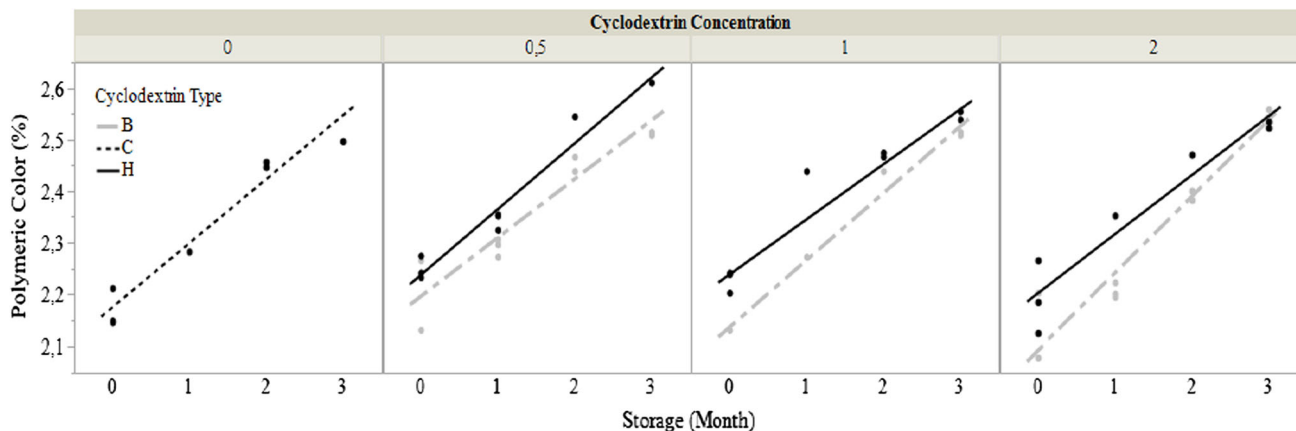


Fig. 2 Polymeric color change of CD-treated and untreated PJ during storage

Total color difference

Total color difference (ΔE) is a color characteristic to understand how the human eye perceives color difference. In this study, ΔE was also monitored to assess the color change in CD-treated and untreated PJ samples during storage. According to the results of this study; none of the CD treatments (different CD type and concentrations) was effective to protect the initial color of clear PJ during storage ($P > 0.05$; Fig. 3). Besides, especially in HP- β -CD treatments, concentration of this CD more than 1% increased more the ΔE values when compared to the CD-untreated PJ (control sample), namely, rather affected negatively the color stability of the juice (Fig. 3, 3rd month). Hot clarification technique and higher storage temperatures more than 4 °C are known as negatively affecting conditions for color stability of clear PJ during storage (Aşkın and Atik 2016; Oziyici et al. 2013).

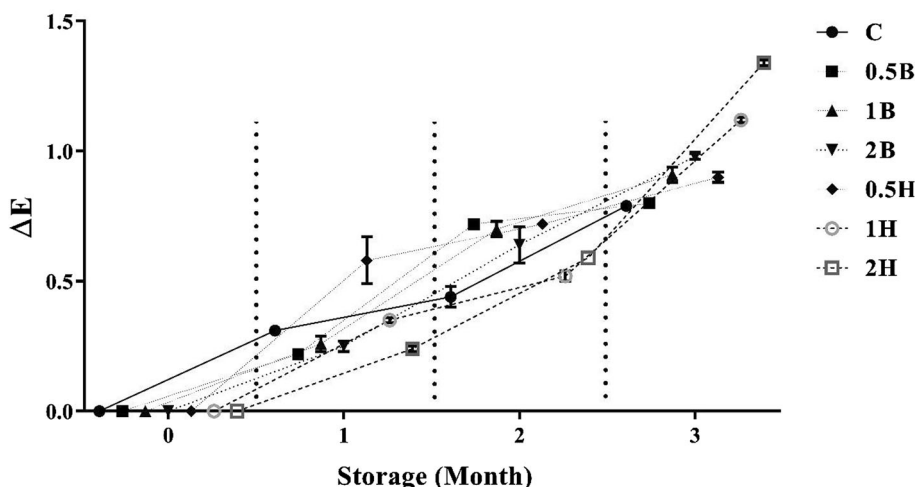
On the other hand, although different CD types and concentrations used in this study were not technically effective in maintaining the color stability of pomegranate

juice (according to the ΔE change), because ΔE values of all PJ samples were so low (max. ΔE value observed < 1.5) it is considered that consumers will not be able to distinguish visual color discoloration of pomegranate juice easily during storage. Because only experienced observers can notice the difference of $1 < \Delta E < 2$ (Mokrzycki and Tatol 2011).

Navarro et al. (2011a) investigated the effect of CD type on sensorial attributes of mandarin juice enriched with pomegranate juice extract and goji berries juice and reported that although β -CD addition was ineffective, HP- β -CD addition lowered losses of some quality attributes as well as the sensorial color intensity compared to the control juice (no CD added).

There are different studies in the literature reporting contribution of the use of β -CD on the overall quality of some fruit juices. For example; Andreu-Sevilla et al. (2011a) studied the effect of the presence or absence of β -CD on sensory quality, volatile compounds and color of pear juice and found that a concentration of 15 mM (approximately 1.7%), β -CD increased significantly the global

Fig. 3 Total color difference of all samples during storage



quality, color intensity of pear juice without decreasing its aroma. Andreu-Sevilla et al. (2011b) also studied the effect of natural CDs (α , β and γ) on different properties (such as color, odor and aroma) of pear juice. According to their findings; β -CD was the second one after α -CD causing the least ΔE change due to enzymatic browning of the pear juice during mixing at 25 °C for 40 min.

But in our study, none of the CD treatments (any type or concentration) was found to be sufficient to stabilize the color of clear PJ during storage when compared to the control juice (Fig. 3).

Total phenolic content

Change in TPC of all PJ samples during 3-month storage period is shown in Fig. 4. In this study, we used the β -CD in 0.5, 1 and 2% concentrations but none of the concentrations we have conducted for β -CD showed a protective effect on the phenolic content of the clear pomegranate juice. This can be due to not only due to non-effectiveness of the concentrations we tried in this study but also the complexity of the phenolic compounds that PJ possesses. Navarro et al. (2011b) also reported similar findings to our study. They found that β -CD usage no significant effects on vitamin C, antioxidant activity, carotenoids profile and sensory analysis of pasteurized orange juice during storage at room temperature. In another study, Howard et al. (2013) investigated the stability of chokeberry anthocyanins by β -CD addition and refrigeration and reported that β -CD concentrations less than 3% had no protective effect on anthocyanin stability during storage.

During the storage period, TPC values of all PJ samples were gradually decreased except the 2nd month of the storage. At this month, a slight increase was observed. It is known that β -CD acts as an inhibitor for polyphenol

oxidase (PPO) activity in a single phenolic solution, but in a multi-phenolic solution, addition of β -CD can result both in a large or small decrease or a slight activation of the oxygen uptake which is catalyzed by PPO (Billaud et al. 1995).

On the other hand, only 0.5% HP- β -CD treated PJ had the highest TPC concentration compared to the control juice ($P < 0.05$) and any dose higher than 0.5% in this treatment was not effective to preserve the total phenolic content of PJ ($P > 0.05$).

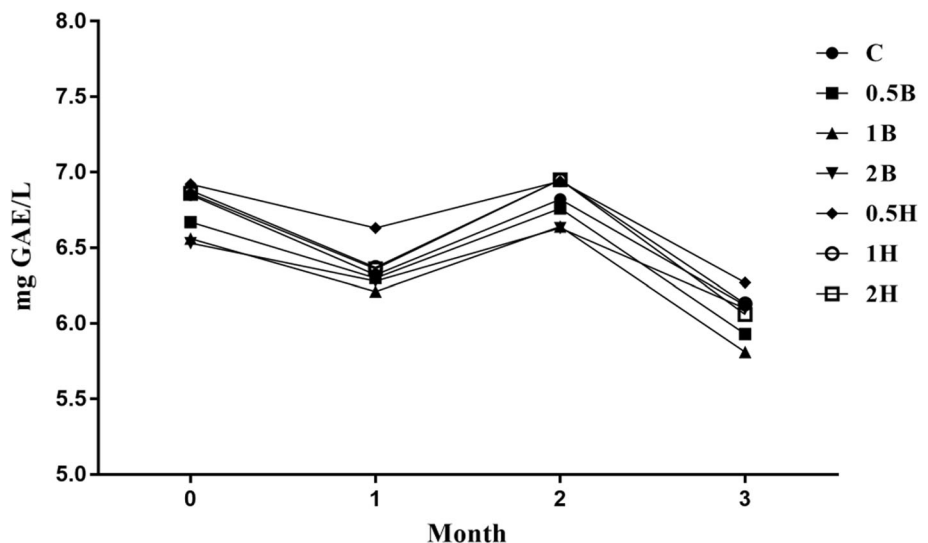
Free radical scavenging activity

Pomegranate juice is reported to have almost three times higher free radical scavenging activity namely, antioxidant activity (AA) than red wine and green tea (Gil et al. 2000). Due to this important functional property of PJ, preserving this functionality during product shelf life is essential for consumers' health.

In order to evaluate the possible protection effect of CD on AA of PJ; change in AA of the samples produced with/without CD addition (in different type and concentration combinations) were monitored during 3-month storage (Fig. 5). The initial IC_{50} levels (14.09–15.82 μ l PJ/mg DPPH) of all PJ samples observed in this study were very low compared to Çam et al. (2009)'s findings (29.8–70.5 μ l PJ/mg DPPH). This difference could be due to the pomegranate fruit varieties (Only Hicaz variety used in this study) and different pomegranate juice production stages (no clarification in Çam et al. (2009)'s study).

When the efficacy of CD treatments was assessed; it was found that among all treatments, only 0.5% HP- β -CD treatment was effective to protect the initial AA of PJ during the whole storage period ($P < 0.05$). But also in this CD-type, higher concentrations used more than 0.5% were

Fig. 4 Change in total phenolic content of all samples during storage



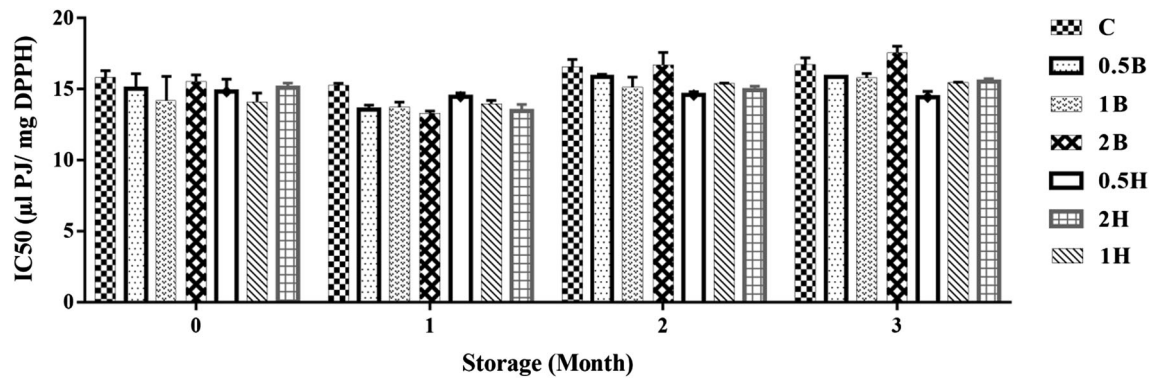


Fig. 5 Change in antioxidant activity values of all samples during storage

decreased the antioxidant activity of clear PJ (Higher IC_{50} values; Fig. 5).

The results that was obtained for the efficiency of HP- β -CD treatment in terms of antioxidant activity of were similar to the results in the study performed by Navarro et al. (2011a). The researchers also found HP- β -CD treatment effective to lower nutritional losses in mandarin juice enriched with pomegranate juice extract and goji berries juice. 1% of HP- β -CD was sufficient to protect antioxidant activity of this fruit juice mixture when compared to β -CD application, as well (Navarro et al. 2011a). In another study focused on the effects of organic farming, pasteurization and β -CD addition on orange juice chemical properties; the addition of β -CD caused no significant protective effect on antioxidant capacity and approximately 40% of the initial level for this parameter was lost in the pasteurized orange juices at the end of 145-day storage period at room temperature (Navarro et al. 2011b).

Conclusion

Effectiveness of cyclodextrin treatment on the quality of clear pomegranate juice (CPJ) during shelf life was studied by using the production parameters that are widely used in fruit juice industry. For this purpose, CPJ was subjected to different cyclodextrin types (β -CD and HP- β -CD) and their different concentrations (0.5, 1 and 2%). 25 °C of temperature was chosen as the storage temperature as this is the temperature at which fruit juices generally remain kept on the market shelves. According to the results; the best treatment was 0.5% of HP- β -CD treatment, to maintain total phenolic content and antioxidant activity of PJ. However, both different CD types and concentrations that was used in this study, were not effective in terms of color stability of the PJ.

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