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Technical paper

Changes in the Phenolic Content and Free Radical-scavenging Activity of Vacuum Packed Walnut Kernels during Storage

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In this study, the effects of storage temperature, O_2 permeability of packaging materials and variety on phenolic content and free radical-scavenging activity of vacuum-packaged walnut kernels were studied over a 12 months storage period. Methyl gallate (23.70 − 93.75 mg/kg), ellagic acid (137.95 − 569.22 mg/kg), and an ellagic acid pentoside (270.59 − 637.17 mg ellagic acid equivalent/kg) were identified in walnut varieties. While a slight decrease in the amount of ellagic acid was observed during 12 months storage, decreases in the amount of ellagic acid pentoside, total phenolic content and free radical-scavenging activity were severe. The present study concluded that it is possible to protect the phenolic content and antiradical activity of walnut kernels by packaging in Polyamide/Polyethylene laminate pouches having an oxygen permeability lower than 63.40 ± 0.40 (mL/m^2/24h at 23°C) under vacuum at 20°C up to twelve months.

Keywords: walnut, phenolic content, free radical-scavenging activity, storage

Introduction

Walnut (*Juglans regia* L.) is a well known nut due to its high polyunsaturated fatty acid (PUFA) content. Higher intake of PUFA has some positive effects on human health (Abbey *et al.*, 1994; Iso *et al.*, 2002). However, high PUFA content limits the shelf life of food products due to susceptibility to oxidation. Oxidation, which results in an undesirable rancid taste, makes walnut unacceptable for the consumer (Jensen *et al.*, 2003). Although walnut possess high PUFA content, walnut is readily preserved due to their high antioxidant capacity. Most of the antioxidant capacity of walnuts results from phenolic compounds, especially ellagitannins (Fukuda *et al.*, 2003), tocopherols (Kornsteiner *et al.*, 2006) and phenolic acids (Coloric *et al.*, 2005). The antioxidant capacity of walnut can be evaluated in two major fractions of walnut (walnut oil and defatted matter). While the contribution of walnut oil to the total antioxidant capacity of walnut is less than 5%, most of the antioxidant capacity in walnut is present in the defatted walnut matter. Ellagitannins in defatted matter of walnut are major contributors to antioxidant capacity (Arranz *et al.*, 2008). In addition, the slight astringent flavor of walnut fruit has been associated with the presence of these compounds (Deshpande *et al.*, 1986).

Ellagitannins are readily hydrolyzed by acid, bases, temperature or certain enzymes. In hydrolysis of ellagitannins, ester bonds are hydrolyzed, and hexahydroxydiphenoyl group spontaneously lactonizes into ellagic acid (EA). EA is a dimeric derivative of gallic acid, and has been used as a chemical marker compound for ellagitannins. EA is present in many plants of economic importance, particularly in fruits and nuts, either in its free forms as EA, EA-derivatives, or bound as water-soluble ellagitannins (Clifford and Scalbert, 2000). Ellagitannins in foods change to free EA and EA-derivatives during processing and storage (Viriot *et al.*, 1993). Interest in EA has increased over the past few years because of its properties as micronutrient. EA and its derivatives play an important role in human nutrition and are implicated with numerous biological properties, including antioxidant (Zafrilla *et al.*, 2001), anticancer (Narayanan

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et al., 1999), antiatherosclerotic (Anderson *et al.*, 2001), anti-inflammatory (Masamune *et al.*, 2005), gastroprotective (Beserra *et al.*, 2011) and antibacterial (Akiyama *et al.*, 2001) activities. EA is now used as a food additive in Japan, functioning as an antioxidant (Amakura *et al.*, 2000). Antioxidant activities of 34 different compounds belonging to phenolic acids, flavonols, flavanols, flavanones, anthocyanidins/anthocyanins and synthetic antioxidants were compared using β-carotene bleaching method and DPPH method. EA and α-tocopherol had high antioxidant activity according to the β-carotene bleaching and DPPH methods (Fukumoto and Mazza, 2000). Fukuda *et al.* (2004) found that EA proportion in the phenolic fraction extracted from walnut was 158 g/kg. The total EA content of walnut and pecan nut was recorded as 0.59 and 0.33 g/kg dry weight, respectively (Daniel *et al.*, 1989). Li *et al.* (2006) declared that amounts of free EA in Combe and Lake walnut varieties were 0.32 g/kg and 0.25 g/kg, respectively. Decreases in the total EA content of the four raspberry cultivars ranged from 14% to 21% during the long-term frozen storage period (365 days) (Ancos *et al.*, 2000). Zafrilla *et al.* (2001) found that while EA derivatives in raspberry jams remained quite stable with processing and during 6 months of jam storage, content of free EA increased 3-fold during the storage period.

The objective of this study was to determine the phenolic composition and free radical-scavenging activity of popular commercial walnut varieties grown in Turkey. In addition, the effects of storage temperature, O_2 permeability of packaging materials and variety on the phenolic content and free radical-scavenging activity of walnut kernels during storage were investigated.

Materials and Methods

Samples In this study, phenolic content of seven popular walnut varieties (Yalova-1, Yalova-3, Yalova-4, Kaman-5, Şebin, Bilecik and Şen-1) grown in Turkey were determined. Yalova-4, Şebin, Bilecik and Şen-1 varieties were obtained from Atatürk Central Horticultural Research Institute in Yalova, in the northwest region of the Anatolia. Yalova-1 and Yalova-3 varieties were obtained from local orchards in Denizli, in the southwest region of the Anatolia. Kaman-5 variety was obtained from a local orchard in Kırşehir, in central Anatolia. The walnut fruits were harvested in 2004 and 2005. After harvesting, ~ 2 kg walnut samples (3 replicates for each variety) were immediately transported to the laboratory and air-dried. The samples were stored in the shell, packed in polyethylene bags and frozen to −30°C, until the analysis.

Chemicals and reagents Methanol and n-hexane for extraction (all analytic grade), and acetonitrile and water for chromatography (HPLC grade) were obtained from Merck (Darmstadt, Germany). Standards of gallic acid, EA, methyl gallate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical, α-tocopherol and butylhydroxytoluene (BHT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Storage experiment Just two walnut varieties (Yalova-1 and Yalova-3 varieties harvested in 2005) having high phenolic content were selected from total seven walnut varieties to avoid excessive number of experimental run for storage experiments. Selected walnut varieties were used to evaluate the effects of storage temperature, O_2 permeability of packaging materials and variety on phenolic content during storage. As soon as the walnuts reached the laboratory, the walnuts were manually shelled. The walnut kernels were packed under vacuum (700 mmHg) in two different packaging materials: Polyamide/Polyethylene laminate pouches (90M PA/PE) having 90 μm total thickness with an oxygen permeability of 63.40 ± 0.40 mL/m^2/24h (23°C) and Polyamide/Polyethylene laminate pouches (150M PA/PE) having 150 μm total thickness with an oxygen permeability of 38.05 ± 0.85 mL/m^2/24h (23°C). The samples were stored in the dark at 10°C, 20°C, and 30°C for 12 months. At 0, 2, 4, 6, 8, 10 and 12 months of storage, the samples were withdrawn for chemical analyses.

Preparation of methanolic extract of walnut kernel Defatted ground walnut with n-hexane was placed in a vacuum oven (40°C, 2 h) to remove n-hexane. Nearly 0.25 g of defatted ground walnut was put into a centrifuge tube and extracted by shaking with 9.75 mL of 80:20 (v/v) methanol:water for 2 h in dark at room temperature. After this, the mixture was centrifuged at 8750 g (Sigma, Osterode am Harz, Germany) for 10 min at 20°C and then, supernatant was transferred into an amber bottle. The above procedure was repeated twice using the residue and then, supernatants were combined. Pooled supernatants were evaporated in a rotary vacuum evaporator (Buchi B114, Flawil, Switzerland) ($T \leq$ 45°C). The residue was dissolved in dimethyl sulfoxide (2 mL) and the volume was adjusted to 25 mL with methanol.

Determination of phenolic content by HPLC Chromatographic analyses for phenolic compounds were carried out using an HPLC system (Shimadzu, Kyoto, Japan) that consisted of a LC-10 AD-VP gradient pump, a Rheodyne 7725i valve furnished with 20 μL loop, a SPD-M10A photodiode array detector, CTO-10AS column oven, DGU-14A degasser and a SCL-10A system controller. The method reported by Coloric *et al.* (2005) was used with some modifications. Methanolic extract of walnut kernel was diluted with methanol (HPLC grade) (1:1, v/v). Diluted extract was filtered through a 0.45 μm PTFE membrane filter (Millipore, Bedford, USA) and analyzed by HPLC. Separation of phenolic compounds was carried out using a Symetry C18 (250 × 4.6 mm id, particle size 5 μm) column (Waters, USA)

at 1 mL/min flow rate. Detection was made at 280 nm and 25°C. The method utilizes a binary mobile phase consisting of (A) 2 % acetic acid in water and (B) 0.5 % acetic acid in water:acetonitrile (1:1, v/v). Gradient program was as follows: 0 min 90 % A; 50 min 45 % A. The compounds appearing in chromatograms were identified on retention times and spectral data by comparison with standards. In addition, mass spectrometry (MS) was used for characterization of unidentified peak on chromatogram. The MS data were acquired using Waters Micromass ZQ Mass Detector. The ionization conditions and energy levels for both electrospray negative and positive modes were as follows: desolvation temperature, 350°C; source temperature, 100°C; capillary volt, 3260 eV, cone volt 26 eV.

Determination of total phenolic content (TPC) TPC of methanolic extracts of walnut kernels was determined by the Folin-Ciocalteu method (Li *et al.*, 2006). 1 mL methanolic extract was diluted to 10 mL with methanol. Each dilution (0.2 mL) was mixed with 1 mL of the folin-ciocalteu reagent and 0.8 mL of 75 g/L sodium carbonate solution. The mixture was allowed to stand at room temperature for 60 min, and then the absorbance was measured at 765 nm in a Shimadzu 1601 UV/VIS spectrophotometer (Kyoto, Japan). A standard curve was generated with gallic acid (r^2 = 0.9987). Results were expressed as mg gallic acid equivalent (GAE) per kg walnut.

Free radical-scavenging activity using DPPH method Free radical-scavenging effects of walnut methanolic extracts were measured according to Pyo *et al.* (2004). 1 mL walnut methanolic extract was diluted to 5 mL with methanol. An aliquot of diluted walnut methanolic extract (0.1 mL) was added to 3.9 mL of DPPH solution (0.025 g/L in methanol). The mixture was left for 120 min in dark at room temperature until the reaction reached a plateau. The absorbances of sample and control at 515 nm were measured by spectrophotometer (Shimadzu, 1601 UV/VIS) after 120 min. The inhibitory percentage of DPPH was calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_S) / A_0] \times 100$$

Where A_0 is the absorbance of the control, and A_S is the absorbance of the sample. α-tocopherol and BHT dissolved in methanol (100 mg/L) were also analyzed for comparison.

Statistical analysis Statistical analyses were performed using Minitab 15.0 (Minitab Inc,. State College,PA, USA). Storage experiment was carried out in three replicates, and data were obtained from factorial design. A general linear model was used to compare differences in means among groups. Duncan method was used to evaluate differences between means.

Results and Discussion

In this study, methyl gallate and EA was detected on HPLC chromatogram by congruent retention time and UV spectrum with those of the authentic standard (Fig. 1). In addition, there is a major unidentified peak on HPLC chromatogram. UV spectrum of this peak (λ_{max} (nm): 361) was similar to characteristic shape of UV spectrum of EA. In addition, the peak was identified using MS and exhibited intense deprotonated a molecular ion $[M\text{-}H]^-$ at *m/z* 433 in the negative mode. Therefore, the compound corresponding to this peak was presumed to be an EA-pentoside, and results

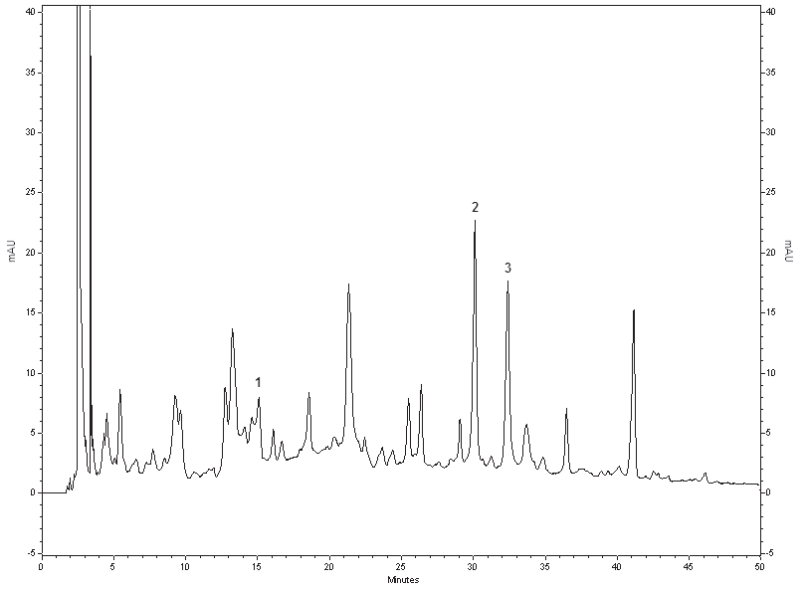

Fig. 1. A typical HPLC chromatogram of walnut (peak assigment: 1, methyl gallate; 2, EA-pentoside; 3, EA).

were expressed as mg EA equivalent per kg walnut. UV and MS data similar to our results was previously identified in muscadine grapes as EA-xyloside by Lee *et al.* (2005) and in raspberry fruits as EA-pentoside by Mullen *et al.* (2003). The amounts of these phenolic compounds in walnut kernels were shown in Table 1. EA, EA-pentoside and methyl gallate in walnut ranged from 137.95 to 569.22 mg/kg walnut, 270.59 to 637.17 mg ellagic acid equivalent (EAE)/kg walnut and 23.70 to 93.75 mg/kg walnut, respectively. Yalova-1, Yalova-3 and Şen-1 varieties had the high EA and EA-pentoside contents. Similar results for EA were found for Combe (0.32 g/kg) and Lake (0.25 g/kg) walnut varieties by Li *et al.* (2006). However, our results regarding with EA were generally higher than the results reported by Coloric *et al.* (2005) (32.6 − 97.7 mg/kg).

TPC of walnut varieties ranged from 9313.5 to 31808.8 mg GAE/kg walnut. While Yalova-1 variety had the highest TPC, Yalova-4 had the lowest TPC. The TPC of Yalova-1 and Yalova-3 varieties were found to be higher than the findings of Anderson *et al.* (2001) and Arranz *et al.* (2008). The results for other walnut varieties (Yalova-4, Şebin, Bilecik, Şen-1 and Kaman-5) were similar to the data reported by Anderson *et al.* (2001) and Arranz *et al.* (2008). In addition, the TPC of six different walnut varieties grown in Portugal were reported to vary between 58.78 and 95.06 mg GAE/g by Pereira *et al.* (2008) which were higher than the results obtained in our study.

As seen in Table 1, walnut methanolic extracts were found to be most potent free radical-scavenger (38.79 − 85.15 % of inhibition). The highest free radical-scavenging activity was found for methanolic extracts of Yalova-1 and Yalova-3. They were also the richest in EA, EA-pentoside and TPC. Yalova-4 had the lowest TPC and exhibited the weakest free radical-scavenging activity.

Effects of storage on EA, EA-pentoside and TPC EA, EA-pentoside and TPC of walnut varieties decreased with increased storage time and temperature. However, the decrease in EA-pentoside (Fig. 2) and TPC (Fig. 4) was higher than decrease in EA (Fig. 3) during storage. While the EA-pentoside and TPC of walnuts remained almost steady during storage at 10°C, there were appreciable decreases at 20°C. However, the EA-pentoside and TPC of walnuts stored at 30°C significantly decreased. For the EA-pentoside and TPC of walnuts, the differences among storage temperatures were statistically significant ($P < 0.01$). However, there is no difference between 10°C and 20°C, except 30°C. The decrease in EA-pentoside at 10°C, 20°C and 30°C after 12 months storage period ranged from 2.59% to 7.00%, 10.00% to 20.92%, and 22.70% to 42.45%, respectively, and the decrease in the TPC ranged from −0.19% to 2.20%, 2.96% to 9.10%, and 22.76% to 36.655%, respectively. The EA-pentoside and TPC of walnuts decreased dramatically during the initial 2 − 4 months, the decrease in the TPC after these months was slower compared to that of initial 2 − 4 months. This may be due to the reaction between phenolic content in walnut skin and residual oxygen inside the package. How-

Table 1. Phenolic contents and free radical-scavenging activities of walnut varieties grown in Turkey.

Variety	Methyl gallate (mg/kg)	EA-pentoside (mg EAE/kg)	EA (mg/kg)	TPC (mg GAE/kg)	DPPH (% inhibition)
2004					
Yalova-1	65.5 ± 0.76^{bA}	611.15 ± 5.73^{aA}	569.22 ± 23.38^{aA}	31808.8 ± 22.4^{aA}	82.23 ± 2.21^{aA}
Yalova-3	66.47 ± 9.84^{bA}	434.58 ± 0.1^{bA}	329.97 ± 15.35^{bA}	21440.3 ± 61.2^{bA}	65.28 ± 3.17^{bB}
Şebin	23.70 ± 1.2^{c}	304.36 ± 12.15^{c}	182.38 ± 9.08^{c}	10623.7 ± 55.7^{e}	39.38 ± 1.73^{d}
Bilecik	93.75 ± 13.21^{aA}	270.59 ± 34.61^{cA}	257.23 ± 34.4^{bcA}	17237.0 ± 1525^{cA}	52.12 ± 8.62^{cA}
Kaman-5	33.64 ± 1.25^{cB}	346.04 ± 10.59^{cB}	273.61 ± 36.18^{bcA}	13611.0 ± 364.9^{dB}	50.00 ± 7.00^{cA}
2005					
Yalova-1	45.53 ± 1.4^{abB}	637.17 ± 41.64^{aA}	392.5 ± 12.73^{aB}	30296.0 ± 456.8^{aA}	85.15 ± 1.05^{aA}
Yalova-3	53.16 ± 2.58^{aA}	479.30 ± 5.44^{bA}	254.5 ± 17.12^{bA}	21828.0 ± 604.5^{bA}	75.46 ± 0.24^{abA}
Yalova-4	41.15 ± 4.26^{bc}	296.53 ± 13.34^{d}	142.71 ± 0.78^{c}	9313.5 ± 825.1^{c}	38.79 ± 6.66^{c}
Bilecik	50.85 ± 2.77^{abB}	379.97 ± 19.77^{cA}	137.95 ± 7.38^{cA}	11549.6 ± 1624^{cA}	41.91 ± 7.50^{cA}
Şen-1	31.91 ± 3.17^{c}	485.17 ± 6.95^{b}	266.49 ± 43.99^{b}	18738.4 ± 778.6^{b}	63.99 ± 6.66^{b}
Kaman-5	53.82 ± 5.37^{aA}	467.11 ± 24.96^{bA}	202.04 ± 8.79^{bcA}	19261.8 ± 2132^{bA}	65.92 ± 8.07^{bA}
α-tcopherol					19.64 ± 0.84
BHT					20.50 ± 0.61

Data are expressed as mean ± SD. Numbers followed by a different superscript lowercase letter within the same column for same year are significantly different ($P < 0.05$). Numbers followed by different superscript uppercase letter between the years for the same variety are significantly different ($P < 0.05$).

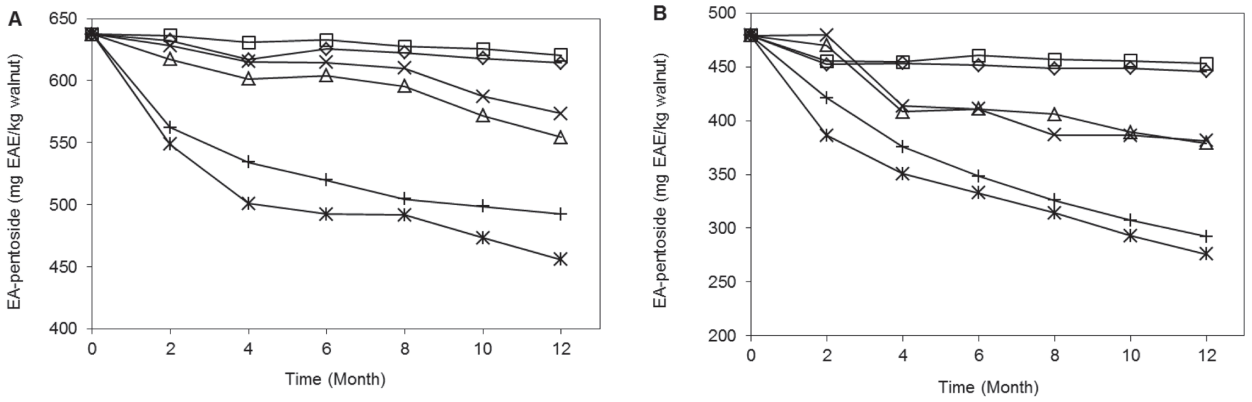

Fig. 2. Changes in EA-pentoside content of walnuts stored in different conditions (A, EA-pentoside results of Yalova-1 variety during storage; B, EA-pentoside results of Yalova-3 variety during storage) (—◇— 90M PA/PE 10°C; —□— 150M PA/PE 10°C; —△— 90M PA/PE 20°C; —×— 150M PA/PE 20°C; —✳— 90M PA/PE 30°C; —+— 150M PA/PE 30°C—◇—).

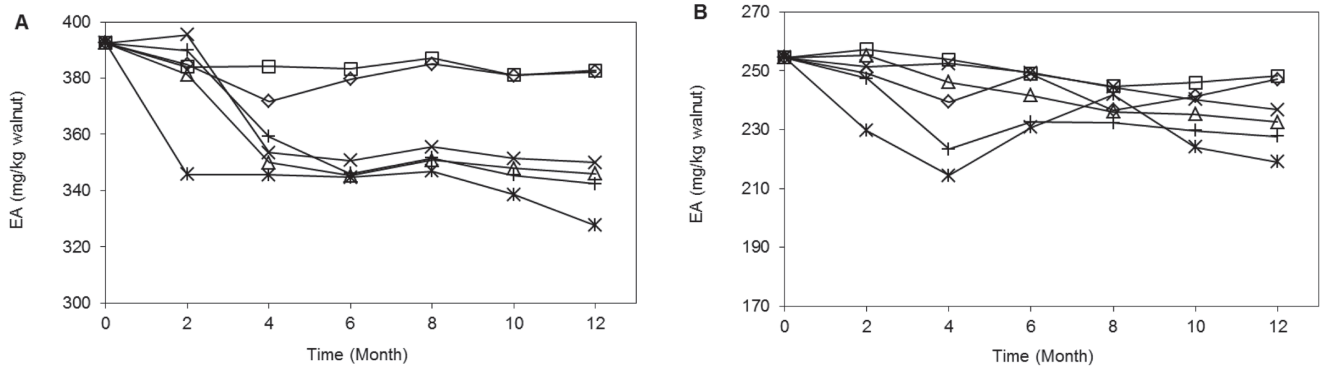

Fig. 3. Changes in EA content of walnuts stored in different conditions (A, EA results of Yalova-1 variety during storage; B, EA results of Yalova-3 variety during storage) (—◇— 90M PA/PE 10ºC; —□— 150M PA/PE 10ºC; —△— 90M PA/PE 20ºC; —×— 150M PA/PE 20ºC; —✳— 90M PA/PE 30ºC; —+— 150M PA/PE 30ºC).

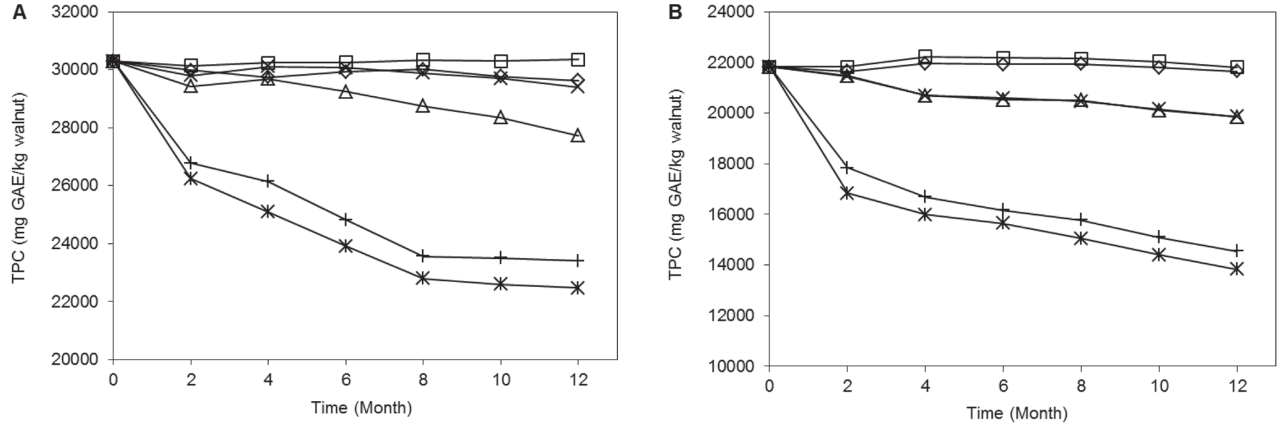

Fig. 4. Changes in TPC of walnuts stored in different conditions (A, TPC results of Yalova-1 variety during storage; B, TPC results of Yalova-3 variety during storage) (—◇— 90M PA/PE 10°C; —□— 150M PA/PE 10°C; —△— 90M PA/PE 20°C; —×— 150M PA/PE 20°C; —✳— 90M PA/PE 30°C; —+— 150M PA/PE 30°C).

ever, the decline in EA content of walnuts during storage showed small fluctuations during storage. The decrease in EA content at 10°C, 20°C and 30°C after 12 month storage period ranged from 2.49% to 2.89%, 6.95% to 11.85%, and 10.55% to 16.52%, respectively. The differences among temperatures were found to be statistically insignificant ($P > 0.05$). Ancos *et al.* (2000) reported that decreases in the total EA content of four raspberry cultivars ranged from 14% to 21% during the long-term frozen storage (365 days). The decreases in free EA content of walnuts in the present

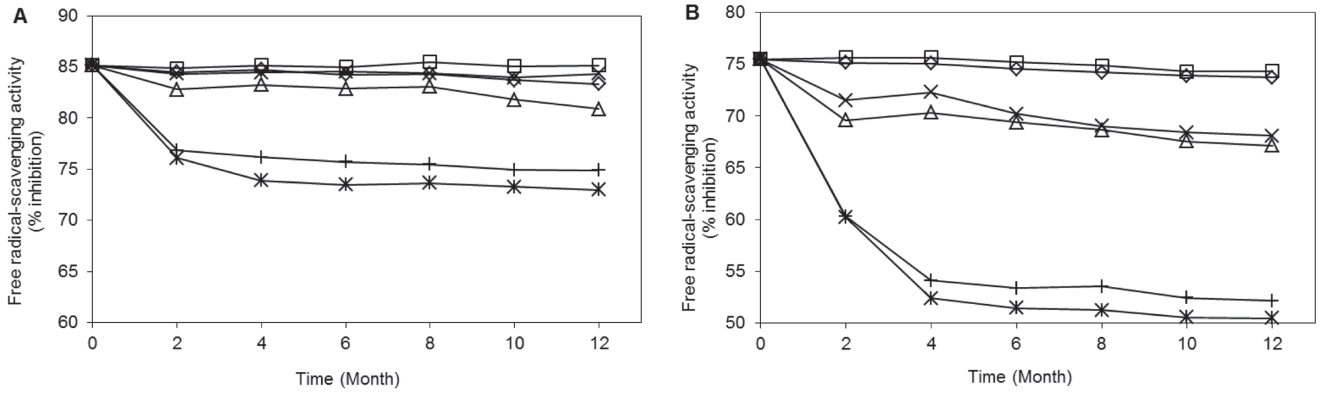

Fig. 5. Changes in free radical-scavenging activities of walnuts stored in different conditions (A, free radical-scavenging activities of methanolic extracts of Yalova-1 variety during storage; B, free radical-scavenging activities of methanolic extracts of Yalova-3 variety during storage) (◇ 90M PA/PE 10°C; □ 150M PA/PE 10°C; △ 90M PA/PE 20°C; ✕ 150M PA/PE 20°C; ✳ 90M PA/PE 30°C; + 150M PA/PE 30°C).

study were lower than the findings of Ancos *et al.* (2000). This might be related to a more severe cellular disruption in raspberry fruits by freezing, which could be produced by release of oxidoreductasic ionic forms of the enzyme polyphenol oxidase linked to the cellular wall (Ancos *et al.*, 2000). Zafrilla *et al.* (2001) declared that while five different EA-derivatives remained quite stable during 6 months of raspberry jam storage at 20°C, the content of free EA increased 3-fold during the storage period. Our results are opposite to findings of Zafrilla *et al.* (2001). Our results showed that EA, EA-pentoside and TPC were decreased during storage, but the decreases in EA during 12 month storage were lower compared to that in EA-pentoside and TPC. We expected that the EA would have been oxidized during storage. However EA content of walnuts was slightly decreased after storage. This situation could be explained by a release of EA from ellagitannins during storage.

The declines in EA-pentoside and TPC of Yalova-1 at the end of 12 months storage period were lower than that of Yalova-3, while the declines in EA of Yalova-1 were higher than that of Yalova-3. Rates of EA, EA-pentoside and total phenolic losses for Yalova-1 were 2.49 − 16.52%, 2.53 − 28.43%, and −0.19 − 25.81%, respectively. Rates of EA, EA-pentoside and total phenolic losses for Yalova-3 were also 2.49 − 13.95%, 4.46 − 42.45%, and −0.26 − 36.64%, respectively. For the EA, EA-pentoside, and TPC, whereas the difference between walnut varieties was found to be statistically significant ($P < 0.01$), the differences between packaging materials were found to be statistically insignificant ($P > 0.05$).

Effects of storage on free radical-scavenging activity

Free radical-scavenging activities of walnut methanolic extract also decreased with increased storage time and temperature (Fig. 5). While there was almost no change in the free radical-scavenging effects of methanolic extracts of walnuts during storage at 10°C, there was a slight drop at 20°C. However, there was a significant decrease at 30°C and this decrease was high during the initial 2 − 4 months. For the % inhibition values of methanolic extracts of walnuts, the differences among temperatures were found to be statistically significant ($P < 0.01$), but the differences between 10°C and 20°C were insignificant ($P > 0.05$), except 30°C. The decrease in % inhibition values of walnut methanolic extracts at 10°C, 20°C and 30°C after 12 month storage period ranged from 0.03% to 2.36%, 0.99% to 12.41%, and 13.75% to 49.48%, respectively. Rates of losses in antiradical activities for Yalova-1 and Yalova-3 were also 0.03 − 16.71% and 1.55 − 49.48%, respectively.

The differences between varieties were found to be significant ($P < 0.01$).The declines in free radical-scavenging activity of Yalova-3 during 12 months storage period were higher than that of Yalova-1. This situation was similar to that observed in EA-pentoside and TPC. High decrease in phenolic content and antiradical activity of Yalova-3 variety may be due to the tissue properties or higher PUFA content that these associated with oxidation. However, the differences between packaging materials were insignificant ($P > 0.05$). Highest loss in free radical-scavenging activity (49.48 %) were found in Yalova-3 variety stored at 30°C for 12 months in 90M PA/PE film pouches. However, these walnuts exhibited approximately 2-fold stronger free radical-scavenging activity than α-tocopherol and BHT. DPPH results have strong correlation with EA, EA-pentoside and TPC ($P < 0.01$). Correlation coefficients between DPPH and EA, DPPH and EA-pentoside, and DPPH and TPC were 0.75, 0.88, and 0.91, respectively.

Conclusion

The results of the present study demonstrate that Turkish walnut varieties contained high phenolic content and free radical-scavenging activity. Especially, Yalova-1 and Yalova-3 have the high phenolic content and antiradical activity. Walnut kernel were stored for up to 12 month under vacuum in PA/PE plastic containers at 10°C and 20°C without any major changes in phenolic content and free radical-scavenging activity. In contrast, EA-pentoside, TPC and free radical-scavenging activity significantly decreased during storage at 30°C. The most important decreases were observed in first 4 months of storage. The decreases in phenolic content and antiradical activity of Yalova-3 were higher than those of Yalova-1 during storage. Our results showed that phenolic content and antiradical activity were decreased during storage, but the decrease in EA was lower than that in EA-pentoside, TPC and free radical-scavenging activity. Highest correlation was found between DPPH and TPC. The results show that it is possible to preserve phenolic contents and antioxidant activity of walnut kernels packed in PA/PE plastic material having oxygen permeability lower than 63.40 ± 0.40 $ml/m^2/24h$ at (23°C) under vacuum at 20°C for a period of 12-month storage.

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