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ORIGINAL PAPER

Shelf life extension of walnut kernel: effect of temperature and vacuum packaging storage

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Abstract

Walnut is one of the most important dry fruits with high nutritional value that serves as a natural source of antioxidants. However, there have always been concerns regarding the preservation of walnut kernels due to the high oxidation. In this study, the effect of temperatures (4 ℃ and 25 °C) and vacuum packaging (5% vacuum, and non-vacuum control) were investigated on the quality of walnut kernels of two commercial genotypes (stone and paper) during 6 month. The total phenol and flavonoid contents, and antioxidant capacity decreased, and consequentially kernel browning occurred with increased hue angle value and decreased lightness values. During the storage the activities of PPO and POD increased but the activity of CAT decreased, indicating the redox leaned to oxidation. Low temperature and vacuum packaging both slowed down the oxidative metabolism, and suppressed reduction of antioxidants, and delayed the discoloration/browning of walnut kernels.

Keywords Antioxidant capacity · Phenolic content · Flavonoids content · Enzyme activity · Storage conditions

Introduction

Walnut (*Juglans regia* L) is one of the most important nuts in the world. Many countries have been developing walnut cultivation due to the importance of this tree and the high demand on the market [1]. According to paleontological studies, various species of walnut have existed since ancient times, but most of its species are found in Eastern Asia, the Middle East, the Carpathian Mountains in the southeast of Europe, the Himalayas, North Africa, Greece, and North America [1].

Walnut is one of the most important sources of essential fatty acids, fiber, plant proteins, vitamins, minerals, magnesium, potassium, and arginine amino acid [2]. Due to high levels of antioxidant compounds such as vitamin E, tocopherol and polyphenols [3, 4], walnut is beneficial to human health by regulating cholesterol levels and positively affecting the cardiovascular disease [5]. The walnut kernel as a dry product degrats very quickly by chemical and microbial agents. The chemical degradation is caused by significant amounts of fat (about 64 to 71%) and unsaturated fatty acids, including oleic acid, linoleic acid, and linolenic acid. So there are always concerns about quality degradation of the walnut kernel, such as oxidation, which results in poor taste and darkening of the color [2].

Temperature and air presence are among the most important factors affecting the postharvest quality of nuts during storage. Fu et al. and Spaccarotella et al. [2, 5] reported that increased temperature and oxygen increase sensitization of products in terms of fat oxidation (rancidity and volatile matter production) and sensory properties. Christopoulos and Tsantili [3] investigated the effect of temperature and packaging atmosphere on total antioxidants and color of walnut during storage. They reported that low temperatures and packaging under N_2 or CO_2 prevented an increase in antioxidant and browning. Furthermore, Tsantili et al. [7] reported low temperatures (1 °C instead of 20 °C) and packaging with N_2 atmosphere prevented the reduction of phenolic and antioxidant compounds in eight pistachio cultivars.

Currently, there is little comprehensive information available about Iranian walnut kernel traits such as antioxidant

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properties (phenol and flavonoids), walnut color and the effect of these traits on the quality of walnut kernel during the storage period. Therefore, the aim of the present study is to investigate the changes in antioxidants, phenol, flavonoid, color and enzyme activity, including polyphenol oxidase, peroxidase and catalase of walnut kernel during storage.

Materials and methods

Pre-storage preparation and packaging of samples

Walnut fruits, from the highly marketable commercial genotypes, Stone and Paper, were collected from a commercial grove in the mountainous region (Rabar), Kerman Province (Longitude 56° 45′ to 57° 16′ and latitude 29° 27′ to 38° 54′), Iran. Fully matured fruit (hull separated easily from the shell) were hand-picked. After cleaning, the fruits were dried for four days in shade with natural air circulation. The walnuts were transferred to the laboratory and their shells were removed using a walnut cracker. The walnut kernels, 25 g, were packaged immediately in polyethylene bags with a thickness of 0.087 mm. Samples were treated with a vacuum machine (GSM-DZ410-610, city and country) with a vacuum level of 5%. The same number of samples were air packaged as control. Vacuum packed samples and the controls were each divided to two groups, and stored at 4 °C and 25 °C (control) for 6 months. The chemical, enzymatical, and colour analysis were conducted monthly. The schematic illustration of the sample preparation procedures is shown in Fig. 1.

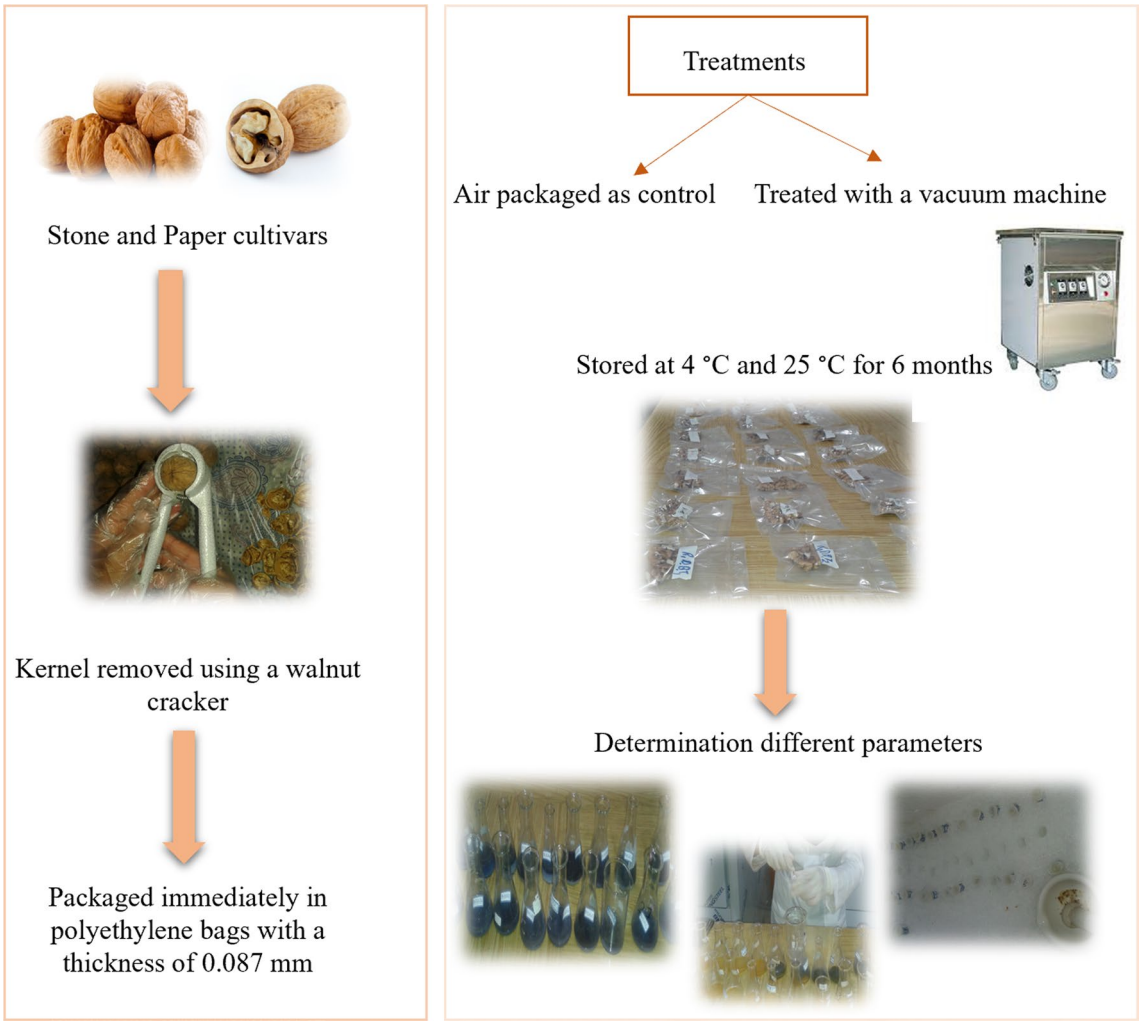

Fig. 1 A schematic illustration of the sample preparation and treatments of the present study

Total phenolic content (TPC) total flavonoids and antioxidant activity (TAC)

To prepare the ethanol extract, 1.0 g of kernel samples homogenized by 10 ml of 50% ethanol and after 30 min, it was centrifuged (11,000×*g*) for 10 min. The supernatant was diluted again by 10 ml of 50% ethanol and was applied to analyze total phenol, total flavonoids content and antioxidant capacity.

The TPC values of methanolic extracts were determined using a modified Folin–Ciocalteu colorimetric method [9]. The reaction mixture contained 0.5 ml of diluted walnut extracts, 2.5 ml of the Folin–Ciocalteu reagent (10:1) and 2 ml of sodium carbonate (7.5% w/v). A spectrophotometer (CECIL CE250) was used to measure the absorbance at 750 nm after 90 min at room temperature (25 °C). With the use of gallic acid as the standard, a reference curve was constructed. Milligrams of gallic acid equivalents (GAE) per gram of the extract were used to represent the findings.

Total flavonoids (TF) were determined using a colorimetric assay according to the methods of Ghasemi, et al. [10]. 0.5 ml of extract solution mixed with 1.5 ml of 96% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of sodium acetate (1 M) and 2.8 ml of distilled water. After keeping the samples at room temperature for 30 min, the absorbance of the mixture was read at 415 nm using a spectrophotometer (CECIL CE250). The data were presented in the form of mg of quercetin equivalents (QE) per gram of fresh weight (mg g^{-1} F.W).

A properly diluted extract sample of 0.1 ml was added to a screw-cap tube containing 3.9 ml of DPPH solution(2,2-diphenyl-1-picryhydrazyl, 60 µM in methanol) for the radical scavenging assay. After 30 min of incubation in the dark at room temperature (25 °C), a decrease in absorbance at 515 nm was observed in comparison to the blank [11]. The antioxidant activity was calculated as follows: antioxidant activity (%) = [1 − (Absample/Abs-control)] × 100.

Polyphenol oxidase (PPO) activity and Peroxidase (POD) activity

Polyphenoloxidase activity was determined with some modification [12]. 25 mM phosphate buffer (pH 7), 0.1 mM pyrogallol, and 0.1 ml enzyme extract and blank without pyrogallol were all included in the 3 ml reaction mixture. At 420 nm, the formed purpurogallin's absorbance was measured. One-unit POD activity was defined as an absorbance change of 0.01 units per minute.

The assay solution (3 ml) contained 0.1 ml enzyme extract, 20 mM guaiacol, 40 mM H_2O_2, and 50 mM potassium phosphate buffer (pH 7.0) for POD activity measurement. The addition of the enzyme extract sparked the reaction. Every 20 s, the reaction solution's absorbance read at 470 nm. One-unit POD activity was defined as an absorbance change of 0.01 units per minute [13].

Catalase (CAT) activity

For the CAT activity assay, 0.1 ml of enzyme extract was added to a solution of 3 ml containing 5.9 mM H_2O_2 and 50 mM phosphate buffer (pH 7.0). Every 20 s, the reaction solution's absorbance read to 240 nm. One-unit CAT activity was defined as an absorbance change of 0.01 units per minute [13].

Colour

Kernel colour was measured by a chromatometer (CR-400; Minolta, Japan). The recorded CIE-L*a*b* values together with converted hue angle (h°) (McGuire, [14] and whiteness index (WI) (Boun and Huxsoll, [15] were used to represent kernel colour, where L* indicates lightness from 0 (black) to 100 (white), h° indicates color changes from 90 (yellow) to 0 (red), and WI indicates whiteness from 100 (colourful) to 0 (white). WI and h° were calculated by following equations:

$$WI = 100 - \left[\left(100 - L^{*}\right)^{2} + a^{*2} + b^{*2}\right]^{1/2}$$

$$h^{\circ} = \tan^{-1}\left(b^{*}/a^{*}\right)$$

Statistical design

With three replications and a completely random design, the experiment was carried out. Means were compared with SAS software (Version 9.4) using a Duncan test. Graphs were plotted with Excel. Results were expressed as mean values ± standard error. R software was used to draw clustering heatmap in multivariate analysis.

Results

Antioxidant capacity (TAC)

Antioxidant levels of the samples varied according to the storage conditions and type of genotypes, The initial antioxidant capacities (TAC) were 35.68% and 38.21%, respectively, in Stone and Paper cultivars. Figure 2A and B shows that the antioxidant capacities of both genotypes that decreased during the storage period, but air-containing packages and samples stored at 25 °C decreased more. At the end of the 6-month period, in both genotypes, the control samples (containing air and stored at 25 °C) showed approximately a 30% reduction in the antioxidant capacity,

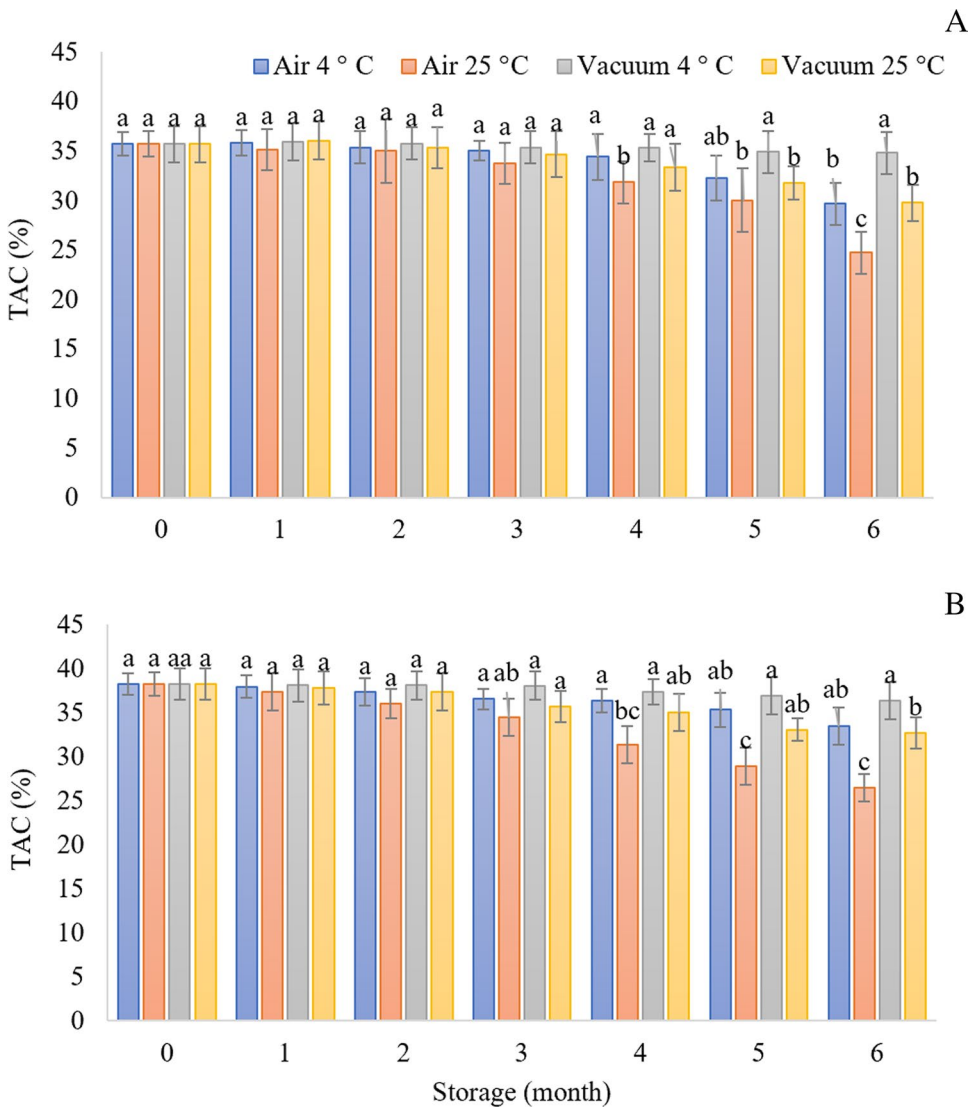

Fig. 2 **A** Stone. **B** Paper. Total antioxidant capacity (TAC) of the walnut kernel in different temperature and packaging during 6 months storage

but the antioxidant capacity of the samples stored at 4 °C and vacuum packaging decreased only by 4%.

Total phenol and flavonoid compounds

The effects of storage conditions and storage time on the level of phenolic and flavonoid compounds of walnut genotypes are presented in Tables 1 and 2. The two genotypes showed a significant difference in phenol content ($p > 0.05$), as the phenol contents in the Stone and Paper genotypes before the experiment were 15.67 and 16.73 mg/100 g gallic acid, respectively. According to Table 1, total phenol content decreased gradually over time, but the samples at 4 °C and vacuum packaging significantly maintained phenolic compounds ($p > 0.05$) so that the highest phenol content was found in them and the lowest content was found in the control samples (temperature 25 °C and air package). However, the genotypes under analysis did not show significant differences in terms of flavonoids content, The flavonoids content before and after storage was about 1.17 and 1.22 mg/100 g quercetin, respectively, in stones and paper genotypes. The flavonoid composition of the samples with a similar pattern to phenol shows a decreasing trend during the storage period (Table 2), especially at the last two storage times. As our findings suggest, the samples treated with vacuum packaging and temperature of 4 °C had significantly higher flavonoid contents than those treated at a temperature of 25 °C and with the air-containing packaging. The control samples (25 °C and air-containing packaging) showed 40% and 30% decline in the flavonoid contents of the two genotypes at the end of the experiment period, while the treated samples showed only 14% and 18% reduction of the flavonoid contents, implying a significant difference between the treated samples and the control samples.

Polyphenol oxidase (PPO) enzyme and peroxidase (POD)

The polyphenol oxidase enzyme, gradually increased over the course of six months in the two genotypes, This increase was higher in the control samples (25 °C and air-packed), and was significant at the fifth and sixth time periods (Table 3). At the end of the experiment, the enzymatic activity in the control samples was 1.4 times higher than that of the treated samples. At the end of the experiment, the highest and lowest levels of PPO enzymatic activity were observed in samples stored under air-containing conditions and 25 °C and the samples treated under vacuum and at 4 °C. Other applied conditions (storage at 25 °C, vacuum packaging, air-containing packaging, and storage at 4 °C) showed moderate results. The type of genotypes, storage duration,

Table 1 Effect of genotype, temperature, air presence and storage time on the phenolic (TP) of walnut kernel

Genotype	T (°C)	Packing	Storage (month)						
			0	1	2	3	4	5	6
Stone	4	Vacuum	15.67 ± 2.2a	15.68 ± 2.3a	15.64 ± 2.2a	15.56 ± 2.1a	15.46 ± 2.2ab	14.8 ± 2.4ab	14.8 ± 1.4ab
		Air	15.67 ± 2.2a	15.54 ± 2.1a	15.13 ± 2.2ab	14.42 ± 2.8ab	13.5 ± 2.4abc	12.7 ± 2.3abc	12.33 ± 3.1a–c
	25	Vacuum	15.67 ± 2.2a	15.12 ± 2.1ab	14.9 ± 3ab	14.2 ± 2.0ab	13.41 ± 2.0abc	12.5 ± 2.6a-c	11.60 ± 1.9a–c
		Air	15.67 ± 2.2a	15.30 ± 2.1ab	14.49 ± 3.1ab	13.15 ± 3.7abc	11.97 ± 3.9abc	10.1 ± 3.8bc	9.03 ± 3c
Paper	4	Vacuum	16.73 ± 1.3a	16.8 ± 1.4a	16.73 ± 1.9a	16.6 ± 1.9a	16.6 ± 1.8a	16.3 ± 1.2ab	16.15 ± 1.0ab
		Air	16.73 ± 1.3a	16.7 ± 2.1a	16.42 ± 2.8ab	16.21 ± 1.7ab	15.54 ± 1.4a-c	13.6 ± 2.7abc	13.36 ± 2.4a-c
	25	Vacuum	16.73 ± 1.3a	16.8 ± 2.7a	15.58 ± 2.2a-c	15.18 ± 2.8a-c	13.78 ± 1.1a-c	13.4 ± 0.9a-c	13 ± .09bc
		Air	16.73 ± 1.3a	16.4 ± 1.3ab	15.6 ± 2.3a–c	14.72 ± 2.2a–c	13.33 ± 1.5a-c	12.2 ± 0.9cd	9.6 ± 0.7d

For each parameter, values followed by the same letter in do not differ significantly according to Duncan's range test (p 0.05)

Table 2 Effect of genotype, temperature, air presence and storage time on the flavonoid (TF) compounds of walnut kernel

Genotype	T (°C)	Packing	Storage (month)						
			0	1	2	3	4	5	6
Stone	4	Vacuum	1.17 ± 0.03a	1.19 ± 0.03a	1.17 ± 0.03a	1.1 ± 0.02a	1.13 ± 0.05ab	1.01 ± 0.02a–c	1 ± 0.02a–c
		Air	1.17 ± 0.03a	1.17 ± 0.1a	1.12 ± 0.1ab	1 ± 0.1a–c	1 ± 0.1a–c	0.94 ± 0.1a–c	0.8 ± 0.09a–c
	25	Vacuum	1.17 ± 0.03a	1.17 ± 0.03a	1.08 ± 0.1ab	1.01 ± 0.1a–c	0.95 ± 0.2a–c	0.9 ± 0.1a–c	0.86 ± 0.1a–c
		Air	1.17 ± 0.03a	1.14 ± 0.7ab	1.03 ± 0.2a-c	0.93 ± 0.2a–c	0.77 ± 0.1bc	0.66 ± 0.1c	0.64 ± 0.1c
Paper	4	Vacuum	1.22 ± 0.02a	1.22 ± 0.1a	1.2 ± 0.01ab	1.19 ± 0.02ab	1.13 ± 0.01a-c	1.02 ± 0.01c-f	0.96 ± 0.1d-g
		Air	1.22 ± 0.02a	1.2 ± 0.01a	1.19 ± .01ab	1.05 ± .01b–f	1 ± .01def	0.91 ± .15fgh	0.86 ± .16 g–i
	25	Vacuum	1.22 ± 0.02a	1.22 ± 0.02a	1.17 ± .01abc	1.07 ± .02a–f	1 ± .01def	0.94 ± .015fgh	0.91 ± .15f–h
		Air	1.22 ± 0.02a	1.11 ± 0.1a–e	0.95 ± 0.1fgh	0.9 ± 0.09fgh	0.79 ± .09hi	0.75 ± .07i	0.75 ± .07i

For each parameter, values followed by the same letter in do not differ significantly according to Duncan's range test (p 0.05)

Table 3 Effect of genotype, temperature, air presence and storage time on PPO enzymatic activity of walnut kernel

Genotype	T (°C)	Packing	Storage (month)						
			0	1	2	3	4	5	6
Stone	4	Vacuum	3.37 ± 0.46c	3.43 ± 0.52c	3.44 ± 0.48c	3.45 ± 0.49c	3.48 ± 0.56c	3.50 ± 0.50c	3.79 ± 0.4c
		Air	3.37 ± 0.46c	3.37 ± 0.46c	3.4 ± 0.47c	3.44 ± 0.59c	3.94 ± 0.26bc	3.95 ± 0.35bc	4.13 ± 0.34bc
	25	Vacuum	3.37 ± 0.46c	3.37 ± 0.48c	3.48 ± 0.48c	3.72 ± 0.45c	3.98 ± 0.41bc	4.12 ± 0.59bc	4.16 ± 0.62bc
		Air	3.37 ± 0.44c	3.38 ± 0.44c	3.43 ± 0.65c	3.93 ± 0.43bc	4.24 ± 0.39bc	4.9 ± 0.36ab	5.55 ± 0.71a
	4	Vacuum	1.71 ± 0.10d	1.71 ± 0.17d	1.71 ± 0.17d	1.71 ± 0.11d	1.82 ± 0.18d	1.84 ± 0.19d	1.9 ± 0.27 cd
Paper		Air	1.71 ± 0.10d	1.77 ± 0.10d	1.77 ± 0.17d	1.78 ± 0.36d	1.77 ± 0.45d	1.79 ± 0.48d	2.28 ± 0.44b-d
	25	Vacuum	1.71 ± 0.10d	1.71 ± 0.10d	1.83 ± 0.10d	1.95 ± 0.65cd	2.06 ± 0.77bcd	2.22 ± 0.88bcd	2.69 ± 0.9bc
		Air	1.71 ± 0.10d	1.77 ± 0.17d	1.77 ± 0.16d	1.82 ± 0.2d	1.96 ± 0.24cd	2.81 ± 0.26b	4.11 ± 0.3a

For each parameter, values followed by the same letter in do not differ significantly according to Duncan's range test (p 0.05)

storage temperature, and air presence affected POD enzyme activity as was the case with PPO enzyme ($p > 0.05$). The peroxidase(POD) enzyme with a similar pattern of polyphenol oxidase (PPO) (Table 3) showed an increasing trend over a period of six months, especially during the fourth, fifth, and sixth time periods. The effect of packing and storage temperature on PPO enzyme showed a significant difference ($p > 0.05$) so that packaging under vacuum and at temperature of 4 °C plays an important role on reducing peroxidase activity and preventing the loss of quality (Table 4).

Catalase enzyme

Both genotypes showed the same pattern in terms of catalase enzyme activity. By storage, the enzymatic activity was gradually reduced in contrast to previous enzymes, and this reduction occurred more rapidly in the control samples (1.4-fold) than the treatment samples. At the end of storage period, the highest enzymatic activity was observed for samples stored at 4 °C and under vacuum conditions. The temperature of 4 °C and the vacuum packaging significantly maintained the activity of the catalase enzyme until the fifth round of the experiment, and at each sampling time the CAT enzyme was at a higher level than that in the control sample (25 °C) and the air-containing packaging),.The control samples in both genotypes showed a decrease in the enzymatic activity from the second storage time (Fig. 3A, B).

Color

The color value of kernel before storage in the two paper and stone genotypes were L*(55.74, 37.71), h (80.71–74.76) and WI (46.24–26/51), respectively. The two genotypes had significant differences in terms of L*, hue, and WI indices. Tables 5 and 6 shows the changing trend of color values (L*, WI) over a period of 6 months, which is more evident during the last two-time periods. There was a significant difference between the different storage conditions in the Stone genotypes, the lowest level of L* was observed in samples stored at 25 °C and air-containing packages. Figure 4A and B shows the reduction of angle hue in the stone and paper genotypes, which, like L* and WI, this color parameter decreased at the end of storage period. Therefore, based on the results, the storage time leads to a decrease in color values (L*, hue, and WI). This reduction in the visual characteristics depends on the temperature and storage environment. At the temperature 4 °C and a vacuum packaging, L*, WI, and hue values are higher than the corresponding values at temperature of 25 °C and the air-containing packaging, which indicates the destructive effects of the high temperature and the presence of air on the visual characteristics, marketability, and the quality of the walnut kernel.

Multivariate analysis of biochemical traits

Regarding the clustering heatmap, there was no relationship between two genotypes (Fig. 5). Cluster I included some of the treatments in stone genotype. In cluster I, P2D1M2 through P1D2M1 was characterized by fruit with low CAT and POX enzymes and medium to high carbohydrate and antioxidant. Cluster II included all of the treatments in paper genotype and this genotype had very low to low POX in fruit. The third cluster, which included P1D1M7 and P2D1M6 showed low PPO and CAT enzymes and very low POX enzymes and high carbohydrate in fruit.

Discussion

According to the results of this study, the two genotypes showed significant differences in antioxidant content and the Paper genotypes had higher antioxidant capacity. The results obtained in this study are less than those reported by other researchers. Other also reported that antioxidant capacity was differentiated among pecan and pistachio genotypes [6]. Akbari et al. [16], in a study of six genotypes of walnut,

Table 4 Effect of genotype, temperature, air presence and storage time on POD enzymatic activity of walnut kernel

Genotype	T (°C)	Packing	Storage (month)						
			0	1	2	3	4	5	6
Stone	4	Vacuum	9.34 ± 2.0c	9.08 ± 2.0c	9.32 ± 2.6c	9.39 ± 2.8c	9.52 ± 2.1c	9.52 ± 2.3c	10.6 ± 1.9c
		Air	9.34 ± 2.0c	9.27 ± 1.9c	9.39 ± 2.1c	9.73 ± 3.3c	9.98 ± 2.5c	11.36 ± 2.9c	11.87 ± 2.7bc
	25	Vacuum	9.34 ± 2.0c	9.26 ± 2.2c	9.22 ± 2.8c	9.72 ± 2.5c	10.41 ± 2.2c	11.66 ± 2.1bc	12.50 ± 1.8bc
		Air	9.34 ± 2.0c	10.04 ± 2.3c	10.77 ± 2.1c	12.2 ± 1.7bc	13.24 ± 3a-c	16.3 ± 3.1ab	17.37 ± 3.5a
	4	Vacuum	8.0 ± .84def	8.0 ± .84def	7.77 ± .93ef	7.7 ± .8f	8.04 ± .74def	8.2 ± .79def	8.88 ± 1.1b–f
Paper		Air	8.0 ± .84d–f	7.87 ± .80d–f	8.32 ± .78c-f	8.42 ± 1c–f	8.83 ± 1b-f	9.24 ± 1.2b-f	9.98 ± 1.7a–e
	25	Vacuum	8.0 ± .84 d-f	8.09 ± .83def	8.54 ± .70c–f	8.85 ± .65b.-f	8.89 ± .70b–f	10.73 ± 1abc	11.13 ± 1ab
		Air	8.0 ± .84d–f	8.45 ± .86c–f	9.26 ± .90b–f	9.58 ± 1b-e	10.24 ± 1.2a–d	11 ± 1.6ab	12.2 ± .2a

For each parameter, values followed by the same letter in do not differ significantly according to Duncan's range test (p 0.05)

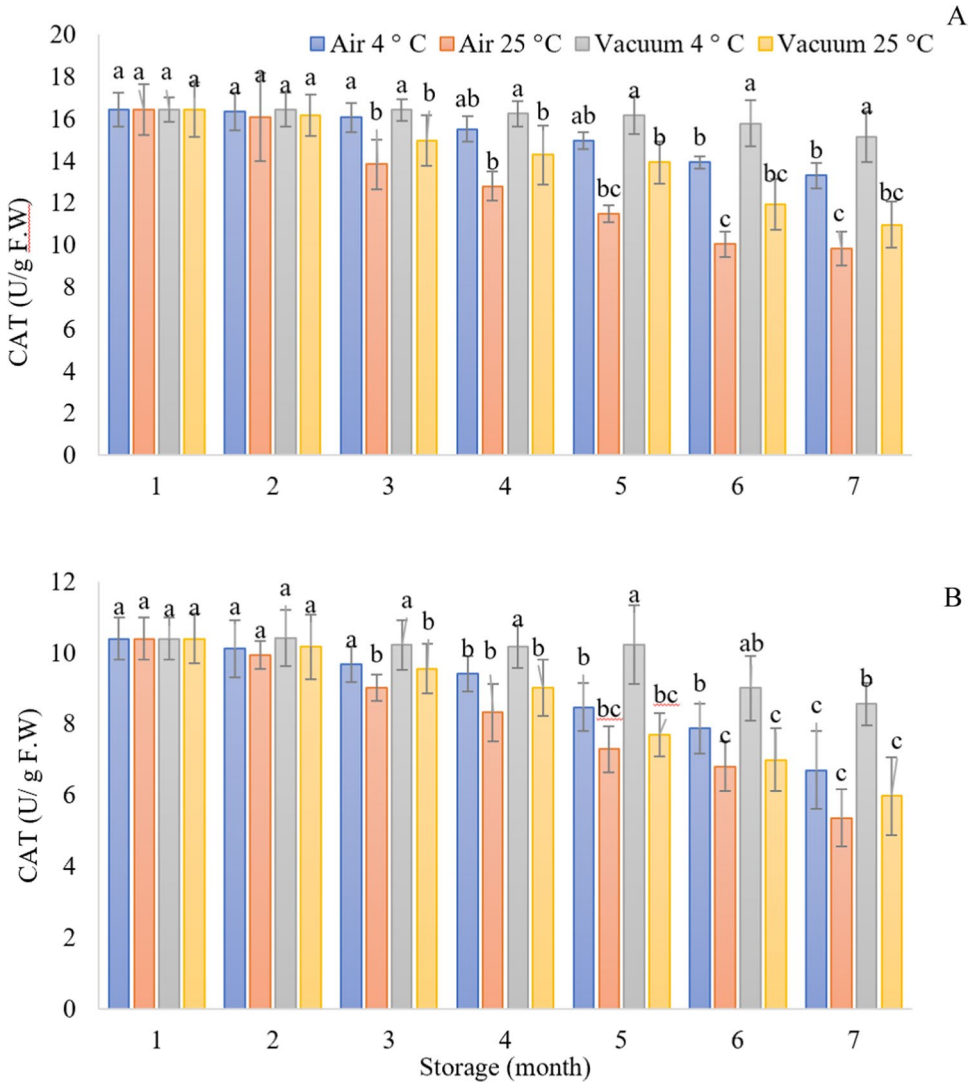

Fig. 3 Paper genotype (**A**) and Stone genotype (**B**). Catalase activity of the walnut kernel in different temperature conditions and different packing environment during 6 months

Table 5 Effect of genotype, temperature, air presence and storage time on the color parameters (L*) of the walnut kernel

Genotype	T (°C)	Packing	Storage (month)						
			0	1	2	3	4	5	6
Stone	4	Vacuum	55.74 ± .95a	55.79 ± .94a	55.47 ± .90ab	55.95 ± .60a	55.5 ± .79a	54.40 ± .69a-c	54.36 ± .63a-c
		Air	55.74 ± .95a	55.76 ± .95a	55.1 ± .99a-c	54.40 ± 1a-c	54.26 ± 1.1a-c	53.80 ± 1a-c	53 ± 1 cd
	25	Vacuum	55.74 ± .95a	55.58 ± .67a	55.5 ± .90a	54.71 ± .61abc	54.04 ± .94abc	53.73 ± .78abc	53.1 ± .90bcd
		Air	55.74 ± .95a	55.73 ± .95a	55.57 ± .97a	54.84 ± 1abc	54.06 ± 1.1abc	51.5 ± 1.1de	50 ± 1.3e
Paper	4	Vacuum	61.37 ± 3.2a	61.37 ± 3.2a	61.35 ± 3.0a	61.24 ± 2.6a	61.13 ± 2.8a	61.17 ± 3.5a	61.01 ± 2.9a
		Air	61.37 ± 3.2a	61.37 ± 3.2a	61.36 ± 2.9a	60.45 ± 2.6a	59.48 ± 2.1a	59.43 ± 3.2a	57.12 ± 2.1a
	25	Vacuum	61.37 ± 3.2a	61.37 ± 3.2a	61.35 ± 3.2a	61.27 ± 2.7a	59.44 ± 3.0a	59.34 ± 2.0a	57 ± 2.01a
		Air	61.37 ± 3.2a	61.37 ± 3.2a	60.7 ± 3.4a	60.16 ± 3.4a	58.25 ± 2.0a	55.57 ± 2.5ab	54.48 ± 2.3b

For each parameter, values followed by the same letter in do not differ significantly according to Duncan's range test (p 0.05)

reported antioxidant capacity between 53.83 and 90.38%. Christopoulos and Tsantili [11] examined antioxidant compounds of some of the important genotypes of walnut (Chandler, Hartley, and Loli) and reported an antioxidant potential of about 94.4 to 181.2 µmol of TAE per dry weight. These differences seem to be related to environmental conditions, measurement method and various genotypes of walnut. The results of the present study indicated that during storage the

Table 6 Effect of genotype, temperature, air presence and storage time on the color parameters (WI) of the walnut kernel

Genotype	T (°C)	Packing	Storage (month)						
			0	1	2	3	4	5	6
Stone	4	Vacuum	46.42 ± 1.5a	46.42 ± 1.9a	46.42 ± 1.7a	46.45 ± 2.0a	46.2 ± 1.5a	46.1 ± 2.0a	46.42 ± 1.5a
		Air	46.42 ± 1.5a	46.46 ± 1.5a	46.35 ± 1.2a	46 ± 1a	45.5 ± .95a	44.9 ± .90ab	46.42 ± 1.5a
	25	Vacuum	46.42 ± 1.5a	46.42 ± 1.6a	46.46 ± 1.2a	46.06 ± 1.18a	45.9 ± 1.5a	45.4 ± 1a	46.42 ± 1.5a
		Air	46.42 ± 1.5a	46.47 ± 1.5a	46.26 ± 1.5a	45.58 ± 2.0a	45.1 ± 2.4ab	44.4 ± 2.7ab	46.42 ± 1.5a
	4	Vacuum	51.2 ± 3.7a	51.2 ± 3.6a	51.18 ± 3.7a	51.1 ± 2.7a	51.1 ± 2.7a	51 ± 2.7a	51.2 ± 3.7a
Paper		Air	51.2 ± 3.7a	51.2 ± 3.6a	51.12 ± 2.8a	50.7 ± 2.9a	49.7 ± 3.6a	49.7 ± 2.0a	51.2 ± 3.7a
	25	Vacuum	51.2 ± 3.7a	51.2 ± 3.6a	51.2 ± 3.3a	50.5 ± 3.0a	49.8 ± 3.1a	49.5 ± 2.0a	51.2 ± 3.7a
		Air	51.2 ± 3.7a	51.16 ± 3.6a	50.28 ± 3.3a	49.4 ± 2.1a	47.8 ± 2.2ab	47.3 ± 2.1ab	51.2 ± 3.7a

For each parameter, values followed by the same letter in do not differ significantly according to Duncan's range test (p 0.05)

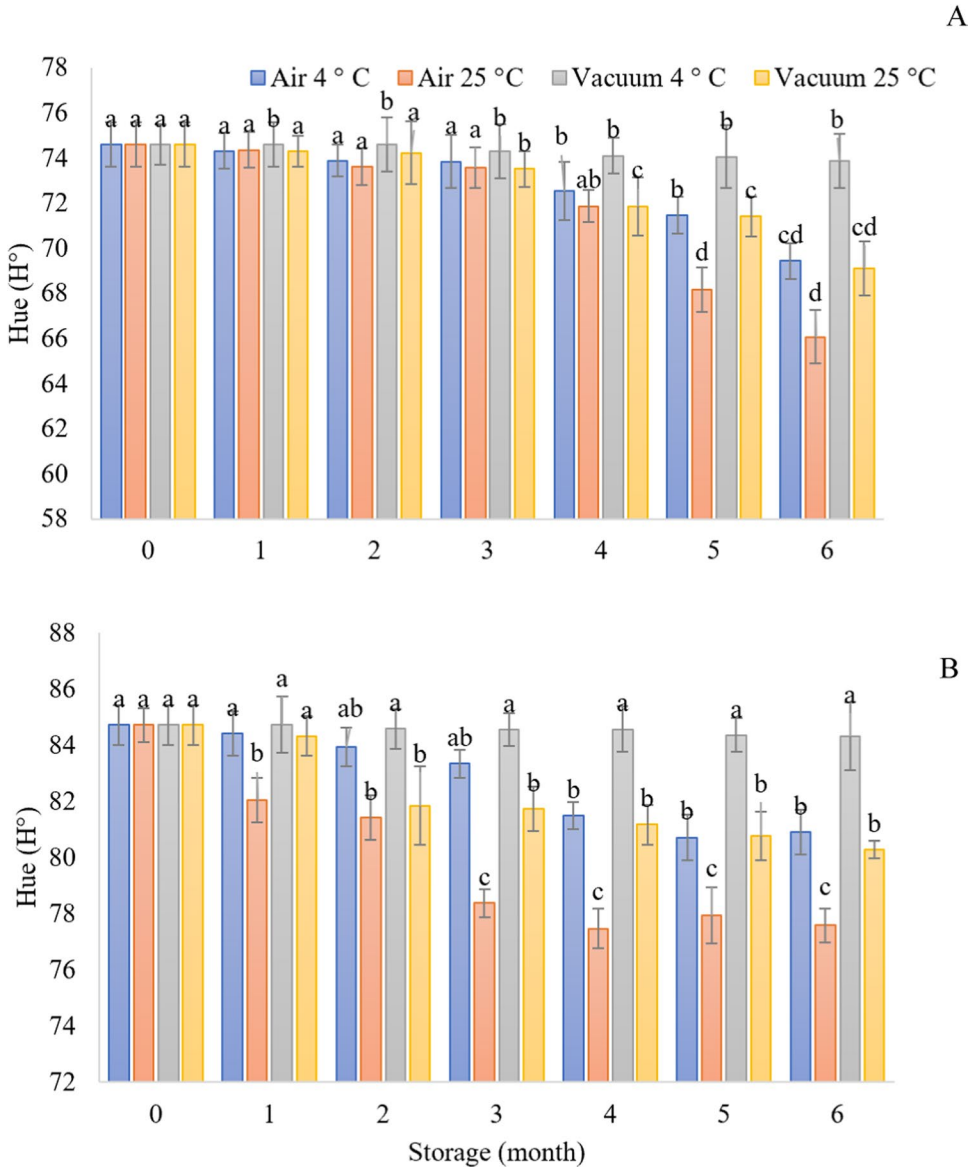

Fig. 4 Paper genotype (**A**) and Stone genotype (**B**). Parameter of color [Hue angle (h°)] of the walnut kernel in different temperature conditions and different packing environment during 6 months

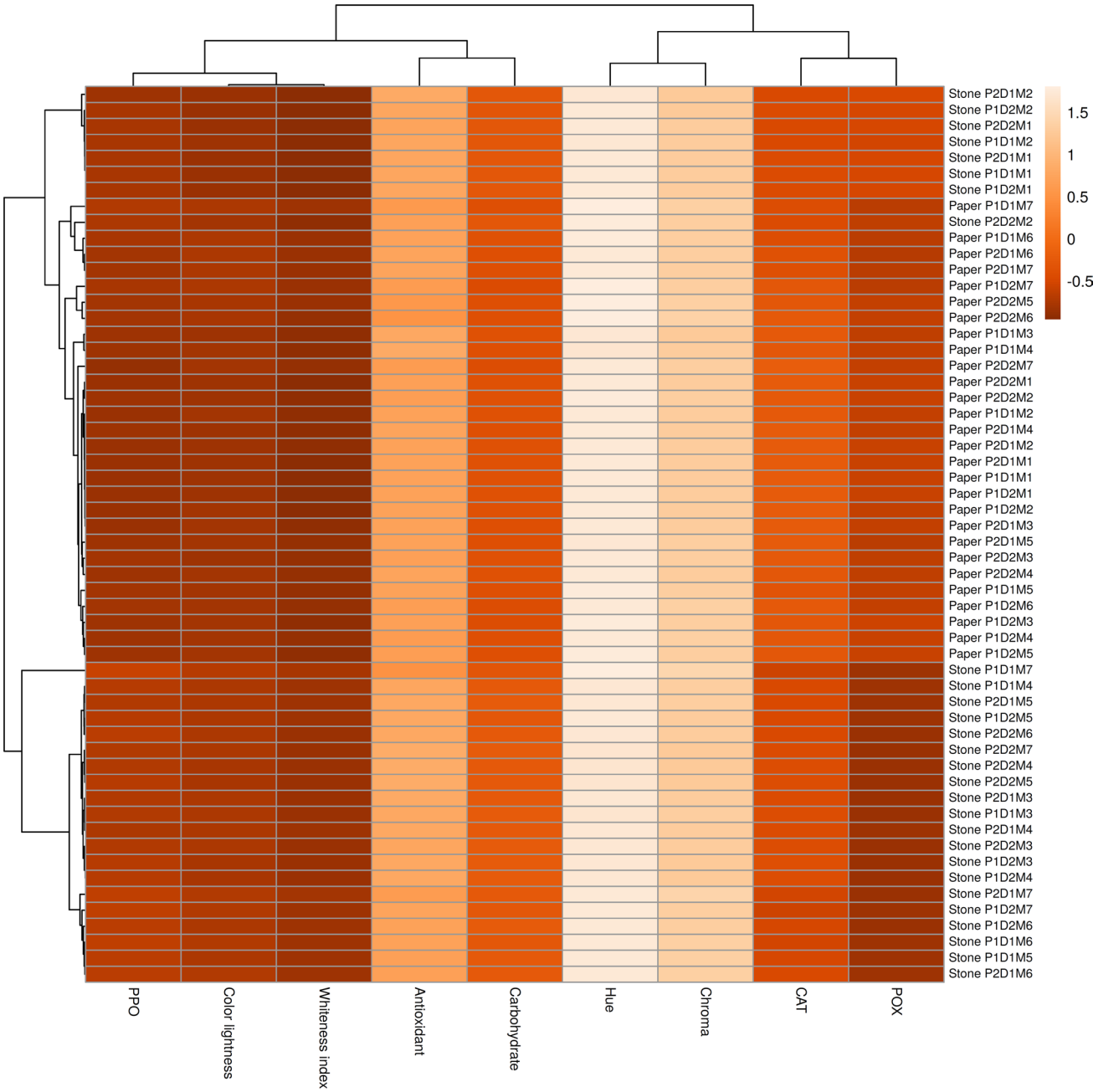

Fig. 5 Cluster analysis of walnut cv. Stone based on physical and chemical properties of fruit under two packaging modes,two temperature conditions [gradient from low (brown), medium (orange) to high (cream)]. P = packaging method (P1 = Vacume, P2 = Air), D = temperature (D1and 2: 4and 25 °C), M = storage time (M1–6: 1, 2, 3, 4, 5 and 6 months, respectively)

antioxidant capacity of two genotypes gradually decreased following a similar pattern and the samples stored at the temperature of 25 °C, in an air-containing package showed the highest reduction in antioxidant potential and nutritional value. The results of this study confirm this point the treatment at 4 °C temperature and vacuum packaging has a significant role in the preservation of antioxidant capacity and showed a significant difference compared to control conditions (at temperature of 25 °C and air-containing packaging). In another study, a vacuum system with less air access during a 12-week storage period inhibited the oxidative process better in pistachio [17]. The authors also stated that high temperatures during storage are necessary for lipid oxidation (above 20 ℃).

The phenol and flavonoid compounds impact the color, taste, flavor and health benefits of nut. Therefore they could be used as an important trait for the assessment of overall walnut quality [2, 18]. The temperature of 4 °C and the

vacuum packaging lead to effective preservation of phenol and flavonoids Phenol and flavonoids content show a decreasing trend over the course of the 6-month period with a similar pattern. This process is most evident in the last time periods. The results of this study are in the range of data reported by other researchers [11]. Bakkalbaşi et al. [19] conducted a study on several walnut genotypes and showed that total phenol content decreased significantly during walnut kernel storage. Also, studies on peanuts show that the samples kept at a temperature of 20 °C maintained their quality better than those stored at 35 °C [20]. The prevention of the reduction of phenolic compounds can be attributed to the limitation of oxidation reactions by low temperatures and air scavenger, since phenolic compounds are susceptible to chemical or enzymatic oxidation [20]. Generally during storage, antioxidants, especially phenolic compounds and flavonoids are susceptible to oxidation and thus a reduction in nutritional value. It was also observed that low temperature and reduced access to air play a significant role in preserving the quality and antioxidant compounds (phenol and flavonoids), as well as reducing fat oxidation in stored samples over a period of 6 months.

Enzymatic browning is one of the most important parameter affecting fruits and nuts quality. Walnut kernel, because of its high levels of fats and high phenolic compounds, is subject to enzymatic and chemical browning and thus reduced quality. The results of this study showed that high temperatures and the presence of air play an important role in increasing the activity of polyphenol oxidase. Accordingly, it can be concluded that by increasing the temperature and access to air, the pathway of polyphenol oxidase becomes more active, and this increased activity results in the reduction of antioxidant compounds, followed by increased fat and phenol oxidation. As a result, phenolic compounds are converted to quinones, and the formation of quinone causes browning. Like PPO, the level of POD activity was very low before storage, but it showed an increasing trend during storage with a similar pattern to polyphenol oxidase enzyme and showed significance differences especially during the fifth and sixth storage periods. If hydrogen peroxide is present, the brown color is caused by the POD enzyme, which oxidizes one electron [19, 20]. However, polyphenol oxidase can induce peroxidase activity by producing hydrogen peroxide during phenol oxidation process [19, 20]. The analysis of the relationship between peroxide value, antioxidant compounds, and enzymatic activity was investigated by measuring the correlation between traits. It was shown that increased fat oxidation is associated with reducing antioxidant compounds followed by increased PPO and POD enzymes. POD undergoes single-electron oxidation, which is the cause of the brown color if hydrogen peroxide is present. Plant tissues only contain very trace amounts of hydrogen peroxide. However, by producing hydrogen peroxide during phenol oxidation, PPO may increase POD activity.PPO causes monophenols to be hydroxylated into o-diphenols and then o-quinones under molecular oxygen, which in turn produce brown pigments. However, it is essential that PPO immediately participate in tyrosine digestion, preventing the accumulation of tyramine, and in the biosynthesis of hydroxycoumarin esculetin in walnut tree green leaves [19, 20]

Catalase is one of the antioxidant enzymes in the plant, which has a significant impact on oxidative stresses and increases the antioxidant activity of the cell and reduces the risk of free radicals [21]. Data in this experiment showed that the catalase enzyme activity was opposite to the peroxidase and polyphenol oxidase activity in the sense that decreasing the storage time decreased catalase enzyme, while activity of peroxidase and polyphenol oxidase enzymes increased. There is no complete information available about enzyme activity of the walnut kernel or nuts during the storage time. However, based on the findings of this study, it can be stated that the catalase enzyme also helps to maintain the quality of the walnut kernel during storage.

According to these results, the storage conditions and storage time can have a significant effect on the visual features of the walnut kernel. In general, the color of the walnut kernel is classified as bright to amber. In the present study, the samples showed a significant difference in the type of kernel color, and this color difference can be related to genetic factors. The paper genotypes showed the highest value (h*, WI, and L*) before and after the experiment. L* values in both genotypes before and after storage were higher than 40, which can ensure the quality of the color according to the walnut industry standards [25]. During the storage period, the color parameters (W, h*, L*) showed a decreasing trend. This decrease in the samples kept in air-containing packages and at a temperature of 20 °C was more intense. These results were in agreement with [23, 24]. Enzymatic activity or phenolic chemical oxidation could be the cause of the browning of walnut kernels and customers prefer the walnut pellicle because of its higher color index [25, 26]. Additionally, the walnut pellicle color index is important because it may indicate the rate of pellicle browning [27]. The oxidative stress brought on by a greater accumulation of reactive oxygen species (ROS) and the activation of the lypoxygenase enzyme, both of which increased lipid oxidation and peroxidation as well as nonenzymatic browning, may be the cause of the browning of the walnut pellicle during drying [28]. In fact, in this experiment, the highest browning level (minimum WI, L* and h*) in the samples stored at 25 °C and air containing packages with the highest peroxidase activity and polyphenol oxidase, as well as the lowest amount of antioxidant and phenolic compounds was observed, confirming the strong correlation between these factors. The present work showed the lowest values (L, Hue, and WI) for samples stored under ambient temperature

conditions and air-containing packaging followed with the highest loss in phenol (TP). This study's findings are in line with those of Christopoulos et al. [3] that reported Hue, L* and WI were gradually decreasing during storage.

Conclusion

This study explored the effect of storage conditions and type of genotype on TAC, TP, TF concentrations, enzymatic activity (PPO, POD and CAT), and walnut kernel color. The Paper genotypes showed higher TAC, TP, TF and CAT than the Stone genotype, while the POP and PPO contents of Stone genotype were higher than that of the Paper genotype. The results showed that all of the variables under investigation were affected by the treatment conditions during the 6-month period. The TAC, TP, TF and CAT enzymes showed a decreasing trend while the PPO and POD increased. According to these results, walnut kernel vacuum packaging and 4 °C have an important role in preventing phenol oxidization and preserving the quality of walnut kernel for 6 months. The present work showed that the deterioration of antioxidants was reduced during storage when nuts were kept in vacum and at the lower temperature.

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Author contributions AS: doing the research, formal analysis, writing—original draft, SR: investigation, supervision, writing, review & editing, PS-A:writing—review, editing, visualization and validation.

Data availability Data will be available upon request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. D. Hassani, R. Dastjerdi, R. Haghjooyan, A. Soleimani, M. Keshavarzi, J. Atefi, M. Mozaffari, R. Rezaee, A. Fahadan, A. Rahmanian, Genetic improvement of Persian walnut (*Juglans regia* L.) in Iran. In *VII* International Walnut Symposium 1050, 95–102 (2013).
2. M. Fu, Q. Qu, X.X. Yang, X. Zhang, Effect of intermittent oven drying on lipid oxidation, fatty acids composition and antioxidant activities of walnut. LWT **65**, 1126–1132 (2016). https://doi.org/10.1016/j.lwt.2015.10.002
3. M.V. Christopoulos, E. Tsantili, V. Papageorgiou, M. Komaitis, D. Rouskas, Effects of package atmosphere and temperature on phenolics, total antioxidant capacity and colour in kernels of 'Franquette' walnuts during 8-month storage. Acta Hortic. **858**, 75–81 (2010)
4. B.W. Bolling, D.L. McKay, J.B. Blumberg, The phytochemical composition and antioxidant actions of tree nuts. Asia Pac. J. Clin. Nutr. **19**(1), 117 (2010)
5. K.J. Spaccarotella, P.M. Kris-Etherton, W.L. Stone, D.M. Bagshaw, V.K. Fishell, S.G. West, F.R. Lawrence, T.J. Hartman, The effect of walnut intakeon factors related to prostate and vascular health in older men. Nutr. J. (2008). https://doi.org/10.1186/1475-2891-7-13
6. C.M. Ortiz, A.R. Vicente, R.P. Fields, F. Grillo, J.M. Labavitch, I. Donis-Gonzalez, C.H. Crisosto, Walnut (*Juglans regia* L.) kernel postharvest deterioration as affected by pellicle integrity, cultivar and oxygen concentration. Postharvest Bio. Technol. **156**, 110948 (2019). https://doi.org/10.1016/j.postharvbio.2019.110948
7. E. Tsantili, K. Konstantinidis, M.V. Christopoulos, P.A. Roussos, Total phenolics and flavonoids and total antioxidant capacity in pistachio (*Pistachia vera* L.) nuts in relation to cultivars and storage conditions. Sci. Hortic. **129**, 694–701 (2011)
8. AOAC, Peroxide value of oils and fats Official methods of analysis of AOAC international. AOAC: Maryland (2000).
9. P. Siddhuraju, P.S. Mohan, K. Becker, Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem. **79**(1), 61–67 (2002)
10. K. Ghasemi, Y. Ghasemi, A. Ehteshamnia, S.M. Nabavi, S.F. Nabavi, M.A. Ebrahimzadeh, F. Pourmorad, Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (*Juglans regia* L.) green husks. J. Med. Plants Res. **5**(7), 1128–1133 (2011)
11. M.V. Christopoulos, E. Tsantili, Effects of temperature and packaging atmosphere on total antioxidants and colour of walnut (*Juglans regia* L.) kernels during storage. Sci Hortic. **131**, 49–57 (2011)
12. M. Kar, D. Mishra, Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. **57**, 315–319 (1976)
13. B. Chance, A.C. Maehly, Assay of catalases and peroxidases. Methods Enzymol. **11**, 764–755 (1955)
14. R.G. McGuire, Reporting of objective color measurements. HortScience **27**, 1254–1255 (1992)
15. H.R. Boun, C.C. Huxsoll, Control of minimally processed carrot (*Daucus carota*) surface discoloration caused by abrasion peeling. J. Food Sci. **56**, 416–422 (1991)
16. V. Akbari, R. Heidari, R. Jamei, Fatty acid compositions and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Iran. Adv. Herb. Med. **1**(1), 36–41 (2014)
17. A. Leufven, N. Sedaghat, N.M. Habibi, Influence of different packaging systems on stability of raw dried pistachio nuts at various conditions. Iran. Food Sci. Technol. Res. J. **34**, 29–38 (2008)
18. H. Prabhakar, S. Sharma, F. Kong, Effects of postharvest handling and storage on Pecan quality. Food Rev. Int. (2020). https://doi.org/10.1080/87559129.2020.1817066
19. E. Bakkalbaşi, Ö.M. Yilmaz, O. Yemiş, N. Artik, Changes in the phenolic content and free radical-scavenging activity of vacuum packed walnut kernels during storage. Food Sci. Technol. Res. **19**(1), 105–112 (2013)
20. G. Adiletta, A. Magri, D. Albanese, L. Liguori, M. Sodo, M. Di Matteo, M. Petriccione, Overall quality and oxidative damage in packaged freshly shelled walnut kernels during cold storage. J. Food Meas. Charact. (2020). https://doi.org/10.1007/s11694-020-00589-9
21. M.V. Christopoulos, E. Tsantili, Participation of phenylalanine ammonia-lyase (PAL) in increased phenolic compounds in fresh cold stressed walnut (*Juglans regia* L.) kernels. Postharvest Biol. Technol. **104**, 17–25 (2015). https://doi.org/10.1016/j.postharvbio.2015.03.003

22. A. Sheikhi, S.H. Mirdehghan, H.R. Karimi, L. Ferguson, Effects of passive- and active-modified atmosphere packaging on physiochemical and quality attributes of fresh in-hull pistachios (*Pistacia vera* L.cv. Badami). Foods **8**(564), 1–15 (2019)
23. X. Yuan, S. Huang, H. Ma, N. Huang, N. Ye, Differential responses of walnut cultivars to cold storage and their correlation with postharvest physiological parameters. Hortic. Environ. Biotechnol. **60**, 345–356 (2019). https://doi.org/10.1007/s13580-019-00126-8
24. R.P.F. Guiné, C.F.F. Almeida, P.M.R. Correia, Influence of packaging and storage on some properties of hazelnuts. J. Food Meas. Charact. **9**(1), 11–19 (2014). https://doi.org/10.1007/s11694-014-9206-3
25. R.T. Habashi, S. Zomorodi, A. Talaie, S.K. Jari, Evaluation of shelf life of walnut kernel coated by antioxidants in combination with packaging under different storage conditions. J. Postharvest Technol. **7**(3), 87–95 (2019)
26. S. Wang, M. Monzon, J.A. Johnson, E.J. Mitcham, J. Tang, Industrial-scale radio frequency treatments for insect control in walnuts. II: heating uniformity and energy efficiency. Postharvest Biol. Technol. **45**, 240 (2007)
27. I.R. Donis-González, S.M. Bergman, G.M. Sideli, D.C. Slaughter, C.H. Crisosto, Color vision system to assess English walnut (*Juglans Regia*) kernel pellicle color. Postharvest Biol. Technol. **167**, 111199 (2020). https://doi.org/10.1016/j.postharvbio.2020.111199
28. S. Pakrah, M. Rahemi, A. Nabipour, F. Zahedzadeh, F. Kakavand, K. Vahdati, Sensory and nutritional attributes of Persian walnut kernel influenced by maturity stage, drying method, and cultivar. J. Food Process. Preserv. **45**(6), 1–12 (2021). https://doi.org/10.1111/jfpp.15513