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ORIGINAL PAPER

Overall quality and oxidative damage in packaged freshly shelled walnut kernels during cold storage

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Abstract

In this study, the effect of passive- and active-modified atmosphere packaging (PMA and MAP with 70% N_2 and 30% CO_2) using semipermeable and barrier films on physicochemical and enzymatic properties and quality of freshly shelled walnut kernels stored at 6 °C for 18 days were investigated. Changes in physicochemical such as free fatty acidity content, peroxide value, colour and enzymatic traits such as polyphenol oxidase, lipoxygenase were tracked in the fresh kernels during cold storage in different packaging systems. The MAP system inhibited polyphenol oxidase activity, delayed browning of tegumented kernels, and helped in maintaining a high total polyphenol content. The PMA system preserved membrane integrity by inhibiting lipoxygenase activity and malondialdehyde accumulation with values of 3.26 ± 0.05 nmol hydroperoxides min^{-1} g^{-1} dry weight and 10.90 ± 0.50 nmol g^{-1} dry weight, respectively after 18 days of cold storage. Moreover, this system inhibited free fatty acid formation and it was characterised by lower peroxide values with 5.17 ± 0.04 mEq O_2 kg^{-1} oil at the end of storage. Principal component analysis provided a global view of the differential qualitative responses of fresh walnut kernels in different packaging systems during cold storage. Our results show that packaging systems could be utilised to extend postharvest shelf life, improve qualitative traits, and inhibit enzyme-mediated browning of fresh kernel walnuts during cold storage.

Keywords Walnut kernels · Modified atmosphere packaging system · Physico-chemical traits · Antioxidant enzymes

Introduction

The global production of walnuts (*Juglans regia* L.) is approximately 3.8 million tons and Italy ranks 19th in the world producing 13.000 tons of walnut (FAOSTAT 2017). Lateral fruit-bearing cultivars obtained by breeding and different training systems have led to significant increases in walnut production in different countries to meet the increased market demand for walnut [1].

Shelled walnut is a fruit widely appreciated for its many beneficial properties [2]. Several compounds such as polyphenols, tocopherols, tocotrienols, ellagic acid, and melatonin have been identified in the tegument and kernel of walnuts [2, 3]. Indeed, kernels have a high oil content (more than 60% of its weight) and are very rich (more than 70%) in polyunsaturated essentials fatty acids (PUFA). These mainly constitute of linoleic (omega-3) and linolenic (omega-6) acids, which are known to reduce blood pressure, total and low-density lipoproteins (LDLs) associated cholesterol, and consequently, mitigate the risk of cardiovascular diseases [4].

The consumption of freshly shelled walnut kernels is less widespread, although these are greatly appreciated due to their characteristic flavour and nutritional value, which is lost from walnuts as a result of postharvest treatments [5]. Walnuts, at harvest time, show high water activity which contributes to the growth of mould and facilitates the activity of polyphenol oxidase and lipase involved in browning reactions and fatty acid hydrolysis, respectively [6]. The

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walnut economic losses are due to the progress in lipid oxidation, that produces an off-flavour, undesirable rancid taste, and browning reactions during storage [6, 7].

Lipid oxidation is influenced by many factors such as storage temperature, presence of light, and oxygen levels [8]. Dried walnut kernels represent the most used form of this fruit, because they can be stored for several months at room temperature, while fresh walnuts have a short shelf life due to their perishable nature [5]. Physicochemical and biological activities of the walnuts are highly influenced by their processing and storage conditions as demonstrated by Ahad et al. [9].

In this context, several studies have tested the influence of packaging with different modified atmospheres on lipid oxidation of nuts using nitrogen and carbon dioxide (CO_2) flushing, vacuum packaging, and oxygen absorber systems [10, 11]. Jensen at al. [6] showed that the optimal storage condition for packaged walnut kernels was 11 °C or lower, eventually combined with an oxygen absorber. Furthermore, a packaging material with low oxygen permeability such as ethylene vinyl alcohol combined with nitrogen flushing has been used to maintain the high quality of walnut kernels [11]. Mexis et al. [8] investigated the effect of the O_2 barrier imposed by the packaging material, lighting conditions, and temperature on the quality of shelled walnuts as a function of storage time. They demonstrated that the use of polyethylene (PE)–air, polyethylene terephthalate/polyethylene (PET/PE)–N_2, and polyethylene terephthalate–silicon oxide/polyethylene (PET–SiO_x/PE)–N_2 at 20 °C in the dark was helpful in maintaining walnut quality for ca. 2, 4–5, and 12 months, respectively.

Furthermore, Javanmard [12] studied the effects of PE/PET bag (98 µm thickness) with different O_2/CO_2 compositions in packaged shelled walnuts at different temperatures during a period of three months. They have shown that optimal packaging conditions required to maximize the quality of stored walnuts include: 1.46% O_2, 10% CO_2, and a temperature of 4 °C.

Ma et al. [13] investigated the effects of chlorine dioxide and sodium diacetate in controlled atmosphere (2% O_2 + 25% CO_2) storage on deterioration of fresh walnuts. They demonstrated that controlled atmosphere combined with chlorine dioxide was the optimal treatment and kept quality of fresh walnuts for 135 days at 0 ± 1 °C, with the lowest mold incidence, the highest firmness and contents of fat and melatonin, as well as the maximum peroxidases activity.

Other studies have demonstrated that raw ground almonds could maintain their physicochemical and nutraceutical traits, when stored in containers with an oxygen barrier and oxygen absorbers running at 4 and 20 °C. Hulled pistachio nuts were shown to retain their freshness, physical properties, and sensory attributes in the MAP system (100% CO_2) combined with a storage temperature of 5 °C, thereby extending their shelf-life [12, 14].

Furthermore, MAP systems with 30% CO_2, 70% N_2, and 4 ppm O_2 was helpful in maintaining the overall quality and safety of fresh in-hull pistachios for 30 days at 4 °C, compared to conventional or vacuum packaging [7].

However, to the best of our knowledge, no scientific studies on the physico-chemical changes in fresh shelled kernel walnut during cold storage conditions were carried out. The aim of this study was to investigate the changes in physico-chemical, qualitative, and enzymatic traits of packaged freshly shelled walnut kernels stored at cold temperature (6 ± 0.5 °C). Furthermore, the knowledge about optimum storage conditions for fresh shelled walnuts obtained from this work provides important information to minimize post-harvest losses and prolong the shelf-life of this product.

Materials and methods

Fruit samples and experimental design

Fruit were collected from Chandler walnut trees grown under standard commercially used conditions in a commercial orchard at Nola (Napoli, southern Italy; 41° 04′ N, 14° 19′ E). Ripe fruit were harvested and transported to a farm, where walnut shells were removed by the use of husking machines. Subsequently, selected fruit were transported under cold conditions to the Food Technology Laboratory of the University of Salerno and stored at 4 °C before processing. The hard shells of nuts were manually removed, and fresh kernels were mixed to obtain a homogeneous initial sample. This was afterwards segregated into three groups for the different treatments. The moisture content of fresh kernels was measured to be 240 ± 10 g kg^{-1}, as expressed on the basis of fresh kernel weight.

Fresh walnut kernels (about 50 g) were packaged in bags in two different conditions: passive-modified atmosphere (PMA) using a semipermeable film and in modified atmosphere packaging (MAP) with N_2 (70%) and CO_2 (30%) using a barrier film. Semipermeable films (polyolefins) used for the PMA experiments were characterized by the following parameters: thickness 15 µm; transmission rate of CO_2 41,000 $cm^3\,m^{-2}\,day^{-1}$; transmission rate of O_2 10,000 $cm^3\,m^{-2}\,day^{-1}$ at 23 °C and 0% RH; and moisture transmission vapour 25 g $m^{-2}\,day^{-1}$ at 38 °C and 100% RH. The barrier film (polyamide/polyethylene) used for the MAP process had a thickness of 140 µm; transmission rate of CO_2 < 130 $cm^3\,m^{-2}\,day^{-1}$; transmission rate of O_2 < 40 $cm^3\,m^{-2}\,day^{-1}$ at 23 °C and 75% RH; and moisture transmission vapour < 2 g $m^{-2}\,day^{-1}$ at 23 °C and 85% RH. Unsealed fresh walnut kernels exposed to air (C) were also evaluated as controls. In this case, the fresh sample (50 g)

was placed in sanitized cellulose trays (15 × 15 × 3 cm) without packaging film. All samples were stored at 6 °C for 18 days. The experimental design has been reported in Table 1. Three biological replicates were used from each packaging system on 0, 4, 7, 10, 14, and 18 days post packaging. Two technical replicates were performed on each biological replicate.

Physicochemical features of walnut kernels

Weight loss, colour, pH, moisture content

Weight loss was determined during the storage period by monitoring the weight of kernels in the package before and after storage. Weight loss was expressed as the percentage of the loss of kernel weight with respect to the initial weight and was determined in triplicates [15].

CIE L*a*b* colour coordinates (lightness L*, red/green index a*, and yellow/blue index b*) of the tegument (T) and flesh (K) of kernels were measured using a colourimeter (Chroma Meter II Reflectance CR-300 Minolta, Japan) with a 10 mm triple flash mode aperture.

Colour was also assessed in terms of hue angle (H°) and chroma (C*) according to Fratianni et al. [16]. Furthermore, the browning index (BI, denoting the development of brown pigments on the tegument and flesh) was calculated according to the following equation [12]:

$$\mathrm{BI} = \frac{[100 * (X - 0.31)]}{0.17}, \quad (1)$$

where

Table 1 Experimental design used in this study

Item	Values/characteristics
Factors	
A: packaging	Active-modified atmosphere (MAP, 70% N_2 and 30% CO_2)
	Passive-modified atmosphere (PMA)
	Unpacked
B: storage time (days)	0 (At harvest), 4, 7, 10, 14, 18
Factors level	
A = 3	Treatment simple effect
B = 6	Storage time simple effect
A × B = 18	Treatment × storage time interactions
Replications	
3	
Storage conditions	
Storage temperature	6 ± 1 °C

$$\mathrm{X} = \frac{a - 1.75L}{5.645L + a - 3.012b}. \quad (2)$$

The pH of ground kernel was measured with a digital pH meter (Crison 2001, Crison, Barcelona, Spain).

The moisture content of fresh shelled walnut kernels was measured according to AOAC official method 934.01 [17].

Free fatty acid content (TA) and peroxide value (PV)

Free fatty acid content (TA) and peroxide value (PV) were measured using freshly extracted cold walnut oil. Walnut kernels were ground with a coffee mill (KSW 445 CB, Bomann, Germany) and a 10 g aliquot of the powder was mixed with 100 mL of ethyl ether. The mixture was subsequently left in the dark at room temperature for 24 h. After that, mixture was filtered, and the solvent removed by a rotary vacuum evaporator (IKA RV-8, Staufen, Germany) operated at 40 °C. The extracted oil was dried by adding powdered anhydrous sodium sulphate.

TA was measured by neutralisation with 0.1 m NaOH, according to the AOAC official method 940.28 [18] and expressed as % (w/w) of oleic acid (OL). PV of walnut oil was determined by iodometric titration with 0.01 N $N_2S_2O_3$, according to 965.33 of AOAC [18] and expressed as milliequivalents of active oxygen per kg of oil (mEq O_2 kg^{-1} oil).

Headspace composition

On each day of sampling day, the headspace gas composition in each package was determined using a O_2/CO_2 gas analyzer (CheckMate3, PBI-Dansensor, Ringsted, Denmark) with a 0.1% accuracy. Gas composition was measured in three biological replicates for each film monitored.

Total phenolic content

Total phenolic content (TP) was determined by homogenizing fresh walnuts (2.5 g) in a methanolic solution (methanol/water 80/20 v/v). The homogenate was centrifuged at 14,000 × *g* for 5 min and the supernatant was used to estimate TP according to the Folin–Ciocalteu method [19]. TP was expressed as mg gallic acid equivalents of kg dry weight basis (DW, mg GAE kg^{-1}).

Enzyme extraction and activity assays

Polyphenol oxidase activity

Polyphenoloxidase activity (PPO) was determined according to the modified method described by Adiletta et al. [20]. Crude enzyme extract was obtained by homogenizing frozen

kernel powder (0.5 g) in sodium phosphate buffer (0.1 m, pH 6.4) and 0.02 g of polyvinylpolypyrrolidone (PVPP). PPO activity was detected by monitoring the increase in absorbance at 398 nm with a buffer containing 0.5 m catechol dissolved in sodium phosphate buffer (0.1 m, pH 6.4) and crude enzyme extract (200 µL). PPO activity was expressed as molar change in mmol catechol g^{-1} DW min^{-1}.

Lipoxygenase activity

Lipoxygenase activity (LOX) was evaluated by the method described by Petriccione et al. [21] with some modifications. Frozen kernel powder was blended in 3 mL of reaction mixture containing potassium phosphate buffer (0.05 m, pH 7.8), sodium–EDTA (0.001 m, pH 7), and 2% PVPP and centrifuged at 12,500 × *g* for 10 min at 4 °C. The reaction was carried out in the presence of sodium phosphate buffer (0.1 m, pH 6), linoleic acid sodium salt (0.005 m) and crude enzyme extract (10 µL). LOX activity was estimated as the increase in absorbance at 234 nm and was expressed as nmol hydroperoxides g^{-1} DW min^{-1}.

Malondialdehyde content determination

Malondialdehyde (MDA) content was evaluated according to Adiletta et al. [22] with some modifications. Frozen kernel powder (1 g) was blended with 10% (w/v) trichloroacetic acid (10 mL) and 0.5% thiobarbituric acid dissolved in 0.25 m HCl. The solution was placed in a boiling water bath at 95 °C for 30 min and immediately cooled. The mixture was centrifuged at 12,000 × g for 10 min and the supernatant obtained was spectrophotometrically assayed for absorbance at 450, 532, and 600 nm. The MDA content was expressed as nmol g^{-1} DW.

Statistical analysis

All results were expressed as mean ± standard deviation. Principal component analysis (PCA) was used to describe the influence of various packaging systems on the different analysed parameters for identification of the components responsible for the main variations in walnut kernels during cold storage. All analysis were performed using the SPSS software package, version 20.0 (SPSS, Inc., Chicago, IL, USA). Statistically significant differences among packaged and unpackaged shelled walnut kernels were analysed by Duncan's test. Significant differences ($p \leq 0.05$) were indicated with different letters. Correlations among the tested parameters were analysed using Pearson's correlations ($p \leq 0.05$ and ≤ 0.01).

Results and discussion

Effect of packaging system on the weight loss and headspace composition of fresh shelled walnut kernels

Fresh shelled walnut kernels are susceptible to quality deterioration and reduced shelf life [23]. Packaging systems are useful postharvest technologies to preserve the freshness and extend the shelf life of fresh and minimally processed fruit under different storage conditions [24]. Several studies have demonstrated that air humidity in the headspace of the packaging was useful for maintaining a higher water content in fruit, thereby reducing dehydration and minimising weight loss [25, 26].

The air-stored unpackaged samples (C) showed a faster decrease in moisture with the greatest weight loss percentage, compared to packaged ones during 18 days of cold storage (Fig. 1a). Minimal losses in weight were observed in the two different packaging systems, with values of 0.25% and 0.40% in MAP and PMA at the end of storage, respectively. The highest weight loss observed in air-stored unpackaged samples could be attributed to the transpiration phenomena, that is driven by the difference in water vapour pressure between the product surface and the environment. Furthermore, the differences found between MAP and PMA packaging systems could be due to the different moisture transmission vapour of these films, which influences the transpiration process [27]. In particular, the higher permeability or moisture vapour of the film used in PMA led to a lower water vapour pressure in the packaging atmosphere.

The higher weight loss has been observed in control sample compared to packed ones as reported by Shayanfar et al. [14] in fresh pistachio nuts. Sheikhi et al. [28] reported that weight loss of de-hulled pistachio stored at 0 ± 0.5 °C for passive-MAP and control treatments varied by 0.5% and 10.9% of initial weight respectively after 105 days of storage. In whole walnuts and shelled kernels, the weight loss was 29% and 17.5%, respectively in samples stored in air at 1 °C and 90% RH after 40 days [5]. Furthermore, a previous study highlighted that in shelled walnut a reduction of 50% in water content after 3 months of storage independent of storage conditions was observed [6].

MAP packaging is known to influence respiratory activity, rate of oxidation, and rate of degradation of valuable flavour and colour components in several fruit [26, 29–31].

In the context of the respiratory activity, the mixture of gas used for the MAP system seems to inhibit the respiration of the kernels, since no significant changes in CO_2 % were observed during storage and only a small increase of O_2 (up to 0.56%) was observed at the end of the storage period.

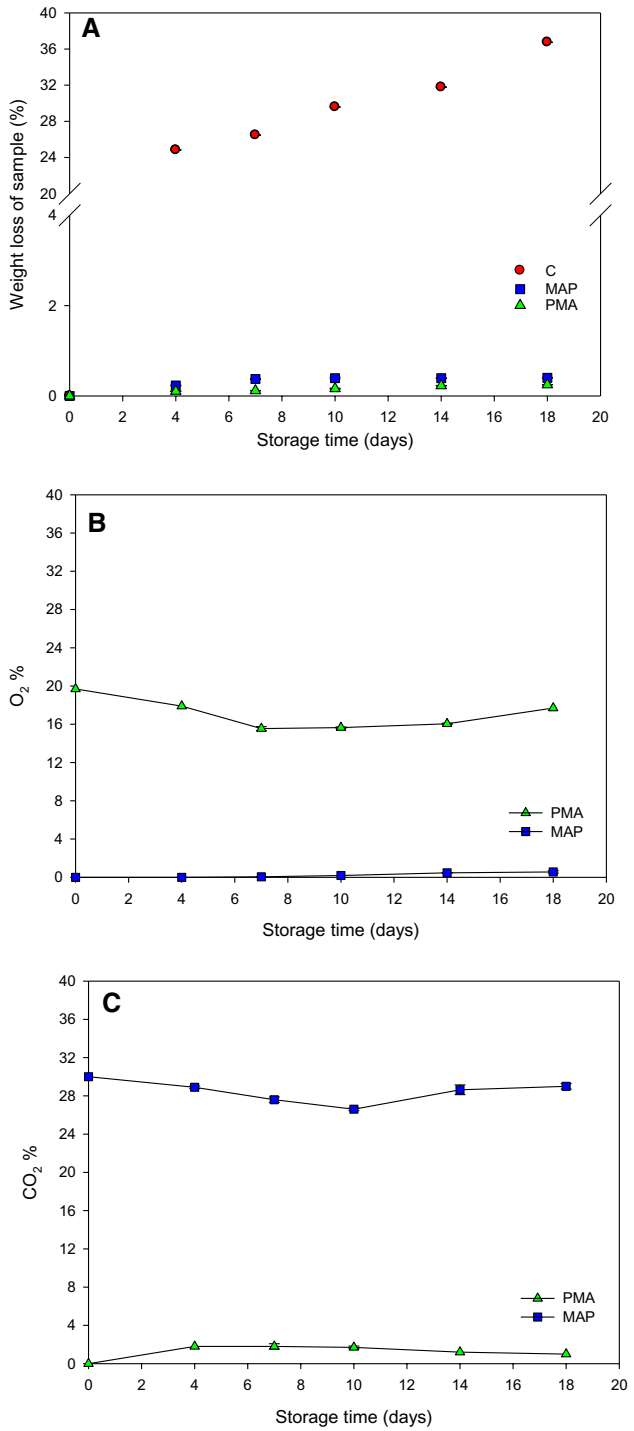

Fig. 1 Weight loss of samples (**a**) and gas composition [O_2 percentage (**b**) and CO_2 percentage (**c**)] in shelled kernels from the walnut cultivar 'Chandler' packaged in semipermeable (PMA) and barrier (MAP) films and stored at 6 °C for 18 days. Error bars indicate the standard deviation

On the contrary, the PMA system registered a decrease of O_2 from 19.7 to 17.7% at 18 days of storage (Fig. 1b) and an increase of CO_2 up to 2% (Fig. 1c) as a result of the respiration of walnut kernels and gas transmission rate of the semipermeable films employed.

Shayanfar et al. [14] demonstrated that in pistachio nuts packed under MAP conditions was observed a slighter weight loss compared to control samples due to less respiration rate in the modified atmosphere which results in less weight loss of the fresh product.

Evaluation of colour, total phenolic content, and PPO activity in fresh shelled walnut kernels

Colour is one of the most important sensory attributes of fruit that can affect consumer acceptance [32, 33].

At time 0, the recorded colour parameters of tegument (T) (Table 2A) and kernel (K) (Table 2B) indicated a light yellow and white colour, respectively. During storage, L* and H° values decreased in packaged and air-stored unpackaged samples (Table 2), denoting colour deterioration of kernels, probably due to enzymatic browning [5, 13].

In order to evaluate the extent of browning during cold storage, the BI parameter was calculated. Significant differences ($p < 0.05$) in browning were recorded among packaged and unpackaged samples during the storage period. In the MAP system, the absence of O_2 seems to slow down the browning phenomena. For these samples, in fact, the percentage increase in BI after 18 storage days was 29.54 and 18.97% for the tegument (T) and kernel (K), respectively, in contrast to the 41.36 and 91.76% decrease in unpackaged samples and 68.48 and 22.10% decrease recorded for PMA samples. High extents of browning was observed for the control and PMA samples, due to contact with O_2 and PPO-mediated oxidation of phenolic compounds in the brown pigments [34].

The MAP system was observed to be effective in the reduction of kernel browning, in agreement with other studies on 'Franquette', and 'Xifu No. 1' walnut and on fresh in-hull pistachios [13, 35].

Similar to the browning results, PPO activity increased in all walnut kernels during the storage; with the highest increase detected for PMA system, followed by that in the unpackaged control samples (Table 3). The absence of O_2 in the MAP system seems effective in hindering the PPO activity, that increased by 37% at the end of storage period compared to the initial value. A good correlation between PPO activity and BI (T) was found with R^2 values of 0.843, 0.867, and 0.624 for C, PMA- and MAP-packaged samples, respectively.

The amount of total phenols detected in fresh walnut samples was about 9300 mg GAE kg^{-1} DW. Similar values were detected by Christopoulos and Tsantili [36] who reported a total phenolic content in 'Chandler', 'Hartley', and 'Ioli' walnuts ranging from 1100 to 2200 mg GAE 100 g^{-1} DW.

Table 2 Effect of different packaging systems (PMA: semipermeable film and MAP: barrier film) on colour parameters in walnut kernels, compared to air-stored unpackaged ones (C)

A						
Systems	Storage time	L* T	a* T	b* T	Hue° T	BI T
C	0	73.67 ± 2.76^{e}	1.01 ± 0.83^{a}	27.23 ± 1.74^{b}	87.80 ± 1.74^{d}	44.58 ± 2.03^{a}
	7	65.85 ± 0.04^{c}	2.62 ± 0.01^{b}	28.72 ± 0.13^{b}	84.82 ± 0.05^{c}	57.83 ± 0.37^{b}
	18	60.69 ± 0.02^{b}	2.39 ± 0.02^{b}	28.36 ± 0.07^{b}	85.18 ± 0.04^{c}	63.02 ± 0.18^{b}
PMA	0	73.67 ± 2.76^{e}	1.01 ± 0.83^{a}	27.23 ± 1.74^{b}	87.80 ± 1.74^{d}	44.58 ± 2.03^{a}
	7	56.48 ± 2.05^{a}	5.94 ± 0.06^{d}	26.85 ± 0.85^{a}	77.51 ± 0.31^{a}	69.57 ± 0.59^{c}
	18	56.77 ± 3.60^{a}	5.92 ± 1.06^{d}	28.54 ± 1.04^{b}	78.33 ± 1.63^{a}	75.11 ± 11.11^{c}
MAP	0	73.67 ± 2.76^{e}	$1{,}01 \pm 0.83^{a}$	$27{,}23 \pm 1.74^{b}$	87.80 ± 1.74^{d}	44.58 ± 2.03^{a}
	7	79.41 ± 0.34^{f}	2.27 ± 0.10^{b}	28.95 ± 0.51^{b}	85.52 ± 0.26^{c}	45.78 ± 0.95^{a}
	18	67.03 ± 1.72^{d}	4.25 ± 0.98^{c}	28.45 ± 2.05^{b}	81.37 ± 2.54^{b}	57.75 ± 1.93^{b}
B						
Systems	Storage time	L* K	a* K	b* K	Hue° K	BI K
C	0	78.58 ± 2.76^{bc}	-2.18 ± 0.55^{a}	20.04 ± 0.89^{a}	96.17 ± 1.33^{e}	26.20 ± 1.04^{a}
	7	72.62 ± 0.03^{a}	0.07 ± 0.00^{d}	26.79 ± 0.07^{c}	89.85 ± 0.00^{b}	44.26 ± 0.16^{d}
	18	75.42 ± 0.01^{ab}	1.21 ± 0.04^{e}	30.20 ± 0.05^{d}	87.70 ± 0.08^{a}	50.24 ± 0.16^{e}
PMA	0	78.58 ± 2.76^{bc}	-2.18 ± 0.55^{a}	20.04 ± 0.89^{a}	96.17 ± 1.33^{e}	26.20 ± 1.04^{a}
	7	$76.48 \pm 0.51^{a–c}$	-0.89 ± 0.06^{bc}	20.50 ± 0.21^{a}	92.50 ± 0.20^{cd}	29.19 ± 0.19^{b}
	18	$77.36 \pm 1.66^{a–c}$	$-0.71 \pm 0.42^{b–d}$	22.27 ± 1.03^{b}	91.85 ± 1.15^{cd}	31.99 ± 1.38^{c}
AMA	0	78.58 ± 2.76^{bc}	-2.18 ± 0.55^{a}	20.04 ± 0.89^{a}	96.17 ± 1.33^{e}	26.20 ± 1.04^{a}
	7	83.56 ± 1.86^{d}	-1.35 ± 0.24^{b}	19.76 ± 0.75^{a}	93.39 ± 1.09^{d}	27.63 ± 2.24^{a}
	18	80.70 ± 2.47^{cd}	-0.52 ± 1.00^{cd}	22.55 ± 0.40^{b}	91.34 ± 2.56^{bc}	31.17 ± 2.74^{c}

These include lightness (L*), red/green index (a*), yellow/blue index (b), hue angle (H°), and browning index (BI) in the tegument (T) (A) and kernel (K) (B) of shelled 'Chandler' walnuts during 18 days of cold storage at 6 °C. Means followed by the same letter do not differ significantly ($p < 0.05$; Duncan's test)

Table 3 Effect of different packaging systems (PMA: semipermeable film and MAP: barrier film) on enzymatic traits in packaged walnuts, compared to air-stored unpackaged one (C)

Systems	Storage time	TA	PV	TP	LOX	MDA	PPO
C	0	0.14 ± 0.01^{a}	2.60 ± 0.01^{a}	9299.8 ± 219.3^{a}	1.3 ± 0.01^{a}	1.04 ± 0.21^{a}	7.64 ± 0.74^{a}
	7	0.48 ± 0.05^{d}	5.17 ± 0.04^{c}	9839.2 ± 399.2^{a}	1.42 ± 0.01^{b}	2.50 ± 0.37^{b}	10.57 ± 0.77^{b}
	18	0.63 ± 0.07^{e}	9.13 ± 0.03^{e}	$10{,}009.0 \pm 612.2^{a}$	1.84 ± 0.17^{c}	3.21 ± 0.55^{c}	18.90 ± 1.93^{c}
PMA	0	0.14 ± 0.01^{a}	2.60 ± 0.01^{a}	9299.8 ± 219.3^{a}	1.3 ± 0.01^{a}	1.04 ± 0.21^{a}	7.64 ± 0.74^{a}
	7	0.26 ± 0.02^{b}	3.08 ± 0.01^{b}	$17{,}548.7 \pm 411.5^{b}$	2.60 ± 0.01^{d}	5.60 ± 0.78^{d}	18.80 ± 0.11^{c}
	18	0.45 ± 0.02^{c}	5.17 ± 0.04^{c}	$22{,}059.7 \pm 217.1^{d}$	3.26 ± 0.05^{e}	10.90 ± 0.50^{f}	29.55 ± 0.31^{d}
MAP	0	0.14 ± 0.01^{a}	2.60 ± 0.01^{a}	9299.8 ± 219.3^{a}	1.3 ± 0.01^{a}	1.04 ± 0.21^{a}	7.64 ± 0.74^{a}
	7	0.86 ± 0.10^{f}	7.14 ± 0.08^{d}	$19{,}521.0 \pm 1396.6^{c}$	2.61 ± 0.01^{d}	7.62 ± 0.08^{e}	10.17 ± 0.43^{b}
	18	0.96 ± 0.06^{g}	13.51 ± 0.06^{f}	$27{,}649.0 \pm 632.1^{e}$	3.85 ± 0.01^{f}	13.24 ± 0.17^{g}	10.46 ± 0.22^{b}

The traits include titratable acidity (TA, %), peroxide value (PV mEq O_2 kg^{-1} oil), total polyphenol content (TP, mg GAE kg^{-1} DW), lipoxygenase activity (LOX nmol hydroperoxides min^{-1} g^{-1} DW), malondialdehyde content (MDA nmol g^{-1} DW), and polyphenol oxidase (PPO mmol of catechol min^{-1} g^{-1} DW) of shelled kernels of 'Chandler' walnut during 18 days of cold storage at 6 °C. Means followed by the same letter do not differ significantly ($p < 0.05$; Duncan's test)

In our study, the TP content was influenced by packaging conditions during storage. In particular, kernels packaged in the MAP system showed a trend of significant increase, compared to those stored in the PMA system. No significant change was observed in the air-stored unpackaged sample throughout storage.

Based on our findings, the increase in TP during storage could be due to an increase in PAL activity, involved in the

biosynthesis of phenolic compounds through the phenylpropanoid pathway, in walnut during cold storage, as demonstrated in other studies [5, 37].

Evaluation of oxidative damage in fresh shelled walnut kernels

Walnuts are susceptible to oxidation due to their high fat content and high percentage of PUFA. This could be prevented using optimal packaging conditions, thereby significantly extending the shelf life of fresh walnuts [9, 13].

In addition, total acidity is an important quality parameter in most fruit with a high percentage of fat in their composition [37].

Packaging systems affected the TA during storage. At the end of storage, TA values were approximately 5.5-, 3.5-, and 6.8-fold higher than initial values in control, PMA, and MAP samples, respectively. Several studies have demonstrated that an increase in TA could be due to the enzymatic hydrolysis of triacylglycerides, that release fatty acids associated with the development of soapy flavour or off-flavours in fresh walnuts during storage [36, 38].

LOX is an enzyme which catalyses the oxygenation of PUFA to form fatty acid hydroperoxides [39, 40].

For all evaluated samples, an increase of LOX activity was observed during the storage period. In particular, at the end of storage, the MAP samples showed a higher LOX value than the PMA and C walnut samples. The control walnut kernels registered the lowest LOX value, probably due to low moisture content (3%) at the end of storage. As reported by Li et al. [3] and Drapon [41], there is a significantly positive correlation between LOX activity and a_w and moisture content in foodstuff.

Lipid oxidation of walnut kernels was also evaluated by estimating the MDA content, which is considered to be one of the final products of PUFA peroxidation.

As observed for LOX, the highest activity of MDA was found to increase faster in MAP samples (Table 3) reaching a value, after 18 days, of 13.24 nmol g^{-1} DW, compared to values of 10.90 and 3.21 nmol g^{-1} DW in PMA and control samples, respectively. In order to confirm the previous results, lipid oxidation of kernel walnut was also evaluated by measuring the peroxide value (PV) as an index of primary oxidation products [8]. The low initial PV (< 25 mEq O_2 kg^{-1} oil) of fresh kernels indicate good product quality in terms of degree of lipid oxidation. In fact, PV at time 0 was 2.60 mEq O_2 kg^{-1} oil and this value is in agreement with those found in 'Yalova' kernels [42].

As for LOX and MDA content, the amount of hydroperoxides detected in all the samples increased in walnut kernels packaged using the MAP system. In fact, for these samples, PV increased during cold storage reaching a value of 13.51 mEq O_2 kg^{-1} oil, as compared to 9.13 and 5.17 mEq O_2 kg^{-1} oil in C and PMA kernels, respectively.

A possible explanation of the high lipid oxidation observed in the MAP system could be the high percentage of CO_2 in the atmosphere which, dissolving in the kernel matrix, caused a lowering of pH to 5.1, as compared to 6.1 and 5.7 in control and PMA samples, respectively.

As reported by Yesiloglu and Demirkan [43], although the optimum pH value required for lipase activity of walnut seed was 9, in the pH range of 3–8 the enzymatic activity increased significantly, thereby decreasing the pH. Therefore, the lower pH values in MAP samples could be responsible of a higher lipase activity, leading to a high content of free fatty acids that represent the primary substrate of LOX [39].

Evaluation of packaging conditions by PCA and Pearson's correlation

PCA is a standard tool for modern multivariate data analysis. It reduces a large data matrix to a few composite variables, and it provides a way of identifying statistical patterns in data, expressing the different parameters in a way that highlights their similarities and differences [44].

A PCA approach was applied to evaluate the different packaging conditions in fresh shelled kernel walnut during cold storage by assessing some physico-chemical, qualitative, and enzymatic traits.

The eigenvalues of the covariance matrix showed that the set of the two principal components (PCs) accounted for 76.62% of the total variance in the dataset (48.51 and 28.11% for PC1 and PC2, respectively, Fig. 2). LOX ($r^2 = 0.955$), TP ($r^2 = 0.981$), and MDA ($r^2 = 0.943$) were positively correlated to PC1; whereas, pH ($r^2 = -0.972$) and WL ($r^2 = -0.738$) were negatively correlated to the same. BI (K) ($r^2 = 0.753$), BI (T) ($r^2 = 0.381$), PPO ($r^2 = 0.309$), PV ($r^2 = 0.887$), and TA ($r^2 = 0.749$) showed a positive correlation to PC2.

The multivariate space of the first two PCs showed that during storage the average PC scores in samples packaged in the MAP system shifted from negative values to positive ones along PC1. As cold storage progressed, the air-stored unpackaged and PMA samples displayed a shift from negative values to positive ones along PC2.

BI (K), weight loss, and pH were positively correlated to air-stored unpackaged samples (C_7, C_{18}), while BI (T), PPO activity, PV, and TA were positively correlated to the samples packaged in the PMA system. Furthermore, MDA content, LOX activity, and TP content were positively correlated to the samples packaged in the MAP system.

The Pearson matrix underlined the inter-correlations among the physico-chemical, qualitative, and enzymatic traits, showing some positive and negative correlations (Fig. 3). MDA was positively correlated to LOX

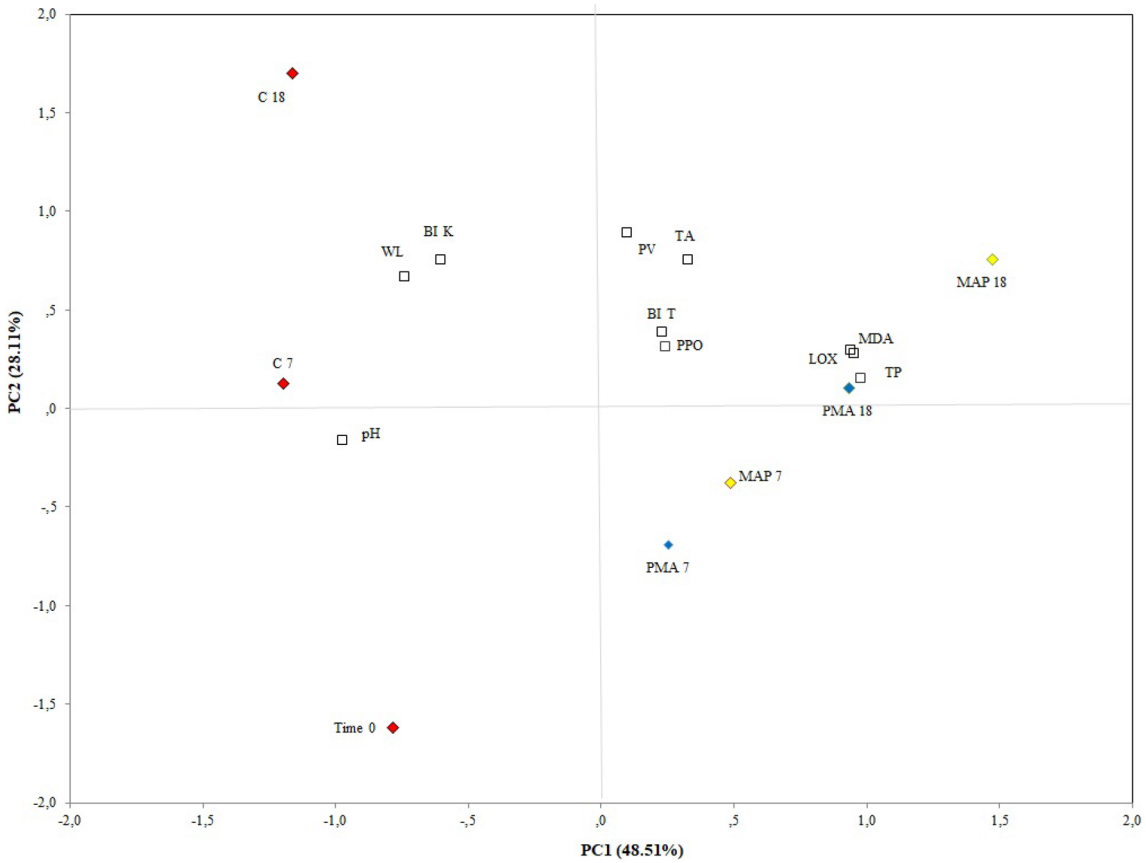

Fig. 2 Principal component analysis of the physicochemical, nutraceutical, and enzymatic traits in shelled kernels from the walnut cultivar 'Chandler' packaged in semipermeable (PMA) and barrier (MAP) films, at harvest (time 0), and after 7 days (time 7) and 18 days (time 18) of storage at 6 °C [*TA* free fatty acid content, *WL* weight loss, *TP* total polyphenol content, *PV* peroxide value, *BI* browning index for tegument (T) and kernel (K), *MDA* malondialdehyde content, *PPO* polyphenol oxidase, *LOX* lipoxygenase]

($r^2 = 0.979$; $p \leq 0.01$, Fig. 3). This suggested that during fresh walnut kernel storage, the lipid oxidation was due to LOX activity, with an increase in MDA content. LOX was negatively correlated with pH ($r^2 = -0.971$; $p \leq 0.01$), which indicates that pH strongly affected the enzyme activity. Furthermore, PPO activity was positively correlated to BI (T) ($r^2 = 0.838$; $p \leq 0.01$), suggesting that browning reactions were responsible for colour changes and polyphenol oxidation in fresh shelled kernels walnut (Fig. 3).

PCA is a valuable tool in consolidating the large amount of information that is obtained from antioxidant enzymes and qualitative analyses of kiwifruit and loquat during storage and shelf life [45, 46]. Several studies have demonstrated that this type of multivariate analysis is widely used to highlight the effects of storage conditions on physicochemical, qualitative, and enzymatic changes that occur during storage in different fruit crops such as ready to eat pomegranate arils, wine grape, kiwifruit and loquat [45–48].

Conclusion

Freshly shelled kernels are greatly appreciated by consumers due to their characteristic flavour and nutritional value; however, they are highly perishable due to metabolic processes that continue after harvest and even during storage. Two packaging systems, namely PMA and MAP, were tested for suitability in the storage of fresh walnut kernels. Air-stored unpackaged (control) kernels showed a faster weight and moisture loss because of the natural drying process. Comparing the two packaging systems, PMA delayed the lipid peroxidation with lower LOX activity and MDA content, and consequently led to a lower PV and free fatty acid formation. On the other hand, the MAP system improved the colour changes in tegument of kernels with a lower BI, associated with low PPO activity and high TP content. Proper packaging systems, that prevent qualitative decay, browning reactions, and lipid oxidation, should be optimized in shelled or unshelled, fresh or dried walnut fruit.

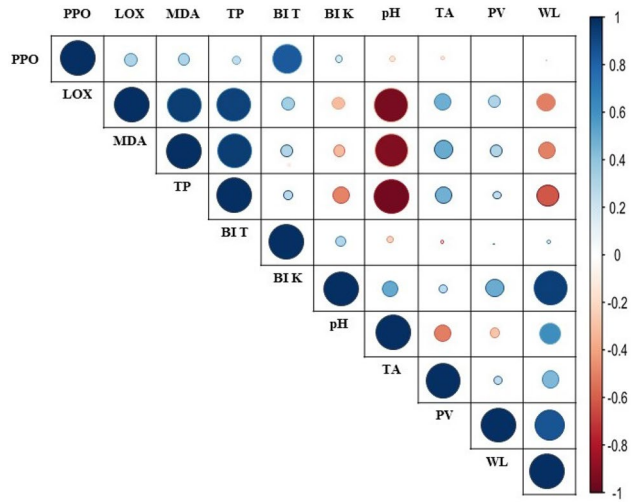

Fig. 3 Pearson's correlation matrix for all the analysed traits [*TA* free fatty acid content, *WL* weight loss, *TP* total polyphenols content, *PV* peroxide value, *BI* browning index for tegument (T) and kernel (K), *MDA* malondialdehyde content, *PPO* polyphenol oxidase, *LOX* lipoxygenase, pH]. The circle size and colour intensity are proportional to the value of each correlation coefficient. Positive and negative correlations are displayed in blue and red, respectively (Color figure online)

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