



## Walnut (*Juglans regia* L.) kernel postharvest deterioration as affected by pellicle integrity, cultivar and oxygen concentration

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### ABSTRACT

Increased demand for convenient, healthy foods has promoted the commercialization of shelled, more perishable walnut kernels. In this work for two of the major commercial walnut cultivars ('Chandler' and 'Howard') we determined the influence that disruption of the integrity of the seed coat pellicle during shelling operations trigger postharvest deterioration. Commercially mature 'Chandler' and 'Howard' nuts were subjected to Gentle (GS, < 4% pellicle area damaged per kernel) or Harsh Shelling (HS, 20–22% of pellicle area damaged) and stored in air at 25 or 35 °C (accelerated aging) for three or six weeks. During this period, which simulated current marketing and retail display, we evaluated kernel color changes (Dried Fruit Association of California 'DFA' scale,  $L^*$  and  $Hue$ ), ethanol-soluble phenolic antioxidants, oil-free fatty acids (FFA), and peroxide value (PV). The kernel color changed from 'light' to 'amber' during storage, as demonstrated by the decrease in extra light and light kernels and by the reduced lightness ( $L^*$ ) and  $Hue$  values. Pellicle browning (amber) incidence was common on HS kernels, which also lost more phenolic antioxidants during storage. Minimizing pellicle damage by GS operations reduced triglyceride hydrolysis and peroxidation. Kernel quality loss was largely dependent on cultivar; browning oxidation, and lipid hydrolysis and oxidation were faster in 'Howard' than in 'Chandler'. Searching for a practical and direct postharvest technology, in absence of proper temperature control, to reduce the rate of kernel deterioration, we tested controlled atmospheres (CA) at different  $O_2$  concentrations (0.0, 3.0, 6.0 or 21.0 kPa) on both cultivars. Overall, commercially shelled 'Howard' and 'Chandler' (kernels) will benefit from retail packaging with oxygen concentrations equal to or lower than 3.0 kPa during warm retail display. This information will be useful for processors, distributors and produce handlers to protect snack-friendly, ready-to-eat walnuts.

### 1. Introduction

Growing scientific evidence suggests that nuts help maintain cardiovascular health and prevent some types of disease (Alasalvar and Bolling, 2015; Bamberger et al., 2017). This has contributed to a rapid expansion in walnut planting, production, consumption, and trade.

The edible walnut kernel consists of the embryo (meat) and a seed coat or pellicle. The seed is further protected by a hard shell (outer portion of the ovary) and green fleshy husk or hull (involucre and sepals). After mechanical harvest, walnut hulls are removed

immediately (hulling) and the nuts are dried down to 8% moisture content. As the season progresses, shelled nuts are cracked (shelled) and sold mainly as halves or pieces (kernels), with a smaller proportion sold in-shell.

In recent years walnut distribution chains expanded in terms of both volume and geographical destinations. In turn reaching the market with premium quality kernels has become more challenging (Chang et al., 2016). Premium quality defined as large size and light kernels without any 'off flavor'. There is a strong economic incentive to appraise the impacts that each step in the supply chain has on nut postharvest

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quality. Unfortunately, most transportation and handling are carried out without proper temperature control, especially during display at retail stores. Walnut pellicle browning is a primary deteriorative change that reduces kernel quality. While non-enzymatic Maillard reaction products contribute to browning in some nut species (Wall and Gentry, 2007), walnut browning has been more closely linked to oxidation of phenolic compounds by polyphenoloxidases (PPOs) (Escobar et al., 2008; Taranto et al., 2017). Non-enzymatic phenolic oxidation may contribute to postharvest browning of dried nuts, but is often overlooked and seldom examined. Loss of phenolic-yielding quinone polymerization products reduces nut visual quality and antioxidant capacity (Labavitch, 2004; Christopoulos and Tsantili, 2015).

If kernel pellicle browning compromises appearance and some health benefits of walnuts, lipid degradation leading to rancidification is the major cause of flavor rejection (Shahidi and John, 2013). Although all nut species are prone to rancidity, walnuts are particularly susceptible, due to their elevated oil content (~65 to 70%) and high proportion of poly-unsaturated fatty acids (PUFAs) (Martínez et al., 2006, 2010).

Changes in eating habits caused by urbanization and consumer trends are increasing the demand for shelled walnuts (Contini et al., 2016), which are a convenient, healthy and nutritious snack food (Workman et al., 2018). However, ready-to-use shelled nuts are more susceptible to pellicle browning and rancidity. The greater perishability of shelled walnuts may be due to the removal of the protective shell (Christopoulos and Tsantili, 2015; Evans et al., 2016), though disrupting the seed coat (pellicle) during shelling operations may also favor deteriorative reactions.

The negative effects of physical damage on the shelf life of high-water content commodities such as fruits and vegetables are well documented (Castro Ibañez et al., 2017). In fresh fruit, wounding increases respiration, ethylene production and, by eliminating natural surface barriers, greatly enhances susceptibility to fungal rots and browning (Ansah et al., 2018). In contrast, few studies have evaluated the influence of surface physical damage on the postharvest performance of products with low water activity ( $A_w$ ) and restricted metabolism, such as walnuts. To our knowledge, the influence of walnut seed coat (pellicle) integrity on pellicle browning and lipid oxidation has not been examined previously. Our hypothesis was that mechanical pellicle damage during walnut shelling operations contributes to favor kernel browning and lipid deterioration reactions. We evaluated the influence of walnut seed coat (pellicle) integrity on pellicle browning, kernel phenolic antioxidants, oil hydrolysis and peroxidation in two of the most widespread cultivars ('Chandler' and 'Howard') during accelerated aging. We also assessed the influence of cultivar on the extent of pellicle browning of walnuts that were shelled using current commercial processes and stored under recommended and suboptimal CA conditions (0, 3, 6 or 21 kPa  $O_2$ ).

## 2. Materials and methods

### 2.1. Effect of walnut pellicle integrity on browning, antioxidants and oil quality

Five-year-old 'Chandler', and 'Howard' trees growing at the UC Davis Plant Sciences experimental field (38° 32' 42" N / 121° 44' 21" W) were used to quantify kernel color quality performance under shelling treatments. The commercial orchard is trained in a minimum pruning center leader and spaced at 8 × 8 m in a square planting. The orchard has full-coverage micro-sprinklers and ground cover except for a ~ five-foot-wide, herbicide-sprayed strip in the tree row. A standard irrigation management was designed to keep the trees at no less than ~ 2.0 bars below the proposed walnut baseline ([http://informatics.plantsciences.ucdavis.edu/Brooke\\_Jacobs/index.php](http://informatics.plantsciences.ucdavis.edu/Brooke_Jacobs/index.php)), while avoiding prolonged periods at or wetter than the baseline.

Walnut cultivars were harvested at commercial maturity (onset of

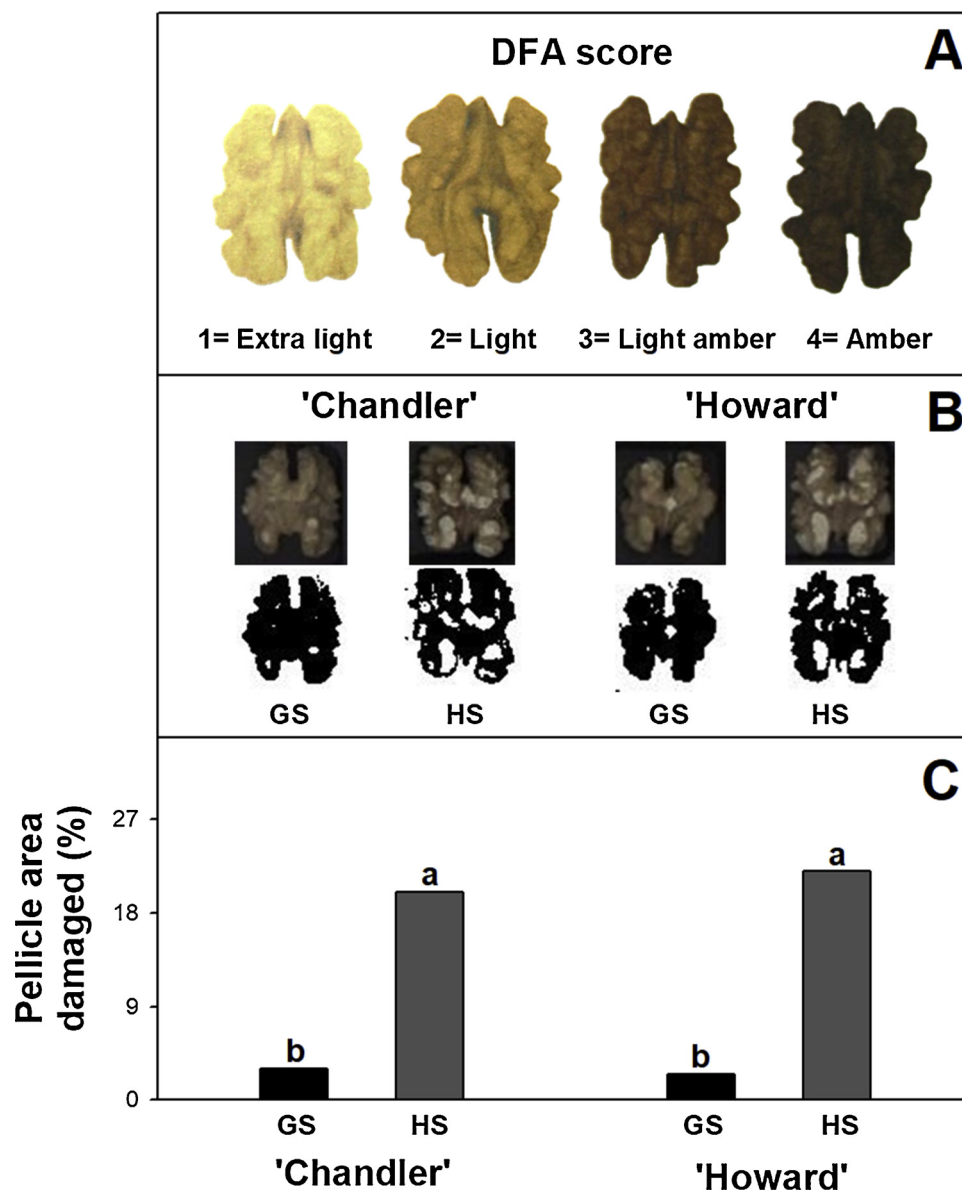
hull split) ~ 12 days after physiological maturity (determined as packing tissue brown; PTB) and randomly divided into two groups. Initial moisture content on these samples, of two different cultivars, varied from ~20% to 12% and took up to eight hours to reach the 8.0% moisture content target. The end of the drying process was determined by measuring weight changes in few samples in the lot and by moisture sensor readings. At harvest, 100 walnuts per treatment-replication were collected and hull removed immediately prior to drying. In-shell nuts were dried at 43 °C to a moisture content (MC) of 8.0% (w/w). After drying, the first group of in-shell nuts was cracked gently by hand to prevent physical damage (gently shelled; GS), using small hammers and tweezers to extract and select only visible undamaged halves. The second group was passed through a small-sized commercial shelling processor (JEM Walnut Sheller, CA, USA) that compromised kernel pellicle integrity to a degree like that of commercial operations (harsh shelling; HS). The kernels were placed in halves in plastic trays obtained from the Dried Fruit Association of California (DFA). Each tray has 100 slots, sized to hold kernel halves, and was spray-painted black to provide a uniform background color. Samples were scanned and the acquired images were used to determine the percentage of the outermost region of the dorsal side damaged using the software ImageJ (Schneider et al., 2012) and expressed as the percentage of pellicle damaged area per kernel. At least 600 kernels were used for each cultivar/treatment combination. Samples were packed in red mesh 45 × 14 cm produce bags with a white header and stored at 35 °C and 50% RH for 0, three and six weeks to induce accelerated aging (Greve and Labavitch, 1985 and Labavitch, 2004). Five independent lots consisting of 20 kernels (observations) each were prepared for each cultivar, treatment and storage time. Samples were collected, color was evaluated, and then the samples were frozen and stored at -80 °C for subsequent EtOH phenolics evaluation, oil extraction and analysis. In all measurements, five replications of 20 kernels (observations) each were evaluated for each cultivar, treatment and storage time.

#### 2.1.1. DFA color distribution and score

Individual kernel color was evaluated using two different methods; a scaled-score subjective evaluation following the Dried Fruit Association (DFA) guidelines (United States Standards for Grades of Shelled Walnuts, 2017) and the Minolta Chroma Meter (Konica Minolta, NJ, USA) provided an objective measurement. The DFA is based on a chart for color evaluation that classified kernels into one of four categories (Fig. 1A): 'Extra light' (1), 'Light' (2), 'Light Amber' (3) and 'Amber' (4). The percentage of high-quality color kernels in the samples was calculated as the percentage of extra light and light kernels in the sample. Same for percentage of amber kernels using light amber and amber kernels. Color was also evaluated with the chroma meter, to remove human bias and error by expressing color as a numerical value (McGuire, 1992). Measurements on each individual kernel were taken in the center of the outer side of walnut kernel half, closest to the shell.

#### 2.1.2. EtOH-soluble phenolics (TP)

For phenolic extract preparation, three kernels were ground in a mill and 2 g of the resulting material were extracted in 25 mL ethanol, vortexed and centrifuged at 14,000 × g for 10 min at 4 °C. The supernatant was saved and the pellet was re-extracted with 25 mL ethanol and centrifuged again. Both supernatants were pooled, taken to 50 mL with ethanol, and used to evaluate EtOH-soluble phenolics as described (Singleton et al., 1999). Briefly, 50 µL Folin-Ciocalteu reagent diluted 1:1 in water was pipetted into test tubes containing 100 µL sample extract and taken to 1.4 mL with water. Samples were vortexed and after three min at 20 °C, 100 µL 20% (w/v)  $Na_2CO_3$  dissolved in 0.1 mol/L NaOH was added. Samples were vortexed, incubated at 20 °C for one h and the absorbance at 760 nm was measured. A standard curve of gallic acid (GA) was created and the results were expressed as mg GA equivalents  $kg^{-1}$  fresh weight.



**Fig. 1.** (A) Dried Fruit Association of California (DFA) color scale for walnuts. (B) Raw and skeletonized black (pellicle on) and white (pellicle off) Walnut Computer Vision System images of 'Chandler' and 'Howard' walnuts subjected to a gentle (GS, low damage) or harsh (HS, commercial damage) shelling operation. (C) Percentage of pellicle area damaged on 'Chandler' and 'Howard' walnut kernels subjected to a GS or HS operation. Different letters indicate significant differences between treatments based on a Tukey test at a significance level of  $\alpha = 0.05$ .

### 2.1.3. Oil extraction

For oil extraction, the kernels were ground mechanically and passed through a three mm sieve. Samples were put in a five cm diameter stainless cylinder over Whatman N°1 filter paper and covered with a layer of filter paper and a stainless-steel disc. The extraction was conducted using a manual hydraulic lab scale press (Carver Model 3851, IN, USA) as described (Martínez et al., 2013). The oil obtained was filtered through a series of cartridge filters from 100 to 2 mm pore size to eliminate solids in suspension. Samples were weighed and put in plastic tubes flushed with  $N_2$  and stored at  $-80^\circ C$  until analysis.

### 2.1.4. Free fatty acids (FFA)

FFA were evaluated according to AOCS (2009). Measurements were made in triplicate and results were expressed in percentage of oleic acid.

### 2.1.5. Peroxide value (PV)

The PV was evaluated according to AOCS (2009). Five grams of oil

were dissolved in chloroform (30 mL). After that 30 mL of acetic acid and one mL saturated potassium iodide solution were added, and the solution mixed for one min. After incubation for five min in darkness, 50 mL distilled water and 0.5% (w/v) starch solution was added. The samples were titrated with 0.02 mol/L sodium thiosulphate until the blue color disappeared. The PV was expressed as reactive oxygen in meqO<sub>2</sub>/kg walnut oil. Measurements were done in triplicate.

### 2.2. Browning of harsh-shelled walnuts during storage under different O<sub>2</sub> concentrations

Walnut samples were collected from twelve-year-old 'Chandler' and 'Howard' trees growing side by side with trees spaced  $10 \times 10$  m. The orchard, which is planted with more than two cultivars, is located in Los Molinos ( $40.02245^\circ N$ ,  $-122.09981^\circ E$ ) and orchard management, including irrigation, canopy handling, and nutrition, was according to UC Davis guidelines. Walnut samples, harvested at commercial maturity and after shelling at Crain Walnut Shelling Inc. (Los Molinos, CA,

USA), were obtained immediately after drying and delivered to Davis. Upon arrival at Davis (CA), the walnuts were carefully shelled using the Model 900 Versa-Cracker (JEM Equipment Manufacturing, CA, USA). Samples of 45 damaged kernels per treatment-cultivar were stored at 35 °C and 50% RH in one-liter plastic containers with continuous gas flow under four CA conditions: air, 6 kPa O<sub>2</sub>:94 kPa N<sub>2</sub>, 3 kPa O<sub>2</sub>:97 kPa N<sub>2</sub> or 100 kPa N<sub>2</sub>. The gas mixtures and flow rates were established using a small gas mixing board with micro-metering valves and were monitored daily with a Bridge Analyzer (MAP Analyzer, Bridge Analyzers, Inc., OH, USA) and ADM Flowmeter (Agilent Technologies, Singapore). Each treatment had three replicates of 15 kernels (observations) per cultivar, treatment and storage time. Samples were collected immediately before kernel color evaluations. Individual kernel color was evaluated subjectively using a scaled score following current DFA guidelines as described in section 2.1.1. The percentage of amber (light amber and amber) walnut kernels in the samples was calculated by adding kernels classified as DFA 3 and 4 as a percentage of the total.

### 2.3. Statistical analysis

A completely randomized design was used, with five replications each of *GS* and *HS*. The percentage of extra-light and light kernels determined using the DFA color score was arcsine-transformed prior to statistical analysis to compensate for its discrete nature. The data was subjected to analysis of variance (ANOVA). Means were tested with the Tukey test for paired comparison, at a significance level of  $\alpha = 0.05$ , using the SYSTAT v. 12 software (Systat Software, Inc., Chicago, USA). Changes in the percentage of light amber and amber kernels under low oxygen were plotted as a function of time. Each line was calculated by linear regression (Origin Pro 8.5 software).

## 3. Results and discussion

### 3.1. Effects of shelling on walnut quality

#### 3.1.1. Surface color

The intensity of the shelling operation had a significant impact on pellicle integrity of 'Chandler' and 'Howard' kernels, detected as white area (embryo) on photos of half kernels (Fig. 1B). *GS* nuts had < 4% of the pellicle area damaged, in contrast to *HS* kernels, in which the damaged area was 20–22 % (Fig. 1C). Based on our kernel quality surveys, the incidence of mechanical pellicle damage induced by *HS* treatment is not uncommon among California commercial operations.

Kernel pellicle lightness ( $L^*$ ) was used to monitor browning in response to the shelling treatments during storage. When kernels reached  $L^*$  values below 46.0, they lost commercial color quality (Crisosto personal communication). In 'Chandler', the  $L^*$  values were not significantly different between storage dates by week 3. At week 6,  $L^*$  values significantly decreased. However, cultivar pellicle browning was not affected by the shelling treatment (Table 1). In 'Howard' kernels, there was already significant browning at week 3, while on *GS* kernels, most color changes occurred by week 6. Thus, pellicle disruption accelerated tissue browning during accelerated aging faster on 'Howard' than on 'Chandler' kernels. The Hue angle showed a similar trend to that of  $L^*$  values: a greater tendency to brown in 'Howard' kernels, which was exacerbated by *HS* (Table 1).

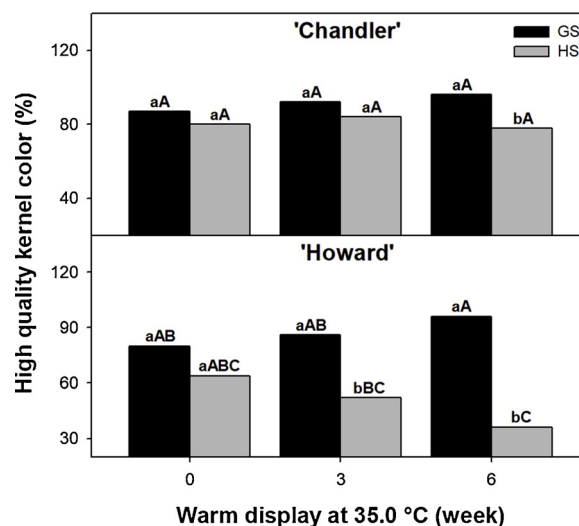
The commercial importance of walnut shelling treatment intensity on color changes was also depicted by changes in the percentages of kernels in the high-quality color category during storage (Fig. 2). Prior to storage and after three weeks, the percentage of high-quality color 'Chandler' kernels was high (84–92 %), however, after six weeks, the percentage of extra light and light nuts was reduced to 78% of *HS* kernels but remained unchanged in *GS* kernels.

In 'Howard', there were already significant differences in the percentage of high-quality kernels between treatments at the beginning of

**Table 1**

Lightness ( $L^*$ ) and hue angle in 'Chandler' and 'Howard' walnut kernels subjected to gentle (*GS*) or harsh shelling (*HS*) during warm display for 0, 3 or 6 weeks at 35.0 °C and 50% RH. Different letters indicate significant differences between shelling treatments (lower case) and storage time (upper case) based on a Tukey test at a level of significance of  $\alpha = 0.05$ .

			Time at 35.0 °C (week)		
			0	3	6
Lightness ( $L^*$ )	'Chandler'	<i>GS</i>	49.6 aA	49.5 aA	45.1 aB
		<i>HS</i>	49.2 aA	48.2 aAB	46.8 aAB
	'Howard'	<i>GS</i>	49.8 aA	47.1 aAB	46.2 aB
		<i>HS</i>	47.1 aAB	43.5 bB	43.0 bB
Hue (°)	'Chandler'	<i>GS</i>	77.2 bB	75.4 aC	75.6 aC
		<i>HS</i>	79.2 aA	75.0 aC	74.3 bBC
	'Howard'	<i>GS</i>	77.2 aA	74.6 aA	74.5 aA
		<i>HS</i>	76.0 aA	74.4 aB	70.4 bA



**Fig. 2.** Proportion of extra-light and light kernels in 'Chandler' and 'Howard' walnuts subjected to a gentle (*GS*) or harsh (*HS*) shelling operation during warm display for 0, three or six weeks at 35.0 °C and 50% RH. Different letters indicate significant differences between shelling treatments (lower case) and storage time (upper case) based on a Tukey test at a level of significance of  $\alpha = 0.05$ .

the experiment (80% versus 64%), suggesting that pellicle damage can trigger browning in this cultivar very rapidly upon shelling. Most importantly, in the *HS* 'Howard' walnuts, the percentage of kernels within the high-quality color category decreased dramatically to 36% after six weeks storage. Thus, current commercially important walnut cultivars have significant differences in browning tendency and in the effects caused by pellicle damage. 'Howard' had greater susceptibility to pellicle browning and was more affected by *HS* shelling than 'Chandler'. Future studies to establish the genetic and biochemical basis for such differences may be valuable. Historically, the California industry has been dominated by 'Chandler', but in the last decade, other cultivars from the UC Davis breeding program such as 'Howard', 'Tulare', 'Solano' and 'Ivanhoe' are becoming important components of the California walnut industry. Currently, 40% of California industry plantings are 'Chandler' and 28% are lateral-bearing cultivars such as 'Howard' and 'Tulare' released from the UC Davis breeding program (McGranahan and Leslie, 2009). The remaining 12% is older cultivars such as 'Serr', 'Franquette', 'Eureka' and 'Hartley'. New lateral-bearing cultivars from the UC Davis breeding program such as 'Ivanhoe', 'Solano' and 'Durham' are being planted across California and evaluated for their storage performance based on pellicle browning and rancidity. Thus, further detailed studies should be pursued to understand





Fig. 3. Appearance of 'Chandler' and 'Howard' walnut kernels subjected to a gentle (GS) or harsh (HS) shelling operation after warm display for six weeks at 35.0 °C and 50% RH.

browning susceptibility among genotypes. It is worth noting that the browning induced by physical disruption of the pellicle was not restricted to regions near the injured areas: browning damage extended throughout the kernel surface (Fig. 3). Thus, wounding on dried nuts (~8% water content), like on fresh fruit (80 to 90% water content), may induce browning responses not only at the primary injured site, but also at a relatively distant area (Iakimova and Woltering, 2018). Wound responses in metabolically active tissues include complex strategies such as programmed cell death (PCD) to restrict the primary damage and to build a physical barrier of dead cells between injured and healthy tissue. Very little information is available for dried nuts, in which metabolic activity is restricted, and future work to better understand this response is needed.

### 3.1.2. Phenolics

In addition to the health benefits associated with polyunsaturated fatty acids (PUFAs), walnuts also have high concentrations of both lipophilic and hydrophilic antioxidants such as tocopherols and phenolic compounds, respectively (Sánchez-González et al., 2017; Zhang et al., 2009). Walnuts are rich in phenolic compounds such as ellagitannins, quinones, phenylpropanoids and dicarboxylic acid derivatives (Grace et al., 2014; Slatnar et al., 2015). Because phenolic compounds are involved in wound responses in fruit and vegetables with high water content, there have been many studies on their role in deterioration processes, but very limited information regarding dried fruit. In walnut, phenolics are present throughout kernel (embryo) development and their concentrations are eight times greater in the embryo pellicles than in the embryos (Arcan and Yemenicioglu, 2009). The total phenolics (TP) concentrations found in the present study are consistent with those previously reported for walnuts grown in Argentina (Tapia et al., 2013) and Turkey (Arcan and Yemenicioglu, 2009). At arrival, 'Howard' GS kernels had ~23% more phenolic compounds than GS 'Chandler' prior to storage and remained at this concentration (4472 mg GAE kg<sup>-1</sup> FW) during warm storage (Fig. 4). However, 'Howard' HS kernels started with low concentrations (2724 mg GAE kg<sup>-1</sup> FW) compared to 'Chandler' with no significant changes during storage. Contrary, HS and GS 'Chandler' kernels had no significant differences in TP at arrival. In 'Chandler' GS kernels, the TP remained at 3452 mg GAE kg<sup>-1</sup> FW, without significant changes during storage. In contrast, TP in 'Chandler'

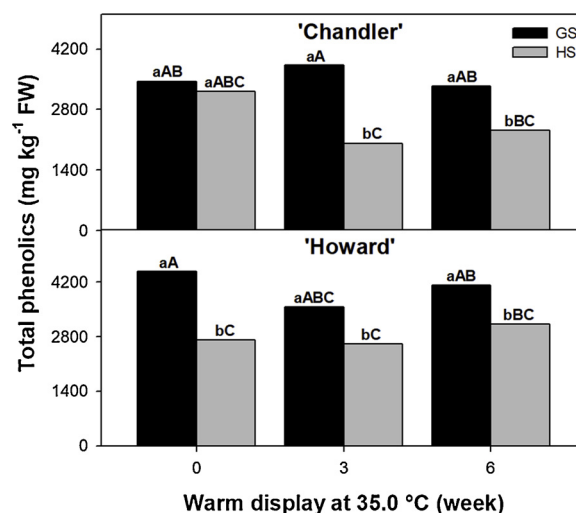


Fig. 4. Ethanol-soluble total phenolics in 'Chandler' and 'Howard' walnut kernels subjected to a gentle (GS) or harsh (HS) shelling operation during warm display for 0, three or six weeks at 35.0 °C and 50% RH. Different letters indicate significant differences between shelling treatments (lower case) and storage time (upper case) based on a Tukey test at a level of significance of  $\alpha = 0.05$ .

HS kernels dropped to 2019 mg GAE kg<sup>-1</sup> after three weeks and remained low thereafter. Thus, 'Chandler' and 'Howard' kernels with little mechanical damage to the pellicle had significantly more TP than commercially damaged pellicle kernels. In fresh vegetables, tissue browning is related mainly to enzymatic reactions through PPOs (Korbel et al., 2013). In walnut, two PPO genes (*JrPPO1* and *JrPPO2*) have been identified (Martínez-García et al., 2016). These genes have been shown to participate in pathogen resistance in walnut vegetative tissues (Araji et al., 2014), but their role in pellicle browning in kernels with low water content during storage after drying remains to be confirmed. Enzyme activities and biological processes can be very different in dried walnut pellicle and moist green tissues. Loss of cell compartmentation during harvest in fresh produce, as in walnut during processing, is a primary cause of tissue browning. In walnut, damaged

pellicle cells disintegrate, forming phenolic-rich vesicles (Wu et al., 2009). In addition, the catalytic activity of PPO in an environment containing ~8% water is expected to be much less than in fully-hydrated leaf or fruit tissues. PPO was almost totally inhibited in mango fruit dehydrated below  $A_w$  values of 0.65 (Korbel et al., 2013). Phenolic compounds also oxidize in some food matrices through non-enzymatic oxidation involving the redox cycle of  $Fe^{3+}/Fe^{2+}$  and  $Cu^{2+}/Cu^+$  (Danilewicz et al., 2008). The relevance of this pathway in nut species has not been investigated. Interestingly, this reaction was favored for aromatic compounds containing catechol rings or galloyl groups, which are common among walnut phenolics. Finally, involvement of non-enzymatic Maillard reactions in tissue browning can occur in some nut species, especially during heat treatments (Wall and Gentry, 2007). The kernel browning observed was directly associated with loss of phenolics, indicating that color change is most likely due to degradation of aromatic moieties. However, it would be useful to determine the role of reactions between sugars and nitrogen compounds in tissue browning, if any. Overall, maintaining walnut pellicle integrity greatly reduced kernel browning.

### 3.1.3. Oil quality

Lipid deterioration is a complex process including both hydrolytic and/or oxidative reactions (Labavitch, 2004).

The level of free fatty acids (FFA) increased significantly in both cultivars during warm storage (Fig. 5). In both cultivars, shelling triggered an increase in FFA to similar values after six weeks storage. However, FFA concentrations were always greater in *HS* kernels than in *GS* kernels. Lipid hydrolysis releases free fatty acids (FFA), which may cause 'off flavors'. In addition, FFA are more susceptible to subsequent oxidation (Hamilton, 1994). The lipid hydrolysis of triacylglycerols to fatty acids and alcohols is catalyzed by lipases, which occur in walnut kernels (Yeşilöğlü and Demirkan, 2010). In almond, lipases may act at moisture contents > 6%, providing a window for oxidation action (Lin et al., 2012). Whether or not lipases are active in dried kernels is not known. We speculate that mechanical damage and disruption of oil body membranes in the kernel or high exposure to oxygen during standard commercial shelling may facilitate lipid hydrolysis, producing high FFA concentrations. Lipid peroxidation is also a common problem in stored nuts, leading to oxidative rancidity. Peroxides are the main initial breakdown products formed when fats, especially unsaturated fats, react with oxygen. Rancid 'off flavor' will develop as alcohols,

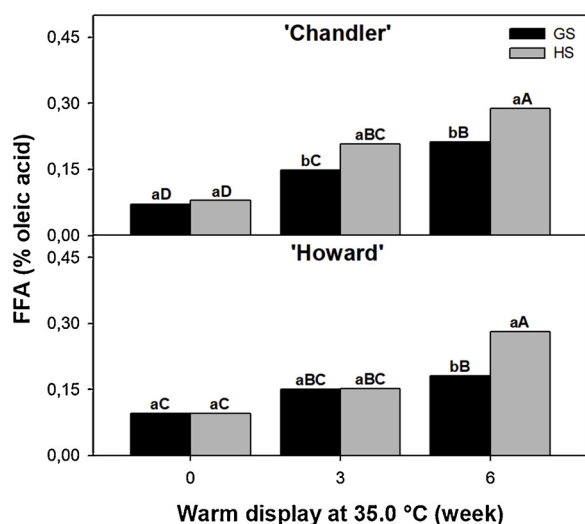


Fig. 5. Free Fatty Acids (FFA) in 'Chandler' and 'Howard' walnut kernels subjected to a gentle (*GS*) or harsh (*HS*) shelling operation during warm display for 0, three or six weeks at 35.0 °C and 50% RH. Different letters indicate significant differences between shelling treatments (lower case) and storage time (upper case) based on a Tukey test at a level of significance of  $\alpha = 0.05$ .

Table 2

Peroxide value (PV) in 'Chandler' and 'Howard' walnut kernels subjected to gentle (*GS*) or harsh shelling (*HS*) treatments during warm display for 0, 3 or 6 weeks at 35.0 °C and 50% RH. Different letters indicate significant differences between shelling treatments (lower case) and storage time (upper case) based on a Tukey test at a level of significance of  $\alpha = 0.05$ .

		Peroxide value (meqO <sub>2</sub> kg <sup>-1</sup> )		
		Time at 35.0 °C (week)		
		0	3	6
'Chandler'	GS	0 aC	0.22 aBC	1.58 aA
	HS	0 aC	0.28 aB	1.62 aA
'Howard'	GS	0 aC	0.19 aBC	1.08 bB
	HS	0 aC	0.29 aBC	2.64 aA

ketones, alkanes, aldehydes and other end products are generated (Li et al., 2007). We determined the degree of oil peroxidation by evaluating the oil peroxide value (PV). After three weeks storage, the PV was very low, with no significant differences between treatments or cultivars. At the last sampling date, the PV was 1.6 meqO<sub>2</sub> kg<sup>-1</sup> in 'Chandler', with no differences between shelling treatments (Table 2). In 'Howard', *HS* kernels had significantly greater PV (2.6) than *GS* kernels (1.1). Although the absolute levels reached were still relatively low, physical damage to the pellicle did activate lipid peroxidation. The increased peroxidation in damaged kernels could simply result from greater oxygen diffusion through to the embryo. However, this would not explain the marked differences in PV between cultivars. 'Howard' kernels had lower concentrations of tocopherols than 'Chandler'; this could make 'Howard' kernels more sensitive to oxidation (Kafkas et al., 2017). In this context, the disruption of the pellicle that provides a barrier to oxygen diffusion would affect primarily the most susceptible cultivar. Lipid oxidation could take place non-enzymatically via direct oxygen attack at the sites of fatty acid unsaturation or enzymatically through lipoxygenases (LOXs). A recent study in *Arabidopsis* indicated that both enzymatic and non-enzymatic oxidation reactions are involved in seed senescence (Oenel et al., 2017). In wet walnut homogenates, inhibiting LOX retarded oil peroxidation. However, the actual contribution of LOXs to lipid oxidation in dry kernels remains to be determined. Pellicle physical damage in specific areas caused whole pellicle browning, loss of phenolic antioxidants, and activated lipid peroxidation. Minimizing mechanical damage to walnut pellicles during shelling and reducing lipid deterioration processes throughout postharvest handling are vital to protect kernel quality. While the partial removal of the natural seed coat barrier may favor such changes, the fact that pellicle disruption increased not only lipid oxidation, but also their hydrolysis, and favored browning of *HS* kernels well beyond the site of damage are intriguing observations that deserve further evaluation to understand the mechanisms involved. In addition, this study identified significant cultivar differences in the response to harsh shelling operations in walnuts.

### 3.2. Color of harsh-shelled walnuts during storage under different O<sub>2</sub> concentrations

This additional fast market life, low-oxygen experiment's results were used to validate the benefits of low oxygen on kernel browning development in commercially shelled kernels. Development of color browning in 'Chandler' and 'Howard', expressed as the percentage of light amber and amber kernels, associated significantly with oxygen exposure under warm conditions. Very little has been published on the benefits of low oxygen on kernel color, as most low-oxygen work has focused on rancidity (Gama, et.al, 2018). One exception examined long-term warm (20 °C) and cold (0 °C) storage in modified packaging created by flushing with nitrogen or carbon dioxide. In these early studies,

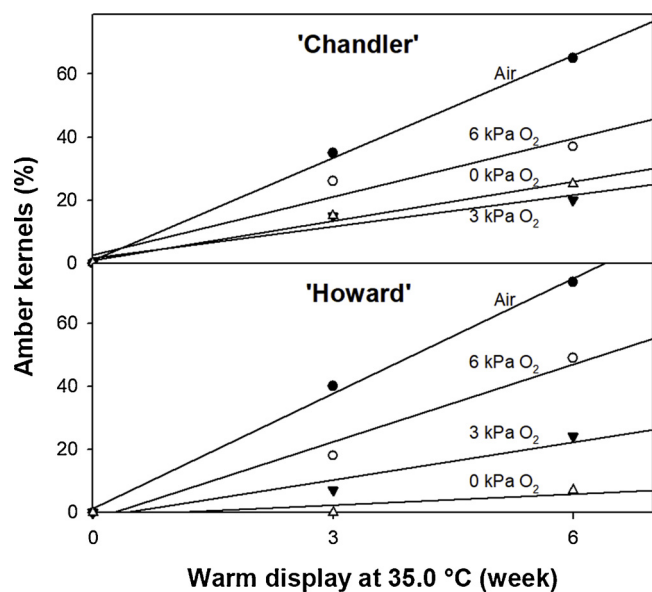


Fig. 6. Regression curves showing the percentage of amber (light amber and amber) 'Chandler' and 'Howard' walnut kernels subjected to a harsh (commercial damage) shelling operation and stored for 0, three or six weeks at 35.0 °C and 50% RH in air or Controlled Atmosphere (CA) containing 0, three or six kPa oxygen.

where oxygen varied from 0.01 to 10% during the 12 months of evaluation, a delay in amber color development was reported for 'Chandler' and 'Hartley' grown in Greece (Christopoulos and Tsantili, 2010 and 2011).

In our work, using steady oxygen concentrations on 'Chandler' (kernel browning non-sensitive) and 'Howard' (kernel browning sensitive), but under high temperature (35 °C) conditions, a strong positive correlation was found between percentage of amber kernels and oxygen exposure during warm storage ( $R^2 = 0.75$  to 0.99). Oxygen concentrations below six kPa protected kernels from browning for three weeks. In both cultivars, there was ~20% amber kernels under 6 kPa  $O_2$  compared to almost 40% amber kernels under air. Kernels stored at 3 kPa or 0 kPa  $O_2$  had 10% or fewer amber kernels. (Fig. 6). This data corroborates the benefits of low oxygen reported in previous work (Christopoulos and Tsantili, 2010 and 2015b). Thus, kernel browning can be avoided by using low-temperature handling and low-oxygen packaging, especially when proper temperature management is not available. In addition, the results show the benefits of reducing  $O_2$  depending on the cultivar considered. Reducing oxygen below 3 kPa has greater impact on browning prevention in 'Howard' kernels than in 'Chandler'. Previous works have reported that depending on their structural features phenolic compounds may show large variation in their susceptibility to both enzymatic and non-enzymatic oxidation (Danilewicz et al., 2008). Thus, variations in the profile of these compounds may contribute to explaining the distinct responses observed between 'Howard' and 'Chandler' to low  $O_2$  atmospheres. In the case of enzymatic oxidation it is also plausible to speculate that the cultivars' PPO's may differ in their catalytic properties as has been the case for isozymes from different plant sources (Taranto et al., 2017). Future work to address this point would be of great interest.

#### 4. Conclusions

Reducing walnut pellicle damage during shelling delays postharvest kernel browning, prevents antioxidant turnover in shelled walnuts, and protects walnut oil stability. 'Chandler' and 'Howard' kernel pellicle color changed from 'light' to 'amber' during storage, as evidenced by the reduction in lightness ( $L^*$ ) reducing the percentage of kernels in the

high-quality color category. To reveal the mechanism of color and rancidity losses, we measured the effects of shelling with low mechanical disruption of the pellicle. This improved the stability of phenolic antioxidants and reduced triglyceride hydrolysis and oxidation, reflected in the lower FFA and PV values. Thus, walnut pellicle damage during shelling should be minimized or kernels should be stored at low temperatures to avoid browning, loss of bioactive compounds and oil deterioration. The negative biological effects of pellicle damage were cultivar-dependent, greater in 'Howard' than in 'Chandler'. The benefits of low oxygen during warm storage support the hypothesis that deterioration in kernel color and rancidity are linked to oxidation processes and can be reduced by low-temperature handling and low-oxygen packaging, especially when proper temperature management is not available. CA storage experiments under different  $O_2$  partial pressures showed a beneficial effect of oxygen equal to or lower than 3 kPa to reduce deterioration of 'Howard' kernels. This information may be useful for processors and distributors of ready-to-eat walnuts.

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