Optimizing Walnut Storage Conditions: Effects of Relative Humidity, Temperature, and Shelling on Quality after Storage

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Abstract. With increasing walnut production in California, walnuts are stored for longer times. It is increasingly important to optimize storage conditions, wherever possible, to reduce quality degradation. We examined the effects of temperature (5, 15, and 25 °C) and relative humidity (20%, 40%, and 60% in year 1 and 40%, 60%, and 80% in year 2) on the rate of quality degradation of four walnut varieties. The relationship between water activity and moisture content was investigated for each variety. In addition, the effects of harvest timing (early vs. late) and storage as shelled or in-shell product were investigated. Later harvested walnuts had darker kernel color ($P < 0.001$), and walnuts stored as kernels (shelled) had higher rates of peroxide formation and free fatty acid development than walnuts stored in-shell. Temperature had a significant effect on quality with faster degradation at higher temperatures. There was a significant interaction between temperature and relative humidity effects on quality. The effects of relative humidity were often not significant at storage temperatures of 5 °C but were apparent at 15 °C and at 25 °C. Managing relative humidity during walnut storage is difficult under typical commercial storage conditions; however, when low temperature storage is used, quality is preserved even when relative humidity is not controlled, although storage at 80% relative humidity should be avoided. To reduce the rate of color darkening and rancidity development during commercial storage, operators should emphasize storage at lower temperatures, at least below 15 °C.

Walnuts (*Juglans regia*) contain high amounts of the essential omega-3 fat, α -linolenic acid, and a ratio of linoleic acid to α -linolenic acid that is ideal for decreasing cardiovascular risk (Simopoulos, 2004). These polyunsaturated fatty acids are prone to oxidation over time and in adverse environmental conditions, leading to off-flavor development and rancidity. Light-colored walnut kernels receive a premium, but they are also susceptible to undesirable kernel darkening over time as the pellicle of the walnut (the skin) becomes a darker shade of brown, most likely due to oxidation of phenols (Escobar et al., 2008). Kernel darkening, off-flavor development, negative textural changes, and rancidity can lead to decreases in the value of

stored walnuts and eventually complete loss of marketability. As walnut acreage and yields have increased in recent years, the length of storage and potential for quality degradation have also increased, making it more important to optimize storage conditions to extend postharvest quality and storage life. There is also interest in expanding market opportunities for walnuts which generally require high quality and long shelf life.

Rancidity development in nuts occurs via oxidation and hydrolysis. Oxidative rancidity can be due to auto-oxidation, photo-oxidation, or enzymatic-oxidation, whereas hydrolytic rancidity is caused by the reaction of water with lipids in the presence of a catalyst, such as light and heat (Shahidi and John, 2013). Oxidation of the triacylglycerides found in walnuts results in the formation of hydroperoxides, an intermediary oxidation product that can then be broken down further into secondary and tertiary products, namely aldehydes, ketones, furans, acids, and hydrocarbons. Hydrolysis of triacylglycerides yields free fatty acids.

The walnut harvest occurs once a year in autumn, when the nuts are mature for harvest, using a mechanical shaker. Some orchards are shaken twice to maximize yield. Walnuts are stored in-shell, generally under ambient conditions, and are cracked to yield shelled kernels throughout the year. Shelled kernels

can also be stored for additional lengths of time and are more frequently kept under refrigerated conditions. Storage of this shelled or in-shell product can range from weeks to upwards of 12 months, and storage conditions vary throughout the industry. The very large volumes of harvested walnuts make it costly to use refrigerated storage conditions. There is a need to quantify the potential benefits to refrigerated storage.

Moisture content is used by the industry to determine if walnuts are at an optimal dryness level before storage. Water activity is the ratio of the vapor pressure of water in a food to the vapor pressure of pure water or the amount of unbound water in a substance that is available for metabolic activity (Labuza, 1980). General trends for water activity relating to shelf life, texture parameters, browning relationships, enzymatic activity, lipid oxidation, and microbial activity have been discussed for many foodstuffs (Nelson and Labuza, 1992). Multiplying the water activity by 100 yields the equilibrium relative humidity, describing how a substance equilibrates to the relative humidity of its surroundings. However, water activity has not been commonly used to manage walnut storage.

Currently, the walnut industry uses analysis of free fatty acids and peroxide values in extracted walnut oil to quantify the degree of hydrolytic and oxidative rancidity, respectively—and therefore, changes in the quality of their stored walnuts. However, these methods can be inaccurate in quantifying the extent of oxidation because peroxides can be produced and then consumed throughout the oxidation process. In addition, industry-wide threshold values have not been established.

The objectives of this study were to 1) understand the effects of temperature, relative humidity, storage type (in-shell or shelled), and storage time on walnut quality and rancidity development and 2) assess the role of water activity in walnut quality poststorage.

Materials and Methods

Survey of industry. An anonymous survey of walnut processors in California was completed to understand storage practices. The survey included questions on the quantity of walnuts stored, varieties stored, and storage conditions used (temperature and relative humidity). Responses were received from walnut storage processors responsible for 30% of all stored walnuts in California.

Storage of samples. Walnuts of the Chandler, Howard, Tulare, and Vina varieties were procured from Diamond Nuts in Stockton, CA, soon after harvest and commercial drying (average kernel moisture content of 5.3% to 6.7%), and stored at 0 °C until all varieties arrived. Each year, two harvests were obtained from the same orchard for each variety to test the variation in harvest time. For each variety, the first harvest occurred in September, and the second harvest occurred in October. Walnuts were dried with commercial dryers using standard practices. Half of the walnuts were shelled by the

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processor for our storage as kernels, and the other half were obtained and stored in-shell. Walnuts were sorted to remove undeveloped or broken nuts and to evenly distribute sizes among storage treatments. Sorted walnuts were divided into nine groups for each variety and storage type (in-shell or shelled), and placed into mesh bags for storage.

Walnuts were stored at 5, 15, or 25 °C in sealed stainless-steel bins fitted with an inlet at the top and outlet port. Within each temperature, walnuts were stored at three relative humidities (RHs). RH was established according to methods from Forney and Brandl (1992). Briefly, the air was humidified by bubbling it through solutions with varying concentrations of glycerol, which then flowed into the bins and then out using the ports. Flow rates were adjusted to achieve the desired RH. RH and temperature loggers (Onset Computer Corporation, Bourne, MA) were placed in each bin, and the data were monitored continually. If the RH of the bin increased above the target RH, zeolite-drying beads (Rhino Research, Bangkok, Thailand) were placed in the bin to decrease the RH. The level of the glycerol-water solutions was monitored to replenish what water was lost due to evaporation.

In year 1, within each temperature, bins were stored at 20%, 40%, and 60% RH, for a total of nine storage treatments. In the second year, the same procedures were repeated, but within each temperature, bins were maintained at 40%, 60%, and 80% RH.

The initial quality of each variety at each harvest was evaluated before placing the nuts in storage according to methods detailed subsequently. Every 3 months during 12 months of storage, walnuts were removed from their controlled environments and analyzed for quality.

After 9 months of storage in year 2 of the experiment, mold growth was observed on the walnut kernels stored at 15 °C and 80% RH after cracking. The mold was isolated, cultured, and identified as both *Penicillium* species and *Aspergillus melleus*. Because these fungi can produce harmful mycotoxins, they were removed from the experiment and autoclaved before disposal. Subsequent analysis excluded this data for that reason. There were no other issues with mold growth in any other storage conditions, indicating that this combination of relative humidity and temperature might be ideal for these fungal species to grow.

Sample preparation for analysis. Sixty walnuts were removed per treatment. For the shelled walnuts (stored as kernels), kernels of similar size and color were matched in pairs so that each walnut “half” was put together to make one full walnut. Walnuts stored in-shell were hand-cracked using a hammer. The shells were discarded, and every analysis going forward was completed on the shelled kernel pieces (both for walnuts stored as shelled kernels and those stored in-shell, then shelled).

Moisture content and water activity. Ten walnuts at a time were chopped and sorted to <0.7-mm pieces using a sieve (Advantech, New Berlin, WI); these walnut pieces were used for determination of water activity and

moisture content. Approximately 2 g of sample was measured into disposable water activity sample cups (Meter Group, Pullman, WA). Water activity of the sample was measured using an AquaLab 4TE Duo moisture analyzer from Meter Group. Approximately 5 g of walnut pieces were weighed in an aluminum weigh boat (VWR, Radnor, PA) and reweighed after drying for 48 h in a dry oven at 105 °C. Percent moisture content (MC, wet basis) was calculated by the following formula:

$$\% \text{ MC} = \frac{[\text{wet weight (g)} - \text{dry weight (g)}]}{\text{wet weight (g)}} \times 100$$

For each storage treatment, storage type and variety, water activity and moisture content measurements were completed in triplicate with 10 nuts per replicate. The mean and standard deviation were taken.

Kernel color. Thirty walnuts were used for both color analyses. The color was determined using a chroma meter on the surface of the kernel (Konica Minolta Sensing Americas, Inc, Ramsey, NJ) using the CIELAB color space. The L^* value (representing the darkness to lightness of the sample) was used to determine kernel darkening. The walnut industry currently grades all walnuts using a categorical color chart created by the U.S. Department of Agriculture for use by the Dried Fruit and Tree Nut Association of California. The chart shows photos of walnuts in four categories ranging from lightest to darkest; extra light, light, light amber, and amber.

To understand how the color chart (categorical and subjective) relates to the colorimeter L^* value (numerical and objective), the measurements for the same walnuts in year 1 were compared over all data. This included four varieties, five time points of analysis, two harvests, and storage in-shell and shelled. Each treatment featured an average of 60 colorimeter readings, two per walnut, and 30 color chart readings. To obtain a composite color chart reading per treatment, the categories were given numerical values (extra light = 1, light = 2, amber = 3, light amber = 4), and the mean values were summed to get a single value (deemed the “color score”) for each treatment, variety and evaluation time.

Oil extraction. The same thirty nuts used for color analysis were stored at –80 °C until used for oil analysis. At the time of extraction, walnuts were removed from the –80 °C freezer, and three groups of 10 walnuts were placed on plastic trays to thaw. Once thawed, walnut oil was extracted in an unheated stainless-steel test-cylinder-outfit of a bench-top hydraulic laboratory press (model #3925; Carver Inc., Wabash, IN). Ten walnuts yielded 15 mL of oil that was then transferred to a 15 mL freezer-safe tube, flushed with nitrogen gas, then stored at –80 °C.

Peroxide and free fatty acid analysis. Vials of stored walnut oil were thawed, and 5 g of oil was weighed into 250 mL erlenmeyer flasks (Fisher Scientific, Waltham, MA). Oil was analyzed for free fatty acids according to the American Oil Chemists’ Society (AOCS) Official Method (Cd 3d-63; Firestone, 1973)

by calculating the amount of KOH used to neutralize the oil. Peroxide value was measured by titrating with sodium thiosulfate according to the AOCS official method (Cd 8-53; Firestone, 1997).

Relationship between water activity and moisture content. After the first year of the experiment, the relationship between water activity and moisture content at 25 °C was evaluated for each variety using a salt slurry method (Schmidt and Lee, 2012). Freshly harvested walnuts from each variety were collected from the first harvest. Nuts were held at 20 °C and ≈60% RH for less than 1 month before use. Six replicates of 5 to 10 shelled walnuts were placed in plastic weigh boats and stored in sealed plastic containers containing a saturated salt solution in the base of the container. Weigh boats were placed atop glass jars to avoid contamination with the salt solution. Five salt solutions were used to obtain RH levels in the range of 32.8% to 81.0% (Supplemental Table 1). Salt solutions were chosen based on the desired RH level (Greenspan, 1977). Samples were weighed daily until an equilibrium was reached, and then the moisture content and water activity were measured.

Statistics. The mean and standard deviation was used for objective measurements (peroxide value, free fatty acid content, moisture content, water activity, and L^* value). Data were analyzed using analysis of variance (ANOVA) and Pearson’s product-moment correlations using R and R Studio Software. In addition to base statistical analysis, ggplot2 and dplyr packages were used (R Development Core Team, 2013). ANOVA was used to evaluate main effects and interactions for all four varieties. The effects of temperature and relative humidity during storage of in shell walnuts on oil oxidation is shown for all four varieties; however, other detailed results are shown for Chandler walnuts only because it is the most common variety in California.

Results

The survey results indicated that in-shell walnuts are stored at temperatures between 1 and 25 °C, and many are held at ambient conditions. Ambient temperatures between harvest in October and the following September in some walnut-storing regions of California can get as high as 35 °C during the day and often cool significantly at night. Outdoor RH in these regions can get as high as 90% RH during the winter months. Shelled kernels are often stored in refrigerated conditions (5 to 15 °C), but most operators do not control RH. After receiving survey results in the first year, the experiment was altered to remove the 20% RH and add a storage condition (80% RH) that would be more indicative of what walnuts stored in California year-round could be subjected to if processors used ambient conditions.

The correlation between L^* value and color score was highly significant ($P < 0.001$; Fig. 1). A higher L^* value corresponded to a lower color score value (meaning a higher percentage of walnuts in lighter categories were

Fig. 1. The relationship between mean subjective color score as determined by categorical color chart (U.S. Department of Agriculture Walnut Color Chart) and mean objective L* value as quantified by chromameter for experimental nuts from year 1. The L* value represents the darkness to lightness of the sample, with 100 being the lightest and 0 being the darkest. To obtain a composite color chart reading per treatment, the categories were given numerical values (extra light = 1, light = 2, amber = 3, light amber = 4), and the values for the 30 nuts were summed to get a single value (deemed the “color score”) for each treatment, variety, and evaluation time.

present in that group) and a lower L* value represents a higher color score (higher percentage of darker walnuts). L* value and color score both accurately depicted kernel darkening. All further kernel darkening results are presented with the more quantitative L* value in this manuscript.

Because we used 20%, 40%, and 60% RH in year 1 of the experiment, and 40%, 60%, and 80% RH in year 2 of the experiment, an ANOVA could not be completed on all conditions for both years. To understand the significance of year, we ran an ANOVA for both

years of the study, excluding the 20% and 80% RH conditions that were unique to each year. Table 1 shows the results of this joint multiple year ANOVA. Moisture content, peroxide value, and color were significantly different between the 2 years of our study and were slightly higher in year 2 (data not shown). The main effect means and temperature $\times$ RH interaction means for walnuts stored in year 1 are shown in Supplemental Table 2, and those stored in year 2 are shown in Table 2.

Harvest significantly impacted L* value and color score ($P < 0.001$), as well as free

fatty acid development over the 2 years of the study ($P < 0.05$; Table 1). In year 2, second harvest Chandler walnuts were darker at harvest (No Storage) and remained darker than the first harvest walnuts following storage for 12 months under different temperature and RH conditions (Fig. 2). Across all storage conditions and times, the harvest 2 walnuts developed higher free fatty acids than the harvest 1 walnuts, but there were no differences between harvests for peroxide values in year 2 (Table 2). In contrast to the overall results, there were no significant differences in free fatty acids between the first and second harvest walnuts within storage conditions after 12 months in year 2 (Supplemental Fig. 1).

Storage type. Across both years, storage as shelled kernels or in-shell walnuts (storage type) did not significantly impact kernel darkening or water activity but did impact moisture content ($P < 0.001$), peroxide value ($P < 0.001$), and free fatty acid development ($P < 0.01$, Table 1). In year 1, walnuts stored as kernels had slightly higher moisture content, and had higher peroxide values and free fatty acids (Supplemental Table 2). In year 2, moisture content was not significantly different between storage types, but peroxide value and free fatty acid values were higher in walnuts stored as kernels, and the differences were bigger than in year 1 (Table 2). There was a significant interaction between storage type, storage time, and temperature across both years (Table 1) and a trend toward higher peroxides in shelled product (kernels) from most storage conditions in year 2 (Fig. 3). However, significantly higher free fatty acids were seen primarily in product stored at 25 °C and 80% relative humidity (Fig. 3).

Temperature and RH. Over the 2-year study, temperature of storage significantly

Table 1. Joint multivariate analysis of variance for years 1 and 2 of the experiment, including only data from the 40% and 60% relative humidity (RH) storage conditions that were common among the 2 years.

	Water activity	Moisture content	Peroxide value	Free fatty acids	L* value	Color score
Year		***	***		***	**
Harvest				*	***	***
Variety		***	***	***	***	***
Storage type		**	***	**		
Storage time	***	*	***	***	***	***
Temp	***	***	***	***	***	***
RH	***	***		***		*
Variety: storage type						
Variety: storage time						
Variety: temp						
Variety: RH				*		
Storage time: temp			***	***		
Storage type: storage time			***			
Storage time: RH				***		
Storage type: temp			***	**		
Temperature: RH		*	**	***		
Storage type: RH						
Storage type: storage time: temperature			**			
Storage time: temp: RH	***			***		
Storage type: storage time: RH						
Storage type: temp: RH			**			

²Relationship determined using multivariate analysis of variance.

*, **, ***Significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 2. Main effect means and interaction between temperature and relative humidity (RH) for walnuts harvested in year 2 and stored under various conditions.

	Water activity	Moisture content	Peroxide value	Free fatty acids	L* value
Variety					
Chandler	0.454 a	3.99 a	0.865 b	0.202 b	45.60 a
Howard	0.477 a	3.65 a	0.718 c	0.181 c	43.63 b
Tulare	0.445 a	3.76 ab	0.948 a	0.238 a	45.36 a
Vina	0.461 a	4.03 a	0.813 bc	0.216 b	36.20 c
Harvest					
1	0.464 a	3.85 a	0.838 a	0.199 b	46.18 a
2	0.455 a	3.87 a	0.840 a	0.220 a	39.17 b
Storage type					
Shelled	0.465 a	3.87 a	1.008 a	0.241 a	42.67 a
In shell	0.454 a	3.85 a	0.6764 b	0.178 b	42.70 a
Temp (°C)					
5	0.511 a	4.10 a	0.351 c	0.0870 c	43.59 a
15	0.419 b	3.77 b	0.737 b	0.119 b	43.14 a
25	0.445 b	3.69 b	1.401 a	0.402 a	41.34 ab
RH (%)					
40	0.370 c	3.34 b	0.871 a	0.082 c	42.08 ab
60	0.441 b	3.71 b	0.920 a	0.199 b	42.89 a
80	0.577a	4.58 a	0.694 b	0.387 a	43.14 a
Storage time					
0	0.406 c	3.96 b	0.000 d	0.038 d	44.98 a
3	0.398 c	3.55 b	NA	NA	44.72 a
6	0.490 b	4.42 a	0.645 c	0.159 c	43.83 b
9	0.527 a	3.85 b	0.831 b	0.207 b	41.28 c
12	0.418 c	3.59 b	1.065 a	0.269 a	40.64 c
Temp/RH					
5/40	0.425 cde	3.74 c	0.370 e	0.062 e	42.33
5/60	0.467 bc	3.91 bc	0.370 e	0.081 e	44.00
5/80	0.638 a	4.65 a	0.314 e	0.119 d	44.44
15/40	0.316 f	3.22 c	0.828 d	0.068 e	42.99
15/60	0.399 def	3.58 c	0.741 d	0.122 d	42.81
15/80	0.582 a	4.77 a	0.451 e	0.263 c	43.79
25/40	0.368 ef	3.07 c	1.418 b	0.116 d	40.90
25/60	0.458 bcd	3.64 c	1.648 a	0.392 b	41.85
25/80	0.510 b	4.37 ab	1.154 c	0.697 a	41.26

Means followed by different letters within main effects and columns were significantly different ($P < 0.05$). NA = sample not available.

influenced all aspects of quality, whereas RH affected water activity, moisture content, and free fatty acids and had a small effect on kernel color (Table 1). The ANOVA across years

did not show an effect of RH on peroxide values; however, it should be remembered that the more extreme RH levels (20% and 80%) were removed from the ANOVA due to dif-

ferences between the years. When the full range of RH was included for year 2, RH had a significant effect on peroxide values, with lower peroxide value at 80% RH compared with 40% and 60% RH, as well as water activity, moisture content, free fatty acids, and color (Table 2). Similarly, RH levels between 20% and 60% had no effect on peroxide values in year 1 (Supplemental Table 2).

In both years, temperature during storage had a large influence on peroxide formation, and a smaller effect on development of free fatty acids, while RH had a smaller effect on both peroxides and free fatty acids (Table 2, Supplemental Table 2). In year 2, Chandler walnuts stored at 25 °C for 6 months developed the highest levels of peroxides and free fatty acids (Fig. 4). Walnuts stored at 5 or 15 °C had similar and significantly higher peroxide values from at harvest values and lower values than in walnuts stored at 25 °C after 6 months of storage (Fig. 4). It seemed that these temperatures were low enough to retard oil oxidation for shorter periods of time; however, the differences became apparent after 12 months of storage when a storage temperature of 15 °C resulted in increased peroxide formation, with values similar to those of walnuts stored at 25 °C (Fig. 5). Peroxide formation was slowed at 5 °C, even after 12 months of storage (Fig. 5).

Increases in walnut free fatty acids were strongly affected by RH at 5 °C (6 months storage) and 25 °C (6 and 12 months storage) (Figs. 4 and 5). All walnuts stored at 40% RH had low quantities of free fatty acids after 6 and 12 months of storage, regardless of temperature (Figs. 4 and 5). Walnuts stored at 60% RH had similar free fatty acid levels to those stored at 40% RH except at 25 °C where 60% RH resulted in significantly higher free fatty acid levels.

Temperature amplified the differences in free fatty acids due to higher RH in storage (Table 2). Peroxide formation appears to be inhibited by higher RH in storage (Table 2). After 12 months, peroxide values were lower in nuts stored at 25 °C with 80% RH compared with 40% RH (Fig. 5). It is clear that temperature and RH do not work in isolation from each other.

Walnut color in storage followed a similar trend to peroxide values, with storage at 25 °C leading to faster kernel darkening which was apparent as early as 6 months (Table 2). After 12 months, the difference in color between walnuts stored at 5 or 15 °C was apparent, and kernels stored at 25 °C had continued to darken (data not shown).

Variety. Across the 2 years and specifically for year 2, variety was significantly different for all measurements except water activity (Tables 1 and 2; Supplemental Table 2). In year 2, across all storage times and conditions, peroxide value and free fatty acids were highest in Tulare and lowest in Howard walnuts (Table 2). In year 1, these trends only held for free fatty acids (Supplemental Table 2). There were few consistent differences in peroxide values and free fatty acids between varieties stored in different environmental conditions, but Chandler and Vina

Fig. 2. Influence of harvest timing on kernel darkening (L* value) after 12 months of storage of in-shell and shelled Chandler walnuts harvested in year 2. Lower L* value indicates a darker kernel. Bars with different letters within harvests are significantly different ($P < 0.05$). *Samples lost due to fungal contamination.

Fig. 3. Influence of storage type (in-shell or shelled), temperature, and relative humidity on formation of peroxides and free fatty acids after 12 months of storage for Chandler walnuts harvested in year 2, harvest 1. Bars with different letters within storage type are significantly different ($P < 0.05$).

walnuts had higher peroxide values when stored at 25 °C with 40% RH, and higher free fatty acids when stored at 25 °C with 60% or 80% RH (Fig. 6). Tulare walnuts had slightly higher peroxide values after storage at 5 °C with 60% or 80% RH.

The relationship between moisture content and water activity for each variety was investigated (Fig. 7). All varieties followed relatively similar trends, although decreases in kernel moisture content at lower water activity were smaller for Howard and Tulare walnuts. Varieties differed in the specific relationship between water activity and moisture content. Howard

and Chandler kernels followed together closely, but Tulare and Vina differed greatly with Tulare having higher moisture content and Vina having lower moisture content at a particular water activity. For example, at a constant water activity of 0.6 (corresponding to storage at around 60% RH), the moisture content of Vina would be 3.7%, with Chandler and Howard around 4.4% to 4.6% and Tulare at 5.0% moisture content. A moisture content of 4%, a common level for walnut kernels immediately after harvest and drying, would relate to a broad range of water activities among varieties (Fig. 7), with 0.42, 0.51, 0.54, and 0.63 water activity

for Tulare, Chandler, Howard, and Vina walnuts, respectively. However, the lower water activity in Tulare did not appear to result in lower overall peroxide values or free fatty acids, and the higher water activity values for Vina did not result in higher peroxides and free fatty acids (Table 2). It is important to recognize that walnut moisture content quickly equilibrates with the RH of the environment in which they are stored. In our study where walnuts were stored under a wide range of RH levels, walnut moisture content remained within the range of 3.0% to 4.8%.

Discussion

Walnut color. Light-colored walnuts receive a premium price and kernels darken over time in storage, leading to quality loss. Kernel darkening was impacted by variety, harvest, and year, in agreement with the findings of López et al., (1995). Kernel darkening also depends on preharvest or at-harvest factors, such as irrigation (Fields, 2018), moisture content and hull presence (Khir et al., 2014), and damage to the pellicle during shelling (Ortiz et al., 2019). In addition to color, moisture content, and peroxide value differed by year, demonstrating that these characteristics can be influenced by conditions not associated solely with storage. The second harvest of walnuts was darker than the first and darkened further during storage. In storage, kernel color was not affected by storage in-shell or as shelled walnuts. Kernel darkening increased with increasing temperatures, but RH in storage was not a factor in kernel darkening. Overall, the variety and the harvest timing had the greatest influence on kernel color, and storage conditions and time had a much smaller effect.

Walnut oil degradation. In our study, the different walnut varieties accumulated peroxides and free fatty acids at different rates during storage. In an earlier study of oil composition in four walnuts varieties (Chandler, Hartley, Franquette, and Ioli), Christopoulos and Tsantili (2015) found that the ratio of omega-6 to omega-3 fatty acids impacted the amount of oil decomposition, yet unlike our results, the varieties followed consistent trends across all storage treatments of 1 or 20 °C under air, nitrogen, or carbon dioxide. It is possible that their atmosphere treatments had a stronger effect than differences in RH in our study. Bakkalbaşo et al. (2012) found that variety and temperature had a greater impact on lipid oxidation than packaging. Although we found differences in varieties, the trends were not consistent, and no variety had higher or lower oxidation products across all treatments, indicating that perhaps some varieties do better than others under certain environmental conditions. For example, compared with other varieties, Chandler and Vina had higher peroxides under 25 °C and 40% RH, and also had higher free fatty acids at 25 °C, but with 60% and 80% RH. Tulare had higher peroxides at 5 °C with 60% or 80% RH.

Fig. 4. Effect of temperature and relative humidity on formation of peroxides and free fatty acids in walnut kernels after 6 months of storage of shelled Chandler walnuts harvested in year 2, harvest 1. Bars with different letters are significantly different ($P < 0.05$).

Fig. 5. Effect of temperature and relative humidity on formation of peroxides and free fatty acids after 12 months of storage in shelled Chandler walnuts harvested in year 2, harvest 1. Bars with different letters are significantly different ($P < 0.05$). *Samples lost due to fungal contamination.

The industry currently uses peroxide value and free fatty acids content to characterize rancidity development in walnuts; however, these do not always provide the most accurate depictions of the extent of lipid oxidation that has occurred. During oxidation, peroxides are a primary oxidation product, meaning that they can be self-consumed or further metabolized into secondary or tertiary oxidation by-products

(Österberg et al., 2001; Velasco et al., 2010). Therefore, a low occurrence of peroxides in a sample might not accurately enumerate the amount of oxidation that has occurred. Nearly 70 years ago, Musco and Cruess (1954) stated that peroxide values are “not reliable and subject to error” and were concerned that thresholds did not exist. Tappel et al. (1957) recommended better measurements

than peroxide values. Lin et al. (2012) also expressed dissatisfaction with the usage of peroxide values because it is an intermediate oxidation product. They found similar results to our study when examining oxidation of almonds during storage; increased temperature led to an increase in peroxide values, but with increased RH, peroxide values decreased. We found a significant difference in peroxide values between walnuts stored in 40% RH and those stored in 80% RH at both 25 and 5 °C, with higher values at lower RH within a temperature after 12 months. Lin et al. (2012) described this as oxidation being “promoted by heat, but limited by humidity,” and because free fatty acids increased with both temperature and RH, the relationship between free fatty acids and peroxide values was described as “disconnected.” In peanuts stored at 15, 25, or 35 °C, Evranuz (1993) also found that temperature had a significant effect on peroxide value. Peanuts of different moisture content (1.4%, 2.2%, 2.8%, and 3.9%) were also stored at 35 °C to investigate the effect of moisture content. Low (1.4%) and high (3.9%) moisture content peanuts had more rapid oxidation than peanuts at 2.2% and 2.8% moisture content, although the effect at other temperatures, and therefore the interaction between temperature and moisture content, was not investigated (Evranuz, 1993).

Although storage temperature and RH influenced the rate of accumulation of free fatty acids and peroxides, the levels remained low and may not be commercially significant. This may, in part, be due to the rigorous sorting of shelled kernels selected for our study that removed any kernels that had been damaged in the shelling process. Higher levels of oxidation with similar trends between storage conditions would be expected under commercial conditions. Although they provide

Fig. 6. Peroxide value and free fatty acid content in four varieties of walnuts harvested in year 2, harvest 1 and stored in-shell under different temperatures and relative humidity conditions after 12 months of storage. Bars with different letters within a storage temperature are significantly different ($P < 0.05$). *Samples lost due to fungal contamination.

Fig. 7. Relationship between water activity and moisture content at 25 °C for four walnut varieties. Walnut kernels were stored in a sealed container with salt solutions selected to achieve a relative humidity ranging from 8% to 93% to achieve a range of moisture content and water activity levels. Each point represents six replicates of 5 to 10 walnuts each.

an indication of how much degradation has occurred, measurement of oxidation should not rely solely on peroxide values and free fatty acids. Alternative methods should be developed in the future for use by both researchers and the industry.

Water activity and moisture content. The relationships of water activity to shelf life, food stability, microorganism growth, and browning reactions have been well documented (Labuza, 1980) and used by various food industries. After comparing water activity and moisture content for individual walnut varieties, we saw that varieties responded to the RH of their environment in different ways. Walnut moisture content, peroxide values, free fatty acids, and color can all be influenced by oil composition and the amount and type of specific lipids. The degree of unsaturation in walnut oils is influenced by several factors, including genotype, growing environment, nut maturity, and the interactions of those qualities (Greve et al., 1992). Further work is needed to understand how lipid profile impacts these relationships, especially given that all walnut varieties are dried to a set moisture content upon harvest and placed in storage, frequently together with other varieties. If the RH of that storage is not managed to reflect the target moisture content of the nuts, they will equilibrate to the water activity (or equilibrium relative humidity) of the surrounding air. Due to the differences in oil content and lipid composition, which are commonly observed between varieties and especially different harvest dates and years within varieties, rates of decomposition under the same storage conditions may vary between walnut samples. Walnut kernel water activity would be more reflective of the metabolic activity within the stored walnuts and can be measured with routine equipment, similar to moisture content. Because the relationship between water activity and moisture content varies by variety, it is

important to measure both and understand the differences between the two.

Managing RH during walnut storage is difficult; however, when low temperature storage is used, quality is preserved even when RH is not controlled, although storage at 80% RH should be avoided. Storage at 5 °C is particularly recommended for any walnut variety to be stored longer than 6 months in air to avoid development of rancidity and excessive darkening. Given the large volumes of walnuts harvested each year and the capital cost to implement refrigerated storage, processors can also consider storage under low oxygen environments, whether in packages or chambers, to reduce the rate of quality degradation (Ortiz et al., 2019).

Literature Cited

- Bakkalbışoğlu, E., Ö.M. Yılmaz, I. Javidipour, and N. Artık. 2012. Effects of packaging materials, storage conditions and variety on oxidative stability of shelled walnuts. *Lebensm. Wiss. Technol.* 46:203–209, doi: 10.1016/j.lwt.2011.10.006.
- Christopoulos, M.V. and E. Tsantili. 2015. Oil composition in stored walnut cultivars—quality and nutritional value. *Eur. J. Lipid Sci. Technol.* 117:338–348, doi: 10.1002/ejlt.201400082.
- Escobar, M.A., A. Shilling, P. Higgins, S.L. Uratsu, and A.M. Dandekar. 2008. Characterization of polyphenol oxidase from walnut. *J. Amer. Soc. Hort. Sci.* 133:852–858, doi: 10.21273/JASHS.133.6.852.
- Evranuz, E.Ö. 1993. The effects of temperature and moisture content on lipid peroxidation during storage of unblanched salted roasted peanuts: Shelf life studies for unblanched salted roasted peanuts. *Intl. J. Food Sci. Technol.* 28:193–199, doi: 10.1111/j.1365-2621.1993.tb01264.x.
- Fields, R.P. 2018. Maturity and excess irrigation trigger amber kernel color in “Howard” Walnut, but not in “Chandler.” PhD Diss., University of California, Davis.
- Firestone, D. (ed.). 1973. Official Method Cd 3d-63. Acid value. Official methods and recommended practices of the American Oil Chemists’ Society. 5th ed. AOCS Press, Champaign, IL.
- Firestone, D. (ed.). 1997. Method Cd 8-53. In: Official Methods and Recommended Practices of the American Oil Chemists’ Society. 4th ed. American Oil Chemists’ Society Press, Champaign, IL.
- Forney, C.F. and D.G. Brandl. 1992. Control of humidity in small controlled-environment chambers using glycerol-water solutions. *HortTechnology* 2:52–54, doi: 10.21273/HORTTECH.2.1.52.
- Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. *J. Res. Natl. Bur. Stnd. Section A. Physics and Chem.* 81A:89, doi: 10.6028/jres.081A.011.
- Greve, L.C., G. McGranahan, J. Hasey, R. Snyder, K. Kelly, D. Goldhamer, and J.M. Labavitch. 1992. Variation in polyunsaturated fatty acids composition of Persian walnut. *J. Amer. Soc. Hort. Sci.* 117:518–522, doi: 10.21273/JASHS.117.3.518.
- Khair, R., G.G. Atungulu, Z. Pan, J.F. Thompson, and X. Zheng. 2014. Moisture-dependent color characteristics of walnuts. *Intl. J. Food Prop.* 17:877–890, doi: 10.1080/10942912.2012.675610.
- Labuza, T.P. 1980. The effect of water activity on reaction kinetics of food deterioration. *Food Technol.* 34:36–41.
- Lin, X., J. Wu, R. Zhu, P. Chen, G. Huang, Y. Li, N. Ye, B. Huang, Y. Lai, H. Zhang, and W. Lin. 2012. California almond shelf life: Lipid deterioration during storage. *J. Food Sci.* 77:C583–C593, doi: 10.1111/j.1750-3841.2012.02706.x.
- López, A., M.T. Pique, A. Romero, and N. Alea. 1995. Influence of cold-storage conditions on the quality of unshelled walnuts. *Intl. J. Refrig.* 18: 544–549, doi: 10.1016/0140-7007(96)81781-6.
- Musco, D.D. and W.V. Cruess. 1954. Food rancidity, studies on deterioration of walnut meats. *J. Agr. Food Chem.* 2:520–523, doi: 10.1021/jf60030a006.
- Nelson, K.A. and T.P. Labuza. 1992. Relationship between water and lipid oxidation rates, p. 93–103. In: *Lipid Oxidation in Food*. American Chemical Society, Washington, D.C.
- Ortiz, C.M., A.R. Vicente, R.P. Fields, F. Grillo, J.M. Labavitch, I. Donis-Gonzalez, and C.H. Crisosto. 2019. Walnut (*Juglans regia* L.) kernel postharvest deterioration as affected by pellicle integrity, cultivar and oxygen concentration. *Postharvest Biol. Technol.* 156:110948, doi: 10.1016/j.postharvbio.2019.110948.
- Österberg, K., G.P. Savage, and D.L. McNeil. 2001. Oxidative stability of walnuts during long term in shell storage. *Acta Hort.* 591–597, doi: 10.17660/ActaHortic.2001.544.82.
- R Development Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <<http://www.R-project.org/>>.
- Schmidt, S.J. and J.W. Lee. 2012. Comparison between water vapor sorption isotherms obtained using the new dynamic dewpoint isotherm method and those obtained using the standard saturated salt slurry method. *Intl. J. Food Prop.* 15:236–248, doi: 10.1080/10942911003778014.
- Shahidi, F. and J.A. John. 2013. Oxidative rancidity in nuts, p. 198–229. In: L.J. Harris (ed.). *Improving the Safety and Quality of Nuts*. Woodhead Publishing.
- Simopoulos, A.P. 2004. Health effects of eating walnuts. *Food Rev. Intl.* 20:91–98, doi: 10.1081/FRI-120028832.
- Tappel, A.L., F.W. Knapp, and K. Urs. 1957. Oxidative fat rancidity in food products II. Walnuts and other nut meats. *J. Food Sci.* 22:287–295, doi: 10.1111/j.1365-2621.1957.tb17012.x.
- Velasco, J., C. Dobarganes, and G. Márquez-Ruiz. 2010. Oxidative rancidity in foods and food quality, p. 3–32. In: L.H. Skibsted, J. Risbo, and M.L. Andersen (eds.). *Chem. Deterioration Physical Instability Food Beverages*. Woodhead Publishing Ltd., Cambridge.

Supplemental Fig. 1. Influence of harvest timing on kernel free fatty acid content after 12 months of storage of in-shell and shelled Chandler walnuts harvested in year 2. Bars with different letters within harvests are significantly different ($P < 0.05$). *Samples lost due to fungal contamination.

Supplemental Table 1. Saturated salt slurries used to achieve target relative humidity values for isotherm studies with four walnut varieties.

Saturated salt slurry	Relative humidity generated at 25 °C
Magnesium chloride	32.78 ± 0.16
Lithium nitrate	47.06 ± 0.18
Sodium bromide	57.57 ± 0.40
Potassium iodide	68.86 ± 0.24
Sodium chloride	75.29 ± 0.12

Supplemental Table 2. Main effects means and interaction between temperature and relative humidity (RH) for walnuts harvested in year 1 and stored under various conditions, including 20%, 40%, and 60% RH.

	Water activity	Moisture content	Peroxide value	Free fatty acids	L* value
Variety					
Chandler	0.390 b	2.83 b	0.818 a	0.112 b	47.56 a
Howard	0.381 c	2.84 b	0.675 b	0.089 c	40.21 c
Tulare	0.403 a	2.97 b	0.685 b	0.191 a	41.78 b
Vina	0.403 a	3.49 a	0.647 b	0.116 b	30.99 d
Harvest					
1	0.384 b	2.97 b	0.718 a	0.121 b	42.08 a
2	0.404 a	3.10 a	0.694 a	0.133 a	38.17 b
Storage type					
Shelled	0.398 a	3.10 a	0.868 a	0.138 a	39.90 a
In-Shell	0.389 ab	2.96 b	0.570 b	0.119 b	40.23 a
Temp (°C)					
5	0.433 a	3.33 a	0.288 c	0.94 c	41.45 a
15	0.387 b	2.97 b	0.613 b	0.111 b	40.05 b
25	0.360 c	2.79 c	1.258 a	0.181 a	38.69 c
RH (%)					
20	0.353 c	2.65 c	0.743 a	0.099 c	39.48 b
40	0.376 b	2.99 b	0.717 a	0.113 b	40.44 a
60	0.452 a	3.24 a	0.698 a	0.174 a	40.28 a
Storage time					
0	0.401 ab	3.42 a	0.000 d	0.046 c	44.48 a
3	0.388 b	3.11 a	—	—	41.34 b
6	0.380 b	2.83 b	0.633 c	0.122 b	40.76 b
9	0.423 a	2.88 b	0.705 b	0.124 b	38.68 d
12	0.384 b	3.29 a	0.819 a	0.139 a	39.47 c
Temp/RH					
5/20	0.370 e	2.96 c	0.296d	0.080 d	41.29 ab
5/40	0.431 c	3.39 abc	0.252 d	0.076 d	41.49 a
5/60	0.500 a	3.64 a	0.317 d	0.127 c	41.59 a
15/20	0.337 g	2.53 e	0.669 b	0.085 d	39.63 cd
15/40	0.358 ef	2.91 cd	0.634 bc	0.097 d	40.33 bc
15/60	0.467 b	3.46 ab	0.538 c	0.151 bc	40.20 c
25/20	0.353 f	2.47 e	1.264 a	0.133 c	37.48 e
25/40	0.338 g	2.66 de	1.267 a	0.167 b	39.49 cd
25/60	0.389 d	3.23 bc	1.242 a	0.243 a	39.08 d