Impact of Room Temperature Storage on Pecan Kernel Color, Carotenoids, Polyphenols, and Physicochemical Properties

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ABSTRACT

This study aimed to investigate the changes in pecan kernel color, carotenoids, polyphenols, and physicochemical properties during five months of storage at room temperature for five different pecan varieties. The results showed the pecan kernel color darkened, with a shift towards more red and less yellow during the storage. Additionally, the dorsal side of the kernel had lighter color than the ventral side. Total carotenoids deceased from 92.0 – 118.8 to 45.2 – 101.9 μg/100g whole kernel, while total polyphenols had no significant differences (18.1 – 27.0 mg GAE (gallic acid equivalent)/g whole kernel) commonly. Three phenolics (gallic acid, catechin, and ellagic acid) were 6.7 – 9.6, 31.4 – 46.7, and 11.7 – 16.2 mg/100g whole kernel and increased during storage. Pecan kernel moisture loss was significant, while total lipids remained unchanged. Five texture parameters (hardness, toughness, slope, fracturability, and break) showed irregular changes. Genotypic variation was observed in all five varieties, although the storage was the main factor affecting compositions.

KEYWORDS: *Carya illinoinensis*, pecan shelf life, polyphenol, carotenoids, pecan color, pecan texture

INTRODUCTION

Pecan (*Carya illinoinensis*) is one of the edible nut trees in the hickory species and are primarily produced in Mexico and the United Sates.[1] The U.S. is the leading country for pecan production, consumption, and exportation around the world. Pecan trees have been cultivated across multiple states, which are a notable component of the U.S. agricultural economy with economic value reaching up to $700 M.[2] However, per capita consumption of pecan kernels in the U.S. was only 0.46 lbs. in 2018.[3] There is a room to increase the consumption of pecan kernels based on the 0.87 lbs of per capita consumption in almonds (the most produced tree-nuts).[3] There are several factors influencing pecan kernel consumption. Of them, sensory quality (appearance, flavor, and texture) and nutritional quality are the top two crucial ones. The pecan kernel nutritional quality characteristics include unsaturated fatty acids and redox-active metabolites, such as tocopherols, plant sterols, and phenolic compounds.[4,5] Along with health benefits, these compositions might also contribute to sensory qualities like appearance (color) and texture. Pecan flavor (aroma and taste), on the other hand, is determined by its volatile and non-volatile compounds, including sugars and acids.

Pecan kernels can be consumed fresh or added to various food products such as baked products, snacks, and yogurts.[6] For fresh consumption, raw pecan kernels are commonly packed in zip-bags or clamshell containers and sold in super-markets at room temperature. These kernels are prone to developing rancid flavors duo to their high content of unsaturated fatty acids. As a result, maintaining the shelf life of pecan kernels at room temperature is a challenge for both end-users and those involved in the storage, distribution, and transportation of the products. It is crucial for pecan growers and manufacturers to store their products efficiently to prevent sensory and nutritional quality degradation, including undesirable physical and chemical changes, caused

by environmental factors such as temperature. By extending the shelf life of pecan kernels, stakeholders can increase financial returns and make the product more nutritious.

Several variables have been used as pecan kernel quality indicators, including size, color, chemical compositions (moisture, lipids, free fatty acids), and other sensory qualities and physicochemical properties.[2] During storage, the major changes in pecan kernel quality include color darkening, rancid flavor from lipid oxidation, and texture degradation.[2] The color of a pecan kernel is commonly used to evaluate its overall quality and freshness.[2] The appearance, specifically the color, is the first sensory attribute that influences consumer preference and acceptability.[7] Studies have showed that the pecan kernel with a light golden color was rated with the highest liking score, and milky white interior was positively correlated with overall kernel liking.[7] However, research on color and texture changes during storage is limited, and more studies are needed to address this issue. It is preferable if compare those potential breeding lines from a breeding program and popular commercial varieties, because such knowledge about the changes in the pecan kernel taste and texture during storage can provide more information for future new cultivar releases. On the other hand, extensive research has been conducted on the effects of lipid oxidation and resulting rancid flavor.[1,2]

Studies have showed that temperature and storage time are two important variables that impact overall sensory quality and physicochemical properties of pecan kernels, though postharvest procedures such as processing and packaging may have an effect.[1,2] Therefore, this study aimed to examine the effects of storage at room temperature for five months on pecan kernel color, total carotenoids, total polyphenols, individual polyphenols, moisture, total lipids, and texture in five pecan varieties. The selected varieties included one commercial variety (Pawnee), one native selection (known as Prilop of Lavaca or Prilop), and three breeding lines

(1992-09-0041, 1992-09-0045, and 2000-01-0027, shortened as 0041, 0045, and 0027 thereafter) from the USDA-ARS pecan repository and breeding orchards. This study will increase our knowledge of changes in pecan kernel color, chemical compositions, and physical properties during storage at room temperature for different pecan varieties. Furthermore, knowledge of these changes is a valuable addition for potential breeding lines in the USDA-ARS pecan breeding program before release to pecan growers.

MATERIALS AND METHODS

Pecan Kernel Samples and Storage

Five fully matured pecan varieties – an improved (Pawnee), a native selection (Prilop of Lavaca or Prilop), and three breeding lines (1992-09-0041, 1992-09-0045, 2000-01-0027, shortened as 0041, 0045, and 0027 respectively thereafter) were harvested from the USDA-ARS pecan repository and breeding orchards in Somerville and Brownwood (variety 0027), TX in the fall of 2021. The USDA Pecan Repository orchard was created in 1991 to exhibit various pecan cultivars from around the country. All the collections were grafted onto an Apache rootstock and planted as a single clone in clay soil with an average pH of 7.5. One of the collections in the repository orchard (Prilop) was selected for the representative of native variety in this study. The USDA Pecan Breeding Program established two National Pecan Advanced Clonal Test System (NPACTS) orchards in Somerville and Brownwood, Texas, for comparing unreleased breeding lines with standard cultivars. In Somerville NPACTS orchard, 30 unreleased breeding lines and 5 standard cultivars as controls cultivars were arranged in a completely randomized design with 6 replications. In 2010, two of the 30 breeding lines (1992-09-0041 and 1992-09-0045) and a standard cultivar (Pawnee) were grafted onto a VC1-68 rootstock in Somerville. In Brownwood

NPACTS orchard, 49 unleased breeding lines and 3 control cultivars were arranged in a completely randomized design with 5 replications. One of the 49 breeding lines (2000-01-0027) was grafted onto the Apache rootstock in 2007 in Brownwood. Slow-release fertilizer, containing a balanced ratio of N:P:K, was applied in March and September during the first five years of each orchard. Once the trees matured (7 years or older), a 0.24% zinc sulfate solution was applied using a tractor-driving sprayer when leaves are fully expanded.

The nuts were hand-harvested in bulk from the trees when the shucks split. Fresh pecan nuts were placed in a heavy-duty paper bag (a common approach for farmers) and stored in a cold room (16 °C) to dry until the pecan nuts' relative water content was ~5% (in about 1 week). Then, all samples were shipped to Texas Woman's University (TWU) in Denton, Texas. Once received, samples (approximately 3 lbs per variety) were immediately unshelled for this shelf life study.

Pecan nuts were unshelled using a manual nutcracker (Duke's Easy Pecan and Nut Cracker, Model 0100). Then, ~ 25 g of kernel was allocated into individual quarter-gallon zip-bags (Ziploc all-purpose storage bags, made with polyethylene) with six bags for each variety (5 varieties x 6 bags/variety, ~25 g kernels/bag) – that is, one bag for each month of storage (0 – 5-months total) for all five varieties. The kernels labelled 0 month were immediately stored at -20 °C, except samples saved for texture analysis, which was analyzed without -20 °C storage. All remaining bags were stored in an incubator (Water-Jacket Incubator, Forma Scientific, Model 3326, OH, USA) with temperature controlled at 25±0.5 °C and humidity around 50%. When the storage time reached for each month, one bag of kernels per variety was removed from the incubator. Then each variety was divided into two portions. One portion was immediately used for texture analysis (within hours) without any cold storage. Another portion was stored in a

freezer (-20 °C); these samples were used for all analyses (except for texture analysis). By the end of shelf life storage (month 5), all the frozen kernel samples were analyzed for chemical compositions within 3 months.

Pecan Kernel Color Measurement

Kernel color was measured using a CIELAB color space embedded in a spectrophotometer (ColorFlex EZ, A60-1014-593, Version 2.2, Reston, VA, USA) and different colors were categorized by three letters L*, a*, and b*. The L* value represents lightness with a scale of 0 (black) to 100 (white). The a* and b* values represent chromaticity without specific numeric limit. Negative a* corresponds with green, while positive a* corresponds with red. For b* value, negative b* corresponds with blue, while positive b* corresponds with yellow. The colorimeter was calibrated using standard white and black tiles prior to kernel sample color measurement.

For every variety and storage month, three pecan kernel halves were randomly selected from the storage bag and analyzed for the L*, a*, and b* values. One halve was divided into 5 sections: top, middle, bottom, middle-left, and middle-right (each area was distinct to make 5 areas represent the whole halve). These 5 measurements were performed on both ventral and dorsal sides of each pecan halve. In total, 10 measurements were taken for each pecan kernel halve. The analysis was conducted in triplicate (three pieces of halves).

Pecan Kernel Total Carotenoid Measurement

Total carotenoids were measured using a modified method previously described in literature.[8,9] Two randomly selected kernel halves for each pecan variety in each test month were

ground into a fine powder using a mortar and pestle. One gram powder was placed into a 50 mL centrifuge tube, and 5 mL of hexane was added for carotenoid extraction. After a quick vortex, the tube was placed in an ultrasonic bath for 5 min and centrifuged on 4000 rpm for 10 min. The supernatant (hexane fraction) containing carotenoids was collected. The extraction was repeated with 5 mL hexane two more times to produce a total of 15 mL pooled hexane extracts. The 15 mL of pooled extracts were vortexed briefly for 2 sec and filtered through Whatman No.1 filter paper.

A total of 20 µL of each pooled extract and blanks (only hexane solvent) were dispensed into a 96-well microplate. The absorbance was read at 450 nm using a spectrophotometer (BioTek Synergy H1, Agilent, Santa Clara, CA, USA). Prior to carotenoid content calculations, all reads were calibrated with the hexane blank reads. The total carotenoid content was calculated using the following formula:

$$\text{Total carotenoid content (µg/100g kernel)} = \frac{A * V\,(mL) * 10^6}{A_{1cm}^{1\%} * P\,(g)}$$

where A= absorbance at 450 nm; V = total extract volume (mL); P = sample weight (g); $A_{1cm}^{1\%}$ = 2592 (β-carotene Extinction Coefficient in hexane or in petroleum ether).

Pecan Kernel Total Polyphenol Measurement

Total polyphenols were measured using the Folin-Ciocalteu method with minor modifications.[10] Two randomly selected kernel halves of each variety in each month were ground into a fine powder using a mortar and pestle. For polyphenol extraction, 1 g powder of each sample was placed in a 50 mL centrifuge tube and vortexed for 30 sec after adding 10 mL of

petroleum ether and 10 mL of 60% acetone (diluted with DI water). The slurry was then centrifuged at 4000 rpm for 10 min. The bottom liquid (acetone phase) was collected and diluted with the above solution mixture, and repeatedly extracted twice to produce 30 mL pooled acetone. The pooled acetone phases were vortexed for 2 sec and then passed through Whatman No.1 filter paper.

For total polyphenol quantification, 1 mL of pooled acetone extracts was first diluted by mixing 9 mL of 60% acetone. Afterwards, 200 µL of such diluted solution was pipetted into in a 15 mL centrifuge tube containing 500 uL of Folin-Ciocalteu's phenol reagent (2 M; analytical standard grade; MilliporeSigma, St. Louis, MO, USA). The mixture was set for 2 min and then 1 mL of 7% aqueous sodium carbonate (7:93, sodium carbonate: water, w/v) was added. Another incubation of 15 min in a 50 ^{o}C water bath was performed. After cooling down to room temperature, a mixture of 200 µL aliquots of the reacted samples (dilution factor 1700x) was dispensed in a 96-well microplate and was measured using a spectrophotometer at 750 nm.

The quantified sample absorbance readings were converted to total polyphenol content via a gallic acid standard curve. To create this standard curve, a stock solution was first prepared by dissolving 500 mg of gallic acid standard in 100 mL of 10% aqueous acetone in a volumetric flask. Then, the stock solution was diluted into nine known concentration levels: 0.001, 0.002, 0.003, 0.004. 0.005, 0.006, 0.007, 0.008, and 0.020 mg gallic acid equivalent weight (GAE)/mL, plus a blank without gallic acid included. These standard solutions were treated similarly to the samples by incubating with Folin-Ciocalteu's phenol reagent and sodium carbonate solution to complete the color-changing reaction; 750 nm absorbance readings were likewise taken. Those absorbance values were plotted to generate a linear calibration curve which was forced through the origin with r^2>0.99. Using the calibration curve's produced linear equation, the sample

absorbance was expressed as mg of gallic acid equivalent weight (GAE)/100g of whole pecan kernel.

Pecan Kernel Phenolic Analysis with HPLC-UV

Individual phenolic compounds were analyzed with a modified method previously described in the literature.[11] The dried and defatted pecan nutmeals from the completion of the Soxhlet fat extraction (described in section "Pecan Kernel Total Lipid Analysis") were used for phenolic analysis.

To perform phenolic compound extraction, 0.25 g of de-moisturized and defatted pecan kernel powder for each variety and storage month was placed in a 50 mL centrifuge tube. The kernel powder was vortexed for 10 sec with 5 mL of 60% aqueous methanol and then sonicated for 5 min. Samples were centrifuged at 4000 rpm for 10 min; afterwards, the supernatant containing phenolic compounds was collected. The extraction procedure was repeated two more times (first with 3 mL and then with 2 mL of 60% methanol), for a total of 10 mL pooled extracts. The 10 mL pooled extracts were vortexed briefly for 2 sec and then passed through Whatman No.1 filter paper.

For phenolic compound quantification, 4 mL of each pooled extract was filtered through a syringe disc filter (0.45μm, 30mm diameter; Fisher Scientific, Fair Lawn, NJ, USA), and collected in a 4-mL glass vial for high performance liquid chromatography with ultraviolet detection (HPLC-UV). The HPLC (Shimadzu USA Manufacturing, Oregon, USA) configuration included the following parameters: binary pump (LC - 20AR), degassing unit (DGU-20A 3R), autosampler (SIL - 20A) with injection volume set to 20 μL, UV detector (SPD - 20A) at 280 nm, and an Ultra AQ C18 column (5 μm, 250 × 4.6 mm, Restek, Bellefonte, PA, USA). Two

mobile phases were used at a total flow rate of 1.0 mL/min: 5% acetic acid (aqueous phase, pump A) and 100% methanol (organic phase, pump B). Gradient elution involved mobile phase B increasing from 5% to 95% for the first 40 min, followed by 10 min of phase B slowly reverting to 5%. Finally, the system was held at 5% mobile phase B for 10 min to gain baseline stability for the next injection.

Three phenolic chemical standards (MilliporeSigma, St. Louis, MO, USA) were used to generate calibration curves with gallic acid (0.0011 – 0.07 mg/mL), catechin (0.0011 – 0.07 mg/mL), and ellagic acid (0.0017 – 0.1 mg/mL). A gallic acid and catechin standard curve was generated using eight known concentrations. The gallic acid and catechin stock solution (0.07 mg/mL) was prepared by dissolving 3.5 mg of each standard powder in 50 mL of 60% aqueous methanol in a volumetric flask. Then, the stock solution was diluted to eight additional levels of 0.0420, 0.0252, 0.0151, 0.0091, 0.0054, 0.0033, 0.0012, and 0.0011 mg/mL. For ellagic acid, the stock solution (0.1 mg/mL) was prepared by dissolving 2.5 mg of the standard powder in 25 mL of 100% methanol in a volumetric flask. Then, the stock solution was diluted to eight additional levels of 0.060, 0.036, 0.022, 0.013, 0.0078, 0.0047, 0.0028, and 0.0017 mg/mL. The standard solutions were injected and subsequently analyzed in like fashion to the samples. Three linear calibration curves with $r^2>0.99$ were then generated by plotting the peak area against concentration. Sample peak areas were compared to these curves in order to express results as mg of phenolic compound/100g of whole pecan kernel.

Pecan Kernel Moisture Analysis

The Forced Draft Oven method used for moisture analysis was adapted from the AOAC Official Method 977.11 with modification.[12] In brief, five pecan kernel halves of each variety in

each month were randomly selected and crushed into a fine powder using a mortar and pestle. From the pool of nutmeal, 4 g was spread out evenly onto pre-weighed 150 mL disposable aluminum dishes. The filled dishes were placed in a forced draft oven (Stabil - Therm Gravity Oven, Blue M Electric Company, IL, USA) at 70 ^{o}C for 3 hours and then cooled down to room temperature in a desiccator for 1 hr. The cooled dishes were weighed and finally stored in the desiccator for further analysis.

Pecan Kernel Total Lipid Analysis

A modified Soxhlet method from the AOAC Official Method 963.15 was conducted for total lipid quantification.[12] All dried samples from pecan kernel moisture determination (~4 g) were used in this section's total pecan kernel lipid analysis. Each dried pecan kernel sample (2~ 4 g) was transferred onto a sheet of weighed filter paper (Whatman No. 1) and then wrapped by folding the filter. The wrapped sample was placed into a weighed cellulose thimble (25 mm x 80 mm; Sigma Aldrich, St. Louis, MO, USA) and covered with glass wool.

Lipids were extracted from the prepared sample thimble. The prepared thimble was put inside the extraction chamber, on top of which a reflux condenser was attached. A round-bottom flask (250 mL) was filled with 150 mL of petroleum ether and placed underneath an extraction chamber. The flask was placed on a heater set at 50% power, with a rate of 5-6 condensate drips per second. The Soxhlet apparatus was heated for 2 hrs past the first siphon, or extraction chamber draining; the extraction chamber and reflux condenser were subsequently disconnected, and the round-bottom flask was left on the electric heater until the petroleum ether evaporated. Both cellulose thimbles and round-bottom flasks were baked at 70 ^{o}C for 1 hr. The flask was cooled in a desiccator and then weighed in a fume hood.

Pecan Kernel Texture Analysis

In each treatment, unfrozen pecan kernel halves were directly used for texture analysis using a texture analyzer (EZ-SX; Shimadzu, Kyoto, Japan), which was equipped with Shimadzu's Volodkevich Bite Jaw (PN 346-57805-00, only upper jaw used for this study), slotted base plate from the blade shear jig set (PN 346-57807-00), and 100 N load cell (0.5% accuracy). The trigger force was set to 0.5 N, test speed was 1 mm/s, and the distance traveled by the blade was 10 mm.

The test was performed by laying the flatter portion of the pecan kernel halves on the slotted base plate. Each pecan halve was measured lengthwise with a ruler to ensure that the blade cut through the middle of the halve. The reported parameters were hardness (N; highest positive force reached before breaking in halve), toughness (N.mm, area under the curve to hardness), slope (N/mm, the linear segment of the force curve), fracturability (N, force required for first fracture), and break (mm, point at sudden 10% drop in force after reaching max force).[13,14] Three halves per variety per month were measured for these five texture parameters for each halve.

Statistical Analysis

All analyses were replicated three times in order to produce true sample triplicate results. One-way ANOVA (analysis of variance) with Tukey's honestly significant difference (HSD) was performed for all analyses to significantly distinguish sample means. Factorial ANOVA, with independent factors of variety (5 pecan types) and storage month (6 different months – 0-5 months), was conducted using a univariate general linear model. One-way, two-way, and

three-way ANOVA were conducted using SPSS v. 25 software (IBM SPSS Statistics, Armonk, NY, USA). Principal component analysis (PCA) was conducted to differentiate kernel samples concurrently with all variables measured in this study; this was performed with XLSTAT v. 2019 (Addinsoft, New York, NY, USA). Statistical significance was defined as $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Pecan Kernel Color Changes During Storage

Wholesale distributors and retailers ranked the color of pecans as the top quality criteria. The United States Department of Agriculture (USDA) has developed a 4-level color model (Pec-MC-1-1968), including light – golden color, light amber – light brown, amber – medium brown, and dark amber – dark brown.[15] To improve the color scale range for measuring pecan kernel quality, a more advanced 6-level model (light cream, cream, golden, light brown, reddish brown, and dark reddish brown) using the Munsell color rating system (hue, chroma, and value) has been established to measure pecan kernel quality.[16] Studies have showed that pecan kernel color changes from a bright golden to brown or reddish-brown during storage.[17,18] A darker color indicates advanced age and chemical changes such as oxidation and rancidity. However, there are limited studies on the pecan kernel color and physicochemical changes that occur during storage at room temperature with genotype variation.

In this study, pecan kernel color changes during the tested 0- to 5-month time period were visually identifiable, as evidenced in the sample photo (Figure S1). When storage time increased, the color plainly darkened for all five pecan varieties. Other nuances related to color change with time progression were hard to identify with the naked eye. Instrumental measurements reported using the CIELAB color space (L*, a*, b*) provides a more accurate means of color description.

The results for L* value (lightness, a higher L* value will represent a lighter color) for pecan kernel color changes during storage are shown in Table S1. Significant L* value changes during storage were observed. As shown in Figure 1 (A and B), both dorsal and ventral sides for all five varieties had decreasing L* values as storage time progressed; this indicated the kernel darkened over time. For example, Table S1 shows the kernel dorsal side at month 0 had L* values ranging 23.9 – 26.3, while they significantly decreased to 17.7 – 20.0 at month 5. Although this decreasing trend was observed in all five varieties, L* value variety difference was additionally observed. The Prilop and 0045 varieties had the altogether highest L* value (lightest), while variety 0041 had the lowest L* value (darkest); this was determined by comparing the means of all L* data points for each variety (all storage months and both kernel sides averaged together). In addition, the kernel dorsal side was found to be significantly lighter (higher L* value) than the ventral side.

For a* value (-a* towards green, while +a* towards red; a higher value indicates increased redness), as shown in Figure 2A, the dorsal side of all five varieties had significant a* value increases during storage; this indicated the kernel became redder. For example, Table S1 shows that kernel a* at month 0 was 8.8 – 10.6, while it significantly increased to 11.3 – 13.2 after 5 months of storage. In contrast, the ventral side showed a significant increase in a* value in only one variety (Prilop) between 0 and 5 months of storage. The overall kernel ventral side mean (disregarding variety and storage month) was significantly redder (higher a* value) compared to the dorsal. In like fashion, the means of all a* data points for each variety (all storage months and both kernel sides averaged together) were compared, and 0045 was found to have the s highest a* value (redder) by a significant amount.

For b* values (-b* towards a blue color, while +b* towards yellow), as shown in Figure 3 (A and B), b* values decreased during storage for both dorsal and ventral sides of all five varieties, indicating kernel shifted from yellower to bluer over time. For example, Table S1 shows the kernel dorsal side at the beginning (month 0) had b* values ranging from 20.9 – 23.2, while this significantly decreased to 16.2 – 18.7 after 5 months of storage. Although the negative trend was the same for all five varieties, b* value variety difference was identified as well. Variety 0027 had significantly the lowest b* value while Prilop was significantly the highest; this was determined by comparing the means of all b* data points for each variety (all storage months and both kernel sides averaged together). Kernel side difference was similarly significant, with the average of all dorsal side b* values possessing a significantly more yellow color (higher b* value) than the means for the ventral side.

Overall, this study found that after 5 months of storage, the color of pecan kernels changed to a dark reddish with a slight purpleness, as shown by the decline of L* and b* values, and the incline of a* value. The dorsal side had a lighter color than the ventral side in general. Although no literature has documented the reasons for the darker color at ventral side, naturally, dorsal and ventral sides have different groove depth and width, which might cause different color intensity. In addition, pecan kernel necrosis could cause darkened tissue,[19] although we did not perceive this was the case for the kernel samples in this study. In addition, this color change was observed in all five tested pecan varieties, although some differences were noted between the varieties. Variety difference indicates a cultivar's innate resistance to the breakdown of pigments. The color changes of pecan kernel during storage indicate chemical degradation, including degradation of carotenoids and flavonoids that are the most commonly found pigments in the nuts. In addition to degradation, flavonoids could condense to tannins or oxidize to other

phenolic compounds, which can impact pecan kernel color,[2,18] although there are no such studies focusing on the mechanism of flavonoid changes during storage.

Pecan Kernel Storage Total Carotenoids Changes

Carotenoids are triterpene pigments, which contribute to the color yellow, orange, red, and purple in plants. In this study, the results for pecan kernel total carotenoid changes during storage are shown in Table S2 and Figure 4. Overall, the five pecan kernel varieties had total carotenoids ranging from 92.0 – 118.8 μg/100g whole kernel at month 0 (fresh). At storage month 5, total carotenoids decreased to 45.2 – 101.9 μg/100g whole kernel; this decrease was only significant for two varieties (Pawnee and 0041). To compare variety differences, all storage months were averaged within each variety. The grand means were compared and Pawnee was found to have the lowest overall amounts of total carotenoids (68.5 μg/100g), while sample 0027 (114.0 μg/100g) was significantly higher than the other four varieties.

The results from the current study were consistent with the USDA database, which shows the total carotenoids in pecan kernels range from 52.6 – 119.6 μg/100g whole kernels. The phenomenon that the total carotenoids decreased from 92.0 – 118.8 to 45.2 – 101.9 μg/100g whole kernels after 5 months of storage in this study was most likely caused by oxidation. Carotenoids are sensitive to light, heat, and oxygen due to multiple conjugated double bonds in the structure.[20] Xanthophyll is the major carotenoid responsible for the golden yellow color of pecans, which can be oxidized and cause darkening.[2]

Pecan Kernel Storage Total Polyphenol Changes

Beside carotenoids, polyphenol is another group of phytochemicals. The results for total polyphenol changes in pecan kernels during storage are shown in Table S3 and Figure 5. Fresh (month 0) pecan kernels had total polyphenols ranging from 18.1 – 27.0 mg GAE/g whole kernel. At storage month 5, the total polyphenol content for all varieties was between 18.6 – 23.5 mg GAE/g, which was not significantly different from month 0. However, variety difference for total polyphenol content was observed. A comparison of the comprehensive sample means (all storage months averaged together) yielded the following conclusion: varieties of 0041 (25.5 mg GAE/g) and 0045 (25.1 mg GAE/g) were significantly higher in total polyphenols than Prilop (22.7 mg GAE/g), 0027 (19.9 mg GAE/g), and Pawnee (18.6 mg GAE/g).

The results from the current study that the concentration of total polyphenols in pecan kernels ranged between 18.1 – 27.0 mg GAE/g whole kernel is consistent with previous studies, which have showed that the total polyphenols ranged from 8.29 – 20.16 mg GAE/g of whole kernel.[4,21] Furthermore, total polyphenol did not show any significant changes during 5 months of storage in this study, which could be attributed to the total amount of polyphenols did not change, although chemical reactions such as degradation or conjugation of polyphenols could occur. Another reason might be the storage period being too short to have a change. One previous study showed that the leucoanthocyanidin level in pecan kernel skin decreased during 16 weeks of storage at 32 ^{o}C and 50% RH and the researchers explained it could be caused by oxidation, which formed phlobaphene and anthocyanidin, contributing to red-brown discoloration on pecan skin.[22] However, another study in almonds showed that the total phenolic compounds after five years of storage did not change in either hull or shell.[23] In contrast, there is a study showing that total polyphenols in citrus peels increased during long-term storage.[24] The increase of total polyphenol content during storage has resulted from the biodegradation of unextractable

phenolic compounds.[24] Consequently, the results from these studies are inconclusive and further research is needed to fully understand the changes in polyphenol content during storage of pecan kernels.

Pecan Kernel Storage Individual Polyphenol Changes

The content for total polyphenol mainly contains derivatives of catechin, gallic acid, and more than half is composed of ellagic acid.[4,21] A quantitative test on each derivative of phenolic compounds is necessary to determine which derivatives increase or decrease during storage time over total polyphenols.

Changes in pecan kernel gallic acid content are shown in Table S4 and Figure 6. Gallic acid at month 0 ranged from 6.7 – 9.6 mg/100g whole kernel. After 5 months of storage, the gallic acid content increased to 8.6 – 10.6 mg/100g whole kernel. Significant differences were observed between months 0 and 5 for all five varieties. When all storage months were averaged, gallic acid did exhibit a variety difference: Pawnee was significantly the lowest (7.6 mg/100g), whereas Prilop was the highest (9.7 mg/100g).

Table S4 and Figure 7 present the catechin content for fresh (month 0) pecan kernels, which had a range from 31.4 – 46.7 mg/100g whole kernel. At month 5, catechin content increased to 36.9 – 61.4 mg/100g whole kernel; this change was only significant in the Pawnee and 0041 varieties. Variety difference was observed (all storage months). Pawnee (51.8 mg/100g), Prilop (48.1 mg/100g), and 0027 (48.0 mg/100g) had significantly higher catechin content than varieties 0041 (36.2 mg/100g) and 0045 (36.8 mg/100g).

For ellagic acid, fresh kernel (month 0) contained 11.7 – 16.2 mg/100g whole kernel (Table S4 and Figure 8). After 5-month storage, it changed to 11.7 – 21.0 mg/100g whole kernel. Although the ellagic acid showed an increasing trend (13.2 mg/100g at month 0 increased to 16.0/100g at month 5), the increase was variety dependent. Only variety 0041 show a significant change during the 5-month storage. Variety difference was significant. Varieties 0041 and Pawnee contained a significantly higher amount of ellagic acid (16.4 and 15.9 mg/100g, respectively), while Prilop, 0045 and 0027 had a significant lower content (13.9, 13.2, and 12.5 mg/100g, respectively).

The findings of this study indicate that there is a significant increase in the content of gallic acid, catechin, and ellagic acid during 5 months of storage. This is consistent with previous studies in almonds, which showed a significant increase in flavonoids and phenolic acids during 15 months of storage in darkness at 4 ºC and 23 ºC, with a 400-fold increase for hydroxybenzoic acids.[23] In pecan kernels, flaven-3-ols content (catechin) appears to make up a large portion of flavonoid content; gallic acid and ellagic acid are two common forms of hydroxybenzoic acids, which are major phenolic acids in pecan kernel. In contrast, phenolic acids (gallic acid, gentistic, and vanillic acids) in pecan kernel showed significant decrease during 12 weeks of kernels storage at 21 °C, 65% RH.[17] This means different storage conditions, experiment design, and measurement methods make it difficult to compare results between studies. It would be valuable to conduct a quantitative analysis of each phenolic compound to determine which derivatives increase or decrease during storage.

Pecan Kernel Storage Moisture Loss

In addition to the phytochemicals, moisture and lipid content are key factors in determining shelf-life stability in pecan kernels. In foods, the moisture content is related to mold growth and other metabolic activities. Therefore, drying or dehydration processes are commonly used to lower the moisture content in order to increase shelf life.

The results for moisture content of the five pecan varieties with 5 months of storage and two-way ANOVA are shown in Table S5. A trend of moisture loss during storage was observed (Figure 9). The moisture content at month 0 ranged 2.7 – 3.6%. The moisture content decreased to 1.8 – 2.2% after 5-months of storage. Specifically, varieties 0041, 0045, 0027, and 'Prilop' showed significant moisture loss during storage, whereas the Pawnee variety did not.

As expected, the storage life of pecan kernels is closely linked to moisture content reduction. In this study, pecan moisture content was low, in line with previous studies that found unroasted pecan moisture content to be around 3%.[25] Multiple exogenous factors influence the moisture content, such as temperature, humidity, container materials, and storage period. Some detailed information regarding how these factors influence pecan shelf life, especially how type of packaging materials and modified atmosphere impact pecan kernel shelf life has been summarized in a review article.[2] In addition to variety, the storage condition for pecan kernels in this study (temperature: 25 °C; humidity: ~ 50%; bags: polypropylene; storage time: 5 months), was hard to compared to literature, since there is no similar study. It should be pointed out that the storage conditions in this study was very "mild", and it has been reported that the polypropylene storage bags have a better storage stability than pecans stored in direct air contact.[2] While moisture is not the main composition in pecan kernel, pecans are predominately composed of lipids.

Pecan Kernel Storage Total Lipid Changes

Pecan kernel lipid content has been extensively studied.[1,2] Nuts are known to be susceptible to deterioration due to their high amount of lipids and unsaturated fatty acids, which can lead to lipid oxidation and reduce shelf life. The results for total lipid changes in pecan kernel during storage and two-way ANOVA are shown in Table S6. The total lipid percent ranged from 69.5 – 75.6% at month 0 (fresh kernel), as shown in Figure 10. No trend for total lipid changes was identified during 5 months of storage. In contrast, the total lipid percent had a significant difference across varieties. Total lipid percent in varieties Pawnee (69.8%) and Prilop (68.6%) was lower than varieties 0045 (76.3%), 0041 (72.4%), and 0027 (71.6%).

The total lipid content in pecan kernels could up to 78%.[1] The current study found that the fresh samples of five pecan varieties had a total lipid content of 69.5 – 75.6% and no trend in total lipid changes was observed during the 5 months of storage, which was coincident with the results reported for peanuts, with no statistical changes observed during 320 days of storage at 25 ^{o}C.[26]

Pecan Kernel Storage Texture Changes

Texture is a critical quality parameter in the pecan market and crispy and crunchy are preferred. Limited studies have focused on the pecan's texture analysis,[25,27] and few studies have focused on texture quality changes during storage.[28] In this study, five parameters (hardness, toughness, slope, fracturability, and break) were used to characterize the texture of pecan kernels. The results of texture characteristic changes in the pecan kernel during 5 months of storage and two-way ANOVA are indicated in Table S7. No significant differences have been found during storage, although variety variation was presented.

For hardness force (N), there was a significant difference in comparison among varieties. Variety Prilop had the highest hardness force (41.0 N), compared to varieties 0027 (36.4 N), 0041 (34.7 N), 0045 (34.5 N), and Pawnee (34.4 N). Variety 0041 had the highest toughness (66.1 N.mm), followed by variety 0045 (60.1 N.mm). Other three varieties had much smaller values (40.7 – 48.1 N.mm). The slope (the linear segment of the force curve) of variety Prilop (19.1 N/mm) was significantly higher than that of 0041 (14.9 N/mm), Pawnee (14.3 N/mm), and 0045 (13.9 N/mm); however, it had no significant difference with variety 0027 (17.2 N/mm). Varieties Prilop (32.3 N/mm) and 0027 (32.8 N/mm) had significant higher fracturability than that of Pawnee (28.5 N/mm), 0041 (28.4 N/mm), and 0045 (27.1 N/mm). Finally, Varieties 0041 (3.1 mm) and 0045 (3.0 mm) had significant higher break than that of Pawnee (2.4 mm), 0027 (2.3 mm), and Prilop (2.1 mm).

The results from this study that texture changes in pecan kernels during storage were irregular and varied in terms of toughness, slope, fracturability, and break could be associated with multiple reasons. It should be pointed out that measuring pecan kernel texture is challenging due to the non-uniform, irregular, and semi-infinite geometry of the kernels, which can result in unpredictable measurement errors. In addition, kernel sample variance such as diameter and thickness from the same variety has been observed, although it has not been well documented in literature. Although three halves per variety per month have been included for texture analyze in this study, increasing sample size might reduce the variance for texture parameters.

Correlation Analysis for All Variables

Color and texture reflect physicochemical properties of pecan kernels.[2] To investigate the relationship between variables and pecan kernel samples, PCA was conducted. Two outputs

from PCA were selected for result presentation: Pearson's correlation matrix (Table 1) and PCA biplot.

In Table 1, pecan kernel color (L* value, especially for dorsal) was significantly and positively correlated to the total carotenoid content, meaning a darker color (lower L* value) and less yellow (lower total carotenoid content). Pecan kernel color (L* value, especially in the ventral side) was significantly and negatively correlated to the content of gallic acid and ellagic acid, meaning a darker color (lower L* value) related to a higher amount of these two phenolic compounds. In addition, pecan kernel color (L* value for both dorsal and ventral sides) was significantly and positively correlated to moisture content, meaning the higher the moisture content the lighter the color. The results that a positive correlation between the L* value (brightness) of pecan kernel color and carotenoids and a negative correction between the a* value (green/red) and carotenoids indicate that carotenoids play a significant role in the color changes of pecan kernels during storage, causing the color to darken with less yellow and more red. The findings are consistent with previous reports in the literature.[2]

Interestingly, with regards to texture variables, toughness was significantly and positively correlated to the content of total polyphenol and lipids, but negatively correlated to catechin content. However, this study found that hardness did not correlate to any chemicals, likely due to its relation to physical properties. On the other hand, toughness was positively correlated to the total polyphenol and total lipid contents. Lipid content in pecan kernels has been linked to positive mouth sensation,[29] while polyphenols are commonly associated with bitterness and astringent mouth feel. In addition, moisture level is known to impact flavor and texture with a moisture content of 5% being ideal for optimal flavor and texture,[13] and a fragile texture

occurring when the moisture content is less than 4%. However, this study found no significant correlation between moisture level and texture.

Variety Differentiation with PCA

The PCA biplot is shown in Figure 11. The first two PCs (PC1 and PC2) accounted for 55.28% of the total variance, while PC1 vs PC3 accounted for 46.71% (12.67% from PC3), in which two biplots could explain the major components used to differentiate the samples. Roughly, along the PC1 axis, samples were separated by month, and the monthly difference was mainly governed by color and moisture content, since the two variables defined monthly changes during storage. Meanwhile, at the PC2 axis, samples were separated by variety. Variety difference was mainly governed by the chemical compositions (carotenoids, total polyphenol, individual polyphenols, and total lipids).

In details, all five fresh pecan varieties (month 0) and three varieties (Prilop, 0041, 0045) at month 1 were separated from other samples at the positive side of PC1, due to their higher values of L*, b*, and moisture. In contrast, the value of these three variables were low in four varieties (Pawnee, Prilop, 0041, 0027) at month 5 and two varieties (0041 and 0027) at month 4, which were separated at the negative side of PC1. Four other samples (variety 0045 at months 3, 4, and 5, and 0041 at month 2) were separated at the positive side of PC2 for their high value of a*, total polyphenol, lipids, and texture (toughness and break texture). In contrast, these values were low in the other three samples (Pawnee at months 1 and 2, and Prilop at month 2) and they were separated at the negative side of PC2. The remaining four samples (0041 at month 2, 0045 at months 3, 4, and 5) was separated at the positive side of PC3.

Overall, the PCA results indicated that storage was the main factor (across the tested varieties) that differentiated pecan kernel samples due to the darkening of color and loss of moisture. However, variety was the second factor that differentiated pecan kernel samples based on their chemical compositions. The native selection Prilop had the lightest color (highest L* value for brightness) but also had relatively low carotenoid and lipid contents, which may result in lower nutritional value.

It's important to note that multiple variables contribute to the quality of pecan kernels during storage, and that measuring all of them is beyond the scope of this study. Future studies could benefit from measuring variables such as fatty acid profile, volatiles related to rancid flavor, and sensory quality over the shelf life of pecan kernels.

In addition, this study also faced several challenges. The pecan kernel samples varied in size and surface area, and the chemical composition of the kernel differed, making it difficult to maintain consistency in the skin to nutmeat ratio during sample preparation. Kernel samples were ground manually using a mortar and pestle, leading to difficulties in controlling the uniformity of particle size and potentially introducing errors during extraction.

SUPPORTING DATA

Supporting Tables

Supporting Figures

FUNDING INFORMATION

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CONFLICTS OF INTEREST

The authors declare that they do not have any conflict of interest.

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Table 1. Pearson relation (*r*) between the measurements of color and texture and the quantification of chemical compositions.

Variables	Color L* (Dorsal)	Color L* (Ventral)	Color a* (Dorsal)	Color a* (Ventral)	Color b* (Dorsal)	Color b* (Ventral)	Hardness (N)	Toughness (N.mm)	Slope (N/mm)	Fracturability (N)	Break (mm)
Total carotenoids	0.293	**0.462**	**-0.415**	-0.236	0.342	0.356	0.172	0.204	0.132	0.191	0.025
Total polyphenols	-0.006	0.261	0.265	0.187	0.162	0.046	0.046	**0.542**	0.043	-0.104	**0.484**
Gallic acid	**-0.494**	**-0.413**	0.093	**0.589**	-0.342	-0.147	0.299	-0.091	**0.466**	0.095	-0.257
Catechin	-0.186	**-0.452**	-0.152	0.032	-0.335	-0.136	0.007	**-0.738**	0.324	0.069	**-0.708**
Ellagic acid	**-0.560**	-0.339	0.248	-0.031	**-0.549**	**-0.550**	-0.252	0.024	0.007	0.154	0.069
Moisture	**0.780**	**0.785**	**-0.702**	**-0.575**	**0.792**	**0.655**	0.111	0.337	-0.354	0.088	0.347
Total lipid	0.049	0.210	0.356	-0.084	0.101	-0.121	-0.161	**0.538**	**-0.450**	-0.278	**0.603**

Numbers in bold indicate significant correlation ($p \leq 0.05$).

FIGURE CAPTIONS

Figure 1. Interaction plot illustrates changes in pecan kernel lightness (L* value) for dorsal side (A) and ventral side (B) measured using a CIELAB color space in a spectrophotometer. The L* value represents lightness with a scale of 0 (black) to 100 (white). The error bars represent the standard deviations of the mean values with 10 measurements in triplicates. Mean score and standard deviation for each point could be found in Table S1.

Figure 2. Interaction plot illustrates changes in pecan kernel green/red channel (a* value) for dorsal side (A) and ventral side (B) measured using a CIELAB color space in a spectrophotometer. The a* values approaching higher value indicates redder color. The error bars represent the standard deviations of the mean values with 10 measurements in triplicates. Mean score and standard deviation for each point could be found in Table S1.

Figure 3. Interaction plot illustrates changes in pecan kernel blue/yellow channel (b* value) for dorsal side (A) and ventral side (B) using a CIELAB color space in a spectrophotometer. The b* values approaching higher value indicates yellower color. The error bars represent the standard deviations of the mean values with 10 measurements in triplicates. Mean score and standard deviation for each point could be found in Table S1.

Figure 4. Interaction plot illustrates change in pecan kernel total carotenoids (μg/100g whole kernel). The error bars represent the standard deviations of the mean values with triplicate analyses. Mean score and standard deviation for each point could be found in Table S2.

Figure 5. Interaction plot illustrates changes in pecan kernel total polyphenol content (mg GAE/g whole kernel). The error bars represent the standard deviations of the mean values with triplicate analyses. Mean score and standard deviation for each point could be found in Table S3.GAE = Gallic acid equivalent.

Figure 6. Interaction plot illustrates changes in pecan kernel gallic acid (mg/100g whole kernel). The error bars represent the standard deviations of the mean values with triplicate analyses. Mean score and standard deviation for each point could be found in Table S4.

Figure 7. Interaction plot illustrates changes in pecan kernel cetechin (mg/100g whole kernel). The error bars represent the standard deviations of the mean values with triplicate analyses. Mean score and standard deviation for each point could be found in Table S4.

Figure 8. Interaction plot illustrates changes in pecan kernel ellagic acid (mg/100g whole kernel). The error bars represent the standard deviations of the mean values with triplicate analyses. Mean score and standard deviation for each point could be found in Table S4.

Figure 9. Interaction plot illustrates changes in pecan kernel moisture content (%). The error bars represent the standard deviations of the mean values with triplicate analyses. Mean score and standard deviation for each point could be found in Table S5.

Figure 10. Interaction plot illustrates changes in pecan kernel lipid content (%). The error bars represent the standard deviations of the mean values with triplicate analyses. Mean score and standard deviation for each point could be found in Table S6.

Figure 11. Principal component analysis (PCA) biplot with mean scores for18 measured variables as loading values and five pecan kernel samples with 5-month storage (0 – 5 months) as score values. M = month. Letters L*, a*, and b* represent different colors using a CIELAB color space.