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Changes in bioactive compounds content and antioxidant capacity of pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)] during storage

Variaciones en el contenido de compuestos bioactivos y capacidad antioxidante de la nuez pecanera [*Carya illinoinensis* (Wangenh. K. Koch)] durante su almacenamiento

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Abstract
Pecan nut kernels are rich in health-promoting substances such as unsaturated fatty acids, tocopherols, and phenolic compounds. Due to their importance in the human diet, it is essential to evaluate the effects of drying, and storage temperature and time, on these phytochemicals. Moisture, water activity, lipid content, bioactive compounds concentration, and antioxidant capacity of wet and dry pecan nuts (Western variety) were determined. Kernels and in-shell nuts were stored (240 days) at 4 ºC and 25 ºC, monitoring the changes in tocopherols concentration, phenolic content, and antioxidant capacity. Wet and dry kernels exhibited similar fatty acids profile and γ-tocopherol concentration (20.37±0.73 and 19.27±1.62 mg·100 g^{-1} kernels in dry basis), whereas phenolic content and antioxidant capacity decreased due to the drying process (33.2 and 22.3%, respectively). Throughout storage, condensed tannins concentration of kernels and in-shell nuts reduced by 31.5 and 41.8%, while DPPH antioxidant capacity improved 216.4 and 188.4%, respectively. These results evidenced that drying and storage time are the most significant variables regarding pecan nuts postharvest processing; nonetheless, further research related to the relationship between condensed tannins degradation and the increments in DPPH antioxidant capacity is needed.
Keywords: Pecan nuts, storage, phenolic compounds, antioxidant capacity.

Resumen
La almendra de nuez pecanera es rica en compuestos con alto potencial funcional como ácidos grasos insaturados, tocoferoles y compuestos fenólicos. Debido a su importancia en la dieta, resulta esencial evaluar los efectos del tratamiento de secado, así como tiempo y temperaturas de almacenamiento sobre estos fitoquímicos. En este sentido, se determinaron humedad, actividad de agua, contenido de lípidos y bioactivos, así como capacidad antioxidante (CA) de nuez pecanera (variedad Western) húmeda y seca. Posteriormente, se almacenaron (240 días) almendras y nuez en cáscara a 4 y 25 ºC, monitoreando los cambios en la concentración de γ-tocoferol (γ-T), contenido de fenólicos (CF) y CA. Las almendras húmedas y secas presentaron similitudes en el perfil de ácidos grasos y γ-T (20.37±0.73 y 19.27±1.62 mg·100 g^{-1} almendra en base seca), mientras que, CF y CA disminuyeron 33.2 y 22.3% debido al proceso de secado. Durante el almacenamiento, la concentración de taninos condensados (TC) se redujo 31.5 y 41.8% en almendras y nuez en cáscara, en cambio, CA aumentó 216.4 y 188.4%, respectivamente. Estos resultados demuestran que el secado y el tiempo de almacenamiento son variables importantes en el procesamiento postcosecha de la nuez pecanera; siendo necesario investigar a profundidad la relación entre TC y CA.
Palabras clave: Nuez pecanera, almacenamiento, compuestos fenólicos, capacidad antioxidante.

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1 Introduction

Pecan nuts [*Carya illinoinensis* (Wangenh. K. Koch)] are indigenous from Mexico and the United States, together they provide 92% of the worldwide pecan nut production (Bello-Huitle *et al*., 2010; Corral-Escárcega *et al*., 2017; International Nut & Dried Fruit Council, 2019). The pecan nut kernel, or edible part, has been recognized as a source of bioactive compounds; its lipid fraction contains mono- and polyunsaturated fatty acids, tocopherols, and phytosterols that help diminish plasma cholesterol concentration and act as antioxidants preventing coronary heart disease (Atanasov *et al*., 2018; Fernandes and Cabral, 2007; Gong *et al*., 2017; U.S. Department of Agriculture, 2017), whereas its non-lipid fraction comprises a significant amount of phenolic compounds with reported biological and pharmacological properties (antibacterial, anticancer, anti-inflammatory, antioxidant, antiviral, immunomodulatory, and cardioprotective) (Flores-Martínez *et al*., 2016; Jia *et al*., 2018; Smeriglio *et al*., 2017).

Several research groups have focused on investigating the phytochemical profile of pecan nut kernels from different varieties (Flores-Córdova *et al*., 2017; Robbins *et al*., 2014) or growing location (de la Rosa *et al*., 2014; Domínguez-Avila *et al*., 2013), while their bioactive compounds changes during maturation are becoming an area of great interest (Bouali *et al*., 2014; Carrasco-Del Amor *et al*., 2017; Jia *et al*., 2018). However, a gap is found regarding the preservation of pecan nuts with outdated investigations directed to evaluate how processing impacts on kernels sensorial quality rather than on their phytochemical composition. For instance, Herrera (1994) investigated sensorial changes in dry pecan nut kernels collected at different harvest dates; Erickson *et al*. (1994) associated physical and chemical analyses to sensory changes of raw and roasted kernels stored at different conditions, and Kanamangala *et al*. (1999) analyzed shelf-life of reduced-lipid kernels stored at ambient temperature. There are other studies directed to extend pecan nuts shelf-life by applying edible coatings (Baldwin and Wood, 2006), evaluating different packaging materials (Oro *et al*., 2008), or using electron-beam irradiation (Villarreal-Lozoya *et al*., 2009). Therefore, the objective of this research was to investigate the impact of common practices related to pecan nuts processing (drying, storage temperatures, along with kernels and in-shell storage) on their bioactive compounds content (fatty acids, tocopherols, and phenolic compounds) and antioxidant capacity throughout storage.

2 Materials and methods

2.1 Pecan nut samples

Pecan nuts (*Carya illinoinensis*) from the autumn 2017 harvest (Western variety) were donated by a producer from Cajeme, Sonora, Mexico (27°29'38"N, 109°56'20"W). In-shell wet pecans were collected directly from the orchard after shucks split, whereas in-shell dry pecans were obtained after a drying process in the producers' facility.

2.2 Chemicals

Methanol (MeOH), tetrahydrofuran (THF), and water (H_2O) HPLC grade were purchased from Sigma-Aldrich (USA) along with fatty acid methyl esters, δ-, γ-, and α-tocopherols, gallic acid, catechin, Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), fluorescein sodium salt, 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), and sodium phosphate dibasic (Na_2HPO_4). Ethanol (EtOH), sodium nitrite ($NaNO_2$), and sodium hydroxide (NaOH) were purchased from DEQ (Mexico). Hexane, sulfuric acid (H_2SO_4), and aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_2O$) were acquired from Fermont (Mexico). Acetone and sodium carbonate anhydrous (Na_2CO_3) were obtained from Avantor Performance Materials (Mexico) and CTR Scientific (Mexico), respectively. Working solutions were protected from light and stored at -20 ºC.

2.3 Storage experiment

Wet and dry in-shell pecans were visually inspected removing damaged or germinated nuts and classified into different experimental groups: wet and dry kernels stored at 4 ºC, and in-shell dry pecan nuts stored at 4 and 25 ºC. A portion of 30 g of kernels and their equivalent of in-shell nuts (100 g) were placed in 12×15 cm polyethylene bags (Filmpack S.A. de C.V., Mexico) and vacuum-sealed (EVD 4, TORREY, Mexico) until sampling day. Moisture, water activity (a_w), lipid content, fatty acids profile, tocopherols,

total phenolic content (TPC), total flavonoids (TF), condensed tannins (CT), and antioxidant capacity were determined for wet and dry samples. TPC, TF, CT, and antioxidant capacity of wet and dry kernels were evaluated at days 0, 15, 30, 45, 60, 90, 120, 150, 210, and 240 while in-shell nuts were analyzed at day 0, 15, 60, 150, and 240. Tocopherols content of kernels and in-shell pecan nuts was determined at days 0 and 240.

2.4 Moisture content

Moisture (2.5 g) was determined according to the 920.151 AOAC Official Method (AOAC, 1996) and expressed as g per 100 g of pecan nut kernels (dry basis, db).

2.5 Water activity (a_w)

Pecan nut kernels were ground using a laboratory mortar to determine a_w with a LabMaster-a_w instrument (Novasina, Switzerland) at 25 ºC.

2.6 Lipid content

Oil extraction was performed as reported by Villarreal-Lozoya *et al*. (2007) with modifications; pecan nut kernels were ground in a laboratory mortar, then samples (3.0 g) were defatted with hexane (1:10 w/v) using a homogenizer adapted with a S 25 N-25G-ST dispersing tool (IKA® T25 Ultra-turrax, IKA, Germany) for 1.5 min at 6,000 rpm. Next, samples were centrifuged (3.273 g, 15 min, 20 ºC) (Allegra X-12 Centrifuge, Beckman Coulter Inc., USA) and supernatants collected, this procedure was repeated three times. Pooled supernatants were concentrated using a medium-scale vacuum evaporator (2.5 h, 45 ºC) (Genevac RKCM-12060-SNN, SP Scientific, England). The extracted oil was used to determine lipid content gravimetrically in an air oven at 60 ºC for 24 h following the AOAC 960.39 procedure (AOAC, 1996). Lipid content was expressed as g per 100 g of pecan nut kernels db. Lipid fractions were stored (-20 ºC) in 8 mL amber vial with N_2 in the head-space and sealed with parafilm until analyses.

2.7 Fatty acids profile

Lipid fraction of pecan nut kernels were analyzed using the methodology reported by Christie (1989). Samples (0.1 g) were dissolved in toluene (1:10 v/v), then 2 mL of 2% MeOH:H2SO4 were added. Next, the mixture was heated in a water bath at 80 ºC for 30 min, the fatty acids methyl esters obtained were recovered with hexane (5 mL), vortexed, and centrifuged (3.273 g, 15 min, 20 ºC). Aliquots of hexane (1 μL) were used for the identification and quantification of fatty acid methyl esters of pecan nut lipid fraction. Samples were analyzed using a gas chromatograph system with flame ionization detector (GC-FID) (Agilent 7890A, Agilent Technologies, USA) equipped with a 0.25 μm, 0.25 mm × 30 m DB-23 capillary column (Agilent Technologies, USA). The temperature of injection and detector were 250 and 230 ºC, respectively. The mobile phase was N_2 at a flow rate of 1 mL/min. Fatty acids profile was identified by the retention time of myristic, palmitic, heptadecanoic, stearic, arachidic, palmitoleic, cis-9-heptadecenoic, oleic, behenic, eicosenoic, linoleic, and α-linolenic standards (CAS 124-10-7, 112-39-0, 1731-92-6, 112-61-8, 1120-28-1, 1120-25-8, 1981-50-6, 112-62-9, 929-77-1, 2390-09-2, 112-63-0, 301-00-8, respectively). Results were expressed as g of fatty acid methyl ester per 100 g of pecan nut kernels db.

2.8 Tocopherols content

Tocopherols were quantified using a modified procedure reported by Andrés *et al*. (2014). Lipid fraction samples (25 mg) were reconstituted in a mobile phase consisted of MeOH:Tetrahydrofuran: H_2O (67:27:6) (1 mL). Aliquots (40 μL) were injected into a Waters HPLC system with a photodiode array detector (HPLC-PDA) equipped with an In-Line Degasser AF, a 600 Controller, a 717 Plus Autosampler, and a 2996 Photodiode Array Detector (Waters Corporation, USA). Tocopherols separation was achieved using a 5 μm, 4.6 mm × 250 mm Waters Spherisorb ODS2 column (Waters Corporation, USA) set at 25 ºC and an isocratic elution at a flow rate of 1 mL/min. Standard curves were prepared (0.3 - 3.5 mM) for the quantification of δ-, γ-, and α-tocopherol (CAS 119-13-1, 54-28-4, and 59-02-9, respectively), and the detector was set at 290 nm. Results were expressed as mg of tocopherol per 100 g of pecan nut kernels db.

2.9 Phenolic compounds and condensed tannins extraction

A non-lipid fraction was obtained from the extraction of pecan nut lipid fraction by removing the remaining hexane using a tube-scale vacuum evaporator (2.5 h, 45 ºC) (Genevac EZ-2.3, SP Scientific, England) and

sieving the pellet. Samples were stored at -20 ºC in 6×15 cm polyethylene bags and vacuum sealed until aqueous and methanolic extraction. Aqueous extracts were used to determine TPC, TF, and antioxidant capacity whereas CT was evaluated in methanolic extracts.

2.9.1 Aqueous extraction of phenolic compounds

Phenolic compounds from non-lipid fraction were extracted as reported by Wu *et al*. (2004) and Vazquez-Flores *et al*. (2017). The extraction method was selected after comparison against other procedures (data not showed). Samples of non-lipid fractions (0.3 g) were placed in 15 mL centrifuge tubes along with an extraction solution (Acetone:H_2O:CH_3COOH, 70:29.5:0.5) in a 1:10 w/v proportion and sonicated in an ultrasonic bath (Ultrasonic Cleaner 97043-970, VWR, China) for 30 min with agitation every 10 min. Next, samples were centrifuged (3.273 g, 15 min, 20 ºC) and supernatants collected. The extraction process was performed twice. After evaporation of the pooled supernatants, using the tube-scale vacuum evaporator (2.5 h, 45 ºC), aqueous extracts were diluted to 5 mL with distilled water in a volumetric flask. Aqueous extracts were stored in 15 mL centrifuge tubes protected from light at -20 ºC until analyses.

2.9.2 Methanolic extraction of condensed tannins

Methanolic extraction was performed according to Villarreal-Lozoya *et al*. (2007). Non-lipid fraction (0.1 g) and 1% MeOH:HCl (1:30 w/v) were placed in a water bath (20 min, 30 ºC) and vortexed at 0, 10, and 20 min of reaction. Afterwards, supernatants were collected by centrifugation (3.273 g, 15 min, 20 ºC). This procedure was performed once. Methanolic extracts were diluted using 5 mL volumetric flasks with 1% MeOH:HCl and stored in 15 mL centrifuge tubes protected from light at -20 ºC until analyses.

2.9.3 Total phenolic content

TPC analysis was based on the Folin-Ciocalteu method reported by Singleton and Rossi (1965) and adapted by Villarreal-Lozoya *et al*. (2007). The Folin-Ciocalteu solution (6.25:100 v/v) was prepared using nanopure water. Samples from aqueous extraction (13 μL) were loaded in a 96-well flat bottom plate (Costar® Assay Plate #9017, Corning, USA) along with 221 μL of Folin-Ciocalteu solution and led to react for 3 min in the dark. Next, 26 μL of 0.5 M Na_2CO_3 were added and allowed to react for 2.5 h in darkness. After this time, absorbance was measured at 765 nm using a microplate reader (Synergy HT Plate Reader, Bio-Tek Instruments Inc., VT). TPC was determined by comparing absorbance of the samples against a standard curve of gallic acid (0.1-1.0 mg·mL^{-1}). Results were expressed as μmol gallic acid equivalents per g of pecan nut kernels (μmol GAE·g^{-1} db).

2.9.4 Total flavonoids

TF were determined as reported by de la Rosa *et al*. (2011) adapted for microplate reader measurements. Aliquots from aqueous extracts (100 μL) were loaded in a 48-well flat bottom plate (Nunc^{TM} Multidish 48-well plate #150787, Thermo Scientific, Denmark) followed by 420 μL of distilled water and 30 μL of 0.5 M NaNO2. After 5 min, 0.75 M $AlCl_3$ (30 μL) was added and led to react for 3 min. Later, 0.5 M NaOH (30 μL) was pipetted and the plate was incubated (30 min, 25 ºC) in darkness. Absorbance measurements were made at 510 nm and a standard curve of catechin (0.1-0.5 mg·mL^{-1}) was used to report TF as μmol catechin equivalents per g of pecan nut kernels (μmol CE·g^{-1} db).

2.9.5 Condensed tannins

CT were quantified using the HCl-vanillin method reported by Price *et al*. (1978) and modified by Herald *et al*. (2014). A stock solution of vanillin was prepared by dissolving 1.0 g of vanillin in 100 mL of 4% MeOH:HCl. Before analysis, vanillin solution was diluted (1:1 v/v) with 8% MeOH:HCl and placed in a water bath at 30 ºC. Methanolic extracts (30 μL) were pipetted in a 96-well flat bottom plate followed by vanillin dilution (150 μL) and incubated at 30 ºC for 20 min. A 1% MeOH:HCl solution was used as blank and absorbance measurements were made at 500 nm. A standard curve of catechin (1.0-3.5 mg·mL^{-1}) was made to measure CT content of methanolic extracts. Results were expressed as μmol CE·g^{-1} db.

2.10 Antioxidant capacity

To spectrophotometric screening of antioxidant capacity of pecan nuts, two *in vitro* methods based on different mechanisms were selected: DPPH that determines antioxidants ability to transfer one electron to reduce free radicals, and ORAC that measures quenching ability of antioxidants by hydrogen

donation (Granato *et al*., 2018; Shivakumar and Kumar, 2018).

2.10.1 DPPH radical scavenging capacity (DPPH)

DPPH was performed according to Villarreal-Lozoya *et al*. (2007). To optimize reaction time, preliminary experiments were performed employing the diluted solution of DPPH (data not showed). A DPPH stock solution was prepared by dissolving 0.01 g of DPPH in 25 mL of MeOH. Prior to each analysis, the DPPH stock solution was diluted (1.5:10 v/v) in MeOH. Aqueous extracts were pipetted (26 μL) into 96 well flat bottom plate. To avoid light exposure, 234 μL of diluted DPPH was added using the dispenser module of the microplate reader. Absorbance measurements were made at 515 nm and registered every minute until 15 min of reaction. Trolox was used for the standard curve (0.02-0.10 mg·mL^{-1}) to express the results as μmol trolox equivalents per g of pecan nut kernels (μmol TE·g^{-1} db).

2.10.2 Oxygen radical absorbance capacity (ORAC)

As described by Held (2005), fluorescein solutions, AAPH solution, and samples dilutions were prepared in 0.075 M phosphate buffer (pH 7.4). Prior to analysis, the fluorescein stock solution (0.003 M) was diluted (1:5000 v/v) and an AAPH solution was prepared (0.15 M). Samples of aqueous extracts (25 μL) were placed in a 96-well black round bottom plate (Costar® Assay Plate #3792, Corning, USA) in addition to fluorescein dilution (150 μL) and incubated (30 min, 37 ºC) in darkness. After incubation, AAPH solution was added (25 μL) and fluorescence measured at 485 nm for excitation and 520 nm for emission employing a microplate reader. Fluorescence decrement was read every minute during 90 min. Trolox was used to prepare the standard curve (0.025-0.0025 mg·mL^{-1}) and results were expressed as μmol TE·g^{-1} db.

2.11 Statistical analysis

All measurements along with pecan nut oil extraction were done by triplicate. To determine the effect of storage conditions on bioactive compounds content of pecan nuts, an One-way ANOVA followed by Tukey statistical test for multiple means comparison along with Pearson correlation analysis were performed (α=0.05), using Minitab 18 software (Minitab® 18.1, USA).

3 Results and discussion

3.1 Effect of drying processing on pecan nuts composition

Moisture, a_w, lipid content, fatty acids profile, tocopherols and phenolic compounds content, along with antioxidant capacity of wet and dry kernels are shown in Table 1. The drying process, intended to preserve quality attributes of pecan nuts and avoid lipid oxidation (Kader, 2013; Olguín Rojas *et al*., 2019), decreased the moisture content of wet kernels from 7.10±0.89 to 2.28±0.08 g·100 g^{-1} db with a consequent reduction of a_w (from 0.89 to 0.49) and an increment of lipid content (from 67.36±0.07 to 69.60±0.23 g·100 g^{-1} db). The moisture, a_w, and lipid content of dry kernels were in the range of values reported by Fernandes *et al*. (2017), Flores-Córdova *et al*. (2016), and the U.S. Department of Agriculture (2017).

The major fatty acids identified in pecan nut kernels were oleic and linoleic acids representing more than 85.0% of their total lipid content: wet samples contained a higher concentration of monounsaturated fatty acids, specifically oleic acid (17.9%), while no differences between wet and dry kernels were observed in total saturated and polyunsaturated fatty acids. However, myristic acid was higher in wet samples (11.8%) and α-linolenic acid on dry samples (16.9%). The effect of drying has been studied in other nuts such as macadamia nuts (Phatanayindee *et al*., 2012) and walnuts (Fu *et al*., 2016) which also presented a reduction on oleic acid, whereas α-linolenic acid was not affected. The maintenance of α-linolenic acid concentration was attributed to a decrement in lipoxygenase (LOX) activity (Fu *et al*., 2016).

Regarding tocopherols group, only γ-tocopherol was identified showing a similar content in wet and dry kernels. These values (20.37±0.73 and 19.27±1.62 mg·100 g^{-1} kernels db) were comparable to concentrations reported by Yao *et al*. (1992) for Western Schley pecan nuts and Fernandes *et al*. (2017) for cold-pressed pecan oil (28.43±1.46 and 27.27±1.78 mg·100 g^{-1} oil for wet and dry samples, respectively).

The drying process produced a decrement on phenolic compounds content (TPC 33.2%, TF 54.0%, and CT 63.4%) and antioxidant capacity (DPPH 22.3% and ORAC 45.9%) of pecan nuts.

Table 1. Effect of drying processing on moisture, aw, lipid content, fatty acids profile, tocopherols content, phenolic compounds concentration, and antioxidant capacity of Western pecan nut kernels.

	WET		DRY	
Moisture g·100 g^{-1}	7.10 ± 0.89	a	2.28 ± 0.08	b
***a*$_w$**	0.84 ± 0.01	a	0.49 ± 0.01	b
Lipid content g·100 g^{-1}	67.36 ± 0.07	b	69.60 ± 0.23	a
Fatty acids profile g·100 g^{-1}				
Myristic	0.08 ± 0.00	a	0.07 ± 0.00	b
Palmitic	5.67 ± 0.21	a	5.40 ± 0.36	a
Heptadecanoic	0.05 ± 0.00	a	0.05 ± 0.00	a
Stearic	1.83 ± 0.07	a	1.71 ± 0.12	a
Arachidic	0.08 ± 0.00	a	0.08 ± 0.01	a
Total SFA	**7.71 ± 0.29**	a	**7.31 ± 0.49**	a
Palmitoleic	0.05 ± 0.00	a	0.05 ± 0.00	a
Cis-9-Heptadecenoic	0.00 ± 0.00	b	0.04 ± 0.00	a
Oleic	35.63 ± 1.36	a	30.18 ± 2.03	b
Behenic	0.68 ± 0.04	a	0.70 ± 0.05	a
Eicosenoic	0.17 ± 0.00	a	0.18 ± 0.01	a
Total MUFA	**36.53 ± 1.41**	a	**31.14 ± 2.10**	b
Linoleic	24.99 ± 0.96	a	27.75 ± 1.86	a
α-Linolenic	0.99 ± 0.04	b	1.17 ± 0.08	a
Total PUFA	**26.13 ± 1.00**	a	**28.92 ± 1.94**	a
Tocopherols content mg·100 g^{-1}				
δ-Tocopherol	*ND*		*ND*	
γ-Tocopherol	20.37 ± 0.73	a	19.27 ± 1.62	a
α-Tocopherol	*ND*		*ND*	
Phenolic compounds concentration				
Total phenolic content μmol gallic acid EQ·g^{-1}	109.52 ± 4.13	a	82.20 ± 3.85	b
Total flavonoids μmol catechin EQ·g^{-1}	31.91 ± 1.34	a	20.72 ± 1.54	b
Condensed tannins μmol catechin EQ·g^{-1}	298.09 ± 20.11	a	182.44 ± 5.58	b
Antioxidant capacity				
DPPH μmol trolox EQ·g^{-1}	62.07 ± 2.71	a	50.75 ± 2.69	b
ORAC μmol trolox EQ·g^{-1}	182.14 ± 2.00	a	124.85 ± 5.42	b

Concentrations were expressed in g of pecan nut kernels in dry basis. *a*$_w$, water activity; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected; EQ, equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl method; ORAC, oxygen radical absorbance capacity method. Means with different letters within rows are significantly different according to ANOVA Tukey statistical test (α=0.05).

Christopoulos and Tsantili (2012) along with Wang *et al*. (2018) also reported losses in phenolic compounds of wet walnuts and hazelnuts after drying. Wang *et al*. (2018) proposed that moderate temperatures might promote phenolic compounds oxidation by enhancing enzymatic activity, while phenolic compounds could be released at high temperatures. Despite this, TPC concentrations were comparable to the values reported by de la Rosa *et al*. (2014) and Flores-Córdova *et al*. (2017); besides TF and CT concentrations were 2.1 and 3.1 folds higher than those reported for pecans from Chihuahua, Mexico (de la Rosa *et al*., 2014). Antioxidant capacity evaluated by DPPH was 2.9 folds higher in wet and dry samples compared to those reported by Jia *et al*. (2019) for Western pecan nuts, whereas ORAC values were comparable to the total antioxidant capacity reported for commercial pecans (Wu *et al*., 2004).

3.2 Effect of storage conditions on pecan nuts composition

The storage of wet and dry kernels along with in-shell nuts at 4 and 25 ºC impacted differently their retention percentage of γ-tocopherol. Better preservation of γ-tocopherol was observed in dry kernels and in-shell pecan nuts than wet kernels. At day 240, retention percentage of dry kernels was 73.1% while in-shell pecans maintained 58.3 and 69.3% of their initial concentration at 4 and 25 ºC, respectively. In contrast, wet kernels retained only 52.8% of their γ-tocopherol concentration after 210 days of storage at 4 ºC; further analyses were not possible due to wet samples deterioration by mold growth. Yao *et al*. (1992) reported a comparable concentration for dry pecan nut kernels stored at 24 ºC, while Momchilova *et al*. (2017) observed higher retention of tocopherols in dry kernels than in in-shell dry hazelnuts; this was related to the maintenance of moisture in in-shell hazelnuts inducing an increment on lipids oxidation rate. Wet samples presented the highest moisture and a_w values that have been associated to oxidative rancidity of nuts (Shahidi and John, 2013; Troller and Christian, 2014) and might promote degradation of tocopherols.

The effect of storage conditions on the phenolic content and antioxidant capacity of pecan nuts is shown in Figure 1. Wet kernels maintained a higher concentration of phenolic compounds and antioxidant capacity throughout storage with a significant increment of TPC (8.7%), TF (7.1%), CT (22.2%), and ORAC (21.3%) on day 15. Similar to these results, Christopoulos and Tsantili (2012) observed an increase in phenolic compounds of wet walnut kernels after 20 days of storage at 1 ºC; the authors associated this effect with a rise in phenylalanine ammonia-lyase (PAL) activity due to chilling stress. Likewise, Corral-Escárcega *et al*. (2017) proposed the abiotic stress as a technique to increase phenolic compounds content in different food matrices. A further study of Christopoulos and Tsantili (2015) related PAL activity to the synthesis of 4-hydroxybenzoic, 2,4-dihydroxybenzoic, syringic, and vanillic acids while no relation to ellagic acid was found. Identification of 4-hydroxybenzoic, syringic, and ellagic acids have been previously reported in pecan nut kernels by Robbins *et al*. (2015). Hence, the increment in phenolic content of wet pecan nut kernels might be attributed to the synthesis of phenolic acids. Even though wet kernels exhibited higher concentrations of phenolic compounds, they developed mold growth after 210 days of storage, with dry samples being more stable during storage. In-shell pecan nuts stored at 4 ºC preserved a higher content of phenolic compounds compared to other dry samples. This could be related to polyphenols migration from shells to kernels (Santerre, 1994). Also, in-shell preservation at low temperatures has been reported to extend nuts shelf-life by preventing skin darkening and lipids oxidation (Gardea *et al*., 2011; Kader, 2013; Shahidi and John, 2010).

Total phenolic content of wet and dry kernels along with in-shell nuts stored at 4 ºC remained above 85.54±1.46, 68.14±3.97, and 74.26±2.97 μmol GAE·g^{-1} db, respectively. These were the lowest concentrations observed during pecan nuts storage, representing 78.1, 62.2, and 67.8% of their initial concentration. No changes in the TPC of in-shell samples stored at 25 ºC were observed (Fig. 1A). Wet kernels preserved 98.8% of their initial total flavonoids content after 210 days of storage, whereas TF increased by 39.4% in dry kernels and 23.9% on in-shell samples at day 240 (Fig. 1B). Contrarily, a marked decrement in condensed tannins was observed during pecan nuts storage (Fig. 1C). Wet and dry kernels lost 35.2 and 31.5% of CT concentration, respectively, by the end of the storage. CT of in-shell samples were highly influenced by storage temperature decreasing 41.8 and 53.3% at 4 and 25 ºC, respectively, at day 240. This decrement could be caused by condensed tannins polymerization throughout storage. Senter and Forbus (1978) reported non-enzymatic polymerization of condensed tannins in pecan nut kernels from Stuart and Western Schley varieties stored at 32 ºC for 112 days, while do Prado

et al. (2013) associated red coloration of spray-dried extracts of pecan shells with phlobaphenes formation. The determination of CT is based on a reaction between the vanillin reagent and the terminal unit of condensed tannins (Vazquez-Flores *et al*., 2017), thus a possible explanation for the reduction of CT in stored pecan nuts might be that polymerization of condensed tannins decreases the number of terminal units available to react with vanillin reagent.

Regarding the antioxidant capacity, a similar trend was found between kernels and in-shell pecan nuts: DPPH incremented after 90 days of storage (Fig. 1D). Dry and wet kernels reached their highest antioxidant capacity at days 90 and 120 improving 216.4 and 224.7% compared to their initial concentrations. Likewise, DPPH values of in-shell nuts increased by 188.4 and 199.8% in samples stored at 4 and 25 ºC, respectively, towards the end of the storage.

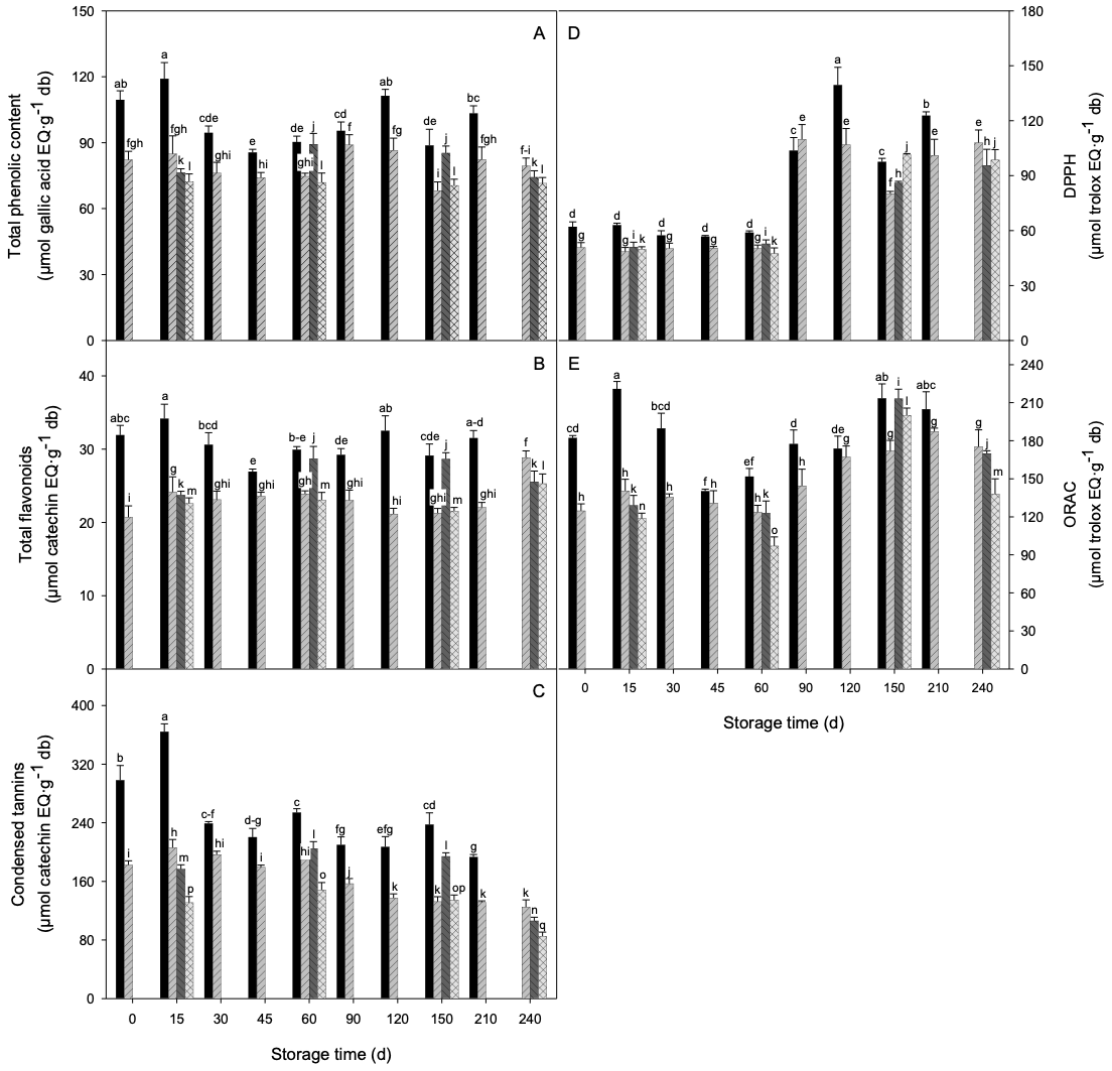

Fig. 1. Changes in total phenolic content (A), total flavonoids (B), condensed tannins (C), and antioxidant capacity by DPPH (D) and ORAC (E) of Western pecan nuts during 240 days (d) of storage at different conditions: wet kernels at 4°C (■), dry kernels at 4°C (▨), in-shell nuts at 4°C (▩), and in-shell nuts at 25°C (▩). Concentrations were expressed as μmol equivalents (EQ) per g of pecan nut kernels in dry basis (db). DPPH, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method; ORAC, oxygen radical absorbance capacity method. Means with different letters within storage conditions are significantly different according to ANOVA Tukey statistical test (α=0.05).

Table 2. Pearson correlation coefficients (r) for the relation of condensed tannins content and antioxidant capacity by DPPH and ORAC of Western kernels and in-shell pecan nuts stored for 240 days (α=0.05).

		Antioxidant capacity			
		DPPH		ORAC	
Condensed tannins	T (°C)	*p-value*	r	*p-value*	r
Kernels					
WET	4	*0.001*	-0.576	*0.105*	0.297
DRY	4	*0.000*	-0.774	*0.000*	-0.739
In-shell					
DRY	4	*0.006*	-0.651	*0.606*	-0.145
DRY	25	*0.008*	-0.633	*0.568*	-0.154

DPPH, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method; ORAC, oxygen radical absorbance capacity method.

ORAC antioxidant capacity differed between pecan nut samples (Fig. 1E) increasing after 150 days of storage in dry kernels (150.1%) and in-shell nuts (170.8 and 160.3% at 4 and 25 ºC, respectively); while in wet kernels the highest value was observed at day 15 (121.3%). Pearson correlation coefficients were calculated to associate the decrement in condensed tannins content and the increment in antioxidant capacity of pecan nuts (Table 2). A negative moderate linear relationship was observed between CT and DPPH of kernels and in-shell pecan nuts, suggesting that antioxidant capacity evaluated by DPPH increases along with the decrement of CT concentrations. As complex polyphenols, condensed tannins are characterized by their amphipathic nature and degree of polymerization, both closely related to their chemical properties (Macías-Cortés *et al*., 2020; Vazquez-Flores *et al*., 2017): Nicoli *et al*. (2002) along with Bors and Michel (2002) reported that the antioxidant capacity of larger polyphenols is related to an increase in their number of reactive hydroxyl groups that allow charge delocalization, while Shivakumar and Kumar (2018) mentioned that condensed tannins possessed higher radical scavenging activity within phenolic compounds. Hence, CT polymerization during storage could be linked to the increment of DPPH antioxidant capacity due to the enhancement of electron-transfer reactions capable to stabilize DPPH radicals. On the contrary, associations between ORAC and CT values were not statistically significant. This method is based on a competitive reaction between antioxidants and a fluorescent probe for the stabilization of AAPH radicals by hydrogen donation (Roy *et al*., 2010), which could explain the differences in the antioxidant capacity measurements observed in this study. These findings are an indicative of the antioxidant properties of pecan nuts; at earlier stages of the storage predominate hydrogen donor reactions while, after 90 days of storage, radical-scavenging reactions increase probably due to polymerization of condensed tannins.

Conclusions

From the results of this study, drying processing and storage time appear to have the highest impact on phytochemical composition and antioxidant capacity of pecan nuts. The drying process decreased kernels initial content of phenolic compounds (33.2%) and antioxidant capacity (22.3%); however, helped to preserve TPC and TF concentration along 240 days despite storage temperature and kernel or in-shell storage. Throughout storage, a decrement in CT content (31.5 and 41.8%) along with a significant increment of DPPH antioxidant capacity (188.4 and 216.4%) were observed in kernels and in-shell nuts. These results indicate the importance of storage conditions on pecan nuts bioactive compounds and their health-related properties. Nevertheless, more research is needed to better understand the polymerization reactions and changes of *in vivo* antioxidant capacity that occurred during pecan nuts storage.

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References

Andrés, V., Villanueva, M. J., Tenorio, M. D. (2014). Simultaneous determination of tocopherols, retinol, ester derivatives and β-carotene in milk- and soy-juice based beverages by HPLC with diode-array detection. *LWT - Food Science and Technology 58*, 557-562. https://doi.org/10.1016/j.lwt.2014.03.025

AOAC (1996). Official Methods of Analysis. *AOAC International.*

Atanasov, A. G., Sabharanjak, S. M., Zengin, G., Mollica, A., Szostak, A., Simirgiotis, M., Huminiecki, Ł., Horbanczuk, O. K., Nabavi, Seyed M. N., Mocan, A. (2018). Pecan nuts: A review of reported bioactivities and health effects. *Trends in Food Science and Technology 71*, 246-257. https://doi.org/10.1016/j.tifs.2017.10.019

Baldwin, E. A., Wood, B. (2006). Use of edible coating to preserve pecans at room temperature. *HortScience 41*, 188-192.

Bello-Huitle, V., Atenco-Fernández, P., Reyes-Mazzoco, R. (2010). Adsorption studies of methylene blue and phenol onto pecan and castile nutshells prepared by chemical activation. *Revista Mexicana de Ingeniería Química 9*, 313-322.

Bors, W., Michel, C. (2002). Chemistry of the antioxidant effect of polyphenols. *Annals of the New York Academy of Sciences 957*, 57-69. https://doi.org/10.1111/j.1749-6632.2002.tb02905.x

Bouali, I., Trabelsi, H., Herchi, W., Martine, L., Albouchi, A., Bouzaien, G., Sifi, S., Boukhchina, S., Berdeaux, O. (2014). Analysis of pecan nut (*Carya illinoinensis*) unsaponifiable fraction. Effect of ripening stage on phytosterols and phytostanols composition. *Food Chemistry 164*, 309-316. https://doi.org/10.1016/j.foodchem.2014.05.029

Carrasco-Del Amor, A. M., Aguayo, E., Collado-González, J., Guy, A., Galano, J. M., Durand, T., Gil-Izquierdo, Á. (2017). Impact of processing conditions on the phytoprostanes profile of three types of nut kernels. *Free Radical Research 51*, 141-147. https://doi.org/10.1080/10715762.2017.1288909

Christie, W. W. (1989). The preparation of derivatives of fatty acids. *Gas Chromatography and Lipids: A Practical Guide*. Pp. 37-38. Oily Press. https://doi.org/10.1016/0031-9422(89)80324-3

Christopoulos, M. V., Tsantili, E. (2012). Storage of fresh walnuts (*Juglans regia* L.) - Low temperature and phenolic compounds. *Postharvest Biology and Technology 73*, 80-88. https://doi.org/10.1016/j.postharvbio.2012.06.001

Christopoulos, M. V., Tsantili, E. (2015). Participation of phenylalanine ammonia-lyase (PAL) in increased phenolic compounds in fresh cold stressed walnut (*Juglans regia* L.) kernels. *Postharvest Biology and Technology 104*, 17-25. https://doi.org/10.1016/j.postharvbio.2015.03.003

Corral-Escárcega, M. C., Ruiz-Gutiérrez, M. G., Quintero-Ramos, A., Meléndez-Pizarro, C. O., Lardizabal-Gutiérrez, D., Campos-Venegas, K. (2017). Use of biomass-derived from pecan nut husks (*Carya illinoinensis*) for chromium removal from aqueous solutions. Column modeling and adsorption kinetics studies. *Revista Mexicana de Ingeniería Química 16*, 939-953.

de la Rosa, L. A., Alvarez-Parrilla, E., Shahidi, F. (2011). Phenolic compounds and antioxidant activity of kernels and shells of mexican pecan (*Carya illinoinensis*). *Journal of Agricultural and Food Chemistry 59*, 152-162. https://doi.org/10.1021/jf1034306

de la Rosa, L. A., Vazquez-Flores, A. A., Alvarez-Parrilla, E., Rodrigo-García, J., Medina-Campos, O. N., Ávila-Nava, A., González-Reyes, S., Pedraza-Chaverri, J. (2014). Content of major classes of polyphenolic compounds, antioxidant, antiproliferative, and cell protective activity of pecan crude extracts and their fractions. *Journal of Functional Foods 7*, 219-228. https://doi.org/10.1016/j.jff.2014.02.008

do Prado, A. C. P., Manion, B. A., Seetharaman, K., Deschamps, F. C., Barrera Arellano, D., Block,

J. M. (2013). Relationship between antioxidant properties and chemical composition of the oil and the shell of pecan nuts [*Carya illinoinensis* (Wangenh) C. Koch]. *Industrial Crops and Products 45*, 64-73. https://doi.org/10.1016/j.indcrop.2012.11.042

Domínguez-Avila, J. A., Alvarez-Parrilla, E., González-Aguilar, G. A., Villa-Rodríguez, J., Olivas-Orozco, G. I., Molina Corral, J., Gómez-García, M. C., De la Rosa, L. A. (2013). Influence of growing location on the phytochemical content of pecan (*Carya illinoinensis*) oil. *Journal of Food Research 2*, 143-152. https://doi.org/10.5539/jfr.v2n5p143

Erickson, M. C., Santerre, C. R., Malingre, M. E. (1994). Oxidative stability in raw and roasted pecans: Chemical, physical, and sensory measurements. *Journal of Food Science 59*, 1234-1238.

Fernandes, G. D., Gómez-Coca, R. B., Pérez-Camino, M. del C., Moreda, W., Barrera-Arellano, D. (2017). Chemical characterization of major and minor compounds of nut oils: Almond, hazelnut, and pecan nut. *Journal of Chemistry 2017*, 1-11. https://doi.org/10.1155/2017/2609549

Fernandes, P., Cabral, J. M. S. (2007). Phytosterols: Applications and recovery methods. *Bioresource Technology 98*, 2335-2350. https://doi.org/10.1016/j.biortech.2006.10.006

Flores-Córdova, M. A., Sánchez-Chávez, E., Chávez-Mendoza, C., García-Hernández, J. L., Preciado-Rangel, P. (2016). Bioactive compounds and phytonutrients in edible part and nutshell of pecan (*Carya illinoinensis*). *Cogent Food & Agriculture 2*, 1-12. https://doi.org/10.1080/23311932.2016.1262936

Flores-Córdova, M. A., Sánchez, E., Muñoz-Márquez, E., Ojeda-Barrios, D. L., Soto-Parra, J. M., Preciado-Rangel, P. (2017). Phytochemical composition and antioxidant capacity in Mexican pecan nut. *Emirates Journal of Food and Agriculture 29*, 346-350. https://doi.org/10.9755/ejfa.EJFA-2016-08-1075

Flores-Martínez, H., León-Campos, C., Estarrón-Espinosa, M., Orozco-Ávila, I. (2016). Process optimization for the extraction of antioxidants from mexican oregano (*Lippia graveolens* HBK) by the response surface methodology (RSM) approach. *Revista Mexicana de Ingeniería Química 15*, 773-785.

Fu, M., Qu, Q., Yang, X., Zhang, X. (2016). Effect of intermittent oven drying on lipid oxidation, fatty acids composition and antioxidant activities of walnut. *LWT - Food Science and Technology 65*, 1126-1132. https://doi.org/10.1016/j.lwt.2015.10.002

Gardea, A. A., Martínez-Téllez, M. A., Yahia, E. M. (2011). Pecan (Carya illinoiensis (Wangenh.) K. Koch.). *Postharvest Biology and Technology of Tropical and Subtropical Fruits* (Vol. 4). Pp. 143-166. Woodhead Publishing Limited. https://doi.org/10.1533/9780857092618.143

Gong, Y., Pegg, R. B., Carr, E. C., Parrish, D. R., Kellett, M. E., Kerrihard, A. L. (2017). Chemical and nutritive characteristics of tree nut oils available in the U.S. market. *European Journal of Lipid Science and Technology 119*, 1-15. https://doi.org/10.1002/ejlt.201600520

Granato, D., Shahidi, F., Wrolstad, R., Kilmartin, P., Melton, L. D., Hidalgo, F. J., Miyashita, K., Camp, J., Alasalvar, C., Ismail, A. B., Elmore, S., Birch, G. G., Charalampopoulos, D., Astley, S. B., Pegg, R., Zhou, P., Finglas, P. (2018). Antioxidant activity, total phenolics and flavonoids contents: Should we ban in vitro screening methods? *Food Chemistry 264*, 471-475. https://doi.org/10.1016/j.foodchem.2018.04.012

Held, P. (2005). Performing oxygen radical absorbance capacity assays with synergy^{TM}HT. *Application Note, Biotek*, 9. Retrieved from http://www.biotek.com/resources/docs/ORAC_Assay_Application_Note.pdf

Herald, T. J., Gadgil, P., Perumal, R., Bean, S. R., Wilson, J. D. (2014). High-throughputmicro-plate HCl-vanillin assay for screening tannin content in sorghum grain. *Journal of the Science of Food and Agriculture 94*, 2133-2136. https://doi.org/10.1002/jsfa.6538

Herrera, E. A. (1994). Early harvest and oven drying temperatures influence pecan kernel flavor. *Hortscience 29*, 671-672.

International Nut & Dried Fruit Council. (2019). *Nuts & Dried Fruits Statistical Yearbook* 2018/2019. https://doi.org/10.1002/9781118464663

Jia, X., Luo, H., Xu, M., Wang, G., Xuan, J., Guo, Z. (2019). Investigation of nut qualities of pecan cultivars grown in China. *Journal of Plant Sciences 7*, 117-124. https://doi.org/10.11648/j.jps.20190705.13

Jia, X., Luo, H., Xu, M., Zhai, M., Guo, Z., Qiao, Y., Wang, L. (2018). Dynamic changes in phenolics and antioxidant capacity during pecan (*Carya illinoinensis*) kernel ripening and its phenolics profiles. *Molecules 23*, 1-17. https://doi.org/10.3390/molecules23020435

Kader, A. A. (2013). Impact of nut postharvest handling, de-shelling, drying and storage on quality. *Improving the Safety and Quality of Nuts*. Pp. 22-34. Woodhead Publishing Limited. https://doi.org/10.1533/9780857097484.1.22

Kanamangala, R. V., Maness, N. O., Smith, M. W., Brusewitz, G. H., Knight, S., Chinta, B. (1999). Reduced lipid pecans: Chemical alterations and implications for quality maintenance during storage. *Journal of the American Society for Horticultural Science 124*, 389-398.

Macías-Cortés, E., Gallegos-Infante, J. A., Rocha-Guzmán, N. E., Moreno-Jiménez, M. R., Medina-Torres, L., González-Laredo, R. F. (2020). Microencapsulation of phenolic compounds: Technologies and novel polymers. *Revista Mexicana de Ingeniera Quimica 19*, 491-521. https://doi.org/10.24275/rmiq/Alim642

Momchilova, S. M., Taneva, S. P., Zlatanov, D., Antova, G. A., Angelova-Romova, M. J., Blagoeva, E. (2017). Fatty acids, tocopherols and oxidative stability of hazelnuts during storage. *Bulgarian Chemical Communications 49*, 65-70.

Nicoli, M. C., Lerici, C. R., Manzocco, L., Mastrocola, D., Calligaris, S. (2002). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science & Technology*, 11(9-10), 340-346. https://doi.org/10.1016/s0924-2244(01)00014-0

Olguín Rojas, A., Vázquez-León, L. A., Salgado-Cervantes, M. A., Fernandez-Barbero, G., Díaz-Pacheco, A., García-Alvarado, M. A., Rodriguez-Jimenes, G. C. (2019). Water and phytochemicals dynamic during drying of red habanero chili pepper (*Capsicum chinense*) slices. *Revista Mexicana de Ingeniería Química 18*, 851-864. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Olguin

Oro, T., Ogliari, P. J., Dias de Mello Castanho Amboni, R., Barrera-Arellano, D., Mara Block, J. (2008). Evaluación de la calidad durante el almacenamiento de nueces Pecán [*Carya illinoinensis* (Wangenh.) C. Koch] acondicionadas en diferentes envases. *Grasas y Aceites 59*, 132-138. https://doi.org/10.3989/gya.2008.v59.i2.501

Phatanayindee, S., Borompichaichartkul, C., Srzednicki, G., Craske, J., Wootton, M. (2012). Changes of chemical and physical quality attributes of macadamia nuts during hybrid drying and processing. *Drying Technology 30*, 1870-1880. https://doi.org/10.1080/07373937.2012.703275

Price, M. L., Scoyoc, S. Van, Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry 26*, 1214-1218. https://doi.org/10.1021/jf60219a031

Robbins, K. S., Gong, Y., Wells, M. L., Greenspan, P., Pegg, R. B. (2015). Investigation of the antioxidant capacity and phenolic constituents of U.S. pecans. *Journal of Functional Foods 18*, 1002-1013. https://doi.org/10.1016/j.jff.2015.05.026

Robbins, K. S., Ma, Y., Wells, M. L., Greenspan, P., Pegg, R. B. (2014). Separation and characterization of phenolic compounds from U.S. pecans by liquid chromatography-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry 62*, 4332-4341.

Roy, M. K., Koide, M., Rao, T. P., Okubo, T., Ogasawara, Y., Juneja, L. R. (2010). ORAC and DPPH assay comparison to assess antioxidant capacity of tea infusions: Relationship between total polyphenol and individual catechin content. *International Journal of Food Sciences and Nutrition 61*, 109-124. https://doi.org/10.3109/09637480903292601

Santerre, C. R. (1994). Pecan processing. *Pecan Technology*. Pp. 49-67. Champman & Hall.

Senter, S. D., Forbus, W. R. (1978). Leucoanthocyanidin oxidation in pecan kernels: relation to discoloration and kernel quality. *Journal of Food Science 43*, 128-134.

Shahidi, F., John, J. A. (2010). Oxidation and protection of nuts and nut oils. *Oxidation in Foods and Beverages and Antioxidant Applications* (Vol. 1). Pp. 274-305. Woodhead Publishing Limited. https://doi.org/10.1533/9780857090331.2.274

Shahidi, F., John, J. A. (2013). Oxidative rancidity in nuts. *Improving the Safety and Quality of Nuts*. Pp. 198-229. Woodhead Publishing Limited. https://doi.org/10.1533/9780857097484.2.198

Shivakumar, A., Kumar, M. S. Y. (2018). Critical review on the analytical mechanistic steps in the evaluation of antioxidant activity. *Critical Reviews in Analytical Chemistry 48*, 214-236. https://doi.org/10.1080/10408347.2017.1400423

Singleton, V. L., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture 16*, 144-158.

Smeriglio, A., Barreca, D., Bellocco, E., Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology 174*, 1244-1262. https://doi.org/10.1111/bph.13630

Troller, J. A., Christian, J. H. B. (2014). Lipid oxidation, changes in texture, color, and nutritional quality. *Water Activity and Food*. Pp. 69-85. https://doi.org/10.1016/b978-0-12-700650-5.50010-x

U.S. Department of Agriculture. (2017). *USDA National Nutrient Database for Standard Reference.*

Vazquez-Flores, A. A., Wong-Paz, J. E., Lerma-Herrera, M. A., Martinez-Gonzalez, A. I., Olivas-Aguirre, F. J., Aguilar, C. N., Wall-Medrano, A., Gonzalez-Aguilar, G. A., Alvarez-Parrilla, E., de la Rosa, L. A. (2017). Proanthocyanidins from the kernel and shell of pecan (*Carya illinoinensis*): Average degree of polymerization and effects on carbohydrate, lipid, and peptide hydrolysis in a simulated human digestive system. *Journal of Functional Foods 28*, 227-234. https://doi.org/10.1016/j.jff.2016.11.003

Villarreal-Lozoya, J. E., Lombardini, L., Cisneros-Zevallos, L. (2007). Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. *Food Chemistry 102*, 1241-1249. https://doi.org/10.1016/j.foodchem.2006.07.024

Villarreal-Lozoya, J. E., Lombardini, L., Cisneros-Zevallos, L. (2009). Electron-beam irradiation effects on phytochemical constituents and antioxidant capacity of pecan kernels [*Carya illinoinensis* (Wangenh.) K. Koch] during storage. *Journal of Agricultural and Food Chemistry 57*, 10732-10739. https://doi.org/10.1021/jf901719s

Wang, W., Jung, J., McGorrin, R. J., Traber, M. G., Leonard, S. W., Cherian, G., Zhao, Y. (2018). Investigation of drying conditions on bioactive compounds, lipid oxidation, and enzyme activity of Oregon hazelnuts (*Corylus avellana* L.). *LWT 90*, 526-534. https://doi.org/10.1016/j.lwt.2018.01.002

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry 52*, 4026-4037. https://doi.org/10.1021/jf049696w

Yao, F., Dull, G., Eitenmiller, R. (1992). Tocopherol quantification by HPLC in pecans and relationship to kernel quality during storage. *Journal of Food Science 57*, 1194-1197. https:

//doi.org/10.1111/j.1365-2621.1992. tb11297.x