Short note

Chemical evaluation and antioxidant capacity of Western and Wichita pecan nut cultivars [*Carya illinoinensis* (Wangenh.) K. Koch]

L.A. Medina-Juárez
D.M.A. Molina-Quijada
S. Agustin-Salazar
L.A. Rascón Valenzuela
C.C. Molina-Domínguez
N. Gámez-Meza*

Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora Hermosillo, Sonora, Mexico

The proximal composition of Western and Wichita cultivars of pecan nut [*Carya illinoinensis* (Wangenh.) K. Koch] was evaluated, as well as, the phenol content, total flavonoids, condensed tannins and the antioxidant capacity of kernel cake extracts. The presence of phenolic acids and flavonoids in the kernel cake was demonstrated by MS^2 analysis. In addition, the tocopherol content, fatty acids profile, and oxidative stability of the pecan nut oil were determined. Pecan nut presented an oil high content (60 - 73%) in both cultivars. The most representative fatty acids were oleic acid (50 - 60%) and linoleic acid (28 - 37%). A significant concentration of γ–tocopherol was also found. Kernel cake presented a high antioxidant capacity due to the phenolic, flavonoid and tannin contents in both cultivars. The results indicated that pecan nut, in addition to being an oil-rich fruit, possesses phytochemicals with antioxidant capacity.

Keywords: Antioxidant capacity; *Carya illinoinensis*; Phenolic compounds; Pecan nut; Pecan nut oil.

1. INTRODUCTION

There are over 40 types of nuts in the world, including almonds (*Prunus amygdalus*), hazelnut (*Corylus avellana*), macadamia (*Macadamia* spp), pistachio (*Pistachia vera* L.), walnut (*Juglans regia*) and pecan nut (*Carya illinoinensis*) [1]. Pecan nut [*Carya illinoinensis* (Wangenh.) K. Koch] belongs to the Juglandaceae family and is native of southern United States and northern Mexico. The seed is considered an oilseed of commercial importance. Currently, the United States and Mexico occupy first and second place, respectively, as world producers of pecans. Western and Wichita are among the primary cultivated varieties [2].

Nuts are rich in vitamins, minerals, monounsaturated fatty acids, fibre and numerous phytochemicals, particularly phenolic compounds that have not been completely identified or characterised [3]. Pecan nuts have gained attention due to their numerous health benefits. Those benefits are not only attributed to hydrophilic compounds (such as phenolic antioxidants), but also, lipophilic compounds that are believed to contribute to antioxidant properties [4].

Recent studies have reported some phenolic compounds present in various parts of the nut fruit, primarily phenolic acids and catechins [5 - 8]. Several biological activities have been attributed to phenolic compounds; however, their antioxidant capacity is the most studied [4, 6, 7].

Oil, another important component of the nuts, can be affected by the oxidation reaction. Oil oxidation depends on factors such as fatty acid composition, ultraviolet radiation, temperature, presence of metallic ions, oxygen, and water activity [9].

(*) CORRESPONDING AUTHOR:
Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora, Blvd. Rosales y Blvd. Luis Encinas, CP 83000, Hermosillo, Sonora, Mexico.
Phone: +52 6622 592179
Fax: + 52 6622 592169
E-mail: nohemi.gamez@unison.mx

Consumers identify the pecan nut as a healthy product, considered as having nutritional properties, even though the nutritional information on Mexican nuts is scarce. However, about 60% of the references found on the market do not have a nutritional label, as well as information regarding the benefits associated with their phenolic compounds. Adequate information on the labels could encourage consumption, especially in those consumers that are more likely to value the nutritional attributes.

The objective of this study was to determine the chemical composition of the kernel cake and oil of two pecan nut cultivars (Western and Wichita) from three harvests (2010, 2011, and 2012), and evaluate the antioxidant capacity of kernel cake extracts to determine its potential as a functional food.

2. MATERIALS AND METHODS

2.1. RAW MATERIAL

Two export cultivars pecan nut, Western and Wichita, were selected. The nuts were harvested in northeast Mexico (29°00'12.8"N, 111°33'53.5"W at 69 m elevation) in October during three harvests: 2010, 2011 and 2012. Firstly, the husk (exocarp and mesocarp) was removed from the nuts, and then the nuts were dried into a silo with constant aeration (35 to 40°C) for 3 to 5 days, until reaching a moisture of 3 to 4%.

2.2. CHEMICALS

All chemicals used in this study, like heptadecanoic acid (PubChem CID: 10465), gallic acid (PubChem CID: 370), catechin (PubChem CID: 9064), α–tocopherol (PubChem CID: 2116), γ–tocopherol (PubChem CID: 92729), δ–tocopherol (PubChem CID: 92094), Trolox (PubChem CID: 40634), were purchased from Sigma–Aldrich (St. Louis, MO). Methanol (PubChem CID: 887), ethanol (PubChem CID: 702), hexane (PubChem CID: 8058), formic acid (PubChem CID: 284), isopropanol (PubChem CID: 3776), Folin–Ciocalteu, acetone (PubChem CID: 180), ABTS (PubChem CID: 35687), DPPH (PubChem CID: 2735032), were purchased from J.T. Baker (Toluca, Edomex, México).

2.3. PROXIMATE ANALYSIS

Pecan nut proximate composition was determined following the official AOAC methods [10] for raw protein (984.13), lipids (920.39), ash (942.05), raw fibre (962.09) and moisture (930.15).

2.4. PECAN NUT OIL EXTRACTION

Nuts were peeled manually, ground in a blender (Osterizer, Model 4122, Mexico) and defatted using hexane (1:10 w/v) under agitation (320 rpm) for 1 hour, the extraction was repeated twice (for both cultivars) [11]. The oil was separated from the kernel cake by filtration; the remaining hexane was removed using a rotary evaporator (Labconco, Kansas City, MO, USA). The oil was subject to reflux with nitrogen and stored at -20°C until its analysis.

2.5. PECAN NUT OIL PHYSICOCHEMICAL ANALYSIS

The extracted oils were subject to the following determinations, according to the official methods of the American Oil Chemists´ Society (AOCS) [12]: free fatty acids (FFA, Ca 5a-40), peroxide value (PV, Cd 8b-90), *p*–anisidine value (*p*–AV, Cd 18-90), and oxidative stability index (OSI, Cd 12b-92) by Rancimat (Metrohm 679; Herisau, Switzerland).

2.5.1. Pecan Nut Oil Fatty Acid Profile

Oil samples were saponified and methylated according to the official AOCS (Ce 2-66) procedure for the fatty acids profiles determination. Fatty acid methyl esters (FAMEs) were analysed in a gas chromatograph (Agilent 7890A, Santa Clara, CA, USA) attached to a flame ionization detector and fitted with a silica capillary column P-2560 (100 m × 0.25 mm i.d. × 0.25 µm film thickness, Supelco, Bellefonte, PA, USA). Nitrogen was used as carried gas (29.5 cm/s). Temperature for the column was maintained at 180°C for 10 min and subsequently increased to 220°C (3°C/min) for 17 min. Injector and detector temperatures were taken to 250°C. FAME peaks were identified by comparison of retention times with their respective standards (Sigma Chemical Co., St. Louis, MO). Quantification was performed using heptadecanoic acid (C17:0) as internal standard. Results were expressed as g FAME/100 g FAMEs (AOCS Ce 1h - 05).

2.5.2. Tocopherols Quantification

Tocopherol content was determined according to AOCS method (Ce 8–89). The analysis was conducted using an HPLC equipped with an isocratic pump (Varian, ProStar 210 model, Walnut Creek, CA, USA) and a UV–Vis detector (Varian, model 9050, Walnut Creek, CA, USA). Separation of tocopherols was conducted in a normal phase Supelcosil Si column (15 cm × 4.6 mm × 5 µm, Supelco, Bellefonte, PA, USA) conditioned with a Si precolumn (2 cm × 4.6 mm × 5 µm particle size, Supelco, Bellefonte, PA, USA). Hexane-isopropanol (99.5:0.5 v/v) was used as mobile phase (flow rate: 1.0 mL/min). The injection volume was 50 mL (2 g sample in 25 mL hexane). Tocopherols were analysed at a wavelength of 292 nm, with an elution time of 15 min. Identification of tocopherols was performed by a comparison of retention

times of α–tocopherol, γ–tocopherol and δ–tocopherol standards (Supelco-Sigma, Aldrich Química, Mexico). Quantification was performed using calibration curves for each standard.

2.6. CHEMICAL CHARACTERIZATION OF KERNEL CAKE

After separation, the residual kernel cake was uniformly distributed in a container, kept in dark in a fume extractor to eliminate any remaining solvent.

2.6.1. Determination of Total Phenols and Total Flavonoids

Extraction of phenols and flavonoids kernel cake was performed following the methodology described by Yilmaz and Toledo [11]. One gram of sample in 10 mL of 70% methanol was used for every washing. The total phenolic compounds were determined spectrophotometrically by the Folin-Ciocalteu method at 765 nm [13]. The results were reported as gallic acid equivalents (GAE)/g dry sample [7]. Flavonoids were determined following the technique of De la Rosa *et al*. [6]. Samples were analysed spectrophotometrically (Varian, model Cary 100 Bio, Walnut Creek, CA, USA) at 498 nm. The results were expressed as milligrams of catechin equivalents (mg CE)/g dry sample.

2.6.2. Determination of Condensed Tannins

Extraction of condensed tannins was performed following the method described by Villarreal-Lozoya *et al*. [14], and the quantification was conducted spectrophotometrically at 500 nm, using 1 mL of acidified extract of kernel cake. The results are expressed as milligrams of catechin equivalents (mg CE)/g dry sample.

2.6.3. Identification Main Phenolic Compounds by Mass Spectrometry

Mass spectra of the samples were obtained using a Varian 500-MS IT mass spectrometer (Walnut Creek, CA, USA) equipped with an electrospray ionisation source and an ion trap mass analyser, which was controlled using MS WorkStation v.16 software (Agilent Technologies, Santa Clara, CA, USA). The mass spectrometer was operated in negative mode in the 100 - 2000 m/z interval using an infusion rate of 10 µg/mL, a voltage of -17 kV, a capillary temperature of 350°C and helium, as an auxiliary gas, at a flow rate of 0.8 mL/min. Fragmentation patterns of ions of interest were obtained by tandem MS. Identification of compounds present in the samples was performed by a comparison of the primary ion fragments and their relative abundance with information reported in literature [5].

2.6.4. Antioxidant Capacity

Two methods were used to measure the antioxidant capacity. The first method is based on the stabilisation reaction of $ABTS^{\bullet+}$ cation radical, measuring the absorbance at 754 nm [15]. The second method was stabilisation of $DPPH^{\bullet}$, which produces a colour change in solution in the presence of antioxidants. The reaction was conducted for 30 min and subsequently was read spectrophotometrically at 515 nm. The results are expressed as micromoles of Trolox equivalents (µM TE)/g [15].
It is important to employ more than one type of *in vitro* antioxidant assay to help develop a more complete capacity, because two significantly different mechanisms are involved with antioxidant action: a hydrogen atom transfer (HAT) and/or a single electron transfer (SET) [4].

2.7. STATISTICAL ANALYSIS

Statistical analysis using SigmaStat Version 3.5 (p <0.05) was performed. An analysis of variance (ANOVA) was conducted for all analysed variables for both cultivars, and among the harvests. To verify the dependence between variables, the Pearson correlation coefficient was used. All analyses were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. PROXIMATE ANALYSIS

Pecan nut, for both varieties among harvests, was mainly composed by lipids (62.54 - 75.19%), followed by protein (9.24 - 16.13%). The differences observed among harvests could result from agroclimatic changes in the crop, such as high temperatures (41 ± 3°C) during the months prior to the 2012 harvest (August, September) [16]. Biochemical composition have been reported for various cultivars of commercial pecan nuts for Western-Schley and Wichita [17], identifying similar values of lipids (71.19 and 71.97%, respectively) and protein (9.55 and 10.42%, respectively).
Pecan nuts composition depends on the different types of stress caused by adverse external conditions, biotic and abiotic, which affect its growth and development. These external conditions trigger a wide range of responses in the plant, such as alterations in the genetic expression and cellular metabolism or changes in rate of growth and crop yield, manifesting primarily in its biochemical composition [18]. For this reason, it is important to know the chemical composition of pecan nuts grown in different countries or regions.

3.2. OIL CHEMICAL ANALYSES

3.2.1. Fatty Acids Profile and Tocopherol Content

The fatty acid profile indicated that oleic and linoleic acids were most abundant in pecan oils for both cultivars (Table I). Other studies on pecan nuts cultivated in Texas (USA) have identified similar content of oleic (53 - 71%) and linoleic (15 - 36%) acids [14]. Venkatachalam *et al*. [17] reported these two fatty acids in 24 pecan nut cultivars from USA. Oleic acid content varied from the origin state, 52.52% for Desirable cultivar (Texas), 70.23% for Western cultivar (Arizona) and 74.09% for Wichita cultivar (Arizona). Linoleic acid contents ranged from 17.69% for Wichita cultivar (Arizona) to 37.52% for Desirable cultivar (Texas). This demonstrates the wide variability of these fatty acid contents depending on the cultivation area.

Western and Wichita Mexican pecan nuts showed a higher content of γ-tocopherol, followed by α-tocopherol and a low amount of δ-tocopherol (Table I). Prado *et al*. [8] reported the presence of α-tocopherol and γ-tocopherol in pecan nut oil from nuts cultivated in Brazil, while δ-tocopherol was not identified.

The presence of tocopherols in pecan nut oil contributes to its lipophilic antioxidant capacity. The efficacy of pecan bioactive compounds, specifically γ–tocopherol, against LDL oxidation has been shown in both *in vitro* and *in vivo* studies, finding that pecan meals resulted in an increase in plasma antioxidant activity as assessed by both hydrophilic and lipophilic levels, as well as by a decrease in oxidised LDL over time [4].

3.2.2. Oxidative Stability

Western and Wichita oils showed 0.021 - 0.032% FFA contents, 0.44 - 0.64 meq O_2/kg of PV and 0.16 - 0.24 *p*-AV, values below to the maximum permitted values for vegetable oils [19]. Miraliakbari and Shahidi [3] reported higher *p*-AV values (0.821) for Brazilian pecans.

The oxidative stability evaluation demonstrated that Wichita oil displayed a greater stability than Western oil (Table I). OSI values in Wichita oil could be associated with the tocopherol content. Other studies on pecans have reported OSI values from 3.9 to 7.0 h (110°C) [9]. Prado *et al*. [8] reported OSI values between 7.4 and 11.2 h (110°C) in pecan nuts.

Oil oxidative stability, quality, nutritional value, sensory properties, and potential health effects could be largely determined by its chemical composition. This study shows again that pecan nuts are cultivated for use as oil crops and are important sources of energy and essential dietary nutrients as well as phytochemicals [9].

Table I - Composition of the fatty acid methyl esters (g FAME/100 g FAMEs), tocopherols and oxidative stability of pecan nut oil

Compound	We 2010	We 2011	We 2012	Wi 2010	Wi 2011	Wi 2012
Myristic (14:0)	0.09±0.00a	0.11±0.01a	0.09±0.01a	0.08±0.00a	0.11±0.01a	0.08±0.00a
Palmitic (16:0)	8.17±0.28a	7.66±0.34a	7.69±0.15a	8.03±0.16a	7.73±0.13a	7.14±1.07a
Stearic (18:0)	2.28±0.02a	2.44±0.02a	2.31±0.17a	2.52±0.23a	2.36±0.21a	2.07±0.13a
Oleic (18:1)	50.47±1.20a	53.93±1.30a	51.44±1.25a	51.79±1.19a	54.04±1.06a	60.77±1.45b
Linoleic (18:2)	37.44±1.32b	34.08±1.34a	36.34±1.13b	36.09±1.12b	34.05±1.01b	28.29±0.35a
Linolenic (18:3)	1.29±0.02a	1.37±0.11a	1.32±0.12a	1.22±0.04a	1.33±0.08a	1.26±0.01a
α–Tocopherol	nd	4.50±0.12a	4.76±0.35a	4.79±0.62a	8.01±0.41b	4.34±0.24a
γ–Tocopherol	237.31±3.13b	220.20±1.64a	290.14±6.08c	306.28±12.54a	312.46±11.57ab	332.04±9.48b
δ–Tocopherol	2.28±0.08	nd	nd	nd	4.10±0.11	nd
OSI	5.91±0.37a	5.07±0.32a	5.11±0.30a	6.51±0.31a	7.50±0.38b	8.74±0.62c

We: Western; Wi: Wichita. Tocopherols in ppm (nd: not detected. Limit of detection 2 ppm); OSI: Oxidative stability index (h, 120°C); Values in each row with a different letter (a-c) represent significant differences ($p < 0.05$) in each cultivar.

3.3. CONTENT OF PHENOLS, FLAVONOIDS, AND TANNINS IN PECAN KERNEL CAKE

Results indicated that kernel cakes, both cultivars, have significant phytochemical content (Table II). De la Rosa *et al*. [6] reported lower values of phenols (11.7 - 12.5 mg GAE/g fresh sample), flavonoids (5.8 - 6.4 mg CE/g fresh sample) and condensed tannins (20.3 - 26.7 mg CE/g fresh sample) to those reported in this study. The differences in geographical regions may be one of the causes of different phenol contents, and therefore secondary metabolites content because genetic level and phytochemicals formation is highly influenced by environmental factors [20].

Significant correlations ($R > 0.98$) between ABTS assay and total phenolics in both cultivars were found. These results indicate that the presence of the phenolic compounds contributes to their antioxidant potential. High correlations were also found between tannin contents and free radical scavenging activity measured by both assays ($R > 0.82$).

Table II - Chemical characterization of pecan nut kernel cake [*Carya illinoinensis* (Wangenh.) Koch]

Analysis	We 2010	We 2011	We 2012	Wi 2010	Wi 2011	Wi 2012
TF	16.81±1.34[a]	19.95± 1.33[b]	16.80±1.27[a]	18.16±1.91[a]	19.90±1.68[a]	18.27±1.46[a]
TFv	14.76±1.39[a]	13.90± 0.37[a]	14.79±1.07[a]	16.36±1.56[b]	13.34±1.17[a]	14.17±1.23[ab]
Tannins	8.32±0.14[a]	7.92±0.35[a]	8.94±0.86[a]	9.10±0.88[a]	7.55±0.68[a]	8.47±0.69[a]
Antioxidant capacity of extracts of pecan nut kernel cake						
ABTS	213.77±23.67[a]	276.65±12.85[b]	203.62±2.03[a]	211.36±13.73[a]	269.46±4.65[b]	210.73±9.81[a]
DPPH	243.45±25.63[a]	287.67±9.25[b]	267.33±10.62[a]	245.26±16.37[a]	281.27±24.08[a]	276.48±15.63[a]

We: Western; Wi: Wichita. TF: Phenols in mg of gallic acid equivalent/g. TFv: Flavonoids and tannins in mg of catechin equivalent/g. Antioxidant capacity in µM TE/g. Values in each row with different letters (a-c) show significant differences ($p < 0.05$) in each cultivar.

3.3.1. Phenolic Compounds Profile

Ions of interest observed in the full scan were subject to second-order fragmentation (MS^2) by which over 50 substances were identified for both cultivars. The identification was made by comparing the primary ion fragments present in the paste samples with information in literature. As observed in Tables III and IV, the profile of compounds in both cultivars is similar. These substances were classified in the following groups according to their nature: phenolic acids, flavonoids, glycosylated phenolic acids, glycosylated flavonoids and (epi)catechin dimers and trimers [4-6, 21-32]. Most of the substances identified belong to the group of phenolic acids. 2,4-dihydrobenzoic acid, cinnamic acid, protocatechuic acid, coumaric acid, caffeic acid and gallic acid were observed. The common characteristic presented by phenolic acids upon analysis by mass spectrometry is decarboxylation of acids, which records losses of 28 and 44 amu, which are attributed to CO and CO_2, respectively.
Compounds belonging to the flavonoids group were also identified; among them, the presence of the isoflavonone genistein, flavonol kaempferol, flavan-3-ol catechin, flavonol quercetin and the flavanone hesperitin are highlighted. Fragmentations that the aforementioned substances undergo are different because of the structural characteristics of their rings; thus, catechin with molecular ion 289 m/z generated ion fragments of 137 and 161 m/z, which were caused by retro Diels-Alder (RDA) fragmentation mechanisms and fragmentation by heterocyclic rupture of the C ring, respectively [33].

Table III - Identification main phenolic compounds from methanolic extract of pecan nut kernel cake of the Western cultivar

Substance	Ion precursor [M-H] (*m/z*)	Ion products ESI-MS^n (*m/z*)
Protocatechin aldehyde [21]	137	109 (100)
Cinnamic acid [22]	147	119 (19)
Protocatechin acid [21]	153	109 (100), 137 (41)
Coumaric acid [5]	163	119 (100)
Gallic acid [21]	169	125 (100)
Caffeic acid [5]	179	135 (59), 161 (100)
Quinic acid [23]	191	93 (8), 111 (181), 146 (72), 162 (100), 189 (78)
Syringic acid [24]	197	153 (86), 182 (100)
Genistein [25]	269	183 (57), 241 (49)
(Epi)catechin [21]	289	179 (9), 203 (37), 205 (42), 245 (100)
Ellagic acid [21]	301	185 (37), 217 (5.4), 229 (100), 257 (95)
Hesperetin [30]	303	257 (44), 301 (100)
Methylellagic acid [6]	315	300 (94), 301 (45)
Caffeic acid-hexoside [4]	341	179 (100)
Oleuropein [26]	377	341 (100)
Ellagic acid pentsoide [4]	433	300 (100), 301 (86)
Methylgalloylellagic acid [4]	447	300 (13), 301 (3), 315 (100)
Myricetin 3-*O*-pentoside [25]	449	315 (100), 316 (55)
Quercetin 3-*O*-hexoside [22]	463	300 (58), 301(100)
Methylellagic acid hexoside [27]	477	300 (36), 315 (100)
Myricetin-3-*O*-hexoside [25]	479	301 (33), 315 (42), 316 (17), 461 (100)
HHDP-hexoside [24]	481	275 (25), 301 (100)
B type proanthocyanidin, (epi)catechin dimer [28]	577	245 (7), 289 (77), 407 (100), 425 (56)
Galloyl ellagic acid pentoside [28]	585	301 (75), 433 (50)
Dimer of [epi]gallocatechin-[epi]catechin [28]	593	305 (17), 347 (32), 403 (100), 423 (13)
Dimer of caffeic acid-hexoside [29]	683	341 (100)
Trimer of [epi]catechin [28]	865	407 (100), 575 (1), 577 (40), 695 (86)

The relative abundance of ion products is presented in parenthesis. HHDP: Hexahydroxydiphenic acid.

Other substances, such as glycosides such as ellagic acid pentoside, myrcetin 3-*O*-pentoside, quercetin 3-*O*-hexoside, methoxykaempferol-3-*O*-glucoside, and HHDP-hexoside, were also identified in kernel cake, which generated precursor ions with m/z of 433, 449, 463, 477, and 481, respectively. These glycosides underwent neutral losses of 132 and 162 amu (pentosides and hexosides) because of the removal of the carbohydrate residue.

Dimers and trimers of catechin were found in the kernel cake, in both Western and Wichita cultivars. The (epi)catechin dimer displayed a fragmentation pattern as noted by Tala *et al.* [28], in which an ion fragment of 425 m/z generated by an RDA reaction is present, that led to the loss of 152 amu; likewise, an ion with 289 m/z belonging to the monomeric catechin was detected. The trimeric proanthocyanidin (865 m/z) generated a characteristic ion of 695 m/z because of an RDA and dehydration of the molecule ([M-H]$^-$ - 152 amu [RDA] -18 amu [-H_2O]) as well as an ion with 577 m/z generated by the loss of a catechol group [28].

Robbins *et al.* [4] determined the content of phenolic compounds and their antioxidant capacity in 18 pecan cultivars of the United States; as in this investigation, those authors used MS^2 analysis to identify compounds, identifying a profile similar to the profile reported in this study. This result was obtained despite extraction performed with a system of methyl ether, water and acetic acid, using the nut and husk of the pecan as the sample.

3.3.2. Antioxidant Capacity

Kernel cake, for both cultivars, displayed significant antioxidant capacity (Table II), which is attributed to phenolic compounds content [8]. De la Rosa *et al.* [6] reported lower antioxidant capacities in the kernel cake of pecans grown in Chihuahua (Mexico); on the other hand, Villarreal-Lozoya *et al.* [14] reported greater antioxidant capacities in those cultivated in Texas (USA), than those obtained in this study. Antioxidant capacity depends on the complexity of the concentration and variation of individual phenolic compounds that can lead to some interactions among its components, such as synergism or additive or antagonist effects [15].

Table IV - Identification main phenolic compounds from methanolic extract of pecan nut kernel cake of the Wichita cultivar

Substance	Ion precursor [M-H] (*m/z*)	Ion products ESI-MS^n (*m/z*)
2,4-dihydroxybenzoic acid [5]	137	108 (100)
Protocatechic aldehyde [21]	137	109 (15), 136 (100)
Cinnamic acid [22]	147	119 (45)
Protocatechic acid [21]	153	108 (81), 109 (29), 137 (100)
Coumaric acid [5]	163	119 (30)
Gallic acid [21]	169	125 (100)
Caffeic acid [5]	179	135 (30), 179 (100)
Quinic acid [23]	191	93 (16), 111 (25), 146 (100), 189 (97)
Genistein [25]	269	157 (13), 183 (100)
Kaempferol [31]	285	183 (100), 185 (32)
(Epi)catechin [21]	289	137 (11), 161 (34), 179 (20), 203 (40), 205 (61), 227 (22), 245 (100)
Carboxylic acid for brevifolin [27]	291	203 (34), 247 (69)
Ellagic acid [21]	301	185 (51), 217 (100), 229 (89), 257 (80)
Quercetin [22]	301	229 (19), 257 (40), 300 (32), 301 (100)
Hesperetin [30]	303	257 (19), 301(100)
Methylellagic acid [6]	315	300 (100), 301 (36)
Caffeic acid-hexoside [4]	341	179 (100)
Oleuropine derivative [26]	377	179 (59), 341 (100)
Ellagic acid pentoside [4]	433	300 (100), 301 (77)
Methylgalloylellagic acid [4]	447	301 (10), 315 (100)
Myricetin 3-*O*-pentoside [25]	449	301 (31), 315 (100), 316 (72)
Quercetin 3-*O*-hexoside [22]	463	300 (42), 301 (100)
Valoneic acid bilactone [4]	469	301 (100), 425 (37)
Methoxykampferol-3-*O*-hexoside [32]	477	300 (20), 315 (100)
Myricetin 3-*O*-hexoside [25]	479	272 (81), 301 (100), 316 (34), 417 (29)
HHDP- hexoside [24]	481	275 (11), 301 (100)
B type proanthocyanidin (dimer) Dimer of (epi)catechin [28]	577	245 (42), 289 (86), 407 (100), 425 (58)
Galloyl ellagic acid pentoside [28]	585	301 (100)
Dimer of caffeic-*O*-hexoside acid [29]	683	341 (100)
Trimer of (epi)catechin [28]	865	407 (9), 575 (11), 577 (43), 695 (100)

The relative abundance of ion products is presented in parenthesis. HHDP: Hexahydroxydiphenic acid.

Primary antioxidants promote the removal or inactivation of free radicals in the initiation and propagation stages of the oxidation while synergists, substances with little or no antioxidant activity, may increase the activity of primary antioxidants when used in an appropriate combinations [34]. Synergist behaviour is commonly attributed to natural extracts due to its complex composition.

4. CONCLUSIONS

Mexican pecan nuts, Wichita and Western cultivars, are a significant source of oil, composed primarily of oleic and linoleic acids, with an important amount of γ–tocopherol. Furthermore, pecan kernel cake, in addition to being an important source of oil and protein, possesses a high content of phenols, flavonoids and tannins in both cultivars, which are related to an antioxidant capacity. The results indicated that pecan nut, besides being an oil-rich fruit, possesses phytochemicals with antioxidant capacity.

Acknowledgement
The authors thank Viñedos Alta S.A. de C.V. (www.grupoalta.com) for the financial support to this study. D.M.A. Molina-Quijada and S. Agustin-Salazar thanks the National Council for Science and Technology (CONACYT) for the scholarship grant.

REFERENCES

[1] M. Delgado. Nogal, un cultivo en expansión. Inforural (2017). http://www.inforural.com.mx/nogal-un-cultivo-en-expansion/ (accessed January 12, 2017).

[2] D.L. Ojeda-Barrios, O. A. Hernández-Rodríguez, G. R. López-Ochoa, J. J. Martínez-Téllez. Evolución de los sistemas de producción de nuez en México. Tecnociencia Chihuahua *3*, 115-120 (2009).

[3] H. Miraliakbari, F. Shahidi. Oxidative stability of tree nut oils. J. Agric. Food Chem. *56*, 4751-4759 (2008).

[4] K.S. Robbins, Y. Gong, M.L. Wells, P. Greenspan, R.B. Pegg. Investigation of the antioxidant capacity and phenolic constituents of U.S. pecans. J. Funct. Foods *15,* 11-22 (2015).

[5] M. Gültekin-Özgüven, F. Davarcı, A.A. Pasli, N. Demir, B. Özçelik. Determination of phenolic compounds by ultra-high liquid chromatography-tandem mass spectrometry: Applications in nuts. LWT - Food Sci. Technol. *64,* 42-49 (2015).

[6] L.A. de la Rosa, E. Alvarez-Parrilla, F. Shahidi. Phenolic compounds and antioxidant activity of kernels and shells of Mexican pecan (*Carya illinoinensis*). J. Agric. Food Chem. *59,* 152-62 (2011).

[7] A.C.P. Prado, H.S. Da Silva, S.M. da Silveira, P.L.M. Barreto, C.R.W. Vieira, M. Maraschin, S.R.S Ferreira, J.M. Block. Effect of the extraction process on the phenolic compounds profile and the antioxidant and antimicrobial activity of extracts of pecan nut [*Carya illinoinensis* (Wangenh) C. Koch] shell. Ind. Crops Prod. *52,* 552-561 (2014).

[8] A.C.P. Prado, B.A. Manion, K. Seetharaman, F.C. Deschamps, D. Barrera Arellano, J.M. Block. Relationship between antioxidant properties and chemical composition of the oil and the shell of pecan nuts [*Carya illinoinensis* (Wangenh) C. Koch]. Ind. Crops Prod. *45,* 64-73 (2013).

[9] F. Shahidi, H. Miraliakbari, Tree Nut Oils. Pecan, in: F. Shahidi (Ed.), Bailey's Ind. Oil Fat Prod., Sixth Edit, John Wiley & Sons, Inc., Publication, Hoboken, New Jersey 182-183 (2005).

[10] AOAC, Official Methods of Analysis, in: 15th. Ed., Association of Official Analytical Chemists, Arlington, Virginia. (2002).

[11] Y. Yilmaz, R.T. Toledo. Major Flavonoids in Grape Seeds and Skins: Antioxidant Capacity of Catechin, Epicatechin, and Gallic Acid. J. Agric. Food Chem. *52,* 255-260 (2004).

[12] AOCS, Official Methods and Recommended Practices of the American Oil Chemists' Society, in: 4th ed., American Oil Chemists Society, Champaign, IL., USA (2009).

[13] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. *299*, 152-178 (1999).

[14] J.E. Villarreal-Lozoya, L. Lombardini, L. Cisneros-Zevallos. Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. Food Chem. *102,* 1241-1249 (2007).

[15] A.C.P. Prado, A.M. Aragão, R. Fett, J.M. Block. Antioxidant Properties of Pecan Nut [*Carya illinoinensis* (Wangenh.) C. Koch] Shell Infusion. Grasas y Aceites *60,* 330-335 (2009).

[16] AGROSON, Red estatal de estaciones meteorológicas de Sonora, red estatal del clima. (2012). http://agroson.org.mx/ (accessed August 13, 2016).

[17] M. Venkatachalam, H.H. Kshirsagar, N.P. Seeram, D. Heber, T.E. Thompson, K.H. Roux, S.K. Sathe. Biochemical composition and immunological comparison of select pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. J. Agric. Food Chem. *55,* 9899-9907 (2007).

[18] D.L. Ojeda-Barrios, A. Abadía Bayona, Javier Lombardini, Leonardo Abadía, S. Vázquez, J. Martínez. Aspectos nutricionales relacionados a estrés abiótico en nogal pecanero, in: Mem. Científica N° 4, XIV Simp. Int. Nogal Pecanero. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Centro de Investigación Regional del Noroeste, Hermosillo, Mexico, 2013:22-28.

[19] T. Wang, L.A. Johnson. Refining high-free fatty acid wheat germ oil. J. Am. Oil Chem. Soc. *78,* 71-76 (2001).

[20] G. Del Valle Leguizamón, A. González León, R. Báez Sañudo. Grape anthocyanins (*vitis vinifera* l.) and their relation to color. Rev. Fitotec. Mex. *28,* 359-368 (2005).

[21] J.A. John, F. Shahidi. Phenolic compounds and antioxidant activity of Brazil nut (*Bertholletia excelsa*). J. Funct. Foods *2,* 196-209 (2010).

[22] D. Kammerer, A. Claus, R. Carle, A. Schieber. Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. J. Agric. Food Chem. *52,* 4360-4367 (2004).

[23] L.L. Saldanha, W. Vilegas, A.L. Dokkedal. Characterization of flavonoids and phenolic acids in *Myrcia bella* cambess. Using FIA-ESI-IT-MSn and HPLC-PAD-ESI-IT-MS combined with NMR. Molecules *18,* 8402-8416 (2013).

[24] P. Mena, L. Calani, C. Dall'Asta, G. Galaverna, C. García-Viguera, R. Bruni, A. Crozier, D. Del Rio. Rapid and comprehensive evaluation of (Poly)phenolic compounds in pomegranate (*Punica granatum* L.) Juice by UHPLC-MSn. Molecules, *17*, 14821-14840 (2012).

[25] L.-Z. Lin, M.H. Arnly. A Screening method for the identification of glycosylated flavonoids and other phenolic compounds using a standard analytical approach for all plant materials. J Agric. Food Chem. *55*, 1084-1096 (2007).

[26] S.M. Cardoso, S.I. Falcao, A.M. Peres, M.R.M. Domingues. Oleuropein/ligstroside isomers and their derivatives in Portuguese olive mill wastewaters. Food Chem. *129*, 291-296 (2011).

[27] K.S. Robbins, Y. Ma, M.L. Wells, P. Greenspan, R.B. Pegg. Separation and characterization of phenolic compounds from U.S. pecans by liquid chromatography–tandem mass spectrometry. J. Agric. Food Chem. *62*, 4332-4341 (2014).

[28] V.R.S. Tala, V.C. Da Silva, C.M. Rodrigues, A.E. Nkengfack, L.C. dos Santos, W. Vilegas. Characterization of proanthocyanidins from Parkia biglobosa (Jacq.) G. Don. (Fabaceae) by flow injection analysis - Electrospray ionization ion trap tandem mass spectrometry and liquid chromatography/electrospray ionization mass spectrometry. Molecules *18*, 2803-2820 (2013).

[29] M.N. Clifford, W. Wu, J. Kirkpatrick, N. Kuhnert. Profiling the chlorogenic acids and other caffeic acid derivatives of herbal chrysanthemum by LC–MSn. J. Agric. Food Chem. *55*, 929-936 (2007).

[30] R. Erenler, O. Sen, H. Aksit, I. Demirtas, A.S. Yaglioglu, M. Elmastas, I. Telci. Isolation and identification of chemical constituents from *Origanum majorana* and investigation of antiproliferative and antioxidant activities. J. Sci. Food Agric. *96*, 822-836 (2016).

[31] Y. Zhao, X. Li, X. Zeng, S. Huang, S. Hou, X. Lai. Characterization of phenolic constituents in *Lithocarpus polystachyus*. Anal. Methods *6,* 1359-1363 (2014).

[32] M.J. Simirgiotis. Antioxidant capacity and HPLC-DAD-MS profiling of chilean peumo (*Cryptocarya alba*) fruits and comparison with german peumo (*Crataegus monogyna*) from Southern Chile. Molecules *18*, 2061-2080 (2013).

[33] C.M. Rodrigues, D. Rinaldo, L.C. dos Santos, P. Montoro, S. Piacente, C. Pizza, C.A. Hiruma-Lima, A.R.M. Sousa Brito, W. Vilegas. Metabolic fingerprinting using direct flow injection electrospray ionization tandem mass spectrometry for the characterization of proanthocyanidins from the barks of *Hancornia speciosa*. Rapid Commun. Mass Spectrom *21*, 1907-1914 (2007).

[34] N.A. Santos, A.M.T.M. Cordeiro, S.S. Damasceno, R.T. Aguiar, R. Rosenhaim, J.R. Carvalho Filho, I.M.G. Santos, A.S.Maia, A.G. Souza. Commercial antioxidants and thermal stability evaluations. Fuel *97*, 638-643 (2012)

Received: March 6, 2017
Accepted: May 17, 2017