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Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars

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Abstract

Six pecan cultivars were analyzed for their antioxidant capacity (AC), total phenolics (TP), condensed tannin (CT), HPLC phenolic profile, tocopherol and fatty acid composition. Kernels which included the outer brown testa or pellicle, and shells which is the hard cover that surrounds the kernel, were evaluated for each cultivar. Strong correlations were found in kernels between AC and TP for both DPPH ($r^2 = 0.98$) and AC_{ORAC} ($r^2 = 0.75$) antioxidant assays. AC_{ORAC} values ranged from 372 to 817 μmol trolox equivalents/g defatted kernel, corresponding to Desirable and Kanza cultivars, respectively. CT ranged from 23 to 47 mg catechin equivalents/g defatted kernel and TP from 62 to 106 mg of chlorogenic acid equivalents/g defatted kernel. After a consecutive basic-acid hydrolysis, gallic acid, ellagic acid, catechin and epicatechin were identified by HPLC. The TP, AC and CT were 6, 4.5 and 18 times higher, respectively, for shells compared to kernels. The presence of phenolic compounds with high antioxidant capacity in kernels and shells indicates pecans can be considered an important dietary source of antioxidants.
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1. Introduction

Pecans [*Carya illinoinensis* (Wangenh.) K. Koch], native from North America, belong to the Juglandaceae family which also includes walnuts (*Juglans* sp.) (Hall, 2000). Pecan is distributed over an area of geographic and climatic variation extending from northern Illinois and southeastern Iowa to the gulf coast of United States (Grauke, 1991). Recent studies have shown that pecan kernels may improve human serum lipid profile and lower low density lipoprotein levels, due to their high monounsaturated fatty acid content (Rajaram, Burke, Connell, Myint, & Sabate, 2001). Wu et al. (2004) screened common foods and vegetables across the US and reported pecan kernels have the highest antioxidant capacity (AC) and total extractable phenolic content (TP) within the nut group and ranked pecans among the foods with highest phenolic content. According to several studies, phenolics have antiradical activity and are thought to reduce incidence of chronic diseases, including Alzheimer, Parkinson, some types of cancer, and other degenerative diseases (Awika & Rooney, 2004; Li et al., 2005; Mertens-Talcott & Percival, 2005; Zhao, Wang, Chen, & Agarwal, 1999).

Senter, Horvat, and Forbus (1980) screened kernels from Stuart and Schley cultivars for phenolic acid content using a gas chromatographic method revealing the presence of hydroxybenzoic acid derivatives. Gallic acid constituted 138 μg/g of defatted kernel and accounted for 78% of the phenolic acid constituents.

Proanthocyanidins or condensed tannins (CT) have also been reported in pecan kernels (Polles, Hanny, & Harvey, 1981). These type of compounds have biological activities such as antioxidant and antimutagenic properties, which are affected by the degree of polymerization, the monomer structure, and the bond type between monomers (Grimmer,

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Parbhoo, & McGrath, 1992). Gu et al. (2002) reported the presence of B type proanthocyanidins (C4–C8 and C4–C6 bonds) in pecan kernels. Prodelphinidins (3-*O*-gallates) were also found including epigallocatechin, epicatechin-3-*O*-gallate, and the more common flavan-3-ols, catechin and epicatechin. However, no reference was made to cultivar type, growth location, storage or processing conditions. In another study Gu et al. (2004) characterized the degree of polymerization of CT and reported a total proanthocyanidin content of 494.1 ± 86.2 mg/100 g FW in pecan kernels (~0.5% w/w). Monomers, dimers, trimers, tetramers through hexamers, heptamers through decamers, and polymers above 10 subunits were present in amounts of 17.2, 42.1, 26, 101, 84, and 223 mg/100 g, respectively.

Despite the studies mentioned above, the influence of pecan cultivars on phytochemical content and antioxidant capacity has not been characterized. This information is key for breeding programs as well as for the food industry in the selection of cultivars with enhanced nutraceutical properties. The objective of the present study was to characterize six different pecan cultivars for their nutraceutical constituents, including phenolic compounds, antioxidant capacity, vitamin E content and fatty acid profiles.

2. Materials and methods

2.1. Pecan samples

Pecan nuts were mechanically harvested during Fall (September through November) 2004. Desirable, Kanza, Kiowa, Nacono, Pawnee, and Shawnee cultivars were chosen due to their commercial relevance or, in case of the recently-released Nacono (Thompson & Grauke, 2001), for its prospective increase diffusion and production. All cultivars but Kiowa were grown at the USDA Experiment Station located in Brownwood, Texas (BW). Kiowa and one additional batch of Desirable nuts were harvested from a commercial orchard located near Caldwell, Texas (CW). After harvest, approximately 20 kg of nuts per cultivar were transported to the laboratory and stored in a cold room set at −5 °C. For analysis, 4.5 kg of nuts per cultivar were mechanically cracked and the kernel which included the brown outer testa or pellicle was separated from the shell which is the hard cover that surrounds the kernel. After removal of rotten and necrotic kernels, healthy pecan halves were stored at −80 °C in freeze-resistant plastic bags.

2.2. Chemicals

Solvents were HPLC grade and purchased from Fisher Scientific (Houston, TX). Folin–Ciocalteu reagent, vanillin reagent, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, fluorescein sodium salt (FL), and phenolic standards were purchased from Sigma Chemical Co. (St. Louis, MO). AAPH (2,2′-Azobis (2-amidino-propane)-dihydrochloride) was obtained from Wako Chemicals (Richmond, VA). Nanopure water was used in all solutions.

2.3. Pecan sample preparation

Kernels were chopped using a food processor and then defatted with hexane (1:20 w/v) using an Ultraturrax T25 homogenizer (IKA Works, Wilmington, NC). After homogenizing, samples were filtered with a Buchner funnel and slow filtration rate filter paper (Fisherbrand 09-801F, Fisher Scientific, Houston, TX). The cake was defatted two more times and the remaining powder was dried at 35 °C under vacuum for 2 h. The powder was flushed with nitrogen and stored in a sealed container at −20 °C until analyses. Oil was obtained after evaporating hexane with a rotavapor. The oil was flushed with nitrogen and stored at −20 °C until analyses.

Defatted pecan powder (1 g) was placed in 50 mL falcon tubes and homogenized with 20 mL of acetone: water (70:30, v/v) solution. Falcon tubes were capped, placed in an oscillatory shaker at 5 °C and shaken overnight. After shaking, slurries were centrifuged at 18,000 g and supernatants were collected, flushed with nitrogen and stored at −20 °C. A similar extraction protocol was followed to determine TP, AC and CT of pecan shells from each cultivar. Another extraction from shells using only water was performed to assess potential CT leaching from shell to kernel.

According to the literature, phytochemicals in high oil content samples such as pecans may be reported on per gram extract, per gram sample or per gram defatted sample, being the last two more common. In the present study phytochemical content was reported on gram defatted sample basis.

2.4. Extractable phenolic and condensed tannin content

Extractable phenolic compound content (TP) analysis was performed as explained by Swain and Hillis (1959) and adapted for microplate reader measurements. Acetone:water (70:30, v/v) extracts were diluted with water and 13 μL was loaded in each well of a 96 well flat bottom plate (Costar #3595, Corning, Inc., Corning, NY). Water was added to six wells to be used as blanks. Using a multi-channel micropipette, 208 μL of nanopure water was added to each well followed by 13 μL of Folin–Ciocalteu reagent. Mixture was allowed to react for 3 min. After time elapsed, 26 μL of 1N Na_2CO_3 was added. Plates were sealed using two layers of parafilm and allowed to react for 2 h, after which absorbance was read at 725 nm in the microplate reader. A Synergy HT plate reader (Bio-Tek Instruments, Inc., Winooski, VT) was used with different 96-well plates depending on absorbance measurements. A standard curve was made with chlorogenic acid to express TP as mg chlorogenic acid equivalents/g of defatted kernel (mg CAE/g). Six replicates of each sample were evaluated.

Procyanidins or CT were evaluated using the vanillin assay (Price, Vanscoyoc, & Butler, 1978). An aliquot of 0.5 g of defatted kernel was placed in a centrifuge tube and 15 mL of 1% HCl in methanol was added to each sample. Each tube was vortexed and placed in a water bath at 30 °C with constant shaking for 20 min and vortexing every 10 min. After incubation, tubes were centrifuged and supernatants were extracted. Aliquots of the supernatants were placed in two separate assay tubes, one for the sample determination and the other for blank determination. Samples and blanks were incubated for exactly 20 min after adding 5 mL of the vanillin reagent (0.5 g of reagent and 200 mL of 4% HCl methanol) to samples and 4% HCl in methanol to the blanks. After 20 min, absorbance was read at 500 nm from of each sample and blank using a HP 8452 A diode array spectrophotometer (Hewlett Packard, Palo Alto, CA). Samples absorbance was rectified with the blank standard and compared against a standard curve made with catechin. Results were expressed as mg catechin equivalents/g of defatted sample (mg CE/g). Analyses were done using six replicates per cultivar.

2.5. Phenolic hydrolysis and HPLC analysis

To determine phenolic profile, extracts used for AC and TP assays were also analyzed by high performance liquid chromatography (HPLC). Acetone from extracts was evaporated under vacuum using a SpeedVac concentrator (Thermo, Marietta, Ohio) and 1 mL of water residue was transferred to an assay tube and diluted with 1 mL of 8 N NaOH. Samples were flushed with nitrogen, capped and allowed to react for 16 h in the dark. After basic hydrolysis, 1.33 mL of 6 M HCl was added to the tubes and flushed with nitrogen, capped and heated using a block heater (Fisher Scientific, Houston TX) at 85 °C for 45 min. After acid hydrolysis, 1 mL of samples was filtered with a 0.2 μm PTFE filter (Fisher Scientific, Houston, TX) and 20 μL was injected into the HPLC system. The HPLC system was equipped with two Waters 515 gradient pumps (Waters Corp., Mildford, MA) and coupled with a Waters 717 autosampler (Waters Corp., Mildford, MA). An Atlantis C18 column (5 μm particle size, 4.6 mm × 150 mm; Waters Corp., Milford, MA) coupled with a guard column of the same chemistry was used to separate phenolic compounds. A photodiode array detector was used to scan absorbance from 190 nm to 500 nm. Peak spectra, retention times, and standard spikes were used for determination of compounds. Nanopure water, acidified to pH 2.3 with 2 M HCl (solvent A) and acetonitrile (solvent B) were used as mobile phases. Solvent gradient was used as follows: from 0 to 5 min isocratic 85% A flow, from 5 to 30 min a linear gradient of 85% A to 100% B, and from 35 to 40 min isocratic conditions of 100% B. After termination of the cycle, 30 min of column equilibration (85% A) were allowed prior next injection. Standard curves of the identified compounds were elaborated by dissolving standards in methanol and injecting them into the HPLC. Samples and standard curves were analyzed by triplicate.

2.6. Antioxidant capacity

2.6.1. DPPH assay

DPPH free radical was used to measure antioxidant capacity (AC_{DPPH}) as described by Brand-Williams et al. (Brand-Williams, Cuvelier, & Berset, 1995) and adapted for microplate reader measurements. Extracts were diluted in methanol and 13 μL was pipetted into each well of a 96 well flat bottom plate (Costar #3595, Corning, Inc., Corning, NY). Using a multichannel pipette, 247 μL of DPPH aliquot in methanol was added. Plates were then tightly sealed with two layers of parafilm to prevent evaporation and placed in the dark at 20 °C. After 24 h, microplates were read at 515 nm in a plate reader (Synergy HT, Bio-Tek Instruments, Inc., Winooski, VT) and readings of blanks with water were subtracted from each sample. A standard curve was prepared using trolox as reference reagent. AC was expressed as μg trolox equivalents/g of defatted sample (μg TE/g).

2.6.2. ORAC assay

A modification of the procedure described by Wu et al. (2004) was used for hydrophilic AC (AC_{ORAC}). All reagents were dissolved in 75 mM phosphate buffer pH 7.4. Each well of clear-bottom 96-well black plates (Costar #3631, Corning, Inc., Corning, NY) was loaded with 25 μL of extracts aliquot and incubated at 37 °C for 45 min prior to analysis. Fluorescein sodium salt (FL) was used as protein probe and AAPH as free radical source. FL stock solution (FL_1) was prepared by diluting 112.5 mg of FL powder in 50 mL of phosphate buffer using a volumetric flask. A second FL solution (FL_2) was made by diluting 100 μL of FL_1 in 10 mL of buffer. After preparation, FL_1 and FL_2 solutions were stored at 2 °C. Prior to analysis a third solution (FL_3) was made by dissolving 400 μL of FL_2 in 25 mL of buffer. AAPH solution was prepared after incubation of buffer at 37 °C for 45 min and prompt dissolution of 260 mg of AAPH pellets. After priming injectors, 200 μL of FL_3 were injected in each well followed by 75 μL aliquots of APPH solution.

Fluorescence readings were done in a plate reader (Synergy HT, Bio-Tek Instruments, Inc., Winooski, VT) using excitation and emission wavelengths of 485 nm and 520 nm, respectively. Each well was read at ~1 min intervals during 50 min. The loss of fluorescence was recorded and processed by analytical software package KC-4 v. 3.4. (Bio-Tek Instruments, Inc., Winooski, VT) Samples were compared against trolox standard and a blank curve made from normalized data using the area under the curve. Results were expressed as μmol trolox equivalents/g defatted kernel (μmol TE/g). In addition, the specific antioxidant capacity was defined in this study as the ratio of

total antioxidant capacity / total phenolic content (e.g. AC_{ORAC}/TP) and expressed as μmol trolox equivalents/mg chlorogenic acid equivalent. The specific antioxidant capacity provides information of the effectiveness of phenolics to neutralize free radicals. A higher specific antioxidant capacity means phenolic compounds have a higher capacity to stabilize free radicals.

2.7. Fatty acid profile

Fatty acid methyl esters were analyzed in a Varian CP 3800 gas chromatograph (Palo Alto, CA) coupled with a Varian CP-8200 autosampler and a flame ionization detector (FID). A Varian FAME fused silica capillary column (100 m × 0.25 mm, Varian CP-Select CB) was used to determine lipid profile. Oven temperature gradient was set from 0–30 min at 185 °C and from 30–45 min at 235 °C with an increase of 20 °C/min. FID temperature was set at 270 °C, and helium, air and hydrogen flows at 1.6, 300 and 35 mL/min, respectively. Fatty acid derivatization was performed using the protocol described by Misir, Laarveld, and Blair (1985). Approximately 0.15 g of oil was diluted in 3 mL of diethyl ether and 0.2 mL of 20% tetramethyl ammonium hydroxide in water was added and allowed to react for 5 min. After time elapsed, 0.5 mL of methanol was added and vortexed for 1 min. After phase separation, 1 mL of the upper organic phase was transferred to a vial and capped for injection into the gas chromatograph.

2.8. Tocopherol content

Tocopherol content was determined as explained by Mendoza, Pons, Bargallo, and Lopez-Sabater (2003). Pecan oil was weighted (~0.5 g) and 2 ml of methanol was added. After the sample was vortexed for 1 min, 500 μL of hexane was added and vortexed again. Samples were then centrifuged and 1 mL of the top layer was extracted and filtered using 0.2 μm PTFE filters. Twenty microliters of the filtered sample was injected into the HPLC. HPLC system was equipped with a Lichrosorb® Spherisorb ODS2 C18 column (5 μm particle size, 4.6 mm × 250 mm) and a guard column of the same chemistry (Waters Corp., Mildford, MA). An isocratic flow of 1 mL/min of methanol 100% was used as mobile phase. Concentration of α-, β-, and γ-tocopherols standard solutions were determined as suggested by AOCS official method Ce 8-89 (1997). Peak spectra at 295 nm and retention times were used for identification and quantification.

2.9. Statistical analysis

To determine statistical difference between means ($p < 0.05$), ANOVA and Tukey's Honestly Significant Differences (HSD) were calculated using SPSS statistical software package v. 11.5 (SPSS Inc., Chicago, IL). Results are expressed as mean values ± standard error.

3. Results and discussion

3.1. Phenolic constituents

The total extractable phenolic content (TP) and condensed tannin content (CT) were significantly affected by pecan cultivar (Table 1). TP ranged from 62 to106 mg CAE/g defatted kernel with Kanza cultivar showing the highest TP value and Desirable BW the lowest. For all cultivars, the following trend was found in TP: Kanza > Nacono ≈ Kiowa ≈ Pawnee ⩾ Shawnee ≈ Desirable CW ⩾ Desirable BW. In previous studies unknown pecan cultivars were reported to have a TP of ~2016 (Wu et al., 2004) and 1284 mg gallic acid equivalents/100 g FW (mg GAE/100 g FW) (Kornsteiner, Wagner, & Elmadfa, 2006) which correspond to 112 and 71 mg chlorogenic acid equivalents/g defatted kernel, respectively, assuming a conversion factor of 0.6 from CAE to GAE (Kyoung Chun & Kim, 2004) and 70% lipid content (Rudolph, Odell, Hinrichs, Hopfer, & Kays, 1992).

The CT content evaluated with the vanillin assay showed differences among cultivars, ranging from 23 to 47 mg CE/g defatted kernel and representing 0.7–1.4% of kernel weight, similar to the values found by Polles et al. (1981). Kanza presented the highest CT values while Pawnee the lowest. Pecan cultivars showed the following descending order in relation to CT: Kanza ⩾ Nacono ≈ Shawnee ⩾ Kiowa > Desirable CW ≈ Desirable BW ≈ Pawnee (Table 1). In general, the ratio CT/TP ranged from 0.31 to 0.56 indicating that CT was an important constituent of the phenolics present in pecan kernels.

HPLC phenolic profiles were determined for all pecan cultivars. Extracts prior to hydrolysis had no identifiable peaks at 280 nm (Fig. 1A). After a basic hydrolysis (NaOH), some phenolic compounds were identified using peak spectra and retention times; however after an additional acid hydrolysis with HCl the chromatogram revealed additional peaks (Fig. 1B). Gallic acid, ellagic acid, catechin and epicatechin were identified using standards. A peak at 13 min (peak 4) had a spectra similar to that of ellagic acid (peak 5) (Fig. 1C and D) and was tentatively identified as an ellagic acid derivative (Breitfellner, Solar, & Sontag, 2002). The present study revealed the presence of gallic acid in the range of 651 to 1300 μg/g defatted kernel and ellagic acid in the range of 2505 to 4732 μg/g defatted kernel, with no significant differences among pecan cultivars for gallic acid ($p > 0.05$) and ellagic acid ($p > 0.05$). These results confirm the presence of hydrolyzable tannins in pecan kernels, containing two types of phenolic acids (i.e., gallo- and ellagitannins). The amounts of catechin and epicatechin in the HPLC phenolic profile were minimal when compared to gallic and ellagic acids (Fig. 1B). This could be associated with the alkaline oxidation of these compounds during basic hydrolysis (Jorgensen, Marin, &

Table 1
Phenolic content, antioxidant capacity, and condensed tannin content of defatted pecan kernels from different cultivars

Cultivar	TP[A] (mg CAE/g)	CT[B] (mg CE/g)	CT/TP ratio	AC_{ORAC}[C] (μmol TE/g)	AC_{DPPH}[D] (mg TE/g)	AC_{ORAC}/TP ratio
Kanza	106 ± 2.3 a[E]	47 ± 1.9 a	0.44	817 ± 67 a	135 ± 4.2 a	7.7
Nacono	76 ± 2.2 b	43 ± 2.7 ab	0.56	688 ± 54 ab	95 ± 4.0 bc	9.0
Kiowa	76 ± 2.5 b	36 ± 3.9 bc	0.47	568 ± 31 bcd	100 ± 2.9 b	7.4
Pawnee	72 ± 0.9 b	23 ± 0.9 d	0.32	562 ± 38 bcd	88 ± 1.8 cd	7.8
Shawnee	71 ± 1.9 bc	38 ± 4.9 b	0.53	623 ± 45 bc	89 ± 2.4 bcd	8.8
Desirable CW	70 ± 2.0 bc	28 ± 0.8 d	0.40	449 ± 14 cd	90 ± 1.4 bcd	6.4
Desirable BW	62 ± 2.3 c	25 ± 1.4 d	0.41	373 ± 11 d	81 ± 1.4 d	6.0
Average[F]	76 ± 1.9	34 ± 1.3	0.45 ± 0.0	583 ± 32	97 ± 6.7	7.6 ± 0.5

[A] Total extractable phenolic content (Folin–Ciocalteu assay).
[B] Condensed tannin content (vanillin–HCl assay).
[C] Antioxidant capacity (ORAC assay).
[D] Antioxidant capacity (DPPH free radical assay).
[E] Values in a column with similar letters are not significantly different (Tukey HSD, $p < 0.05$).
[F] Overall mean values ± SE.

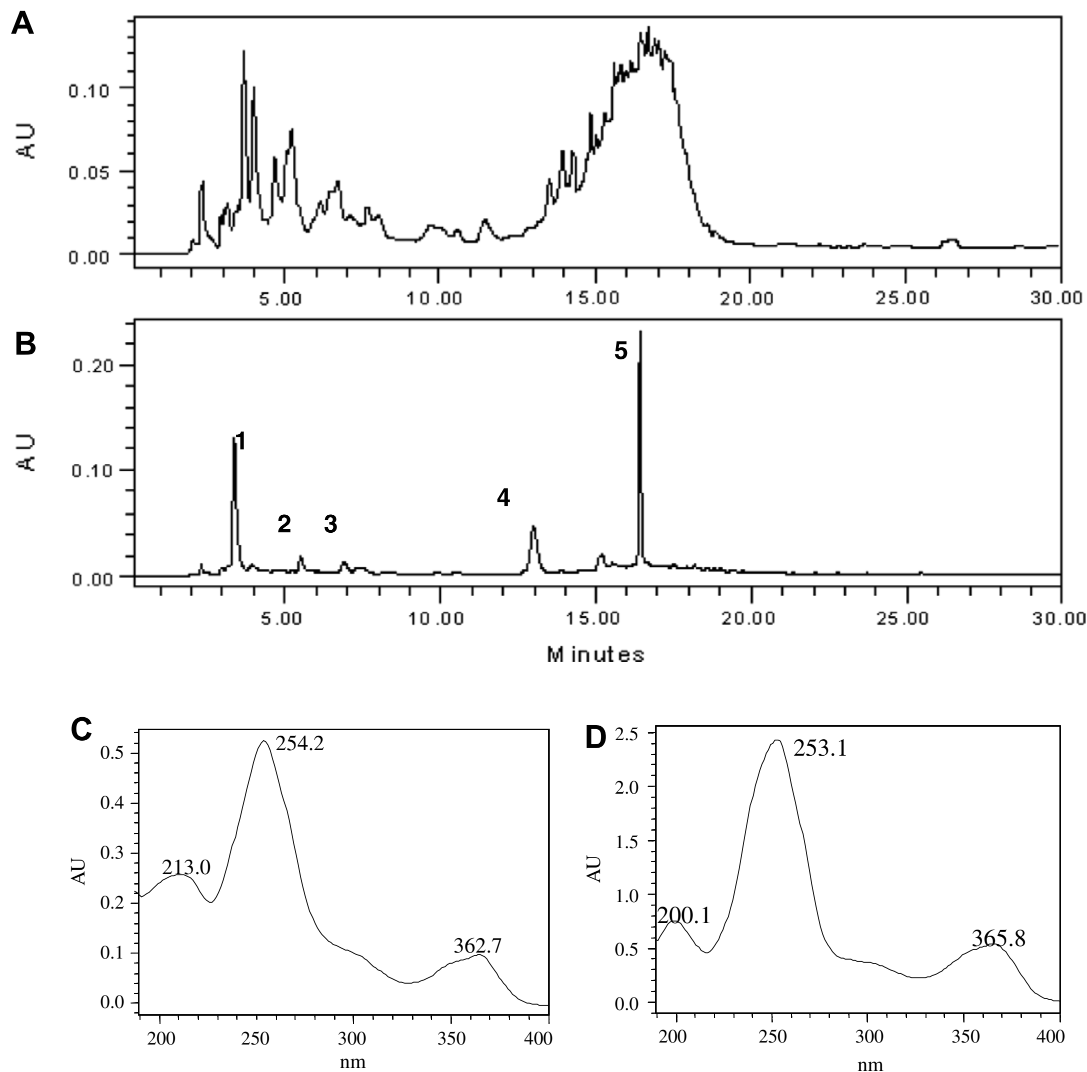

Fig. 1. HPLC chromatograms of (A) non-hydrolyzed and (B) basic-acid hydrolyzed extracts of defatted Kanza pecan kernels. Identified peaks correspond to (1) gallic acid (RT, 2.9 min; λ_{max} 215, 271 nm); (2) catechin (RT, 5.9 min; λ_{max} 202, 277 nm); (3) epicatechin (RT, 6.9 min; λ_{max} 201, 277 nm); (4) ellagic acid derivative (RT, 12.9 min, λ_{max} 213, 254, 363 nm); (5) ellagic acid (RT, 16.2 min, λ_{max} 200, 253, 366 nm); AU = absorbance units; horizontal axis is retention time in minutes (RT). (C) UV-visible spectra for peak 4 and (D) peak 5, nm = nanometer.

Kennedy, 2004). Under the hydrolysis conditions used, precipitation was observed which could be associated to condensed tannins (Hagerman, 2002). Strong alkaline conditions could also affect the recoveries of gallic acid as reported by Mattila and Kumpulainen (2002). The presence of hydroxybenzoic acid derivatives in pecan kernels was reported previously by Senter et al. (1980) using a gas chromatography method. That study reported that the content of gallic acid was 130 μg/g defatted kernel, corresponding to ~78% of the total phenolic acids. The present study reports a 10-fold higher content in gallic acid and identifies ellagic acid for the first time in pecan kernels. In addition to hydrolyzable tannins, the presence of 3-*O*-gallates in pecan kernels reported by Gu et al. (2003) may also be a source of gallic acid under the hydrolysis conditions used.

Phenolic acids, catechins and condensed tannins have been reported to be present mainly in the outer testa or pellicle of the kernels (Polles et al., 1981; Senter, Forbus, & Smith, 1978; Senter et al., 1980) and it is likely that hydrolyzable tannins as well (Jurd, 1956). When analyzing extractable phenolics from nut shells of the same cultivars, the average TP and CT values obtained were 6 and 18 times higher, respectively, than those found in kernels (Table 2). Kanza shells showed the highest values in TP and CT while Desirable CW the lowest values. Forbus and Smith (1971) suggested that tannins leach from shells to kernels during soaking and preconditioning during commercial processing. Another possible source of leaching could be cold room storage prior to cracking and shelling, due to water condensation inside nuts, as a result of small temperature fluctuations. Water extraction of shells for condensed tannin content (CTw) analysis was 10–23 times lower (Table 2) than CT using acetone:water (70:30, v/v) as extraction solvent. Interestingly, CTw for shells showed values similar to those found in kernels using acetone:water solvent (Table 1). It seems that phenolics from shells are mainly formed by condensed tannins ($TP \approx CT$) and their presence may affect the content of kernel phenolics. This should be considered as a factor when studying differences among cultivars.

3.2. Antioxidant capacity

The antioxidant capacity (AC) of pecan kernels was significantly affected by cultivars (Table 1). In both assays, AC_{DPPH} and AC_{ORAC}, defatted kernels of Kanza variety showed the highest AC values and Desirable BW the lowest. Strong correlations were found between AC_{ORAC} and TP ($r^2 = 0.75$) and AC_{DPPH} and TP ($r^2 = 0.98$) (Fig. 2A and B). A strong correlation was found for AC_{ORAC} and CT ($r^2 = 0.75$) as well. AC_{ORAC} ranged from 373 to 817 μmol TE/g defatted kernel and showed an AC_{ORAC}/TP ratio (specific antioxidant capacity, μmol TE/mg CAE) of 6.0 to 9.0 (Table 1). Wu et al. (2004) reported previously an ORAC value of 583 μmol TE/g defatted kernels (unknown variety and assuming a 70% oil content) and a AC_{ORAC}/TP ratio of 8.7.

The range values obtained for CT/TP and AC_{ORAC}/TP imply that proportions of condensed and hydrolyzable tannins differ for each cultivar and this proportion determines the specific antioxidant activity of the phenolics present in each pecan cultivar. Nacono kernels showed the highest AC_{ORAC}/TP ratio among all cultivars studied. A higher specific antioxidant capacity (AC_{ORAC}/TP, μmol TE/mg CAE) implies that phenolic compounds present in the cultivar have a higher capacity to stabilize free radicals. The AC_{DPPH} for pecan shells was ~4.5 times higher than those found in kernels. AC_{DPPH} was highest in Kanza and Kiowa. There was a strong correlation between AC_{DPPH} and TP from nut shells ($r^2 = 0.61$).

Shells represent a large by-product of the pecan industry. Shell percentage in pecan nut varies from 40% to 50% (Worley, 1994). Processing plants have found limited market for pecan shells with minimal profit. In the present study, the high antioxidant capacity observed shows a potential alternative use of pecan shells as a novel source of antioxidants.

Table 2
Phenolic compounds and antioxidant capacity of shells from different pecan cultivars extracted with acetone: water (70:30, v/v) or water

Cultivar	TP[A] (mg CAE/g)	AC_{DPPH}[B] (mg TE/g)	CT[C] (mg CE/g)	CTw[D] (mg CE/g)
Kanza	633 ± 29 a[E]	675 ± 18 a	876 ± 32 a	48 ± 2 a
Nacono	451 ± 6 c	442 ± 7 c	550 ± 23 cde	45 ± 2 a
Kiowa	344 ± 10 de	331 ± 11 d	598 ± 15 e	26 ± 1 b
Pawnee	537 ± 10 b	582 ± 29 b	704 ± 35 bc	49 ± 2 a
Shawnee	506 ± 12 b	444 ± 3 c	827 ± 14 ab	48 ± 2 a
Desirable CW	290 ± 1 e	453 ± 6 c	388 ± 14 ef	29 ± 2 b
Desirable BW	378 ± 17 d	482 ± 30 c	495 ± 21 c	47 ± 1 a
Average[F]	448 ± 45	487 ± 42	634 ± 67	42 ± 1.5

[A] Total extractable phenolic content (Folin–Ciocalteu assay).
[B] Antioxidant capacity (DPPH free radical assay).
[C] Condensed tannin content of acetone:water (70:30 v/v) extracts (vanillin–HCl assay).
[D] Condensed tannin content of water extracts (vanillin–HCl assay).
[E] Values in a column with similar letters are not significantly different (Tukey HSD, $p < 0.05$).
[F] Overall mean values ± SE.

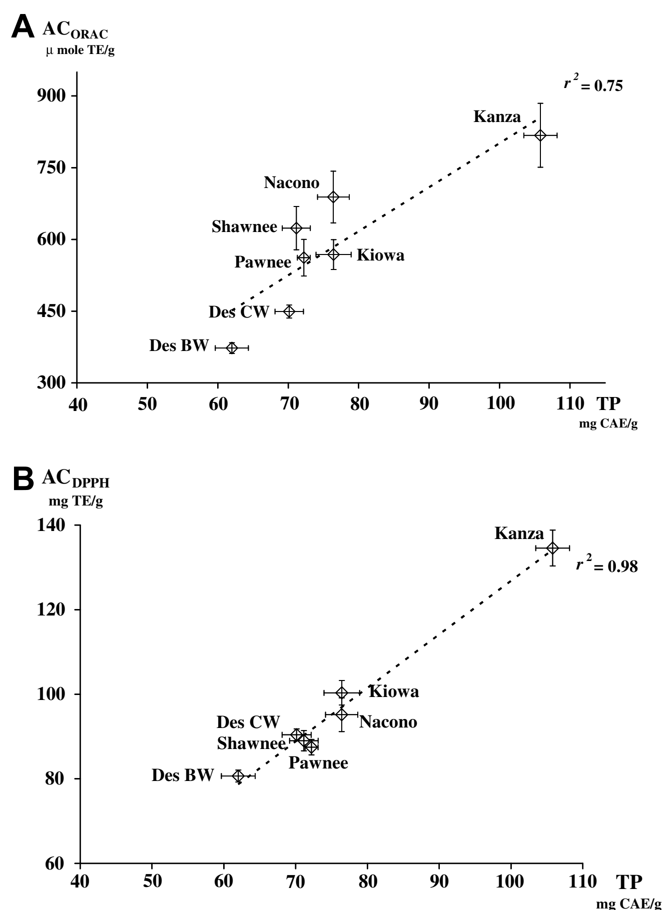

Fig. 2. (A) Correlation between antioxidant capacity measured by ORAC assay (AC_{ORAC}) and total extractable phenolic content (TP) of defatted pecan kernels. (B) Correlation between antioxidant capacity measured by DPPH free radical assay (AC_{DPPH}) and total extractable phenolic content (TP). Each point is the average of 6 replicates per cultivar ± SE.

3.3. Fatty acid profile and tocopherol content

Total lipid content of pecan kernels varies from 65% to 75% of total kernel weight (Toro-Vazquez, Charo-Alonso, & Perez-Briceno, 1999). The oil of the cultivars studied was constituted by oleic, linoleic, palmitic, stearic and linolenic acids forming ~99% of the total lipid content of pecan kernels (Table 3). There was significant variation in fatty acid profile between cultivars. Oleic and linoleic acid ranged across from 53% to 75% and 15% to 36% of lipid composition, respectively (Table 3), while relative content of palmitic, stearic and linolenic acid did not vary significantly across cultivars. A strong inverse correlation between oleic and linoleic acid was found ($r^2 = 0.99$, $y = -0.928x + 84.525$) when pooling data from all cultivars, indicating a possible interconversion of oleic and linoleic acid in a range of pecan kernel varieties (Rudolph et al., 1992).

Desirable kernels grown at two distinct locations (BW and CW) showed differences in their oleic–linoleic composition. Fatty acid profile may depend on environmental conditions, cultivar, maturity and horticultural practices (Grauke, Thompson, Storey, & Sistrunk, 2001; Rudolph et al., 1992). For example, increasing nitrogen fertilization rate increases saturation of monounsaturated fatty acids and increases the polyunsaturation ratio, due to a decrease of monounsaturated fatty acids (Heaton, 1969). Thus, the selection of appropriate cultivation conditions is an important factor to consider when selecting pecan cultivars (Rajaram et al., 2001; Rajaram, Myint, Connell, Burke, & Sabaté, 2000).

Tocopherols are non-polar antioxidants present in pecan kernels (Chun, Lee, Ye, & Eitenmiller, 2002; Fourie & Basson, 1989; Rudolph et al., 1992; Yao, Dull, & Eitenmiller, 1992) and their content make pecan kernels an important nutritional source for the diet (Haddad, Jambazian, Tanzman, & Sabate, 2001). Thus, selection of cultivars with improved tocopherol content is of importance to consumer and industry. There were differences ($p < 0.05$) in the content of γ-tocopherol among the cultivars evaluated ranging from 72 to 135 µg γ-tocopherol/g oil (Table 3), confirming the large variation previously found in pecan kernels (Chun et al., 2002; Demir & Cetin, 1999; Toro-Vazquez et al., 1999). In the present study α- and β-tocopherol were also identified and accounted for less than 5% of total tocopherol content with no significant differences among cultivars ($p > 0.05$, data not shown). Variables such as genetics, environment, maturity and stor-

Table 3
Kernel oil composition and γ-tocopherol content of different pecan cultivars

Cultivar	Palmitic %[A]	Stearic %	Oleic %	Linoleic %	Linolenic %	γ-Toc[B] (µg γ-Toc/g)
Kanza	5	2	71	20	1	105 ± 1 b
Nacono	6	2	53	36	1	135 ± 4 a
Kiowa	5	3	71	20	1	72 ± 6 d
Pawnee	6	3	66	23	1	100 ± 1 bc
Shawnee	6	2	75	15	1	102 ± 2 b
Desirable CW	5	2	71	20	1	84 ± 1 cd
Desirable BW	6	2	59	31	1	126 ± 5 a
Average	6	2	67	23	1	104 ± 8

[A] Fatty acid expressed as percentage of total fatty acid content.
[B] Average γ-tocopherol content, grams of γ-tocopherol per gram of oil ± SE; values in a column with similar letters are not significantly different (Tukey HSD, $p < 0.05$).

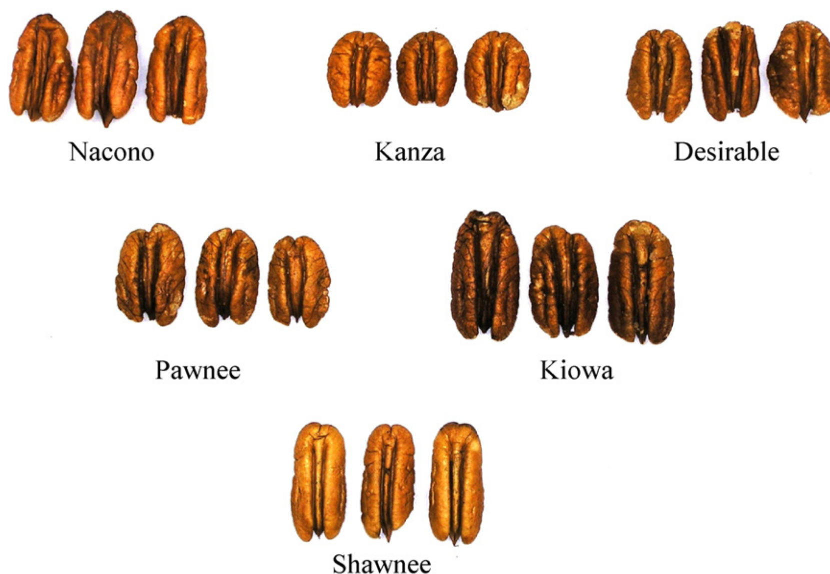

Fig. 3. Pecan kernels from six diferent cultivars harvested in Texas.

age conditions, may affect tocopherol content of pecans (Rudolph et al., 1992). For example, Rudolph et al. (1992) reported a decline of total tocopherol content as harvestable maturity approached and this decline followed distinct degradation rates for different cultivars.

Pecan oils and olive oils have similar lipid composition and tocopherol content (Toro-Vazquez et al., 1999). However, pecan oil seems more stable against rancidity than olive oil, suggesting oxidative protection of other antioxidant compounds in addition to tocopherols. The exact role of tocopherol and phenolic compounds as antioxidants in pecan oil oxidation is still unclear.

In general the phenolic compounds, tocopherols and the high monounsaturated fatty acid profiles suggest an array of potential health benefits which may vary depending on pecan cultivar (Fig. 3). Health benefits of wine and berries have been linked to the content of gallic and ellagic acids and their oligomeric forms (Cerda, Tomas-Barberan, & Espin, 2005; Prior & Cao, 2000; Sadler, Chappas, & Pierce, 2001). The oligomeric forms of phenolic compounds present in pecan kernels and nut shells may differ in their bioavailability. Thus further studies on chemical structure of these phenolic compounds are needed to determine its potential use as a source of dietary antioxidants.

The tendency of pecan trees to bear fruit in 2-year cycles (i.e., large crops followed by little or no crop), can cause differences in compositional attributes of kernels, such as fatty acid composition and tocopherol content (Grauke et al., 2001) and even phenolic compounds. Studies should address the role of crop load on phytochemical composition of pecan kernels. Future research should determine if horticultural practices have any effects on phytochemical content depending on cultivar genetics.

This study can be used as basis for future breeding programs aiming to develop pecan kernels with improved nutritional profile and health benefits.

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