Antioxidant Properties of Pecan Kernels

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Abstract

Pecan kernels from cvs.: Kanza, Nacono, Pawnee, Shawnee, and Desirable were collected and analyzed for their antioxidant capacity (AC), total phenolic content (TP), condensed tannin content (CT), and High-Performance Liquid Chromatography (HPLC) phenolic profile. Values obtained for antioxidant capacity assessed with the Oxygen Radical Absorbance Capacity (ORAC) assay (AC_{ORAC}) followed the trend 'Kanza' > 'Nacono' ≥ 'Shawnee' ≥ 'Pawnee' > 'Desirable'. Similar trends were observed for TP, CT, and AC measured with the DPPH assay. 'Kanza' had the greatest AC_{ORAC} value and 'Desirable' the least, with 817 and 372 µmoles of trolox equivalents/g defatted kernel, respectively. Cultivars differed greatly in their phenolic content; CT ranged from 23 to 47 mg catechin equivalents/g defatted kernel and TP from 62 to 106 mg chlorogenic acid equivalents/g defatted kernel. After basic-acid hydrolysis, gallic and ellagic acids were identified, suggesting the presence of hydrolyzable tannins. The presence of phenolic compounds with high antioxidant capacity indicates that pecan kernels should be added to the list of important dietary sources of antioxidants.

INTRODUCTION

Pecans have been used for centuries by Native Americans (Hall, 2000) and is the only tree nut with commercial importance native to North America. Over 1000 different pecan varieties have been described (Thompson and Young, 1985); however, about 57% of improved acreage is composed of only four varieties (Stuart, Western Schley, Desirable, and Wichita) and about 90% by 33 varieties (Thompson, 1990).

Pecan kernels are commonly used as an ingredient for desserts, candies, or ice cream however, until recently they were not considered of value for their nutritional attributes. Lately, pecan kernels have been observed to be beneficial for human health in numerous ways. They improve serum lipid profile and may play an important role in reducing the risk for heart disease (Rajaram et al., 2000, 2001). Most recently, they have been identified to have phenolic compounds (Wu et al., 2004). This compounds act as antioxidants and have the ability to lower the incidence of chronic diseases such as; Alzheimer's, Parkinson's, some types of cancer, and other degenerative diseases, according to several studies (Mertens-Talcott and Percival, 2005; Tam et al., 2006a), Other studies have indicated pecans are a good source of this important group of phytochemicals (Chun et al., 2002; Kornsteiner et al., 2006; Wu et al., 2004). Wu et al. (2004) reported the antioxidant capacity of over 100 different kinds of foods across the United States. Several nuts, according to this study, ranked among the foods with high antioxidant capacity, with pecans having the highest antioxidant capacity in the nut group. However, despite the studies mentioned above, the influence of pecan cultivars on phytochemical content and antioxidant capacity has not been characterized. This information is essential for breeding programs, as well as the food industry in the selection of cultivars with enhanced nutraceutical properties.

The objective of this study was to characterize five different pecan cultivars for their nutraceutical constituents, including phenolic compounds, antioxidant capacity,

vitamin E content, and fatty acid profiles.

MATERIALS AND METHODS

Five cultivars (Kanza, Nacono, Pawnee, Shawnee, and Desirable) were used for the study. Pecan nuts were collected in the fall season 2004 from 10 trees/cultivar grown at the USDA Experiment Station located in Brownwood, Texas. After harvest, 4.5 kg of nuts per cultivar were mechanically cracked and shelled, and pecan kernels were stored at -80°C. Kernels were chopped using a food processor, defatted with hexane (1:20 w/v), and filtered with a Buchner funnel and slow filtration rate filter paper (Fisherbrand 09-801F, Fisher Scientific, Houston, TX). After the cake was defatted two more times, the powder was dried at 35°C under vacuum for 2 h, then flushed with nitrogen, and stored in a sealed container at -20°C until analyses. Defatted pecan powder (1 g) was then placed in 50 ml falcon tubes and homogenized with 20 ml of acetone:water (70:30) 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.

Extractable phenolic compound content (TP) analysis was performed as explained by Swain and Hillis (1959) and adapted for microplate reader measurements. 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 (Sigma Chemical Co., St. Louis, MO) equivalents per g of defatted kernel (mg CAE/g). Six replicates of each sample were evaluated.

Condensed tannins (CT) were evaluated using the vanillin assay (Price et al., 1978). Results were expressed as mg of catechin (Sigma Chemical Co, St. Louis, MO) equivalents/g of defatted sample (mg CE/g). Analyses were done using six replicates per cultivar.

Antioxidant capacity (AC) was measured using DPPH free radical (AC_{DPPH}) (Brand-Williams et al., 1995) and adapted for microplate reader measurements. A standard curve was prepared using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) (Sigma Chemical Co., St. Louis, MO) as reference reagent. AC was expressed as μg of trolox equivalents per g of defatted sample (μg TE/g). Hydrophilic antioxidant capacity (AC_{ORAC}) was determined by modifying the procedure described by Wu et al. (2004). Fluorescence readings were done using excitation and emission wavelengths of 485 nm and 520 nm, respectively. Each well was read at ~1 min intervals during 50 min. Samples were compared against trolox standard and a blank curve made from normalized data using the area under the curve (AUC). Results were expressed as μmol of trolox equivalents per g of defatted kernel (μmol TE/g).

To determine phenolic profile, extracts used for AC and TP assays were analyzed by high performance liquid chromatography (HPLC). Acetone from extracts was evaporated under vacuum using a SpeedVac concentrator (Thermo, Marietta, OH) and 1 ml of the water residues 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 x 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 (Sigma Chemical Co. St. Louis, MO) 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 to the 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.

Statistical differences between means ($P<0.05$) were determined with ANOVA and Tukey's Honestly Significant Differences (HSD) using SPSS statistical software package v. 11.5 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The total extractable phenolic content (TP) and condensed tannin content (CT) were significantly affected by the cultivar (Fig. 1). TP ranged from 62 to 106 mg CAE/g defatted kernel with 'Kanza' showing the highest TP value and 'Desirable' the lowest. The CT content ranged from 23 to 47 mg CE/g defatted kernel and representing 0.7 to 1.4% of kernel weight. 'Kanza' had the highest CT values while 'Pawnee' the lowest. 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.

The AC was significantly affected by cultivars (Fig. 2). In both assays, AC_{DPPH} and AC_{ORAC}, 'Kanza' showed the greatest AC values and 'Desirable' the least. Strong correlations were found between AC_{ORAC} and TP ($r^2 = 0.75$), AC_{DPPH} and TP ($r^2 = 0.98$) and between AC_{ORAC} and CT ($r^2 = 0.75$) (data not shown). 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 (data not shown). 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 ORAC/TP ratio of 8.7.

The range values obtained for CT/TP and ORAC/TP imply that proportions of condensed and hydrolyzable tannins differed for each cultivar and this proportion determined the specific antioxidant activity of the phenolics present in each pecan cultivar. 'Nacono' kernels had 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 present study revealed the presence of gallic acid at 1132 µg/g of defatted kernel and ellagic acid at 4706 µg/g defatted kernel, with no significant differences among pecan cultivars for either acid (Table 1). The presence of ellagic acid in such high levels suggests not only the occurrence of simple phenolic acid esters, but of more complex structures derived from these compounds, such as hydrolyzable tannins. These structures have been found to have antioxidant and chemopreventive activities and to be present in human plasma after consumption (Cerda et al., 2005; Seeram et al., 2004). The use of hydrolysis treatments and the obtained phenolic compounds suggest the potential presence of compounds such as: epigallocatechin, epigallocatechin gallate, and other flavonoid derivatives, (Lambert et al., 2006; Tam et al., 2006b), as well as hydrolyzable tannins. The specific structures of these phenolic acid oligomers need to be investigated to determine the potential health benefits of pecan kernels.

This study can be used as basis for future breeding programs aiming to develop pecan kernels with improved nutritional profile and health benefits. Further studies should address the role of crop load, horticultural practices, and biotic and abiotic stresses on phytochemical composition of pecan kernels.

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Tables

Table 1. Gallic and ellagic acid content of pecan kernels from five different cultivars after consecutive basic and acid hydrolyses.

Cultivar	Gallic acid[z] (µg GA/g)	Ellagic acid[y] (µg EA/g)
Kanza	1132 ± 321 a[x]	4706 ± 1014 a
Nacono	651 ± 110 a	2505 ± 103 a
Pawnee	877 ± 97 a	3008 ± 690 a
Shawnee	748 ± 209 a	2609 ± 475 a
Desirable	886 ± 391 a	3981 ± 1345 a
Average	950 ± 87	3701 ± 323

[z] Gallic acid content, micrograms of gallic acid per gram of defatted kernel ± S.E.

[y] Ellagic acid content, micrograms of ellagic acid per gram of defatted samples ± S.E.

[x] Mean values followed by the same letter within each column are significantly different (Tukey HSD, $P<0.05$).

Figures

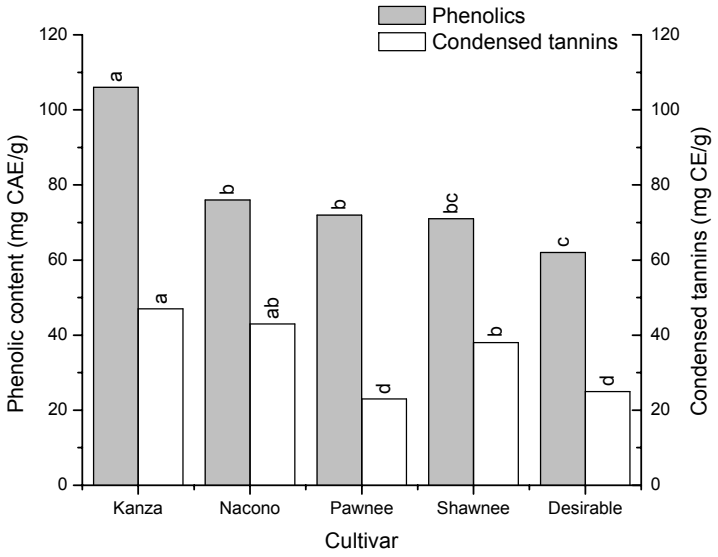

Fig. 1. Total extractable phenolic content (Folin-Coicalteu method) and condensed tannins (vanillin-HCl assay) of kernels of five different pecan cultivars. Extractable phenolic content data are expressed as milligrams of chlorogenic acid equivalents per gram of defatted kernel. Data of condensed tannins are expressed in milligrams of catechin equivalents per gram of defatted kernel.

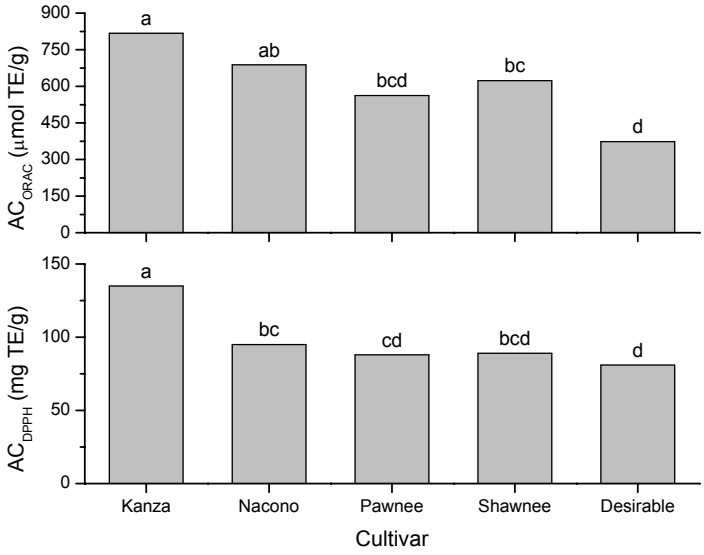

Fig. 2. Antioxidant capacity of kernels of five different pecan cultivars determined with the ORAC (top) and the DPPH assay (bottom).