JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Biochemical Composition and Immunological Comparison of Select Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] Cultivars

MAHESH VENKATACHALAM,[†,‡] HARSHAL H. KSHIRSAGAR,[†] NAVINDRA P. SEERAM,[§] DAVID HEBER,[§] TOMMY E. THOMPSON,[||] KENNETH H. ROUX,[⊥] AND SHRIDHAR K. SATHE*,[†]

Department of Nutrition, Food, and Exercise Sciences, College of Human Sciences, Florida State University, Tallahassee, Florida 32306, Center for Human Nutrition, David Geffen School of Medicine, UCLA Health Sciences, University of California at Los Angeles, Los Angeles, California 90095, USDA-ARS Pecan Breeding and Genetics, Somerville, Texas 77879, and Department of Biological Science, Florida State University, Tallahassee, Florida 32306

On an edible portion basis, pecan moisture, protein, lipid, total soluble sugars, and ash contents ranged from 2.1% to 6.4%, 6.0% to 11.3%, 65.9% to 78.0%, 3.3% to 5.3%, and 1.2% to 1.8%, respectively. With the exception of a high tannin (2.7%) Texas seedling, pecan tannin content was in a narrow range (0.6–1.85%). Unsaturated fatty acids (>90%) dominated pecan lipid composition with oleic (52.52–74.09%) and linoleic (17.69–37.52%) acids as the predominant unsaturated fatty acids. Location significantly influenced pecan biochemical composition. Pecan lipid content was negatively correlated with protein ($r = -0.663$) and total sugar ($r = -0.625$). Among the samples tested using SDS–PAGE a common pattern, with minor differences, in subunit polypeptide profiles was revealed. Rabbit polyclonal antibody-based immunoblotting experiments (Western blot) also illustrated the similarity in polypeptide profiles with respect to immunoreactivity. All tested cultivars registered similar immunoreactivity when their protein extracts (each at 1 mg/mL) were assessed using inhibition ELISAs (mean ± standard deviation = 0.89 ± 0.20; $n = 27$) with the USDA "Desirable" cultivar as the reference standard (immunoreactivity designated as 1.0).

KEYWORDS: Pecans; fatty acids; cultivar; tannin; proximate composition; protein; polypeptide; rabbit polyclonal antibody; immunoreactivity

INTRODUCTION

Pecans have been grown in North America for a very long time. The name "pecan" came from the Algonquin Indian word "pacaan" that also included walnuts and hickories and was used to describe "all nuts requiring a stone to crack". Originating in central and eastern North America and the river valleys of Mexico, pecans were widely used by precolonial residents. Native American tribes in the United States and Mexico used wild pecans as a major food source during autumn. Pecans [*Carya illinoinensis* (Wangenh.) K. Koch] belong to the Juglandaceae family that also includes other tree nuts such as walnuts, hickory nuts, heartnuts, and butternuts (*1*).

Popularly used as a snack food (roasted/salted), pecans are also used in a variety of food products including the widely enjoyed pecan pie, baked goods, candy and confections, dessert toppings, salads, and several main dishes. Pecan nut seeds are a high-energy food (~690 kcal/100 g) as lipids [up to 75% (w/w)] and carbohydrates [up to 18% (w/w)] make up the bulk of the seed kernel weight (*2–4*).

The United States produces more than 80% of the world's pecans. During 1999–2005, the annual pecan production in the United States was 173–400 million pounds (in-shell) with an estimated market value of $201–407 million (*5*). A multistate crop, pecans display a high degree of genetic variability across current commercial cultivars and have a system of heterodichogamy to ensure cross-pollination (*6*). Each cultivar vegetatively propagated as clones is genetically invariant. Starting in late 1800s, the proportion of improved pecan cultivars increased significantly, resulting in over 1000 named and documented pecan cultivars (*7*). Currently improved cultivars account for more than 75% of the pecans produced in the United States (*5, 8*). Despite several reports on the chemical composition of pecan cultivars published over the last 30 years, comparative chemical

* Corresponding author. Tel: (850)-644-5837. Fax: (850)-645-5000. E-mail: ssathe@mailer.fsu.edu.
[†] Department of Nutrition, Food, and Exercise Sciences, Florida State University.
[‡] Present address: Roquette America Inc., Keokuk, IA 52632.
[§] University of California at Los Angeles.
[||] USDA-ARS Pecan Breeding and Genetics.
[⊥] Department of Biological Science, Florida State University.

10.1021/jf0714721 CCC: $37.00 © 2007 American Chemical Society
Published on Web 11/01/2007

Table 1. Pecan Cultivar, History of Origin, and Physical Characteristics[a]

cultivar	origin	nuts/lb	% kernel
Burkett[c]	TX seedling	43	55
Caddo[b]	'Brooks' × 'Alley'	60	56
Cape Fear	open-pollinated seed of 'Schley'	45	54
Cheyenne	'Clark' × 'Odom'	48	58
Choctaw	'Success' × 'Mahan'	37	58
Desirable	'Russell' × 'Success'	38	54
Elliott	FL seedling	67	53
GraCross[c]	TX seedling	42	59
GraTex[c]	'Ideal' × 'Success'	45	64
Kiowa[c]	'Mahan' × 'Desirable'	38	58
Mahan	seedling	32	58
Mohawk[c]	'Success' × 'Mahan'	32	59
Moneymaker	LA seedling	62	50
Oconee[b]	'Schley' × 'Barton'	48	56
Pawnee[b]	'Mohawk' × 'Starking Hardy Giant'	44	58
Schley	MS seedling	54	62
Shawnee	'Schley' × 'Barton'	48	58
Sioux[c]	'Schley' × 'Carmichael'	60	60
Stuart	MS seedling,	51	49
Sumner	GA seedling	39	61
Western Schley	TX seedling	57	58
Wichita	'Halbert' × 'Mahan'	43	62
–[d]	TX seedling		
–[d]	FL seedling		

[a] Compiled from pecan cultivars index, L. J. Grauke and T. E. Thompson, Pecan Breeding Program, National Clonal Germplasm Repository for Pecans and Hickories, USDA-ARS Pecan Breeding, Somerville, TX (http://aggie-horticulture.tamu.edu/carya/). [b] New improved pecan cultivars. [c] Cultivars with no reported chemical composition. [d] Native tree with no name.

composition data on commercially improved pecan cultivars (see **Table 1**) appear to be lacking. The current investigation reports results of chemical analyses, moisture, protein, lipids including fatty acids, ash, tannins, and carbohydrates, for 24 commercially important pecan cultivars collected in the year 2003. The cultivars were also assessed for their immunoreactivity using the rabbit polyclonal antibody- (pAb-) based inhibition ELISA.

Pecans, among several other edible tree nuts, can be allergenic to sensitive individuals. However, pecan allergenic proteins remain to be characterized (*9*). To this end we have recently reported (*10*) development of a pAb-based enzyme-linked immunosorbent assay (ELISA) capable of detecting trace quantities of pecan proteins (range 32–800 ng/mL) in cultivar "Desirable". In the same study we demonstrated that the rabbit pAbs recognized substantially the same polypeptides as those recognized by sera IgE from the patients with anaphylaxis to pecans. In order to determine whether the rabbit pAbs raised against proteins extracted from cultivar Desirable can also be used for the purpose of detecting other pecan cultivars, it was important to assess the immunoreactivity of the selected pecan cultivars in a comparative manner using the anti-Desirable rabbit pAbs as the probe.

MATERIALS AND METHODS

In-shell (i.e., unshelled) pecans were procured from commercial sources and shelled manually prior to analysis. Commercially sold pecans may often represent a composite of seeds from many trees in one or more orchards. USDA Desirable cultivar was the source of antigenic proteins for raising rabbit pAbs. Sources of pecan cultivars are listed in **Table 1**. To prevent rancidity, both in-shell and shelled pecans were flushed with nitrogen and stored in airtight sandwich bags at 4 °C. Electrophoresis chemicals and supplies were from sources as described by Sathe (*11*). Nitrocellulose (NC, 0.2 μm) blotting papers were purchased from Schleicher and Schuell, Inc. (Keene, NH). RIBI adjuvant system (product code R-730) for immunization of rabbits was purchased from Corixa Corp., Hamilton, MT. All other chemicals and supplies, reagent or better grade, were purchased from Fisher Chemical Co., Orlando, FL, unless specified otherwise.

Preparation of Full Fat and Defatted Nut Flours. Pecans were ground in an Osterizer blender (Jarden Consumer Solutions, Boca Raton, FL) to homogeneous flour (16-mesh) and the flour samples stored under nitrogen in airtight containers at 4 °C until further use. Defatted nut flours were prepared by extracting fat as described in Lipid under Analytical Methods.

Rabbit Polyclonal Antibody (pAb) Production. Rabbit pAb production and characterization were as described previously by Venkatachalam et al. (*10*). Rabbit anti-pecan pAbs were raised against proteins extracted from the USDA Desirable cultivar as this cultivar was considered to be a premium market variety at the time, mainly due to the large kernel size, the ease of shelling, and regular bearing habit (*7*).

Analytical Methods. *Moisture (AOAC Official Method 925.40).* A known weight of pecan full fat flour (~1 g) was placed in an aluminum pan and dried in a previously heated vacuum oven (95–100 °C, 25 in. Hg) to a constant weight.

Lipid (AOAC Official Method 948.22). Pecan full fat flours (~10 g/thimble) were defatted in a Soxhlet apparatus using petroleum ether (boiling point range 39.0–53.8 °C) as the solvent [flour to solvent ratio of 1:10 (w/v)] for 8 h. Defatted flours were dried overnight (~10–12 h) in a fume hood to remove residual petroleum ether and weighed to calculate lipid content.

Fatty Acid Analysis. Ether extracts containing nut lipids, described under Lipid above, were subjected to vacuum distillation at ~40 °C using a Rotovap (Büchi Rotavapor R-300; Brinkman Instruments Inc., Westbury, NY) to remove ether. The nut lipids were stored at −20 °C under nitrogen until further analysis. Lipid samples were analyzed for saturated and unsaturated fatty acid composition using gas chromatographic (GC) analysis. A methylation method (*12, 13*) was used to prepare the samples prior to GC analysis. Briefly, a known volume of lipid sample (~8 μL) was suspended in a MeOH/benzene solution (7:3), was treated with acetyl chloride, and was incubated at 100 °C for 1 h. After cooling, 6% potassium carbonate buffer was added, and the samples were centrifuged. A known amount of the supernatant from each sample (~50 μL) was removed and diluted with benzene [1:4 (v/v)] prior to GC analysis.

The GC methodology details are as follows: (a) Instrumentation: Varian 3400CX, flame ionization detector (FID), CP8200 Auto sampler (10 μL syringe). (b) GC conditions: helium carrier gas (industrial, combination trap). Inlet: 260 °C, 2 μL injection volume with 50:1 split. (c) Column: Supelco SP-2380, 30 m × 0.32 mm i.d., 0.25 μm film part no. 2-4116, 1.0 mL/min flow rate. (d) Oven: 90 °C for 2.0 min, ramp at 4 °C/min to 150 °C, ramp at 10 °C/min to 260 °C, hold for 5 min, for a total run time of 33 min. (e) Detector: FID detector at 300 °C, hydrogen (industrial) and air (industrial), helium (industrial) makeup gas. (f) Signal: 5–34 min. (g) Standard: external standard (ESTD), GLC-10 Supelco FAME mix (Sigma Aldrich Co., St. Louis, MO). (h) Integration parameters: initial area reject = 0, threshold = 12.0, autoscaling by the largest peak.

Protein (AOAC Official Method 950.48). A known weight of full fat flour (~0.2–0.25 g) was used in the micro-Kjeldahl nitrogen analysis. Protein (%) was calculated using the formula N (%) × 5.32 (*14*).

Ash (AOAC Official Method 923.03). Pecan full fat flour (~0.1 g) was weighed in a ceramic crucible and heated in a muffle furnace maintained at 550 °C until a constant weight was obtained.

Total Soluble Sugars. Total soluble sugars extracted in distilled–deionized water were analyzed by the method of Dubois et al. (*15*). The glucose standard curve (0–100 μg of glucose) in distilled–deionized water was prepared simultaneously.

Tannin Analysis. Tannin analysis was done using 2% (w/v) vanillin assay as described by Deshpande and Cheryan (*16*). The catechin standard curve (0–1.0 mg/ml) was prepared simultaneously, and tannin content was expressed as catechin equivalents.

Soluble Proteins. Defatted pecan flour samples were extracted at 25 °C for 1 h with buffered saline borate (BSB: 0.1 M H_3BO_3, 0.025 M $Na_2B_4O_7$, 0.075 M NaCl, pH 8.45) using a flour to solvent ratio of 1:10 (w/v) and with continuous vortex mixing. The extracts were

centrifuged (25 °C, 10 min, 16100*g*) and the supernatants used for further analyses. Soluble proteins were estimated by the method of Lowry et al. (*17*) or Bradford (*18*).

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in the presence of a reducing agent β-mercaptoethanol (β-ME) was done according to the method of Fling and Gregerson (*19*) as described by Sathe (*11*).

Isoelectric Focusing. Defatted pecan flour was extracted in 8 M urea [flour to solvent ratio of 1:10 (w/v)] with continuous vortexing for 1 h at room temperature (~25 °C). All samples were then centrifuged (16100*g*, 10 min, room temperature), and supernatants were analyzed for soluble proteins by the Bradford assay as described under Soluble Proteins. Urea isoelectric focusing of proteins was done on 4% linear monomer acrylamide [acrylamide:bisacrylamide = 15.8:1 (w/w)] gels using the Multiphor II flat bed electrophoresis system as per the recommendations of the manufacturer (Amersham Biosciences, Piscataway, NJ). Gels were allowed to set for 2 h and then placed on a flat bed apparatus maintained at 10 °C using a MultiTemp II thermostatic circulator (Amersham Biosciences, Piscataway, NJ). Electrode buffers (500 mL each) were 1 M phosphoric acid (anolyte) and 1 M NaOH (catholyte). Electrode strips [anode end (+) soaked in 1 M phosphoric acid and cathode end (−) soaked in 1 M NaOH] were placed at the two ends, giving a run distance of ~10 cm. Gels were prefocused at 600 V for 45 min. Protein samples were then loaded on sample application pieces placed 3–4 cm from the cathode end (the pH will be neutral to slightly alkaline, thus keeping the proteins in solution). Standard proteins (vial contents reconstituted in 100 μL and 10 μL used) were loaded on reference slots. Proteins (100 μg) were focused at 1000 V for 4 h. Gels were fixed in 20% (w/v) TCA for 1 h followed by washing twice (5 min each) in deionized water. Gels were incubated in the staining solution [freshly prepared by mixing equal volumes of solution A [1% (w/v) copper sulfate, 20% (v/v) acetic acid] and solution B [0.3% (w/v) Coomassie Blue G-250, 90% (v/v) methanol]] for 1 h and destained using 35% (v/v) ethanol containing 10% (v/v) acetic acid until the background was clear.

Western Blotting. Protein transfer onto nitrocellulose (NC) paper and immunoblotting were done as described by Su et al. (*20*). Rabbit antiserum was diluted [1:50000 (v/v)] in Tris-buffered saline [10 mM Tris, 0.9% (w/v) NaCl, 0.05% (v/v) Tween 20) (TBS-T)].

Competitive Inhibition ELISA. Soluble protein extracts prepared from defatted pecan cultivar flours, normalized to 1.0 mg/mL, were assayed for immunoreactivity using competitive inhibition ELISA as described earlier (*10*). Immunoreactivity of the cultivars was expressed as the fraction of the reactivity of the USDA Desirable cultivar used as a reference (arbitrarily assigned a value of 1.0).

Data Analysis and Statistical Procedures. All statistical analyses were performed using SPSS statistical software (version 10; Chicago, IL). All experiments were carried out at least in duplicate, and data are expressed as the mean ± standard error of mean (SEM). One-way ANOVA and Fisher's least significant difference (LSD) test as described by Ott (*21*) were used to determine statistical significance ($p = 0.05$). For correlation analyses between sample sets, Pearson product-moment correlation coefficient (r value) and p-value (two-tailed) were calculated.

RESULTS AND DISCUSSION

Because of the extended time needed to come to bearing age and the many years of productive life of pecan trees, pecan orchards are fairly permanent. Many orchards today consist of older cultivars such as Stuart, Western Schley, Desirable, and Wichita. Although Desirable is the standard cultivar for the Southeastern United States, it is becoming less popular mainly due to its disease susceptibility. Newly established orchards can integrate newer cultivars which perform better than older ones with respect to greater resistance to disease and insect pests. Newer cultivars such as Caddo, Kanza, Oconee, Hopi, Nacono, and Waco offer improved yield, nut seed quality, and superior disease and insect resistance and are therefore commonly planted in newer orchards.

The selected 24 cultivars (**Table 1**) represent wide genetic variability as indicated by the diverse parentage and include several new cultivars for which no chemical composition has been reported. Many of the cultivars are direct selections from the indigenous native populations of North America.

Proximate Composition. Chemical composition (**Table 2**) data were expressed on an edible portion basis. Choctaw (Caldwell, TX, and Byron, GA) and Desirable (Somerville, TX, Fort Valley, GA, and Byron, GA) pecans procured from different locations were also included to assess the effect of cultivation region on seed chemical composition and protein immunoreactivity. Location where pecans were grown had a statistically significant effect on pecan proximate composition (**Table 2**). For example, compare samples Desirable (1), a Texas grown sample, with Desirable (3), a Georgia grown sample. Similarly, chemical composition of Texas grown Choctaw (4) was statistically significantly different when compared to that of Georgia grown Choctaw (5).

Moisture. Moisture content (w/w) of the pecan cultivars tested ranged from 2.1% (Pawnee) to 6.4% (Cape Fear). Moisture content of pecans fluctuates considerably depending on weather conditions, time of harvest, and storage conditions and hence is often not relied upon as an index of seed maturity (*22*). Various studies have shown that pecan kernel moisture decreases significantly as the harvest date is delayed (*23–25*).

Lipids. Total lipid content [range 65.9–78.0% (w/w)] was negatively correlated to protein ($r = -0.663$, $p < 0.001$). As pecan seeds mature, increase in oil content results in simultaneous decrease in proteins, carbohydrates, moisture, and other constituents (*26*). Pecan kernels, depending on the cultivar and growing location, year of production, nitrogen content in fertilizers, and time of harvest, reportedly contain 60–75% lipids. Triglycerides account for more than 95% (w/w) of total pecan lipids with monoglycerides, diglycerides, free fatty acids, and sterols together contributing <1% of total lipids (*2, 27–30*).

Proteins. Pecan seed protein content in the present study ranged from 6.0% to 11.3% (w/w), which is equivalent to 22.9–38.1% (w/w) on defatted flour weight basis. The protein content range (6–11.3%) in the current study was comparable to 9–18% reported by Prasad (*2*). Essential amino acids in pecans account for ~30–34% of total amino acids with lysine being the first limiting amino acid (*4, 31, 32*).

Ash. Significant differences in ash content [range 1.23–1.79% (w/w)], a reflection of mineral content of the kernels analyzed, may be due to varietal differences, growing locations, and production year. Senter (*33*) quantified 16 minerals in the nutmeats of 10 pecan cultivars by atomic absorption and emission spectroscopy and reported significant differences among cultivars in the quantities (mg/100 g dry weight basis) of Cu (0.82–1.44), Fe (1.93–2.65), Cr (0–0.2), Mn (1.73–5.33), B (0.32–0.90), Zn (5.30–10.40), Ba (0.27–0.90), P (340–610), K (330–660), and Ca (0–21.2) but not in the case of Co (<1 ppm), Mo (0.05–0.08), Sr (0.52–0.74), Na (0–0.84), Al (0), and Mg (120–170). Singanusong et al. (*25*) reported location to significantly influence pecan mineral content as illustrated by lower concentrations of K, Mg, Mn, and Ca in the United States grown pecans and higher concentrations of Cu, P, and Zn when compared to pecans grown in Australia.

Carbohydrates. Total carbohydrates (calculated by difference) ranged from 7.4% Wichita (2) to 11.97% Choctaw (5) and compared favorably with reported literature data (range of 13–18%) for total carbohydrate contents of several pecan cultivars (*2–4*). Total soluble sugar range [3.3–5.3% (w/w)] in the present study is also consistent with the 4.1% total sugars

Table 2. Chemical Composition of Pecan Cultivars[a]

cultivar	moisture	lipid	protein	ash	sugar	tannin
Burkett (6)	3.07 ± 0.04	70.37 ± 0.53	11.29 ± 0.30	1.47 ± 0.02	3.44 ± 0.04	1.54 ± 0.02
Caddo (5)	4.25 ± 0.01	73.46 ± 0.45	7.49 ± 0.00	1.31 ± 0.01	3.73 ± 0.14	1.25 ± 0.08
Cape Fear (5)	6.36 ± 0.10	68.16 ± 0.72	7.32 ± 0.01	1.31 ± 0.02	4.27 ± 0.08	1.25 ± 0.09
Cheyenne (4)	3.90 ± 0.01	70.74 ± 0.50	8.84 ± 0.24	1.57 ± 0.03	4.42 ± 0.11	1.51 ± 0.03
Choctaw (4)	3.86 ± 0.19	69.92 ± 0.38	9.84 ± 0.07	1.79 ± 0.01	4.32 ± 0.06	1.25 ± 0.02
Choctaw (5)	5.37 ± 0.07	65.93 ± 0.21	8.88 ± 0.05	1.52 ± 0.02	5.31 ± 0.15	1.02 ± 0.03
Desirable (3)	3.16 ± 0.00	70.56 ± 0.63	10.62 ± 0.22	1.60 ± 0.01	3.84 ± 0.16	0.66 ± 0.03
Desirable (5)	4.93 ± 0.05	72.54 ± 0.52	7.62 ± 0.15	1.46 ± 0.02	4.45 ± 0.12	0.78 ± 0.02
Desirable (1)	6.23 ± 0.16	70.08 ± 0.39	9.54 ± 0.09	1.78 ± 0.04	3.67 ± 0.06	0.94 ± 0.03
Elliott (3)	3.52 ± 0.05	72.73 ± 0.53	8.68 ± 0.06	1.47 ± 0.00	4.09 ± 0.17	0.98 ± 0.06
GraCross (4)	3.63 ± 0.06	75.44 ± 0.39	6.81 ± 0.00	1.31 ± 0.01	3.27 ± 0.07	1.58 ± 0.05
GraTex (4)	4.30 ± 0.05	72.21 ± 0.66	8.82 ± 0.01	1.49 ± 0.04	4.59 ± 0.04	0.90 ± 0.04
Kiowa (4)	3.91 ± 0.11	70.59 ± 0.30	9.42 ± 0.07	1.55 ± 0.02	4.51 ± 0.15	1.49 ± 0.05
Mahan (5)	4.54 ± 0.03	70.67 ± 0.32	7.50 ± 0.18	1.41 ± 0.01	4.21 ± 0.04	1.60 ± 0.04
Mohawk (6)	2.62 ± 0.04	74.39 ± 0.65	8.76 ± 0.00	1.30 ± 0.01	3.96 ± 0.07	1.31 ± 0.04
Moneymaker (5)	6.30 ± 0.02	67.22 ± 0.66	8.61 ± 0.04	1.55 ± 0.03	4.65 ± 0.10	1.59 ± 0.04
Oconee (5)	5.00 ± 0.18	72.32 ± 0.40	7.10 ± 0.00	1.53 ± 0.04	4.05 ± 0.10	0.90 ± 0.05
Pawnee (6)	2.13 ± 0.05	78.07 ± 0.72	6.00 ± 0.04	1.25 ± 0.00	3.67 ± 0.06	0.80 ± 0.01
Florida seedling (7)	5.02 ± 0.09	70.65 ± 0.39	8.18 ± 0.09	1.62 ± 0.05	3.75 ± 0.05	1.33 ± 0.06
Schley (3)	4.17 ± 0.13	74.08 ± 0.73	7.46 ± 0.45	1.45 ± 0.00	4.01 ± 0.26	1.02 ± 0.06
Shawnee (5)	4.91 ± 0.12	70.56 ± 0.41	7.74 ± 0.05	1.53 ± 0.02	4.49 ± 0.10	0.80 ± 0.04
Sioux (5)	4.62 ± 0.10	74.24 ± 0.58	6.97 ± 0.02	1.23 ± 0.03	3.29 ± 0.13	0.93 ± 0.02
Stuart (3)	2.90 ± 0.03	74.00 ± 0.26	7.89 ± 0.10	1.38 ± 0.02	3.56 ± 0.04	1.14 ± 0.04
Sumner (5)	6.32 ± 0.10	67.02 ± 0.55	9.06 ± 0.04	1.38 ± 0.01	4.81 ± 0.19	1.74 ± 0.06
Texas seedling (6)	3.26 ± 0.06	69.47 ± 0.20	8.75 ± 0.08	1.26 ± 0.01	3.85 ± 0.17	2.68 ± 0.05
Western Schley (2)	4.03 ± 0.06	71.19 ± 0.23	9.55 ± 0.13	1.36 ± 0.02	3.97 ± 0.08	1.42 ± 0.05
Wichita (2)	3.37 ± 0.06	71.97 ± 0.49	10.42 ± 0.06	1.42 ± 0.02	3.89 ± 0.03	1.45 ± 0.05
LSD	0.25	1.41	0.4	0.06	0.33	0.13

[a] All values are expressed on a grams per 100 g of edible portion basis and data reported as the mean ± SEM ($n = 3$). The numbers in parentheses indicate the source from where the pecans were procured, and it is assumed that they were mixed samples obtained from different trees: (1) USDA-ARS Pecan Breeding & Genetics, Somerville, TX (2001); (2) Green Valley Pecan Co., Sahuarita, AZ (2003); (3) Lane Packing Co., Fort Valley, GA (2003); (4) Royalty Pecan Farm, Caldwell, TX (2003); (5) USDA, Byron, GA (2003); (6) Concho Valley Co., San Angelo, TX (2003); (7) Florida State University, Tallahassee, FL (2003), single tree.

reported in the USDA database (*4*). According to the literature data, (*4, 34*), sucrose accounts for ~99% and reducing sugars (glucose, fructose) and inositol account for <1% of total sugars. Total sugars and lipids in pecans were negatively correlated ($r = -0.625$, $p < 0.001$). Wood and McMeans (*35*) attributed a decrease in total sugars to an increase in total lipids in developing pecan kernels. These investigators found that embryo and cotyledon expansion in maturing pecans was accompanied by accumulation of fatty acids, a decrease in reducing sugars (fructose, glucose) and inositol, and an increase in sucrose content.

Proximate composition of pecans from the current study generally compared well with those reported in the literature (*2, 4, 30*). A significant difference in the chemical composition of two Choctaw and three Desirable pecan samples is not surprising in view of the known influence of environmental factors on pecan kernel chemical composition (*30, 36*).

Fatty Acid Composition. Fatty acid composition of the pecan cultivars is summarized in **Table 3**. As in most tree nut lipids, pecan lipids are comprised of fatty acids with 16 or more carbons: palmitic ($C_{16:0}$, 4.16–7.36%), palmitoleic ($C_{16:1}$, 0.02–0.12%), stearic ($C_{18:0}$, 1.0–3.15%), oleic ($C_{18:1}$, 52.52–74.09%), linoleic ($C_{18:2}$, 17.69–37.52%), linolenic ($C_{18:3}$, 0.65–1.85%), and arachidonic ($C_{20:0}$, 0.06–0.13%) acids. All cultivars predominantly contained unsaturated fatty acids (>90% of the total fatty acids) with monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), respectively, accounting for 55.92–74.12% and 18.34–39.36% of total fatty acids. These results are consistent with previous reports on fatty acid composition of several pecan cultivars (*2, 4, 36–39*). Regardless of the cultivar, oleic acid and linoleic acid were the main contributors to the MUFAs and PUFAs, respectively. Oleic acid concentration was significantly negatively correlated to linoleic acid concentrations ($r = -0.99$, $p < 0.001$), with oleic plus linoleic acid concentrations remaining in a narrow range (88.47–93.10%). Heaton et al. (*29*) analyzed fatty acid composition of 45 pecan cultivars grown in six different locations (TX, LA, GA, FL, MS, and OK) and found that growing location resulted in only small differences in the fatty acid composition, whereas application of nitrogen fertilizers significantly influenced fatty acid distribution. For example, the $C_{18:2}$:$C_{18:1}$ ratio varied significantly (2.38, 1.07, and 1.65) depending on the soil nitrogen content (low, medium, and high, respectively), while no significant differences were observed in the total lipid content.

High amounts of unsaturated fatty acids in pecan lipids make pecans a heart-healthy food when they are used as part of a balanced food intake. Rajaram et al. (*40*) showed that adding just a handful of pecans to a low fat, cholesterol-lowering diet significantly lowered total cholesterol (5.6%) and LDL (16.5%) cholesterol levels of individuals with normal to mildly elevated blood serum cholesterol levels. In 2003, the U.S. Food and Drug Administration (FDA) approved the qualified health claim for the statement: "Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" (http://www.cfsan.fda.gov/~dms/qhc-sum.html#nuts; INC, 2003). The edible nuts for such a claim included almonds, hazelnuts, peanuts, pecans, some pine nuts, pistachio nuts, and walnuts. The U.S. FDA also noted that "The types of nuts on which the health claim may be based is restricted to those nuts that were specifically included in the health claim petition, but that do not exceed 4 g saturated fat per 50 g of nuts."

Tannins. Acidified MeOH was more efficient than absolute methanol for tannin extraction (**Figure 1A**). In contrast, in the case of walnuts, Sze-Tao et al. (*41*) found that absolute MeOH extracted significantly higher tannins compared to acidified

Table 3. Fatty Acid Composition of Pecan Lipids[a]

cultivar	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
Burkett (6)	0.00 ± 0.00	6.62 ± 0.10	0.02 ± 0.00	3.15 ± 0.11	55.90 ± 0.67	32.57 ± 0.77	1.64 ± 0.08	0.10 ± 0.01
Caddo (5)	0.00 ± 0.00	5.10 ± 0.16	0.03 ± 0.00	1.44 ± 0.10	64.76 ± 0.86	27.65 ± 1.08	0.96 ± 0.06	0.06 ± 0.01
Cape Fear (5)	0.00 ± 0.00	4.16 ± 0.17	0.04 ± 0.00	2.18 ± 0.11	71.86 ± 0.34	20.99 ± 0.33	0.65 ± 0.04	0.12 ± 0.01
Cheyenne (4)	0.00 ± 0.00	7.36 ± 0.20	0.06 ± 0.00	2.36 ± 0.08	60.10 ± 0.57	28.76 ± 0.33	1.28 ± 0.01	0.10 ± 0.00
Choctaw (4)	0.00 ± 0.00	4.73 ± 0.15	0.04 ± 0.00	1.91 ± 0.03	67.16 ± 0.30	25.13 ± 0.40	0.93 ± 0.00	0.10 ± 0.00
Choctaw (5)	0.00 ± 0.00	6.57 ± 0.15	0.02 ± 0.00	1.75 ± 0.09	55.21 ± 0.50	35.06 ± 0.54	1.31 ± 0.03	0.07 ± 0.00
Desirable (3)	0.00 ± 0.00	5.86 ± 0.24	0.02 ± 0.00	1.59 ± 0.04	55.81 ± 0.44	35.12 ± 0.69	1.55 ± 0.03	0.06 ± 0.01
Desirable (5)	0.00 ± 0.00	5.18 ± 0.20	0.03 ± 0.00	1.12 ± 0.00	66.46 ± 0.07	26.15 ± 0.25	0.99 ± 0.02	0.06 ± 0.00
Desirable (1)	0.00 ± 0.00	6.39 ± 0.12	0.03 ± 0.00	1.64 ± 0.05	52.52 ± 0.73	37.52 ± 0.69	1.85 ± 0.03	0.06 ± 0.00
Elliott (3)	0.00 ± 0.00	5.59 ± 0.21	0.03 ± 0.00	1.76 ± 0.11	62.41 ± 0.04	28.90 ± 0.12	1.25 ± 0.01	0.08 ± 0.01
GraCross (4)	0.00 ± 0.00	4.53 ± 0.21	0.04 ± 0.01	2.19 ± 0.12	71.65 ± 0.38	20.70 ± 0.65	0.78 ± 0.08	0.11 ± 0.02
GraTex (4)	0.00 ± 0.00	4.77 ± 0.22	0.04 ± 0.01	1.00 ± 0.11	68.76 ± 0.31	24.35 ± 0.24	1.01 ± 0.05	0.08 ± 0.01
Kiowa (4)	0.00 ± 0.00	5.96 ± 0.17	0.03 ± 0.00	1.61 ± 0.13	61.72 ± 0.04	29.38 ± 0.26	1.24 ± 0.03	0.07 ± 0.01
Mahan (5)	0.00 ± 0.00	5.16 ± 0.08	0.06 ± 0.00	2.05 ± 0.14	54.96 ± 0.15	36.58 ± 0.26	1.07 ± 0.01	0.11 ± 0.00
Mohawk (6)	0.00 ± 0.00	4.92 ± 0.30	0.12 ± 0.09	2.44 ± 0.09	72.34 ± 0.35	19.39 ± 0.30	0.67 ± 0.01	0.12 ± 0.00
Moneymaker (5)	0.00 ± 0.00	5.92 ± 0.16	0.03 ± 0.00	2.07 ± 0.11	63.08 ± 0.51	27.48 ± 0.45	1.35 ± 0.04	0.08 ± 0.00
Oconee (5)	0.00 ± 0.00	5.93 ± 0.15	0.04 ± 0.00	2.65 ± 0.09	55.12 ± 0.38	34.72 ± 0.12	1.44 ± 0.04	0.10 ± 0.01
Pawnee (6)	0.00 ± 0.00	4.68 ± 0.07	0.03 ± 0.00	1.99 ± 0.04	70.36 ± 0.14	21.77 ± 0.13	1.06 ± 0.05	0.10 ± 0.00
Florida seedling (7)	0.00 ± 0.00	5.03 ± 0.16	0.03 ± 0.00	1.25 ± 0.08	68.38 ± 0.98	24.21 ± 1.02	1.04 ± 0.08	0.06 ± 0.00
Schley (3)	0.00 ± 0.00	4.67 ± 0.22	0.06 ± 0.01	1.75 ± 0.03	66.46 ± 1.10	26.09 ± 1.21	0.87 ± 0.05	0.09 ± 0.01
Shawnee (5)	0.00 ± 0.00	5.06 ± 0.24	0.06 ± 0.00	1.86 ± 0.05	69.87 ± 0.32	22.10 ± 0.31	0.97 ± 0.06	0.09 ± 0.00
Sioux (5)	0.00 ± 0.00	5.28 ± 0.20	0.03 ± 0.00	1.40 ± 0.07	69.92 ± 0.38	22.24 ± 0.51	1.09 ± 0.02	0.06 ± 0.00
Stuart (3)	0.00 ± 0.00	5.51 ± 0.10	0.05 ± 0.00	2.01 ± 0.07	58.93 ± 0.36	32.12 ± 0.20	1.25 ± 0.01	0.13 ± 0.00
Sumner (5)	0.00 ± 0.00	5.10 ± 0.24	0.06 ± 0.01	2.14 ± 0.06	72.92 ± 0.04	19.01 ± 0.19	0.66 ± 0.06	0.11 ± 0.00
Texas seedling (6)	0.00 ± 0.00	5.18 ± 0.20	0.03 ± 0.00	1.88 ± 0.11	71.47 ± 0.31	20.40 ± 0.48	0.96 ± 0.02	0.07 ± 0.00
Western Schley (2)	0.00 ± 0.00	4.66 ± 0.11	0.05 ± 0.00	2.46 ± 0.04	70.23 ± 0.42	21.73 ± 0.51	0.73 ± 0.03	0.13 ± 0.00
Wichita (2)	0.00 ± 0.00	5.24 ± 0.17	0.03 ± 0.00	2.21 ± 0.14	74.09 ± 0.32	17.69 ± 0.43	0.66 ± 0.02	0.09 ± 0.00
LSD	0.00	0.52	0.06	0.25	1.38	1.54	0.12	0.02

[a] Reported as the mean ± SEM ($n = 3$). The numbers in parentheses indicate the source from where the pecans were procured (please refer to **Table 2**).

MeOH. The dependence of tannin extraction efficiency on the type of solvent is well documented in the literature (*16, 42*). Extraction time beyond 15 min did not significantly improve assayable tannins (**Figure 1A**), regardless of extraction solvent. Maximum color development occurred within the first 2 min of reagent addition and thereafter remained constant over a period of 1 h. In the case of walnuts, maximum color development was reported after 12–15 min after the addition of 0.5% (w/v) vanillin reagent (*41*). The apparent faster color development in the case of pecans is likely due to the use of higher vanillin reagent concentration [2% versus 0.5% (w/v)] used in the current investigation. For final tannin analyses of all pecan cultivars, we therefore used acidified (1% HCl) MeOH for tannin extraction, 1 h extraction time, and 30 min color development at room temperature. With the exception of one sample, the Texas seedling [2.68% (w/w) tannins], pecan tannins were in a narrow range [0.66–1.74% (w/w)] and in excellent agreement with the reported range of 0.69–1.71% (w/w) for condensed tannins in kernels of 31 pecan cultivars (*43*). This similarity in tannin range between the two studies is remarkable in view of only 12 cultivars being common to both studies. High quantities of tannins are found in the shuck and corky middle portion of the nut and to a lesser extent in the hull and kernel (*43*). Kays and Payne (*44*) found that extractable phenolics varied from 20.2% to 52.6% (w/w) in the packing tissue (dry leaf-like partition between the two halves of the pecan kernel). Therefore, any contamination of the kernels with packing tissue could lead to significant variation in analyzed tannins. As "in-shell" pecans were used in the current study, kernels were carefully removed (manually) to avoid kernel contamination by packing tissues. Analysis of tannins in the packing tissue of the Desirable cultivar indicated 79.01 ± 7.01% (w/w) tannins (*as is* basis). Similarly, Sze-Tao et al. (*41*) observed a high concentration of tannins in the packing tissue of walnuts (~5.9% dry weight basis) when compared to that in nondefatted freshly shelled walnut kernels (0.75% dry weight basis).

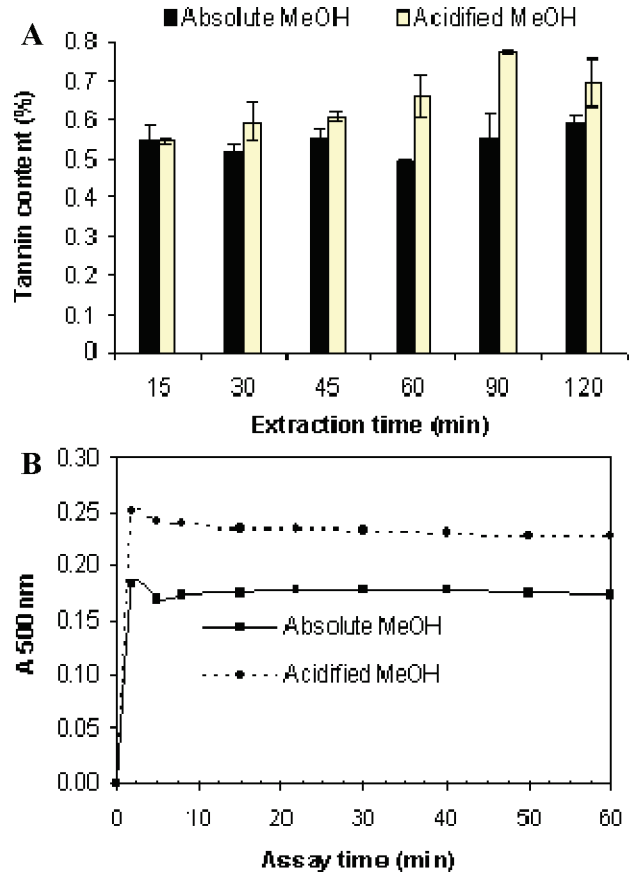

Figure 1. Optimization of pecan tannin extraction and analysis. **(A)** Effect of solvent and extraction time on pecan tannin extraction. **(B)** Time course for color development of tannin extracts assayed using 2% (w/v) vanillin reagent. All data are expressed as mg of catechin equivalents per 100 g of full fat nut seed flour (mean ± SEM).

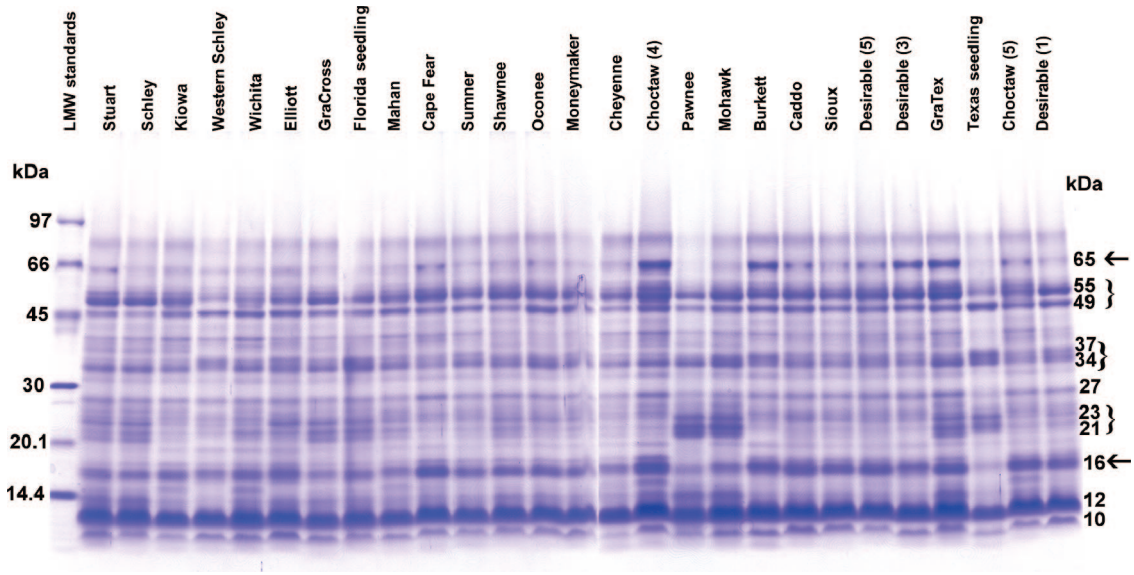

Figure 2. SDS–PAGE in the presence of a reducing agent [2% (v/v) β-ME] for the BSB-extracted pecan proteins. Pecan protein load in each lane was 60 μg. Molecular masses of the individual standards in the LMW standard kit (Pharmacia) are indicated in the left margin. Note the variable relative intensity in polypeptides marked with ← and } in the right margin.

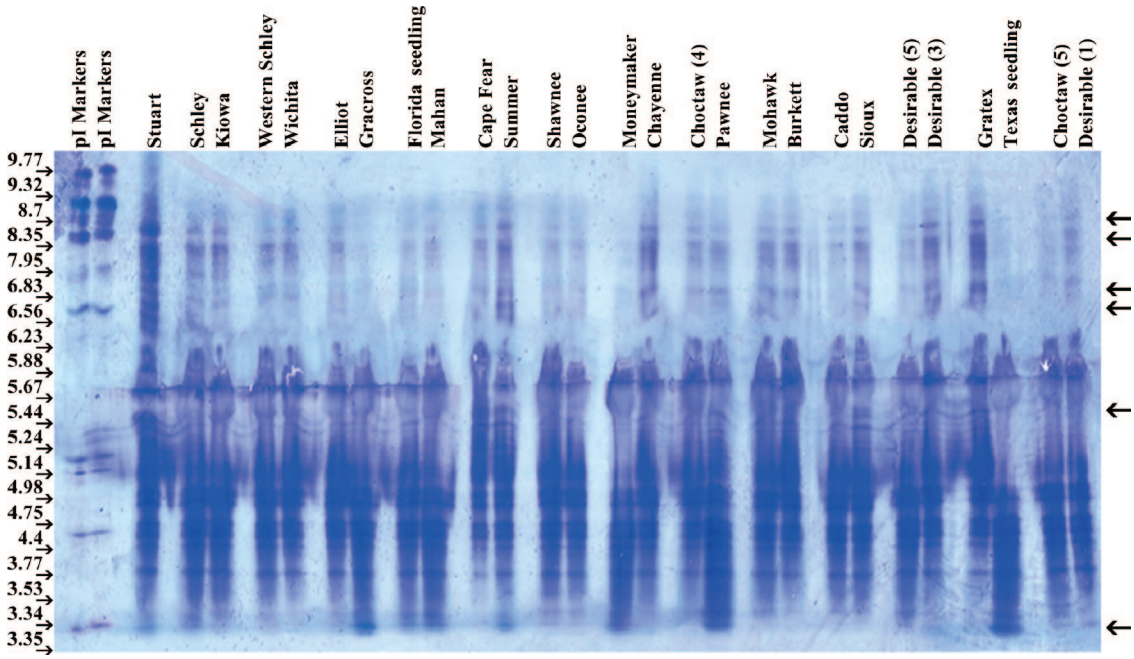

Figure 3. Isoelectric focusing in the presence of 8 M urea of pecan proteins. With the exception of marker proteins (16.25 μg total protein), protein load in each lane was 102 μg. Note the differences in the polypeptide/protein banding patterns indicated by the arrows on the right-hand side of the figure.

Electrophoresis and Immunoassays. Wood and Reily (*45*) reported that kernel development in pecans was characterized by accumulation of dilute acid/alkali-soluble proteins (i.e., glutelins) and a decline in buffer-soluble (i.e., albumins, globulins) and alcohol-soluble proteins (prolamins). Seed maturity and growing conditions may cause shifts in protein solubility fractions. Such shifts may manifest in constituent polypeptide composition of the fractions. It was therefore important to learn whether or not the pecan cultivars used in the present study were different with respect to protein and polypeptides profiles. To develop anti-pecan rabbit pAb-based detection methods, it was important to determine their applicability regardless of the pecan cultivar or variety.

SDS–PAGE profiles of soluble proteins in the presence of 2% (v/v) β-ME did not exhibit significant differences (**Figure 2**). This pattern of relative similarity among cultivars of the same seed species is consistent with the polypeptide profile observed among 60 almond genotypes and hybrids by SDS–PAGE (*46*). Pecan proteins were characterized by a majority of polypeptides in the range 6–80 kDa. On the basis of bandwidth and staining intensity (judged subjectively), all pecan cultivars had several major polypeptides with estimated molecular masses of 55, 49, 34, 34, 29, 16, 12, and 10 kDa (indicated in the right margin). Although SDS–PAGE revealed that the tested cultivars displayed fairly similar polypeptide staining patterns, varying intensities for certain polypeptides were apparent (marked by "←" and "}" in **Figure 2**). Molecular heterogeneity of storage protein polypeptides within a plant species has been observed in several plant seed storage proteins. Factors such as proteolytic processing and glycosylation have

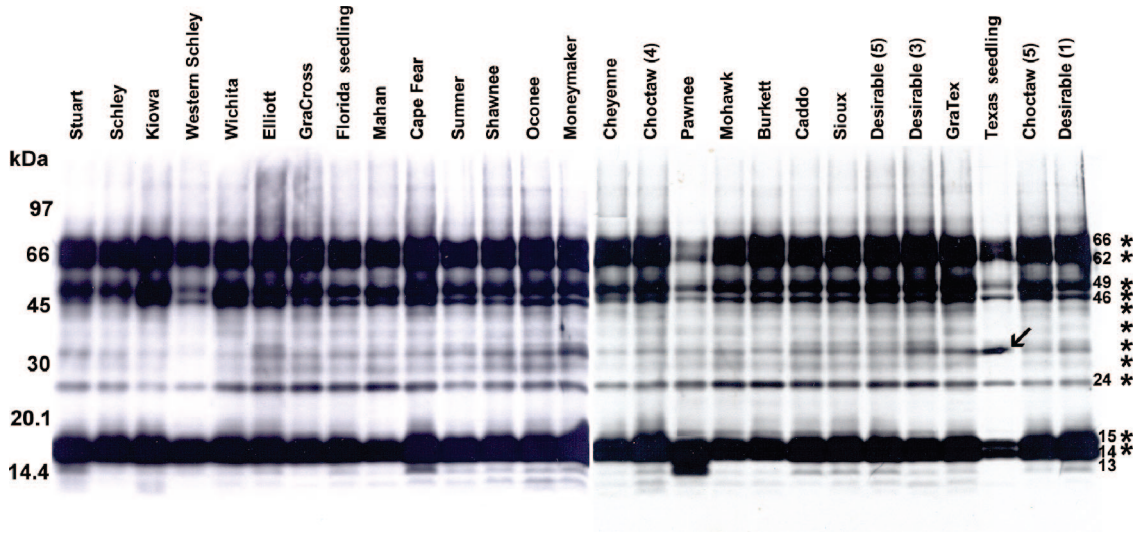

Figure 4. Western blot analysis for pecan cultivars probed with anti-pecan rabbit pAb [1:50000 (v/v) dilution]. Samples were electrophoresed on 8–25% linear acrylamide gradient SDS–PAGE (with 2% β-ME) gels. Ther protein load in each lane was 30 μg. An asterisk indicates pecan polypeptide(s) recognized by sera IgE from patients known to be allergic to pecans.

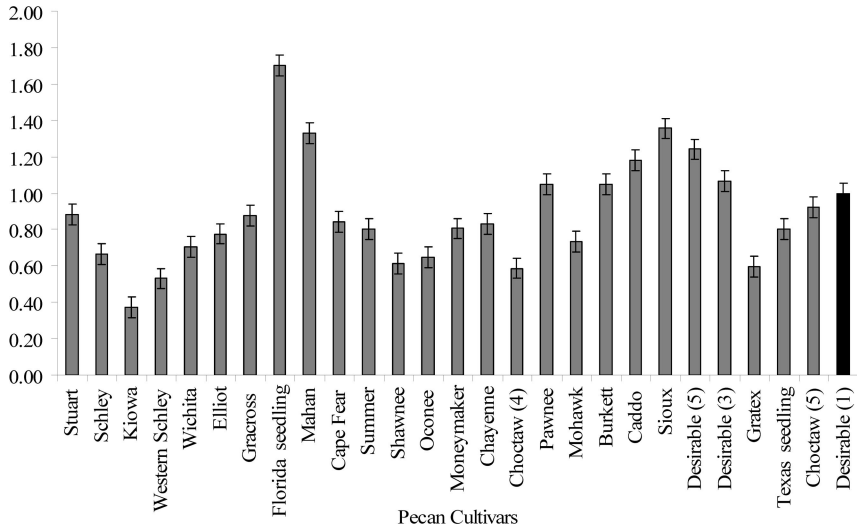

Figure 5. Immunoreactivity of pecan proteins determined by competitive inhibition ELISA assays. All data, expressed as the mean $\pm$ SEM ($n = 9$), are expressed in relation to the immunoreactivity of the USDA Desirable cultivar used as a reference (arbitrarily assigned a value of 1.0). LSD = 0.72. Differences between the means exceeding this value are significant.

been suggested to be responsible for such polypeptide microheterogeneity (*47, 48*). Isoelectric focusing (IEF) was performed in the presence of 8 M urea to further evaluate protein heterogeneity (**Figure 3**). We noted that IEF buffers without sufficient ionic strength were unable to effectively solubilize pecan proteins. The majority of the pecan polypeptides had p*I*s between pH 4.0 and 8.3. Most cultivars had similar p*I* profiles. However, small variations were noted in specific polypeptides/proteins indicated by solid arrows on the right-hand side of **Figure 3**. Since the protein load in each lane was identical and the general qualitative profiles were similar for the cultivars, the observed differences likely represent differences in the amounts and type of proteins in the kernels at the time of harvest. Such microheterogenity may arise due to growing conditions, *in vivo* protein processing, or both.

Commonly consumed tree nuts, including pecans, are associated with food-induced allergies (*9*). Increased pecan consumption therefore may potentially increase the amount of exposure of allergy-prone individuals and the incidences of unintended exposure of previously sensitized (pecan-allergic) individuals to pecans. To minimize such exposure, it is important to have a specific and sensitive assay to detect the presence of pecans in food products. We have recently used anti-pecan rabbit pAbs to develop a sensitive immunoassay to detect pecans in processed foods. In addition to detection, the assay was used to demonstrate the thermal and digestive stability of the pecan peptides and proteins (*10*). Western blotting of BSB-extracted pecan proteins (**Figure 4**) revealed pAb reactivity with several polypeptides in the molecular mass range 10–120 kDa. Many of these polypeptides (indicated by an asterisk) were earlier detected by pecan allergic patient sera IgE (*31*). On the basis of bandwidth and intensity (judged subjectively), the polypeptides with estimated molecular masses of 66–62, 49, 46, 24, and 15–13 kDa appeared to be the most antigenic. The antigenic polypeptide profile was similar in most, but not all, samples tested using Western blotting: small but distinct differences in the banding intensity (judged subjectively) of some of the major antigenic polypeptides were noted in certain cultivars (e.g., Western Schley, Pawnee, and Texas seedling). For instance, although the Western blots and SDS–PAGE generally correlated

with the corresponding bands, the band intensity for 66, 62, 49, and 46 kDa polypeptides in these three cultivars was notably less than the band intensity for the same polypeptides in other cultivars. Interestingly, staining intensity for the 49 kDa polypeptide was about the same, higher, and lower than that for the 46 kDa polypeptide, respectively, in Western Schley, Pawnee, and Texas seedling. On the other hand, Kiowa, Wichita, Elliot, Gracross, and Gratex cultivars registered the highest immunostaining for the 49 and 46 kDa polypeptides among the cultivars tested. Polypeptides of 66 and 62 kDa in a particular cultivar had comparable banding intensity in Western blots as did the polypeptide pair of 14 and 15 kDa. Among the samples tested, Texas seedling also registered higher immunoreactivity for the ~34 kDa band (indicated by the angled arrow) and the lowest band intensity for the 14, 15 kDa doublet (**Figure 4**). Staining intensity of the 13 kDa polypeptide was the highest in Pawnee, among the tested samples.

To assess whether the qualitative variation in the Western blotting assays was related to pAb reactivity of the soluble proteins, all cultivars were also evaluated using inhibition ELISA (**Figure 5**). Any significant difference in detection between different cultivars could adversely affect the use of rabbit pAb-based ELISA for the detection of pecans in foods, as the source of the pecan used is rarely revealed. All samples, normalized to 1.0 mg/mL protein content, reacted in the inhibition ELISAs with a mean ± standard deviation of 0.89 ± 0.20 ($n = 27$) when compared to the USDA Desirable cultivar as the reference (designated as 1.0). Certain cultivars [e.g., Kiowa, Mahan, Cape Fear, Desirable (5), and Desirable (3)] had lower immunoreactivity than the USDA Desirable cultivar used as the reference, but not statistically significantly different.

CONCLUSIONS

Most cultivars had similar biochemical composition with small but significant differences noted in certain samples. On the basis of the assessment techniques, the tested samples were similar, but not identical, with respect to polypeptide composition and immunoreactivity. The results also suggest that anti-pecan rabbit pAbs may be used for qualitative and quantitative detection of pecan proteins in the commercial pecan cultivars tested.

LITERATURE CITED

(1) Rosengarten, F. R. *The Book of Edible Nuts*; Walker and Co.: New York, 1984.

(2) Prasad, R. N. B. Walnuts and pecans. In *Encyclopedia of food science and technology*; Macrae, R., Robinson, R. K., Sadler, M. J., Eds.; Academic Press: London, 1993; *Vol. 7*, pp 4828–4834.

(3) Duke, J. A. *Handbook of nuts*; CRC Press: Boca Raton, FL, 2001; pp 69–72.

(4) USDA. National Nutrient Database for Standard Reference, Release 19, Agricultural Research Service, U.S. Department of Agriculture, 2006.

(5) USDA. Fruit and Tree Nuts Situation and Outlook Yearbook/FTS2006/October 2006, Economic Research Service, U.S. Department of Agriculture, 2006.

(6) Thompson, T. E.; Romberg, L. D. Inheritance of heterodichogamy in pecan. *J. Hered.* **1985**, *76*, 456–458.

(7) Thompson, T. E.; Young, E. F. *Pecan cultivars—past and present*; TX Pecan Growers Assn.: College Station, TX, 1985; 265 pp.

(8) Thompson, T. E. Pecan cultivars: current use and recommendations. *Pecan South.* **1990**, *24*, 12–20.

(9) Roux, K. H.; Teuber, S. S.; Sathe, S. K. Tree nut allergens. *Int. Arch. Allergy Immunol.* **2003**, *131*, 234–244.

(10) Venkatachalam, M.; Teuber, S. S.; Peterson, W. R.; Roux, K. H.; Sathe, S. K. Antigenic stability of pecan [*Carya illinoinensis* (Wangenh.) K. Koch] proteins: Effects of thermal treatments on in vitro digestion. *J. Agric. Food Chem.* **2006**, *54*, 1449–1458.

(11) Sathe, S. K. Solubilization, electrophoretic characterization and in vitro digestibility of almond (*Prunus amygdalus*) proteins. *J. Food Biochem.* **1993**, *16*, 249–264.

(12) Bagga, D.; Capone, S.; Wang, H.-J.; Heber, D.; Lill, M.; Chap, L.; Glaspy, J. A. Dietary modulation of omega-3/omega-6 polyunsaturated fatty acid ratios in patients with breast cancer. *J. Natl. Cancer Inst.* **1997**, *89*, 1123–1131.

(13) Lepage, G.; Roy, C. C. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **1986**, *27*, 114–120.

(14) AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Arlington, VA, 1995.

(15) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

(16) Deshpande, S. S.; Cheryan, M. Evaluation of vanillin assay for tannin analysis of dry beans. *J. Food Sci.* **1985**, *50*, 905–910.

(17) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

(18) Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(19) Fling, S. P.; Gregerson, D. S. Peptide and protein molecular weight determination by electrophoresis using a high-molarity Tris buffer system without urea. *Anal. Biochem.* **1986**, *155*, 83–88.

(20) Su, M.; Venkatachalam, M.; Teuber, S. S.; Roux, K. H.; Sathe, S. K. Impact of gamma-irradiation and thermal processing on the antigenicity of almond, cashew nut and walnut proteins. *J. Sci. Food Agric.* **2004**, *84*, 1119–1125.

(21) Ott, L. *An Introduction to Statistical Methods and Data Analysis*; Duxbury Press, a Division of Wadsworth Publishing Co.: Belmont, CA, 1977; pp 1–208.

(22) Heaton, E. K.; Worthington, R. E.; Shewfelt, A. L. Pecan nut quality: Effect of time of harvest on composition, sensory and quality characteristics. *J. Food Sci.* **1975**, *40*, 1260–1263.

(23) Love, J. E.; Young, W. A. Harvest dates and pecan quality studied. *La. Agric.* **1971**, *14*, 7–9.

(24) Herrera, E. A.; Cardenas, M.; Melendez, G. Effects of early harvesting on pecan moisture content, weight loss, and kernel percent. *Phyton Int. J. Exp. Bot.* **1994**, *55*, 89–96.

(25) Singanusong, R.; Mason, R. L.; Darcy, B. R.; Nottingham, S. M. Compositional changes of Australia-grown Western Schley pecans [*Carya illinoinensis* (Wangenh.) K. Koch] during maturation. *J. Sci. Food Agric.* **2003**, *51*, 406–412.

(26) Worley, R. E. Pecan production. In *Pecan Technology*; Santerre, C. R., Ed.; Chapman and Hall: New York, 1994; pp 13–38.

(27) Wells, J. M.; McMeans, J. L.; Payne, J. A. Pecan oil content and composition as affected by cultivar and orchard conditions. *Proc. Southeastern Pecan Growers Assoc.* **1980**, *71*, 159–162.

(28) Eddy, M.; Storey, J. B. The influence of harvest date on oil and flavor development in Desirable and Wichita pecans. *HortScience* **1988**, *23*, 782–786.

(29) Heaton, E. K.; Marion, J. E.; Woodroof, J. G. Pecan oil is highly unsaturated. *Peanut J. Nut World* **1966**, *45*, 36–38.

(30) Santerre, C. R. Pecan Composition. In *Pecan Technology*; Santerre, C. R., Ed.; Chapman and Hall: New York, 1994; pp 99–110.

(31) Venkatachalam, M. Chemical composition of select pecan [*Carya illinoinensis* (Wangenh.) Koch K.] varieties and antigenic stability of pecan proteins. Ph.D. Dissertation, Florida State University, Tallahassee, FL, 2004, 90 pp.

(32) Meredith, F. I. Amino acid composition and quality in selected varieties of pecans. *Fla. State Hortic. Soc. Proc.* **1974**, *87*, 362–365.

(33) Senter, S. D. Mineral composition of pecan nutmeats. *J. Food Sci.* **1976**, *41*, 963–964.

(34) Fourie, P. C.; Basson, D. S. Sugar content of almond, pecan and macadamia nuts. *J. Agric. Food Chem.* **1990**, *38*, 101–104.

(35) Wood, B. W.; McMeans, J. L. Carbohydrates and fatty acids in developing pecan fruit. *J. Am. Soc. Hortic. Sci.* **1982**, *107*, 47–50.

(36) Rudolph, C. J.; Odell, G. V.; Hinrichs, H. A.; Hopfer, D. A.; Kays, S. J. Genetic, environmental, and maturity effects on pecan kernel lipid, fatty-acid, tocopherol, and protein composition. *J. Food Qual.* **1992**, *15*, 263–278.

(37) Senter, S. D.; Horvat, R. J. Minor fatty acids from pecan kernel lipid. *J. Food Sci.* **1978**, *43*, 1614–1615.

(38) Senter, S. D.; Horvat, R. J. Lipids of pecan nutmeats. *J. Food Sci.* **1976**, *41*, 1201–1203.

(39) Beuchat, L. R.; Worthington, R. E. Technical note: fatty acid composition of tree nut oils. *J. Food Technol.* **1978**, *13*, 355–358.

(40) Rajaram, S.; Burke, K.; Connell, B.; Myint, T.; Sabaté, J. A monounsaturated fatty acid rich pecan-enriched diet favorably alters the serum lipid profile of healthy men and women. *J. Nutr.* **2001**, *131*, 2275–2279.

(41) Sze-Tao, K. W. C.; Schrimpf, J. E.; Teuber, S. S.; Roux, K. H.; Sathe, S. K. Effects of processing and storage on walnut (*Juglans regia* L) tannins. *J. Sci. Food Agric.* **2001**, *81*, 1215–1222.

(42) Deshpande, S. S.; Cheryan, M.; Salunkhe, D. K. Tannin analysis of food products. *CRC Crit. Rev. Food Sci. Nutr.* **1986**, *24*, 401–449.

(43) Polles, S. G.; Hanny, B. W.; Harvey, A. J. Condensed tannins in kernels of 31 Pecan [*Carya illinoinensis* (Wangenh) K. Koch] Cultivars. *J. Agric. Food Chem.* **1981**, *29*, 196–197.

(44) Kays, S. J.; Payne, J. A. Analysis of physical and chemical parameters of the shells of pecan genotypes in reference to the production of phenolic plastics and resins. *HortScience* **1982**, *17*, 978–980.

(45) Wood, B. W.; Reilly, C. C. Pecan kernel proteins and their changes with kernel development. *HortScience* **1984**, *19*, 661–663.

(46) Sathe, S. K.; Teuber, S. S.; Graziel, T. M.; Roux, K. H. Electrophoretic and immunological analyses of almond (*Prunus dulcis* L.) and hybrids. *J. Agric. Food Chem.* **2001**, *49*, 2043–2052.

(47) Millerd, A. Biochemistry of legume seed proteins. *Annu. Rev. Plant Physiol.* **1975**, *2*, 53–72.

(48) Müntz, K. Deposition of storage proteins. *Plant Mol. Biol.* **1998**, *38*, 77–99.

Received for review May 17, 2007. Revised manuscript received September 20, 2007. Accepted September 27, 2007. This work was partly supported by the College of Human Sciences (CHS) Research Initiative Program (scholarships to M.V. and H.H.K.) and by the Council on Faculty Research (COFRs) at Florida State University, Tallahassee, FL. This work was presented in part at the Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 2005 (Abstract 18C-17).

JF0714721