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Chemical Composition of Selected Edible Nut Seeds

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Commercially important edible nut seeds were analyzed for chemical composition and moisture sorption. Moisture (1.47−9.51%), protein (7.50−21.56%), lipid (42.88−66.71%), ash (1.16−3.28%), total soluble sugars (0.55−3.96%), tannins (0.01−0.88%), and phytate (0.15−0.35%) contents varied considerably. Regardless of the seed type, lipids were mainly composed of mono- and polyunsaturated fatty acids (>75% of the total lipids). Fatty acid composition analysis indicated that oleic acid ($C_{18:1}$) was the main constituent of monounsaturated lipids in all seed samples. With the exception of macadamia, linoleic acid ($C_{18:2}$) was the major polyunsaturated fatty acid. In the case of walnuts, in addition to linoleic acid (59.79%) linolenic acid ($C_{18:3}$) also significantly contributed toward the total polyunsaturated lipids. Amino acid composition analyses indicated lysine (Brazil nut, cashew nut, hazelnut, pine nut, and walnut), sulfur amino acids methionine and cysteine (almond), tryptophan (macadamia, pecan), and threonine (peanut) to be the first limiting amino acid as compared to human (2−5 year old) amino acid requirements. The amino acid composition of the seeds was characterized by the dominance of hydrophobic (range = 37.16−44.54%) and acidic (27.95−33.17%) amino acids followed by basic (16.16−21.17%) and hydrophilic (8.48−11.74%) amino acids. Trypsin inhibitory activity, hemagglutinating activity, and proteolytic activity were not detected in the nut seed samples analyzed. Sorption isotherms (A_w range = 0.08−0.97) indicated a narrow range for monolayer water content (11−29 mg/g of dry matter). No visible mold growth was evident on any of the samples stored at A_w < 0.53 and 25 °C for 6 months.

KEYWORDS: Tree nuts; chemical composition; protein; lipids; fatty acids; phytates; tannins; amino acids; storage; sorption isotherm

INTRODUCTION

Edible nuts are cultivated and grown in a variety of growing conditions and climates, are globally popular, and are valued for their sensory, nutritional, and health attributes. Typically rich sources of lipids and proteins, edible nuts also contain certain vitamins and minerals in appreciable amounts. Nut seeds with skins can also be a good source of fiber. Peanuts (or groundnuts) are universally popular and are used as a snack food or as an ingredient in the manufacture of a variety of food products such as peanut butter and peanut brittle. Globally, the most popular and commercially important edible nuts are peanuts (*Arachis hypogaea*) and several tree nuts—almond (*Prunus dulcis*), cashew (*Anacardium occidentale*), Brazil nut (*Bertholetia excelssa*), hazelnut (*Corylus avellana*), macadamia (*Macadamia integrifolia*), pecan (*Carya illinoinensis*), pine nut (*Pinus pinea*), pistachio (*Pistachia vera*), and walnut (*Juglans regia*).

In 2005, the United States accounted for approximately 14.9 and 5.8% of global tree nut and in-shell peanut production, respectively. In 2004, the United States shares of globally exported tonnage of total edible nuts, in-shell groundnut, and shelled groundnuts were 19.9, 10.8, and 18.5%, respectively. During the same year, the United States continued to be the major global importer of shelled cashew nuts (48.55% tonnage and 48.97% U.S. dollar value) and the major exporter of almonds (85.21% value of the global exports), walnuts (35.97%), and hazelnuts (61.47%) (*1*). The United States is also the largest pecan producer with ∼80% of global production (*2*).

Typical published studies on the chemical composition of specific types of edible nuts often result in fragmentary data. For example, a recent publication (*3*) analyzed several samples of edible nuts (peanuts, hazelnuts, and pistachio nuts) grown in Turkey for proximate [moisture, fat, protein, ash, and total carbohydrates (by difference)] and detailed mineral (Na, Mg, K, Ca, Cu, Zn, and Fe) composition. Additional examples include proximate chemical composition of Cambodian nut (*4*), selenium distribution in Brazil nut proteins (*5*), chemical composition of wild peanuts (*6*), the influence of climatic conditions on oil and sugars in peanuts grown in Argentina (*7*), or the influence of elevated CO_2 levels on composition of U.S.-grown peanuts (*8*), to name just a few. The USDA Nutrient Data Bank (*9*), perhaps the most widely used source for the

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nutrient composition of foods (including edible nuts), is mainly based on the best available (but often fragmentary) data at the time of such compilation.

As part of our ongoing investigations on tree nuts the current study was designed to analyze commercially important edible nuts—almond, cashew, Brazil nut, hazelnut, macadamia, pecan, pine nut, pistachio, and walnut—for a variety of chemical components including moisture, total lipids, fatty acid composition, total nitrogen (TN) and nonprotein nitrogen (NPN), ash, total sugars (TS), proteolytic activity (PA), total tannins, phytates, trypsin inhibitory activity (TIA), and hemagglutinating activity (HA). A recently developed high oleic acid Virginia peanut cultivar (VA 98R) was also included for comparative purposes. Sorption isotherm studies were included to determine monolayer water content and to assess keeping quality of the seeds under select A_w (0.08−0.97) and temperature [room temperature (RT), 25 °C] conditions.

MATERIALS AND METHODS

Brazil, cashew, hazelnut, macadamia, and pine nuts were purchased from local grocery stores. Almonds (Nonpareil marketing variety; Almond Board of California, Modesto, CA), pecans (cultivar Desirable, Dr. T. Thompson, USDA-ARS, Pecan Breeding and Genetics, Somerville, TX), pistachio (Paramount Farms, Inc., Los Angeles, CA), walnuts (Blue Diamond Growers, Sacramento, CA), and Virginia peanuts (VA 98R, Dr. Sean F. O'Keefe, VPI&SU, Blacksburg, VA) were gifts. All full-fat seeds were placed in the desired labeled containers, which were flushed several times with nitrogen gas; the lids were tightly placed on the containers, and the samples were stored at −20 °C until further use.

Unless otherwise specified, all analyses were done at room temperature (RT, ∼25 °C). When needed, samples were powdered using either a mortar and pestle or an Osterizer blender (Galaxie model 869-18R) and passed through a 40 mesh sieve for homogeneity. Ground samples were stored in airtight containers after they had been gently flushed with nitrogen gas, at −20 °C, until further analysis.

Analytical Methods. *Moisture (AOAC Official Method 925.40) (10).* An accurately weighed sample (∼1 g) was placed in an aluminum pan and the sample dried in a previously heated vacuum oven (Barnstead lab-Line, Melrose Park, IL; model 3608-5; 95−100 °C, 25 in. of Hg) to a constant weight.

Lipid (AOAC Official Method 948.22) (10). A known weight of the sample (∼10 g/thimble) was defatted in a Soxhlet apparatus using petroleum ether (boiling point range = 38.2−54.3 °C) as the solvent (flour-to-solvent ratio of 1:10 w/v) for 8 h. Defatted samples were dried overnight (∼10−12 h) in a fume hood to remove residual traces of petroleum ether and the samples weighed to calculate lipid content.

$$\text{lipid (\%)} = \frac{[\text{initial wt of full fat flour (g)} - \text{final wt of defatted flour (g)}] \times 100}{\text{initial wt of full fat flour (g)}}$$

Defatted samples were homogenized using a Sorvall blender (speed setting at 6−8) and stored in plastic screw-capped bottles at −20 °C until further analysis.

Fatty Acid Analysis (AOAC Official Method 996.06) (10). Ether extracts containing nut lipids, described under Lipid above, were subjected to vacuum distillation at ∼40 °C using a Rotovap (Büchi Rotavapor R-3000, Brinkman Instruments Inc., Westbury, NY) to remove ether. The nut lipids were stored at −20 °C under nitrogen until further analysis. Lipids were analyzed for total, saturated, and unsaturated fatty acids. Lipids were acid hydrolyzed and subjected to direct methylation prior to gas chromatographic (GC) analysis. The GC methodology details are as follows.

(a) Instrumentation: Agilent 6890N, flame ionization detector (FID), 7683 series autoinjector (10 μL syringe).

(b) GC conditions: helium carrier gas (ultrapure, combination trap). *Inlet:* 250 °C, 1 μL injection volume with 100:1 split.

(c) Column: Supelco SP-2340, 60 m, 0.25 μm i.d., 0.20 μm film thickness, 240 °C maximum temperature, 0.8 mL/min flow rate, average velocity = 19 cm/s, pressure = 20.4 psi.

(d) Oven: 100 °C for 8.0 min ramp at 12 °C/min to 180 °C, hold for 3 min, ramp at 1 °C/min to 200 °C, hold for 5 min, ramp at 3 °C/min to 240 °C, hold for 5 min, for a total run time of 61 min.

(e) Detector: FID detector at 285 °C, hydrogen (ultrapure, hydrocarbon/moisture trap) and air (ultrapure, combination trap), helium (same as above) makeup gas.

(f) Signal: 7−45 min.

(g) Standards: external standard (ESTD), GLC-90 NuChek Prep (Elysian, MN) (5/03, and 13.0 mg/mL) and (6/03, 10.8 mg/mL); internal standard (ISTD), C11:0 triundecoin (triglyceride) NuChek (3/03, 1.150 mg/mL) and (6/03, 1.011 mg/mL).

(h) Integration parameters: initial area reject = 0; initial peak width = 0.030, threshold = 12.0, autoscaling by the largest peak.

All data were corrected for recoveries and expressed as grams per 100 g of lipid.

Protein (AOAC Official Method 950.48) (10). The micro-Kjeldahl method was used to determine total proteins. Briefly, 0.1 g of sample was placed in a micro-Kjeldahl flask. A catalyst (mixture of 0.42 g of $CuSO_4$ + 9.0 g of K_2SO_4), a few glass beads (to prevent sample bumping), and 15 mL of concentrated H_2SO_4 (36 N) were added to each sample. Sample digestion was done at 410 °C for 45−75 min (until a clear green solution was obtained, which ensured complete oxidation of all organic matter). The digest was diluted with 50 mL of distilled water, and the micro-Kjeldahl flask was attached to the distillation unit. After the addition of 45 mL of 15 N NaOH, sample distillation was commenced and released ammonia was collected into a boric acid solution containing the indicators methylene blue and methyl red. Borate anion (proportional to the amount of nitrogen) was titrated with standardized 0.1 N H_2SO_4. A reagent blank was run simultaneously. Sample nitrogen content was calculated using the formula

$$\text{\% N} = \frac{(\text{mL of } H_2SO_4 \text{ for sample} - \text{mL of } H_2SO_4 \text{ for blank}) \times \text{normality of } H_2SO_4 \times 1.4007}{\text{wt of sample (g)}}$$

Protein (%) = total N (%) × appropriate factor for sample (*10*). The conversion factors used were 5.18 for almond, 5.46 for peanut, and 5.3 for the rest.

Soluble Proteins (11). Samples were extracted in a suitable buffer with continuous vortexing for 1 h and centrifuged (16100*g*, 15 min, RT); the supernatant was collected, and soluble proteins were analyzed. Bovine serum albumin (Sigma Chemical Co., St. Louis, MO) dissolved in sample extraction buffer was used as the standard protein.

Non-protein Nitrogen (NPN) (12, 13). To 0.1 g of sample was added 1.5 mL of a 10% (w/v) aqueous trichloroacetic acid (TCA) solution, and the sample was extracted with continuous vortexing for 1 h. Supernatant was collected after centrifugation (16100*g*, 15 min, RT) and analyzed for nitrogen according to AOAC method 950.48 (micro-Kjeldahl method, *10*).

Ash (AOAC Official Method 923.03) (10). Accurately weighed sample (∼0.1 g) was placed in a ceramic crucible (previously heated and cooled until constant weight was obtained) and subjected to ashing in a muffle furnace maintained at 550 °C until a constant final weight for ash was achieved.

Total Soluble Sugars (14). A known weight of the sample (∼0.1 g) was extracted with 1 mL of distilled deionized water containing 1 mM NaN_3 for 1 h and centrifuged (16100*g*, 10 min, RT), and the supernatant was collected. To 100 μl of the suitably diluted supernatant was added 100 μL of distilled deionized water followed by 200 μL of lead acetate (20% w/v), and the sample was vortexed to thoroughly mix the contents. To this mixture was added 200 μL of Na_2SO_4 (20% w/v), and the sample was vortexed, followed by centrifugation (16100*g*, 10 min, RT). A known volume (microliters) of the suitably diluted supernatant was made up to 400 μL with distilled deionized water, and 10 μL of 80% (w/v) phenol and 1 mL of concentrated H_2SO_4 were added; the contents were mixed thoroughly using a vortex mixer. Samples were allowed to cool to room temperature, and the absorbance was read at 490 nm.

Table 1. Proximate Composition of Edible Nut Seeds[a]

nut	moisture	lipid	protein	ash	sugars
almond (*Prunus dulcis*)	9.51 ± 0.08	43.36 ± 0.62	19.48 ± 0.51	2.48 ± 0.05	2.11 ± 0.11
Brazil nut *(Bertholetia excelssa)*	3.07 ± 0.37	66.71 ± 1.17	13.93 ± 0.40	3.28 ± 0.01	0.69 ± 0.04
cashew nut (*Anacardium occidentale*)	4.39 ± 0.04	43.71 ± 1.13	18.81 ± 0.06	2.66 ± 0.21	3.96 ± 0.08
hazelnut (*Corylus avellana*)	4.19 ± 0.04	61.46 ± 0.57	14.08 ± 0.34	2.03 ± 0.14	1.41 ± 0.05
macadamia nut (*Macadamia integrifolia*)	2.10 ± 0.12	66.16 ± 0.92	8.40 ± 0.71	1.16 ± 0.04	1.36 ± 0.05
pecan (*Carya illinoinensis*)	7.40 ± 0.08	66.18 ± 0.53	7.50 ± 0.24	1.88 ± 0.07	1.55 ± 0.04
pine nut (*Pinus pinea*)	1.47 ± 0.29	61.73 ± 0.55	13.08 ± 0.75	2.50 ± 0.15	1.82 ± 0.07
pistachio (*Pistachia vera*)	5.74 ± 0.03	45.09 ± 0.27	19.80 ± 0.49	3.21 ± 0.03	1.52 ± 0.07
walnut (*Juglans regia*)	2.70 ± 0.20	64.50 ± 0.45	13.46 ± 0.47	1.82 ± 0.02	2.06 ± 0.23
Virginia peanut (*Arachis hypogaea*)	7.09 ± 0.09	42.88 ± 0.13	21.56 ± 0.26	1.60 ± 0.06	0.55 ± 0.02
LSD ($p = 0.05$)	0.60	2.47	1.62	0.34	0.32

[a] All values are expressed on a grams per 100 g of edible portion basis (as-is basis), and data are reported as mean ± standard error of mean ($n = 3$).

A glucose standard curve (0−100 μg of glucose) was prepared simultaneously. Total sugars were expressed as glucose equivalents.

Amino Acid Composition. Total amino acid composition was determined using a Pico-Tag Column Amino Acid Analyzer (Waters Chromatography Division, Milford, MA). Accurately weighed sample was hydrolyzed in 600 μL of 6 M HCl in the presence of nitrogen (18 h, 110 °C). The protein hydrolysate was treated with a 2:2:1 v/v/v ethanol/triethylamine/water solution and dried. The dried sample was then derivatized with a 7:1:1:1 v/v/v/v ethanol/triethylamine/water/PITC (99.9%) solution, held for 20 min at 25 °C in a nitrogen atmosphere, and dried. Fifty microliters of 5 mM sodium acetate buffer (pH 7.6, 40 °C) containing 6% (v/v) acetonitrile was added to the dried sample, and aliquots were used for analysis by HPLC. Norleucine was used as an internal standard to calculate percent recovery of amino acids. Tryptophan content was separately determined by the colorimetric method (no. 3) of Spies and Chambers (*15*). Amino acid composition was reported as grams of amino acid per 100 g of protein.

Tannins (16). A known weight of the sample (~0.1 g) was extracted for 1 h in absolute methanol (MeOH) as well as acidified (1% HCl, v/v) MeOH with continuous vortexing followed by centrifugation (15000*g*, 10 min, RT). Aliquots of the supernatant were immediately analyzed for tannin using a 4% (w/v) vanillin assay. A catechin standard curve (0−1 mg/mL) was prepared simultaneously. Tannin content was expressed as catechin equivalents.

Phytate Analysis (17). A known weight of the sample (~0.5 g) was extracted in 3% (w/v) TCA and centrifuged (15000*g*, 10 min, RT), and aliquots were used for phytate analysis. A standard curve (5−100 μg of Fe^{3+} ion/200 μL of 3% w/v TCA) was prepared simultaneously.

Trypsin Inhibitor Activity (TIA) (18). TIA was determined according to the method approved for soy products with suitable modifications. Defatted soybean flour (Williams 82) was included as a reference standard for comparative purposes. A known weight of sample and standard (~25 mg) was extracted with 1.5 mL of 0.01 N NaOH in a microcentrifuge tube for 3 h with constant vortexing provided. When necessary, the pH of the suspension was adjusted between 8.4 and 10.0 using 0.1 N NaOH. Sample was centrifuged (16100*g*, 10 min, RT) and supernatant used for analysis. The final assay volume was 1.5 mL (instead of the 10 mL recommended in the original procedure). Suitable blank and 0 sample (no TIA) were run simultaneously. One trypsin unit was defined as an increase of 0.01 absorbance unit (AU) at 410 nm per 10 mL of reaction mixture (therefore, a factor of 10/1.5 = 6.67 was used in calculations). Samples were diluted suitably to obtain 40−60% trypsin inhibition (typically 40−60% of 0 sample AU) under the assay conditions to reduce the relative standard deviation. TIA was expressed as trypsin inhibitor units per milligrams of sample (TIU/mg of sample).

$$\text{TIU/mg of sample or standard} = \frac{(\text{0 sample AU} - \text{sample AU} - \text{blank AU}) \times \text{dilution factor} \times \text{vol for extraction (mL)}}{0.01 \times 6.67 \times \text{volume of diluted sample used for assay } (\mu\text{L}) \times \text{sample wt (g)} \times 1000}$$

Hemagglutinating Activity (HA) (Sigma Chemical Co., St. Louis, MO) (19). A microtiter plate assay was used to determine the HA of the sample. A soybean lectin (soybean, product L1395) was used as the reference standard. The HA activity of the sample extracted in 0.01 M phosphate-buffered saline, pH 6.8, was determined under standard agglutination conditions defined as agglutination of a 2% suspension of fresh human blood (group A) erythrocytes after 1 h of incubation at 25 °C. Activity was determined from serial dilutions of sample extracts in 0.01 M phosphate-buffered saline, pH 6.8. One hemagglutinating unit (HU) was defined as the least amount of hemagglutinin that produced positive evidence of agglutination. Hemagglutination units in the diluted sample were the highest dilution (HD) showing positive evidence of hemagglutination. Hemagglutinating activity of the sample was expressed as HU per gram of sample.

$$\text{HA (HU/g of sample)} = \frac{\text{HD} \times \text{dilution factor} \times \text{volume for extraction (mL)}}{\text{wt of sample (g)}}$$

Proteolytic Activity (PA). Defatted seed flour was extracted with 0.05 M Tris-HCl buffer, pH 8.1, for 30 min at RT with continuous vortexing followed by centrifugation (16100*g*, 15 min, RT), and aliquots of supernatant were analyzed for PA using fluorescein isothiocyanate (FITC) labeled casein (FITC-casein) as the substrate as described by Wolfe et al. (*20*). Appropriate sample and reagent blanks were simultaneously included in the assays.

Moisture Sorption Isotherms. A combined adsorption−desorption working isotherm was determined by static gravimetric method. Seed samples (~10 g per plate) were stored, as is, in each of five chambers equilibrated with saturated salt solutions to obtain constant relative humidity environments ranging from 8 to 97% at 25 °C (*21*). Samples were weighed every week until equilibrium was reached. The total moisture content of each sample was determined according to AOAC Official Method 950.4 (*10*) as described under Analytical Methods. Adsorption isotherms were obtained by plotting equilibrium moisture content (EMC) (grams of H_2O per gram of dry solids) versus water activity (A_w). Experimental data were fitted using Guggenheim−Anderson−de Boer (GAB) and Brunauer−Emmet−Teller (BET) model isotherms using nonlinear regression program (Water Analyzer Plot) developed by Prof. T. P. Labuza, University of Minnesota, Minneapolis, MN.

Statistics. All analyses were done at least in duplicate, and data are reported as the mean ± standard error of the mean (SEM). When appropriate, data were analyzed for significance using ANOVA and Fisher's least significant difference (LSD at $p = 0.05$).

RESULTS AND DISCUSSION

Proximate Composition. *Moisture.* The proximate compositions of the nine major tree nuts and Virginia peanut are summarized in **Table 1**. As expected, the seeds had low moisture content (ranging from 1.47% for pine nuts to 9.51% for pecans). Low moisture content is important for keeping quality and shelf life of seeds as low moisture (and low A_w) decreases the probability of microbial growth, unwarranted fermentation, premature seed germination, and many undesirable biochemical changes normally associated with these processes. Moisture

Table 2. Fatty Acid Composition of Edible Nut Seed Oils[a]

FA	almond	Brazil nut	cashew nut	hazelnut	macadamia nut	pecan	pine nut	pistachio	walnut	Virginia peanut	LSD
TS	**9.09 ± 0.02**	**25.35 ± 0.06**	**21.12 ± 0.04**	**9.11 ± 0.06**	**18.18 ± 0.18**	**8.35 ± 0.04**	**24.10 ± 0.01**	**14.24 ± 0.21**	**11.76 ± 0.05**	**12.86 ± 0.06**	**0.34**
6:0	0.00 ± 0.00	0.14 ± 0.01	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.05	0.03 ± 0.03	0.32 ± 0.01	0.00 ± 0.00	0.07
8:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
10:0	0.00 ± 0.00	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.04	0.02 ± 0.02	0.05 ± 0.01	0.00 ± 0.00	0.07
11:0	0.00 ± 0.00	0.11 ± 0.02	0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.02	0.08 ± 0.00	0.24 ± 0.03	0.00 ± 0.00	0.05
12:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
14:0	0.06 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.90 ± 0.01	0.04 ± 0.00	0.00± 0.00	0.09 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.01
15:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
16:0	7.36 ± 0.02	15.11 ± 0.08	10.70 ± 0.02	5.78 ± 0.01	8.88 ± 0.03	5.90 ± 0.04	5.33 ± 0.00	12.65 ± 0.16	8.14 ± 0.11	6.20 ± 0.01	0.23
17:0	0.05 ± 0.00	0.08 ± 0.00	0.12 ± 0.00	0.05 ± 0.00	0.00 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.00
18:0	1.56 ± 0.01	9.51 ± 0.05	9.33 ± 0.03	3.12 ± 0.05	4.26 ± 0.07	2.24 ± 0.00	2.41 ± 0.00	1.09 ± 0.00	2.84 ± 0.04	2.06 ± 0.01	0.12
20:0	0.06 ± 0.00	0.25 ± 0.00	0.63 ± 0.00	0.14 ± 0.00	2.95 ± 0.06	0.12 ± 0.00	15.75 ± 0.02	0.11 ± 0.00	0.10 ± 0.00	1.03 ± 0.00	0.07
21:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.05
22:0	0.00 ± 0.00	0.06 ± 0.01	0.12 ± 0.00	0.00 ± 0.00	0.79 ± 0.02	0.00 ± 0.00	0.11 ± 0.02	0.08 ± 0.00	0.00 ± 0.00	2.22 ± 0.04	0.06
24:0	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.00	0.00 ± 0.00	0.32 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.28 ± 0.02	0.02
TM	**61.60 ± 0.02**	**29.04 ± 0.02**	**61.68 ± 0.03**	**83.10 ± 0.03**	**77.43 ± 0.16**	**66.73 ± 0.03**	**27.55 ± 0.05**	**51.47 ± 0.14**	**15.28 ± 0.05**	**81.49 ± 0.06**	**0.27**
14:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
16:1	0.66 ± 0.00	0.29 ± 0.00	0.54 ± 0.00	0.15 ± 0.00	18.69 ± 0.15	0.07 ± 0.00	0.11 ± 0.00	1.18 ± 0.00	0.09 ± 0.00	0.07 ± 0.00	0.17
18:1	60.93 ± 0.03	28.75 ± 0.02	61.15 ± 0.03	82.95 ± 0.04	58.51 ± 0.01	66.66 ± 0.03	27.44 ± 0.05	50.29 ± 0.14	15.19 ± 0.05	81.28 ± 0.06	0.20
20:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
22:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.00	0.00
TP	**29.31 ± 0.00**	**45.61 ± 0.04**	**17.19 ± 0.07**	**7.79 ± 0.03**	**4.39 ± 0.02**	**24.92 ± 0.01**	**48.35 ± 0.04**	**34.29 ± 0.07**	**72.96 ± 0.00**	**5.66 ± 0.00**	**0.13**
18:2	29.21 ± 0.00	45.43 ± 0.04	16.88 ± 0.07	7.55 ± 0.02	1.81 ± 0.02	23.68 ± 0.02	46.84 ± 0.05	33.43 ± 0.07	59.79 ± 0.01	3.87 ± 0.00	0.14
18:3	0.10 ± 0.00	0.18 ± 0.00	0.32 ± 0.00	0.24 ± 0.00	2.58 ± 0.00	1.24 ± 0.01	1.51 ± 0.02	0.86 ± 0.00	13.17 ± 0.01	1.79 ± 0.00	0.03

[a] All values are expressed as grams per 100 g of lipid. Data are reported as mean ± standard deviation ($n = 2$). FA, fatty acid; TS, total saturated fatty acids; TM, total monounsaturated fatty acids; TP, total polyunsaturated fatty acids; almond, *Prunus dulcis*; Brazil nut, *Bertholetia excelssa*; cashew, *Anacardium occidentale*; hazelnut, *Corylus avellana*; macadamia, *Macadamia integrifolia*; pecan, *Carya illinoinensis*; pine nut, *Pinus pinea*; pistachio, *Pistachia vera*; walnut, *Juglans regia*; Virginia peanut, *Arachis hypogaea*.

content results of the present investigations are consistent with reported moisture contents for almonds (*22*, *23*), New Zealand macadamia nuts (*24*), Mexican pecans (*25*), Turkish pine nuts (*26*), pistachio (*27*−*29*), Turkish walnuts (*3*), Portuguese walnuts (*30*), commercial walnuts (*31*), New Zealand hazelnuts (*32*), Turkish hazelnuts (*33*), almonds, hazelnuts, pecans, pine nuts, pistachios, and walnuts (*34*), high oleic acid peanut lines (*35*), and the data for moisture contents of several edible nut seeds in the USDA Nutrient Data Bank (*9*).

Lipid. The lipid content of the analyzed samples ranged from 42.88% for peanuts to 66.71% for Brazil nuts. Brazil nut, hazelnut, macadamia, pecan, pine nut, and walnut registered higher (>60%) lipid content as compared to 42.88−45.09% lipids in almond, cashew nut, pistachio, and peanut. Ruggeri et al. (*34*) have similarly reported a wide range for lipid content in edible nuts [almonds (four cultivars, 52.5−57%), hazelnuts (one cultivar, 64.1%), pecans (one cultivar, 71.7%), pistachio (one cultivar, 56.1%), and walnuts (four cultivars, 61.3−73.8%)] grown in Italy and one commercial sample of pine nuts (50.3%). One study of commercial nut samples from Irish markets has reported lipid contents of 40.8, 49.2, 59.2, 37.9, and 50.8%, respectively, for almond, hazelnut, macadamia, peanut, and walnut (*36*). Garcia-Lopez et al. (*37*) found 19 almond cultivars (7 Spanish, 3 Italian, 1 Australian, and 4 U.S.) exhibited a wide range (53.10−61.70%) of total lipid content, whereas Sathe (*23*) reported the lipid contents of five major marketing varieties (Mission, Neplus, Peerless, Carmel, and Nonpareil) to be in a narrow range (53.59−56.05% lipids). Results from the present study compare favorably with several published reports for lipid content of edible nuts including Indonesian, Indian, Brazilian, and Thailand cashew nut (*38*), Nigerian cashew nut (*39*), Turkish hazelnut (*40*, *41*), New Zealand macadamia nut (*24*), pecan (*42*), Mexican pecan (*25*), pistachio nut (57−62% lipids) (*27*−*29*, *43*), walnut (*3*, *30*, *31*, *44*), and high oleic acid peanut lines (*35*).

Protein. The protein content of pecans was the lowest (7.50%) and that of Virginia peanuts the highest (21.56%), a finding consistent with several published studies (*3*, *9*, *22*, *25*, *27*, *28*, *30*−*35*, *42*, *45*−*47*). However, at least one paper has suggested a different protein content (32.9%) in pine nuts (*34*). A recent exhaustive study on Spanish chestnut chemical composition (*48*) noted a considerable variation in protein content (ranging from 1.4 to 9.6%) depending on the growing location and cultivar.

Ash. Ash content ranged from 1.16% (macadamia) to 3.28% (Brazil nut). Other investigators have reported similar ash contents for a variety of edible nuts (*3*, *22*, *27*, *28*, *30*−*34*, *42*).

Sugar. Total soluble sugars ranged from 0.55% (peanut) to 3.96% (cashew nut). Our results for sugar content are consistently lower than those reported in the USDA Data Bank (*9*). The sugar content of nut seeds is known to vary considerably, depending on growing conditions, seed maturity, cultivar, and growth location. Nanos et al. (*49*) noted 4.4% sugar in almonds, which was comparable to the finding in the current study (2.11%). On the other hand, Alasalvar et al. (*33*) have reported a sugar content of 3.58% for Tombul nuts, a Turkish cultivar of hazelnuts, which is much higher than the one (1.41%) in the present study. Similarly, Maskan and Karatas (*27*) reported 13.5% sugar in pistachios grown in Gazientep, Turkey.

Fatty Acid Composition. The fatty acid composition (**Table 2**) of the tree nuts is consistent with the corresponding data in the USDA data bank (*9*). All seeds contained predominantly monounsaturated fatty acids (MUFAs) plus polyunsaturated fatty acids (PUFAs) (ranging from 74.65% for Brazil nut to 91.65% for pecan). Depending on the seed type, there was a significant variation in total MUFAs. For example, MUFAs contributed only 27.55 and 29.04% of total fatty acids in pine nut and Brazil nut, respectively, when compared to the significantly higher percentages in Virginia peanut VA 98R (81.49%) and hazelnut (83.10%). Our results for fatty acid composition are consistent with the corresponding reported data for Indonesian, Indian, Brazilian, and Thai cashews (*38*, *50*); Mexican pecans (*25*); almond, hazelnut, pecan, pine nut, pistachio, and walnut (*34*); high oleic acid peanut lines (*35*); almond, Brazil nut, hazelnut, and walnut (*51*); and hazelnut oil (*52*).

Table 3. Tannins, Total Nitrogen (TN), Non-protein Nitrogen (NPN), Phytate, Trypsin Inhibitory Activity (TIA), Hemagglutinating Activity (HA), and Proteolytic Activity (PA) in Nut Seeds[a]

nut[b]	tannins[c]	tannins[d]	TN	NPN	phytates
almond	0.07 ± 0.00	0.29 ± 0.02	4.06 ± 0.10	0.30 ± 0.00 (7.39)[e]	0.35 ± 0.10
Brazil nut	0.01 ± 0.00	0.01 ± 0.00	2.97 ± 0.08	0.35 ± 0.01 (11.78)	0.19 ± 0.05
cashew nut	0.03 ± 0.00	0.04 ± 0.00	3.76 ± 0.05	0.20 ± 0.04 (5.32)	0.29 ± 0.08
hazelnut	0.04 ± 0.00	0.23 ± 0.01	2.79 ± 0.05	0.14 ± 0.02 (5.02)	0.23 ± 0.07
macadamia nut	0.01 ± 0.00	0.01 ± 0.00	1.73 ± 0.14	0.14 ± 0.01 (8.09)	0.15 ± 0.04
pecan	0.84 ± 0.03	0.88 ± 0.00	1.48 ± 0.05	0.06 ± 0.00 (4.05)	0.18 ± 0.05
pine nut	0.01 ± 0.00	0.01 ± 0.00	2.65 ± 0.15	0.18 ± 0.02 (6.79)	0.20 ± 0.03
pistachio	0.02 ± 0.00	0.22 ± 0.00	4.06 ± 0.09	0.32 ± 0.01 (7.88	0.29 ± 0.08
walnut	0.34 ± 0.01	0.18 ± 0.01	2.58 ± 0.09	0.04 ± 0.01 (1.55)	0.20 ± 0.06
Virginia peanut	0.16 ± 0.02	0.29 ± 0.01	4.10 ± 0.05	0.15 ± 0.00 (3.66)	0.17 ± 0.04
LSD ($p = 0.05$)	0.04	0.03	0.32	0.05[f]	0.22

[a] All values are expressed on a grams per 100 g of edible portion basis (as-is basis), and data are reported as mean ± standard error of mean ($n = 3$). HA, TIA, and PA, under the assay conditions, were not detectable. [b] For botanical names see **Table 2** footnote. [c] Total tannins extracted in absolute MeOH. [d] Total tannins extracted in acidified MeOH (1% HCl). [e] Number in the parenthesis represents NPN as percent of the corresponding TN. [f] The LSD for NPN is for the NPN data (and not for the percentage in parentheses).

Regardless of the seed type, $C_{18:1}$ (oleic acid) and $C_{18:2}$ (linoleic acid) were the predominant contributors toward the makeup of the MUFAs and PUFAs, respectively, as well as the total lipids in the seed. Significant variations in oleic acid and linoleic acid in tree nut lipids have been noted by several researchers. In the current study we found pistachios to contain 50.29% oleic acid, whereas others have noted oleic acid in pistachio lipids to be 55−66% (*29*), 65−71% (*43*), 58% (*34*), and 56−74% (*28*). Part of this variation may be due to cultivar differences. For example, Küçüköner and Yurt (*28*) reported Ohadi pistachios contained 56% oleic acid, whereas Uzun, Kirmizi, Siirt, and Halebi pistachios had a much higher (69−74%) amount of oleic acid. Similarly, oleic acid values for pine nut lipids also seem to differ widely. For example, as opposed to 27.4% oleic acid found in the current study, Ruggeri et al. (*34*) have reported a higher (39.1%) oleic acid content for pine nuts. Nanos et al. (*49*) have reported Ferragnes and Texas almonds to contain 74.7−80.8 and 72.7−74.8% oleic acid in almonds, a much higher quantity compared to the 60.93% oleic acid in the current study. The linoleic acid content of nut lipids was similarly noted to vary over a wide range. For example, the linoleic acid content of almonds in the present study was 29.21% and was within the range ∼8−39% of total lipids in the published literature (*9*, *23*, *34*, *37*, *49*). Linoleic acid in the current study for pistachio lipids was 33.43%, whereas others have reported 13.20% (*9*), 14−18% (*29*), and 15−19% (*43*) for the same. For pecans, the linoleic acid content was 23.68% (current study), which is consistent with 20.63% reported in the USDA Nutrient Data Bank (*9*), both of which are lower than the 32.9% reported by Wakeling et al. (*42*). Among the nut seeds evaluated, walnuts appeared to be distinctly different with respect to fatty acid profile due to much higher linoleic acid content (59.79%) of walnut lipids when compared to the rest (ranging from 1.81% for macadamia to 46.84% for pine nut). Walnuts in the current study were also found to contain a significant amount of linolenic acid (13.17%) compared to the rest of the samples analyzed (the range was from 0.1% for almonds to 2.58% for macadamia). The linolenic acid content of walnuts in the present study is consistent with the findings of other researchers including 9−13% in 6 Portuguese walnut cultivars (*30*), 11.2−13.5% in 10 walnut cultivars from New Zealand (*44*), and 11.58% in 1 sample from Ireland that was bought in a health food store in Cork (*36*); for several walnut oils procured from seven different countries (China, France, Hungary, India, Italy, Spain, and the United States) and analyzed for fatty acids, the range for linoleic acid was 57.3−64.1% of total lipids (*53*). Certain studies have reported intermediate amounts of linolenic acid, for example, 5.8% (*54*) and 4.7−7.0% (*3*), in walnuts. With respect to palmitoleic acid, macadamia nuts were distinct in the sense that they contained a significantly higher amount (18.7%) than the rest (the range was from 0.07% for pecans to 1.18% for pistachios). The palmitoleic acid content in macadamia lipids found in the current investigation is comparable to the reported palmitoleic acid contents of 17.3% (*36*), 23.0% (*54*), and 17−34% (*24*). The available data therefore suggest that genetic factors as well as environmental factors strongly influence the triacylglycerol (TAG) fatty acid composition. Two recent papers (*46*, *55*) on hazelnut TAG composition further support this observation. The investigators found the American hazelnut cultivars to be richer in saturated fatty acids, whereas the French, German, and English cultivar TAGs were richer in polyunsaturated fatty acid (linoleic acid). In addition, the study also reported that besides the cultivar (genetic factors), environmental factors such as the year of production and growing location also strongly influenced the nutmeat TAG composition (*55*). Evaluation of 19 Portuguese hazelnut cultivars (Vila Real region), however, exhibited lesser variations in proximate (ranges for moisture, crude protein, lipids, ash, and carbohydrates were, respectively, 3.5−6.4, 9.3−12.7, 59.3−69.0, 2.4−3.4, and 12.1−21.1%) as well as fatty acid composition or esterified fatty acid composition (palmitic, oleic, and linoleic acid were, respectively, in the ranges of 4.84−6.75, 76.71−82.81, and 7.20−11.37% of total lipids) (*55*). The fatty acid composition of tree nuts is important from several perspectives including (1) nutritional quality [the MUFAs and PUFAs (notably the ω-3 and ω-6 fatty acids) being considered more desirable than the saturated fatty acids]; (2) possible health benefits offered by MUFAs and PUFAs, especially in relation to blood serum lipid profile (notably the decrease in undesirable low-density cholesterols VLDLs and LDLs); (3) flavor-desirable flavors often attributed to several fatty acids in the nut seeds; (4) contribution to texture; and (5) importance in keeping quality (shelf life), especially the propensity for generating off-flavors upon oxidation of MUFAs and PUFAs.

Non-protein Nitrogen (NPN). NPN is arbitrarily defined as nitrogenous materials that are nonproteinaceous in character. Protein is defined as a polymer with a molecular mass of >10 kDa, a suggestion made by Pirie (*56*) and Synge (*57*). In the present study we found NPN to be in the range from 1.55% (walnut) to 11.78% (Brazil nut) of the total nitrogen in the seed meal (**Table 3**). Wolf (*13*) has reported the NPN of almond meal to be 4.8 ± 0.3% ($n = 4$) of total nitrogen. He also noted

Table 4. Total Amino Acid Composition of Edible Nut Seeds[a]

amino acid	almond	Brazil nut	cashew nut	hazelnut	macadamia nut	pecan	pine nut	pistachio	walnut	Virginia peanut	LSD
Asx	9.18 ± 0.08	7.69 ± 0.18	8.53 ± 0.16	9.25 ± 0.02	8.69 ± 0.51	9.33 ± 0.46	8.63 ± 0.00	8.55 ± 0.27	9.11 ± 0.71	12.07 ± 0.26	1.18
Glx	26.78 ± 1.09	20.26 ± 0.09	22.43 ± 0.13	23.88 ± 0.04	23.65 ± 1.04	21.06 ± 0.37	20.45 ± 0.02	23.02 ± 0.43	21.03 ± 0.63	21.11 ± 0.17	1.90
Ser	3.67 ± 0.18	4.00 ± 0.53	5.21 ± 0.21	4.69 ± 0.10	4.30 ± 0.13	5.21 ± 0.02	5.47 ± 0.06	6.25 ± 0.11	5.33 ± 0.18	4.81 ± 0.17	0.74
Gly	6.88 ± 0.07	4.75 ± 0.08	4.55 ± 0.25	4.73 ± 0.05	4.87 ± 0.33	4.73 ± 0.38	4.39 ± 0.04	4.93 ± 0.35	4.89 ± 0.03	6.43 ± 0.30	0.80
His (1.9/1.6)	2.97 ± 0.14	2.92 ± 0.07	2.68 ± 0.02	2.65 ± 0.09	2.45 ± 0.28	2.80 ± 0.11	2.23 ± 0.16	2.38 ± 0.10	2.43 ± 0.04	2.54 ± 0.07	0.44
Arg	10.09 ± 0.29	12.91 ± 0.38	9.84 ± 0.04	12.51 ± 0.13	12.53 ± 0.13	12.45 ± 0.14	15.41 ± 0.01	9.15 ± 0.19	13.80 ± 0.07	11.04 ± 0.19	0.65
Thr (3.4/0.9)	2.60 ± 0.10	2.27 ± 0.29	3.22 ± 0.26	2.95 ± 0.05	2.81 ± 0.34	2.90 ± 0.23	2.43 ± 0.10	2.97 ± 0.25	3.00 ± 0.39	2.21 ± 0.11	0.82
Ala	4.85 ± 0.10	4.30 ± 0.30	4.44 ± 0.06	5.12 ± 0.06	4.51 ± 0.09	5.06 ± 0.02	5.00 ± 0.02	4.78 ± 0.24	4.69 ± 0.02	4.58 ± 0.20	0.49
Pro	5.09 ± 0.38	5.21 ± 0.20	5.37 ± 0.04	4.81 ± 0.09	6.77 ± 0.81	5.50 ± 0.35	5.27 ± 0.02	5.53 ± 0.63	5.50 ± 0.67	5.81 ± 0.16	1.47
Val (3.5/1.3)	4.41 ± 0.12	4.71 ± 0.05	5.65 ± 0.08	4.66 ± 0.04	4.31 ± 0.03	4.72 ± 0.03	4.52 ± 0.05	5.69 ± 0.08	4.61 ± 0.08	3.95 ± 0.02	0.68
Met (2.5/1.7)	0.81 ± 0.20	8.98 ± 0.14	2.27 ± 0.11	1.90 ± 0.00	2.15 ± 0.05	2.52 ± 0.02	2.93 ± 0.02	1.88 ± 0.04	2.14 ± 0.05	1.31 ± 0.04	0.30
Cys	0.30 ± 0.10	0.75 ± 0.14	0.54 ± 0.02	0.52 ± 0.03	0.84 ± 0.23	0.45 ± 0.01	0.67 ± 0.01	0.53 ± 0.03	0.46 ± 0.06	0.33 ± 0.07	0.32
Ile (2.8/1.3)	3.79 ± 0.12	3.21 ± 0.02	4.15 ± 0.02	3.69 ± 0.01	3.26 ± 0.03	4.08 ± 0.04	3.65 ± 0.03	4.10 ± 0.11	4.00 ± 0.03	3.45 ± 0.04	0.19
Leu (6.6/1.9)	7.19 ± 0.19	7.89 ± 0.08	8.00 ± 0.05	7.40 ± 0.04	6.55 ± 0.04	7.51 ± 0.06	7.30 ± 0.01	7.56 ± 0.23	7.76 ± 0.01	7.03 ± 0.03	0.35
Phe (6.3/1.9)	5.46 ± 0.11	4.06 ± 0.07	4.83 ± 0.03	4.54 ± 0.03	3.34 ± 0.04	5.09 ± 0.02	3.59 ± 0.02	4.92 ± 0.24	4.63 ± 0.04	5.38 ± 0.12	0.33
Tyr	2.21 ± 0.45	2.47 ± 0.14	2.43 ± 0.13	2.82 ± 0.12	4.31 ± 0.19	3.01 ± 0.01	3.73 ± 0.12	2.40 ± 0.10	3.41 ± 0.02	3.40 ± 0.19	0.64
Lys (5.8/1.6)	3.06 ± 0.30	2.95 ± 0.06	4.59 ± 0.08	2.93 ± 0.01	4.10 ± 0.03	3.17 ± 0.01	3.54 ± 0.08	4.64 ± 0.69	2.71 ± 0.06	3.88 ± 0.07	0.83
Trp (1.1/0.5)	0.70 ± 0.01	0.71 ± 0.02	1.31 ± 0.08	0.98 ± 0.01	0.59 ± 0.04	0.47 ± 0.01	0.84 ± 0.05	0.78 ± 0.06	0.55 ± 0.02	0.73 ± 0.01	0.13

LEAA[b]	almond	Brazil nut	cashew nut	hazelnut	macadamia	pecan	pine nut	pistachio	walnut	Virginia peanut
first	Met/Cys	Lys	Lys	Lys	Trp	Trp	Lys	Trp	Lys	Thr
second	Lys	Trp	Thr	Thr	Lys	Lys	Thr	Lys	Trp	Met/Cys
third	Trp	Thr		Trp	Thr	Thr	Trp	Thr	Thr	Trp

LEAA[c]	almond	Brazil nut	cashew nut	hazelnut	macadamia	pecan	pine nut	pistachio	walnut	Virginia peanut
first	Met/Cys									Met/Cys
second										
third										
E/T (%)	30.97	37.67	36.68	31.69	29.54	33.23	31.00	34.88	31.82	30.44

AAD[d] (%)	almond	Brazil nut	cashew nut	hazelnut	macadamia	pecan	pine nut	pistachio	walnut	Virginia peanut
hydrophobic	39.44	44.54	41.09	38.33	37.16	40.10	38.13	40.66	39.21	38.96
hydrophilic	8.48	8.74	10.86	10.46	11.42	11.11	11.62	11.61	11.74	10.41
acidic	35.96	27.95	30.96	33.13	32.34	30.39	29.08	31.57	30.13	33.17
basic	16.12	18.77	17.10	18.08	19.07	18.42	21.17	16.16	18.94	17.45

[a] All amino acid (AA) values are expressed as grams per 100 g of protein, and data are reported as mean ± standard deviation ($n = 2$). For botanical names, see **Table 2** footnote. All samples are corrected for 100% recovery using norleucine as an internal standard. Numbers in parentheses represent essential amino acid scores compared to the FAO/WHO recommended pattern for [b]pre-school child (2–5 years) and [c]adult, respectively, and the LEAA value represents corresponding limiting essential amino acid (recommendations by the joint FAO/WHO expert Consultation, 1989). E/T (%) represents essential to total amino acid ratio. [d] Amino acid distribution.

that the volume of TCA solution was not critical in such determination but that the molarity of the TCA was important. Wolf found that almond nitrogen solubility was at its minimum in the TCA range of 0.4−1.0 M. In the present investigation, we selected 10% TCA (~1.84 M) on the basis of careful examination of almond NPN data [Table 3 and Figures 1 and 3 in Wolf (*13*)]. These figures and the table indicated that there was no qualitative change in the polypeptide profile of NPN analyzed by SDS-PAGE. From the table it was also clear that the yield of meal nitrogen was essentially the same (1.9% of meal nitrogen) whether 0.6 M TCA (the concentration recommended by Wolf for NPN extraction) or 2 M TCA was used. Additional studies in our laboratory by Sze-Tao and Sathe (*31*) and Venkatachalam (*58*) have also suggested 10% TCA to be adequate for pecan NPN extraction. However, additional work needs to be done to learn more about the NPN constituents in edible nuts. Specifically, it would be useful to know the relative contribution of free amino acid (and small polypeptide) nitrogen in comparison with the nitrogen content of compounds not related to amino acids and peptides. The importance of the amino acid and peptide nitrogen is in their possible contribution to Maillard browning for color and Strecker degradation for flavor development during processing. Certain free amino acids, notably asparagine, may also contribute to acrylamide formation when edible nuts are subjected to thermal processing treatments such as roasting and baking.

Amino Acid Composition. Amino acid composition data are summarized in **Table 4**. Hydrophobic amino acids dominated the seed protein composition with a range from 37.16% for macadamia to 44.54% for Brazil nut. Acidic amino acids (Glx + Asx) were the next most prominent group with a range from 27.95% (Brazil nut) to 35.96% (almond) with basic (ranging from 16.12% for almonds to 21.17% for pine nuts) and hydrophobic amino acids (ranging from 8.48% for almonds to 11.61% for pistachios) following, in that order. These results compare favorably with the USDA amino acid composition data for edible nuts (*9*). The results for amino acid composition are also consistent with several published reports including those for Spanish almonds (*22*), New Zealand hazelnuts (*32*), Turkish Tombul hazelnut (*33*), Chilean hazelnut (*59*), almond, hazelnut, pecan, pine nut, pistachio, and walnut (*34*), and walnut (*31*). When compared with the FAO/WHO recommended essential amino acid amounts for a 2−5-year-old child, lysine was the first essential limiting amino acid in Brazil nut, cashew nut,

hazelnut, pine nut, and walnut, whereas sulfur amino acids (Met + Cys) were the first limiting essential amino acid in almonds. Tryptophan was the first limiting amino acid in macadamia, pecan, and pistachio, and threonine was the limiting essential amino acid in Virginia peanut. Ruggeri et al. (*34*) reported lysine to be the first essential limiting amino acid in almond, hazelnut, pecan, pine nut, pistachio and walnut. However, compared to the FAO/WHO recommended essential amino acid pattern for an adult, only almond and peanut were deficient in sulfur amino acids (Met + Cys), whereas all other tree nuts seem to contain adequate amounts of all of the essential amino acids.

Arginine is a precursor of nitric oxide (NO), and NO has many bioactivities including vasodilatation, antioxidative, and antiplatelet effects with implications for cardiovascular disease (CVD) risks (*60*, *61*). The report by Wells et al. (*60*) in linking arginine and CVD risks is based on extensive and nationally applicable NHANES III database for adult subjects 25 years and older ($n = 13401$) and is adjusted for a number of variables including dietary fiber intake, age, sex, race, smoking status, body mass index, diabetes status, physical activity, and hypertension. The antioxidative and anti-inflammatory effects of nut components (*62*) may offer additional benefits with respect to lowering CVD risk, reduction in oxidative load on cells, and possible protective effects against inflammatory conditions (such as rheumatoid arthritis). The nut samples analyzed are a rich source of arginine (ranging from 9.15 g/100 g of protein in pistachio to 15.41 g/100 g of protein in pine nuts), equivalent to 1812 and 2016 mg of arginine/100 g of edible nuts and comparable to 2140 mg/110 g of sirloin steak and 2150 mg/182.55 g of whiting fish, and 2140 mg of arginine/68.39 g of peanuts, estimated to be supplied by some of the high-arginine foods in the U.S. food supply (*60*). The high arginine content of edible nuts coupled with wide variations in MUFAs and PUFAs offers opportunities for judicious selection of edible nuts as a part of a well-balanced food intake with potential for several health benefits. The potential for possible human health benefits as a result of amino acid composition of nut consumption must also be viewed in relation to non amino acid components present in the nut seeds that include fiber, minerals, certain vitamins, and secondary metabolites (notably phenolic compounds). Consuming whole seeds (such as almonds, walnuts, and pecans) provides dietary fiber, and adequate dietary fiber consumption in a well-balanced food intake is considered to be a desirable practice. In view of high lysine levels negatively influencing in vivo arginine uptake by cells (*61*), low lysine levels in edible nut seeds may therefore not necessarily be a negative attribute. Using partially delipidated nut meats or incorporating adequate amounts of full fat nut meats with other suitable foods/food ingredients may thus be utilized to develop high-protein foods provided other nutrients present in the seeds are retained during partial removal of lipids (e.g., vitamin E in the case of almonds).

Tannins. Both absolute MeOH and acidified MeOH (1% v/v HCl) were used to extract nonpolar and polar tannins, respectively (**Table 3**). Both solvents extracted about the same amount of total tannins in the case of Brazil nut, macadamia, and pine nut, indicating the tannins in these seeds to be mainly nonpolar in nature. Higher tannin extraction by acidified methanol from almonds, cashew nut, hazelnut, pecan, pistachio, and peanut suggests the presence of measurable amounts of polar tannins in these seeds. In the case of walnuts, absolute MeOH extracted higher (0.34%) tannin amounts as opposed to those extracted (0.18%) using acidified MeOH, indicating walnut tannins to be composed of both polar and nonpolar tannins. Lower extraction efficiency of walnut tannins by acidified methanol is consistent with our earlier report on walnut tannins (*16*). On the basis of the relative proportion of extracted tannins by absolute MeOH versus acidified MeOH, it would appear that almonds, hazelnut, and pistachio tannins contain significant proportions of polar tannins. Wu et al. (*63*) analyzed several (100+) commonly consumed foods in the United States, including edible nuts, for total phenolics as well as antioxidant capacities of the lipophilic and hydrophilic antioxidant compounds in those foods. The total antioxidant capacity (TAC, expressed as micromoles of Trolox equivalent) per serving of the edible nuts (26.8 g) (ranging from 204 for pine nuts to 5095 for pecans) was comparable to other high-antioxidant-containing fruits and vegetables such as tomatoes (415), beets (1886), cranberry (8983), and blackberry (7701) on a per-serving basis. With the recent and renewed interest in the chemistry and particularly bioactivity of phenolic compounds commonly found in plant foods, further investigations into the chemical and biological nature of tree nut phenolics are warranted.

Phytates. The phytate content (**Table 3**) of the seeds in the current investigation ranged from 1.5 mg/g (macadamia) to 3.5 mg/g (almond) and was consistent with those recently reported by Chen (*64*). Chen noted the inositol hexakisphosphate (IP_6) range to be from 3.98 mmol/kg (macadamia) to 14.28 mmol/kg (almond). Using 660.3 g/mol as the molecular weight of IP_6 (free acid form) suggested by Chen, the values reported by Chen correspond to 2.63 mg/g (macadamia), 4.99 mg/g (whole cashew), 4.48 mg/g (dry-roasted peanut), 4.52 mg/g (pecan halves), 6.70 mg/g (walnuts), and 9.23 mg/g (almond). Reddy and Sathe (*65*) have summarized similar values for the phytate content of edible nut seeds. Harland et al. (*66*), however, have reported a considerably higher amount of IP_6 content in the oil of roasted blanched almonds (2.111%), dry-roasted cashew nuts (1.229%), shelled dried hazelnuts (2.340%), dried macadamia nuts (0.947%), dry-roasted ground peanut (2.008%), shelled dried pecans (1.907%), pistachio nuts (2.835%), shelled dried black walnuts (4.029%), and shelled dried English walnuts (1.385%). Because both Chen (*64*) and Harland and co-workers (*66*) used an HPLC method to quantify the IP_6 contents, we anticipated the two data sets to be comparable. It is unclear why the phytate values reported by Harland and co-workers are substantially higher than those reported by Chen as well as those in the current study.

TIA and HA. Neither the TIA nor the HA was detectable under the experimental conditions in the seeds tested. Many seeds high in protein and lipids, notably legumes and several oilseeds, typically contain appreciable amounts of both TIA and HA (e.g., soybeans). Lack of TIA and HA in the seed samples analyzed was unexpected, especially in the case of peanuts.

Proteolytic Activity. None of the seeds tested positive for neutral or slightly alkaline proteolytic activity. The sensitivity of the fluorometric method used is in the sub-nanogram range (*67*). It should be emphasized here that the method used in the current investigation for the detection of proteolytic activity did not determine the presence or absence of acid proteases. The lack of detectable proteolytic activity suggests that future attempts to learn about the presence of intrinsic proteases should perhaps target acid proteases and metalloproteases. Lack of neutral proteases in the seeds may be advantageous during seed protein purification as the use of neutral to slightly alkaline buffers (with sufficient ionic strength) may permit efficient protein solubilization.

Moisture Sorption Isotherms. Sorption isotherms depend on experimental conditions such as water activity (A_w), monolayer water (M), equilibrium moisture content (EMC) at a

Table 5. Brunauer–Emmett–Teller (BET) and Guggenheim–Anderson–de Boer (GAB) Model Analysis of Sorption Isotherm Data for Edible Nut Seeds Stored in an A_w Range of 0.08–0.97 at 25 °C Compared to Literature Data

	BET model[a]	GAB model[a]						GAB model[b]			
nut[c]	*M*	*M*	*C*	*K*	R^2	MRE	SD	*T*	*M*	*K*	*C*
almond	0.029	0.045	133.46	0.681	0.98	5.93	0.006	25	0.027–0.028	0.909–0.919	11.24–18.32
Brazil nut	0.011	0.015	12.38	0.774	0.95	15.75	0.005				
cashew nut	0.020	0.029	27.94	0.783	0.96	8.46	0.008	27	0.047–0.051	0.772–0.823	9.32–9.40
hazelnut	0.020	0.033	41.00	0.650	0.83	8.10	0.006	25–30	0.015–0.018	0.913–103.24	0.980–8.1
macadamia nut	0.014	0.020	11.98	0.765	0.96	11.25	0.005	5	0.053–0.06	0.882–0.846	13.51–14.13
pecan	0.015	0.020	28.29	0.697	0.99	5.09	0.003	5	0.024–0.025	0.796–0.832	10.42–25.43
pine nut	0.020	0.029	31.97	0.703	0.99	6.51	0.004				
pistachio	0.024	0.032	14.92	0.818	0.93	15.52	0.014				
walnut	0.017	0.027	17.20	0.650	0.99	6.56	0.004				

[a] Results from the data from the present study calculated using linear (BET) and nonlinear regression plots (GAB) using Water Analyzer software. [b] Literature data for unprocessed nut seed studies compiled by Güzey and co-workers (*71*). *M*, monolayer moisture (grams of water per gram of dry solids); *K*, constant of the GAB model related to temperature effect; *R*, regression coefficient; *C*, constant (BET and GAB model) related to the enthalpy of sorption; MRE, mean relative error; SD, standard deviation; *T*, storage temperature (°C). [c] For botanical names, see **Table 2** footnote.

particular temperature and humidity, and energy constants and are often used in the design of appropriate food packaging and storage. A number of models have been developed to explain the interrelationships of the experimental parameters (*68*). The two widely used models for such calculations in foods are the BET and GAB models. In the current study, the EMC of the tree nuts stored in different humidity environments (A_w range = 0.082−0.97) at room temperature (25 °C) was determined and used to calculate BET and GAB model coefficients, including the monolayer water content (*M*), and are summarized in **Table 5**. Equilibrium moisture adsorption/desorption was achieved within 4 weeks of storage at room temperature. With the exception of Brazil nut, macadamia, and pistachio, the mean relative error (MRE) for the GAB was typically <10% for the nut seed samples tested, thereby suggesting the GAB model to be useful in describing equilibrium isotherms. These results are consistent with the reported satisfactory use of the GAB model in describing sorption characteristics of almonds (*69*), cashew nuts (*70*), and various edible nut seed isotherms compiled by Güzey and co-workers (*71*). The results of the current study indicate a low monolayer water content (*M*) in a narrow range of 11 mg/g of solids (Brazil nut) to 29 mg/g of dry solids (almond). The *M* values from the GAB model for the data (**Table 5**, compare *M* values in columns 2 and 3) were greater than those obtained from the BET model, consistent with various reports and the mathematical and physical interpretations thereof made by Timmermann (*72*). The *M* values obtained in the present study were also consistent with previous sorption studies on certain edible nut seeds [**Table 5**; note the *M* values under GAB model data compiled by Güzey and co-workers (*71*)]. The low *M* values indicate that the intact seeds do not readily adsorb moisture, a property that is helpful for long shelf life. Over the period of 6 months of storage, no visible mold/yeast growth was apparent on any of the samples stored at $A_w \leq 0.53$. However, at $A_w = 0.973$ by week 4, mold/yeast growth was seen in all samples, except almonds and pecans, with cashew nut, pistachio, and macadamia nuts having more growth (judged subjectively) than others, and by week 8, all samples had visible mold/yeast growth. At $A_w = 0.753$, by week 16, all tree nuts started exhibiting mold/yeast growth. These data suggest that storage of the tree nut seeds at A_w <0.53 is preferable for long shelf life.

Conclusions. Chemical composition analyses of globally important edible nut seeds indicated the seeds to be typically low in moisture and high in proteins and lipids. The seed lipids are a significant source of MUFAs and PUFAs. Almond, hazelnut, pecan, pistachio, walnut, and peanut contained appreciable amounts of tannins. The NPN represented a range of 1.55% (walnut) to 11.78% (Brazil nut) of the TN. The analyzed seeds did not contain detectable TIA, HA, or neutral/slightly alkaline proteinase activities, under the assay conditions used. The seed proteins contain all of the essential amino acids compared to the needs of an adult human. However, compared to the needs of a 2−5-year-old child, the edible nut seed samples analyzed were deficient in methionine, threonine, lysine, or tryptophan. All analyzed seeds contained high amounts of arginine. When stored at 25 °C and low A_w (<0.53), there was no visible mold/yeast growth on the seeds.

ABBREVIATIONS USED

A_w, water activity; BET, Brunauer−Emmet−Teller; CVD, cardiovascular disease; EMC, equilibrium moisture content; GAB, Guggenheim−Anderson−de Boer; HA, hemagglutinating activity; HU, hemagglutinating unit; LDL, low-density lipoprotein; *M*, monolayer water content; MUFA, monounsaturated fatty acid; NPN, non-protein nitrogen; PA, proteolytic activity; PUFA, polyunsaturated fatty acid; RT, room temperature (25 °C); TAG, triacylglycerol; TCA, trichloroacetic acid; TIA, trypsin inhibitor activity; TN, total nitrogen; VLDL, very low-density lipoprotein.

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