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Original article

Nutritional and anti-nutritional compositions of defatted Nebraska hybrid hazelnut meal

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Summary Defatted meal of hybrid hazelnuts may have potential food and value-added industrial applications. The proximate composition, mineral elements, amino acid profile and anti-nutritional factors (total phenolics, tannins, condensed tannins and phytates) of defatted meals of ten Nebraska hybrid hazelnut genotypes were investigated. Excluding the amino acid profile, the nutritional and anti-nutritional compositions of the meals differed significantly, with high protein, carbohydrate, neutral and acid detergent fibre concentrations and energy value. Mineral concentrations were higher than those previously reported. K was the most abundant mineral element, followed by P, Ca and Mg. Seven essential amino acids accounted for approximately 27.8% of the total amino acid contents. Tryptophan was not detected. All essential amino acid contents were significantly lower than the reference levels recommended for human and animal consumption. The high levels of anti-nutritional factors, such as total phenolics, tannins, condensed tannins and phytate in the meals, could reduce protein and mineral bioavailability.

Keywords Amino acid, anti-nutritional factors, defatted meal, hybrid hazelnuts, mineral elements, proximate composition.

Introduction

Hazelnuts (*Corylus* spp.), also known as filberts, are an important commercial crop in many countries. The United States is the third largest hazelnut producer in the world, behind Turkey and Italy. In the USA, commercial hazelnut production occurs largely in Oregon, which produces three per cent of the world's hazelnuts. Currently, all commercial hazelnut cultivars come from the European hazelnut (*Corylus avellana*) because they produce nuts of higher quality, larger size and with thinner shells. However, the European hazelnut cannot tolerate the harsh winters of the Upper Midwest, nor are they resistant to eastern filbert blight (a native disease of hazelnuts in the eastern USA that is uniformly fatal to the European hazelnut). On the other hand, native American species are cold tolerance and disease resistance, but the nuts produced are smaller and, therefore, of less commercial value. Commercial-quality hybrid hazelnut shrub cultivars, which combine the superior qualities of the European hazelnut with disease resistance and cold hardiness of the American species, show great potential as an oilseed crop in the Upper Midwest (Hammond, 2006). Recently, hybrid hazelnuts have emerged as a promising oilseed crop in Nebraska requiring relatively low inputs and marginal soils. A 9 ac hybrid hazelnut planting at Arbor Day Farm in Nebraska City, NE, USA was established in 1996 and currently is one of the largest plantings of hybrid hazelnuts in the east of the Rocky Mountains. There are 5200 genetically distinct hybrid hazelnut shrubs grown from open-pollinated seedlings derived from a breeding programme at Badgersett Research Corporation, Canton, MN, USA. These commercial-quality hybrid hazelnut shrub cultivars combine the superior qualities (larger size and thinner shells) of the European hazelnut with disease (eastern filbert blight) resistance and cold hardiness of the American species.

Evaluation of the top 25 producing Nebraska hybrid hazelnuts suggested that a significant opportunity existed for developing hazelnuts as an economically feasible, sustainable and environmentally friendly oilseed crop for food, feed and industrial applications (Xu *et al.*, 2007). Hybrid hazelnuts have high and valuable quantities of nutrients in the form of oil, protein, fibre and minerals (Xu & Hanna, 2010a). Their unique fatty acid profile and physicochemical properties, together with being rich in bioactive nutrients (phytosterol and tocopherol), make hazelnut oil ideal for food and industrial applications (Xu & Hanna, 2010b). Further, it is of great interest to identify uses for the defatted

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meal, which is a by-product of oil extraction. The high nutritive value of the defatted hazelnut meal may offer opportunities for uses as human food and animal feed supplements. Defatted hazelnut meals have been utilised as a substitute for the fish and soybean meals for poultry diet and fish feeds (Yalcin *et al*., 2005; Buyukcapar & Kamalak, 2007; Emre *et al*., 2008). However, like most plant protein sources, defatted hazelnut meal could contain anti-nutritional factors, such as phenolics, tannins, condensed tannins and phytates, which have been shown to negatively affect feed intake, body weight gain and feed conversion (Erener *et al*., 2009). Anti-nutritional factors generally refer to naturally occurring allelochemical substances, or their metabolites, which interfere with protein and mineral utilisation and affect the health and production of animals (Francis *et al*., 2001). Therefore, the objective of this study is to provide information on nutritional and anti-nutritional compositions of the Nebraska hybrid hazelnut meal after oil extraction for animal feed applications.

Materials and methods

Materials

Hybrid hazelnuts were hand harvested in the fall of 2008 from Arbor Day Farm, Nebraska City, NE. The top 10 producing genotypes from a total of 5199 hybrid hazelnuts planted from 99 different material lines were selected for further processing. The top producing genotypes were identified by establishing four transects, randomly located perpendicular across all rows and ranged in length from approximately 27 to 165 m in the planting to provide an estimated average yield of the plantation. Nuts were mechanically de-hulled, sorted by size and cracked using mechanical crackers. Kernels were ground into powder using a coffee grinder and dried for 8 h prior to hexane extraction of the oil. The remaining defatted meals were used to determine selected nutritional and anti-nutritional compositions.

Proximate analyses and fibre fractions

Hazelnut meals were dried at 60 °C for 24 h prior to all analyses. Analyses of crude protein, oil and ash were performed using the methods as described by Association of Official Analytical Chemists (AOAC, 1990). Crude protein content was measured by combustion method (AOAC 990.03) using an FP-528 Nitrogen/Protein Analyzer with DSP Control (Leco Corp. St. Joseph, MI, USA). The N in the samples was converted into nitric oxide by combustion, which further was reduced to molecular nitrogen. Nitrogen gas was measured with a thermal conductivity detector, using helium as a reference. Crude protein content was calculated by multiplying nitrogen concentration by a conversion factor of 6.25. The oil content was measured using a Soxhlet apparatus (AOAC method 948.22). The oil was extracted from the dried, ground samples using 50 mL of hexane for 8 h. After the extraction, the hexane was evaporated and the collection cups containing the extracted oil were placed in a vacuum oven at 95 °C for 1 h to remove all traces of hexane. The cups were cooled and weighed. Ash content was determined by a burning method (AOAC method 950.49). The dried sample was weighed into a porcelain crucible, placed in muffle furnace preheated to 600 °C and held at that temperature for 2 h. The crucible was then transferred into desiccators, cooled and weighed. Carbohydrate content was determined by subtracting the total percentage of other components from 100. Neutral detergent fibre and acid detergent fibre of the meals were analysed using reflux methods (Van Soest, 1963; Van Soest *et al*., 1991). Defatted samples were mixed with either neutral detergent solution or acid detergent solution in Berzelius beakers and were boiled at a constant steady reflux for 60 min. The mixtures were then filtered through pre-weighed Whatman 541 filters. The filters and residues were dried at 100 °C for 12 h and then cooled and weighed. Energy values were measured using a bomb calorimetry (model 1108 Adiabatic oxygen chamber (bomb) and the 1241 calorimeter; Parr Instrument Company, Moline, IL, USA). Energy values of the samples were determined based on the change in temperature of the calorimeter system, the heat capacity of the calorimeter system and the mass (g) of sample used.

Mineral compositions

The mineral elements, namely Ca, Cu, Fe, Mg, Mn, P, K, Na, Se and Zn, were measured using a GV Instrument Platform XS inductively coupled plasma mass spectrometer (ICP-MS) (Manchester, UK). Dried samples (0.25 g) were digested in 10 mL of concentrated HNO_3 and 0.05 mL HF in 50 mL Teflon vessels using a microwave digestion system at 170 °C for 30 min with 1600 W. The samples were then cooled, filtered through 0.45-μm glass fibre filter paper and diluted with deionised water to 50 mL for analyses for all elements except Se. For Se, a 5 mL volume of digest was transferred to a digestion tube and 5 mL of concentrated HCl (Trace Metal Grade, Fischer Scientific, Pittsburgh, PA, USA) was added. The contents were heated in a block heater for about 10 min at 100 °C for 1 h, cooled to room temperature and then diluted and analysed on a CETAC HGX-200 hydride generator (Omaha, Nebraska) and ICP-MS.

Amino acid profile

The amino acid profiles of hybrid hazelnut meals were determined using ion-exchange chromatography. Hazel-

nut meals were hydrolysed in 6 M HCl for 20 h at 110 °C. For determining sulphur-containing amino acids (cysteine and methionine), the samples were first oxidised with performic acid prior to hydrolysis with 6 M HCl. The hydrolysed samples were filtered quantitatively into 200-mL volumetric flasks and were diluted to volume with deionised water. Sample solutions (15 mL) and 2.5 mL of norleucine internal standard (2.5 μmols mL^{-1}) were pipetted into a 250-mL boiling flask and evaporated to dryness in a Buchi vacuum rotary evaporator (R114; BUCHI Corporation, New Castle, DE, USA). Samples then were transferred to 50-mL volumetric flasks and diluted to volume with lithium diluents and then were filtered through 13 mm Swinney and 0.2 μm Gelman membrane filters prior to analysis. The amino acids were separated, identified and quantified using a Waters HPLC system (Waters Corporation, Milford, MA, USA) consisting an autosampler, a 3 × 250 mm stainless steel and 10 μm cation-exchange resin Pickering Li^+ column at 42 °C, and Waters 474 fluorescence detector. The excitation wavelength and emission wavelength were 330 nm and 450 nm, respectively. Amino acids were measured by postcolumn derivitisation with ortho-phthalaldehyde. An amino acid standard was used for identification, while norleucine was used as an internal standard for qualification. The mobile phase was gradient elution buffers, namely Li275 (Lithium eluant, pH 2.75), Li750 (Lithium eluant, pH 7.50) and RG003 (Lithium column regenerant) (Pickering Laboratories, Inc. Mountain View, CA, USA), at a flow rate of 0.3 mL min^{-1}.

Anti-nutritional compositions

The levels of potential anti-nutritional factors (phenolics and phytate) in the defatted meals from the 10 hybrid hazelnut cultivars were measured. Total phenolics, tannins and condensed tannins were extracted according to the method of FAO/IAEA (2000). Dried meal (200 mg) was mixed with 10 mL of aqueous acetone (70%) and subjected to ultrasonic treatment for 20 min at room temperature, followed by centrifugation for 10 min at approximately 3000 ***g*** at 4 °C. The supernatant was collected and kept on ice. The pellet was re-extracted with two 5 mL aliquots each of 70% aqueous acetone as described earlier, and the supernatants were pooled. Total phenolics and tannins concentrations were determined by the Folin–Ciocalteu method as described by Makkar *et al.* (1993). For total phenolics, the extract was mixed with Folin–Ciocalteu reagent and 20% sodium carbonate solution. After 40 min, the absorbance was recorded at 725 nm using a Shimadzu UV-1800 spectrophotometer (Columbia, MD, USA). Tannin content was different between total phenolics and simple phenolics. Insoluble matrix, polyvinyl polypyrrolidone (PVPP), was used to bind tannin–phenolics. Simple phenolics were measured by the Folin–Ciocalteu method, as mentioned earlier. Both total phenolics and tannins were expressed as tannic acid (TA) equivalent on a dry matter basis. Determinations of condensed tannins were performed according to the method by Porter *et al.* (1986).The tannin extract was diluted with 70% acetone, followed by addition of butanol–HCl and ferric reagents. The tubes were vortexed and held in a water bath (100 °C) for 60 min. After that, they were cooled, and absorbance was recorded at 550 nm. Condensed tannins were expressed as leucocyanidin equivalent (% in dry matter).

Phytate was analysed using a rapid colorimetric procedure (Latta & Eskin,1980). Phytate first was extracted with HCl, and then the extract was passed through an AG1-X8 anion-exchange resin to remove inorganic phosphorus and other interfering compounds. Phytate content was measured based on the reaction between ferric ion and sulfosalicylic acid in the Wade reagent. Absorbance was read at 500 nm.

Statistics analyses

Three replications were used to obtain average values and standard deviations for all tests. All results were analysed with SAS version 9.2 statistical software (SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was used to evaluate the difference in nutritional and anti-nutritional compositions. Probability (P) < 0.05 indicates significance, while $P > 0.05$ indicates nonsignificance.

Results and discussion

Proximate compositions and fibre fractions

Proximate compositions, in terms of oil, protein, ash, total carbohydrate and energy value, and fibre fractions including neutral detergent fibre (NDF) and acid detergent fibre (ADF) of 10 defatted hybrid hazelnut meals are presented in Table 1. All data are reported on a dry basis. Oil content (3.52%) of defatted meal was significantly ($P < 0.05$) lower than that of pristine meal (55.1%). Protein was the predominate component in the defatted hazelnut meals with an average content of 48.2% and ranging from 41.3% to 54.4%. This was approximately threefold higher than their pristine counterparts (18.0%) and was similar to the protein content in soybean meal (Kaushik *et al.*, 1995; Xu & Hanna, 2010a). The high protein content made the defatted hazelnut meal a good supplement for low-protein cereal flours for human consumption and livestock feed. Further, three of the ten genotypes tested (10-57, 18-12 and 29-142) had protein contents over 50%, and the protein contents in two of them (10-57 and 29-142) were significantly ($P < 0.05$) higher than the

Table 1 Proximate composition of defatted hybrid hazelnut meals (dry basis)

Genotype	Crude oil (%)	Crude protein (%)	Ash (%)	Total carbohydrate (%)	Energy value (Cal g^{-1})	NDF (%)	ADF (%)
20-January	3.26 ± 0.15^{c}	41.3 ± 0.55^{h}	8.02 ± 0.51^{ab}	47.5 ± 0.57^{a}	4349 ± 0.1^{c}	24.9 ± 0.89^{cde}	17.3 ± 0.67^{a}
October-50	4.34 ± 0.02^{a}	49.9 ± 0.97^{bc}	7.81 ± 0.44^{ab}	37.9 ± 0.26^{ef}	4468 ± 9.2^{a}	26.1 ± 0.33^{bcd}	17.0 ± 1.17^{a}
October-57	3.64 ± 0.41^{abc}	54.4 ± 0.32^{a}	7.03 ± 0.45^{b}	34.9 ± 0.27^{g}	4390 ± 13.4^{b}	21.9 ± 1.05^{e}	13.5 ± 0.30^{bc}
November-54	4.21 ± 0.05^{ab}	48.1 ± 0.41^{de}	7.57 ± 0.30^{ab}	40.1 ± 0.30^{d}	4341 ± 13.4^{c}	27.8 ± 1.84^{bc}	16.9 ± 0.37^{a}
13-34	3.40 ± 0.32^{bc}	46.7 ± 0.94^{ef}	8.62 ± 0.14^{ab}	42.2 ± 0.01^{c}	4243 ± 12.7^{e}	28.7 ± 1.84^{b}	10.4 ± 0.11^{d}
16-177	4.08 ± 0.31^{abc}	44.6 ± 0.87^{g}	8.46 ± 1.19^{ab}	43.0 ± 0.27^{b}	4367 ± 27.6^{bc}	23.4 ± 1.56^{de}	14.3 ± 0.49^{b}
18-12	3.87 ± 0.35^{abc}	50.6 ± 0.21^{b}	7.11 ± 1.10^{ab}	38.5 ± 0.27^{e}	4474 ± 23.3^{a}	22.0 ± 0.99^{e}	14.1 ± 0.03^{b}
25-60	3.89 ± 0.09^{abc}	45.3 ± 0.54^{gf}	8.64 ± 0.50^{a}	42.2 ± 0.04^{bc}	4372 ± 16.3^{bc}	27.5 ± 1.46^{bc}	17.4 ± 1.14^{a}
28-105	1.93 ± 0.02^{d}	48.7 ± 1.05^{cd}	7.92 ± 0.58^{ab}	41.5 ± 0.24^{cd}	4302 ± 0.7^{d}	23.1 ± 1.39^{de}	12.1 ± 1.22^{c}
29-142	2.55 ± 0.29^{d}	52.9 ± 0.85^{a}	7.50 ± 0.86^{ab}	37.0 ± 0.14^{f}	4390 ± 24.1^{b}	33.9 ± 0.41^{a}	13.5 ± 0.30^{bc}
Mean	3.52 ± 0.02	48.2 ± 0.42	7.86 ± 0.45	40.4 ± 0.03	4369 ± 14.1	25.9 ± 1.18	14.7 ± 0.58
Range	1.93–4.34	41.3–54.4	7.03–8.64	34.9–47.5	4243–4474	21.9–33.9	10.4–17.4

NDF, neutral detergent fibre; ADF, acid detergent fibre.
Data are expressed as mean of three replications.
Means followed by the same letter within a column indicate no significant ($P > 0.05$) difference by Ducan's multiple range test.

others. Carbohydrate was the second major component in the defatted meals and had an average content of 40.4% and ranged from 34.9% to 47.5%, followed by ash (average value of 7.86% and range of 7.03% to 8.64%). Nebraska hybrid hazelnut-defatted meals had comparable protein, oil and ash contents with their Turkish counterparts, which reportedly are 44.8% protein, 3.36% oil and 6.68% ash, respectively (Doğan & Erdem, 2010).Further, Nebraska hazelnut-defatted meals had an average energy value of 4.36 kcal g^{-1}, ranging from 4.24 to 4.47 kcal g^{-1}. The high protein and carbohydrate contents contributed to the high energy value of the meals, making them an excellent energy source for animals.

Neutral detergent fibre and acid detergent fibre are good indicators of fibre contents and are the most common measures of fibre used for animal feed analyses to evaluate the fibrosity and energy value. The NDF content of the Nebraska hazelnut meal ranged from 21.9% to 33.9%, while the ADF content ranged between 10.4% and 17.4%. Bonvehi & Coll (1993) studied NDF and ADF contents of six Spanish varieties of Tarragona hazelnuts. NDF and ADF levels in their meals ranged from 7.77 to 10.95% and 5.18 to 7.30%, respectively, which were almost two times lower than those in our meals. The value of forage as animal feed is greatly influenced by NDF and ADF contents. The higher NDF and ADF revealed that more energy would be available to the ruminant animals (Belewu *et al*., 2008).

Macro- and micro-essential mineral elements

Table 2 shows concentrations of the different macro and micromineral elements in the Nebraska hybrid hazelnut-defatted meals. K was the most abundant mineral element, followed by P, Ca, Mg, Na, Mn, Fe, Cu, Zn and Se. Mineral concentrations in our meals were higher than those reported for Turkish varieties (Köksal *et al*., 2006; and Alasalvar *et al*., 2009), owing to defatting processing involved in our samples. However, the relative concentrations of the mineral elements in our meals and in Turkish meals were similar. The effect of genotypes on mineral contents was significant ($P < 0.05$): Ca, Mg, Na and Cu were highest in genotype 25-60, K, P and Se were highest in genotype 28-105, Fe and Mn were highest in genotype 29-142 and Zn was highest in genotype 18-12.

Macro and micromineral elements play crucial roles in cell growth and metabolism and therefore are vital to overall mental and physical well-being (Dini *et al*., 2005). High Ca and P contents in the nut meals made them an attractive source for Ca and P supplementations. In addition, the nut meals had a mineral profile of high Ca, Mg and K levels associated with low amounts of Na, which was the most beneficial for overall good health and diabetes and coronary heart disease prevention (Segura *et al*., 2006).

As far as essential micro-elements, they are needed only in trace amounts but are essential to a vast array of body processes. Our nut meals also contained higher Cu, Fe, Mn contents but lower Se and Zn levels than those in Turkish varieties, as reported by Köksal *et al*. (2006) and Simsek & Aykut (2007). Nebraska defatted meal served as excellent sources of Mn, Fe, Cu and Se.

Amino acid profile

The preliminary findings indicated that the effect of genotypes on amino acid profile was not significant

Table 2 Mineral compositions of defatted hybrid hazelnut meals (dry basis)

Genotype	Ca (mg/100 g)	Cu (mg/100 g)	Fe (mg/100 g)	Mg (mg/100 g)	Mn (mg/100 g)	P (mg/100 g)	K (mg/100 g)	Se (μg/100 g)	Na (mg/100 g)	Zn (mg/100 g)
20-January	566.7 ± 22.7^{d}	4.0 ± 0.7^{d}	8.3 ± 1.2^{ab}	490.5 ± 47.1^{de}	9.2 ± 0.9^{d}	1031.8 ± 27.0^{g}	1849.7 ± 136.0^{bc}	114.8 ± 32.5^{ab}	223.9 ± 35.6^{b}	0.0 ± 0.0^{c}
October-50	753.1 ± 71.7^{bc}	2.9 ± 0.4^{d}	6.8 ± 0.8^{b}	508.1 ± 71.1^{cde}	3.3 ± 0.1^{g}	1340.0 ± 83.3^{ab}	1924.5 ± 287.0^{bc}	100.2 ± 18.8^{ab}	234.6 ± 43.6^{b}	0.2 ± 0.01^{c}
October-57	868.2 ± 37.1^{ab}	6.0 ± 0.9^{bc}	7.6 ± 0.2^{ab}	466.2 ± 23.6^{e}	5.6 ± 0.1^{f}	1197.5 ± 33.0^{de}	1563.1 ± 162.8^{c}	99.5 ± 52.6^{ab}	213.0 ± 21.8^{b}	0.6 ± 0.2^{c}
November-54	634.1 ± 4.4^{dc}	7.1 ± 1.0^{ab}	7.7 ± 0.9^{ab}	510.9 ± 38.8^{cde}	6.0 ± 0.3^{ef}	1212.0 ± 65.1^{cde}	1844.7 ± 210.4^{bc}	74.4 ± 1.6^{b}	219.4 ± 39.5^{b}	2.2 ± 0.0^{b}
13-34	848.9 ± 35.3^{ab}	3.2 ± 0.3^{d}	7.5 ± 0.9^{ab}	575.4 ± 52.4^{bcd}	10.0 ± 0.2^{d}	1165.4 ± 49.0^{ef}	2189.1 ± 264.5^{ab}	93.9 ± 1.8^{ab}	226.1 ± 39.7^{b}	2.6 ± 0.0^{b}
16-177	878.8 ± 66.6^{ab}	4.4 ± 0.7^{dc}	6.9 ± 0.6^{b}	594.8 ± 36.5^{abc}	6.8 ± 0.1^{e}	1061.8 ± 33.8^{fg}	2192.4 ± 237.3^{ab}	88.7 ± 21.1^{ab}	207.4 ± 24.5^{b}	0.0 ± 0.0^{c}
18-12	629.2 ± 7.8^{dc}	4.5 ± 0.7^{dc}	7.8 ± 0.4^{ab}	483.3 ± 12.1^{de}	12.7 ± 0.7^{c}	1222.9 ± 45.9^{bcde}	1724.0 ± 85.2^{bc}	59.2 ± 19.7^{b}	206.9 ± 17.3^{b}	4.2 ± 0.4^{a}
25-60	973.2 ± 65.8^{a}	8.1 ± 1.3^{a}	7.5 ± 0.7^{ab}	683.2 ± 44.4^{a}	13.7 ± 0.2^{b}	1330.9 ± 51.2^{abc}	2077.4 ± 205.7^{b}	98.4 ± 18.7^{ab}	315.2 ± 33.4^{a}	3.6 ± 0.8^{a}
28-105	553.2 ± 82.6^{d}	4.5 ± 0.4^{dc}	5.0 ± 0.5^{c}	615.5 ± 3.8^{ab}	15.4 ± 0.4^{a}	1450.1 ± 71.2^{a}	2577.6 ± 59.1^{a}	140.1 ± 8.8^{a}	239.9 ± 26.7^{b}	0.0 ± 0.0^{c}
29-142	882.3 ± 79.4^{ab}	2.7 ± 0.1^{d}	9.1 ± 0.2^{a}	459.2 ± 34.1^{e}	15.8 ± 0.5^{a}	1291.6 ± 5.4^{bcd}	1598.7 ± 170.0^{c}	86.3 ± 12.7^{ab}	227.1 ± 17.7^{b}	0.0 ± 0.0^{c}
Mean	758.8 ± 151.8	4.75 ± 1.82	7.43 ± 1.1	538.7 ± 74.5	9.9 ± 4.4	1230.4 ± 128.1	1954.1 ± 311.2	95.5 ± 21.9	231.3 ± 31.4	1.34 ± 1.66
Range	553.2–973.2	2.7–8.1	5.0–9.1	459.2–683.2	3.3–15.8	1031.8–1450.1	1563.1–2577.6	59.2–140.8	206.9–315.2	0.0–4.2

Data are expressed as mean of three replications.
Means followed by the same letter within a column indicate no significant ($P > 0.05$) difference by Ducan's multiple range test.

($P > 0.05$). Table 3 presents amino acid profile of a representative Nebraska hybrid genotype (16-177) defatted meal compared with those from Turkish varieties (Emre *et al.*, 2008) and those from defatted soybean meal (Shiau *et al.*, 1990), because defatted soybean meal is the most common and stable supply for animal feed (Shiau *et al.*, 1990; Kikuchi, 1999; Opapeju *et al.*, 2006). Sixteen amino acids were identified in our meals. Total amino acid contents were 62.5/100 g crude protein. Of the 16 amino acids, seven amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) were essential amino acids and accounted for 27.8% of the total amino acid content. The dominant essential amino acid was leucine (4.56/100 g protein), followed by phenylalanine (2.92/100 g), valine (2.71/100 g), isoleucine (2.15/100 g), lysine (2.06/100 g), threonine (1.94/100 g) and methionine (0.97/100 g). However, another important essential amino acid, tryptophan, was not detected in our meal. In addition, all other essential amino acid contents were significantly lower than the reference levels recommended by WHO/FAO and required level of European sea bass (Emre *et al.*, 2008). On the other hand, the nonessential amino acids represented approximately 72.2% of total amino acid contents. Glutamic acid presented the highest content (15.6/100 g protein), followed by arginine (8.37/100 g protein) and aspartic acid (6.87/100 g protein).

Except for cystine, glutamic acid and methionine, all other amino acids in our hazelnut meals were significant by ($P < 0.05$) lower than their Turkish counterparts studied by Emre *et al.* (2008). Further, most amino acid levels in our hazelnut meals were significantly ($P < 0.05$) lower compared with those in defatted soybean meal with exceptions of serine, threonine and tyrosine. Our hazelnut meal even had higher serine content than soybean meal, while there was no significant ($P > 0.05$) difference for threonine and tyrosine levels. Based on these analyses, the Nebraska hybrid hazelnut meals contain incomplete proteins, and, therefore, are not a good single protein source.

Anti-nutritional factors

Although our analyses suggested that hazelnut meal had high nutritional value, the presence of high concentrations of certain anti-nutritional factors limits their utilisation and palatability. Therefore, the evaluation of anti-nutritional factors in the defatted meal is necessary from a nutritional point of view. The levels of several anti-nutritional factors, such as total phenolics, tannins, condensed tannins and phytates, in 10 Nebraska hybrid hazelnut-defatted meals are tabulated in Table 4. Nebraska hybrid hazelnut-defatted meals had an average total phenolics of 10.7 mg TA g^{-1}, tannins of 7.53 mg TA g^{-1} and condensed tannins of

Table 3 Amino acid profile of one representative hybrid hazelnut (genotype 16-177) defatted meal compared with that from Turkish varieties and from defatted soybean meal

Amino acid	Nebraska hazelnut-defatted meal (g/100 g protein)	Turkish defatted meal* (g/100 g protein)	Defatted soybean meal† (g/100 g protein)	FAO/WHO‡ (g/100 protein)	Requirement by European sea bass* (g/100 g protein)
Alanine	3.10 ± 0.06^b	5.51^a	5.93^a		
Arginine	8.37 ± 0.10^b	10.6^a	7.08^c		4.1
Aspartic acid	6.87 ± 0.10^c	16.0^a	12.6^b		
Cystine	1.39 ± 0.01^a	1.54^a	–		
Glutamic acid	15.6 ± 0.39^b	15.9^b	22.3^a		
Glycine	2.99 ± 0.11^c	5.52^b	5.77^a		
Histidine	1.69 ± 0.01^c	2.10^b	2.58^a	1.9	
Isoleucine§	2.15 ± 0.04^c	3.95^b	5.26^a	2.8	
Leucine§	4.56 ± 0.15^b	9.11^a	8.93^a	6.6	
Lysine§	2.06 ± 0.01^c	3.90^b	5.77^a	5.8	4.8
Methionine§	0.97 ± 0.01^b	1.04^b	1.55^a		
Phenylalanine§	2.92 ± 0.01^b	5.99^a	5.19^a		
Serine	3.17 ± 0.11^b	4.21^a	1.28^c		
Threonine§	1.96 ± 0.11^b	3.75^a	2.26^b		2.6
Tryptophan§	–	–	–	1.1	
Tyrosine	1.90 ± 0.01^b	4.13^a	1.72^b	3.4	
Valine§	2.71 ± 0.08^c	5.26^b	5.85^a	3.5	
Total essential amino acids	17.4	33	34.8		
Total amino acids	62.5	98.5	94.1		

–, indicated not detected.

Data are expressed as mean of three chromatographic injections. Mean ± SD followed by the same letter within a row and same unit indicate no significant ($P > 0.05$) difference by Ducan's multiple range test

*Source: Emre *et al.* (2008).

†Source: Shiau *et al.* (1990).

‡Source: FAO/WHO/UNU (1985).

§Essential amino acids.

0.64% leucocyanidin equivalent. The effects of the genotype on the phenolic matters were significant ($P < 0.05$). Genotypes 1-20, 16-177 and 29-142 had the highest concentrations of total phenolic, tannins and condensed tannins among the 10 genotypes tested, whereas the lowest levels of total phenolics (8.71 mg TA g^{-1}), tannins (5.56 mg TA g^{-1}) and condensed tannins of 0.25% leucocyanidin equivalent were found in genotype 10-57. The total phenolic content in our hazelnut meal was comparable with that (11.2 mg g^{-1}) of extracted soybean meal (Chivandi *et al.*, 2005) but significantly higher than that (0.125 mg g^{-1}) of Turkish hazelnut-defatted meal (Erener *et al.*, 2009). Tannin content in our meals also was significantly ($P < 0.05$) higher than their Turkish counterpart (Erener *et al.*, 2009).

Tannins are known to be a major cause of the astringency experienced when they were tasted in their unprocessed forms (Enujiugha & Ayodele-Oni, 2003). More importantly, tannins can form insoluble complexes with protein, thereby interfering with protein bioavailability and reducing the utilisation of energy, and also may inhibit the activities of digestive enzymes (Siddhuraju *et al.*, 1995; Enujiugha, 2003). Erener *et al.* (2009) found that diet consumption and body weight gain decreased when animals were fed diets containing tannins and phenolics. As most phenolics and tannins are water soluble, soaking and thermal processing, such as cooking, toasting and extrusion, effectively reduces their levels (Enujiugha, 2003; Mukhopadhyay *et al.*, 2007). It is worthy of note that although phenolics and tannins have a negative effect on utilisations of energy and nutrients, they currently are all considered to be health-promoting factors at low concentrations (Siddhuraju *et al.*, 2001).

Phytate was another anti-nutritional factor in our hazelnut meals, and genotype had a significant ($P < 0.05$) effect on its level. The phytate contents ranged from 18.5 to 33.0 mg g^{-1}, with an average concentration of 26.4 mg g^{-1}, which was higher than that (10 mg/100 g) of Spanish hazelnuts reported by Prieto *et al.* (2010). A high phytate content could significantly reduce the overall bioavailability of certain essential minerals including Ca, Mg, Fe and Zn (Enujiugha & Ayodele-Oni, 2003). This is attributable to the ability of phytate to chelate these mineral elements to

Table 4 Anti-nutritional factors of defatted hybrid hazelnut meals (dry basis)

Genotype	Total phenolics (mg TA g^{-1})	Tannins (mg TA g^{-1})	Condensed tannins (% leucocyanidin equivalent)	Phytates (mg g^{-1})
1-20	12.3 ± 1.13^{ab}	8.94 ± 0.87^{a}	1.53 ± 0.12^{a}	24.7 ± 1.20^{cd}
10-50	9.30 ± 0.56^{d}	6.50 ± 0.63^{c}	0.32 ± 0.11^{fg}	25.6 ± 1.06^{c}
10-57	8.71 ± 0.34^{d}	5.56 ± 0.30^{d}	0.25 ± 0.12^{g}	33.0 ± 0.99^{a}
11-54	10.6 ± 0.65^{c}	7.80 ± 0.75^{b}	0.63 ± 0.09^{d}	31.5 ± 1.46^{a}
13-34	11.5 ± 0.09^{bc}	6.78 ± 0.18^{c}	0.26 ± 0.05^{g}	18.5 ± 1.24^{f}
16-177	12.9 ± 0.40^{a}	9.12 ± 0.39^{a}	0.83 ± 0.04^{c}	28.7 ± 0.86^{b}
18-12	9.17 ± 0.33^{d}	6.54 ± 0.30^{c}	0.51 ± 0.02^{de}	21.5 ± 1.38^{e}
25-60	9.51 ± 0.09^{d}	6.83 ± 0.13^{c}	0.41 ± 0.07^{ef}	32.3 ± 1.31^{a}
28-105	10.8 ± 0.40^{c}	7.80 ± 0.67^{b}	0.38 ± 0.12^{efg}	25.2 ± 0.96^{cd}
29-142	12.7 ± 0.41^{a}	9.46 ± 0.46^{a}	1.28 ± 0.04^{b}	23.2 ± 0.76^{de}
Mean	10.7 ± 1.54	7.53 ± 1.31	0.64 ± 0.44	26.4 ± 4.8
Range	8.71–12.9	5.56–9.46	0.25–1.53	18.5–33.0

Data are expressed as mean of three replications.
Means followed by the same letter within a column indicate no significant ($P > 0.05$) difference by Ducan's multiple range test.

form insoluble complexes. Enujiugha (2003) found that osteomalacia developed when certain legumes and cereals rich in phytate were fed to growing animals. Additionally, phytates are known to be primary storage forms of P, which reduce P bioavailability (Siddhuraju *et al.*, 1995). In spite of some negative effects, phytate also may have possible health benefits in human nutrition, such as in the management of diabetes and obesity (Eleyinmi *et al.*, 2008). As was found with phenolics and tannins, soaking and thermal processing effectively reduces phytate level (Enujiugha, 2003).

Conclusions

This study is one part of our ongoing investigations of value-added opportunities for Nebraska hybrid hazelnuts for food, feed and industrial applications and focus on nutritional and anti-nutritional compositions of Nebraska hybrid hazelnut-defatted meal. The meals were found to be rich in protein, carbohydrate, energy value, NDF and ADF, which make them a good source for livestock feed. Mineral concentrations in our meals were higher than previously studied Turkish ones. All essential amino acid contents in our meals were significantly ($P < 0.05$) lower than the reference levels recommended for human and animal consumption. The high levels of anti-nutritional factors may reduce protein and mineral bioavailability. Although Nebraska hybrid hazelnut-defatted meals are nutritionally incomplete, they remain a potential resource for human and animal consumption, when supplemented with some essential amino acids and when the relative concentrations in anti-nutritional factors are reduced via thermal processing. Further studies will be conducted in these fields.

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