The effect of ultrasound on some properties of pulse hulls

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Abstract The aim of this study was to understand the effects of different ultrasound conditions on dietary fiber, phytic acid, total phenolics content, water and oil binding capacity, antioxidant capacity and phenolic acid composition of hull from green and red lentils (*Lens culinaris*), faba bean (*Vicia faba*), and pea (*Pisum sativum*). The highest and the lowest total dietary fiber content was observed in red lentil and faba bean hulls, respectively. Insoluble dietary fiber contents of the untreated pulse hulls were above 66% regardless of the pulse type. Phytic acid content of the pulse hulls ranged between 1.37 and 1.66 mg/g and was not affected from either the pulse type or ultrasound treatment. High and significant correlations were found between total phenolics content and Trolox equivalent antioxidant capacity both of which showed higher values for lentil hulls. Bound (hydrolyzable) extracts contained higher amounts of phenolic acids compared to free form extracts. Protocatechuic, *p*-coumaric and ferulic acids either in free or bound (hydrolyzable) form were present in hull from all the pulses. Ultrasound treated samples showed significantly lower total phenolic content and TEAC values compared to control for lentil hulls, while no consistent trend was observed for phenolic acids with regard to the ultrasound conditions.

Keywords Ultrasound · Pulse · Phenolics · Dietary fiber · Phytic acid

Introduction

Pulses, which are the second most noteworthy food source in the world after cereal grains, are defined as “dry seeds of leguminous plants which are distinguished from leguminous oil seeds by their low fat content” by The Codex Alimentarius Commission. They belong to Leguminosae family which consists of 650 genera and over 18,000 species (Tiwari et al. 2011). Pulses have low glycemic index and consumption of pulses is associated with reduced risk of hypercholesterolemia, cardiovascular diseases, breast cancer and improved bone health (Xu and Chang 2007).

They are consumed whole as food in many parts of the world due to being a rich and cheap source of protein and a good source of B group vitamins and minerals (Hoover et al. 2010). However, fractionation of pulses as starch, protein, and fiber has gained attention in recent years due to changing worldwide agricultural practices and product development applications. Pulse fractions have the potential of being used individually not only in baked goods, extruded products, gluten free products and baby foods but also in meat and beverage industries for various aims as value added ingredients (Toews and Wang 2013; Tosh and Yada 2010; Boye et al. 2010).

Pulses contain various bioactive substances that are not considered as nutrients, but exert beneficial metabolic effects on human body upon consumption. These bioactive components include dietary fibers, resistant starches, polyphenols, and phytosterols and they are mainly concentrated in the hull portion of the pulses (Singh et al.

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2017a, b). Many studies have been reported related to the dry or wet fractionation of starch and protein from pulses (Pelgrom et al. 2013; Ruiz-Ruiz et al. 2012; Naguleswaran and Vasanthan 2010; Betancur-Ancona et al. 2004). However, limited information is available on the isolation of hull or fiber fraction and its functional and bioactive properties.

Ultrasound has been widely studied to enhance the extraction of various components such as protein, oil, and bioactives. It has been suggested that ultrasound increases the mass transfer rate in two ways. On one hand, it produces micro steaming which has a mechanical effect on the surface of raw materials that destroy the surface and reduce the particle size. On the other hand, the high temperature and pressure facilitate the destruction of material surface which in turn results in increased area and increased amounts of compounds that release into solvent (Tao and Sun 2015). This study is a part of a research project which investigates the potential of ultrasound for fractionation of pulses into starch, protein and hull (bran fraction). The objectives of the present study were to evaluate the effects of different ultrasound conditions on some functional and bioactive properties of the pulse hulls such as dietary fiber, phytic acid, water and oil binding capacity, total phenolics, antioxidant activity and phenolic acid composition.

Materials and methods

Material

Faba bean (*Vicia faba*), pea (*Pisum sativum*) and red and green lentils (*Lens culinaris*) were procured from local markets in dry and whole seed form.

Ultrasound treatment

Pulse seeds (50 g) were steeped overnight in 0.05% sodium bisulphite solution (pH 10.5) at a ratio of 1:3 (w/v) at 35 °C in an incubator (IS-971R, Jeio Tech, Seoul, Korea). The pulses then rinsed and milled in a Waring blender with 450 mL cold (4 °C) water for 1 min. The suspension was transferred to a beaker, placed on a magnetic stirrer, and was continuously stirred during sonication procedure. Control sample was gently stirred on a magnetic stirrer for 30 min and no other treatment was employed. Ultrasonication was carried out with an ultrasonic processor (Sonics VCX 750, Connecticut, USA) at a constant frequency of 22 kHz using a horn type 25 mm diameter probe which was immersed in the slurry to a depth of 8.5 cm from the surface. Amplitudes of 40, 70 and 100% corresponding to 100, 200–250, 400–460 W were applied for 3.5, 2.5 and 1.5 h, respectively with pulse durations of 5 s on and 25 s

off. The temperature was continuously controlled with a temperature probe and was not allowed to exceed 45 °C. Both control and ultrasound-treated suspensions were sieved through 100 µm particle size sieve and washed repeatedly with water until the water washings become clear. The material above this 100 µm particle size sieve was defined as hull fraction. The starch amount of the hull fraction was assured to be below 10% by repetitive washing and employing total starch analysis (AACC Method No.: 76-13) (AACC 2000). Hull fraction dried in an oven at 40 °C. Further extraction and filtration steps were made for starch isolation which is another part of the study.

Determination of proximate composition

Moisture (Method No.: 44-15A), crude fat (Method No.: 30-20), ash (Method No.: 08-1) and crude protein (Method No.: 46-12) were analyzed according to the standard methods of AACC (AACC 2000). N was converted to protein by the factor of 5.7.

Determination of dietary fiber and phytic acid content

Soluble, insoluble and total dietary fiber contents were analyzed with Megazyme enzyme kits (Megazyme International, Bray, Co., Wicklow, Ireland) according to the AACC method (Method No.: 32-07) (AACC 2000). Phytic acid content was determined spectrophotometrically according to the colorimetric AOAC method (Method No.: 986.11) (AOAC 2000).

Determination of water and oil absorption

Water and oil absorption of the pulse hulls was determined according to the method outlined by Sharma et al. (2004) with slight modifications. Briefly, 1 g of hull was suspended in 10 mL of water or oil and continuously mixed for 15 min. Then, the mixture was centrifuged at 3200 rpm for 15 min and the water or oil retained by the hull was measured by weighing the supernatant.

Extraction of free and bound (hydrolyzable) phenolics and antioxidants

The free and bound (hydrolyzable) phenolics and antioxidants were extracted according to the method reported by Tuncel and Yılmaz (2011) with some modifications.

Extraction of the compounds in free (soluble) form

Three g of pulse hull was mixed with 40 mL of acetone:methanol mixture (50:50, v/v) and 25 µL concentrated

HCl and was shaken in an incubator (Jeio Tech, IS-971R, Seoul, Korea) at 45 °C for 1 h. Then, the mixture was centrifuged at 7800 rpm for 10 min, the supernatant was evaporated at 40 °C until dryness. The residue was redissolved in a mixture containing 1 mL ethanol, 1 mL acetone and 4 mL distilled water. The extract was filtered through 0.45 µm membrane filter and was stored at 4 °C until analyses.

Extraction of the compounds in bound (hydrolyzable) form

The residue remained from the free form extraction was hydrolyzed with 40 mL of 4 N sodium hydroxide for 3 h at 4 °C with constant agitation in an incubator (Jeio Tech, IS-971R, Seoul, Korea). Afterwards, the pH of the mixture was adjusted to 1.5–2.0 with 6 N ice cold HCl. The acidic mixture transferred to a separating funnel after filtering through filter paper and the target compounds were extracted with 150 mL of ethyl acetate. The ethyl acetate fraction was dried over anhydrous sodium sulfate and was evaporated to dryness at 40 °C. The residue was redissolved in a mixture containing 1 mL ethanol, 1 mL acetone and 4 mL distilled water. The extract was filtered through 0.45 µm membrane filter and stored at 4 °C until analyses.

Determination of total phenolics

Total phenolics content of the pulse hulls was measured using Folin–Ciocalteu assay. Free and bound form extracts (100 µL) was combined with 900 µL of deionised water, 5 mL of 0.2 N Folin–Ciocalteu reagent and 4 mL of sodium carbonate (75 g/L), respectively. The mixture was vortexed for 1 min and kept in a dark cabinet for 2 h at room temperature. Measurements were conducted with a UV–Vis spectrophotometer (Agilent, Model 8453, Waldbronn, Germany) at 765 nm. The results were the average of three replicates and expressed as mg of gallic acid equivalents per gram pulse hull (mg GAE/g).

Determination of Trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacity (TEAC) of the free and bound form extracts was measured according to Re et al. (1999). To obtain ABTS (2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid)) radical cation (ABTS⁺), 0.0384 g ABTS was weighed in a 10 mL volumetric flask, dissolved in small quantity of deionised water, 2 mL of 12.25 mM potassium persulfate was added to the content and brought to volume. The mixture was allowed to stand in a dark cabinet for 12–16 h at room temperature before use. Phosphate buffered saline (PBS)

(pH 7.4) was used either to dilute ABTS radical solution to 0.700 ± 0.020 absorbance at 734 nm or to dilute extracts to obtain an appropriate inhibition rate. Extracts in 3 different concentrations (and in duplicate) were added to the radical solution after recording the initial absorbance and the decrease in absorbance was monitored during 6 min. The results were calculated by plotting the percentage inhibition as a function of concentration of the extracts and Trolox standards for the reference data. Calibration curve was performed with standard Trolox solutions (5–20 µmol final concentration). The results were expressed as µmol Trolox equivalent antioxidant capacity per g pulse hull (µmol Trolox eq./g).

Determination of phenolic acid composition

Phenolic acid profile was analyzed chromatographically according to the method outlined in our previous study (Yılmaz and Tuncel 2015) excluding the solid phase purification step. The separation was carried out with an Agilent Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm i.d., 5 µm particle size) at a flow rate of 1 mL/min. Sample injection volume was 5 µL and the operation temperature was 23 °C. Mobile phase was consisting of A (methanol: water: formic acid (10: 88: 2, (v/v))) and B (methanol: water: formic acid (90: 8: 2, (v/v))). A gradient program was used as follows: 100% A; 0–20 min, changed to 80% A; 25–50 min, to 50% A; 50–54 min, followed by isocratic elutions of 50% A; 54–64 min, 0% A; and 64–70 min, 100% A. The UV detector was set at 280 nm. Gallic (GA), protocatechuic (protoCA), *p*-hydroxy benzoic (*p*-hydBA), vanillic (VA), caffeic (CA), chlorogenic (ChA), syringic (SRA), *p*-coumaric (*p*-COU), ferulic (FA), sinapic (SNA), *o*-coumaric (*o*-COU), rosmarinic (RA) and *trans*-cinnamic (*tr*-CIN) acids were analyzed. The results were the average of 3 replicates and expressed as µg/g pulse hull.

Statistical analysis

Data were analyzed with MINITAB Version 16.1 (StatSoft, Inc., Tulsa, OK, USA) for Windows. Two statistical models (Eqs. 1, 2) were used to evaluate the data.

The effects of pulse type and ultrasound conditions on the investigated parameters were evaluated with the two-way analysis of variance (ANOVA) model

$$Y_{ijk} = \mu + P_i + S_j + (PS)_{ij} + e_{ijk} \quad (1)$$

where Y_{ijk} is the measured value of k for pulse type i and ultrasound treatment j , μ is the general population mean, P_i is the effect of pulse type, S_j is the effect of ultrasound treatment, $(PS)_{ij}$ is the effect of pulse type $\times$ ultrasound treatment interaction and e_{ijk} is the random error.

Also, the effect of ultrasound treatment for a given pulse type (such as green lentil and 40% amplitude, as one factor) were evaluated using the one-way ANOVA model

$$Y_{ij} = \mu + \alpha_i + e_{ij} \quad (2)$$

where Y_{ij} is the j th observation value of the i th pulse, α_i is the effect of ultrasound condition for the given pulse and e_{ij} is the random error.

When significant effects were observed ($P < 0.05$), Tukey's test was performed for multiple comparisons. Data were presented as mean $\pm$ SE. All results presented are the means of three replicates. The free and bound phenolic acids were evaluated individually for each pulse type.

Results and discussion

Proximate composition

The composition of hulls from different pulses is presented in Table 1. Moisture and starch content of the hulls were below 10%. Crude ash contents were ranged between 2.11 and 2.23%. Crude fat content of the hulls were notably low. Crude protein content of lentils were almost twice as higher than faba bean and pea hulls (Table 1). Protein content up to 8% in hulls was an expected result since no additional protein precipitation step was involved during fractionation process.

Dietary fiber content of the pulse hulls

Although there was no statistical difference among samples, the highest and the lowest soluble dietary fiber (DF) was observed in green and red lentil hulls, respectively (Table 2). The effect of ultrasound was insignificant on soluble DF, which ranged between 1.23 and 3.05% ($P > 0.05$). DF is defined as the macromolecules that resist digestion by human endogenous enzymes and is essentially composed of plant cell wall components such as polysaccharides and lignin. Soluble DF develops significant viscosity, influences absorption by delaying gastric emptying, changing the absorption site and delaying small bowel transit time which, in turn, leads modified post-prandial glucose and lipid levels (Tharanathan and Mahadevamma 2003).

Insoluble DF content of the pulse hulls, which varied between 63.66 and 72.47%, was notably higher than that of the soluble DF (Table 2). Insoluble DF has beneficial effects due to reducing intestinal transit time and contact time for faecal mutagens (Tharanathan and Mahadevamma 2003). Although the results were not remarkably different from each other, insoluble DF content of the ultrasound treated pulse hulls were significantly lower than the untreated control for red lentil and pea hulls ($P < 0.05$). On the other hand, Li et al. (2014) reported that 40 min ultrasound assisted extraction resulted in 6% higher soluble DF yield in apple pomace compared to acid hydrolysis alone and attributed this result to the enhancement of mass transfer due to the increased access of extraction solvent to the cells. Also Wang et al. (2014) found that ultrasound treatment increased the efficiency of enzymatic extraction of arabinoxylan from wheat bran. Total DF followed the same trend with insoluble DF with regard to the effect of ultrasound. The highest and the lowest total DF content was observed in red lentil and faba bean hulls, respectively.

Phytic acid content of the pulse hulls

Phytate occurs as an insoluble mineral complex and its content may vary depending on the variety, environmental conditions, type of soil, irrigation conditions, year and place of the cultivation (Oomah et al. 2011; Urbano et al. 2000). Phytic acid content of the pulse hulls ranged between 1.37 and 1.66 mg/g (Table 2). Owing to the notably low content of phytic acid in pulse hulls, phytic acid analysis was also conducted on the whole pulse seeds. Phytic acid contents of green lentil, red lentil, faba bean and pea seeds were 4.34, 3.23, 5.89 and 3.99 mg/g, respectively, which means that the phytic acid content of the seeds were almost fourfold higher than the hulls. This result can be attributed to the location of phytate in seed and more likely to the aqueous soaking step during processing. It has been reported that phytate accumulates in different parts of the cereals and legumes (Gupta et al. 2015). For instance, phytate is stored in the endosperm portion of pulses within the protein bodies (Oomah et al. 2011). However, it is found in bran fraction such as aleurone and pericarp in rice and wheat, while it is seen in

Table 1 Proximate composition of the pulse hulls

	Green lentil hull	Red lentil hull	Faba bean hull	Pea hull
Moisture (%)	9.20 $\pm$ 0.43	9.35 $\pm$ 0.06	9.57 $\pm$ 0.32	9.71 $\pm$ 0.14
Crude ash (% d.w.)	2.11 $\pm$ 0.05	2.23 $\pm$ 0.01	2.16 $\pm$ 0.02	2.23 $\pm$ 0.02
Total starch (% d.w.)	<10	<10	<10	<10
Crude fat (% d.w.)	0.18 $\pm$ 0.06	0.91 $\pm$ 0.01	0.15 $\pm$ 0.01	0.28 $\pm$ 0.09
Crude protein (% d.w.)	8.58 $\pm$ 0.08	8.64 $\pm$ 0.07	4.53 $\pm$ 0.10	4.50 $\pm$ 0.02

d.w. dry weight

Table 2 Some properties of the pulse hulls

Pulse type	Ultrasound amplitude	Soluble dietary fiber (%)	Insoluble dietary fiber (%)	Total dietary fiber (%)	Phytic acid (mg/g)	Water absorption (g/100 g)	Oil absorption (g/100 g)
Green lentil hull	Control	2.90 ± 0.37	68.42 ± 0.86 ^{ABC}	71.32 ± 0.48 ^{AB}	1.48 ± 0.08	464.2 ± 6.9 ^{CDE}	196.3 ± 13.8 ^{ABC}
	40%	2.58 ± 0.03	68.05 ± 1.29 ^{ABC}	70.64 ± 1.25 ^{AB}	1.49 ± 0.07	463.1 ± 20.2 ^{CDE}	181.6 ± 4.6 ^{BC}
	70%	3.01 ± 0.27	67.95 ± 1.30 ^{ABC}	70.96 ± 1.02 ^{AB}	1.37 ± 0.01	481.3 ± 12.9 ^{BCDE}	182.0 ± 0.1 ^{BC}
	100%	2.65 ± 0.02	68.20 ± 0.98 ^{ABC}	70.86 ± 0.95 ^{AB}	1.47 ± 0.02	439.5 ± 11.8 ^{DEF}	163.6 ± 3.1 ^C
Red lentil hull	Control	1.54 ± 0.21	71.80 ± 0.08 ^A	73.34 ± 0.12 ^A	1.66 ± 0.15	524.1 ± 2.3 ^{ABCD}	225.1 ± 0.1 ^{ABC}
	40%	1.93 ± 0.18	70.55 ± 1.24 ^{AB}	72.48 ± 1.06 ^{AB}	1.44 ± 0.01	535.5 ± 10.8 ^{ABC}	230.7 ± 1.9 ^{ABC}
	70%	1.62 ± 0.05	68.31 ± 0.58 ^{ABC}	69.94 ± 0.63 ^{AB}	1.43 ± 0.01	538.4 ± 2.5 ^{ABC}	234.0 ± 7.7 ^{ABC}
	100%	1.23 ± 0.01	69.15 ± 0.81 ^{ABC}	70.39 ± 0.82 ^{AB}	1.45 ± 0.01	476.6 ± 16.7 ^{BCDE}	222.7 ± 9.2 ^{ABC}
Faba bean hull	Control	3.05 ± 0.43	66.77 ± 1.60 ^{ABC}	69.82 ± 1.16 ^{AB}	1.58 ± 0.14	372.6 ± 17.3 ^F	195.0 ± 13.6 ^{ABC}
	40%	1.97 ± 0.12	64.38 ± 1.35 ^{BC}	66.35 ± 1.47 ^B	1.48 ± 0.05	420.9 ± 24.3 ^{EF}	197.0 ± 9.5 ^{ABC}
	70%	1.91 ± 0.44	68.31 ± 2.47 ^{ABC}	70.23 ± 2.91 ^{AB}	1.37 ± 0.09	440.8 ± 35.6 ^{DEF}	209.3 ± 16.2 ^{ABC}
	100%	2.31 ± 0.01	66.17 ± 0.52 ^{ABC}	68.48 ± 0.53 ^{AB}	1.44 ± 0.05	412.8 ± 14.9 ^{EF}	186.4 ± 17.3 ^{ABC}
Pea hull	Control	2.10 ± 0.97	72.47 ± 0.10 ^A	74.57 ± 0.87 ^A	1.59 ± 0.14	579.2 ± 14.6 ^A	226.1 ± 12.1 ^{ABC}
	40%	2.70 ± 0.61	63.66 ± 0.54 ^C	66.37 ± 0.06 ^B	1.47 ± 0.02	554.6 ± 2.5 ^{AB}	246.3 ± 13.0 ^{AB}
	70%	1.88 ± 0.67	69.45 ± 0.94 ^{ABC}	71.33 ± 0.26 ^{AB}	1.55 ± 0.09	595.6 ± 4.0 ^A	259.8 ± 36.5 ^{AB}
	100%	2.53 ± 0.56	67.26 ± 1.42 ^{ABC}	70.29 ± 0.85 ^{AB}	1.44 ± 0.02	575.2 ± 7.6 ^A	264.1 ± 17.0 ^A
<i>P</i>		0.141	0.005	0.006	0.535	0.000	0.093

Means followed by different letters in the same column are significantly different by Tukey ($P < 0.05$)

endosperm portion of corn (Gupta et al. 2015). On the other hand it is well known that some processes such as cooking, fermentation, germination and soaking reduces phytate content (Urbano et al. 2000). As indicated in the method section, pulse seeds (both control and processed) were soaked in 0.05% sodium bisulphite solution (pH 10.5) and incubated at 35 °C for overnight in our study. Also the ultrasound process was employed in aqueous medium and the hulls were washed with water repetitively to get rid of the starch. It has been reported that soaking of chickpea for 12 h reduces phytate content by 55.71% and the pH of the water does not have a significant effect on the level of phytate (Ertaş and Türker (2014). Also, Khalil (2001) reported that phytic acid losses in guar and faba bean brans were 45 and 27%, respectively after soaking the seeds at room temperature for 12 h.

Besides, neither the effect of pulse type nor the effect of ultrasound treatment was significant on phytic acid content of the pulse hulls ($P > 0.05$). Further, none earlier study has reported the effect of ultrasound on phytic acid content.

Water and oil absorption of the pulse hulls

The highest and the lowest water absorption was observed in pea and faba bean hulls, respectively ($P < 0.05$). On the other hand, pea and red lentil hulls showed higher oil

absorption compared to the other pulses. The effect of ultrasound was insignificant on oil absorption and although it was significant for water absorption no consistent trend was observed with regard to the ultrasound conditions (Table 2).

Phenolics and antioxidants of the pulse hulls

Total phenolics

Total phenolic content of the pulse hulls was remarkably lower than those reported earlier (Xu and Chang 2008; Boudjou et al. 2013). On the other hand, Marathe et al. (2011) also reported low phenolic content (~0.9 mg GAE/g) in whole lentil and pea seeds with 80% aqueous methanol extraction. Researchers also indicated that lentils, black pea, pigeon pea, moth bean and lablab bean (brown) though having dark color seed coat, had total phenolic content lower than 1.2 mg GAE/g. Also, Xu and Chang (2007) investigated how phenolics and antioxidants of 8 commonly consumed legumes were affected by 6 frequently used solvent systems and reported that the total phenolic contents of green pea and lentil seeds extracted by different solvents ranged from 1.04 to 1.53 mg GAE/g and from 1.02 to 7.53 mg GAE/g, respectively (Xu and Chang 2007). Besides, taking into account of the polar nature of

some phenolics, low phenolic content of the pulse hulls used in this study may also be related with the soaking and washing steps during extraction. Xu and Chang (2008) reported significant losses in total phenolic content of soaked peas and lentils with regard to the raw samples.

Additionally, it was observed that the total phenolic content of the bound hull extracts was higher than that of the free ones for lentils. However, the amount of total phenolics in faba bean and pea hulls were almost negligible either in free or bound form extracts (Table 3). The highest total phenolic content was observed in red lentil hull. Further, the level of total phenolics was generally higher in control samples when compared to the ultrasound treated ones. Similarly, Hromádková et al. (2008) also reported higher total phenolics content in the extracts obtained by classical extraction procedures than in those obtained by sonication for wheat bran. Also, Ahmad-Qasem et al. (2013) found very similar total phenolics results with conventional and ultrasound assisted extraction in olive leaves.

TEAC

Trolox equivalent antioxidant capacity (TEAC) of the pulse hulls was in the same trend with the total phenolics results. This was also consistent with the Pearson correlations between total phenolics and TEAC, which were 0.755

($P = 0.000$), 0.980 ($P = 0.000$), and 0.986 ($P = 0.000$) for free form extracts, bound form extracts, and total, respectively. The high correlation values indicate that total phenolics in pulse hulls were responsible for their antioxidant capacity. Lentil hulls were richer in antioxidant capacity compared to the other pulse hulls. The highest and the lowest TEAC was observed in red lentil and pea hulls, respectively. In general, the antioxidant capacity of the pulse hulls was found to be notably low in an agreement with Marathe et al. (2011) who found very weak antioxidant potential in 30 different varieties of commonly consumed legumes in India. On the other hand, these results may also be related to the loss of polar antioxidants due to the soaking and washing procedures. Vadivel et al. (2011) investigated the effect of indigenous processing methods of immature wild type legume grains and reported significant reductions of total free phenolic content, antioxidant activity and type II diabetes related enzyme inhibition properties with soaking and cooking treatments. Researchers attributed these results to the leaching out of phenolics by increased seed coat permeability and/or to the degradation of phenolics by high temperature during cooking. Similarly, Xu and Chang (2008) also reported 6–34% loss of antioxidant activity in soaked chickpea, yellow pea, green pea and lentil when compared to the raw samples.

Table 3 Total phenolics and Trolox equivalent antioxidant capacities (TEAC) of the pulse hulls

Pulse type	Ultrasound amplitude	Total phenolics (mg GAE/g pulse hull)		TEAC (μmol Trolox eq./g pulse hull)	
		Free	Bound	Free	Bound
Green lentil hull	Control	0.30 ± 0.02^A	4.05 ± 0.44^{AB}	1.85 ± 0.76^A	24.75 ± 1.35^{BC}
	40%	0.22 ± 0.02^{AB}	2.14 ± 0.16^D	0.53 ± 0.16^B	18.44 ± 1.36^{DE}
	70%	0.21 ± 0.01^B	2.24 ± 0.37^{CD}	0.51 ± 0.04^B	16.42 ± 0.67^E
	100%	0.21 ± 0.01^B	2.71 ± 0.12^{CD}	0.57 ± 0.17^B	20.48 ± 2.12^{CDE}
Red lentil hull	Control	0.24 ± 0.01^{AB}	5.16 ± 0.10^A	1.16 ± 0.06^{AB}	33.17 ± 0.17^A
	40%	0.21 ± 0.01^B	4.27 ± 0.34^{AB}	0.61 ± 0.04^B	28.44 ± 1.14^{AB}
	70%	0.18 ± 0.01^B	4.57 ± 0.12^A	0.62 ± 0.03^B	31.36 ± 0.63^A
	100%	0.21 ± 0.01^B	3.33 ± 0.34^{BC}	0.68 ± 0.19^{AB}	22.03 ± 0.03^{CD}
Faba bean hull	Control	0.17 ± 0.01^B	1.00 ± 0.09^E	0.33 ± 0.10^B	6.42 ± 0.65^F
	40%	0.17 ± 0.01^B	0.46 ± 0.08^E	0.27 ± 0.05^B	4.17 ± 1.01^{FG}
	70%	0.16 ± 0.01^B	0.86 ± 0.01^E	0.25 ± 0.01^B	7.03 ± 0.13^F
	100%	0.17 ± 0.01^B	0.65 ± 0.22^E	0.24 ± 0.06^B	3.93 ± 0.62^{FG}
Pea hull	Control	0.21 ± 0.01^B	0.28 ± 0.01^E	0.24 ± 0.01^B	0.62 ± 0.04^G
	40%	0.21 ± 0.02^B	0.27 ± 0.05^E	0.28 ± 0.01^B	0.65 ± 0.01^G
	70%	0.20 ± 0.03^B	0.27 ± 0.05^E	0.23 ± 0.03^B	0.63 ± 0.14^G
	100%	0.19 ± 0.01^B	0.24 ± 0.02^E	0.26 ± 0.01^B	0.90 ± 0.04^G
<i>P</i>		0.000	0.000	0.004	0.000

Means followed by different letters in the same column are significantly different by Tukey ($P < 0.05$)

GAE gallic acid equivalents

Moreover, antioxidant capacity of the control samples was higher than the sonicated ones for lentil hulls. However, almost no notable variation was observed in faba bean and pea hulls with regard to the effect of ultrasound (Table 3).

Phenolic acids

Phenolic acids that were found in free and bound (hydrolyzable) form extracts of the noted pulse hulls were shown in Tables 4 and 5, respectively. Syringic, sinapic, rosmarinic and *tr*-cinnamic acids could not be detected in any of the extracts among the analyzed phenolic acids. Protocatechuic, *p*-coumaric and ferulic acids were found in all pulse hulls either in free or bound (hydrolyzable) form. Although gallic acid was also detected in almost every sample, it was not evaluated because of the inconsistent results due to presence of co-eluting impurities. Robbins (2003) reported that caffeic, *p*-coumaric, vanillic, ferulic, and protocatechuic are acids that present in nearly all plants, however, other phenolic acids are found in selected foods or plants. *P*-hydroxybenzoic and chlorogenic acids were detected only in red and green lentil hulls, respectively. Further, *o*-coumaric acid could not be detected in lentils while it was found in faba bean and pea hulls.

Moreover, it can be clearly seen that bound (hydrolyzable) extracts contained more phenolic acids either qualitatively or quantitatively. It has been reported that only a minor fraction of phenolic acids exists in free form, instead, the majority of them are linked through ester, ether, or acetal bonds either to structural components of the

plant (such as proteins, cellulose or lignin) or to larger polyphenols (such as flavonoids) or smaller organic molecules (such as glucose, maleic or tartaric acids) or other natural products (such as terpenes) (Robbins 2003; Adom and Liu 2002). Similarly, Parmar et al. (2017) reported that base hydrolysis of kidney bean showed the presence of catechin, chlorogenic acid, protocatechuic acid, and *p*-coumaric acid.

Statistical evaluation of the phenolics acids was conducted individually for each pulse type due to the unequal number of observations. Although the pulse types could not be compared with each other statistically, it can be seen that lentil hulls showed higher phenolic acid content among the others (Table 5). Although some trends were observed with regard to the effect of ultrasound, the results were not robust enough to generalize. For instance, ferulic acid contents of sonicated red lentil and pea hulls were significantly lower than control, while no consistent variation was observed with regard to the increasing ultrasound amplitude for the other pulse hulls (Table 5). The very low amount of phenolic acids also makes it difficult to evaluate the effect of the process. Higher results would have led to more reliable discussion. For instance, hydrolyzable protocatechuic and *p*-hydroxybenzoic acid contents of red lentil hull, which were higher quantitatively, showed a linear decreasing trend with increasing ultrasound amplitude (Table 5). The effect of ultrasound on phenolic acids of pulses has seldom been reported in the literature. Qiao et al. (2013) reported that protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and ferulic acid were stable while caffeic and sinapic acids degraded

Table 4 Phenolic acids in free form extracts of the pulse hulls (μg/g)

Pulse type	Ultrasound amplitude	proto-CA	VA	<i>p</i> -Cou
Green lentil hull	Control	1.00 ± 0.01 ^A	0.64 ± 0.01	n.d.
	40%	0.76 ± 0.07 ^B	0.76 ± 0.01	n.d.
	70%	0.62 ± 0.03 ^B	0.80 ± 0.07	n.d.
	100%	0.66 ± 0.03 ^B	0.72 ± 0.01	n.d.
Red lentil hull	Control	n.d.	n.d.	1.69 ± 0.01 ^A
	40%	n.d.	n.d.	1.36 ± 0.02 ^C
	70%	n.d.	n.d.	1.34 ± 0.01 ^C
	100%	n.d.	n.d.	1.46 ± 0.01 ^B
Faba bean hull	Control	0.79 ± 0.08 ^{AB}	0.73 ± 0.02	n.d.
	40%	0.60 ± 0.02 ^B	0.80 ± 0.02	n.d.
	70%	0.93 ± 0.10 ^A	0.81 ± 0.05	n.d.
	100%	0.63 ± 0.03 ^{AB}	0.72 ± 0.05	n.d.
Pea hull	Control	0.98 ± 0.24	0.82 ± 0.15	n.d.
	40%	1.24 ± 0.24	0.99 ± 0.18	n.d.
	70%	1.38 ± 0.03	0.77 ± 0.09	n.d.
	100%	1.46 ± 0.24	0.72 ± 0.04	n.d.

Means followed by different letters in the same column (for each pulse hull type) are significantly different by Tukey ($P < 0.05$)

n.d. not detected, *proto-CA* protocatechuic acid, *VA* vanillic acid, *p-Cou* *p*-coumaric acid

Table 5 Phenolic acids in bound form extracts of the pulse hulls ($\mu\text{g/g}$)

Pulse type	Ultrasound amplitude	protoCA	p-OH-BA	VA	CA	ChA	p-Cou	FA	o-Cou
Green lentil hull	Control	21.62 $\pm$ 1.57	n.d.	2.69 $\pm$ 0.05	3.02 $\pm$ 0.28 ^A	2.02 $\pm$ 0.12 ^B	2.56 $\pm$ 0.25	6.04 $\pm$ 0.84 ^{AB}	n.d.
	40%	17.19 $\pm$ 0.56	n.d.	2.08 $\pm$ 0.14	2.53 $\pm$ 0.23 ^{AB}	6.85 $\pm$ 1.79 ^{AB}	2.81 $\pm$ 0.27	4.32 $\pm$ 0.31 ^B	n.d.
	70%	17.20 $\pm$ 1.23	n.d.	2.19 $\pm$ 0.36	2.24 $\pm$ 0.20 ^{AB}	4.30 $\pm$ 0.03 ^B	2.53 $\pm$ 0.25	9.89 $\pm$ 0.98 ^A	n.d.
Red lentil hull	100%	19.74 $\pm$ 2.06	n.d.	2.66 $\pm$ 0.20	1.96 $\pm$ 0.15 ^B	11.86 $\pm$ 2.23 ^A	2.70 $\pm$ 0.25	9.80 $\pm$ 1.44 ^A	n.d.
	Control	27.49 $\pm$ 2.95 ^A	53.73 $\pm$ 0.10 ^A	n.d.	n.d.	n.d.	n.d.	11.23 $\pm$ 1.02 ^A	n.d.
	40%	22.28 $\pm$ 1.77 ^{AB}	52.59 $\pm$ 5.37 ^A	n.d.	n.d.	n.d.	n.d.	4.24 $\pm$ 0.07 ^B	n.d.
Faba bean hull	70%	20.15 $\pm$ 0.95 ^{AB}	49.82 $\pm$ 3.57 ^A	n.d.	n.d.	n.d.	n.d.	4.78 $\pm$ 0.35 ^B	n.d.
	100%	15.25 $\pm$ 1.94 ^B	26.56 $\pm$ 2.71 ^B	n.d.	n.d.	n.d.	n.d.	4.91 $\pm$ 0.24 ^B	n.d.
	Control	9.58 $\pm$ 2.22	n.d.	0.85 $\pm$ 0.05	n.d.	n.d.	2.43 $\pm$ 0.59	5.56 $\pm$ 0.12 ^A	1.04 $\pm$ 0.23
Pea hull	40%	7.48 $\pm$ 0.33	n.d.	0.84 $\pm$ 0.06	n.d.	n.d.	2.08 $\pm$ 0.39	3.34 $\pm$ 0.11 ^B	1.09 $\pm$ 0.18
	70%	7.71 $\pm$ 0.75	n.d.	1.08 $\pm$ 0.08	n.d.	n.d.	2.53 $\pm$ 0.44	6.37 $\pm$ 0.41 ^A	1.10 $\pm$ 0.25
	100%	9.29 $\pm$ 0.33	n.d.	0.91 $\pm$ 0.01	n.d.	n.d.	2.47 $\pm$ 0.68	3.61 $\pm$ 0.03 ^B	1.17 $\pm$ 0.13
Pea hull	Control	n.d.	n.d.	n.d.	4.24 $\pm$ 0.52	n.d.	1.84 $\pm$ 0.16	8.15 $\pm$ 0.31 ^A	2.22 $\pm$ 0.66
	40%	n.d.	n.d.	n.d.	6.12 $\pm$ 0.24	n.d.	4.27 $\pm$ 0.78	5.72 $\pm$ 0.82 ^{AB}	1.48 $\pm$ 0.15
	70%	n.d.	n.d.	n.d.	5.28 $\pm$ 0.53	n.d.	4.73 $\pm$ 1.47	6.78 $\pm$ 0.29 ^{AB}	1.98 $\pm$ 0.29
Pea hull	100%	n.d.	n.d.	n.d.	4.75 $\pm$ 0.41	n.d.	1.93 $\pm$ 0.28	4.26 $\pm$ 0.63 ^B	1.05 $\pm$ 0.08

Means followed by different letters in the same column (for each pulse hull type) are significantly different by Tukey ($P < 0.05$)

n.d. not detected

protoCA protocatechuic acid, p-OH-BA p-hydroxybenzoic acid, VA vanillic acid, CA caffeic acid, ChA chlorogenic acid, p-Cou p-coumaric acid, FA ferulic acid, o-Cou o-coumaric acid

under ultrasound treatment in a model system. Researchers also indicated that the degradation reaction was affected from the nature of the solvent and the temperature. Similarly, Singh et al. (2017a, b) found that either ultrasound assisted extraction or the solvent system affect the phenolic acids. For instance, they reported that ultrasound assisted extraction increased chlorogenic acid content of mung bean hull in acetone and water extracts, whereas, a decrease was observed in methanol extract with regard to the conventional solvent extraction. Controversially, Ahmad-Qasem et al. (2013) found that chromatograms from the extracts obtained with ultrasound assisted and conventional procedures were very similar and ultrasound application did not promote the formation of new phenolic compounds or induce phenolic acid degradation.

Conclusion

As a conclusion, it was found that the effect of ultrasound on soluble DF content was insignificant and although it was significant for insoluble and total DF, the differences were not practically important. Phytic acid content of the pulse hulls was almost $\frac{1}{4}$ of the whole seeds and ranged between 1.37 mg/g and 1.66 mg/g. Besides, neither the effect of pulse type nor the effect of ultrasound treatment was significant on phytic acid content ($P > 0.05$). Water and oil absorption capacities of the pulse hulls did not remarkably affected from the ultrasound conditions, while the highest capacities observed in red lentil and pea hulls. Lentil hulls showed higher total phenolic content and antioxidant capacity when compared to the other pulse hulls. Further, the highest total phenolic content and TEAC were observed in red lentil hull. Ultrasound treated samples resulted in significantly lower total phenolic and TEAC values compared to control for lentil hulls ($P < 0.05$). Total phenolics, TEAC, and phenolic acids were higher in bound (hydrolyzable) extracts compared to free ones in all samples. No consistent trend was observed with regard to the effect of ultrasound conditions on phenolic acids.

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