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Effect of tempering moisture and infrared heating temperature on the nutritional properties of desi chickpea and hull-less barley flours, and their blends

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ABSTRACT

The impact of infrared heating surface temperature and tempering moisture on the nutritional properties of desi chickpea, hull-less barley, and their blends were examined. Specifically, this included changes to the level of anti-nutritive factors (*i.e.*, trypsin/chymotrypsin inhibitors, total phenolics and condensed tannins), amino acid composition and *in vitro* protein digestibility. Results indicated that both temperature and the tempering/temperature treatment caused a reduction in levels of all anti-nutritional factors for both flours, and the effect was more prominent in the tempering-temperature combination. The amino acid composition of both flours was not substantially changed with tempering or infrared heating. The amino acid scores (AAS) of chickpea and barley flours, as determined by the first limiting amino acid using the FAO/WHO reference pattern found in the case of barley to be limiting in lysine with an AAS of ~0.9, whereas for chickpea flour, threonine was limiting and had an AAS of ~0.6. The *in vitro* protein digestibility of chickpea samples was found to increase from 76% to 79% with the tempering-heat (135 °C) combination, whereas barley flour increased from 72% to 79% when directly heated to 135 °C (without tempering). *In vitro* protein digestibility corrected amino acid score (IV-PDCAAS) was found to increase from 65% to 71% for chickpea flour and 44% to 52% for barley flour, respectively with tempering-temperature (135 °C) combination indicating that tempering with infrared heating can improve the nutritional value of both flours. The addition of chickpea flour to the barley flour acted to improve the nutritional properties (IV-PDCAAS), to an extent depending on the concentration of chickpea flour present.

1. Introduction

Barley and chickpeas represent cereal and pulse crops, respectively, that are widely consumed as staple foods around the world. They also represent dominant crops grown in countries, such as Ethiopia, whose population is greatly impacted by food insecurity and cases of malnutrition. Often in developing countries, animal-derived proteins are scarce or too expensive leaving a large percentage of the population relying on plant-based proteins for their diet. Often the diet does not contain complementary protein sources creating deficiencies in both indispensable amino acids and protein quality. Barley tends to be rich in carbohydrates (77.72%) and proteins (9.91%), but low in fat (1.16), whereas chickpeas (desi or kabuli) tend to be rich in carbohydrates (62.95%), proteins (20.47%), and fat (6.04%) (USDA, 2016). When considered in isolation, both crops present deficiencies of certain amino acids that diminish their respective protein quality estimates. For instance, barley tends to be high in prolamin-type (alcohol soluble) proteins that are rich in sulphur containing amino acids but limiting in lysine, whereas chickpeas are high in globulin-type (salt soluble) and albumin (water soluble) proteins that are rich in the opposite (Helm et al., 2004; Singh & Jambunathan, 1982). However, when consumed together, the two crops offer the potential to provide a balance of indispensable amino acids needed for growth and development.

Despite their nutritional importance, both barley and chickpea contain “anti-nutritive factors” that can adversely impact protein digestion (*e.g.*, trypsin and chymotrypsin inhibitors, phenolic compounds and tannins), starch digestion (*e.g.*, α-amylase inhibitor) and/or mineral absorption (*e.g.*, phytates and oxalates). These factors, however, can be

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significantly reduced or eliminated through processing by physical means (*e.g.*, dehulling and air classification) (Tiwari & Singh, 2012), fermentation (Khattab & Arntfield, 2009), germination (Vidal-Valverde et al., 1994), canning (Pedrosa et al., 2015), roasting (Sharma & Gujral, 2011), extrusion (Alonso, Aguirre, & Marzo, 2000) or infrared heating (Fasina, Tyler, Pickard, Zheng, & Wang, 2001; Khattab & Arntfield, 2009). Depending on the level and degree of processing, protein digestibility and amino acid compositions can be altered to impact the overall nutritional value or the protein quality of the respective flours (Khattab, Arntfield, & Nyachoti, 2009; Siddhuraju & Becker, 2001).

Although there are multiple methods available to determine protein quality, regulatory jurisdictions vary as it relates to the required method for protein content claims. For instance, in Canada, the Protein Efficiency Ratio is required to support a claim (Health Canada, 1981), whereas in the United States, the Protein Digestibility Corrected Amino Acid Score (PDCAAS) is used (FAO/WHO, 1991). While both of these methods of protein quality determination require the use of a rodent bioassay, there has been ongoing research into the development and validation of an *in vitro* method of protein quality analysis (Nosworthy, Franczyk, Zimoch-Korzycka, Appah, Utioh, Neufeld and House, 2017; Tavano, Neves, & da Silva Junior, 2016).

The current study was undertaken to examine the effect tempering and infrared heating has on the nutritional properties of desi chickpea and hull less barley flours, and their blends. Specifically, to examine changes in a select number of anti-nutritive factors that impact protein digestion (*i.e.*, trypsin/chymotrypsin inhibitors and total phenolics/tannins), protein digestibility (*in vitro* PDCAAS) and the amino acid composition. The approach the World Food Program of the United Nations takes to addressing some food insecurity issues is to source crops locally from farmers and to process them regionally to produce food aid type products with PDCAAS values > 0.70. Infrared heating is one process available in sub-Sahara Africa available for processing, and as such was used in this study as a means to improve the protein quality of both the chickpea and barley crops.

2. Materials and methods

2.1. Materials

Desi chickpeas (var.: CDC Consul, dehulled and split, grown in Elbow, SK in 2014) were purchased for this study from Diefenbaker Seeds (Saskatoon, SK) whereas the hull-less barley (var: CDC McGwire grown in Star City, SK in 2014) was donated. All seeds were stored dry, in large plastic sealed containers at room temperature (21-23 °C). All chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) and were of reagent grade unless otherwise stated. The water used in this research was produced from a Millipore Milli-Q™ water purification system (Millipore Corp., Milford, MA, USA).

2.2. Preparation of flour materials

In this study, chickpeas and barley grains were left un-tempered and tempered to 20% moisture prior to infrared heating. For tempering, ~8 kg of seeds were placed in sealed polyethene bags containing Milli-Q water, at the amount specified by the following equation based on initial seed moisture according to AACC Official Method 26–95.01 (AACC, 1999):

$$W(H_2O) = \frac{W_S \times [M_E - M_O]}{100 - M_O} \quad (1)$$

where, W_s represents the weight of samples used for tempering, M_o (Chickpea: M_o = 8.27%; barley: M_o = 8.60%) and M_E (~20%) are original and end moisture levels (%) found in un-tempered and tempered seeds, respectively. All tempering was carried out at room temperature and atmospheric pressure. A preliminary moisture uptake experiment was conducted over time (0.5 to 8 h) to find an equilibrium for both seeds, which was reached after 1 h at the 20% moisture level.

Infrared heating was carried out at InfraReady Products Ltd. (Saskatoon, SK) using a laboratory scale micronizer (Model A 156379-B0, FMC Syntron ® Bulk Handling Equipment, Homer City, PA, USA). The micronizer was made of a burner (Model type R 1603-2 pat, Rinnai, Japan) to generate heat, a Syntron feeder (Model F010, Riley Automatic Ltd., Derby, England) to feed and control the volume of processing seeds, and a Syntron magnetic feeder (Mode BF2 A, FMC Corporation, Homer City, PA, USA) to convey seeds passing the heating area. The burner was 19 cm above the conveyor and the magnetic feeder was 152 cm long. The surface temperature of the seeds was monitored using a hand-held IR thermometer (Oakton, Vernon Hills, IL, USA). Approximately 2 kg of each tempered and un-tempered sample was processed in order to reach the surface temperatures of 115 and 135 °C. Each heating treatment was carried out under the same condition three times to achieve three processing replicates. All processed seeds were dried to moisture levels < 10% using the laboratory scale micronizer. Un-tempered and unheated seeds served as another control.

All seeds were then ground into coarse flour using a disc mill (Glen Mills Inc., Clifton, NJ, USA) and then into finer flour using a UDY Cyclone Sample Mill with 0.1 mm mash (UDY Corporation, Fort Collins, CO, USA). Flours were stored in polyethylene Ziploc bags at 4 °C in a cold room.

In the case of blends, only the chickpea and barley flours tempered to 20% moisture and heated to a seed surface temperature of 135 °C (from one processing run) were used based on protein quality and anti-nutritional results (see Results). The flours were blended in polyethylene bags by hand mixing at six ratios (w/w) of chickpea to barley at 0: 100, 20: 80, 40: 60, 60: 40, 80: 20 and 100: 0. The flours were stored under the same condition as the original flour. Blends were analyzed for their proximate composition, functionality and protein quality. Data were reported as the mean ± standard deviation (n = 3) following the same methods mentioned below.

2.3. Proximate analysis

Proximate analysis of all flours was carried out according to the Association of Official Analytical Chemists (AOAC) methods 925.10, 923.03, 920.85 and 984.13A for moisture, ash, crude fat and crude protein (%N × 6.25 for chickpeas and blended flours and 5.7 for barley flour), respectively (AOAC, 2003). Ash, fat, and protein levels are reported on a percent dry weight basis (d.b.). For all composition analyses, measurements were made in duplicate on triplicate processing samples, and reported as the mean ± one standard deviation (n = 3).

2.4. Anti-nutritional properties

For all anti-nutritional compound analyses, measurements were made in duplicate on triplicate processing samples for the chickpea and barley flours only (*i.e.*, no blends), and reported as the mean ± one standard deviation (n = 3).

2.4.1. Total phenolic content

The total phenolic content within the flours was determined according to Singleton and Rossi (1965) and Chiremba, Taylor, and Duodu (2009). This method uses spectrophotometry to determine the change of absorbance of phenolic groups due to their reaction with Folin Ciocalteu reagent. In brief, 2 g of the flour treatments was extracted with 5 mL of 1% HCl in methanol by vortexing for 15 s every 20 min for 2 h (Vortex mixer, VWR, Mississauga, ON, Canada), followed by centrifugation at 1050 × *g* (VWR Clinical 200 centrifuge, VWR International, Mississauga, ON) for 10 min to recover the supernatant. This extraction procedure was repeated 2 more times and the supernatant from each time of extraction was pooled for later analysis. A standard catechin (monohydrate (+)-catechin, Catalog No. ALX-385-017-G001, Enzo Life Sciences Inc., Farmingdale, NY) curve was

obtained by preparing a 1 mg/mL catechin stock solution, followed by diluting with water to obtain catechin concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL. 0.5 mL of each standard concentration was transferred to a 50 mL volumetric flask with 2.5 mL of Folin Ciocalteu phenolic reagent. Then 7.5 mL of 20% (w/w) Na_2CO_3 was quickly added and brought to the volume with water. The samples were determined in duplicate by using 0.5 mL of sample extract instead of 0.5 mL of catechin dilutions and following the same procedure. The absorbance was measured at 760 nm using a spectrophotometer (Thermo Scientific spectrophotometer, Madison, WI, USA) exactly 2 h after the addition of Na_2CO_3. The total phenol content was calculated using Eq. (2), and the results were expressed per mg of catechin equivalent/g of flour on a dry basis.

$$\text{Total phenolic content (g catechin equivalent per g flour)} = \frac{(\text{Abs}_1 - b)}{a} \times 15\ \text{mL of extract} \div 2\ \text{g of sample} \quad (2)$$

where, Abs_1 refers to the absorbance of sample, b refers to the intercept of the standard curve and a refers to the slope of the standard curve.

2.4.2. Condensed tannins

The level of condensed tannins within the flours was determined according to the vanillin assay reported by Price, Van Scoyoc, and Butler (1978). A working vanillin reagent for analysis was prepared daily by mixing the stock solution of 1% vanillin in methanol and 8% HCl in methanol at the volume ratio of 1:1. In brief, 0.2 g of each sample was extracted using 10 mL absolute methanol for 20 min followed by centrifugation at 3000 ×*g* for 10 min with a VWR Clinical 200 centrifuge (VWR International, Mississauga, ON). A 0.3 mg/mL of catechin solution was prepared every day by dissolving 3.0 mg catechin in 10 mL methanol. A standard curve was made by making a serial dilution using that catechin solution. 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mL of the catechin solution was transferred to a set of five tubes and the volume was adjusted to 1 mL with methanol. Another set of tubes were prepared in the same way. Both sets of tubes were incubated in a 30 °C water bath. 5.0 mL of the working reagent was added to one set of tubes and 5.0 mL of 4% HCl was added to the other set of tubes in 1 min interval. The two sets of tubes were incubated at 30 °C for exactly 20 min and then the absorbance was measured at 500 nm using a spectrophotometer (Thermo Scientific spectrophotometer, Madison, WI, USA). The standard absorbance *vs.* mg catechin curve was made according to the results obtained. 1 mL of the supernatant of samples were transferred into 3 tubes and two of them reacted with 5 mL of working reagent and the third one incubated with 5 mL of 4% HCl in the same procedure described above. The level of condensed tannins was determined using Eq. (3), and the results were expressed on per gram of flour on a dry basis.

$$\text{Tannins (mg of catechin equivalent per g of flour)} = \frac{(\text{Abs}_1 - \text{Abs}_0 - b)}{a} \times 10\ \text{mL of extract} \div 0.2\ \text{g of sample} \quad (3)$$

where, Abs_1 refers to the absorbance of samples with working reagent and Abs_0 refers to the absorbance of the solution with HCl, b refers to the intercept of the standard curve and a refers to the slope of the standard curve.

2.4.3. Trypsin inhibitor activity

The trypsin inhibitor activity was determined according to AACC method 22–40.01 with modifications. In brief, 0.1 g of flour was weighed and extracted with 25 mL 0.01 N NaOH for 3 h, followed by centrifugation at 3000 ×*g* for 20 min at 4 °C using a Sorvall RC 6+ Centrifuge (Thermo Fisher Scientific Inc., Waltham, MA, USA). The pH of the supernatant was adjusted to 9.0 using 1 N HCl. Then, 0, 0.6, 1.0, 1.4 and 1.8 mL of extract was transferred to five 15 mL centrifuge tubes, volume adjusted to 2.0 mL with water. Each tube was incubated in a water bath at 37 °C with 2 mL of trypsin solution for 10 min. 5 mL of Na-Benzoyl-D, L-arginine 4-nitroanilide hydrochloride (DL-BAPNA) substrate solution pre-warmed at 37 °C was added into each test tube and then they were incubated for another 10 min, before stopping the reaction by adding 1 mL of 30% acetic acid. The solution was then filtered through Whatman No. 2 paper (GE Healthcare UK Limited, Buckinghamshire, UK). A blank was prepared for each sample concentration in a similar manner, except the acetic acid was added prior to the addition of the trypsin solution. The absorbance of the sample and blank at each concentration was measured at 410 nm measured using a spectrophotometer (Thermo Scientific spectrophotometer, Madison, WI, USA). The trypsin inhibitor activity was calculated using Eq. (4).

$$\text{Trypsin inhibitor unit per mg sample} = \frac{\text{TIU}}{\text{mL of extract taken}} \times \frac{25\ \text{mL of extract}}{100\ \text{mg of sample}} \times \text{D} \quad (4)$$

where, TIU refers to the trypsin inhibitor unit, arbitrarily defined as an increase of 0.01 absorbance unit, at 410 nm, of the sample relative to the corresponding concentration blank. D refers to the dilution factor, which preferably provides an inhibition between 40 and 60%. The result was expressed as TIU per mg of the sample and converted to dry basis.

2.4.4. Chymotrypsin inhibitor activity

Chymotrypsin inhibitory activity within the flour was determined according to the method reported by Makkar, Siddhuraju, and Becker (2007). In brief, 1 g of flour was accurately weighed into a beaker and was extracted by stirring with 10 mL borate buffer (pH 7.6) for an hour. The mixture was then transferred to a centrifuge tube and centrifuged using a VWR Clinical 200 centrifuge (VWR International, Mississauga, ON) at 3000 ×*g* for 10 min. 0, 0.25, 0.5 and 0.75 mL of supernatant were transferred to two sets of tubes (sets 1 and 2). The volumes were adjusted to 1 mL with the borate buffer mentioned above. Then 1 mL of chymotrypsin solution was added to each tube and the tubes were incubated in a 37 °C water bath for 10 min. Then 2 mL of pre-warmed casein was added to one set of tubes (set 1). The casein was prepared daily by taking 1 g of casein powder into 80 mL borate buffer (pH 7.6) and stirred at 35 °C until it was completely dissolved and the pH was kept at 7.6. The volume was brought up to 100 mL with borate buffer. The tubes with casein were incubated at 37 °C in a water bath for exactly 10 min. 6 mL of trichloroacetic acid reagent (18 g of trichloroacetic acid, 18 g of anhydrous sodium acetate and 20 mL of glacial acetic acid in 1 L of distilled water) was added to each tube after incubation. Then 2 mL of casein was added to the set of tubes (set 2). All the tubes were left to stand for 30 min before filtered through Whatman No. 2 paper (GE Healthcare UK Limited, Buckinghamshire, UK). The absorbance of filtered solutions was measured at 275 nm in a quartz cuvette against the blank. One chymotrypsin unit was defined as the increase by 0.01 absorbance unit at 275 nm of the reaction mixture. The chymotrypsin inhibitory activity was calculated following Eq. (5):

$$\text{Chymotrypsin inhibitor unit per mg sample} = \frac{\text{CIU}}{\text{mL of extract taken}} \times \frac{10\ \text{mL of extract}}{\text{mg of sample}} \times \text{D} \quad (5)$$

where, CIU was the absorbance divided by 0.01. D stands for the dilution factor, to keep the percent of inhibition within the range of 40% to 60%. The result was expressed as TIU per mg of the sample and converted to a dry basis.

2.5. Protein quality

2.5.1. Amino acid composition

The amino acid composition of treated and untreated chickpea and

barley flours, and blends were determined at POS Bio-Sciences Corp. (Saskatoon, SK) using a Pico-tag™ amino acid analysis system (Waters Corporation, Milford, MA, USA) and a high performance liquid chromatography (HPLC). All the 18 amino acids were determined. The determination of 15 amino acids followed the method reported by Bidlingmeyer, Cohen, Tarvin, and Frost (1987). In brief, the flours containing ~20 mg protein were prepared and mixed with 15 mL 6 N hydrochloride acid in Pyrex tubes, followed by flushing with N_2. The tubes were then capped and kept at 110 °C for 20 h to hydrolyze the proteins into amino acids for HPLC separation and determination. The determination of tryptophan followed the AOAC method 988.15 (2005) with slight modification. The samples were first hydrolyzed by 10 M NaOH and kept in a boiling water bath for 20 min and then were put in the oven at 110 °C for 16 h followed by HPLC determination. Tryptophan was determined by reverse phase liquid chromatography with UV detection. The concentration of sulphur amino acids, methionine, and cysteine, was determined following AOAC method 985.28 (2005) using ion-exchange chromatography with modification. The 1-octanol was not included in the procedure. The cold performic acid was used for cysteine and methionine oxidation and they were kept for reaction at 4 °C overnight. The sulphur amino acids were oxidized with performic acid and hydrolyzed with 6 M HCl at 110 °C for 18 h.

2.5.2. Determination of the amino acid score

Amino acid score refers to the ratio of 1 g of the target protein to the reference protein. The amino acid composition of the reference protein was recommended by FAO/WHO using the amino acid requirement for children 2 to 5 years of age (amino acid, mg/g protein): Histidine, 19; Isoleucine, 28; Leucine, 66; Lysine, 58; Methionine + Cysteine, 25; Phenylalanine + Tyrosine, 63; Threonine, 34; Tryptophan, 11; Valine, 35 (FAO, 1991). The amino acid score of the flour represents the most limiting essential amino acid.

2.5.3. In vitro protein digestibility

The *in vitro* protein digestibility was determined by the pH drop of the solution digested by a multi-enzyme solution according to Tinus, Damour, Van Riel, and Sopade (2012). This solution was prepared every day by mixing 31 mg chymotrypsin, 16 mg trypsin and 13 mg protease with 10 mL water and kept at 37 °C. Its pH was adjusted to 8.0 ± 0.05 with 0.1 M NaOH and HCl. 62.5 ± 0.5 mg protein (~0.2 g of chickpea flour or ~0.6 g of barley flour) was mixed with 10 mL water and added to 8 mL of water preheated to 50 °C. The mixture was stirred for 1 h at 37 °C. The pH of the solution was adjusted to 8.0 ± 0.05 with 0.1 M NaOH and HCl before adding 1 mL of the multi-enzyme solution mentioned above. The pH of the protein solution was recorded every 30 s for 10 min and the *in vitro* protein digestibility (IVPD) was calculated following the equation below:

$$\textit{In vitro}\text{protein digestibility (\%)} = 65.66 + 18.10 \times \Delta pH_{10\,min} \quad (6)$$

where ΔpH_{10min} refers to the change in pH from initial 8.0 to the end of 10 min.

2.5.4. In vitro Protein Digestibility Corrected Amino Acid Score (IV-PDCAAS)

The IV-PDCAAS was calculated as the product of the amino acid score and *in vitro* protein digestibility.

2.6. Statistics

A one-way analysis of variance with a Tukey's *Post Hoc* test was performed to determine statistical differences between treatments [(a) un-tempered non-micronized; (b, c) un-tempered, heated to 115 or 135 °C; and (d, e) tempered to 20% moisture, heated 115 or 135 °C] for each parameter. Note: triplicate measurements were made for each parameter measured, using triplicate processing runs. The mean values from each processing run were used in the ANOVA analysis, and in the calculation of the mean ± one standard deviation (n = 3). For the un-tempered non-micronized flours (control), triplicate measurement values were used for the ANOVA analysis. Blended flours with chickpea to barley ratio of 20: 80, 40: 60, 60: 40 and 80: 20 along with the original chickpea and barley flour tempered and heated at 135 °C were also analyzed by one-way analysis of variance followed by a Tukey's *Post Hoc* test. All statistical analysis was performed using the Minitab 17 statistical software (Minitab Inc., State College, PA).

3. Results and discussion

3.1. Composition

The proximate composition of desi chickpea flour was found to be comprised of ~25.4% (d.b.) protein, ~3.0% (d.b.) ash and 5.5% (d.b.) crude fat, whereas barley flour was found to be comprised of ~11.4% (d.b.) protein, ~1.9% (d.b.) ash and 2.3% (d.b.) crude fat. The effect of tempering moisture and infrared heating temperature did not impact the proximate composition. The results of proximate composition fell within the range reported in previous studies on different varieties of chickpeas with 24.1 to 26.2% of protein, 3.1 to 3.6% of ash and 4.2 to 5.0% of fat, on a dry weight basis (Ma et al., 2011; Marconi, Ruggeri, Cappelloni, Leonardi, & Carnovale, 2000; Milán-Carrillo, Reyes-Moreno, Armienta-Rodelo, Carábez-Trejo, & Mora-Escobedo, 2000), and for barley with 9.5 to 13.3% of protein, 1.5 to 1.6% of ash, 1.7 to 2.3% of fat (Abraha, Uhlen, Abay, Sahlstrøm, & Bjørnstad, 2013; Emami, Meda, & Tyler, 2011; Fasina, Tyler, Pickard, & Zheng, 1999).

3.2. Anti-nutritional properties

The presence of anti-nutritional compounds can have an adverse effect on protein digestibility and mineral absorption. Trypsin and chymotrypsin are endopeptidases that hydrolyze proteins at different sites. Trypsin and chymotrypsin inhibitors in plants are low molecular weight proteins that are used to protect seeds against bacteria and dormancy (Guillamón et al., 2008). Trypsin or chymotrypsin inhibitors can bind to lysine and arginine residues in trypsin and hydrophobic residues in chymotrypsin, respectively, to reduce the hydrolytic capacity of those enzymes (Dantzger et al., 2015). Phenolic compounds are able to cross-link proteins to inhibit their unfolding, making proteins less soluble and less susceptible to proteolytic digestion (Sreerama, Sashikala, Pratape, & Singh, 2012; Vidal-Valverde et al., 1994). Tannins belong to the phenol family and they can easily bind to proline and histidine (Boye, Wijesinha-Bettoni, & Burlingame, 2012), leading to a decrease in protein digestibility.

3.2.1. Total phenolic and condensed tannins content

The concentration of total phenolic content (TPC) of chickpea and barley was determined and the results are shown in Table 1. Results from the one-way ANOVA provide evidence that levels of total phenolics within chickpea and barley flours significantly decreased after treatment ($p < 0.05$). The total phenolics declined slightly from 1.3 mg catechin equivalent (CE)/g of flour (d.b.), for the un-tempered non-micronized chickpea sample, to 1.2 mg catechin equivalent/g of flour for the un-tempered, heated to 115 °C chickpea sample. A significant reduction was found in other treated chickpea samples including the un-tempered sample heated to 135 °C, and the tempered samples heated to 115 or 135 °C ($p < 0.05$). The lowest TPC of chickpea samples was found in the chickpea samples that were tempered and then heated to 135 °C, reaching a reduction of ~16%. The TPC of barley was higher than that of chickpea samples. The TPC was significantly decreased from 2.1 mg CE/g of flour for the untreated barley sample to ~1.7 CE/g of flour in the treated barley flours ($p < 0.05$) with the highest reduction of ~23%. But different treatments did not show a significant difference. The reduction of TPC has also been found in studies using infrared heating or other heat

Table 1
The concentration of anti-nutritional compounds in untreated and treated flours prepared from desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	Total phenolics (mg catechin equivalent/g of flour, d.b.)	Condensed tannins (mg catechin equivalent/g flour, d.b.)	Trypsin inhibitor activity (TIU/mg of flour, d.b.)	Chymotrypsin inhibitor activity (CIU/mg of flour, d.b.)
a) Desi chickpeas				
• Un-tempered; non-micronized	1.28 ± 0.02^{a}	0.16 ± 0.08^{a}	16.35 ± 1.90^{a}	11.21 ± 0.37^{a}
• Un-tempered; heated to 115 °C	1.18 ± 0.06^{ab}	0.08 ± 0.08^{a}	14.91 ± 1.37^{a}	7.55 ± 1.15^{b}
• Un-tempered; heated to 135 °C	1.08 ± 0.08^{b}	0.08 ± 0.06^{a}	9.85 ± 0.49^{b}	4.38 ± 0.51^{c}
• Tempered to 20% moisture; heated to 115 °C	1.08 ± 1.07^{b}	0.09 ± 0.16^{a}	3.73 ± 0.55^{c}	2.18 ± 0.16^{d}
• Tempered to 20% moisture; heated to 135 °C	1.07 ± 0.06^{b}	0.09 ± 0.15^{a}	2.74 ± 0.53^{c}	1.55 ± 0.16^{d}
b) Hull-less barley				
• Un-tempered; non-micronized	2.09 ± 0.07^{A}	1.99 ± 0.44^{A}	1.25 ± 0.28^{A}	0.50 ± 0.09^{A}
• Un-tempered; heated to 115 °C	1.69 ± 0.06^{B}	1.07 ± 0.04^{B}	0.80 ± 0.11^{B}	0.46 ± 0.05^{A}
• Un-tempered; heated to 135 °C	1.61 ± 0.05^{B}	1.05 ± 0.10^{B}	0.64 ± 0.09^{B}	0.28 ± 0.02^{B}
• Tempered to 20% moisture; heated to 115 °C	1.79 ± 0.08^{B}	0.76 ± 0.19^{B}	0.74 ± 0.08^{B}	0.29 ± 0.02^{B}
• Tempered to 20% moisture; heated to 135 °C	1.61 ± 0.10^{B}	0.85 ± 0.13^{B}	0.52 ± 0.06^{B}	0.16 ± 0.04^{B}

Data with different superscript letters in the same column indicate significant differences ($p < 0.05$).

treatments. Xu and Chang (2008) found that boiling and steaming significantly decreased the total phenolic content in maize flour by 98%. The TPC in barley decreased after extrusion at 150 or 180 °C with a moisture of 15% or 20% (Sharma, Gujral, & Singh, 2012). The decrease of total phenolic content could be attributed to the breakdown of phenolic compounds (Xu & Chang, 2008). However, there have been studies reporting an increase in TPC in peanut hulls under far-infrared heating, chickpeas under microwave roasting, infrared heating in soybeans *etc.* (Jogihalli, Singh, & Sharanagat, 2017; Lee et al., 2006; Žilić et al., 2014), where they attributed the increase to the breakdown of cellular compounds and the liberation of bound phenolics. The different reaction of TPC against heat treatments are possibly associated with the variety considering the TPC in black, red and pinto dry beans showed an increase, decrease and no significant difference respectively after infrared treatment (Oomah et al., 2014). Sogi, Siddiq, Roidoung, and Dolan (2012) also mentioned that storage time affected the susceptibility of phenolics to infrared treatment.

The levels of condensed tannins were determined and the results are shown in Table 1. Although the content of tannins was reduced by half after treatment, only barley samples were observed to have a significant reduction in the content of condensed tannins ($p < 0.05$). The levels of condensed tannins were significantly decreased from 2.0 mg CE/g of flour for the untreated barley samples to ~1.1 mg CE/g of flour for the heated barley samples without tempering and to ~0.8 mg CE/g of flour for the barley samples tempered before heated ($p < 0.05$). The low levels of tannins in the chickpea are associated with removing the seed coat which contains 79–86% of the total tannins of the seeds (Yadav & Chen, 2007), the breeding practices which focused on reducing the levels of this anti-nutritional factor as well as the environment effect (Nikolopoulou, Grigorakis, Stasini, Alexis, & Iliadis, 2006). The decline in total phenolic compounds and condensed tannins suggests that they are heat liable. Infrared heating is an effective way to reduce the levels of those compounds.

3.2.2. Trypsin and chymotrypsin inhibitor activity

The trypsin inhibitor activity within untreated and treated desi chickpea and hull-less barley flour is given in Table 1. In the case of chickpea flour, trypsin inhibitor activity declined from ~16.3 TIU/mg of flour (d.b.) for un-tempered non-micronized seeds, to 14.9 and 9.3 TIU/mg of flour (d.b.) for seeds brought to a surface temperature of 115 °C and 135 °C, respectively ($p < 0.05$). The addition of 20% tempering plus heating to either a surface temperature of 115 °C or 135 °C resulted in a further decline in trypsin inhibitor activity to ~3.7 and ~2.7 TIU/mg of flour (d.b.) ($p < 0.05$). Tempering and infrared heating both have been shown to be effective in reducing trypsin inhibitor activity in various previous studies (Al-Bakir, Sachde, & Naoum, 1982; Khattab et al., 2009; Márquez & Alonso, 1999). Khattab and Arntfield (2009) reported an 89 to 94% decrease in trypsin inhibitor activity in different varieties of cowpeas, kidney beans and peas after infrared heating, which was much more effective than other methods of physical treatment including boiling, roasting, microwave cooking and autoclaving. Al-Bakir et al. (1982) reported that soaking for 24 h alone was effective in reducing trypsin inhibitor activity in chickpea and cooking at 121 °C for 30 min after soaking was able to completely inactivate trypsin inhibitor activity. They also showed in the same study that trypsin inhibitor in chickpea was heat-labile and was susceptible to longer heat treatment. In the present study, levels in hull-less barley declined from ~1.2 TIU/mg of flour (d.b.) for un-tempered non-micronized seeds to levels ranging between 0.5 and 0.8 TIU/mg of flour (d.b.) depending on the processing conditions ($p < 0.05$). However, no statistical differences were evident among the various processing treatments ($p > 0.05$).

The chymotrypsin inhibitor activity showed a similar trend as trypsin inhibitor activity (Table 1). In chickpea flour, the chymotrypsin inhibitor decreased significantly from 11.2 to 7.5 CIU per mg of flour and 4.38 per mg of flour in 115 °C and 135 °C micronized flours

respectively ($p < 0.05$), with a further decrease to 2.18 and 1.55 with the addition of tempering ($p < 0.05$). In contrast to chickpea flour, barley flour showed low chymotrypsin inhibitor activity of 0.50 units in the un-tempered and non-micronized flour and both infrared heating and tempering showed the ability to decrease chymotrypsin inhibitor activity. The chymotrypsin inhibitor activity in barley flour decreased to 0.46 in the 115 °C-heated barley sample and a significant decrease to 0.28 units per mg of flour in the 135 °C-heated barley sample. In the tempered barley flours, both 115 and 135 °C micronized samples showed a significant decline in chymotrypsin inhibitor activity with 0.29 and 0.16 units per mg of flour respectively ($p < 0.05$). The trypsin and chymotrypsin inhibitors are low molecular weight proteins and tend to be heat-labile. The increase in temperature can lead to an unravelling of protein structure and a loss of biological function. The greater decrease in inhibitor activity in the tempered flours is thought to be due to the soluble nature of the respective inhibitors. The dissolved proteolytic enzyme inhibitors can be removed with the removal of water (Vidal-Valverde et al., 1994).

3.3. Protein quality

3.3.1. Protein quality of desi chickpea and barley flours

The amino acid composition (g/100 g of flour as is) for untreated and treated desi chickpea and hull-less barley flour is given Table 2. Overall for both flour types, the amino acid levels were relatively constant regardless of whether heat or tempering + heat was applied. The concentration of essential amino acids is given in Table 3 for untreated and treated desi chickpea and hull-less barley flour, along with the 1990 FAO/WHO reference pattern for children 2–5 years of age. In the case of hull-less barley flours, threonine and lysine concentrations were lower than the reference pattern. The low lysine content was expected since it is well known that cereals are deficient in this important amino acid. Concentrations of valine, the sulphur amino acids (methionine + cysteine), isoleucine, leucine, phenylalanine + tyrosine, histidine and tryptophan were all higher than the reference pattern. For desi chickpea, the concentration of threonine was the lowest compared to the reference pattern, followed by tryptophan and the sulphur amino acids (methionine + cysteine); whereas the concentration of valine, isoleucine, leucine, phenylalanine + tyrosine, histidine and lysine were greater than the required amounts. Typically, it is well known that pulses are deficient in sulphur-containing amino acids, which is interesting since the present results contradict this. The reason could be the decrease of threonine or the increase of sulphur-containing amino acids.

There are a few possible reasons for the deficient threonine content in the desi chickpeas. [1] In Canada, pulses are typically grown in rotation with canola crops, which often require the use of sulphur-based fertilizers to enhance the growth. As a result, it was hypothesized that during rotation, residual sulphur in the soil may be incorporated into the chickpea and metabolized into sulphur amino acids. [2] Another probable reason may be associated with the biosynthesis of lysine, threonine, methionine and leucine from asparagine in plants (Galili, Amir, Hoefgen, & Hesse, 2005). Within this pathway, there are two crucial enzymes involved with regulating methionine and threonine synthesis: cystathionine γ-synthase (CGS), which leads to the methionine synthesis, and threonine synthase (TS) which catalyzes threonine synthesis. Depending on the environmental stresses (*e.g.*, drought), one CGS pathway tends to out compete the other leading to differing levels of methionine. Shen, Foster, and Orcutt (1989) found that the concentration of methionine was higher in drought-stressed flatpea. Methionine levels in a hybrid Bermuda-grass were also found to increase from the control by 57% and 250% after 6 and 18 d of drought, respectively (Du, Wang, Yu, & Huang, 2012). Taylor, Chapman, Beyaert, Hernández-Sebastià, and Marsolais (2008) also found that the deficiency of storage proteins in common bean lead to an increased synthesis of sulphur amino acids, especially cysteine, where content of sulphur amino acids (methionine + cysteine) rised above the FAO recommendation of 25 mg/g protein (equals to 2.5 g/100 g protein).

Based on the amino acid scores (Table 4), desi chickpeas and hull-less barley flours (untreated or treated) were found to be limiting in threonine and lysine, respectively. Thus, the amino acid scores for the limiting amino acids ranged between 0.83 and 0.89 for chickpea flour, and 0.61 and 0.69 for barley flour depending on the level of heat or tempering + heat (Table 4). *In vitro* protein digestibility (IVPD) for untreated and treated desi chickpea and hull-less barley flour is given Table 4. The addition of heat and tempering increased the IVPD in barley samples from 72% to ~77% ($p < 0.05$). In the case of chickpea samples, there was no significant improvement and the IVPD of the chickpea samples was around 73 to 79% ($p < 0.05$). The cause of the close digestibility results obtained from non-treated chickpea and barley flour could be due to differences in the composition of anti-nutritional factors. Barley flour was found to have a higher content of total phenolic and tannins which would act to cross-link proteins to reduce their digestibility, whereas chickpea flour showed a higher enzyme inhibitor activity, which may lead to a similar inhibition of protein digestibility. Another reason might be the limitation of the protocol. The equation: $IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH_{10min}$ (Eq. (6)) set a starting IVPD results of 65.66, *i.e.*, even with no change in pH, a 65.66%

Table 2
Amino acid composition (g per 100 g of flour, as is basis) of untreated and treated desi chickpea and hull-less barley flours, with and without tempering and infrared heating to different surface temperatures. Treatments include: (A) un-tempered, non-micronized; (B) un-tempered, heated to 115 °C; (C) un-tempered, heated to 135 °C; (D) tempered to 20% moisture, heated to 115 °C; and (E) tempered to 20% moisture, heated to 135 °C.

	ASP	GLU	SER	GLY	HIS	ARG	THR	ALA	PRO	TYR	VAL	MET	CYS	ILE	LEU	PHE	LYS	TRP
Desi chickpeas																		
A	3.01	4.45	1.56	0.99	0.80	2.51	0.74	1.01	1.06	0.69	1.03	0.30	0.33	1.04	1.89	1.44	1.77	0.26
B	3.06	4.53	1.57	1.00	0.81	2.57	0.75	1.04	1.09	0.70	1.04	0.30	0.34	1.05	1.93	1.47	1.79	0.27
C	2.98	4.43	1.54	0.98	0.82	2.53	0.74	1.02	1.07	0.68	1.01	0.31	0.33	1.02	1.88	1.43	1.74	0.27
D	3.03	4.53	1.54	0.97	0.83	2.53	0.72	1.01	1.07	0.69	1.02	0.31	0.33	1.03	1.88	1.46	1.76	0.26
E	3.20	4.74	1.64	1.02	0.88	2.67	0.78	1.04	1.09	0.72	1.04	0.31	0.33	1.04	1.90	1.44	1.76	0.27
Hull-less barley																		
A	0.69	2.65	0.53	0.43	0.28	0.54	0.30	0.45	1.22	0.26	0.51	0.13	0.20	0.36	0.74	0.54	0.39	0.13
B	0.75	2.65	0.54	0.47	0.27	0.68	0.31	0.48	1.20	0.33	0.53	0.15	0.23	0.36	0.75	0.54	0.44	0.18
C	0.73	2.63	0.54	0.46	0.26	0.67	0.31	0.47	1.20	0.32	0.52	0.15	0.22	0.35	0.74	0.53	0.42	0.18
D	0.74	2.69	0.55	0.47	0.27	0.66	0.32	0.47	1.22	0.32	0.54	0.14	0.22	0.36	0.75	0.55	0.43	0.18
E	0.75	2.73	0.57	0.48	0.27	0.67	0.32	0.49	1.25	0.33	0.54	0.15	0.22	0.36	0.76	0.55	0.43	0.18

Abbreviations: ASP, aspartate; THR, threonine; SER, serine; GLU, glutamate; PRO, proline; GLY, glycine; ALA, alanine; CYS, cysteine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; and TRP, tryptophan.

Table 3
Essential amino acid concentration (mg/g protein) of untreated and treated flours prepared from desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	THR	VAL	M + C[a]	ILE	LEU	P + T[b]	HIS	LYS	TRP
a) Desi chickpeas									
• Un-tempered; non-micronized	30	41	25	42	76	86	32	71	10
• Un-tempered; heated to 115 °C	30	41	25	42	76	86	32	71	11
• Un-tempered; heated to 135 °C	30	41	26	41	76	85	33	70	11
• Tempered to 20% moisture; heated to 115 °C	29	41	26	41	75	86	33	70	10
• Tempered to 20% moisture; heated to 135 °C	30	40	25	40	73	83	34	68	10
b) Hull-less barley									
• Un-tempered; non-micronized	27	47	30	33	68	73	26	36	12
• Un-tempered; heated to 115 °C	29	49	35	33	69	81	25	41	17
• Un-tempered; heated to 135 °C	29	49	35	33	69	79	24	39	17
• Tempered to 20% moisture; heated to 115 °C	29	50	33	33	69	80	25	40	17
• Tempered to 20% moisture; heated to 135 °C	29	49	33	33	69	80	24	39	16
1990 FAO/WHO reference pattern	34	35	25	28	66	63	19	58	11

Notes:
Data represent the mean value of one processing run.
[a] Methionine + cysteine
[b] Phenylalanine + tyrosine.

of IVPD could be obtained.

In vitro protein digestibility corrected amino acid score (IV-PDCAAS) for both untreated and treated flours are shown in Table 4. In the case of hull-less barley flour, all the IV-PDCAAS values of treated samples increased to 0.52 compared to the un-tempered and non-micronized sample with the value of 0.44. However, the difference between different heat or moisture + heat levels was not significant ($p > 0.05$). The low IV-PDCAAS of barley flour is due to the low levels of lysine present in the flour. In the case of desi chickpeas, the significant increase in the IV-PDCAAS value was only found in the sample tempered to 20% moisture and heated at 135 °C, where the IV-PDCAAS increased from 0.65 to 0.71 ($p < 0.05$). The high IV-PDCAAS of chickpea flour was not surprising considering its balanced amino acid profile.

Nosworthy, Neufeld, Frohlich, Young, Malcolmson, and House (2017) determined the protein quality for a range of cooked pulses (soaked for 16 h, followed by boiling for 6–10 min depending on the pulse type), including red kidney beans, navy beans, and whole green lentils. From the 2010 crop year, the authors reported that kabuli chickpeas were found to be limiting in tryptophan (AAS 0.61), had a true protein digestibility of 85% and had a PDCAAS score of 0.52. This differed significantly with the current study (2014 crop year) where tryptophan was not found to be limiting in the desi chickpea, which resulted in much higher PDCAAS scores (0.65–0.71). In comparison, Nosworthy, Neufeld, et al. (2017) reported PDCAAS scores for red kidney beans (0.55), navy beans (0.67), whole green lentils (0.63), split red lentils (0.54), split green peas (0.50) and black beans (0.53), with all being limiting in the sulphur containing amino acids (methionine and cysteine). In contrast, the authors reported PDCAAS scores for split yellow peas (0.64) and pinto beans (0.59), which were similar to chickpea, limiting in tryptophan. PDCAAS scores for rolled oats and whole wheat were reported to be 0.57 and 0.40, respectively (FAO/WHO, 1991).

PDCAAS scores are used for labelling purposes in the US regulatory system, to allow for the marketing of food products intended for people > 1 year of age (*i.e.*, non-infants) (Marinangeli et al., 2017).

Table 4
Amino acid scores, limiting amino acid score, *in vitro* protein digestibility, and IV-PDCAAS values for untreated and treated flours prepared from desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures, in reference to the FAO/WHO reported values (1990).

Treatment	THR	VAL	M + C[1]	ILE	LEU	P + T[2]	HIS	LYS	TRP	Limiting amino acid score	*In vitro* protein digestibility (%)	IV-PDCAAS (×100)
a) Desi chickpeas												
• Un-tempered; non-micronized	0.87*	1.18	1.01	1.49	1.15	1.36	1.69	1.23	0.95	0.85	76.46 ± 0.84^{ab}	64.99 ± 0.71^{b}
• Un-tempered; heated to 115 °C	0.87*	1.17	1.01	1.48	1.16	1.36	1.69	1.22	0.97	0.86	73.23 ± 2.89^{b}	63.00 ± 2.49^{b}
• Un-tempered; heated to 135 °C	0.88*	1.16	1.03	1.47	1.15	1.35	1.74	1.21	0.99	0.85	76.20 ± 0.79^{ab}	62.94 ± 0.65^{b}
• Tempered to 20% moisture; heated to 115 °C	0.85*	1.17	1.03	1.47	1.14	1.37	1.75	1.22	0.95	0.83	77.12 ± 2.03^{ab}	65.46 ± 1.72^{b}
• Tempered to 20% moisture; heated to 135 °C	0.89*	1.15	0.99	1.44	1.11	1.33	1.79	1.17	0.95	0.89	79.28 ± 2.84^{a}	70.94 ± 2.54^{a}
b) Hull-less barley												
• Un-tempered; non-micronized	0.81	1.33	1.21	1.18	1.02	1.16	1.35	0.61*	1.08	0.61	72.30 ± 0.76^{B}	44.44 ± 0.46^{C}
• Un-tempered; heated to 115 °C	0.84	1.40	1.41	1.19	1.05	1.28	1.32	0.70*	1.52	0.69	75.47 ± 1.78^{AB}	52.33 ± 1.24^{AB}
• Un-tempered; heated to 135 °C	0.85	1.39	1.38	1.17	1.05	1.26	1.28	0.68*	1.53	0.66	78.59 ± 0.77^{A}	53.26 ± 0.52^{A}
• Tempered to 20% moisture; heated to 115 °C	0.87	1.42	1.32	1.18	1.04	1.27	1.31	0.68*	1.50	0.68	76.96 ± 1.51^{A}	50.94 ± 1.00^{B}
• Tempered to 20% moisture; heated to 135 °C	0.85	1.40	1.34	1.16	1.04	1.26	1.29	0.67*	1.48	0.68	76.92 ± 0.91^{A}	52.12 ± 2.54^{AB}

Notes:
Data represent the mean value of one processing run.
Data with different superscript letters in the same column indicate significant differences ($p < 0.05$).
* Indicates the first limiting amino acid.
[1] Methionine + cysteine.
[2] Phenylalanine + tyrosine.

Based on the standard serving sizes of pulses (90 g) and cereals (110 g) (Corrected for the PDCAAS values; g proteins x PDCAAS), the amount of protein must be between 10% and 19.9% of the daily protein requirement (50 g) for non-infant foods to be labelled a "*Good source of protein*", whereas at levels > 20% can be labelled as an "*Excellent source of protein*". Based on this criterion, the desi chickpea flour in the present study could be labelled as an "Excellent source of protein" since the % of the daily reference value is between 30 and 34% depending on the processing treatment (Table 5). In the case of barley flour used in the present study, it can be listed as a "Good source of protein", since the % of the daily reference value is between 11 and 13% depending on the processing treatment (Table 5).

In Canada, protein labelling is based on the use of the protein efficiency ratio (PER) and a protein rating methodology. Typically PER values are determined using a rat bioassay, which involves feeding the rats a known amount of test protein for 28 days, and are determined by dividing the amount of weight gained by the rat by the total amount of protein consumed. These PER values are then normalized to the PER of casein (2.5) to increase the consistency when comparing values (Marinangeli et al., 2017). Although Canadian Food Inspection Agency (CIFS) allows the use of PDCAAS values in its estimation using the formulae: $PER = [PDCAAS_{(sample)} / PDCAAAS_{(Casein)}] \times 2.5$, using a PDCAAS score for casein of 1.00 (Marinangeli et al., 2107), this calculation is only used for estimation purposes, and is not permitted to extrapolate to foods (which would need to undergo proper validation – PER testing) (Marinangeli et al., 2017), nor does CIFA recognize the use of the *in vitro* PDCAAS over *in vivo*. However, Nosworthy and House (2017) obtained a strong correlation ($R^2 = 0.9898$) relating the IV-PDCAAS values to PDCAAS using a single protein source including casein, pea, faba bean and lentil protein isolates. And also for processed (baked, extruded and cooked) red and green lentil flour with correlations with an R^2 value of 0.9971 (Nosworthy et al., 2018). In the present study, calculated $PER_{IV\text{-}PDCAAS}$ values ranged between 1.62 and 1.77, and between 1.11 and 1.33 for desi chickpea and barley flour, respectively (Table 5). PER values reported in the literature vary somewhat depending on how the seeds were processed. However, present values were reported within a similar range. For instance, Nosworthy, Neufeld, et al. (2017) reported for soaked/boiled kabuli chickpeas to have a PER value of 2.32; Nosworthy et al. (2018) reported red lentils to range between 0.79 and 1.14 depending on the type of processing (extruded, cooked or baked); and Nosworthy, Franczyk, et al. (2017) found PER values for raw and extruded flour of 2.55 and 2.62.

The protein rating system uses those PER values combined with relative serving sizes for determination. In the current study, the Canadian Nutrient file (Health Canada, 2016) was used to estimate the serving size for a 250 mL serving of flour, which is equivalent to 97.2 g and 156.4 g of chickpea and barley flour, respectively. This serving sized is based on the Reasonable Daily Intake (RDI) value which corresponds to the average serving of a food consumed under normal food habits of Canadians (Marinangeli et al., 2017). The protein rating is determined by multiplying the crude protein content in the serving size by the PER value. If the Protein Rating ranges between 20.0 and 39.9 then the food is considered to be a "*Source of protein*", whereas if > 40.0 then it's considered an "*Excellent source of protein*". Based on the present results, the desi chickpea flour was determined to be an "*Excellent source of protein*", whereas the barley flour could only be considered a "*Source of protein*" (Table 5).

3.3.2. Protein quality of desi chickpea-barley blends

Based on the anti-nutritional data and protein quality of the flours alone, flours tempered to 20% moisture and heated to 135 °C were selected for blending experiments at different chickpea: barley ratios. The amino acid composition (g/100 g flour), the concentration of essential amino acids (mg/g protein) and amino acid scores (based on the FAO reference pattern) for the chickpea: barley blended flours are given in

Table 5
Percentage of daily protein reference requirement, calculated protein efficiency ratio and protein rating for flours prepared from desi chickpeas and hull-less barley, with and without tempering and infrared heating to different surface temperatures.

Treatment	Crude protein (CP) (g/100 g)	%Daily reference values – US. Regulatory system			Protein efficiency ratios & protein ratings – Canadian regulatory system			
		IV-PDCAAS	Corrected CP per serving (90 g pulses 110 g cereals)	%Daily reference value	Calculated $PER_{IV\text{-}PDCAAS}$	g/250 mL	CP (g/250 mL serving)	Protein rating (250 mL serving)
a) Desi chickpeas								
• Un-tempered; non-micronized	25.4	0.65	14.9	29.7	1.62	97.2	24.7	40.1
• Un-tempered; heated to 115 °C	25.1	0.63	14.2	28.5	1.58	97.2	24.4	38.4
• Un-tempered; heated to 135 °C	26.2	0.63	14.8	29.7	1.57	97.2	25.5	40.1
• Tempered to 20% moisture; heated to 115 °C	25.9	0.65	15.3	30.5	1.64	97.2	25.2	41.2
• Tempered to 20% moisture; heated to 135 °C	26.6	0.71	17.0	34.0	1.77	97.2	25.9	45.9
b) Hull-less barley								
• Un-tempered; non-micronized	11.4	0.44	5.6	11.1	1.11	156.4	17.8	19.8
• Un-tempered; heated to 115 °C	10.7	0.52	6.2	12.3	1.31	156.4	16.7	21.9
• Un-tempered; heated to 135 °C	10.0	0.53	5.9	11.7	1.33	156.4	15.6	20.8
• Tempered to 20% moisture; heated to 115 °C	11.4	0.51	6.4	12.8	1.27	156.4	17.8	22.7
• Tempered to 20% moisture; heated to 135 °C	11.2	0.52	6.4	12.8	1.30	156.4	17.5	22.8

Table 6
Amino acid composition (g per 100 g of flour, as is basis) of desi chickpea and barley blended flours. Flours were tempered to 20% moisture and heated at 135 °C. Samples include: (A) chickpea: barley = 0: 100; (B) chickpea: barley = 20: 80; (C) chickpea: barley = 40: 60; (D) chickpea: barley = 60: 40; (E) chickpea: barley = 80: 20; and (F) chickpea: barley = 100: 0.

	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	TRP
A	0.75	0.32	0.57	2.73	1.25	0.48	0.49	0.22	0.54	0.15	0.36	0.76	0.33	0.55	0.27	0.43	0.67	0.18
B	1.08	0.38	0.83	3.19	1.48	0.58	0.55	0.22	0.62	0.16	0.51	1.02	0.43	0.75	0.55	0.66	0.97	0.17
C	1.57	0.48	1.05	3.57	1.40	0.68	0.69	0.26	0.72	0.19	0.63	1.23	0.47	0.94	0.66	0.93	1.31	0.20
D	1.97	0.55	1.23	3.89	1.38	0.77	0.79	0.26	0.81	0.21	0.76	1.46	0.57	1.10	0.78	1.18	1.67	0.22
E	2.41	0.64	1.47	4.32	1.33	0.88	0.92	0.30	0.93	0.25	0.90	1.68	0.66	1.31	0.91	1.47	2.11	0.23
F	3.20	0.78	1.64	4.74	1.09	1.02	1.04	0.33	1.04	0.31	1.04	1.90	0.72	1.44	0.88	1.76	2.67	0.27

Abbreviations: ASP, aspartate; THR, threonine; SER, serine; GLU, glutamate; PRO, proline; GLY, glycine; ALA, alanine; CYS, cysteine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; and TRP, tryptophan.

Table 7
Essential amino acid concentration (mg/g protein), amino acid scores, limiting amino acid score, *in vitro* protein digestibility, and IV-PDCAAS values of desi chickpea and barley flours tempered to 20% moisture and heated at 135 °C, blended at different ratios.

Chickpea: barley ratio	THR	VAL	M + C[1]	ILE	LEU	P + T[2]	HIS	LYS	TRP	Limiting amino acid score	*In vitro* protein digestibility (%)	IV-PDCAAS
Essential amino acid concentration (mg/g protein)												
0: 100	29	49	34	33	69	80	25	39	16			
20: 80	25	41	25	34	68	79	37	44	11			
40: 60	25	38	24	33	65	74	35	49	11			
60: 40	26	38	22	36	68	78	36	55	10			
80: 20	26	38	23	37	69	81	37	60	9			
100: 0	30	41	25	41	74	84	34	69	11			
1990 FAO/WHO reference pattern	34	35	25	28	66	63	19	58	11			
Amino acid score												
0: 100	0.86	1.41	1.35	1.18	1.05	1.28	1.30	0.68*	1.50	0.68	76.92 ± 0.91^{A}	52.12 ± 0.62^{A}
20: 80	0.74*	1.18	1.01	1.21	1.03	1.25	1.93	0.76	1.03	0.74	78.27 ± 0.55^{A}	57.92 ± 0.41^{B}
40: 60	0.74*	1.09	0.95	1.19	0.98	1.18	1.83	0.85	0.96	0.74	78.87 ± 0.63^{A}	58.37 ± 0.46^{BC}
60: 40	0.76*	1.08	0.88	1.27	1.03	1.24	1.92	0.95	0.94	0.76	78.51 ± 1.61^{A}	59.67 ± 1.22^{BC}
80: 20	0.77*	1.09	0.90	1.32	1.04	1.28	1.96	1.04	0.86	0.77	80.02 ± 0.73^{A}	61.61 ± 0.56^{C}
100: 0	0.89*	1.16	1.00	1.45	1.12	1.34	1.81	1.18	0.96	0.89	79.28 ± 2.84^{A}	70.94 ± 2.54^{D}

Notes:
Data represent the mean values from triplicate processing runs ± one standard deviation (n = 3). Similar letters within the same column indicate no significant difference ($p < 0.05$).
* Indicates the first limiting amino acid.
[1] Methionine + cysteine.
[2] Phenylalanine + tyrosine.

Tables 6 and 7. For the barley flour alone, lysine was found to be limiting, whereas at ratios between 20:80 and 100:0 chickpea: barley, threonine was found limiting (Table 7). The *in vitro* protein digestibility data was found to be similar regardless of the blending ratio with a mean value of 78.6% ($p > 0.05$) (Table 7). The addition of chickpea to barley flour, however, resulted in an increase from ~52% (100% barley flour) to ~59% IV-PDCAAS values at blending ratios of 20:80, 40:60 and 60:40 chickpea: barley ($p < 0.05$), and then increased further to ~62% (80: 20 chickpea: barley; $p < 0.05$), and then again to ~71% (100% chickpea flour) ($p < 0.05$) (Table 7). Findings from this study indicate that the nutritional properties of barley can be enhanced with the addition of chickpea, however, the protein value would be less than chickpea flour alone.

4. Conclusions

This study demonstrated that tempering and infrared heating is capable of increasing the protein quality of chickpea and barley flours without affecting overall protein content. Reducing anti-nutritive factors such as the phenolic and tannin content and the activity of protease inhibitors of these flours *via* processing was found to increase *in vitro* protein digestibility. This, in conjunction with an increase in the overall amino acid score, contributed to a higher *in vitro* PDCAAS value for processed barley and chickpea flours. Interestingly, while blending chickpea with barley was able to increase the overall protein quality of the barley flour, chickpea alone had a higher *in vitro* PDCAAS score than any blend investigated in this study. This work highlights the functionality of *in vitro* methods for determining protein quality as well as indicating that the quality of chickpea protein may be greater than previously suspected. Because of flours specific to this study, *in vitro* PDCAAS sources indicated that chickpeas (alone) should be tempered to 20% moisture and heated to a surface temperature of 135 °C by infrared heating to obtain scores > 0.70 to ensure sufficient protein quality needed to meet nutritional requirements put forth by the World Food Program. Greater research should be undertaken to better understand environmental effects on the amino acid synthesis pathways in chickpeas as it relates to protein quality.

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