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Impact of Processing on the Protein Quality of Pinto Bean (*Phaseolus vulgaris*) and Buckwheat (*Fagopyrum esculentum* Moench) Flours and Blends, As Determined by in Vitro and in Vivo Methodologies

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ABSTRACT: Blending of protein sources can increase protein quality by compensating for limiting amino acids present in individual sources, whereas processing grain flours by extrusion or baking can also alter protein quality. To determine the effect of baking and extrusion on the protein quality of blended flours from buckwheat and pinto beans, a rodent bioassay was performed and compared to an in vitro method of protein quality determination. Overall, extruded products had higher protein efficiency ratio values, increased digestibility, and greater protein digestibility corrected amino acid score (PDCAAS) values than baked products, with the extruded buckwheat/pinto blend having the greatest PDCAAS value of the experimental diets investigated. A correlation was found between both digestibility and PDCAAS values generated from in vitro and in vivo methods. The use of in vitro digestibility analysis should be investigated as a potential replacement for the current rodent assay for nutrient content claim purposes.

KEYWORDS: *Fagopyrum esculentum Moench, Phaseolus vulgaris, protein efficiency ratio, protein digestibility corrected amino acid score, in vitro protein digestibility*

INTRODUCTION

Protein content has been a leading trend in food product development in recent years. Similarly, a growing desire for non-animal-based protein sources has led to interest in the inclusion of plant-based protein sources, such as cereals and pulses, into foods. Pulses constitute the dried seeds of non-oilseed legume crops, including dried peas, chickpeas, beans, and lentils. The common bean, *Phaseolus vulgaris*, has many varieties and, combined, they comprise the largest pulse crop with global production exceeding 23 MT annually.[1] The pinto bean is the most widely produced and consumed bean variety in the United States. With a protein content ranging from 21.4 to 23.6%,[2] the first limiting amino acid of this pulse can be either tryptophan or the sulfur amino acids cysteine and methionine.[3,4] Beyond serving as a source of protein, beans also contain significant quantities of iron, calcium, folic acid, and multiple vitamins, while being low in fat.[5,6] In contrast, buckwheat, *Fagopyrum esculentum* Moench, is a pseudocereal representing a non-grass plant the seeds of which can be cultivated and used in flour production. Buckwheat also contains no gluten, thereby increasing its appeal to consumers seeking gluten-free foods.[7] The protein content of buckwheat is similar to that of cereals (12−14%); however, buckwheat is rich in lysine, the amino acid typically limiting in cereals, as well as tryptophan and methionine, the limiting amino acids in beans.[7,8] The calculated biological value of buckwheat protein is significantly higher than those of barley, wheat, and corn.[9] Although buckwheat is limiting in leucine,[10] this insufficiency can be ameliorated by combining buckwheat and bean flour, where the amino acid composition of the ingredients can offset the deficiencies found in individual sources. Importantly, the digestibility of buckwheat is lower than that of cereals due to the presence of protease inhibitors, trypsin inhibitors, and tannins.[11,12] Preparation of flours, through processes such as extrusion and baking, could alter the activity and presence of antinutritive factors, thereby increasing overall protein digestibility and bioavailability.

Extrusion is the process by which an ingredient is cooked by being passed by a screw system through a machine that is capable of controlling moisture, temperature, and pressure. This system is commonly used in the production of cereals, snacks, and pastas. This process has been shown to have no effect on protein content,[13,14] but can alter amino acid composition of beans.[15−17] Additionally, extrusion can increase protein digestibility of beans, possibly through reducing the activity of trypsin inhibitors.[13,15,18] Although there have been

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few studies determining the effects of baking on plant protein quality directly, the use of autoclaving as a surrogate of heat treatment is relatively common.[19−21] With autoclaving, a reduction in lysine content of approximately 10% was noted for chickpeas treated for 1 h.[19] A previous investigation of plant protein blends found a significant reduction in lysine content after as little as 5 min of exposure to heating at 121 °C.[20] While a reduction in lysine can be detrimental to the quality of a protein source, it is possible that the excess lysine contained in both beans and buckwheat would be significant enough to offset this loss.

The current study was undertaken to investigate the effect of processing (extrusion and baking) on the amino acid profile of pinto flour, buckwheat flour, and a pinto/buckwheat blend. In accordance with the regulatory requirements of both Canada and the United States, the protein efficiency ratio (PER)[22] and the protein digestibility corrected amino acid score (PDCAAS)[10] were determined via the appropriate rodent models. The digestible indispensable amino acid score (DIAAS) was calculated using true protein digestibility as recommended by the FAO/WHO,[23] and an in vitro measurement of protein quality was determined to compare these values with those obtained via PDCAAS.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care (Protocol F2012-035).

Chemicals. All chemicals and reagents were purchased from Sigma (Oakville, ON, Canada).

Sample Procurement. The whole buckwheat flour and pinto bean flour were procured from Best Cooking Pulses, 110−10th Street N.E., Portage la Prairie, MB, Canada R1N 1B5. The samples were processed by the Food Development Centre (Portage la Prairie, MB, Canada). The samples included the unprocessed flours of pinto bean, whole buckwheat, and a pinto/buckwheat blend (50:50) (untreated) as well as the extrudates and baked products of these flours (treated). Milling was performed on a hammer mill (Fitz mill model D comminutor VHP-506-55B), with screen hole size of 0.020 in., round, with 24% opening. All samples were further screened through a 20 mesh screen on a sifter (Kason, Vibro Screen, K24 3 SS). The hammer mill, sifter, and flour bin were cleaned thoroughly with compressed air after the grinding of each sample. Extrudates were prepared using a Clextral Evolum HT 25 twin-screw extruder. The pinto bean, buckwheat flours, and blends were premixed thoroughly prior to extrusion. The mixed flours/blends were extruded at 45−55 kg/h and 0.7−1.2 kg/h, dry and liquid feed rates, respectively, through a cracker-shaped die. The screw speed was 400−600 rpm. The extrusion barrel temperatures were 30−50, 70−90, and 100−120 °C. After extrusion, samples were milled as described above. The baking process was as follows: 6 kg each of pinto bean, buckwheat, and pinto bean/buckwheat flours were mixed for 2−3 min with 2.6, 3.6, and 2.4 kg of water, respectively. The dough was sheeted to obtain a 2−4 mm thickness. The sheeted dough was cut, transferred to baking trays, and rested for 30 min before baking. Prior to baking, preliminary tests were conducted to establish the optimum baking temperature and time for the crackers. The crackers were baked at 380, 380, and 350 °F for 13 min (pinto bean flour), 380, 360, and 330 °F for 8 min (buckwheat flour), and 380, 360, and 330 °F for 15 min (pinto bean/buckwheat flour). Baking was performed on a Doyon FC2-lll tunnel conveyor oven. The compact in-line triple-tunnel oven has variable belt speed and operating temperature of 300 °C/572 °F in addition to the high-velocity air flow. After baking, samples were milled as described above.

Analytical Procedures. Prior to analysis, all samples were ground in a hand-held electric mill. For all samples, percent crude protein (CP; N × 6.25) was determined through the use of a LECO CNS-2000 nitrogen analyzer (LECO Corp., St Joseph, MI, USA; model 602-00-500), percent dry matter (DM) and ash were determined according to standard procedures.[24] The percent crude fat was determined by extracting crude fat into hexane and by gravimetrics.[24] The amino acid contents of the samples were determined by acid hydrolysis using AOAC Official Method 982.30, whereas methionine and cysteine were determined using AOAC Official Method 45.4.05 (AOAC, 1995).

Tryptophan Analysis. Tryptophan content was determined using alkaline hydrolysis. In brief, 14 mL of double-distilled water and 8.4 g of barium hydroxide were added to approximately 40 mg of sample in a polypropylene flask, which was loosely capped and autoclaved for 20 h at 110 °C. After removal from the autoclave, an additional 30 mL of water was added to the flask, followed by 0.00054 g of α-methyltryptophan and 5 mL of 0.5 M orthophosphoric acid. The resulting solution was then brought to a pH of 3.0 with HCl. Finally, 20 mL of methanol was added and the solution brought to a final volume of 100 mL with water. A sample of the ultimate solution was filtered using a 0.45 μm syringe filter prior to analysis. Samples were analyzed at room temperature on a Varian HPLC using a 125 mm × 4 mm C_{18} Luna column. The mobile phase consisted of 0.3% acetic acid and 0.05% 1,1,1-trichloro-2-methyl-2-propanol in water, brought to a pH of 5 with ethanolamine. Total run time was approximately 34 min with a flow rate of 1 mL/min; excitation/emission was set to 280/356 nm, respectively.

Protein Digestibility-Corrected Amino Acid Score. A rat bioassay was used to determine the PDCAAS of the pulse samples.[10] Amino acid scores were determined by selecting the value of the amino acid with the lowest ratio after comparison of relative abundance to the recommended reference pattern. True protein digestibility was determined using AOAC Official Method 991.29 rat bioassay,[24] using casein as a reference standard, and correcting for endogenous protein losses using previously determined values. Diets were formulated to contain 10% protein, supplied by the test sample, 10% total fat (total of residual pulse fat and supplemental corn oil), and 5% cellulose with the remaining energy derived from corn starch. Vitamins and minerals (AIN-93 formulations; Harlan Teklad, Madison, WI, USA) were added to diets to meet the micronutrient requirements of laboratory rats. Male weanling laboratory rats (n = 10 per treatment; initial weight = 70 g) were individually housed in suspended wire-bottomed cages, and treated as previously described.[25] True protein digestibility (TPD%) was calculated using the following equation:

$$\text{TPD \%} = [(\text{N intake} - (\text{fecal N loss} - \text{metabolic N loss}))/\text{N intake}] \times 100$$

The value for metabolic nitrogen loss was determined as the amount of fecal nitrogen produced by rats consuming a protein-free diet. The PDCAAS was calculated as the product of the amino acid score and TPD%.

Digestible Indispensable Amino Acid Score (DIAAS). DIAAS was calculated using the amino acid reference pattern for children aged 6 months to 3 years, which was used in conjunction with the following equation (FAO/WHO, 2013):

$$\text{DIAAS \%} = 100 \times [(\text{mg of digestible dietary indispensable amino acid in 1 g of the dietary protein})/(\text{mg of the same dietary indispensable amino acid in 1 g of the reference protein}]$$

Although it is suggested that ileal amino acid digestibility be used for the calculation of PDCAAS, the use of fecal digestibility is considered acceptable until such time as a data set of true ileal digestibility is developed.[23]

In Vitro Protein Digestibility-Corrected Amino Acid Score. An in vitro digestibility assay was also performed on each sample provided.[26,27] Initially the equivalent of 62.5 mg of protein of each test sample was added to 8 mL of Milli-Q water, heated to 37 °C, and the pH of the solution was adjusted to 8.0. Simultaneously, a multienzyme cocktail including 3.1 mg/mL chymotrypsin (bovine pancreas ≥ 40 units/mg protein), 1.6 mg/mL trypsin (porcine pancreas 13,000−

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20,000 BAEE units/mg protein), and 1.3 mg/mL protease (*Streptomyces griseus* ≥ 15 units/mg solid) was prepared in 10 mL of Milli-Q water, heated to 37 °C, and adjusted to pH 8. One milliliter of the enzyme cocktail was transferred to the sample solution, and the resultant pH drop was recorded every 30 s for 10 min. The in vitro protein digestibility was calculated as follows, where $\Delta pH_{10\ min}$ is the change in pH in 10 min from the initial pH of about 8.0:

$$IVDP\ \% = 65.66 + 18.10 \times \Delta pH_{10min}$$

The in vitro PDCAAS was calculated as a product of the amino acid score and IVPD%.

Protein Efficiency Ratio. According to Health Canada, PERs are determined over a 28 day growth period for rats consuming feed ad libitum.[22] For the current study, the 28 day growth period included the 9 day protein digestibility study period. Rat weights were recorded throughout the acclimation and balance periods, and feed intake was recorded throughout the study. The PER was calculated as the amount of weight gain (g) divided by the amount (g) of protein consumed over 28 days. Values were adjusted to a standardized 2.5 PER value for the reference casein.

Statistics. Results were compared via one-way ANOVA with post hoc analysis using Tukey's multiple-comparison test, whereas the relationship between in vivo and in vitro digestibilities and corrected amino acid scores was determined via regression analysis (GraphPad Prism, 7.0).

RESULTS AND DISCUSSION

The dry matter, crude protein, and crude fat for the buckwheat and pinto bean products are presented in Table 1. Protein and fat content are provided on an "as-is" basis. Although similar in dry matter percentage, the untreated buckwheat flour had a lower protein and a higher fat content than the untreated pinto flour (12.47 vs 20.99% and 2.55 vs 0.82%, respectively). These values are in agreement with previous work, with buckwheat being approximately 13.3% protein and 3.4% fat, whereas pinto beans consist of 21.42% protein and 1.23% fat.[5] The protein and fat contents of the untreated flour blend were 14.66 and 1.46%, respectively, confirming the efficiency of the blending process. The extrusion process increased the dry matter percentage of treated flours by approximately 7% and reduced fat content by approximately 1.5% for buckwheat, 0.4% for pinto, and 1.1% for the blend. Conversely, the removal of water and fat content by extrusion resulted in an overall increase in protein content of approximately 2% for buckwheat, 4.4% for pinto, and 5.6% for the blend. Similar to extrusion, the baking process increased the dry matter percentage compared to untreated flour. The blended baked buckwheat/pinto flour had a fat content similar to that of the untreated blend, 1.34 vs 1.46% respectively. Baked individual flours were found to have higher protein content than untreated flours, increasing by approximately 7% for buckwheat and 3% for pinto; however, this was not found in the blend, 14.66% untreated and 13.80%, after baking. Overall, it appears that although both extrusion and baking will increase dry matter in a similar fashion for both the individual ingredients and the blend, baked buckwheat has a lower fat content and a higher protein content than extruded buckwheat, while the extruded blend has higher protein and lower fat contents than the baked blend. Interestingly, there was little difference in the protein content between the extruded and baked flours (1.2%), but the fat content was higher in the baked pinto flour, 0.43% for the extrudate versus 0.65% for the baked flour.

The amino acid composition for all ingredients is reported in Table 1. The animals provided the untreated pinto flour and the untreated buckwheat/pinto blend were unable to complete

Table 1. Proximate Analysis and Amino Acid Composition of Buckwheat and Pinto Bean Products[a]

	% DM[b]	% CP[c]	% CF[d]	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Trp
casein		90.80		5.30	3.15	3.99	17.03	8.16	1.23	2.11	0.26	4.91	2.35	4.00	7.05	4.50	4.02	2.25	6.17	2.91	1.19
BWF	89.93	12.47	2.55	1.01	0.41	0.58	1.84	0.48	0.62	0.41	0.24	0.48	0.24	0.33	0.63	0.27	0.43	0.34	0.66	1.09	0.14
PF	88.64	20.99	0.82	2.21	0.90	1.21	2.87	1.04	0.77	0.72	0.19	0.91	0.24	0.69	1.40	0.55	1.02	0.71	1.34	1.17	0.22
BWPF	89.93	14.66	1.46	1.71	0.67	0.92	2.45	0.77	0.71	0.57	0.23	0.75	0.24	0.56	1.10	0.44	0.76	0.52	1.07	1.21	0.19
EBWF	96.86	14.66	1.02	1.16	0.48	0.68	2.04	0.42	0.71	0.47	0.27	0.58	0.28	0.39	0.77	0.51	0.74	0.38	0.73	1.22	0.19
EPF	95.95	25.32	0.43	2.42	0.93	1.31	3.10	1.16	0.83	0.79	0.20	1.01	0.26	0.80	1.60	0.75	1.20	0.77	1.49	1.26	0.25
EBWPF	97.38	20.29	0.38	1.88	0.74	1.01	2.64	0.72	0.77	0.64	0.24	0.81	0.27	0.63	1.25	0.49	0.90	0.60	1.12	1.39	0.25
BBW	96.89	19.41	0.93	1.81	0.72	0.99	2.51	0.85	0.76	0.64	0.21	0.78	0.20	0.60	1.15	0.56	0.91	0.61	0.92	1.19	0.22
BPF	96.58	24.11	0.65	2.49	1.00	1.38	3.13	1.09	0.86	0.83	0.18	1.03	0.24	0.88	1.64	0.68	1.19	0.77	1.37	1.41	0.22
BBPF	98.64	13.80	1.34	1.17	0.47	0.65	2.11	0.51	0.71	0.45	0.25	0.56	0.24	0.41	0.80	0.39	0.60	0.40	0.57	1.16	0.16

[a]BWF, buckwheat flour; EBWF, extruded buckwheat flour; EPF, extruded pinto flour; EBPF, extruded blend; BBW, baked buckwheat flour; BPF, baked pinto flour; BBPF, baked blend. [b]DM, dry matter content. [c]CP, crude protein = nitrogen content (determined by LECO analysis) × 6.25. [d]CF, crude fat determined by hexane extraction.

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Table 2. Protein Digestibility Corrected Amino Acid Scores of Buckwheat and Pinto Bean Products[a]

	Thr	Val	Met+Cys	Ile	Leu	Phe+Tyr	His	Lys	Trp
casein	**1.02**	1.55	1.15	1.57	1.18	1.49	1.30	1.17	1.19
BWF	0.98	1.10	1.51	0.95	**0.76**	0.90	1.44	0.91	1.02
EBWF	0.97	1.13	1.50	0.96	**0.80**	1.35	1.37	0.86	1.16
EPF	1.08	1.14	**0.73**	1.12	0.96	1.22	1.60	1.01	0.88
EBWPF	1.08	1.14	1.00	1.11	**0.93**	1.09	1.55	0.95	1.13
BBW	1.09	1.15	0.84	1.10	0.89	1.20	1.64	**0.81**	1.01
BPF	1.21	1.23	**0.70**	1.30	1.03	1.23	1.68	0.98	0.83
BBPF	1.00	1.16	1.44	1.07	0.87	1.13	1.51	**0.72**	1.05

[a]Bolded values indicate the first limiting amino acid. BWF, buckwheat flour; EBWF, extruded buckwheat flour; EPF, extruded pinto flour; EBPF, extruded blend; BBW, baked buckwheat flour; BPF, baked pinto flour; BBPF, baked blend.

Table 3. Adjusted Protein Efficiency Ratio, Protein Digestibility-Corrected Amino Acid Scores, and in Vitro Protein Digestibility-Corrected Amino Acid Scores of Buckwheat and Pinto Bean Products[a]

	adj PER	AAS[b]	%TPD[c]	PDCAAS[d]	IVPD[e]	in vitro PDCAAS[f]
casein	2.5	1.02	96.41 (1.30)	98.24	84.67 (1.28)	86.27
BWF	2.55	0.76	71.13 (4.23)	54.27	71.72 (0.13)	54.73
EBWF	2.62	0.80	78.42 (1.36)	62.74	77.97 (0.52)	62.38
EPF	1.47	0.73	84.80 (3.16)	61.76	80.23 (0.38)	58.43
EBWPF	2.19	0.93	81.75 (2.99)	76.26	79.96 (0.85)	74.60
BBW	1.60	0.81	63.41 (4.01)	51.66	73.90 (0.13)	60.20
BPF	1.11	0.70	59.95 (5.56)	41.67	76.88 (0.26)	53.44
BBPF	1.6	0.72	69.16 (2.95)	49.51	73.35 (0.13)	52.51

[a]BWF, buckwheat flour; EBWF, extruded buckwheat flour; EPF, extruded pinto flour; EBPF, extruded blend; BBW, baked buckwheat flour; BPF, baked pinto flour; BBPF, baked blend. $n = 10$ for adj PER and %TPD, $n = 2$ for IVPD, and $n = 1$ for AAS, PDCAAS, and in vitro PDCAAS. Numbers in parentheses indicate SD where applicable. PDCAAS is calculated as the product of AAS and %TPD, whereas in vitro PDCAAS is the product of AAS and IVPD. [b]AAS, amino acid score. [c]%TPD, % true protein digestibility. [d]PDCAAS, protein digestibility corrected amino acid score. [e]IVPD, in vitro protein digestibility. [f]In vitro PDCAAS, in vitro protein digestibility corrected amino acid score.

the study due to feed refusal. This was most likely due to the presence of antinutritive factors present in untreated beans such as proteolytic inhibitors,[28,29] hemagglutinins,[30] and tannins,[31] which would have been destroyed or inactivated by the extrusion and baking process.[21,32] The amino acid scores for the remaining ingredients are presented in Table 2. The amino acid score of buckwheat increased after extrusion, with the limiting amino acid remaining leucine. This limitation, as well as the limiting sulfur amino acid content of the extruded pinto flour, is offset in the blend of the two ingredients, resulting in an amino acid score of 0.93, higher than that of either the extruded buckwheat (0.80) or the extruded pinto flour (0.73). Lysine was the first limiting amino acid in baked buckwheat (0.81), and the sulfur amino acids were limiting in baked pinto flour. The baked blend had an amino acid score of 0.72, higher than that of pinto flour but lower than that of buckwheat. Similar to buckwheat, the limiting amino acid in the blend is lysine. It has been well documented that lysine content can be reduced by heating.[19,20] Studies investigating the effect of autoclaving on different blends of plant protein determined that lysine destruction varied on the basis of the duration of heating as well as the blend composition itself.[19,20] Although unanticipated, the results of this study suggest that the lysine content of the baked buckwheat/pinto blend is more susceptible to destruction than the lysine contained in each of the baked flours.

The in vivo and in vitro protein digestibility values of the ingredients are found in Table 3. Untreated buckwheat flour had an in vivo protein digestibility, or true protein digestibility, of 71.1%, lower than previously reported.[11] Extrusion has been shown to increase protein digestibility because it inactivates or destroys antinutritive factors.[13,15,18] This is reflected in this study as extrusion increased the true protein digestibility of the buckwheat flour by 7.3%, from 71.1 to 78.4% ($p = 0.0195$). The extruded pinto flour had a true protein digestibility of 84.8%, with the true protein digestibility of the extruded blend being 81.8%. The investigation of the in vitro digestibility of these proteins provided similar results. The in vitro digestibility of buckwheat increased from 71.7 to 78.0% after extrusion, whereas extruded pinto flour had an in vitro digestibility of 80.2% and that of the blend was 80.0%. Baked buckwheat had lower digestibility than either untreated or extruded buckwheat, 63.4%, with the baked pinto flour and the blend having lower digestibility than their extruded counterparts, 60.0 vs 84.8% and 69.2 vs 81.8%, respectively. This was also reflected in the in vitro analysis, with a lower digestibility in baked flours compared to those extruded. Although both baking and extrusion have been shown to reduce the activity and content of antinutritive factors,[13,15,18−20,32] it is possible that the extrudates had less active antinutritional factors than the baked products, resulting in higher digestibility.

The protein digestibility corrected amino acid score (PDCAAS) is influenced by both the amino acid score and the digestibility of the protein.[10] PDCAAS values are presented in Table 3. The increased digestibility and amino acid score of buckwheat is reflected in an increased PDCAAS value compared to that of untreated buckwheat flour, 62.7 vs 54.3. The blending of extruded buckwheat and extruded pinto flour increased the PDCAAS to 76.3, well above that of either the extruded buckwheat or pinto flours alone. If the amino acid composition and protein digestibility of individual ingredients is available, it is possible to calculate the theoretical PDCAAS value of any given blend of those two ingredients. In the case of the 50:50 blend of the extruded ingredients, the theoretical

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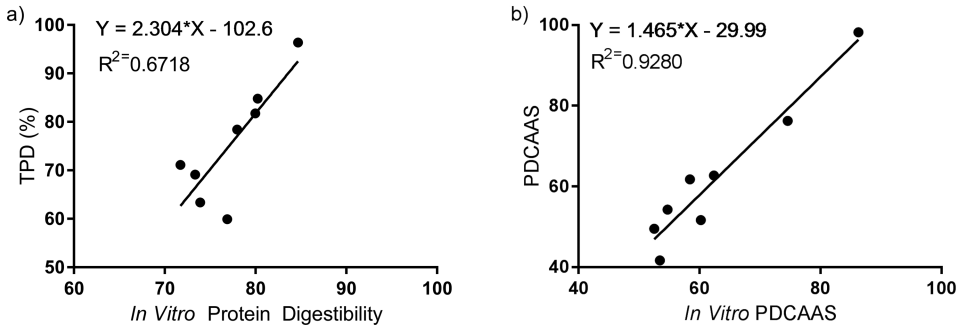

Figure 1. Relationship between the digestibility of the buckwheat and pinto products as determined by in vitro and in vivo methods (a) and relationship between the protein digestibility-corrected amino acid scores calculated using in vitro and in vivo digestibilities (b). TPD, true protein digestibility determined in vivo; PDCAAS, protein digestibility corrected amino acid score; in vitro PDCAAS, in vitro protein digestibility corrected amino acid score.

Table 4. Digestible Indispensable Amino Acid Values of Buckwheat and Pinto Bean Products[a]

	Thr	Val	Met+Cys	Ile	Leu	Phe+Tyr	His	Lys	Trp	DIAAS[b]
casein	1.08	1.21	**1.03**	1.33	1.13	1.74	1.19	1.15	1.49	1.03
BWF	0.76	0.64	1.00	0.59	**0.54**	0.77	0.97	0.66	0.94	0.54
EBWF	0.84	0.72	1.09	0.66	**0.63**	1.28	1.02	0.68	1.18	0.63
EPF	1.00	0.79	**0.57**	0.83	0.81	1.25	1.29	0.87	0.97	0.57
EBWPF	0.97	0.76	**0.75**	0.80	0.76	1.07	1.20	0.79	1.20	0.75
BBW	0.76	0.59	**0.49**	0.61	0.57	0.92	0.99	0.53	0.83	0.49
BPF	0.80	0.60	**0.39**	0.68	0.62	0.90	0.96	0.60	0.64	0.39
BBPF	0.76	0.65	0.92	0.65	0.60	0.95	0.99	**0.50**	0.94	0.50

[a]BWF, buckwheat flour; EBWF, extruded buckwheat flour; EPF, extruded pinto flour; EBPF, extruded blend; BBW, baked buckwheat flour; BPF, baked pinto flour; BBPF, baked blend. Bolded values reflect first limiting amino acid. [b]DIAAS, digestible indispensable amino acid score. DIAAS was calculated using true protein digestibility.

PDCAAS value was 71.62 compared to the actual value of 76.27, whereas the baked blend PDCAAS values were 47.25, calculated, and 49.52, measured. This provides further support to the additivity of the PDCAAS approach for determining protein quality, a characteristic that is lacking in the protein efficiency ratio. The in vitro protein digestibility corrected amino acid score (in vitro PDCAAS) of the untreated buckwheat, 54.7, was lower than that of the extruded buckwheat, 62.4, and that of the extruded blend, 74.6, was higher than that of either extruded buckwheat or pinto, 58.4. With respect to baking, buckwheat flour had the highest PDCAAS and in vitro PDCAAS values, 51.7 and 60.2, when compared to either the baked pinto flour, 41.7 and 53.4, or the baked blend, 49.5 and 52.5. This is due to the combination of low amino acid score for the blend and low digestibility for the baked pinto flour. From these findings it can be suggested that extrusion is more effective at increasing PDCAAS values compared to untreated flour and results in increased digestibility and amino acid score compared to baked flours.

The protein digestibility corrected amino acid score is the method recommended by the FAO for protein quality evaluation.[10] This method requires the use of an animal model to determine the digestibility of a protein; however, there is a continued desire to reduce animal utilization whenever possible. For that reason this study compared digestibility determined via the appropriate rodent model, in vivo, and an in vitro method to determine digestibility.[26,27] A positive correlation was found between true protein digestibility and in vitro protein digestibility, R^2 = 0.6718, suggesting that there is a relationship between these measures of digestibility (Figure 1). The protein digestibility determined in vitro was consistently lower for the extruded samples than that determined in vivo. Although this was not the case for the baked products, should this lower digestibility value be consistent across multiple samples of extruded flours, it could potentially act as a conservative estimate in calculating protein quality because the digestibility of the protein source would always be greater than anticipated once consumed. A larger data set and further investigation are required to confirm this possibility. As PDCAAS is the true measurement of protein quality, not simply protein digestibility, the PDCAAS and in vitro PDCAAS values were also compared. A correlation was found between these two measurements with R^2 = 0.9280 (Figure 1). Although both in vitro PDCAAS and PDCAAS use a common amino acid score, the strong correlation between these values suggests that in vitro PDCAAS could be used as an alternative for PDCAAS under certain circumstances. However, further research and a larger data set are needed to add strength to this idea.

The newest method for determining protein quality, the digestible indispensable amino acid score (DIAAS), was recommended as a replacement for PDCAAS in 2013 by the FAO/WHO.[23] The key differences between these two methods are the use of ileal rather than fecal digestibility, the treatment of individual amino acids and nutrients rather than the protein, and a modified amino acid requirement pattern. Although ileal digestibility is recommended for the calculation of DIAAS, until such time as a comprehensive database of ileal digestibilities becomes available, the use of fecal digestibility is still permitted. In this study the DIAAS values for the experimental samples ranged from 0.39 for the baked pinto flour to 0.75 for the extruded blend, with casein reaching 1.03 (Table 4). In most cases the PDCAAS unit value was greater than the DIAAS value, ranging from an increase of 0.88 unit for the extruded

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blend (PDCAAS of 0.7626, DIAAS of 0.7538) to 4.6 units for extruded pinto flour (PDCAAS of 0.6716, DIAAS of 0.5718). Conversely, casein (PDCAAS of 0.9824, DIAAS of 1.0255) and the baked blend (PDCAAS of 0.4951, DIAAS of 0.5038) had greater DIAAS unit values than PDCAAS, 4.31 and 0.87, respectively, whereas the untreated and extruded buckwheat flours had the same PDCAAS and DIAAS values. The primary reason for these differences, as the same amino acid profile and the same digestibility are being used for both calculations, is the different amino acid requirement patterns. In the case of the untreated and extruded buckwheat the limiting amino acid was leucine and because the leucine requirement remained consistent between PDCAAS and DIAAS, no difference was found. The current recommendation is that no protein claim should be permitted for any source that has a DIAAS value of <0.75.[23] The only sample tested in this study that met this threshold was the extruded blend with a DIAAS of 0.7538. As pulses, including pinto beans, are currently positioned as protein sources in both Canada and the United States, further discussion is warranted regarding whether this cutoff value of 0.75 is appropriate for all protein sources.

The protein efficiency ratio is the protein quality measurement mandated by Health Canada for regulation of protein content claims.[22] Unlike PDCAAS, which determines protein quality through amino acid composition and digestibility, PER relies upon growth, the amount of weight gained based on protein consumption over a 4 week period. The PER data are presented in Figure 2. The PER value for untreated buckwheat

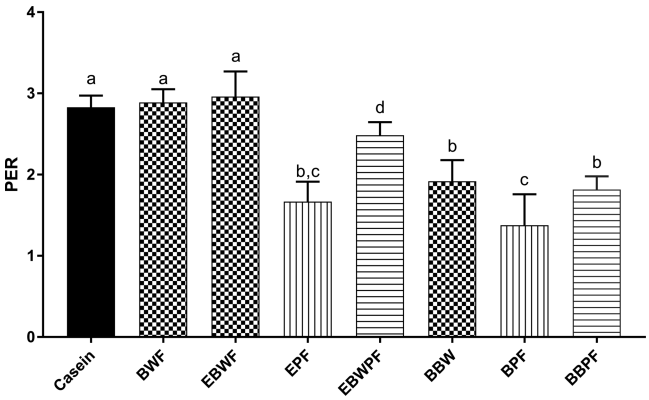

Figure 2. Protein efficiency ratio (PER) values for the buckwheat and pinto bean products. BWF, buckwheat flour; EBWF, extruded buckwheat flour; EPF, extruded pinto flour; EBPF, extruded blend; BBW, baked buckwheat flour; BPF, baked pinto flour; BBPF, baked blend. Mean ± SD (n = 10). Diagonal hatching indicates buckwheat flour, vertical lines indicate pinto flour, and horizontal lines indicate blended flours. Different letters above bars indicate significant differences (p < 0.05).

determined in this study, 2.89, is slightly higher than that previously reported, 2.69.[33] Although there was no difference between the PER values of casein and untreated or extruded buckwheat flour, these flours had the highest PER compared to other extrudates and the baked samples. The extruded blend induced greater growth per gram of protein consumed compared to the extruded pinto flour and any baked product; however, it had a lower PER than the extruded buckwheat flour alone. With respect to the baked products, the baked buckwheat flour and baked blend did not differ from the extruded pinto flour and had higher PER values than the baked pinto flour. To standardize PER determination across laboratories, the untreated PER value undergoes an adjustment relative to the casein PER, and these values are presented in Table 3. In terms of growth potential, these findings indicate that untreated buckwheat is as good as extruded buckwheat; however, baking or blending with pinto flour does not increase growth rates in rodents.

In summary, blending and extrusion of buckwheat flour and pinto flour are capable of increasing overall protein quality. Overall, extruded products had higher PER values, increased digestibility, and greater PDCAAS values than baked products, with the extruded BWPF having the greatest PDCAAS value of the experimental diets. A strong correlation was found between both digestibility and PDCAAS values generated from in vitro and in vivo methods. The use of in vitro digestibility analysis should be investigated as a potential replacement for the current rodent assay for nutrient content claim purposes.

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ABBREVIATIONS USED

PER, protein efficiency ratio; PDCAAS, protein digestibility corrected amino acid score; DIAAS, digestible indispensable amino acid score; in vitro PDCAAS, in vitro protein digestibility corrected amino acid score

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