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Effect of Processing on the *in Vitro* and *in Vivo* Protein Quality of Yellow and Green Split Peas (*Pisum sativum*)

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ABSTRACT: In order to determine the effect of extrusion, baking, and cooking on the protein quality of yellow and green split peas, a rodent bioassay was conducted and compared to an *in vitro* method of protein quality determination. The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) of green split peas (71.4%) was higher than that of yellow split peas (67.8%), on average. Similarly, the average Digestible Indispensable Amino Acid Score (DIAAS) of green split peas (69%) was higher than that of yellow split peas (67%). Cooked green pea flour had lower PDCAAS and DIAAS values (69.19% and 67%) than either extruded (73.61%, 70%) or baked (75.22%, 70%). Conversely, cooked yellow split peas had the highest PDCCAS value (69.19%), while extruded yellow split peas had the highest DIAAS value (67%). Interestingly, a strong correlation was found between *in vivo* and *in vitro* analysis of protein quality (R^2 = 0.9745). This work highlights the differences between processing methods on pea protein quality and suggests that *in vitro* measurements of protein digestibility could be used as a surrogate for *in vivo* analysis.

KEYWORDS: *Extrusion, baking, PER, PDCAAS, DIAAS*

INTRODUCTION

Global production of dry peas, *Pisum sativum*, occurs primarily in three regions. In the years 2008−2012, Canada, Russia, and China were producing 30%, 15%, and 10% of the global dry pea market.[1] By 2014, Canada continued to produce 30% of the world's dry peas, while Russia and Chinese production became equivalent at 13% each.[2] Peas, much like other pulse crops, are nutrient dense and contain significant quantities of minerals, vitamins, fiber, carbohydrates, and protein.[3] Plant based protein sources, such as pea protein, have lower protein quality than animal based protein sources due to limitations in the amino acid profile and lower protein digestibility.[4] While cereals such as wheat and oats are primarily limiting in lysine, pea proteins have inadequate quantities of sulfur amino acids (methionine and cysteine), with respect to the requirements of a human diet.[5] One study of 59 different pea types found the protein content ranged from 13.7−30.7% with an average of 22.3%,[6] while a more recent study of 10 genotypes determined a range of 24.2−27.5%.[7] The reduced protein digestibility found in peas, compared to animal protein, partially stems from the antinutritive factors found in pulses. These antinutritive factors include proteolytic inhibitors, tannins, and phytic acid.[8−10] These antinutritive compounds can be destroyed or inactivated by subjecting peas to processes such as extrusion, cooking, or baking. Furthermore, the processing may influence the amino acid composition of the final product.

Extrusion is a processing method in which the raw material is exposed to high temperatures and water to induce expansion of the resulting dough prior to being forced through a die and cut to size by a rotating blade. The effect of this processing method on protein quality has been investigated in beans where it has been found not to alter total protein content; however there has been evidence of reduced sulfur amino acid content.[11,12] With respect to peas, extrusion has been found to not alter protein content, but the extruded flour had lower quantities of valine, phenylalanine, lysine, and tryptophan compared to raw flour.[3,13] Boiling, a method simulating "home cooking", has been found to increase protein content in beans, fava beans, and chickpeas while reducing protein content in pea samples.[14−17] While direct investigation into the impact of baking on amino acid composition is limited, the use of autoclaves as a heat treatment is relatively common. Certain studies have found that autoclaving of pulses or pulse blends results in lower lysine content but with minimal effect on other amino acids,[18,19] while others did not find any effect of autoclaving on amino acid composition.[20]

This study was undertaken to determine the effects that extrusion, cooking, and baking have on either protein digestibility or the amino acid composition of yellow and green split pea flours. The protein digestibility and amino acid composition are intrinsic to protein quality as measured by the Protein Digestibility Corrected Amino Acid Score (PDCAAS), currently the regulatory standard for protein claims in the United States.[4] The most recent method for determining

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protein quality, the Digestible Indispensable Amino Acid Score (DIAAS), was calculated for the pea samples using true protein digestibility as recommended by the FAO/WHO,[21] while an *in vitro* measurement of protein quality was also determined in order to compare with those obtained via *in vivo* methodology. The Protein Efficiency Ratio (PER), a measurement of growth, was also determined as it is required for regulation of protein content claims in Canada.[22]

■ 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[23] (Protocol Number F2012-035).

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

Sample Procurement and Preparation of Extruded Baked and Cooked Flours. Yellow and green split peas were provided by SaskCan Pulse Trading (Regina, Saskatchewan, Canada) and Thompsons Ltd. (Blenheim, Ontario, Canada) with an additional sample of yellow split peas provided by Diefenbaker Seed Processors (Elbow, Saskatchewan, Canada). Prior to processing, samples of similar peas from different suppliers were combined and thoroughly mixed. Milling of the combined samples was performed on a hammer mill (Jacobson 120-B hammer mill, Minneapolis, MN), with screen hole size of 0.050 in., round. The hammer mill and flour bin were vacuumed thoroughly after milling each sample. Extrudates were prepared using a Clextral Evolum HT 25 twin screw extruder (Firminy, France). The flours were extruded at 36 kg/h with a moisture addition of 0.8 kg/h. The screw speed was 650 rpm. The extrusion barrel temperatures were 30−50 °C, 70−90 °C, and 100−120 °C. After extrusion, samples were milled as described above. The baking process was as follows: 4.5 kg of green pea flour and 4.0 kg of yellow split pea flour were mixed for 4 min with 2.36 and 2.0 kg 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. The dough was baked at 198.3 °C, 198.3 °C, and 165.6 °C for 29 min (green split pea) and 35 min (yellow split pea). Baking was performed on a Doyon FC2-lll tunnel conveyor oven (Menominee, MI, U.S.A). After baking, samples were milled 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). Before boiling, peas were soaked in tap water at a ratio of 1:4 (1.5 kg pea/6 L water) for 16 h with the water being changed prior to cooking. The pea/water mixture was brought to a boil and maintained until done, approximately 25−35 min. After cooking, the samples were drained, freeze-dried, and then milled on a hammer mill (Jacobson 120-B hammer mill, Minneapolis, MN), with screen hole size of 0.050 in., round.

Analytical Procedures. For all samples, percent crude protein (CP; N × 6.25) was determined through the use of a Dumas Nitrogen Analyzer (Dumatherm DT, Gerhardt Analytical Systems, Germany); 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, while methionine and cysteine were determined using the AOAC Official Method 45.4.05 and other amino acids, except tryptophan, were determined using AOAC Official Method 982.30.[24] Tryptophan content was determined as previously described[25]

Protein Digestibility-Corrected Amino Acid Score (PDCAAS). A rat bioassay was used to determine the PDCAAS of the samples.[4] Amino acid scores were determined according to FAO/WHO guidelines. True protein digestibility was determined using the AOAC Official Method 991.29,[24] using casein as a reference standard (Dyets, Bethlehem, PA, U.S.A) and correcting for endogenous protein losses using previously determined values. The casein control for this study was also used in a concurrent study investigating the effect of processing on red lentils, green lentils, and chickpeas. 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 with diets being formulated to contain 10% protein, supplied by the test sample.[26] True protein digestibility (TPD %) was calculated using the following equation:

$$\mathrm{TPD\%} = ((\text{Nitrogen Intake} - (\text{Fecal Nitrogen Loss} - \text{Metabolic Nitrogen Loss}))/\text{Nitrogen Intake}) \times 100$$

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:[21]

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

Ideally, the ileal amino acid digestibility should be used for the calculation of DIAAS; however the use of fecal digestibility is considered acceptable until such time as a data set of true ileal digestibility is developed.[21]

***In Vitro* Protein Digestibility-Corrected Amino Acid Score (*in Vitro* PDCAAS).** An *in vitro* digestibility assay was also performed on each sample as previously described.[25] Briefly, 62.5 mg of protein was incubated at 37 °C for 10 min with a protease cocktail containing trypsin, chymotrypsin, and protease after the sample had been stabilized at a pH of approximately 8.0. The resulting pH drop was recorded for 10 min, and the *in vitro* protein digestibility was calculated as follows

$$\mathrm{IVPD\%} = 65.66 + 18.10 \cdot \Delta \mathrm{pH}_{10\mathrm{min}}$$

($\Delta\mathrm{pH}_{10\ \mathrm{min}} = \mathrm{pH}_{\mathrm{initial}} - \mathrm{pH}_{\mathrm{final}}$, where $\mathrm{pH}_{\mathrm{initial}}$ is the pH after stabilization to approximately pH 8.0, while $\mathrm{pH}_{\mathrm{final}}$ is the pH of the solution 10 min after the addition of the enzyme cocktail.)

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

Protein Efficiency Ratio (PER). According to Health Canada, PER is determined over a 28 day growth period for rats consuming feed *ad libitum*.[22] As recommended, the PER was calculated as weight gain (g) divided by the amount (g) of protein consumed over 28 days. Values were also adjusted to a standardized 2.5 PER value for the reference casein.

Statistics. Protein Efficiency Ratios and True Protein Digestibility were compared via two-way ANOVA with post hoc analysis using Tukey's multiple comparison test. The correlations between *in vivo* and *in vitro* digestibilities and PDCAAS and *in vitro* PDCAAS were determined via linear regression analysis (GraphPad Prism, 7.0, La Jolla, CA, U.S.A).

■ RESULTS AND DISCUSSION

The dry matter, crude fat, and crude protein for untreated and processed lentils are presented on a dry matter basis in Table 1. Untreated yellow and green split peas had similar dry matter (91.30% vs 91.43%), which, in turn, was similar to the previously reported value of 91.38%.[27] Processed yellow and green split peas also showed similar dry matters with extrudates (95.62% vs 95.64%), cooked samples (97.44% vs 99.70%), and baked samples (95.81% vs 97.81%) differing by less than 3%. While the cooked samples had the highest dry matter, this is most likely due to the freeze-drying process used after conventional cooking to remove excess water prior to milling.

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Table 1. Proximate Analysis and Amino Acid Composition of Untreated, Extruded, Cooked, and Baked Yellow and Green Split Pea Flour Presented on a Dry Matter Basis

	%DM[a]	%CF[b]	%CP[c]	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	TRP
casein	93.56	0.21	92.43	8.32	3.58	6.03	21.43	10.44	1.44	3.38	0.83	5.37	1.55	4.10	8.97	5.16	4.91	2.93	7.44	3.33	1.15
yellow pea																					
untreated	91.30	1.50	23.78	2.88	0.85	1.25	3.94	0.96	0.92	1.13	0.25	1.02	0.22	0.87	1.75	0.62	1.04	0.65	1.68	2.02	0.21
extruded	95.62	1.53	24.56	3.13	0.91	1.32	4.20	0.86	0.99	1.20	0.26	1.12	0.22	0.98	1.90	0.70	1.17	0.69	1.75	2.14	0.20
cooked	97.44	2.91	22.88	2.85	0.87	1.30	3.84	0.91	0.88	1.15	0.23	1.10	0.23	0.99	1.97	0.67	1.18	0.64	1.73	2.06	0.19
baked	95.81	2.16	23.35	2.92	0.88	1.27	4.07	0.82	0.97	1.20	0.25	1.12	0.21	0.96	1.87	0.63	1.15	0.68	1.61	2.00	0.22
green pea																					
untreated	91.43	0.35	26.15	3.16	0.93	1.37	4.46	1.01	1.02	1.25	0.31	1.14	0.24	1.01	1.96	0.74	1.22	0.71	1.85	2.38	0.25
extruded	95.64	1.36	25.08	2.97	0.89	1.32	4.27	0.92	0.97	1.21	0.27	1.11	0.25	0.91	1.82	0.70	1.19	0.69	1.68	2.23	0.22
cooked	99.70	2.28	23.91	3.09	0.89	1.36	4.11	0.81	0.95	1.19	0.25	1.15	0.24	1.01	2.05	0.72	1.31	0.67	1.79	2.13	0.23
baked	97.81	2.12	22.77	2.88	0.86	1.30	4.13	0.87	0.96	1.18	0.24	1.00	0.22	0.85	1.81	0.61	1.13	0.69	1.61	2.09	0.22

[a]DM = dry matter content. [b]CP = crude protein = nitrogen content (determined by DUMAS analysis) × 6.25. [c]CF = crude fat determined by hexane extraction.

Untreated yellow split pea had a higher fat content than untreated green split pea (1.50% vs 0.35%); however other work has found the fat content of split peas to be 1.16% and 2.43% for intact peas.[20,27] While the fat content of extruded and baked samples is similar between yellow split and green split peas (1.53% vs 1.36% and 2.16% vs 2.12% respectively), cooked yellow split peas had a higher fat content, 2.91%, than the cooked green split peas 2.28%. All processing methods induced an increase in fat content for both yellow split and green split peas with cooking showing an increase of 1.41% for yellow split and 1.93% for green split peas. The protein content of untreated yellow and green split peas (23.78% and 26.15%) were similar to previously reported results.[6,7,20,27] Interestingly, all processing methods (cooking, baking, and extrusion) increased the protein content of yellow split peas, while cooking and baking reduced the protein content of green split peas. Untreated yellow split peas had a protein content of 23.78% with extrusion resulting in a final protein content of 24.56%, followed by baking, 23.35%, and cooking 22.88%. Conversely untreated green split peas had a protein content of 26.15%, the protein content of the extrudate was 25.08%, cooked protein content was 23.91%, and baked product had a protein content of 22.77%. These results indicate that processing does affect the protein content of yellow and green split peas differentially; however the overall difference is relatively low with a maximum increase of 0.78% between untreated and extruded yellow split peas and a maximum decrease of 3.38% between untreated and baked green split peas.

The amino acid composition for all ingredients is presented in Table 1 with the resulting amino acid scores presented in Table 2. The first limiting amino acid was either tryptophan, in the case of extruded yellow and green split pea (0.72 and 0.81, respectively) and cooked yellow split pea (0.78), or the sulfur amino acids, in the case of cooked green split pea (0.82) and baked yellow and green split pea (0.79 and 0.85, respectively). This is in agreement with previous work demonstrating that split yellow peas are lower in tryptophan while split green peas are lower in sulfur amino acids.[5,28] In both yellow and green split peas, the highest amino acid score was determined in baked samples (0.79 and 0.85, respectively), followed by cooking (0.78 and 0.82), and extrusion (0.81 and 0.72). The amino acid score of the baked peas is similar to that found after autoclaving, where two different autoclaved varietals had amino acid scores of 0.73 (Trapper) and 0.82 (Century).[4] The amino acid score for cooked yellow split peas for this study, 0.78, is similar to what was found in a previous study, 0.73.[28] The value for cooked green split peas differed, 0.82 for this study compared to 0.59 in the previous study.[28] Another study using cooked peas determined an amino acid score of 0.58, suggesting that the amino acid content of cooked peas is variable depending upon processing conditions and varietal selected.[29]

The *in vivo* and *in vitro* protein digestibility values are found in Table 3. The true protein digestibility (%TPD) of yellow split pea was significantly altered by processing ($p < 0.05$), resulting in digestibility values of 91.35% after extrusion, 89.00% after cooking, and 86.77% after baking. A similarly significant trend for %TPD was found in green split peas with extrusion (90.73%) being higher than either cooking (87.58%) or baking (88.51%); however the resulting TPD of cooked green split peas was not significantly different than that of baked. These results are in agreement with previous work,

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Table 2. Amino Acid Score of Extruded, Cooked, and Baked Yellow and Green Split Pea Flour[a]

	Thr	Val	Met+Cys	Ile	Leu	Phe+Tyr	His	Lys	Trp
casein	1.14	1.66	**1.03**	1.59	1.47	1.73	1.67	1.39	1.13
yellow pea									
extruded	1.09	1.30	0.79	1.43	1.17	1.21	1.47	1.23	**0.72**
cooked	1.13	1.37	0.80	1.54	1.30	1.28	1.46	1.30	**0.78**
baked	1.11	1.36	**0.79**	1.48	1.21	1.20	1.52	1.19	0.87
green pea									
extruded	1.04	1.26	0.83	1.29	1.10	1.20	1.45	1.16	**0.81**
cooked	1.10	1.37	**0.82**	1.52	1.30	1.35	1.49	1.29	0.88
baked	1.05	1.24	**0.85**	1.37	1.13	1.20	1.44	1.22	0.88

[a]Bolded values indicate the first limiting amino acid. The reference pattern used to calculate the amino acid scores was as followed (mg/g protein): Thr 34, Val 35, Met+Cys 25, Ile 28, Leu 66, Phe + Tyr 63, His 19, Lys 58, Trp 11.

Table 3. Adjusted Protein Efficiency Ratio, Protein Digestibility-Corrected Amino Acid Scores, and *in Vitro* Protein Digestibility-Corrected Amino Acid Scores of Extruded, Cooked, and Baked Yellow and Green Split Pea Flour[a]

	adj. PER	AAS[b]	TPD[c]	IVPD[d]	PDCAAS[e]	*in vitro* PDCAAS[f]
casein	2.5	1.03	96.11 (1.4)	91.36 (0.72)	99.09	94.10
yellow pea						
extruded	1.5	0.72	91.35 (2.2)[a]	86.93 (0.10)	65.44	62.27
cooked	1.7	0.78	89.00 (1.8)[b]	86.75 (0.63)	69.19	67.44
baked	1.5	0.79	86.77 (2.6)[c]	82.49 (0.54)	68.89	65.49
green pea						
extruded	1.5	0.81	90.73 (2.8)[A]	90.0 (0.81)	73.61	73.03
cooked	1.4	0.82	87.58 (1.6)[B]	86.56 (0.45)	72.00	71.17
baked	1.5	0.85	88.51 (2.2)[B]	82.22 (0.63)	75.22	69.88

[a]*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. TPD was analyzed via Two-Way ANOVA with Tukey's post hoc test. Superscripts with different letters, within either pulse class, are significantly different. PDCAAS is calculated as the product of AAS and %TPD, while *in vitro* PDCAAS is the product of AAS and IVPD. [b]AAS = amino acid score. [c]%TPD = % true protein digestibility. [d]IVPD = *in vitro* protein digestibility. [e]PDCAAS = protein digestibility corrected amino acid score. [f]*In vitro* PDCAAS = *in vitro* protein digestibility corrected amino acid score.

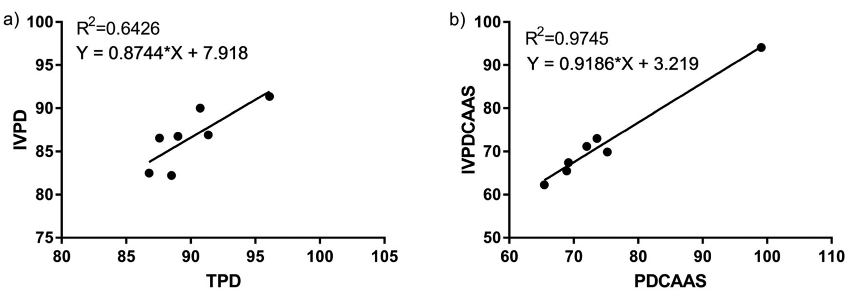

Figure 1. Relationship between the digestibility of extruded, cooked, and baked yellow and green split pea flour determined by *in vitro* and *in vivo* methods (a) and the relationship between the protein digestibility-corrected amino acid scores calculated using *in vitro* and *in vivo* digestibilities (b). TPD = true protein digestibility, IVPD = *in vitro* protein digestibility, PDCAAS = protein digestibility corrected amino acid score, *in vitro* PDCAAS = *in vitro* protein digestibility corrected amino acid score.

which found the %TPD of 87.94% for cooked split yellow peas and 85.15% for cooked split green peas.[28] The digestibility values determined for baked flour (86.77% and 88.51%) are slightly higher than those previously reported for autoclaved peas (83.5%).[4] There was little variation between the *in vitro* protein digestibilities of processed yellow split peas, ranging from 82.49% after baking to 86.93% after extrusion. Green split peas, on the other hand, had a larger range of *in vitro* protein digestibility ranging from 82.22% after baking to 90.0% after extrusion. The *in vitro* digestibility after extrusion was lower in yellow split peas compared to green split peas (86.93% vs 90.0%). In contrast, a previous study found an *in vitro* protein digestibility of 77.18% for extruded pea samples.[8] The *in vitro* digestibility of cooked peas has been previously reported as 94.33%, higher than the results of this study (86.75% and 86.56% for yellow and green split pea), while autoclaved pea had a digestibility of 86.55%, close to the baked results of this study, 82.49% and 82.22% for yellow and green split pea.[20] There are similarities between the general pattern of the *in vivo* and *in vitro* protein digestibility values as extruded was the most digestible followed by cooked and baked for yellow split pea. Although the *in vitro* protein digestibility was lower than that found in the *in vivo* model system in all cases studied, this was not unexpected. The *in vitro* method uses only three digestive enzymes at one pH whereas *in vivo* digestion occurs across a pH range with multiple series of digestive enzymes. For this reason, it is not surprising that the *in vitro* system would underestimate the potential biological capacity for protein digestion.

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Table 4. Digestible Indispensable Amino Acid Values of Extruded, Cooked, and Baked Yellow and Green Split Pea Flour

	Thr	Val	Met+Cys	Ile	Leu	Phe+Tyr	His	Lys	Trp	DIAAS[a]
casein	1.20	1.30	**0.92**	1.34	1.41	2.01	1.52	1.36	1.41	0.92
yellow pea										
extruded	1.09	0.97	**0.67**	1.14	1.07	1.33	1.28	1.14	0.85	0.67
cooked	1.10	0.99	**0.66**	1.20	1.16	1.38	1.24	1.18	0.90	0.66
baked	1.06	0.96	**0.64**	1.12	1.05	1.27	1.25	1.05	0.97	0.64
green pea										
extruded	1.03	0.93	**0.70**	1.03	1.00	1.32	1.25	1.07	0.95	0.70
cooked	1.06	0.98	**0.67**	1.16	1.13	1.43	1.24	1.15	0.99	0.67
baked	1.02	0.89	**0.70**	1.06	1.00	1.28	1.21	1.10	1.01	0.70

[a]DIAAS = Digestible Indispensable Amino Acid Score. DIAAS was calculated using true protein digestibility. Bolded values reflect first limiting amino acid.

The calculated protein digestibility corrected amino acid score (PDCAAS) and the *in vitro* protein digestibility corrected amino acid score (*in vitro* PDCAAS) are presented in Table 3. The two characteristics that can modify PDCAAS are the amino acid score and protein digestibility.[4] The PDCAAS of yellow split peas was 64.55% for extruded product, 68.89% for baked, and 69.19% for cooked, while green split peas had PDCAAS values of 75.22% for baked, 73.61% for extruded, and 72.00% for cooked. Previously the PDCAAS of cooked yellow split pea was found to be 64.3% and that for green split pea was 50.0%, lower than the values determined in this investigation.[28] In this study, the *in vitro* PDCAAS was consistently lower than that of PDCAAS ranging between 5.34% lower for baked green split peas and 0.58% lower for extruded split green peas.

In order for protein quality to be assessed, it requires the use of an *in vivo* animal model to determine the digestibility of the protein source being investigated.[4] It has been suggested that using an *in vitro* method of protein digestion could reduce the use of animals in protein quality regulation, provided that the relationship of the *in vivo* and *in vitro* digestibility was well correlated.[30] This study used a previously described method for determining protein digestibility, a one-step pH drop method, and compared that digestibility with those determined in a rodent *in vivo* system, as presented in Figure 1.[25] The correlation between measures of digestibility, $R^2 = 0.6426$ ($p = 0.0302$), reliably demonstrates that there is a relationship between protein digestibility as calculated in a rodent model and *in vitro*. Since both assays use the same protein source, the same amino acid score is used to determine PDCAAS and *in vitro* PDCAAS. When these protein quality measurements are compared, the correlation becomes much stronger, resulting in an $R^2 = 0.9745$. This correlation is slightly elevated due to the high protein quality of the casein control diet; however when that data point is excluded from the correlation the relationship between PDCAAS and *in vitro* PDCAAS is still $R^2 = 0.76$. This relationship between *in vivo* and *in vitro* protein quality is similar to that found in other plant based protein sources with $R^2 = 0.9898$ and 0.9280.[25,30] These data indicate that an *in vitro* method of determining protein digestibility, and thereby *in vitro* protein quality, is well correlated with *in vivo* results and suggest that *in vitro* PDCAAS could be used as an alternate method for assaying protein quality that does not rely on animal experimentation.

Digestible Indispensable Amino Acid Score (DIAAS) has been presented as a new method for determining protein quality.[21] The difference between DIAAS and PDCAAS is the use of ileal digestibility instead of fecal digestibility, the treatment of individual amino acids as nutrients rather than protein, and the development of a new profile of amino acid requirements. As a comprehensive database of ileal digestibility values is currently lacking, the use of fecal digestibility in the calculation of DIAAS is still acceptable, and has been used in this study.[21] DIAAS values, found in Table 4, were highest for extruded and baked green split peas, both 0.70, while the lowest DIAAS value was 0.64 for baked yellow split peas. For both yellow and green split peas, DIAAS values were highest for extrudates (0.67 for yellow split peas, 0.70 for green split peas), followed by the cooked product (0.66 for yellow split peas compared to 0.67 for green split peas). While baking had lower DIAAS values for yellow split peas, 0.64, baked green split peas had a DIAAS value of 0.70, the same as its extrudate. A study conducted by Nosworthy et al.[28] found a DIAAS value for cooked yellow split peas similar to those in this study (0.73) and a value of 0.46 for green split peas. A comparison between PDCAAS, *in vitro* PDCAAS, and DIAAS demonstrates that in almost all cases the *in vitro* PDCAAS value falls between PDCAAS and DIAAS. This is not the case for extruded yellow split pea, where both PDCAAS and *in vitro* PDCAAS values are lower than DIAAS. An increase in the requirement of sulfur amino acids, from 25 mg/g protein in PDCAAS to 27 mg/g protein in DIAAS, would lead to lower DIAAS values compared with the values obtained in PDCAAS and *in vitro* PDCAAS with the exception of extruded yellow split peas.[4,21]

The measurement of protein quality required by Health Canada for regulation of protein content claims is the Protein Efficiency Ratio (PER).[22] Unlike the previously discussed methods of protein quality determination, PER is a measurement of growth, body weight gain per unit of protein consumed over 4 weeks, rather than protein digestibility and amino acid composition. The PER data is presented in Figure 2. The PER for cooked split yellow peas (2.08) was significantly higher than either its extruded (1.81) or baked (1.85) flours. Interestingly there was no significant difference between preparation method of green split peas (PER of 1.89 for extruded samples, 1.78 for cooked and 1.84 for baked flours). In order to standardize the PER across samples, studies, and laboratories, PER values undergo normalization to the PER of the control diet, casein, as presented in Table 3. These normalized PER data illustrate the similar growth rates between processed yellow and green split pea (PER of 1.4−1.5), with the exception of extruded yellow split pea, which had a higher PER than any test diet (1.7).

In summary, processing peas prior to consumption is necessary to aid digestion and absorption of the nutrients. Depending on the processed used, the protein quality, postprocessing, is directly altered. Generally green split peas had higher protein quality than yellow split peas, as indicated by

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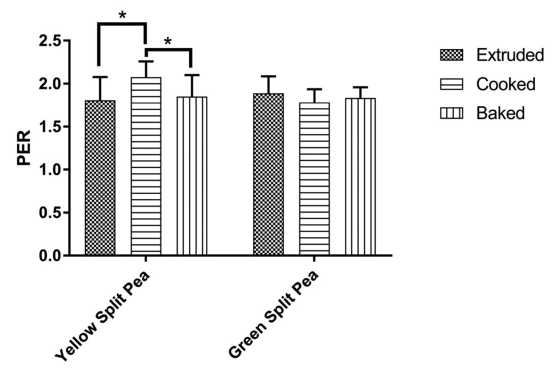

Figure 2. Protein Efficiency Ratio (PER) values of extruded, cooked, and baked yellow and green split pea flour. Hatched bars indicate baked flour, horizontal bars are cooked flour, and vertical bars are extruded flour. Mean ± SD (n = 10). Data were analyzed via Two-Way ANOVA with Tukey's post hoc test. * = $p < 0.05$.

higher PDCAAS and DIAAS values, with cooking being most beneficial for yellow split pea protein quality and baking for green split pea. Application of an *in vitro* method of determining protein digestibility resulted in a good correlation between *in vitro* and *in vivo* protein quality values, *in vitro* PDCAAS and PDCAAS, further supporting the potential use of *in vitro* methods in regulation of protein claims rather than *in vivo* bioassays.

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Notes

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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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