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Effect of processing on the *in vitro* and *in vivo* protein quality of red and green lentils (*Lens culinaris*)

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ABSTRACT

In order to determine the effect of extrusion, baking and cooking on the protein quality of red and green lentils, a rodent bioassay was conducted and compared to an *in vitro* method of protein quality determination. On average, the Protein Digestibility-Corrected Amino Acid Score of red lentils (55.0) was higher than that of green lentils (50.8). Extruded lentil flour had higher scores (63.01 red, 57.09 green) than either cooked (57.40 red, 52.92 green) or baked (53.84 red, 47.14 green) flours. The average Digestible Indispensable Amino Acid Score of red lentils (0.54) was higher than green lentils (0.49). The Protein Efficiency Ratio of the extruded lentil flours (1.30 red, 1.34 green) was higher than that of the baked flour (0.98 red, 1.09 green). A correlation was found between *in vivo* and *in vitro* methods of determining protein digestibility (R^2 = 0.8934). This work could influence selection of processing method during product development.

1. Introduction

Lentils (*Lens culinaris)* are a pulse crop primarily produced in Canada and India (1.99 MT and 1.1 MT in 2014) (FAOSTAT, 2017). Consumed globally, this crop is considered to be rich in protein, fiber, carbohydrates, minerals and vitamins (Ermetice et al., 2006; Iqbal, Khalil, Ateeq, & Khan, 2006). Generally, consumers are demonstrating an increasing interest in plant-based sources of high quality protein. The factors which alter protein quality include protein content, amino acid composition and protein digestibility. The protein content of lentils has been documented to be 28.3%, significantly higher than that of cereals, however the range in lentil protein content has been shown to between 15.9% and 31.4% (Grusak, 2009). In contrast to cereal grains, lentils are rich in lysine but limiting in the sulfur amino acids methionine and cysteine (Sarwar & Peace, 1986). Similar to other pulse crops, lentils contain certain anti-nutritive factors, including trypsin inhibitors and tannins (Wang, Hatcher, Toews, & Gawalko, 2009). These anti-nutritive factors can alter protein bioavailability by inactivating key digestive enzymes (trypsin inhibitors) or complexing with dietary proteins to reduce their digestibility (tannins) (Adsule & Kadam, 1989; Chavan & Kadam, 1989). Processing of lentils provides an opportunity to increase protein digestibility and amino acid availability.

Boiling has been shown to increase the protein content of pulses (Candela, Astiasaran, & Belli, 1997; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010), possibly due to the loss of carbohydrates during the boiling process (Verde, Frias, & Verde, 1992). With respect to lentils, some studies have found no difference in protein content between cooked and uncooked lentils (Candela et al., 1997; Hefnawy, 2011), while others have demonstrated an increase in the protein content after cooking (Wang et al., 2009). While there has been little work on *in vivo* protein digestibility, numerous studies have shown that cooking reduces the activity and concentration of anti-nutritive factors such as trypsin inhibitors, tannins, and phytic acid (Hefnawy, 2011; Sayeed & Njaa, 1985; Wang et al., 2009). As these compounds either inhibit the activity of digestive enzymes or sequester nutrients, thereby making them unavailable for digestion, any reduction in anti-nutritive

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factors would potentially increase dietary protein digestibility and thereby increase the bioavailability of the constituent amino acids.

Extrusion is a process by which ingredients are forced through a die of a particular shape and cut to a certain size by spinning blades after being exposed to expansion inducing temperatures. The effect of extrusion on the nutritional content and anti-nutritive factors has been investigated in beans (Al-Marzooqi & Wiseman, 2009; Arija et al., 1988; Batista, Prudencio, & Fernandes, 2010; Kelkar et al., 2012; Simons et al., 2015) and, to a lesser extent, peas (Alonso, Orúe, & Marzo, 1998; Frias et al., 2011; Roy, Boye, & Simpson, 2010). However, there has been little investigation on the impact of extrusion on the nutritional quality of lentils. Previous work has shown that extrusion reduced trypsin inhibitors by 99.54%, phytic acid by 99.30% and tannins by 98.83% increasing *in vitro* protein digestibility from 39.4% in raw lentil seed to 88.6% after extrusion, without altering protein content (Rathod & Annapure, 2016). Autoclaving is occasionally used to determine the impact of heat on protein quality rather than oven baking (del Cueto & Martinez, 1960; Marquardt, Campbell, Stothers, & Mckirdy, 1974; Srihara & Alexander, 1983; Umoren, Tewe, & Bokanga, 1997). Autoclaved lentils had lower concentrations and activities of trypsin inhibitors, tannins and phytic acid while protein content was not altered (Hefnawy, 2011).

The current study was undertaken to investigate whether processing (extrusion, cooking and baking) alters protein digestibility and/or the amino acid composition of red and green lentils. These two factors influence protein quality as measured by the Protein Digestibility Corrected Amino Acid Score (PDCAAS), currently used in the regulation of protein claims in the United States (FAO/WHO, 1991). The Digestible Indispensable Amino Acid Score (DIAAS), was calculated using true protein digestibility as currently recommended by the FAO/WHO (FAO/WHO, 2013). Additionally, an *in vitro* measurement of protein quality was determined in order to compare these values with those obtained via PDCAAS. As an additional measure of protein quality, the Protein Efficiency Ratio (PER), a bioassay used to assess the efficiency of weight gain in relation to protein consumption in rodents, was also determined due to the fact that it represents the approved method for assessing protein content claims in Canada (Health Canada., 1981).

2. Materials and methods

All procedures were approved by the University of Manitoba's Institutional Animal Care Committee, in accordance with guidelines established by the Canadian Council on Animal Care (CCAC, 2017).

2.1. Chemicals

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

2.2. Sample Procurement and preparation of extruded baked and cooked flours

Samples of red and green lentils were provided by SaskCan Pulse Trading (Regina, Saskatchewan), Thompsons Ltd. (Blenheim, Ontario) with an additional sample of green lentils provided by Diefenbaker Seed Processors (Elbow, Saskatchewan). Prior to processing samples of similar lentils 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 inch (0.127 cm), 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 with a screw diameter of 25 mm L/D ratio of 40. The flours were extruded at 36 kg/hr with a moisture addition of 0.8 kg/hr. 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 kg each of red and green lentil flours were mixed for 4 min with 2 kg water, respectively. Specifically, the mixer (Hobart mixer, model D300DT) was set up with a dough hook attachment. After the water was incorporated, the dough was mixed at a set speed #1 for 1.5 min followed by speed #2 for 2.5 min. In the absence of suitable forming equipment, the dough was extruded into rod-like pieces (Biro, model 6642, attached with a 12.5 mm die, and two blades). Approximately 1.5–1.6 kg of the extruded pieces (≈12 mm diameter) were transferred to standard baking trays (18 × 26 × ½ inches) lined with parchment paper, and rested for approximately 30 min. A tray was placed in the preheated oven (Doyon® FC2-lll tunnel conveyor oven) at 380 °F, 380 °F and 330 °F to establish the bake time. The trays with the cut pieces were baked at set temperatures for 35 min. After the baked pieces cooled to room temperature, they were weighted to calculate loss during baking. The baked samples were milled on a hammer mill (Fitz mill – model #D comminutor VHP-506-55B), with screen hole size of 0.020 inch, round, with 24% opening. All samples were further screened through a 20 mesh screen on a sifter (Kason, Vibro Screen, K24 3 SS).

In the cooking process, lentils were soaked in tap water at a ratio of 1:4 (1.5 kg pulse:6 L water) for 16 h with the water being changed prior to cooking. The lentil/water mixture was brought to a boil and maintained until done, approximately 25–35 min. After cooking, green lentils were rinsed with 4:1 ratio of rinse water (2 × 3 L water aliquots) to halt 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 inch, round.

2.3. 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 (AOAC, 1995). 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, excepting tryptophan were determined using AOAC Official Method 982.30 (AOAC, 1995). Tryptophan content was determined as previously described (Nosworthy, Franczyk et al., 2017)

2.4. Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

A rat bioassay was used to determine the PDCAAS of the samples (FAO/WHO, 1991). Amino acid scores were determined according to FAO/WHO guidelines. True protein digestibility was determined using the AOAC Official Method 991.29 (AOAC, 1995), using casein as a reference standard, and correcting for endogenous protein losses using previously determined values. Male weanling laboratory rats (n = 70, 10 animals per treatment, 6 experimental diets and casein as a control; 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 (House, Neufeld, & Leson, 2010). True protein digestibility (TPD%) was calculated using the following equation:

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

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 %.

2.5. Digestible Indispensable Amino Acid Score (DIAAS)

DIAAS was calculated as recommended by the FAO/WHO using the following equation:

DIAAS% = 100 × [(mg of digestible dietary indispensable amino acid in 1 g of the dietary protein)/(mg of the same dietary indispensable amino acid in 1 g of the reference protein)] (FAO/WHO, 2013). Ideally, the ileal amino acid digestibility should be used for the calculation of PDCAAS, however the use of fecal digestibility is considered acceptable until such time as a dataset of true ileal digestibility is developed (FAO/WHO, 2013).

2.6. 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 (Nosworthy, Franczyk et al., 2017). Briefly, 62.5 mg of protein was incubated at 37 °C for ten minutes with a protease cocktail containing trypsin, chymotrypsin and protease in duplicate. The resulting pH drop was recorded for 10 min and the *in vitro* protein digestibility was calculated as follows (where the $\Delta pH_{10\ min}$ is the change in pH in 10 min from the initial pH of approximately 8.0)

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

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

2.7. Protein Efficiency Ratio (PER)

As mandated by Health Canada, PER is determined over a 28 day growth period for rats consuming feed *ad libitum* (Health Canada, 1981). 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 (analyzed concurrently).

2.8. Statistics

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

3. Results and discussion

3.1. Proximate analysis

The dry matter, crude fat and crude protein for untreated and processed lentil flours are found in Table 1 on an 'as is' basis. Untreated red and green lentils had similar dry matter percentage (92.12% vs 91.38%) which was representative of previous work (Canadian Nutrient File, 2016). Processed red and green lentil flours also showed similar dry matter with the extrudates (95.41% vs 95.13%), cooked samples (99.57% vs 99.47%) and baked samples (97.49% vs 97.32%) being separated by less than 0.3%. The higher percentage of dry matter of the cooked samples was a result of an additional freeze drying operation performed post-boiling. Untreated red lentils had higher fat content than green lentils (1.78% vs 1.13%) and both were greater than the previously reported value of approximately 1% (Canadian Nutrient File, 2016; Wang & Daun, 2006). The protein content of the untreated red and green lentils was 25.13% and 23.93% respectively and was similar to previously reported results of 25–26% (Canadian Nutrient File, 2016; Wang et al., 2009). Processing increased protein content for both red and green lentils with a range of 24.65% for extruded green lentil to 26.86% for extruded red lentils, however in all cases the increase in protein content was less than 2%. These results indicate that processing via extrusion, baking or cooking did not dramatically alter the protein content of these samples.

3.2. Amino acid scores and true protein digestibility

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 score for both red and green lentils was the sulfur amino acids, methionine and cysteine, with values ranging from 0.57, baked green lentil, to 0.68, extruded red lentil. Tryptophan was also limiting in the processed lentil samples with a range of 0.69, extruded red lentil, to 0.78, extruded green lentils. Lentils have been previously described as limiting in sulfur amino acids and tryptophan (Nosworthy, Neufeld et al., 2017; Sarwar & Peace, 1986). After processing, both lentil varieties had the highest amino acid score after extrusion (0.68 and 0.66 for red and green lentils respectively), followed by cooking (0.63 and 0.61) and then baking (0.66 and 0.57). Another study investigating the effect of cooking on amino acid composition determined an amino acid score of 0.59 for cooked red lentils and 0.71 for cooked green lentils (Nosworthy, Neufeld et al., 2017). While these values differ from the current study (0.59 vs 0.63 and 0.71 vs 0.61 for red and green lentils respectively) this may be due to the use of different varieties or crop year as those conditions can alter amino acid composition.

The *in vivo* and *in vitro* protein digestibility values are found in Table 3. The true protein digestibility values of processed red lentil flour was comparable between extruded (92.38%), cooked (90.95%) and baked (88.80%), with processed green lentil flour having a similar relationship (extruded 86.02%, cooked 86.42%, baked 83.05%). Overall, green lentil flour had lower digestibility compared to red lentil flour. These results are similar to previous studies where cooked lentils had true protein digestibilities of 90.60% for red lentils and 87.89% for green lentils compared to this study which found 90.95% and 86.42% respectively (Nosworthy,

Table 1
Proximate analysis and amino acid composition of untreated, extruded, cooked and baked red and green lentil flour presented on an as-is basis.

	%DM[a]	%CF[c]	%CP[b]	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	TRP
Casein	93.56	0.20	86.48	7.78	3.35	5.64	20.05	9.77	1.35	3.16	0.78	5.02	1.45	3.84	8.39	4.83	4.59	2.74	6.96	3.12	1.08
Red Lentil																					
Untreated	92.12	1.78	25.13	2.69	0.78	1.21	3.55	0.60	0.80	0.98	0.22	0.97	0.206	0.77	1.67	0.63	1.09	0.60	1.43	1.88	0.20
Extruded	95.41	1.08	26.86	3.30	0.96	1.47	4.38	0.79	0.97	1.25	0.24	1.18	0.22	0.96	1.88	0.68	1.33	0.79	1.81	2.01	0.20
Cooked	99.57	1.62	26.62	3.31	0.96	1.56	4.41	0.91	0.85	1.23	0.22	1.21	0.21	1.03	2.19	0.71	1.43	0.77	1.83	2.28	0.22
Baked	97.49	2.34	25.93	3.25	1.00	1.54	4.47	0.96	1.00	1.29	0.20	1.13	0.19	1.00	2.05	0.67	1.26	0.80	1.57	2.30	0.20
Green Lentil																					
Untreated	91.38	1.13	23.93	2.81	0.83	1.31	3.86	0.90	0.87	1.15	0.20	1.02	0.19	0.89	1.75	0.67	1.17	0.66	1.61	2.22	0.18
Extruded	95.13	1.48	24.65	3.05	0.89	1.36	4.02	0.73	0.91	1.20	0.21	0.99	0.20	1.02	1.93	0.64	1.24	0.73	1.71	2.14	0.21
Cooked	99.47	2.06	25.67	3.07	0.91	1.45	4.00	0.82	0.90	1.20	0.20	1.19	0.20	1.00	2.05	0.67	1.32	0.71	1.76	2.11	0.21
Baked	97.32	2.21	25.44	2.53	0.79	1.24	3.67	0.53	0.88	1.14	0.18	1.13	0.18	0.89	1.69	0.57	1.06	0.67	1.37	1.88	0.20

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

Table 2
Amino Acid Score of extruded, cooked and baked red and green lentil flour.

	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
Red Lentil									
Extruded	1.05	1.25	**0.68**	1.28	1.06	1.19	1.55	1.16	0.69
Cooked	1.06	1.30	**0.63**	1.38	1.25	1.28	1.53	1.19	0.75
Baked	1.14	1.25	**0.61**	1.37	1.20	1.18	1.63	1.04	0.71
Green Lentil									
Extruded	1.06	1.14	**0.66**	1.48	1.19	1.21	1.55	1.20	0.78
Cooked	1.05	1.33	**0.61**	1.39	1.21	1.23	1.46	1.18	0.74
Baked	0.92	1.27	**0.57**	1.25	1.00	1.02	1.38	0.93	0.72

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.

Neufeld et al., 2017). Autoclaved pulse flour, which has been used as a surrogate for a heat exposed baking process, found lentil digestibility to be between 81.4–85% while this study determined the true protein digestibility of baked flours to be 88.80% for red lentils and 83.05% for green lentils (FAO/WHO, 1991; Porres et al., 2002). Similar results were determined using an *in vitro* method for determination of protein digestibility of red lentil ranging from 85.03% for baked flours to 88.01% for extruded flour and 79.33% in baked green lentils to 84.30% for extruded flour. While the overall pattern is similar between the *in vivo* and *in vitro* measurements, it is worth noting that in all cases the *in vitro* measurement of protein digestibility was lower than that determined by the standard *in vivo* rodent bioassay.Table 4

3.3. *In vivo and in vitro protein digestibility corrected amino acid scores*

The protein digestibility corrected amino acid score (PDCAAS) is presented in Table 3. This measurement of protein quality is influenced by both the amino acid score and protein digestibility (FAO/WHO, 1991). The PDCAAS values ranged from a low of 47.14% (baked green lentils) to 63.01% (extruded red lentils). In both classes, extruded flour had the highest PDCAAS (63.01% for red lentils, 57.09% for green lentils) followed by cooked (57.40% red, 53.93% green) and baked (53.84% red, 47.14% green). In all cases, processed red lentil flour had higher PDCAAS values due to the combination of a higher amino acid score and greater protein digestibility. Previously, the PDCAAS of cooked red lentils has been found to be 53.8% while green lentils were 62.8% (Nosworthy, Neufeld et al., 2017). A study investigating the effect of autoclaving on the PDCAAS of lentils determined a value of 66.4%, primarily due to a higher amino acid score (0.82) compared to that found in this study (average of 0.59 between red and green lentils) (Porres et al., 2002). In this study the *In Vitro* PDCAAS values were consistently lower than PDCAAS with a range of 1.14% (extruded green lentil) to 3.97% (cooked red lentil), however the general pattern of extruded flours having the highest value, and baked flours having the lowest, was maintained.

The current requirements for protein quality determination mandate the use of an *in vivo* bioassay, but a reduction in animal utilization for regulatory purposes is desirable (FAO/WHO, 1991). In order to determine whether an *in vitro* method of protein digestibility would relate to that found *in vivo*, a one-step pH drop method was utilized (Hsu, Vavak, & Satterlee, 1977; Tinus, Damour, Van Riel, & Sopade, 2012; Nosworthy, Neufeld et al., 2017). The relationship between *in vivo* and *in vitro* protein digestibility and measurement of protein quality was determined via correlation analysis and is presented in Fig. 1. A strong correlation was found between *in vitro* protein digestibility and true protein digestibility ($R^2 = 0.8934$). As both methods are using the same protein ingredient, the amino acid score remains the same whether calculating *In Vitro* PDCAAS or PDCAAS, the correlation between these values is stronger than digestibility alone ($R^2 = 0.9971$ for PDCAAS vs *In Vitro* PDCAAS; vs $R^2 = 0.8934$ for IVPD vs TPD). When testing the possibility of whether casein is skewing the correlation analysis, the correlation remains significantly high ($R = 0.9631$) if that data point is removed. These results are similar to previous comparisons between *in vivo* and *in vitro* methods of determining protein quality with $R^2 = 0.9898$ and $R^2 = 0.9280$ (Nosworthy & House, 2017; Nosworthy, Neufeld et al., 2017). Another study compared multiple methods for determining the *in vitro* PDCAAS of chickpea protein fractions to *in* vivo data and found similar results for the method used in the present study ($R^2 = 0.9442$) (Tavano, Neves, & da Silva Junior, 2016). These strong correlations suggest that an *in vitro* method for determining protein quality (*In Vitro* PDCAAS) could be used as a surrogate for *in vivo* evaluation of pulse protein ingredients but a larger dataset is required for further confirmation.

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 red and green lentil flour.

	Adj. Per	AAS[a]	%TPD[b]	IVPD[c]	PDCAAS[d]	*In Vitro* PDCAAS[e]
Casein	2.50	1.03	96.11 (1.4)	91.36 (0.7)	99.09	94.19
Red Lentil						
Extruded	1.05	0.68	92.38 (2.3)	88.01 (2.8)	63.01	60.03
Cooked	1.14	0.63	90.95 (2.2)	84.67 (1.1)	57.40	53.43
Baked	0.79	0.61	88.80 (2.4)	85.03 (0.5)	53.84	51.55
Green Lentil						
Extruded	1.08	0.66	86.02 (2.0)	84.30 (0.0)	57.09	55.95
Cooked	0.98	0.61	86.42 (2.0)	84.03 (1.2)	52.92	51.46
Baked	0.88	0.57	83.05 (1.9)	79.33 (1.0)	47.14	45.03

[a] AAS = amino acid score.
[b] %TPD = % true protein digestibility.
[c] IVPD = *in vitro* protein digestibility.
[d] PDCAAS = protein digestibility corrected amino acid score.
[e] *In Vitro* PDCAAS = *in vitro* protein digestibility corrected amino acid score. n = 10 for Adj. PER and %TPD; n = 2 for IVPD and n = 1 for AAS, PDCAAS, *In Vitro* PDCAAS. Numbers in parentheses indicate SD where applicable. PDCAAS is calculated as the product of AAS and %TPD while *In Vitro* PDCAAS is the product of AAS and IVPD.

Table 4
Digestible Indispensable Amino Acid values of extruded, cooked and baked red and green lentil 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
Red Lentil										
Extruded	1.06	0.94	**0.58**	1.03	0.98	1.33	1.36	1.09	0.83	0.58
Cooked	1.06	0.96	**0.53**	1.10	1.14	1.41	1.32	1.10	0.88	0.53
Baked	1.11	0.90	**0.50**	1.07	1.06	1.27	1.38	0.94	0.82	0.50
Green Lentil										
Extruded	1.00	0.80	**0.53**	1.11	1.02	1.26	1.27	1.05	0.87	0.53
Cooked	0.99	0.93	**0.49**	1.05	1.04	1.29	1.20	1.04	0.82	0.49
Baked	0.84	0.86	**0.44**	0.91	0.83	1.03	1.09	0.79	0.77	0.44

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

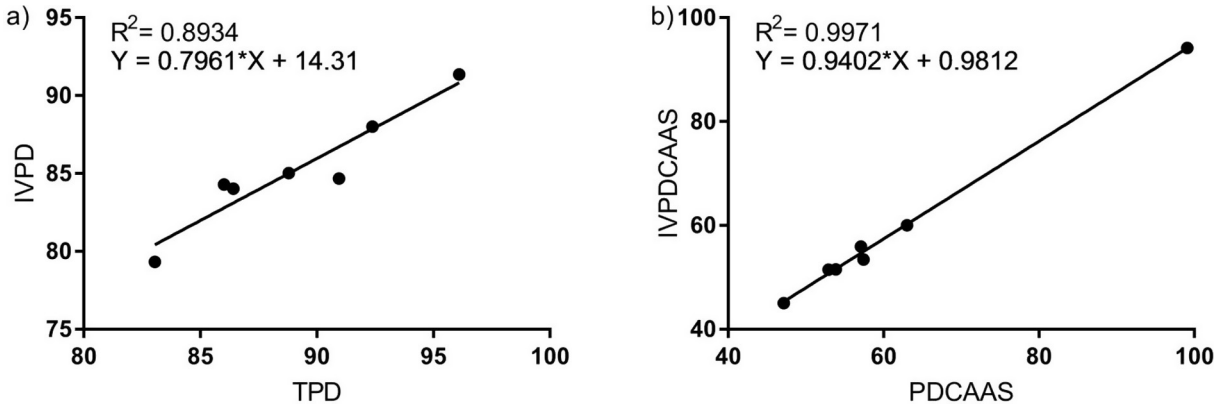

Fig. 1. Relationship between the digestibility, extruded, cooked and baked red and green lentil flours determined by *in vitro* and *in vivo* methods (a) and the relationship between the protein digestibility-corrected amino acid scores calculated using *in vitro* and *in vivo* digestibilities (b). TPD = true protein digestibility, IVPD = *in vitro* protein digestibility, PDCAAS = protein digestibility corrected amino acid score, IVPDCAAS = *in vitro* protein digestibility corrected amino acid score.

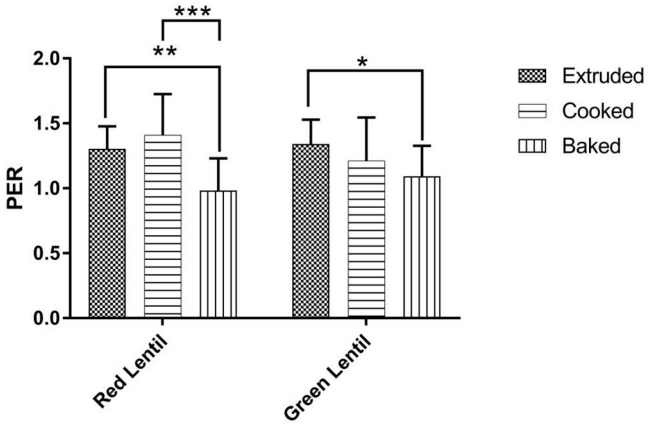

Fig. 2. Protein Efficiency Ratio (PER) values of extruded, cooked and baked red and green lentil flour. Hatched bars indicate baked flour, horizontal bars are cooked flour and vertical bars are extruded flour. Mean ± SD (n = 10). Data were analyzed via Two-Way ANOVA with Tukey's post hoc test. * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

3.4. Digestible Indispensable Amino Acid Score

In 2013 the FAO/WHO presented a new method for determining protein quality, the digestible indispensable amino acid score (DIAAS) (FAO/WHO, 2013). This method was designed to be a replacement for PDCAAS as it utilized ileal digestibility rather than fecal digestibility, individual amino acids were considered nutrients rather than protein and a different amino acid requirement pattern was constructed. As there is little data currently available on the ileal digestibility of amino acids, the FAO/WHO advised that fecal digestibility could be used in the calculation of DIAAS, which was done in this study. The DIAAS values for processed lentil flour ranged from 0.44 for baked green lentil to 0.58 for extruded red lentil. The pattern of DIAAS values were similar to those found in PDCAAS and *In Vitro* PDCAAS with extrusion having the highest value (0.58 for red lentil, 0.53 for green), followed by cooking (0.53 for red, 0.49 for green) and baking (0.50 for red, 0.44 for green). Although there is little DIAAS data currently available, one study found a value of 0.50 for cooked red lentils and 0.58 for cooked green lentils (Nosworthy, Franczyk et al., 2017). In all cases DIAAS values were lower than either PDCAAS or *In Vitro* PDCAAS. As the same amino acid profile and protein digestibility were used in the calculation of DIAAS and PDCAAS, this difference occurs due to the change in the amino acid reference pattern, particularly the sulfur amino acids. In calculating PDCAAS the requirement for sulfur amino acids is 25 mg/g protein while the DIAAS requirement is 27 mg/g protein (FAO/WHO, 1991, 2013). This increase in requirement reduced the amino acid value for DIAAS thereby causing the lower value compared to PDCAAS. Although it is the current recommendation that PDCAAS be replaced with DIAAS it is important to consider the impact of this shift on regulatory decisions. A recent review discussed the potential impacts of a shift to DIAAS on regulatory frameworks and came to the conclusion that using the suggested model, ileal-cannulated pigs, is not practical and the focus should rather be on using fixed estimates of ileal digestibility (similar to those currently in place for folate) or using *in vitro* analyses for determination of protein digestibility rather than *in vivo* (Marinangeli & House, 2017). A comparison between PDCAAS, *in vitro* PDCAAS and DIAAS illustrates the relative relationship between these measurements of protein quality. In all cases PDCAAS has the highest value, DIAAS the lowest and *in vitro* PDCAAS being intermediate. This indicates that, for this sample set, *in vitro* analysis of protein quality provides an average value between both PDCAAS and DIAAS.

3.5. Protein Efficiency Ratio

The protein efficiency ratio (PER) is the measurement of protein quality that is mandated by Health Canada in order to regulate protein content claims (Health Canada, 1981). This measurement is different

than PDCAAS, *In Vitro* PDCAAS or DIAAS in that it is not a measure of either amino acid composition or digestibility, rather it is a measurement of growth, particularly weight gain per unit protein consumed over 28 days. The PER data is presented in Fig. 2. The PER of baked red lentils was 0.98, significantly lower than that of either cooked, 1.41, or extruded, 1.31, red lentil flour. While baked green lentil flour had a lower PER than extruded (1.1 vs 1.3 respectively) there was no difference between cooked and other methods of preparation. As a method of standardization, PER values undergo adjustment relative to the PER of casein; this normalization of PER to a casein value of 2.5 accounts for inter-laboratory and inter-run variation. These values are presented in Table 3. These data indicate that while extrusion increased weight gain compared to baked flours in either lentil class, cooked and freeze dried samples induced greater weight gain in red lentils alone.

4. Conclusions

This study demonstrated that the choice of processing method of red and green lentils directly impacts the overall protein quality of the final product. In the present study red lentils had the highest PER after cooking while green lentils had higher PER after extrusion, indicating that processing can alter PER in a variety specific manner. For both lentils baking had the lowest PER value. We also determined that processing can alter the protein digestibility and amino acid composition of both red and green lentils thereby altering both PDCAAS and DIAAS scores. When comparing processing methods, extrusion resulted in the highest PDCAAS and DIAAS scores for both red and green lentils with baking having the lowest. From these data it can be advised that from a protein quality perspective, extrusion would be the most beneficial method of commercial production of lentil products and for home preparation cooking is more advantageous than baking. When comparing *in vivo* and *in vitro* methods of determining protein quality, a good correlation was found between *In Vitro* PDCAAS and PDCAAS. These results indicate the effect of processing on lentil protein quality and suggest that *in vitro* methods of protein digestibility analysis should be considered as a potential replacement for currently recommended *in vivo* rodent bioassays.

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