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ORIGINAL RESEARCH

Effects of extrusion cooking on the nutritional quality of puffed snacks made from blends of barley and green lentil flours

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Abstract
Increasing demand for nutritionally dense foods warrants the investigation of high fiber and protein ingredients in snack food applications. In this study, blends of barley (22.9% dietary fiber, db) and green lentil (26.4% protein, db) flours were extruded at five blending ratios (barley: green lentil, 100: 0, 75: 25, 60: 40, 45: 55, 0: 100, db), two barrel temperature profiles (60–130 °C and 70–140 °C from feeder to die) and three feed moisture contents (15, 18 and 21%) to produce puffed snacks. Extrusion significantly improved in vitro protein digestibility (IVPD) of all blends by up to 10%. Decreasing feed moisture and increasing die temperature improved IVPD. Blending increased the limiting amino acid score and hence improved the in vitro protein digestibility corrected amino acid score (IVPDCAAS) of extrudates. On average, the blend 45:55 showed the highest average IVPDCAAS (68.62%) among the blends studied and lower glycemic index scores compared to the blend 60: 40. In general, extrusion did not substantially affect the soluble, insoluble or total dietary fiber contents of the blends. All extrudates from blends 60: 40 and 45:55 met the requirement to be labelled as "good source of dietary fiber" in the US.

Keywords Extruded snacks · Protein · In vitro protein digestibility · Amino acid score · Dietary fiber

Introduction

Puffed snacks currently on the market are predominately made from refined cereal flours (e.g., corn and wheat), and they are generally rich in highly digestible carbohydrates and saturated fats, and low in proteins and dietary fibers [1], making them calorie-dense but nutritionally inferior. It is argued that frequent consumption of said snacks might contribute to health problems such as obesity [1]. These puffed snacks cannot meet consumers' increasing demand for healthy foods. Using innovative ingredients [2], such as those rich in proteins and dietary fibers, which refined cereal flours are short of, can address these concerns by improving product nutritional quality.

Among different plants, the outstanding protein content, especially when compared to cereals (e.g., 10–15% for barley), is one of the major advantages of pulses (e.g., 26–31% for lentil) [3, 4]. In addition to the protein quantity, the amino acid compositions of pulse and cereal proteins are also different. Pulses are rich in lysine and limited in sulfur-containing amino acids (i.e., methionine and cysteine), while cereals are generally the opposite. Thus, when blended together, pulses and cereals can complement each other's amino acid composition [5, 6].

Blending whole pulse and cereal flours to replace refined cereal flours in snack food formula can also enhance the content of dietary fibers. Both pulses (e.g., 9–17% for lentil) and cereals (e.g., 11–20% for barley) are major sources of dietary fibers [4, 5, 7, 8]. Pulses, including lentil, have a relatively higher ratio of insoluble to soluble fiber [7], while barley has a relatively lower one [9, 10]. Given the cholesterol and glycemic control benefits of high viscosity soluble fibers (e.g., gel forming beta-glucans), and the regularity/laxative benefits of insoluble fibers [11], formulating new food products that are not only high in dietary fiber but also balanced in soluble and insoluble fiber contents is sensible. Moreover, this approach can potentially offset the relatively higher negative impact of insoluble fiber on overall expansion of pulse-based extruded snacks [10]. Given these advantages, the potential of using cereal and pulse flour blends to produce puffed snacks has seen some interest, while most of

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these studies focusing on extrudate textural and structural properties [12–14]. The nutritional quality of these products has not been as widely studied, especially with the perspective of exploring possible commercial nutrition claims.

The objectives of this study were firstly to assess the effect of extrusion cooking (at three feed moisture content levels and two barrel temperature profiles) on protein (i.e., protein content, amino acid composition, in vitro protein digestibility, in vitro protein digestibility corrected amino acid score) and dietary fiber (i.e., insoluble, soluble and total dietary fiber content, and estimated glycemic index) properties of puffed snacks made from blends of barley and green lentil flours, and secondly to assess the nutritional quality of these extruded snacks for prospective protein and dietary fiber content claims in the US and Canada. This study is a great example of exploring the potential of using the blends of cereal and pulse flours as new fiber- and protein- rich ingredients to replace refined cereal flours in puffed snacks.

Materials and methods

Materials

Hull-less *CD Ratan* barley flour was provided by Against the Grain Farms (Mountain, ON, Canada) and milled by Ottawa Valley Grain Products (Carp, ON, Canada). Dehulled *CDC Richland* green lentil flour was provided by AGT Food and Ingredients (Regina, SK, Canada).

Proximate composition of materials

Crude protein (N×6.25) and moisture contents of barley and green lentil flours were determined in duplicate using a micro-Kjeldahl unit following AACC method 46-13 and 44-19.01, respectively [15]. Crude fat and crude ash contents were measured in duplicate following Min and Ellefson [16] and Marshall [17], respectively. Carbohydrate contents were calculated by subtracting protein, moisture, fat and ash contents from 100%.

Insoluble and soluble dietary fiber contents were measured in duplicate following AACC method 32-07.01 [15] with slight modifications. Sample size was reduced to 0.5 g and the protease digestion time was extended to 1 h. Enzymes were purchased from Megazyme (Total Dietary Fiber test kit, Ireland). Protein and ash contents were subtracted from both insoluble and soluble dietary fiber residues. Total dietary fiber content was calculated as the sum of insoluble and soluble dietary fiber content. Averages and standard deviations were calculated based on the two replications in duplicate dietary fiber measurements ($n = 2 \times 2 = 4$).

Amino acid composition

Methionine and cysteine contents were determined using AOAC method 994.12 [18]. Tryptophan content was determined following ISO method 13904 [19]. Other amino acids were measured using AOAC method 982.30 [20]. All amino acids were calculated from free amino acids.

In vitro protein digestibility

In vitro protein digestibility (IVPD) was determined in triplicate using a pH drop method following Nosworthy et al. [21]. Briefly, the equivalent of 62.5 mg of protein of each flour or extrudate was added to 10 ml Milli-Q water, heated to 37 °C in a beaker-made water bath and adjusted to pH 8.0. Then 1 mL enzyme solution containing trypsin (from porcine pancreas Type IX-S, lyophilized powder, 13,000–20,000 BAEE units/mg protein), alpha-chymotrypsin (from bovine pancreas, Type II, lyophilized powder, P40 units/mg protein) and peptidase (from *Streptomyces griseus* Type XIV, P3.5 units/mg solids) was added to the sample solution and incubated at 37 °C. The pH drop of the initial 10 min of incubation (ΔpH_{10min}) was recorded to calculate IVPD using Eq. (1):

$$IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH_{10min} \quad (1)$$

65.66 and 18.10 are the intercept and slope of the regression line, respectively [39]. Casein (80 mesh, Dyets Inc., Bethlehem, PA, US) was also tested as the control.

Amino acids score and in vitro protein digestibility corrected amino acid score

Following FAO/WHO [22], the amino acid score (AAS) of flours and extrudates was calculated as mg of an amino acid in 1.0 g of test protein per mg of the same amino acid in 1.0g of preschool children (2−5 years) reference pattern. In vitro protein digestibility corrected amino acid score (IVPDCAAS, %) of flours and extrudates were calculated according to Eq. (2) :

$$IVPDCAAS = \text{the lowest amino acid score} \times IVPD. \quad (2)$$

The pre-school children (2–5 years) reference pattern includes the requirements of 9 individual amino acids or groups of amino acids, which are as follows: histidine, 19 (mg/g protein); isoleucine, 28; leucine 66; lysine, 58; methionine + cysteine, 25; phenylalanine + tyrosine, 63; threonine, 34; tryptophan, 11; and valine, 35. The lowest amino acid score is termed as the limiting amino acid score

(LAAS) and the amino acid with the LAAS is referred as the limiting amino acid (LAA).

Identifying the flour blending ratios for extrusion

Flour blending ratios for extrusion were identified based on the amino acid composition and protein quality of flours. Firstly, protein content, amino acid composition and IVPD of barley and green lentil flours were determined. Then, using the data for barley and green lentil flours, the corresponding protein content, amino acid composition, IVPD, AAS and IVPDCAAS values for several different flour blending ratios (for example, 80:20, 75:25, 70:30, 60:40, 50:50, 45:55, 40:60, 35:65, 30:70, 25:75, 20:80, barley: green lentil, db) were calculated. Among the proposed blends, the blend of 60:40 barley: green lentil showed the most balanced AAS (i.e., the highest LAAS) and the highest IVPDCAAS, indicating the best theoretical amino acid composition and protein quality, and was therefore selected for extrusion. In addition to the 60:40 (60Bly40GL) blending ratio, 75:25 (75Bly25GL) and 45:55 (45Bly55GL) ratios were also selected for extrusion acceptable amino acid composition, protein quality and measurable differences between blends are ensured.

Extrusion cooking

Extrusion cooking was performed using a twin-screw extruder (MPF19, APV Baker Ltd., Peterborough, UK), with a constant feed rate of 2.0 kg/h, screw speed of 250 rpm and a die orifice diameter of 5.5 mm. Three feed moisture (FM) levels (15, 18 and 21% on flour weight basis) and two barrel temperature profiles (60/80/100/120/130 °C and 70/90/110/130/140 °C from the feeder to the die exit) were used. The lower and higher temperature profiles are referred to as die temperatures (DT) 130 °C and 140 °C, respectively, throughout the rest of this manuscript. All blends were extruded in duplicate for each extrusion treatment.

Extrudates were collected, cooled down to room temperature and then dried in an air oven (Heratherm OGS100, Thermo Scientific, Langeselbold, Germany) at 40 °C for 15 h. Dried extrudates were milled (ZM 200 Ultra Centrifugal Mill, Retsch, Haan, USA) to pass through a 250 micron sieve (Ring sieve, Retsch, USA). A mixed sample was then created by blending ground extrudates produced from two extrusion runs equally by weight and stored at −40 °C for all tests performed in this study.

Estimated glycemic index

The rate of in vitro starch hydrolysis of the extrudates was determined using the method described by Englyst et al. [23]. Briefly, 100 mg ground extrudate was hydrolyzed using the digestive enzymes pepsin (Sigma, P7000), pancreatin (Sigma-Aldrich, P7545) and amyloglucosidase (3300 U/mL, Megazyme Int., Ireland) at 37 °C. Hydrolyzed starch content was measured at 90 min and total starch hydrolysis (HI, %) was calculated. The hydrolysis index (HI) shows the starch digestion rate while the estimated glycemic index (GI) indicates the digestibility of the extrudate against white bread (GI: 100) The estimated GI value was calculated using Eq. (3) given by Goñi et al. [24].

$$GI = 9.71 + (0.549 \times HI) \quad (3)$$

Statistical analysis

IVPD, IVPDCAAS, IDF, SDF, TDF and GI data was analyzed by SAS software using one-way ANOVA along with Tukey's test. Significant differences were considered at $P < 0.05$.

Results and discussion

Proximate composition of materials

The barley flour had relatively lower protein and ash, but higher fat and total carbohydrate contents compared to the green lentil flour (Table 1). The proximate compositions of barley and green lentil flours were similar to those reported in the literature [7, 9, 25, 26], and the IDF to SDF ratio of barely (1.5) was substantially lower than that of green lentil (8.2), in line with the literature [9, 10].

Protein content and amino acid composition

Flour blends with lower barley to green lentil ratio had higher protein contents following the corresponding protein contents of barley and green lentil flours (Table 2). Protein

Table 1 Proximate compositions of barley and green lentil flours

Composition (% db)	Barley	Green lentil
Crude protein	14.91 ± 0.02	26.42 ± 0.12
Crude fat	3.35 ± 0.25	1.80 ± 0.19
Crude ash	1.95 ± 0.01	2.61 ± 0.01
Carbohydrate	80.39 ± 0.25	69.52 ± 0.30
Total dietary fiber	22.9 ± 1.7	10.1 ± 1.8
Insoluble dietary fiber	13.6 ± 1.5	9.0 ± 1.6
Soluble dietary fiber	9.3 ± 0.7	1.1 ± 0.2

Data is expressed as the average ± standard deviation (n = 4 for dietary fiber tests, n = 2 for other tests)

Table 2 Protein content (%) and amino acid composition (%) of blended flours and extrudates

Blends	DT (°C)	FM (%)	Protein	His	Ser	Arg	Gly	Asp	Glu	Thr	Ala	Pro	Cys	Lys	Tyr	Met	Val	Ile	Leu	Phe	Trp
Bly	*Before extrusion*		14.91 ± 0.02	0.31	0.77	0.69	0.60	0.95	3.84	0.52	0.56	1.82	0.34	0.49	0.53	0.24	0.71	0.54	1.02	0.82	0.17
	130	15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		18	14.74 ± 0.03	0.27	0.66	0.65	0.57	0.95	3.89	0.48	0.58	1.85	0.30	0.51	0.41	0.22	0.76	0.54	1.07	0.84	0.18
		21	14.84 ± 0.02	0.29	0.69	0.71	0.56	1.02	3.94	0.50	0.57	1.89	0.29	0.51	0.44	0.23	0.75	0.55	1.05	0.84	0.18
	140	15	14.66 ± 0.07	0.28	0.68	0.70	0.56	1.08	3.97	0.49	0.57	1.85	0.30	0.52	0.48	0.24	0.77	0.55	1.08	0.84	0.18
		18	14.82 ± 0.40	0.29	0.68	0.69	0.58	1.03	3.97	0.49	0.57	1.87	0.31	0.53	0.47	0.23	0.77	0.55	1.06	0.84	0.18
		21	14.94 ± 0.01	0.28	0.66	0.70	0.56	0.93	3.82	0.49	0.54	1.85	0.30	0.52	0.48	0.24	0.77	0.56	1.05	0.84	0.18
75Bly25GL	*Before extrusion*		17.94 ± 0.13	0.46	0.85	1.11	0.69	1.54	4.10	0.62	0.69	1.69	0.29	0.87	0.58	0.24	0.93	0.72	1.32	0.98	0.19
	130	15	17.90 ± 0.17	0.48	0.86	1.06	0.71	1.60	4.18	0.62	0.71	1.71	0.27	0.87	0.51	0.22	0.93	0.72	1.34	1.00	0.19
		18	17.94 ± 0.19	0.45	0.83	1.12	0.68	1.61	4.02	0.61	0.68	1.68	0.26	0.86	0.55	0.21	0.93	0.73	1.30	0.99	0.19
		21	18.10 ± 0.06	0.46	0.83	1.07	0.70	1.58	4.06	0.62	0.68	1.68	0.27	0.86	0.51	0.22	0.93	0.73	1.31	0.98	0.19
	140	15	17.72 ± 0.08	0.49	0.86	1.07	0.71	1.56	4.16	0.63	0.71	1.71	0.32	0.87	0.56	0.26	0.93	0.72	1.35	1.00	0.18
		18	17.98 ± 0.13	0.50	0.88	1.03	0.72	1.57	4.16	0.62	0.71	1.70	0.31	0.87	0.54	0.25	0.93	0.73	1.31	0.99	0.18
		21	17.89 ± 0.16	0.47	0.89	1.04	0.73	1.55	4.17	0.62	0.71	1.69	0.26	0.88	0.54	0.22	0.93	0.72	1.33	1.00	0.17
60Bly40GL	*Before extrusion*		19.83 ± 0.11	0.61	1.00	1.31	0.79	2.02	4.28	0.71	0.79	1.61	0.33	1.09	0.63	0.28	1.04	0.83	1.51	1.11	0.19
	130	15	19.99 ± 0.12	0.55	1.00	1.34	0.78	1.91	4.28	0.71	0.79	1.60	0.26	1.09	0.60	0.22	1.04	0.83	1.51	1.10	0.19
		18	19.80 ± 0.05	0.62	0.98	1.23	0.79	1.85	4.20	0.70	0.78	1.59	0.28	1.08	0.61	0.23	1.03	0.83	1.52	1.11	0.18
		21	20.01 ± 0.15	0.61	0.96	1.24	0.74	1.81	4.05	0.67	0.76	1.54	0.22	1.06	0.55	0.18	1.00	0.82	1.48	1.09	0.19
	140	15	19.86 ± 0.28	0.60	0.93	1.23	0.75	1.86	4.05	0.67	0.76	1.51	0.29	1.05	0.52	0.23	1.00	0.82	1.51	1.10	0.18
		18	20.12 ± 0.22	0.62	0.97	1.36	0.73	1.90	4.20	0.68	0.78	1.47	0.24	1.10	0.56	0.19	1.04	0.84	1.55	1.11	0.19
		21	20.01 ± 0.09	0.56	0.99	1.28	0.79	1.87	4.25	0.70	0.76	1.60	0.23	1.06	0.62	0.19	1.03	0.82	1.49	1.08	0.20
45Bly55GL	*Before extrusion*		21.46 ± 0.09	0.68	1.09	1.49	0.85	2.27	4.28	0.77	0.86	1.49	0.24	1.24	0.64	0.21	1.10	0.92	1.58	1.15	0.20
	130	15	21.68 ± 0.07	0.68	1.11	1.56	0.83	2.28	4.33	0.78	0.87	1.51	0.25	1.26	0.61	0.21	1.11	0.90	1.63	1.16	0.20
		18	21.44 ± 0.16	0.69	1.09	1.50	0.84	2.23	4.24	0.76	0.85	1.48	0.26	1.24	0.61	0.22	1.09	0.88	1.59	1.14	0.20
		21	21.42 ± 0.14	0.68	1.09	1.53	0.84	2.28	4.29	0.77	0.86	1.49	0.26	1.27	0.59	0.22	1.10	0.91	1.59	1.15	0.20
	140	15	21.23 ± 0.02	0.68	1.11	1.49	0.85	2.28	4.32	0.77	0.88	1.50	0.26	1.27	0.58	0.22	1.11	0.90	1.61	1.16	0.20
		18	21.38 ± 0.04	0.69	1.12	1.58	0.85	2.34	4.37	0.78	0.87	1.53	0.25	1.27	0.64	0.21	1.13	0.93	1.62	1.17	0.20
		21	21.28 ± 0.32	0.68	1.09	1.56	0.83	2.29	4.28	0.77	0.86	1.50	0.25	1.25	0.65	0.21	1.10	0.90	1.60	1.14	0.20
GL	*Before extrusion*		26.42 ± 0.12	0.94	1.46	2.12	1.07	3.07	4.27	1.01	1.05	1.13	0.29	1.72	0.90	0.21	1.20	1.14	1.89	1.35	0.20
	130	15	27.63 ± 0.37	1.05	1.49	2.27	1.11	3.41	4.72	1.05	1.15	1.24	0.22	1.92	0.72	0.21	1.45	1.26	2.14	1.47	0.22
		18	27.86 ± 0.26	1.05	1.47	2.34	1.08	3.41	4.69	1.03	1.14	1.22	0.21	1.92	0.80	0.19	1.43	1.23	2.12	1.45	0.22
		21	27.36 ± 0.02	1.00	1.42	2.20	1.11	3.12	4.50	1.01	1.08	1.20	0.21	1.85	0.79	0.20	1.39	1.21	2.07	1.41	0.21
	140	15	27.61 ± 0.01	1.04	1.51	2.23	1.12	3.44	4.76	1.05	1.16	1.22	0.22	1.94	0.74	0.21	1.44	1.24	2.13	1.46	0.22
		18	27.68 ± 0.10	1.02	1.46	2.30	1.08	3.29	4.58	1.02	1.13	1.21	0.22	1.86	0.76	0.21	1.40	1.22	2.10	1.43	0.22
		21	27.69 ± 0.23	1.03	1.48	2.25	1.10	3.42	4.64	1.04	1.16	1.23	0.22	1.92	0.76	0.20	1.42	1.25	2.12	1.46	0.22

DT die temperature, *FM* feed moisture, *Bly* barley, *GL* green lentil, *His* histidine, *Ser* serine, *Arg* arginine, *Gly* glycine, *Asp* aspartate, *Glu* glutamate, *Thr* threonine, *Ala* alanine, *Pro* proline, *Cys* cysteine, *Lys* lysine, *Tyr* tyrosine, *Met* methionine, *Val* valine, *Ile* isoleucine, *Leu* leucine, *Phe* phenylalanine and *Trp* tryptophan

All data is expressed on a dry basis and protein contents are expressed as the averages ± standard deviations (n = 2) contents of extrudates were similar to their respective flours. Protein contents and amino acid compositions of blended flours and their extrudates were presented in Table 2 as a function of DT and FM. Amino acid compositions of barley and green lentil were found to be complementary to each other, where barley protein is limited in lysine but relatively rich in sulfur-containing amino acids (cysteine and methionine), and green lentil protein generally showed the opposite trend, aligning with the findings of Asif et al. [5]. The lysine content of green lentil flour was 1.72%, which was more than three times as high as that of barley flour. On the other hand, cysteine and methionine contents in barley flour were 0.03–0.05% higher than those in green lentil flour. The amino acid composition of barley and green lentil flours agrees with those reported by Arendt and Zannini [9] and Nosworthy et al. [27].

The amounts of several amino acids (e.g., cysteine, tyrosine, arginine, etc.) decreased after extrusion, which could be due to high temperatures reached during extrusion cooking. Clemente et al. [28] had reported that heat can cause significant amino acid losses, especially for cysteine, methionine and tyrosine, which agrees with the results of this study. The increase in some amino acids (e.g., histidine, arginine, aspartate, etc.) after extrusion were mainly observed for extrudates of GL, which might be attributed to the inactivation of proteinaceous antinutritional factors (e.g., hydrolase inhibitors and lectins) in legumes, as proteinaceous antinutritional factors can release amino acids after heat denaturation [29]. Overall, Table 2 suggested that the amino acid compositions of blend 45Bly55GL were relatively more stable than those of other blends after extrusion, especially compared with Bly and GL. This higher stability might be because many amino acids of barley and green lentil reacted to extrusion cooking in opposing trends. For example, Bly extrudates lost 4–8% threonine after extrusion, while the threonine content of GL extrudates increased by up to 4%, so the higher stability in threonine content of blend 45Bly55GL was possibly a result of neutralization. FM and DT did not show substantial effects on amino acid composition in this study. This might be because the selected ranges of FM (15–21%) and DT (130 and 140 °C) were not wide enough to cover detectable differences. Sosa-Moguel et al. [30] also reported that increasing extrusion DT from 150 to 165 °C at 15% FM did not have significant effect on retention of many amino acids for extrusion of corn and cowpea blends.

In vitro protein digestibility

IVPD values of barley and green lentil flours were 72.90 and 76.28%, respectively, which agree with Bai et al. [31] and Han et al. [32]. IVPD values of 75Bly25GL, 60Bly40GL and 45Bly55GL flours were in the range of 72.36–73.68% and increased slightly with decreasing barley to green lentil ratio. After extrusion, IVPD values of extrudates increased up to 10%, except for higher FM treatments of Bly. This increase could be attributable to the applied heat, shear and pressure during extrusion, leading to changes in protein structure, possibly exposing originally buried sites to be more easily accessible for protease to attack [33]. In addition, extrusion cooking can destroy thermolabile antinutritional factors (e.g., trypsin and chymotrypsin inhibitors) and enhance IVPD [34]. IVPD values for extruded GL were similar to Nosworthy et al. [27] who studied slightly lower extrusion DT (120 °C) and FM (11%).

Decreasing FM increased IVPD values of extrudates (Table 3), which may be due to low FM increased the intensity of extrusion [35]. Increasing DT showed a positive relationship with IVPD for blends 75Bly25GL, 60Bly40GL and 45Bly55GL, aligning with the findings of Ghumman et al. [34]. IVPD values of extrudates of Bly and GL at 140 °C were slightly lower than those at 130 °C, indicating that higher processing temperature did not enhance IVPD. Dietary fibers were reported to adversely affect protein digestibility [36, 37]. This might explain why extrusion cooking had relatively limited beneficial effect on the IVPDs of Bly extrudates (the highest dietary fiber content) when compared to other extrudates. The reason why GL extrudates showed lower IVPD values at higher DT needs more investigation.

Amino acid score and in vitro protein digestibility corrected amino acid score

The AAS of blended flours and extrudates are presented in Table 3. The LAA of Bly and 75Bly25GL flours was lysine, while the LAA of 60Bly40GL, 45Bly55GL and GL flours was tryptophan. The AAS of blended flours followed the trend of blending (e.g., flours with lower barley to green lentil ratio had the higher AAS of lysine). Flour 60Bly40GL (0.86) showed the highest LAAS, followed closely by 75Bly25GL (0.84) and 45Bly55GL (0.84) flours, all of which were higher than Bly (0.56) and GL (0.69) flours. These results indicated that blending provided a more balanced amino acid profile. The LAA of extruded Bly, 75Bly25GL and 45Bly55GL remained the same as their flour blends. However, due to the significant loss of methionine and cysteine during extrusion at high FM (Table 2), the LAA of 60Bly40GL extrudates produced at 21% FM switched to methionine and cysteine. Similarly, due to the loss of cysteine, the LAA of extruded GL changed to methionine and cysteine. Extrudates of 60Bly40GL showed higher LAAS among the five extruded blends, and the one produced at 130 °C and 15% FM had the highest LAAS (0.87), indicating the most balanced amino acid composition using the reference pattern of pre-school children (2–5 years), as required by the FDA. The pre-school children pattern is used

Table 3 Essential amino acid score, in vitro protein digestibility (IVPD) and in vitro protein digestibility corrected amino acid score (IVPDCAAS) values of blended flours and their extrudates

Blends	DT (°C)	FM (%)	His	Ile	Leu	Lys	Met + Cys	Phe + Tyr	Thr	Trp	Val	IVPD (%)	IVPDCAAS (%)
Bly	*Before extrusion*		1.10	1.29	1.03	**0.56**	1.57	1.43	1.02	1.05	1.35	72.90 ± 0.48^{mn}	40.82 ± 0.27^{t}
	130	15	–	–	–	–	–	–	–	–	–	–	–
		18	0.97	1.31	1.10	**0.59**	1.40	1.35	0.96	1.13	1.47	78.45 ± 1.00^{ghi}	46.55 ± 0.59^{qr}
		21	1.03	1.33	1.07	**0.59**	1.40	1.37	0.98	1.08	1.45	74.53 ± 1.61^{lm}	43.99 ± 0.95^{s}
	140	15	1.00	1.33	1.11	**0.61**	1.47	1.43	0.98	1.11	1.49	77.18 ± 0.46^{ijk}	46.98 ± 0.28^{pqr}
		18	1.04	1.33	1.08	**0.62**	1.47	1.40	0.97	1.09	1.48	74.35 ± 0.65^{lmn}	46.23 ± 0.41^{r}
		21	0.98	1.33	1.07	**0.60**	1.45	1.40	0.96	1.11	1.47	72.96 ± 0.89^{mn}	43.58 ± 0.53^{s}
75Bly25GL	*Before extrusion*		1.36	1.43	1.12	**0.84**	1.17	1.38	1.02	0.94	1.48	72.36 ± 0.65^{n}	60.78 ± 0.55^{k}
	130	15	1.42	1.44	1.13	**0.84**	1.11	1.34	1.03	0.95	1.49	80.08 ± 0.84^{efg}	67.42 ± 0.70^{fgh}
		18	1.31	1.45	1.09	**0.83**	1.07	1.36	1.01	0.95	1.48	78.03 ± 0.55^{hij}	64.59 ± 0.46^{i}
		21	1.35	1.44	1.09	**0.82**	1.08	1.31	1.00	0.93	1.47	75.98 ± 0.48^{kl}	62.21 ± 0.39^{jk}
	140	15	1.46	1.44	1.15	**0.85**	1.30	1.40	1.04	0.93	1.51	81.17 ± 0.10^{cdef}	68.65 ± 0.09^{def}
		18	1.47	1.46	1.11	**0.84**	1.25	1.35	1.02	0.92	1.47	80.20 ± 0.42^{efg}	67.11 ± 0.35^{gh}
		21	1.37	1.44	1.13	**0.85**	1.07	1.36	1.03	0.89	1.49	78.57 ± 0.52^{ghi}	66.42 ± 0.44^{h}
60Bly40GL	*Before extrusion*		1.63	1.50	1.15	0.95	1.22	1.39	1.05	**0.86**	1.49	73.44 ± 0.79^{mn}	63.16 ± 0.68^{ij}
	130	15	1.45	1.48	1.15	0.94	0.95	1.35	1.04	**0.87**	1.49	82.55 ± 0.28^{bc}	71.99 ± 0.24^{a}
		18	1.64	1.49	1.16	0.94	1.03	1.38	1.03	**0.84**	1.49	81.35 ± 0.46^{bcde}	68.70 ± 0.38^{def}
		21	1.60	1.45	1.12	0.92	**0.79**	1.30	0.99	0.85	1.43	79.72 ± 0.52^{efgh}	62.98 ± 0.41^{j}
	140	15	1.60	1.47	1.15	0.91	1.06	1.29	0.99	**0.84**	1.44	82.61 ± 0.21^{abc}	69.31 ± 0.18^{cde}
		18	1.62	1.50	1.17	0.94	0.85	1.32	1.00	**0.84**	1.47	81.23 ± 0.31^{cdef}	67.92 ± 0.26^{efg}
		21	1.48	1.45	1.13	0.91	**0.83**	1.35	1.03	0.90	1.47	79.78 ± 0.72^{efgh}	66.22 ± 0.60^{h}
45Bly55GL	*Before extrusion*		1.66	1.52	1.11	1.00	0.85	1.32	1.05	**0.84**	1.46	73.68 ± 0.10^{mn}	61.89 ± 0.09^{jk}
	130	15	1.66	1.49	1.14	1.00	0.85	1.30	1.06	**0.84**	1.47	79.96 ± 0.54^{efgh}	67.26 ± 0.46^{fgh}
		18	1.69	1.47	1.12	1.00	0.90	1.30	1.04	**0.84**	1.45	79.30 ± 0.38^{fgh}	66.69 ± 0.32^{gh}
		21	1.67	1.51	1.13	1.02	0.90	1.29	1.06	**0.85**	1.47	78.45 ± 0.55^{ghi}	66.80 ± 0.47^{gh}
	140	15	1.69	1.52	1.15	1.03	0.90	1.30	1.07	**0.86**	1.49	82.98 ± 0.64^{abc}	71.01 ± 0.54^{ab}
		18	1.70	1.55	1.15	1.02	0.86	1.34	1.08	**0.84**	1.51	82.55 ± 0.86^{bc}	69.61 ± 0.72^{bcd}
		21	1.68	1.51	1.14	1.01	0.86	1.34	1.07	**0.86**	1.48	81.59 ± 0.36^{bcde}	70.32 ± 0.31^{bc}
GL	*Before extrusion*		1.87	1.55	1.08	1.12	0.76	1.35	1.12	**0.69**	1.30	76.28 ± 0.28^{jkl}	52.63 ± 0.19^{ml}
	130	15	2.00	1.62	1.17	1.20	**0.63**	1.26	1.11	0.72	1.50	84.60 ± 0.73^{a}	53.35 ± 0.46^{L}
		18	1.98	1.58	1.15	1.19	**0.57**	1.28	1.09	0.71	1.47	83.34 ± 0.10^{ab}	47.89 ± 0.06^{pq}
		21	1.92	1.58	1.15	1.17	**0.60**	1.27	1.08	0.71	1.46	82.25 ± 0.82^{bcd}	49.56 ± 0.49^{no}
	140	15	1.99	1.60	1.17	1.21	**0.63**	1.26	1.11	0.73	1.49	81.17 ± 0.38^{cdef}	51.41 ± 0.24^{m}
		18	1.94	1.57	1.15	1.16	**0.62**	1.26	1.08	0.72	1.45	80.38 ± 0.42^{defg}	49.84 ± 0.26^{n}
		21	1.96	1.61	1.16	1.20	**0.60**	1.27	1.10	0.71	1.47	80.08 ± 0.28^{efg}	48.26 ± 0.17^{op}

DT die temperature, *FM* feed moisture, *Bly* barley, *GL* green lentil. For abbreviations of amino acids, see Table 1

The limiting amino acid score (LAAS) of each sample is bolded. Theoretical data of blended flours were calculated based on experimental data of Bly and GL flours, following FAO/WHO (1991). IVPD and IVPDCAAS values are expressed as the averages ± standard deviations (n = 3) and values followed by different letters are significantly different ($P < 0.05$)

by the US and acknowledged by Canada to assess protein quality of food products [38, 39].

The IVPDCAAS values of barley and green lentil flour were 40.82 and 52.63%, respectively. Compared to them, blended flours 75Bly25GL, 60Bly40GL and 45Bly55GL had relatively higher IVPDCAAS values (60–64%). Flour blend 60Bly40GL showed the highest IVPDCAAS value (63.45%) because of its higher LAAS resulting from blending. Due to the effect of extrusion cooking on IVPD, IVPDCAAS of extrudates were also improved with extrusion. The IVPDCAAS of 60Bly40GL fell into the range of 63–72%, followed by 45Bly55GL (66–71%), 75Bly25GL (62–69%), GL (47–54%) and Bly (43–47%). Although, the extrudate which had the highest IVPDCAAS was 60Bly40GL produced at 130 °C and 15% FM (71.99%), extrudates of 45Bly55GL showed less variability and the highest average IVPDCAAS (68.62%).

To carry any protein content claims in the US, snack foods need to have at least 5 g PDCAAS (in vivo protein digestibility corrected amino acid score)-corrected protein per 30 g reference amounts customarily consumed (RACC) [38]. For example, a snack with 10% crude protein and 70% PDCAAS value would have 2.1 g (10/100 × 30 × 0.7) PDCAAS-corrected protein per RACC. While the official method for protein claims requires the use of in vivo digestibility assays for measuring PDCAAS and corrected protein levels, IVPDCAAS can be used as a proxy for formulation purposes (but not for regulatory approval) when in vivo PDCAAS values are not available. In the current study, extrudate 45Bly55GL produced at 140 °C DT and 15% FM had the highest IVPDCAAS-corrected protein per RACC (4.2 g). As such, it fell just short of the 5 g mark that is needed for a "good source of protein" claim. Canada has recently permitted the use of PDCAAS values of a food for the estimation of its protein efficiency ratio (PER), where estimated PER equals to PDCAAS×2.5 [10]. CFIA [39] stipulates that the product of the protein content in a food per reference amount (50 g for snacks) and the PER of the food, referred as protein rating, is the key to determine if a food can carry protein claims. The extrudate from blend 45Bly55GL, having the highest IVPDCAAS-corrected protein per RACC, also had the highest protein rating (17.5) based on its IVPDCAAS and estimated PER values. This protein rating is only 2.5 points below the 20 points mark that qualifies to be labelled as "good source of protein" in Canada. The relatively small serving size and the need to balance the carbohydrate: protein ratio for both sensory and nutritional quality of snacks makes it difficult to obtain protein content claims for these foods. However, it should be noted that the IVPDCAAS has been shown to underestimate the PDCAAS value by 2–4% in absolute values [27]. It is to be noted that IVPDCAAS estimates apparent protein digestibility while PDCAAS is calculated from true protein digestibility, thus a IVPDCAAS regression based on true protein digestibility would yield higher values for the same proteins, as it corrects for endogenous losses (protein losses in feces due to nitrogen contributed by dead cells, enzymes, bacteria etc.). Accordingly, the true PDCAAS-corrected protein content of the optimized blends may be slightly higher than those observed in this IVPDCAAS method, and possibly qualify for protein claims.

Based on the superior physical quality [35] and promising IVPDCAAS results of extruded 60Bly40GL and 45Bly55GL blends over other blends, 60Bly40GL and 45Bly55GL extrudates were selected to be tested (as a function of extrusion FM and DT) for their total, insoluble and soluble dietary fiber contents.

Dietary fiber content

In general, extrusion did not substantially affect the insoluble, soluble or total dietary fiber contents of the blends. One exception was the soluble fiber content of the 60Bly40GL extrudate produced at DT 130 °C and FM 15% which had a significantly higher soluble dietary fiber content when compared to the raw 60Bly40GL blend. Among the 60Bly40GL extrudates, the insoluble, soluble and total dietary fiber contents were generally similar, except for the 130 °C and FM 21% whose soluble fiber content was slightly but significantly lower than the rest of the 60Bly40GL extrudates produced at 130 °C. For all the 45Bly55GL extrudates studied, there was no difference in the insoluble, soluble and total dietary fiber contents.

The TDF contents of extruded 60Bly40GL and 45Bly55GL were in the range of 14.9–16.7% and 13.5–15.5%, respectively. All extrudates from these two blends contained more than 2.8 g dietary fiber per 30 g RACC, meeting the requirement of FDA [38] for snacks to be labelled as "good source of dietary fiber" in the US. Four extrudates from blend 60Bly40GL (all three produced at 130 °C DT regardless of their FM, and 21% FM extrudate produced at 140 °C DT) contained more than 6 g or more dietary fiber per 40 g serving size, which can be recognized as very high source of dietary fiber snacks and carry content labels such as "very high dietary fiber" in Canada [39]. Considering the increasing pursuit of consumers to dietary fiber worldwide, 60Bly40GL would be the optional blending ratio since it can achieve dietary fiber claims in both the US and Canada.

Estimated glycemic index

Extrudates 60Bly40GL produced at DT 140 °C and FM 18%, and extrudates 45Bly55GL produced at DT 130 °C and FM 18%, DT 130 °C and FM 18% and DT 140 °C and FM 21% had the lowest GI values, which ranged between 84.65 and 86.56 (Table 4). On average, 45Bly55GL extrudates (average GI = 86.66) had lower GI values when compared to 60Bly40GL extrudates (average GI = 89.95). However, all extrudates of these two blends can be categorized under high glycemic index (GI ≥ 70) foods [40]. As extrusion's effect on starch digestibility is a function of process conditions [41], wider ranges of extrusion temperature and feed moisture content should be explored in the future to better identify the processing conditions that produce low GI snacks.

Table 4 Insoluble, soluble and total dietary fiber contents of 60Bly40GL and 45Bly55GL flours, and their extrudates, along with glycemic index results of extruded 60Bly40GL and 45Bly55GL

Blends	DT (°C)	FM (%)	Dietary fiber (g/100 g)			Glycemic index
			Insoluble	Soluble	Total	
60Bly40GL	*Before extrusion*		11.6 ± 1.3[ab]	4.6 ± 0.4[bcde]	16.2 ± 0.9[abcd]	-
	130	15	11.0 ± 1.3[ab]	5.6 ± 0.4[a]	16.6 ± 1.0[ab]	91.69 ± 1.0[a]
		18	11.8 ± 0.9[ab]	5.5 ± 0.6[ab]	17.2 ± 1.4[a]	88.61 ± 1.7[ab]
		21	12.3 ± 1.0[a]	4.4 ± 0.2[cde]	16.7 ± 0.9[ab]	91.30 ± 0.8[a]
	140	15	10.7 ± 0.7[ab]	5.3 ± 0.4[abc]	15.9 ± 0.8[abcd]	91.24 ± 1.2[a]
		18	10.0 ± 0.5[ab]	5.0 ± 0.2[abcd]	14.9 ± 0.3[abcd]	85.30 ± 0.1[b]
		21	11.4 ± 1.1[ab]	4.9 ± 0.7[abcd]	16.3 ± 1.6[abc]	91.54 ± 0.2[a]
45Bly55GL	*Before extrusion*		9.3 ± 0.9[b]	4.5 ± 0.2[cde]	13.8 ± 1.0[cd]	-
	130	15	10.6 ± 1.5[ab]	3.9 ± 0.2[e]	14.4 ± 1.7[cd]	85.16 ± 1.8[b]
		18	9.4 ± 0.5[b]	4.1 ± 0.5[de]	13.5 ± 0.7[d]	86.56 ± 1.1[b]
		21	11.4 ± 0.9[ab]	4.1 ± 0.1[de]	15.5 ± 0.9[abcd]	88.04 ± 05[ab]
	140	15	10.8 ± 0.4[ab]	4.4 ± 0.2[cde]	15.3 ± 0.3[abcd]	87.90 ± 1.8[ab]
		18	9.6 ± 1.2[b]	4.6 ± 0.5[bcde]	14.2 ± 1.2[bcd]	87.63 ± 0.0[ab]
		21	10.5 ± 1.6[ab]	3.9 ± 0.1[e]	14.4 ± 1.6[bcd]	84.65 ± 0.1[b]

DT die temperature, *FM* feed moisture, *Bly* barley, *GL* green lentil

Data is expressed as the averages ± standard deviations (n = 4 for dietary fiber, n = 2 for glycemic index) on dry basis. Values followed by different letters in each column are significantly different (P < 0.05)

Conclusions

Barley flour with relatively lower protein and lysine, but higher dietary fiber and sulfur-containing amino acids contents, and green lentil flour with the opposite characteristics, were shown to complement each other's protein quality attributes. Amino acid compositions of blend 45Bly55GL were relatively more stable during extrusion than others. Extrusion significantly improved in vitro protein digestibility (IVPD) of all blends by up to 10%. Die temperature had a positive relationship with the IVPDs of blends 75Bly25GL, 60Bly40GL and 45Bly55GL, while feed moisture showed a negative relationship with IVPD.

Blending significantly increased the limiting amino acid score of flours and extrudates. The in vitro protein digestibility corrected amino acid score (IVPDCAAS) also increased to a range of 61–72% after blending. Among extrudates, blend 45Bly55GL showed less variability and the highest average IVPDCAAS (68.62%). Feed moisture and die temperature affected IVPDCAAS of extrudates through their effect on extrudates' IVPD. The effect of extrusion was insignificant on dietary fiber content. All extrudates from blend 60Bly40GL met FDA's requirement to be recognized as "good source of dietary fiber", and most extrudates from the same blend met CFIA's requirement to be recognized as "very high dietary fiber". Despite these high fiber contents, the glycemic index values of the extrudates studied were above 80, and thus they were categorized as high glycemic index foods.

Overall, this study showed that barley and green lentil blends have great potential to produce protein- and fiber- rich puffed snacks, meeting consumers' demand for nutritionally dense snacks. The impacts of extrusion feed moisture and temperature profile on the product were less significant than the blending ratio. Among tested blends, 60Bly40GL and 45Bly55GL showed better and balanced protein and dietary fiber properties. This study can also be generalized to other blends of cereals and pulses, bringing more innovative products and opportunities to the puffed snacks industry.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

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