In vitro protein digestibility of direct-expanded chickpea–sorghum snacks

Esayas K. Bekele1,2 | Robert T. Tyler3 | Carol J. Henry2 | James D. House4 | Matthew G. Nosworthy2

1School of Nutrition, Food Science and Technology, Hawassa University, Hawassa, Ethiopia
2College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
3College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
4Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

Abstract
Blending cereals with pulses provides a balanced protein with higher biological value as their amino acid compositions are complementary. Extrusion not only can improve protein digestibility but also may reduce essential amino acid content. This study investigated the effects of extrusion parameters and blend ratio on in vitro protein digestibility (IVPD) and IVPD-corrected amino acid score (IVPDCAAS) of direct-expanded chickpea–sorghum snacks. Chickpea–sorghum blends (50:50, 60:40, and 70:30 chickpea:sorghum, w/w) were extruded at 10 combinations of moisture content (16%, 18%, and 20%) and barrel temperature (120°C, 140°C, and 160°C), and at 169°C and 15% moisture, the conditions identified in a previous study as producing maximal expansion. Chickpea and sorghum flours were extruded at 140°C and 18% moisture for comparison purposes. The IVPD of raw 50:50, 60:40, and 70:30 chickpea–sorghum blends ranged from 76% to 78%; values for raw chickpea and sorghum flours were 79% and 74%, respectively. Extrusion increased IVPD (P < 0.05) of all flours and blends. An increase in extrusion temperature increased the IVPD of extrudates (P < 0.05), whereas an increase in moisture content had the opposite effect (P < 0.05). The IVPDCAAS of raw 50:50, 60:40, and 70:30 chickpea–sorghum blends were 0.64, 0.72, and 0.73, respectively; values for raw chickpea and sorghum flours were 0.74 and 0.27, respectively. Extrusion increased IVPDCAAS (P < 0.05). The 70:30 chickpea–sorghum blend extruded at the maximal expansion exhibited the highest protein quality indicating this to be the optimal condition for snack production.

KEYWORDS
chickpea, direct-expanded snacks, extrusion, protein quality, sorghum

1 | INTRODUCTION
Sorghum (Sorghum bicolor [L.] Moench) is the number five cereal crop in terms of annual global production, 59 million tons in 2018 (FAOSTAT, 2019). Sorghum is a unique cereal due to its agronomic traits such as drought tolerance and adaptation to both tropical and subtropical conditions (Espinosa-Ramirez & Serna-Saldívar, 2016). Sorghum is also one of the principal staples for millions of people in sub-Saharan Africa (Weerasooriya et al., 2018). The nutrient content of sorghum grain is similar to that of other cereals (Weerasooriya et al., 2018) with a protein content of 9%–17%, carbohydrate content of 77%–89%, and lipid content...
of 2%–6% on a dry-weight basis (Palavecino et al., 2016). Like other cereals, when compared with human nutritional requirements, sorghum protein is deficient in certain essential amino acids, most importantly lysine; however, it contains sufficient levels of the sulfur amino acids, cysteine, and methionine (Mokrane et al., 2010). The in vitro digestibility of raw sorghum protein (40%–77%) is reported to be lower compared with other cereals due to the existence of antinutrients such as tannins (Duodu et al., 2002; Elkonin et al., 2013). In order to overcome its limitations in terms of both amino acid composition and protein digestibility, sorghum-based foods require processing as well as blending with a complementary protein source.

Chickpea (Cicer arietinum L.) is the third most widely grown pulse globally, with production of 17 million tons in 2018 (FAOSTAT, 2019). Chickpea is an important source of protein for human consumption (Liu et al., 2008) and has a protein content of 16%–28% (Chibbar et al., 2010). The most abundant essential amino acids in whole chickpea seed are leucine and lysine, whereas the sulfur-containing amino acids cysteine and methionine are limiting (Wang et al., 2010). The in vitro digestibility of raw chickpea protein has been reported to be 59%–76% (Bhayawant et al., 2018). Chickpea contains antinutrients such as polyphenols and trypsin and chymotrypsin inhibitors that contribute to lower protein digestibility and that can be destroyed by processing (Bessada et al., 2019).

Combining cereals with pulses provides a balanced protein with high biological value (Arribas et al., 2017). In addition, processing methods such as high-temperature extrusion are reported to improve digestibility of various plant-based protein sources (Nosworthy et al., 2017). However, optimization of extrusion conditions to maximize the protein quality of direct-expanded chickpea–sorghum snacks has yet to be reported. Therefore, this study was designed to investigate the effect of extrusion conditions and chickpea–sorghum blend ratio on in vitro protein digestibility (IVPD) and IVPD-corrected amino acid score (IVPDCAAS) of direct-expanded chickpea–sorghum snacks.

## MATERIALS AND METHODS

### 2.1 Raw materials

Kabuli chickpea and sorghum were purchased from Diefenbaker Spice & Pulse (Elbow, SK, Canada) and Sinner Bros. & Bresnahan (Casselton, North Dakota, USA), respectively. Chymotrypsin (from bovine pancreas, 4129 Type II, lyophilized powder, ≥40 units/mg protein), trypsin (from porcine pancreas, Type IX-S, lyophilized powder, 13,000–20,000 BAEE units/mg protein) and protease (from Streptomyces griseus, Type XIV, ≥3.5 units/mg protein) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Ethanol, sodium hydroxide, hydrochloric acid (37% w/w), barium hydroxide, and sodium tetaborate were purchased from Fisher Scientific (Ottawa, ON, Canada). All reagents used were of analytical grade.

### 2.2 Protein and amino acid analysis

Protein content was determined according to AOAC (1997) official method 968.06. Amino acids were determined as described in House et al. (2019). Briefly, samples were hydrolyzed with 6-N hydrochloric acid for 24 h for the quantification of all amino acids, with the exceptions of methionine, cysteine, and tryptophan. For methionine and cysteine, samples were first oxidized with performic acid and then subjected to acid hydrolysis. The amino acids in both hydrolyzed sets were then derivatized and analyzed using the AccQ-Tag method on a UPLC fitted with an AccQ-Tag Ultra C18, 1.7-µm column and an SIL-30 AC autosampler. For the determination of tryptophan, samples were hydrolyzed with barium hydroxide for 20 h and then analyzed using HPLC (ISO protocol 13,904) (ISO, 2016). Amino acid analysis was done in duplicate.

### 2.3 Determination of IVPDCAAS

Amino acid score was determined by comparing the amino acid composition of the target protein to that of the reference protein (FAO/WHO, 1991). The reference amino acid composition was recommended by FAO/WHO (1991) using the amino acid requirements for children 2–5 years of age (amino acid, mg/g protein): histidine, 19; isoleucine, 28; leucine, 66; lysine, 58; methionine + cysteine, 25; phenylalanine + tyrosine, 63; threonine, 34; tryptophan, 11; valine, 35. The FAO/WHO reference pattern for children 2–5 years of age is the amino acid pattern required by the United States FDA for determination of protein content claims (21 CFR 101.9). This reference pattern was selected for the benefit of product developers and regulatory agents. The lowest amino acid score represents the first limiting essential amino acid.

The IVPD was determined for the 10 treatments according to House et al. (2019). Samples containing 62.5 mg protein were heated to 37°C and adjusted to pH 8.0. The stability of the pH was maintained for 10 min, and then a multienzyme cocktail containing trypsin, chymotrypsin, and protease was added. The pH was recorded for 10 min, and the IVPD was determined from the change in pH over 10 min using the following equation:

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in\text{vitro protein digestibility} (\%) = 65.66 + 18.10 \times \Delta pH_{10\,\text{min}}.\]

This pH drop method of analyzing IVPD has been validated in several studies. The IVPDCAAS was calculated as the product of the limiting amino acid score and IVPD (House et al., 2019). IVPD and IVPDCAAS analyses were done in quadruplicate.

### 2.4 Determination of the optimal blend ratio

The optimal chickpea–sorghum blend ratio was determined on the basis of the predicted IVPDCAAS. Using experimentally derived
values for raw chickpea and sorghum flours, the IVPD and amino acid composition of raw chickpea–sorghum blends (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10, chickpea:sorghum, w/w) were calculated. IVPDCAAS of the raw blends was calculated from the IVPD and amino acid score according to House et al. (2019). The 70:30 chickpea–sorghum blend was identified as the point at which the IVPDCAAS plateaued and was therefore designated as optimal (data not shown). With the objective of examining the effect of blending chickpea and sorghum on IVPD and IVPDCAAS, chickpea–sorghum blend ratios of 50:50 and 60:40 also were included in the study for comparative purposes.

2.5 | Extrusion

Extrusion employed a corotating, twin-screw extruder (Model EV-32; Clextral, Firminy, France) according to Bekele et al. (2020). Briefly, chickpea–sorghum blends (50:50, 60:40, and 70:30, chickpea–sorghum, w/w) were extruded by setting the barrel temperature of the last three zones at 120°C, 140°C, or 160°C and the feed moisture content at 16%, 18%, or 20%. The blends also were extruded at the conditions where maximum extrudate expansion was observed, 169°C barrel temperature and 15% feed moisture. The conditions for maximum extrudate expansion were determined according to Bekele et al. (2020). For comparison, chickpea (100%) and sorghum (100%) extrudates were produced at a barrel temperature of 140°C and 18% feed moisture. The screw speed and feed rate were maintained at 396 rpm and 26 kg/h, respectively. Extrudates were dried at 105°C for 5 min using a tunnel drier (Chromalox, Pittsburgh, PA, USA). Each sample was produced in duplicate under each processing condition. Extrudate drying temperature–time was selected because less than 5% available lysine loss was observed in dairy based confectionery heated at 103°C for 5 min (Malec, Llosa, Naranjo, & Vigo, 2005).

2.6 | Statistical analysis

The effects of extrusion and blend ratio on IVPD and IVPDCAAS were analyzed using two-way ANOVA and the Fisher post hoc test. The effects of blend ratio, barrel temperature, and moisture content on IVPD were analyzed using three-way ANOVA and the Fisher post hoc test. Differences were considered significant at \( P < 0.05 \) (Vik, 2013). Statgraphics Centurion version 18.1.12 (Statgraphics Technologies, The Plains, VA, USA) was used for analysis.

3 | RESULTS AND DISCUSSION

3.1 | Amino acid content and amino acid score of chickpea, sorghum, and blends

The amino acid compositions of raw chickpea (100:0), raw sorghum (0:100), and raw chickpea–sorghum blends (50:50, 60:40, and 70:30 chickpea:sorghum, w/w) are presented in Table 1. Measurement of the amino acids was performed in duplicate on the raw samples or extruded samples, so no statistical comparison was undertaken as \( n < 3 \). Amino acid analysis was performed on all samples listed in Table 1, except for the raw 60:40 and 70:30 chickpea–sorghum blends. The amino acid compositions of the raw chickpea–sorghum blends were calculated using the amino acid compositions of raw chickpea (100:0) and raw sorghum (0:100). The amino acid composition of the 50:50 chickpea–sorghum blend was analyzed to compare with the calculated value. The comparison indicated that the measured and calculated values were similar.

Chickpea, sorghum, and chickpea–sorghum snacks exhibited losses of amino acids after extrusion (Table 1). Chickpea, sorghum, and 50:50, 60:40, and 70:30 chickpea–sorghum snacks exhibited 7%, 26%, 19%, 8%, and 8% losses of cysteine, respectively. Sorghum and 50:50, 60:40, and 70:30 chickpea–sorghum snacks displayed losses of 16%, 13%, 8%, and 9% of lysine, respectively. Sorghum and 50:50, 60:40, and 70:30 chickpea–sorghum snacks lost 10%, 7%, 6%, and 11% of arginine, respectively. Sorghum exhibited a loss of tyrosine of 11%. Despite the observed losses of cysteine, tyrosine, and lysine after extrusion, the values remained above the 1991 FAO reference level, except for lysine. The loss of lysine, cysteine, and arginine was attributed to the Maillard reaction (Li et al., 2018). For the most part, the coefficient of variation for amino acid composition was less than 7, except in the case of phenylalanine for raw and extruded sorghum, and isoleucine, tyrosine, and lysine for extruded sorghum where the coefficient of variation was greater than 7.

The amino acid scores for raw chickpea, raw sorghum, and the raw chickpea–sorghum blends were calculated according to the 1991 FAO reference pattern for children 2–5 years of age (FAO/WHO, 1991) (Table 2). The first limiting amino acid for raw sorghum (0:100) was lysine, and its amino acid score was 0.37; for raw chickpea and raw 50:50, 60:40, and 70:30 chickpea–sorghum blends, the first limiting amino acid was tryptophan, and their respective amino acid scores were 0.93, 0.94, 0.94, and 0.84. Statistical comparisons were not done on the amino acid score as \( n < 3 \).

The amino acid scores for chickpea, sorghum, and chickpea–sorghum extrudates also are presented in Table 2. The first limiting amino acid for chickpea and the 70:30 chickpea–sorghum extrudates was tryptophan, with amino acid scores of 0.98 and 0.90, respectively. The first limiting amino acid for sorghum and 50:50 and 60:40 chickpea–sorghum extrudates was lysine, with amino acid scores of 0.31, 0.80, and 0.87, respectively. The coefficient of variation for the amino acid score was less than 7, except for isoleucine, phenylalanine, tyrosine, and lysine of the sorghum extrudate. Other studies reported sulfur-containing amino acids (Jukanti et al., 2012) and threonine (Bai et al., 2018; Wang et al., 2019) as the limiting amino acids for chickpea. Wang et al. (2019) reported that the use of sulfur fertilizer during chickpea cultivation could increase sulfur-containing amino acids, shifting the limiting amino acid from sulfur amino acids to other amino acids. This also could be the reason that tryptophan was found to be the limiting amino acid in this study. In the case of sorghum, a previous...
### TABLE 1  Amino acid compositions of raw and extruded chickpea and sorghum flours and chickpea-sorghum blends (g/100 g protein)

| Chickpea-sorghum blend ratio, w/w | Extrusion conditions | ASP | THR | SER | GLU | PRO | GLY | ALA | CY5 | VAL |
|----------------------------------|---------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 100:0                            | Raw                 | 11.2 (1.5) | 3.5 (2.5) | 5.3 (2.3) | 16.2 (0.8) | 4.1 (4.3) | 3.6 (0.8) | 4.0 (0.7) | 1.5 (2.5) | 4.0 (3.1) |
| 70:30 (C/18)                     | Raw                 | 10.4 (0.6) | 3.5 (1.5) | 5.2 (1.3) | 16.7 (1.0) | 4.8 (3.9) | 3.5 (0.6) | 4.8 (0.8) | 1.6 (1.1) | 4.1 (3.2) |
| 60:40 (C/15)                     | Raw                 | 10.1 (0.2) | 3.5 (1.2) | 5.2 (1.0) | 16.9 (1.2) | 5.2 (3.9) | 3.5 (0.6) | 5.2 (0.9) | 1.7 (0.8) | 4.1 (3.2) |
| 50:50 (C/15)                     | Raw                 | 9.7 (0.2)  | 3.4 (1.0) | 5.2 (0.7) | 17.2 (1.4) | 5.5 (3.9) | 3.5 (0.6) | 5.6 (0.9) | 1.7 (0.6) | 4.1 (3.3) |
| 50:50                            | Raw                 | 9.6 (0.1)  | 3.5 (0.0) | 5.1 (0.0) | 18.1 (0.0) | 5.6 (0.0) | 3.5 (0.0) | 5.5 (0.0) | 1.9 (0.0) | 4.5 (0.0) |
| 0:100                            | Raw                 | 6.9 (3.0)  | 3.3 (1.6) | 4.8 (1.3) | 19.2 (2.4) | 8.4 (4.2) | 3.2 (1.3) | 8.8 (1.3) | 2.0 (2.0) | 4.5 (4.2) |
| 100:0                            | 140 C/18%           | 12.1 (1.2) | 3.7 (1.3) | 5.6 (0.3) | 17.4 (1.1) | 4.3 (3.9) | 3.9 (0.8) | 4.2 (1.2) | 1.4 (1.0) | 4.3 (1.2) |
| 70:30 (C/15)                     | Raw                 | 10.4 (0.0) | 3.5 (0.0) | 5.1 (0.0) | 17.5 (0.0) | 4.8 (0.0) | 3.8 (0.0) | 4.8 (0.0) | 1.5 (0.0) | 4.4 (0.0) |
| 60:40 (C/15)                     | Raw                 | 10.1 (0.0) | 3.5 (0.0) | 5.1 (0.0) | 17.9 (0.0) | 5.1 (0.0) | 3.6 (0.0) | 5.2 (0.0) | 1.5 (0.0) | 4.4 (0.0) |
| 50:50 (C/15)                     | Raw                 | 9.9 (0.0)  | 3.5 (0.0) | 5.1 (0.0) | 18.2 (0.0) | 5.5 (0.0) | 3.6 (0.1) | 5.5 (0.0) | 1.6 (0.0) | 4.5 (0.0) |
| 0:100                            | 140 C/18%           | 7.5 (1.0)  | 3.5 (6.8) | 4.9 (3.1) | 20.3 (0.7) | 8.7 (5.5) | 3.3 (1.3) | 9.0 (1.1) | 1.5 (2.8) | 4.6 (0.8) |

Note. Data (n = 2) are presented as mean with percent coefficient of variation in brackets. Data from the blends are experimental except those labeled with §. The values with °C and % represent barrel temperature and moisture content, respectively.

### TABLE 2  Amino acid scores of raw and extruded chickpea and sorghum flours and chickpea-sorghum blends

| Chickpea-sorghum blend ratio, w/w | Extrusion conditions | THR | MET + CYS | VAL | ILE | LEU | PHE + TYR | HIS | LYS | TRP |
|----------------------------------|---------------------|-----|-----------|-----|-----|-----|-----------|-----|-----|-----|
| 100:0                            | Raw                 | 1.03 (2.5) | 1.24 (0.9) | 1.13 (3.1) | 1.30 (4.0) | 1.04 (1.5) | 1.23 (0.9) | 1.37 (1.6) | 1.15 (1.2) | 0.93 (1.9) |
| 70:30 (C/18)                     | Raw                 | 1.02 (1.5) | 1.31 (0.5) | 1.16 (3.2) | 1.28 (4.0) | 1.17 (2.6) | 1.24 (2.5) | 1.36 (2.1) | 1.01 (1.2) | 0.94 (3.0) |
| 60:40 (C/15)                     | Raw                 | 1.01 (1.2) | 1.34 (0.2) | 1.17 (3.2) | 1.28 (4.0) | 1.22 (2.9) | 1.24 (2.9) | 1.36 (2.5) | 0.96 (1.2) | 0.94 (3.3) |
| 50:50 (C/15)                     | Raw                 | 1.03 (0.0) | 1.54 (0.4) | 1.28 (0.0) | 1.50 (0.0) | 1.44 (0.0) | 1.38 (3.2) | 1.17 (0.0) | 0.91 (0.1) | 0.84 (0.2) |
| 0:100                            | Raw                 | 0.97 (1.6) | 1.64 (1.8) | 1.28 (4.2) | 1.22 (4.1) | 1.74 (4.1) | 1.27 (7.0) | 1.32 (6.2) | 0.37 (2.1) | 0.96 (5.4) |
| 100:0                            | 140 C/18%           | 1.09 (1.3) | 1.20 (0.1) | 1.22 (1.2) | 1.47 (0.7) | 1.11 (0.8) | 1.26 (0.6) | 1.56 (2.5) | 1.17 (0.2) | 0.98 (2.5) |
| 70:30 (C/15)                     | Raw                 | 1.03 (0.0) | 1.32 (0.3) | 1.25 (0.0) | 1.52 (0.0) | 1.29 (0.0) | 1.33 (3.2) | 1.16 (0.0) | 0.92 (0.0) | 0.90 (0.0) |
| 60:40 (C/15)                     | Raw                 | 1.03 (0.0) | 1.36 (2.0) | 1.26 (0.0) | 1.50 (0.0) | 1.30 (0.0) | 1.35 (0.6) | 1.18 (0.0) | 0.87 (0.0) | 0.91 (0.0) |
| 50:50 (C/15)                     | Raw                 | 1.03 (0.0) | 1.37 (1.6) | 1.28 (0.0) | 1.50 (0.0) | 1.43 (0.0) | 1.36 (1.2) | 1.13 (0.0) | 0.80 (0.0) | 0.93 (0.0) |
| 0:100                            | 140 C/18%           | 1.03 (6.8) | 1.48 (2.3) | 1.33 (0.8) | 1.35 (10.2) | 1.87 (1.9) | 1.29 (16.3) | 1.36 (1.9) | 0.31 (8.5) | 1.39 (1.4) |

Note. Bold fonts indicate limiting amino acid scores. Data (n = 2) are presented as mean with percent coefficient of variation in brackets. Data from the blends are experimental except those labeled with §. The values with °C and % represent barrel temperature and moisture content, respectively.

Abbreviations: CYS, cysteine; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine.
study reported that lysine was the first limiting amino acid, with an amino score of 0.4 (Mokrane et al., 2010). Guzman-Ortiz et al. (2015) also observed decreases in levels of amino acids after extrusion of a soybean–corn blend at 160°C and 26% moisture.

3.2 Effect of extrusion on IVPD

The IVPDs of raw and extruded blends are presented in Table 3. Differences (P < 0.05) between extruded and raw samples of the same blend ratio as well as differences (P < 0.05) between blend ratios, but within raw or similar extrusion conditions, are indicated. The IVPD of raw sorghum (74%) was lower (P < 0.05) than those of raw chickpea (79%) and the raw chickpea–sorghum blends (76%–78%). The IVPD of raw sorghum determined in this study falls within the ranges reported by Elkonin et al. (2013), 40%–76%, and by Bhagyawant et al. (2018), 59–76%.

Extrusion increased (P < 0.05) the IVPD of all samples (Table 3). This may be due to denaturation of protein during extrusion, which would expose more polypeptide bonds to proteolytic enzymes, and decreased activity of heat-labile antinutritional factors, protease inhibitors in particular (Patterson et al., 2017). Other studies have reported an increase in IVPD with extrusion of buckwheat at 120°C barrel temperature and maize–soybean at 170°C barrel temperature and 20% feed moisture (Nosworthy et al., 2017; Omosebi et al., 2018). The IVPD of the sorghum extrudate (77%) was lower (P < 0.05) than that of the chickpea extrudate (79%). The IVPD of the 50:50 chickpea–sorghum extrudate (83%) was lower (P < 0.05) than the IVPD of the 60:40 (84%) and 70:30 (85%) chickpea–sorghum extrudates (Table 3).

Studies have reported that one of the explanations for lower digestibility of sorghum is that the prolamin protein (kafirin) forms oligomers or polymers of high molecular weight that are linked together by disulfide bonds and that are resistant to hydrolysis by proteases (Duodu et al., 2002; Nunes et al., 2005). Extrusion increased (P < 0.05) the IVPD of all samples, but the increase in IVPD was less for sorghum.

3.3 Effect of extrusion conditions on IVPD

Extrusion temperature, moisture content and blend ratio had significant (P < 0.05) effects on IVPD (Figure 1). However, the interaction effects were not significant (P > 0.05). IVPD was higher (P < 0.05) for higher extrusion temperatures. In contrast, an increase in moisture content or the proportion of sorghum in the blend resulted in lower (P < 0.05) IVPD. Previous work showed that an increase in extrusion temperature resulted in a concomitant rise in IVPD of a sorghum–maize blend and a flaxseed–maize blend (Licata et al., 2014; Min et al., 2015). Multiple reasons for this phenomenon exist, including the alteration of noncovalent interactions resulting in “opening” of the protein, as well as inactivation of protease inhibitors and other antinutritional factors. Ainsworth et al. (1999) reported that IVPD increased with an increase in extrusion temperature to a point, after which IVPD decreased. The explanation for this was that at higher extrusion temperatures, the extrudate had undergone thermal crosslinking during nonenzymatic browning reactions, resulting in lower IVPD. Similarly, others have reported reductions in IVPD at higher extrusion moisture contents (Chumman et al., 2016; Palanisamy et al., 2019). This might be due to the decrease in shear in the extruder barrel associated with the increase in moisture content. In line with this study, Licata et al. (2014) reported a decrease in IVPD of a sorghum–maize extrudate with an increase in the proportion of sorghum (range of 15%–60%) in the extrudate. This might be due to crosslinking of high molecular weight sorghum proteins. The sorghum–maize blend was extruded at 120°C and 150°C barrel temperature and 21% and 26% moisture content.

### Table 3

| Chickpea–sorghum blend ratio (w/w) | Extrusion conditions | Amino acid score | IVPD | IVPDCAAS |
|-----------------------------------|----------------------|-----------------|------|----------|
| 100:0                             | Raw                  | 0.93            | 79.19 ± 2.77<sup>a</sup> | 0.74 ± 0.03<sup>a</sup> |
| 70:30                             | Raw                  | 0.94            | 77.60 ± 0.56<sup>ab</sup> | 0.73 ± 0.01<sup>b</sup> |
| 60:40                             | Raw                  | 0.94            | 77.00 ± 0.28<sup>ab</sup> | 0.72 ± 0.01<sup>b</sup> |
| 50:50                             | Raw                  | 0.84            | 76.05 ± 0.07<sup>ab</sup> | 0.64 ± 0.01<sup>b</sup> |
| 0:100                            | Raw                  | 0.37            | 73.89 ± 1.15<sup>c</sup> | 0.27 ± 0.01<sup>d</sup> |
| 100:0                            | 140°C/18%            | 0.98            | 83.67 ± 1.06<sup>c</sup> | 0.82 ± 0.01<sup>d</sup> |
| 70:30                            | 169°C/15%            | 0.90            | 84.66 ± 0.64<sup>c</sup> | 0.76 ± 0.01<sup>d</sup> |
| 60:40                            | 169°C/15%            | 0.87            | 84.40 ± 1.41<sup>c</sup> | 0.73 ± 0.01<sup>d</sup> |
| 50:50                            | 169°C/15%            | 0.80            | 82.85 ± 0.13<sup>c</sup> | 0.66 ± 0.01<sup>d</sup> |
| 0:100                            | 140°C/18%            | 0.31            | 77.41 ± 0.39<sup>c</sup> | 0.24 ± 0.01<sup>c</sup> |

Note. IVPD and IVPDCAAS data were analyzed using two way-ANOVA with the Fisher post hoc test (n = 4). Data presented as mean ± standard deviation. Significant differences between extruded and raw samples for the same blend ratio are designated by the symbol <sup>a</sup>. P < 0.05. Significant differences between blend ratios, but within raw or similar extrusion conditions, are designated by different letters, P < 0.05. The coefficient of variation was ≤4 for IVPD and IVPDCAAS data.
Extrusion and IVPDCAAS

3.4 Extrusion and IVPDCAAS

IVPDCAAS calculates protein quality. IVPDCAAS of raw and extruded chickpea and sorghum flours and chickpea–sorghum blends is presented in Table 3. IVPDCAAS values for raw chickpea and sorghum and raw 50:50, 60:40, and 70:30 chickpea–sorghum blends were 0.74, 0.27, 0.64, 0.72, and 0.73, respectively. Raw chickpea exhibited a much higher ($P < 0.05$) IVPDCAAS than did raw sorghum because both the amino acid score (0.93) and protein digestibility (79%) for raw sorghum were lower than the corresponding values (0.93 and 79%) for raw chickpea. Lysine, the limiting amino acid for sorghum, was present in a low amount in raw sorghum (2.2/100-g protein, dry-weight basis) as compared to raw chickpea (6.7/100-g protein, dry-weight basis) and this affected the IVPDCAAS of sorghum. IVPDCAAS for the raw 60:40 and 70:30 chickpea–sorghum blends were higher ($P < 0.05$) than that of the raw 50:50 blend, due to the lower IVPDCAAS for sorghum. Extrusion increased ($P < 0.05$) IVPDCAAS for all samples, with the exception of the sorghum sample. IVPDCAAS for the 70:30 (0.76) and 60:40 (0.73) chickpea–sorghum snacks were higher ($P < 0.05$) than that of the 50:50 (0.66) chickpea–sorghum snack. The IVPDCAAS of the sorghum extrudate (0.24) was markedly lower ($P < 0.05$) than that of the chickpea extrudate (0.82). The decrease in IVPDCAAS observed for the sorghum extrudate was attributed to loss of lysine. Wang et al. (2019) reported that extrusion did not significantly affect IVPDCCAS of sorghum or chickpea. The extrusion conditions (120°C and 150°C barrel temperature and 20% and 24% moisture) were different than in the current study, which might explain the difference in results between the studies. One of the limitations of the current study was that the available lysine content of the extruded snacks was not determined. Knowledge of available lysine content may have strengthened the interpretation of the IVPDCAAS results.

4 CONCLUSIONS

The limiting amino acid was lysine for raw sorghum and tryptophan for raw chickpea and raw chickpea–sorghum blends. Extrusion shifted the limiting amino acids of raw 50:50 and 60:60 chickpea–sorghum blends to lysine. Extrusion increased IVPD of sorghum, chickpea, and chickpea–sorghum blends. Increasing the proportion of chickpea in the chickpea–sorghum blend and the extrusion temperature increased IVPD of the chickpea–sorghum blend, whereas increasing feed moisture content decreased IVPD. The protein quality of chickpea–sorghum extrudates was affected significantly by blend ratio, extrusion, and extrusion conditions. Extrusion improved the protein quality of chickpea–sorghum extrudates but not that of the sorghum extrudate. The study illustrated that blending sorghum with chickpea...
was advantageous from a protein quality point of view. Snacks prepared from 60:40 and 70:30 chickpea-sorghum blends and extruded at the maximal expansion point were found to be preferable in terms of protein quality. Clearly, whole grain sorghum can be blended with whole grain chickpea and used for production of direct-expanded snacks to enhance protein quality.

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AUTHOR CONTRIBUTIONS
Esayas K. Bekele, Robert T. Tyler, and Carol J. Henry designed the study. Esayas K. Bekele carried out the laboratory analysis and data analysis. Esayas K. Bekele, Matthew G. Nosworthy, Robert T. Tyler, James D. House, and Carol J. Henry interpreted the data. All authors contributed to drafting the manuscript and approved the final version.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available upon request from the corresponding author.

ETHICAL STATEMENT
The study does not require any ethical approval.

ORCID
Esayas K. Bekele https://orcid.org/0000-0002-9514-8694
James D. House https://orcid.org/0000-0003-1389-5491
Matthew G. Nosworthy https://orcid.org/0000-0002-3782-1035

REFERENCES
Ainsworth, P., Fuller, P., Plunkett, A., & Ibangolu, S. (1999). Influence of extrusion variables on the protein in vitro digestibility and protein solubility of extruded soy tarhana. *Journal of the Science of Food and Agriculture*, 79(5), 675–678. https://doi.org/10.1002/(SICI)1097-0010(199904)79:5<675::AID-JSFA234>3.0.CO;2-a
AOAC. (1997). *Official methods of analysis*. Gaithersburg, MD: AOAC International.
Arribas, C., Cabellos, B., Sanchez, C., Cuadrado, C., Guillamon, E., & Pedrosa, M. M. (2017). The impact of extrusion on the nutritional composition, dietary fiber and in vitro digestibility of gluten-free snacks based on rice, pea and carob flour blends. *Food and Function*, 8(10), 3654–3663. https://doi.org/10.1039/c7fo00910k
Bai, T., Nosworthy, M. G., House, J. D., & Nickerson, M. T. (2018). Effect of tempering moisture and infrared heating temperature on the nutritional properties of desi chickpea and hull-less barley flours and their blends. *Food Research International*, 108, 430–439. https://doi.org/10.1016/j.foodres.2018.02.061
Bekele, E. K., Nosworthy, M. G., Henry, C. J., Shand, P. J., & Tyler, R. T. (2020). Oxidative stability of direct-expanded chickpea-sorghum snacks. *Food Science and Nutrition*, 8(8), 4340–4351. https://doi.org/10.1002/fsn3.1731
Bessada, S. M. F., Barreira, J. C. M., & Oliveira, M. B. P. P. (2019). Pulses and food security: Dietary protein, digestibility, bioactive and functional properties. *Trends in Food Science & Technology*, 93, 53–68. https://doi.org/10.1016/j.tifs.2019.08.022
Bhagayant, S. S., Gautam, A. K., Narvekar, D. T., Gupta, N., Bhadkaria, A., Srivastava, N., & Upadhyaya, H. D. (2018). Biochemical diversity evaluation in chickpea accessions employing mini-core collection. *Physiology and Molecular Biology of Plants*, 24(6), 1165–1183. https://doi.org/10.1007/s12298-018-0579-3
Chibbar, R. N., Ambigaipalan, P., & Hoover, R. (2010). Molecular diversity in pulse seed starch and complex carbohydrates and its role in human nutrition and health. *Cereal Chemistry*, 87(4), 342–352. https://doi.org/10.1094/cchem-87-4-0342
Duodu, K. G., Nunes, A., Delgadillo, L., Parker, M. L., Mills, E. N. C., Belton, P. S., & Taylor, J. R. N. (2002). Effect of grain structure and cooking on sorghum and maize in vitro protein digestibility. *Journal of Cereal Science*, 35(2), 161–174. https://doi.org/10.1016/s0733-5210(01)00011-x
Elkonin, L. A., Italianskaya, J. V., Fadeeva, I. Y., Bychkova, V. V., & Kozhemyakyn, V. V. (2013). In vitro protein digestibility in grain sorghum: Effect of genotype and interaction with starch digestibility. *Euphytica*, 193(3), 327–337. https://doi.org/10.1007/s10681-013-0920-4
Espinosa-Ramirez, J., & Serna-Saldívar, S. O. (2016). Functionality and characterization of kafirin-rich protein extracts from different whole and decorticated sorghum genotypes. *Journal of Cereal Science*, 70, 57–65. https://doi.org/10.1016/j.jcs.2016.05.023
FAOSTAT. (2019). Food and Agriculture Organization Statistics Database. Retrieved March 21, 2020, from http://www.fao.org/faostat/en/#data/QC
FAO/WHO. (1991). *Protein quality evaluation: Report of the joint FAO/WHO expert consultation*. Rome, Italy: Food and Agriculture Organization of the United Nations.
Ghumman, A., Kaur, A., Singh, N., & Singh, B. (2016). Effect of feed moisture and extrusion temperature on protein digestibility and extrusion behavior of lentil and horsegram. *Lebensmittel-Wissenschaft und Technologie-Food Science and Technology*, 70, 349–357. https://doi.org/10.1016/j.lwt.2016.05.023
Guzman-Ortiz, F. A., Hernandez-Sanchez, H., Yee-Madeira, H., Martinez, E., Robles-Ramirez, M., Rojas-Lopez, M., Berrios, J. D. J., & Mora-Escobedo, R. (2015). Physico-chemical, nutritional, and infrared spectroscopy evaluation of an optimized soybean/com flour extrudate. *Journal of Food Science and Technology-Mysore*, 52(7), 4066–4077. https://doi.org/10.1007/s13197-014-1485-5
House, J. D., Hill, K., Neufeld, J., Franczyk, A., & Nosworthy, M. G. (2019). Determination of the protein quality of almonds (Prunus dulcis L.) as assessed by in vitro and in vivo methodologies. *Food Science and Nutrition*, 7(9), 2932–2938. https://doi.org/10.1002/fsn3.1146
ISO. (2016). *Animal feeding stuffs: Determination of tryptophan content ISO 13904*. Geneva, Switzerland: International Organization for Standardization.
Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (Cicer arietinum L.) as reviewed. *British Journal of Nutrition*, 108(51), 11–26. https://doi.org/10.1017/s0007114512000797
Li, J., Liu, Y., Huang, Y., Yu, Z., Hu, M., Huang, R., & Wu, C. (2018). Investigation of Maillard reaction involvement in the steam processing of panax notoginseng root. *Journal of Pharmaceutical Research*, 17(2), 299–305. https://doi.org/10.4314/jpr.v17i2.15
Licata, R., Chu, J., Wang, S., Coorey, R., James, A., Zhao, Y., & Johnson, S. (2014). Determination of formulation and processing factors affecting slowly digestible starch, protein digestibility and antioxidant capacity of extruded sorghum-maize composite flour. *International Journal of Food Science and Technology*, 49(5), 1408–1419. https://doi.org/10.1111/jifs.12444
