



Research article

Effect of high-temperature, short-time cooking conditions on *in vitro* protein digestibility, enzyme inhibitor activity and amino acid profile of selected legume grains

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ABSTRACT

African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) flours were extruded in a single screw extruder at two extrusion temperatures; 100 °C and 140 °C, and the effect of extrusion cooking temperature on the chemical composition; crude protein, crude fibre, ether extract and nitrogen-free extracts, protein digestibility, enzyme inhibitor activity and amino acid profiles was investigated. The crude protein, amino acid profile and ether extract of the grain legumes were negatively affected ($p < 0.05$) by the extrusion cooking process, with a significant increase in nitrogen-free extracts for all grain legumes, and increased crude fibre of Bambara groundnut and Pigeon pea extrudates. Extrusion cooking of African yam beans and Pigeon pea produced extrudates with significantly lower trypsin, chymotrypsin and amylase inhibitor activity as well as improved protein digestibility. However, extrusion cooking did not modify the chymotrypsin and amylase inhibitor activity of Bambara groundnut extrudates. Extrusion cooking at 140 °C compared to 100 °C significantly reduced the protein quality of extrudates resulting in 22.94–51.27%, 5.11–25.18%, and 7.78–38.42% reduction in amino acid concentration of African yam beans, Bambara groundnut and Pigeon pea, respectively.

1. Introduction

Legume grains (pulses) are the dried fruits or seeds of plants belonging to the family Fabaceae examples of which are lentils, chickpeas, adzuki, black, kidney, lima, navy and pinto beans, and are relied on as staples for subsistence in combination with cereals. Legume grains are high energy-protein ingredients, containing about twice the protein (17%–40%) and an equivalent or higher energy content of cereal grains with total complex carbohydrates ranging from 65–72% in dried legume grains (Iqbal et al., 2006; USDA, 2018). Legume grains also furnish a significant proportion of dietary micronutrients such as iron, zinc, calcium, potassium, magnesium, niacin and folate in diets (De Jager et al., 2019; Messina, 2014). However, grain legumes also contain naturally occurring bioactive compounds such as phytic acid, lectins and enzyme inhibitors, that protect plants from biological stressors whilst conferring antinutritional attributes when used as food/feed (Campos-Vega et al., 2010; Food and Agriculture Organization of the United Nations, 1995; Lajolo and Genovese, 2002). These bioactive compounds impair nutrient digestion and bioavailability especially of protein, starch and trace

minerals (Proietti et al., 2015), lowering the feeding value of legume grains for both humans and livestock.

Enzyme inhibitors are ubiquitous proteins which have evolved as defense strategies in plants. Enzyme inhibitors impede the actions of insect and mammalian gastrointestinal digestive enzymes such as serine proteases (chymotrypsin and trypsin), amylase, lipase, glycosidase and phosphatases (Belitz and Weder, 1990; Payan, 2004).

α -amylase (EC 3.2.1.1) inhibitors impede the hydrolysis of α -1,4 glycosidic linkages in starch and oligosaccharides, which is essential for carbohydrate assimilation. In the event of ingestion of α -amylase inhibitors, a decreased rate of starch hydrolysis is expected, manifesting in reduced postprandial glucose peaks (Payan, 2004; Rahimzadeh et al., 2014) as well as reduction in serum glucose and insulin concentrations as observed in normal and diabetic rats administered a black bean (*Phaseolus vulgaris*) α -amylase inhibitor in a starch meal (Menezes and Lajolo, 1987). In other studies where broiler chicks and pigs were fed transgenic peas containing the α -amylase inhibitor gene, depressed growth and starch digestibility were reported (Collins et al., 2006).

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Trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) inhibitors are the most important known serine protease inhibitors. Ingestion of serine protease inhibitors significantly depresses protein hydrolysis and absorption in mammals (Belitz and Weder, 1990), and elicits physiological responses which include hypertrophy and hyperplasia (increase in size and number of acinar cells) of the pancreas (Ge and Morgan, 1993; Morgan et al., 1986), depressed growth (Grant et al., 2000; Palacios et al., 2004), depression in nutrient retention, especially energy, protein and sulphur-containing amino acids (Li et al., 1998) as well as hypersecretion of serine proteases (Corring et al., 1986; Grant et al., 2000), in pigs, birds, mice and rats.

Due to their proteic nature, enzyme inhibitors are often inactivated under conditions that permit irreversible protein denaturation such as acid, alkali and heat treatment. Heat treatment has proved the most effective processing technique to limit antinutritional factors and improve nutrient digestibility and bioavailability in legume grains (Alonso et al., 2000; Sun et al., 2006). Extrusion cooking; a high-temperature, short-time (HTST) process in which finely particulate forms of a starchy and/or proteinaceous raw material is moistened and conveyed through a heated barrel fitted with a rotating screw which effects compression, exerting shear energy under high pressure - is becoming widely accepted and adopted in the food/feed industry owing to its practicality, high productivity and efficiency, and nutrient retention during the cooking process as a result of limited exposure to high processing temperatures. Products of HTST extrusion possess physical and chemical properties which differ from those of the raw material used. Chemical changes are largely observed in the starch and protein constituents of food/feed ingredients subjected to high-temperature short-time extrusion cooking. These changes arise from thermal degradation of sugars and amino acids and depolymerization of starch, dietary fibre and proteins of these ingredients during extrusion (Camire, 1998). The combination of high temperature, mechanical shear and high pressure employed in extrusion cooking, modify the secondary and tertiary structure of proteins, impacting on full protein functionality. Whilst this process is favorable to the deactivation of thermally labile antinutritional factors such as protease (trypsin and chymotrypsin) inhibitors, it damages amino acids by breaking intermolecular bonds and destroying disulphide bonds/bridges of sulphur amino acids (Avilés-Gaxiola et al., 2018).

Little is known about the effect of HTST extrusion cooking on the chemical properties, protein quality and enzyme inhibitor activity of seemingly "under-utilized" legume grains such as African yam beans (*Sphenostylis stenocarpa*), Pigeon pea (*Cajanus cajan*), and Bambara groundnut (*Vigna subterranean*), limiting expanded use of these legume grains in the preparation of extruded foods and feeds. Therefore, this work focuses on studying the effect of HTST extrusion cooking at different extrusion temperatures in a single-screw extruder, on enzyme susceptibility of protein, serine protease and amylase inhibitory activity and amino acid profile of Bambara groundnut (*Vigna subterranean*), Pigeon pea (*Cajanus cajan*) and African yam beans (*Sphenostylis stenocarpa*) flours.

2. Materials and methods

2.1. Materials, extruder and processing conditions

Bambara groundnut (*Vigna subterranean*), Pigeon pea (*Cajanus cajan*) and African yam beans (*Sphenostylis stenocarpa*) were sourced locally, cleaned and finely milled to pass a 1mm sieve mesh. Moisture content of the flours (approximately 2kg each) was adjusted to 25% and hand mixed before extrusion in a single screw laboratory-scale extruder. Extruder characteristics were: screw diameter – 18.5mm, screw length – 304mm, screw speed - 60 rpm, diameter of the hole in the die plate -12.5mm, feed rate – 1.5 kg/h, and extrusion temperature (measured at the outlet die) -100 °C (low temperature treatment) and 140 °C (high temperature treatment), respectively. After extrusion, extrudates were dried in a

forced air oven at 50 °C overnight (Al-Rabadi et al., 2011), milled and stored in plastic bags at 4 °C.

2.2. Experimental design

The effect of HTST extrusion cooking and extrusion cooking temperatures on protein quality, digestibility and amino acid profile of legume grains was investigated in Bambara groundnut (*Vigna subterranean*), Pigeon pea (*Cajanus cajan*) and African yam beans (*Sphenostylis stenocarpa*). The independent variable considered was extrusion temperature (100 °C and 140 °C), while unextruded (raw) flours served as controls.

2.3. Analytical methods

2.3.1. Chemical analyses

The chemical composition; ether extract, crude protein, crude fibre and nitrogen-free extract of the unextruded and extruded legume grains were determined in triplicates. Dry matter was determined by drying in a forced air oven at 105 °C to constant weight, and ether extract determined by the Soxhlet extraction method with petroleum ether as solvent (AOAC, 2000). Crude fibre was determined gravimetrically after detergent digestion and solubilization of non-fibre fractions and subsequently corrected for ash content (AOAC, 2000). Nitrogen content was determined by the Kjeldahl method (AACC, 2008) and converted to crude protein using 6.25 as the conversion factor, while nitrogen-free extract was calculated as the balance when the sum of percentage values of ether extract, crude protein, ash and crude fibre were deducted from 100%, on dry matter basis.

2.3.2. In vitro protein digestibility

Raw and extruded flours were digested in a multienzyme simulation (Sopade and Gidley, 2009) of gastric and intestinal digestion in monogastrics. Briefly, 125mg of each sample was digested in quadruplicate, with 250µL of artificial saliva containing α-amylase (Megazyme, E-BLAAM (α-amylase from *Bacillus licheniformis*), 250U of α-amylase per mL carbonate buffer) for 15–20 s. Then 1.25 mL of pepsin (Sigma-Aldrich, P7000 (pepsin from hog stomach) 250U/mL of 0.02M aq. HCl) was added and incubated at 37 °C for 30 min in a water bath with intermittent shaking. The digesta was subsequently neutralized with 1.25mL of 0.02M aq. NaOH, then adjusted to pH 6 by the addition of 6.25mL of 0.2M sodium acetate buffer. Thereafter, 1.25mL of pancreatin solution (Sigma-Aldrich, P1750 from porcine pancreas, 2mg pancreatin per ml of acetate buffer (consisting of ~ 8U lipase/mg solid +100U protease/mg solid) per ml of acetate buffer) and 1 mL amyloglucosidase (Megazyme, E-AMGDF (amyloglucosidase from *Apergillus* sp, 28U per ml of acetate buffer) were added and incubated at 37 °C for 4 h. Blanks were similarly setup as described above, but free of the flours to enable estimation of the nitrogen contribution of enzymes and buffers. Each tube was transferred onto ice and subsequently centrifuged at 14,000 × g for 5 min and the supernatant stored at -20 °C till further analysis. Nitrogen in the supernatant was estimated by the Kjeldahl method (Frias et al., 2011; Singh and Jambunathan, 1981) and nitrogen due to the enzymes and buffer was discounted. Protein digestibility calculated as:

$$\text{Protein digestibility} = \frac{\text{nitrogen content of supernatant}}{\text{nitrogen content of the sample}} \times 100 \times 6.25$$

2.3.3. Trypsin, chymotrypsin and amylase inhibitory activity

The ability of crude extracts of the legume grain flours to impede activity of enzyme standards *in vitro* was determined and residual enzyme activity calculated at the end of incubations.

Legume grain flours were first extracted in 100mM Tris-Cl buffer, pH 8, and enzyme - trypsin, chymotrypsin and amylase - inhibitory activities of each extract were determined (Palavalli et al., 2012; Tremacoldi and Pascholati, 2002). Briefly, 50 µL crude extracts were incubated at

37 °C for 10 min with 10mL of trypsin (Sigma-Aldrich T-4799, from porcine pancreas) in 1mM HCl and 0.1M Tris-HCl buffer, pH 8 containing 0.01M CaCl₂ to make a final volume of 500 µL. Thereafter 1mM of chromogenic substrates: N-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA, B-4875, Sigma-Aldrich) or N-Benzoyl-L-tyrosine-p-nitroanilide (BTPNA, B-6760, Sigma-Aldrich), for trypsin and chymotrypsin determinations respectively, were added to the mix and incubated at 37 °C for 10 min. The reaction was stopped by the addition of 500 µL acetic acid to each tube and read at 405 nm. Controls were obtained by adding acetic acid to each tube before incubation with the corresponding chromogenic substrate, while 100% enzyme activity was obtained by replacing the crude extracts in the reaction mix with water. Absorbance values for the control was deducted from the sample containing the crude extract, and further deducted from the absorbance value for 100% activity, allowing for calculation of the residual activity of trypsin and chymotrypsin per unit of crude extract (Tremacoldi and Pascholati, 2002).

Amylase inhibitory activity was determined by incubating crude extracts with the chromogenic substrate 4,6-benzylidene (G₇)-p-nitrophenol (G₁)-α-D-maltoheptaoside (Amylase BPS, Quimica Clinica Aplicada, S.A), and residual amylase activity was calculated. Determinations for enzyme inhibitory activity were conducted in quadruplicates.

2.3.4. Protein quality test: amino acid profiling

Amino acid concentrations of the unextruded and extruded legume grain flours was assayed by a method described by Benitez (1989). Briefly, 600mg of defatted sample was weighed into a sealable glass ampoule, into which 7mls of 6M HCl was added, passed under a nitrogen flow, sealed and digested in an oven at 105 °C for 22 h. After cooling, the hydrolysate was filtered, lyophilized and reconstituted in 5ml acetate buffer (pH 2.0). A parallel alkaline digestion was conducted using 10ml of 4.2M NaOH at 105 °C for 4 h. The filtrate was neutralized to pH 7.0, lyophilized and reconstituted in 5ml borate buffer (pH 9.0) for the determination of tryptophan. Samples (60µl) were then introduced into the amino acid analyzer (Applied Biosystems Inc, California, USA), with norleucine as the internal standard. The amino acids determined are essential amino acids; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, and non-essential amino acids; alanine, aspartate, cystine, glutamate, glycine, proline, serine, and tyrosine. Subsequently, total amino acids, total essential amino acids and total non-essential amino acids were tallied. Amino acid determinations were conducted in triplicates.

2.4. Statistics

Data were subjected to one way analysis of variance (ANOVA) using JASP (Version 0.13.1) computer software. Three orthogonal contrasts were included to study the effect of HTST extrusion cooking; unextruded vs 100 °C and unextruded vs 140 °C, and effect of cooking temperatures; 100 °C vs 140 °C, on all legume grains studied and considered to be significant at p < 0.05.

3. Results

3.1. Effect of HTST extrusion cooking and temperatures on the chemical composition of African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) flours

Figures 1, 2, and 3 show the crude protein, crude fibre, ether extract and nitrogen-free extract composition of African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) flours subjected to HTST extrusion cooking at 100 and 140 °C. Reduction (p < 0.05) in the crude protein and ether extract content as well as increase (p < 0.05) in nitrogen-free extract of flours were recorded in African yam beans, Bambara groundnut and Pigeon pea extruded at 100 °C and 140 °C. A significant increase in crude fibre of flours was recorded for African yam beans, Bambara groundnut and Pigeon pea. However, extrusion at 140 °C compared to 100 °C resulted in a marginal reduction in crude fibre observed for African yam beans (4.38%–4.28%). Increasing extrusion temperature from 100 °C to 140 °C significantly reduced the crude protein of African yam beans, Bambara groundnut and Pigeon pea extrudates by 9.84, 1.77 and 7.02%, respectively. Nitrogen-free extract of African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) extrudates was also increased (p < 0.01) by 8.24 and 8.04% respectively, when extrusion temperature was increased from 100 °C to 140 °C. Extrusion of grain legume flours at 140 °C also significantly lowered the ether extract content of Bambara groundnut by 38.78% compared to 100 °C extrudates, while increasing crude fibre in pigeon pea by 16.52% over corresponding 100 °C extrudates.

3.2. Effect of HTST extrusion cooking and temperatures on in vitro protein digestibility of flours

The effect of HTST extrusion cooking and extrusion cooking temperatures on in vitro enzyme digestibility of proteins of African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) flours in a buffered multienzyme media is

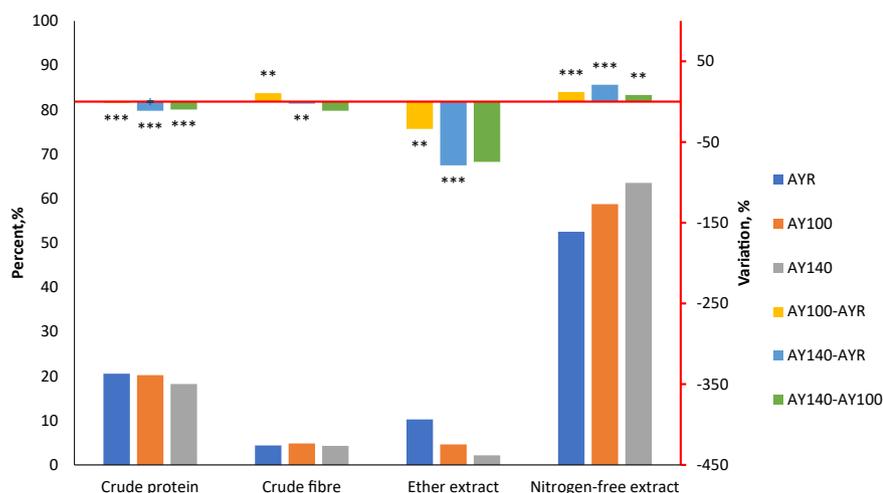


Figure 1. Crude protein, crude fibre, crude fat, and nitrogen free extract of unextruded and extruded African yam beans (*Sphenostylis stenocarpa*) flours (on dry weight basis) and variations due to extrusion and extrusion temperature treatments. AYR - unextruded African yam beans flour; AY100 - African yam beans flour extruded at 100 °C; AY140 - African yam beans flour extruded at 140 °C. Values are means of results obtained in triplicate *** p < 0.001 **p < 0.01. % variation: AY100-AYR = variation due to extrusion at 100 °C, i.e. $\frac{AY100-AYR}{AYR} \times 100$. % variation: AY140-AYR = variation due to extrusion at 140 °C, i.e. $\frac{AY140-AYR}{AYR} \times 100$. % variation: AY140-AY100 = variation due to extrusion temperature, i.e. $\frac{AY140-AY100}{AY100} \times 100$

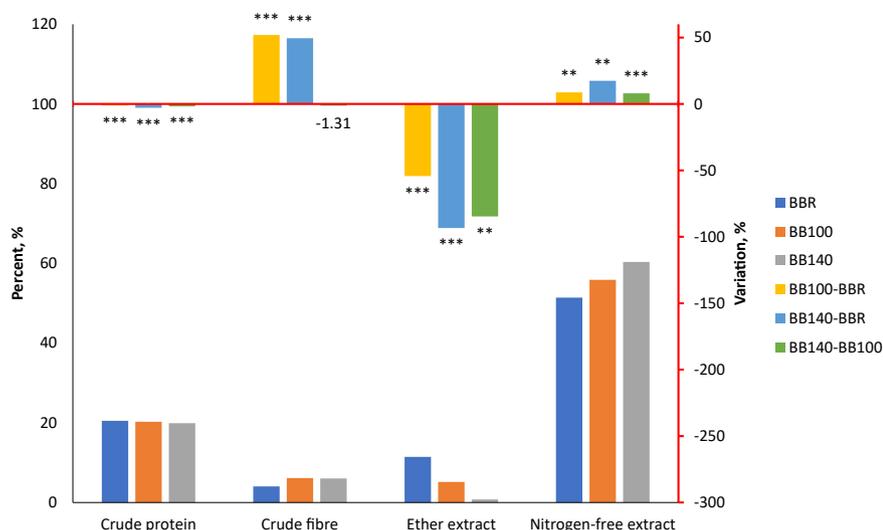


Figure 2. Crude protein, crude fibre, crude fat, and nitrogen free extract of unextruded and extruded Bambara groundnut (*Vigna subterranean*) flours (on dry weight basis) and variations due to extrusion and extrusion temperature treatments. BBR - unextruded Bambara groundnut flour; BB100 - Bambara groundnut extruded at 100 °C; BB140 - Bambara groundnut extruded at 140 °C. Values are means of results obtained in triplicate ***p < 0.001 **p < 0.01. % variation: BB100-BBR = variation due to extrusion at 100 °C, i.e. $\frac{BB100-BBR}{BBR} \times 100$. % variation: BB140-BBR = variation due to extrusion at 140 °C, i.e. $\frac{BB140-BBR}{BBR} \times 100$. % variation: BB140-BB100 = variation due to extrusion temperature, i.e. $\frac{BB140-BB100}{BB100} \times 100$

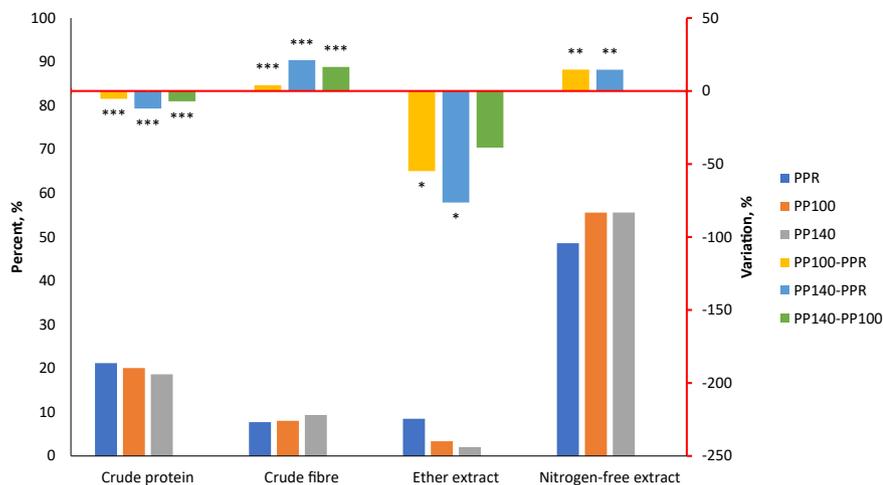


Figure 3. Crude protein, crude fibre, crude fat, and nitrogen free extract of unextruded and extruded Pigeon pea (*Cajanus cajan*) flours (on dry weight basis) and variations due to extrusion and extrusion temperature treatments. PPR - unextruded Pigeon pea flour; PP100 - Pigeon pea extruded at 100 °C; PP140 - Pigeon pea extruded at 140 °C. † Values are means of results obtained in triplicate ***p < 0.001 **p < 0.01 *p < 0.05. % variation: PP100-PPR = variation due to extrusion at 100 °C, i.e. $\frac{PP100-PPR}{PPR} \times 100$. % variation: PP140-PPR = variation due to extrusion at 140 °C, i.e. $\frac{PP140-PPR}{PPR} \times 100$. % variation: PP140-PP100 = variation due to extrusion temperature, i.e. $\frac{PP140-PP100}{PP100} \times 100$

summarized in Figures 4, 5, and 6. *In vitro* digestion of protein was increased by extrusion of African yam beans at 100 °C and 140 °C by 151% and 228%, respectively, with a variation of 30.8% in protein digestion, when flours extruded at 100 °C were compared with flours extruded at 140 °C. Protein digestion was also increased by extrusion of

Pigeon pea at 100 °C and 140 °C by 64.95% and 27.66%, respectively, with a variation of 22.61% when flours extruded at 100 °C were compared to flours extruded at 140 °C. However, no significant effect (p < 0.05) of extrusion cooking and/or extrusion cooking temperatures was observed in Bambara groundnut flours.

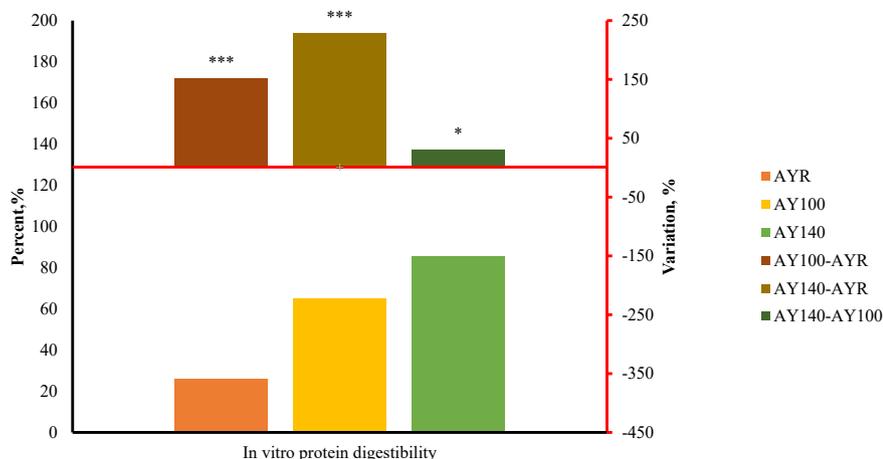


Figure 4. *In vitro* protein digestibility (IVPD) of unextruded and extruded African yam beans (*Sphenostylis stenocarpa*), flours (on dry weight basis) and variations in *in vitro* protein digestibility between temperature treatments. AYR - unextruded African yam beans flour; AY100 - African yam beans flour extruded at 100 °C; AY140 - African yam beans flour extruded at 140 °C. † Values are means of results obtained in quadruplicate ***p < 0.001 *p < 0.05. % variation: AY100-AYR = variation due to extrusion at 100 °C, i.e. $\frac{AY100-AYR}{AYR} \times 100$. % variation: AY140-AYR = variation due to extrusion at 140 °C, i.e. $\frac{AY140-AYR}{AYR} \times 100$. % variation: AY140-AY100 = variation due to extrusion temperature, i.e. $\frac{AY140-AY100}{AY100} \times 100$

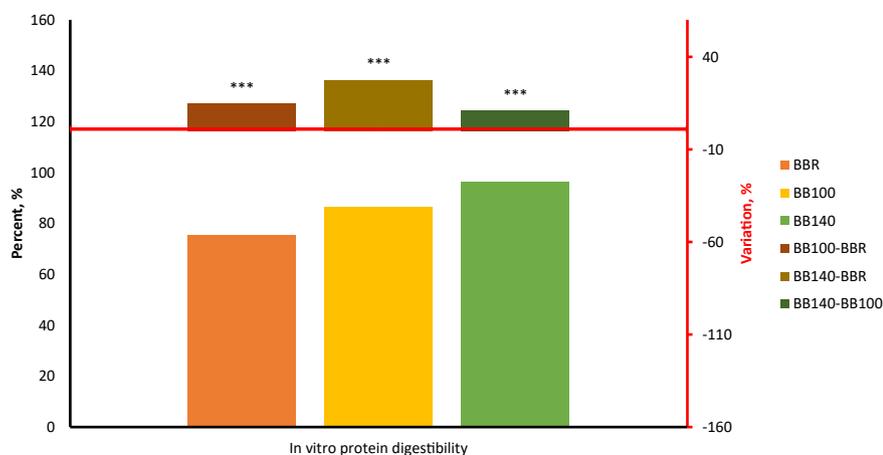


Figure 5. *In vitro* protein digestibility (IVPD) of unextruded and extruded Bambara groundnut (*Vigna subterranean*) flours (on dry weight basis) and variations in *in vitro* protein digestibility between temperature treatments. BBR - unextruded Bambara groundnut flour; BB100 - Bambara groundnut extruded at 100 °C; BB140 - Bambara groundnut extruded at 140 °C. † Values are means of results obtained in quadruplicate ***p < 0.001. % variation: BB100-BBR = variation due to extrusion at 100 °C, i.e. $\frac{BB100-BBR}{BBR} \times 100$. % variation: BB140-BBR = variation due to extrusion at 140 °C, i.e. $\frac{BB140-BBR}{BBR} \times 100$. % variation: BB140-BB100 = variation due to extrusion temperature, i.e. $\frac{BB140-BB100}{BB100} \times 100$

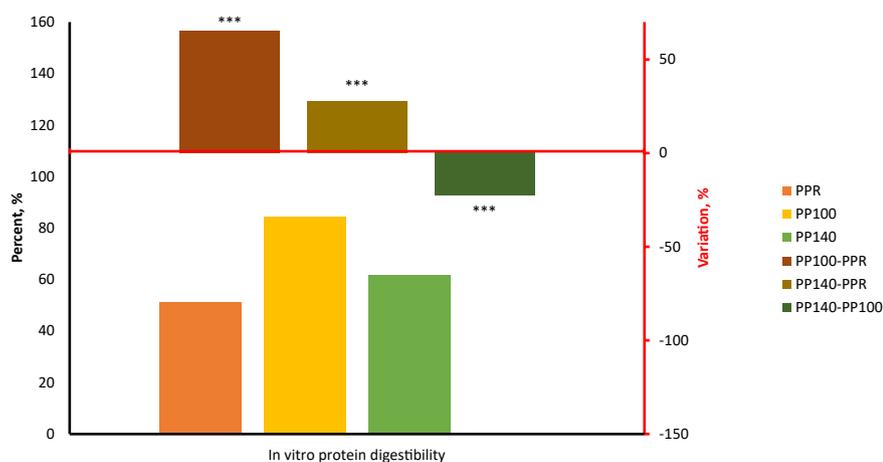


Figure 6. *In vitro* protein digestibility (IVPD) of unextruded and extruded Pigeon pea (*Cajanus cajan*) flours (on dry weight basis) and variations in *in vitro* protein digestibility between temperature treatments. PPR - unextruded Pigeon pea flour; PP100 - Pigeon pea extruded at 100 °C; PP140 - Pigeon pea extruded at 140 °C. † Values are means of results obtained in quadruplicate ***p < 0.001. % variation: PP100-PPR = variation due to extrusion at 100 °C, i.e. $\frac{PP100-PPR}{PPR} \times 100$. % variation: PP140-PPR = variation due to extrusion at 140 °C, i.e. $\frac{PP140-PPR}{PPR} \times 100$. % variation: PP140-PP100 = variation due to extrusion temperature, i.e. $\frac{PP140-PP100}{PP100} \times 100$

3.3. Effect of HTST extrusion cooking and temperatures on enzyme inhibitor activity of flours

Amylase, trypsin and chymotrypsin inhibitor activities were readily detected in crude extracts of the selected legumes to varying degrees; chymotrypsin inhibitor activity > trypsin inhibitor activity > amylase inhibitor activity. Residual enzyme - amylase, trypsin and chymotrypsin activities in the presence of crude extracts of Bambara groundnut, Pigeon pea and African yam beans flours are shown in Figures 7, 8, and 9.

Residual amylase activity in the presence of African yam beans flours was significantly (p ≤ 0.001) increased by 14.64% and 15.58% when extruded at 100 °C and 140 °C, respectively. Extrusion of African yam beans at 140 °C significantly (p < 0.05) influenced residual trypsin and chymotrypsin activities by 10.96% and 144.78% respectively, although extrusion at 100 °C did not improve residual trypsin and chymotrypsin activities when compared with the raw flours. Extrusion of Bambara groundnut had not significant effect on residual amylase and chymotrypsin activities, but significantly increased residual trypsin activity by

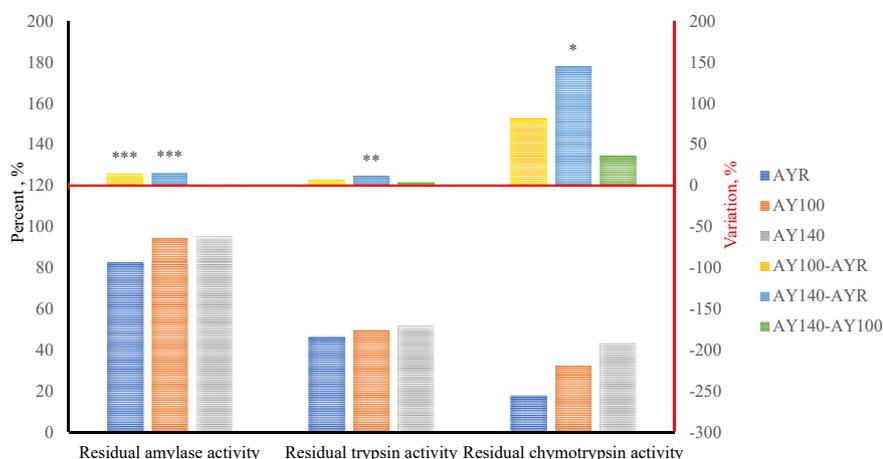


Figure 7. Effect of extrusion temperatures on residual enzyme - amylase, trypsin and chymotrypsin - activity in the presence of African yam beans (*Sphenostylis stenocarpa*) flours (on dry weight basis) and variations in residual enzyme activity between temperature treatments. AYR - unextruded African yam beans flour; AY100 - African yam beans flour extruded at 100 °C; AY140 - African yam beans flour extruded at 140 °C. † Values are means of results obtained in quadruplicate ***p ≤ 0.001 **p ≤ 0.01 *p < 0.05. % variation: AY100-AYR = variation due to extrusion at 100 °C, i.e. $\frac{AY100-AYR}{AYR} \times 100$. % variation: AY140-AYR = variation due to extrusion at 140 °C, i.e. $\frac{AY140-AYR}{AYR} \times 100$. % variation: AY140-AY100 = variation due to extrusion temperature, i.e. $\frac{AY140-AY100}{AY100} \times 100$

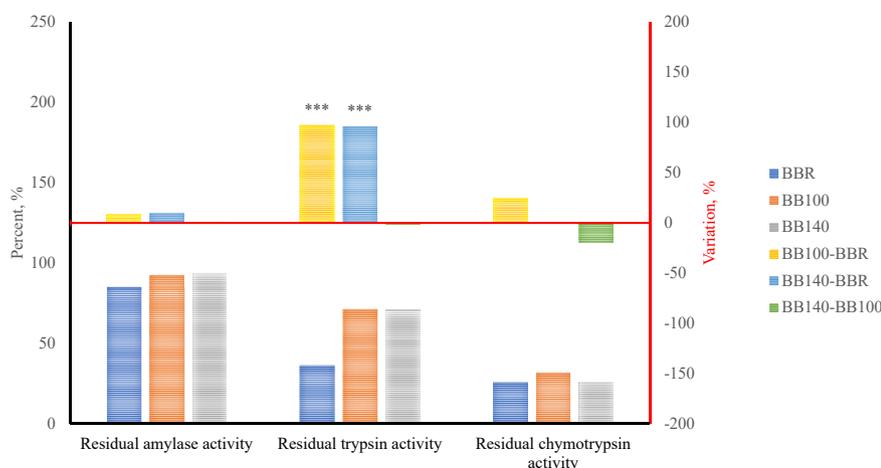


Figure 8. Effect of extrusion temperatures on residual enzyme - amylase, trypsin and chymotrypsin - activity in the presence of Bambara groundnut (*Vigna subterranean*) flours (on dry weight basis) and variations in residual enzyme activity between temperature treatments. BBR - unextruded Bambara groundnut flour; BB100 - Bambara groundnut flour extruded at 100 °C; BB140 - Bambara groundnut flour extruded at 140 °C. † Values are means of results obtained in quadruplicate ***p ≤ 0.001. % variation: BB100-BBR = variation due to extrusion at 100 °C, i.e. $\frac{BB100-BBR}{BBR} \times 100$. % variation: BB140-BBR = variation due to extrusion at 140 °C, i.e. $\frac{BB140-BBR}{BBR} \times 100$. % variation: BB140-BB100 = variation due to extrusion temperature, i.e. $\frac{BB140-BB100}{BB100} \times 100$

97.64 and 96.09% respectively. Assessment of unextruded and extruded pigeon pea flours showed significant increases in residual amylase activity by 15.32% (p ≤ 0.001) and 15.08% (p ≤ 0.01) when extruded at 100 °C and 140 °C respectively, with a significant (p ≤ 0.001) increase of 139.6% in residual trypsin activity observed when flours were extruded at 140 °C. Extrusion of pigeon pea flours at 100 °C and 140 °C also significantly (p < 0.05) increased residual chymotrypsin activity by 72.38% and 73.23% respectively, with no effect of extrusion cooking temperatures (100 °C vs 140 °C) observed on residual amylase, trypsin and chymotrypsin activities in all the grain legumes assessed except in pigeon pea, where extrusion at 140 °C increased residual trypsin activity by 147.3% over flours extruded at 100 °C.

3.4. Effect of HTST extrusion cooking temperatures on amino acid profiles of flours

Tables 1, 2, and 3 summarize the effects of extrusion cooking and extrusion cooking temperatures on the amino acid profile of African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) flours. A significant reduction in concentration of all amino acids due to extrusion cooking at 100 °C (i.e. raw vs 100 °C extrudates), and 140 °C (raw vs 140 °C extrudates) and extrusion cooking temperature (i.e. 100 °C extrudates vs 140 °C extrudates) were observed in African yam beans and Pigeon pea flours. Variations in amino acid concentration of African yam beans due to extrusion at 100 °C and 140 °C were least for tyrosine (3.05%) and glutamate (25.95%) and highest for threonine (17.25%) and methionine (57.86%), respectively, while increasing extrusion temperature from 100 °C to 140

°C resulted in variations in amino acid concentration ranging from 22.94% for alanine to 52.63% for methionine. Similarly, variations in amino acid concentration of Pigeon pea due to extrusion at 100 °C and 140 °C were least for alanine (4.85%) and tyrosine (21.52%) and highest for threonine (24.41%) and cystine (51.35%), respectively, while increasing extrusion temperature from 100 °C to 140 °C resulted in variations in amino acid concentration ranging from 15.26% for threonine to 32.08% for cystine. Extrusion of Bambara groundnut flour at 100 °C significantly reduced the concentration of all amino acids except methionine, cystine, glutamate, proline, serine and tyrosine, while extrusion at 140 °C significantly reduced the concentration of all amino acids except tyrosine. Extruding Bambara groundnut flours at 140 °C rather than 100 °C resulted in significant decline in the concentration of all amino acids except tryptophan, alanine and tyrosine. Variations in amino acid concentrations of Bambara groundnut extruded at 100 °C ranged from 2.17% for arginine to 9.09% for threonine and ranged from 3.93% for tryptophan to 28.03% for cystine in Bambara groundnut extruded at 140 °C, while increasing extrusion temperature from 100 °C to 140 °C resulted in variations in amino acid concentration ranging from 6.21% for serine to 25.18% for cystine. Reduction in TAA, TEAA and TNEAA of African yam bean flours due to extrusion cooking (i.e raw vs 100 °C extrudates and, raw vs 140 °C extrudates) range from 7.07-35.37%, 9.15-39.45% and 5.17-31.36%, respectively. For pigeon pea flours, reduction in TAA, TEAA and TNEAA due to extrusion cooking ranged from 16.49-34.14%, 18.80-38.67% and 14.14-30.30%, respectively. While no significant effect of extrusion cooking was observed on TAA of Bambara groundnut flours, TEAA and TNEAA were reduced by 3.88-9.29% and 1.98-10.74% respectively. In similar fashion, extrusion

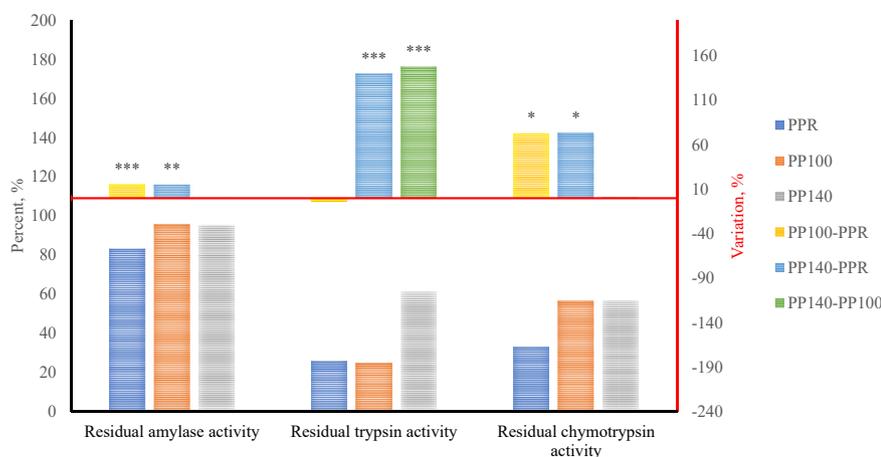


Figure 9. Effect of extrusion temperatures on residual enzyme - amylase, trypsin and chymotrypsin - activity in the presence of Pigeon pea (*Cajanus cajan*) flours (on dry weight basis) and variations in residual enzyme activity between temperature treatments. PPR - unextruded Pigeon pea flour; PP100 - Pigeon pea flour extruded at 100 °C; PP140 - Pigeon pea flour extruded at 140 °C. † Values are means of results obtained in quadruplicate ***p ≤ 0.001 **p ≤ 0.01 *p < 0.05. % variation: PP100-PPR = variation due to extrusion at 100 °C, i.e. $\frac{PP100-PPR}{PPR} \times 100$. % variation: PP140-PPR = variation due to extrusion at 140 °C, i.e. $\frac{PP140-PPR}{PPR} \times 100$. % variation: PP140-PP100 = variation due to extrusion temperature, i.e. $\frac{PP140-PP100}{PP100} \times 100$

Table 1. Effect of high-temperature, short-time extrusion cooking temperatures on amino acid profile and nitrogen content, N% of African yam beans (*Sphenostylis stenocarpa*) flours.

	African yam beans			% Variation			Contrast P- value		
	AYR	AY100	AY140	[1]	[2]	[3]	AYR vs AY100	AYR vs AY140	AY100 vs AY140
Essential amino acids (mg/100mg protein)									
Arginine	1.33 ± 0.01	1.25 ± 0.01	0.84 ± 0.00	-5.90	-36.66	-32.68	***	***	***
Histidine	0.76 ± 0.00	0.69 ± 0.01	0.42 ± 0.02	-8.31	-44.40	-39.37	***	***	***
Isoleucine	0.85 ± 0.00	0.79 ± 0.01	0.55 ± 0.00	-6.77	-35.07	-30.36	***	***	***
Leucine	1.60 ± 0.00	1.44 ± 0.01	1.03 ± 0.05	-9.92	-35.83	-28.76	***	***	***
Lysine	1.31 ± 0.01	1.20 ± 0.01	0.77 ± 0.04	-8.23	-41.07	-35.79	**	***	***
Methionine	0.24 ± 0.01	0.22 ± 0.01	0.10 ± 0.01	-11.20	-57.86	-52.63	**	***	***
Phenylalanine	0.77 ± 0.01	0.70 ± 0.01	0.51 ± 0.02	-9.61	-33.58	-26.52	***	***	***
Threonine	0.67 ± 0.01	0.56 ± 0.01	0.43 ± 0.03	-17.25	-36.48	-23.29	***	***	***
Tryptophan	0.20 ± 0.00	0.18 ± 0.00	0.13 ± 0.00	-11.52	-37.17	-28.97	***	***	***
Valine	0.87 ± 0.01	0.79 ± 0.01	0.43 ± 0.01	-9.28	-50.79	-45.77	***	***	***
Non-essential amino acids									
Alanine	0.76 ± 0.00	0.70 ± 0.01	0.54 ± 0.01	-7.75	-28.94	-22.94	***	***	***
Aspartate	2.18 ± 0.01	2.09 ± 0.01	1.58 ± 0.02	-3.82	-27.61	-24.72	***	***	***
Cysteine	0.24 ± 0.01	0.21 ± 0.00	0.10 ± 0.01	-10.93	-56.62	-51.27	***	***	***
Glutamate	2.75 ± 0.02	2.65 ± 0.01	2.03 ± 0.01	-3.62	-25.95	-23.16	***	***	***
Glycine	0.79 ± 0.01	0.71 ± 0.00	0.51 ± 0.01	-10.24	-35.64	-28.28	***	***	***
Proline	0.62 ± 0.01	0.58 ± 0.01	0.42 ± 0.01	-6.99	-31.81	-26.65	***	***	***
Serine	0.73 ± 0.01	0.70 ± 0.00	0.35 ± 0.00	-3.30	-52.22	-50.58	**	***	***
Tyrosine	0.65 ± 0.01	0.63 ± 0.00	0.45 ± 0.01	-3.05	-30.92	-28.74	*	***	***
Total amino acids	17.30 ± 0.00	16.08 ± 0.05	11.18 ± 0.18	-7.07	-35.37	-30.45	***	***	***
Total essential amino acids	8.60 ± 0.01	7.81 ± 0.04	5.21 ± 0.12	-9.15	-39.45	-33.36	***	***	***
Total non-essential amino acids	8.70 ± 0.01	8.27 ± 0.01	5.98 ± 0.06	-5.17	-31.36	-27.62	***	***	***

Values are mean results obtained in triplicates ± standard deviation, and expressed as g/100g sample.

***p < 0.001 **p < 0.01 *p < 0.05.

AYR - unextruded African yam beans flour; AY100 - African yam bean extruded at 100 °C; AY140 - African yam beans extruded at 140 °C.

% variation: [1] = variation due to extrusion at 100 °C, i.e. $\frac{AY100 - AYR}{AYR} \times 100$

% variation: [2] = variation due to extrusion at 140 °C, i.e. $\frac{AY140 - AYR}{AYR} \times 100$

% variation: [3] = variation due to extrusion temperature, i.e. $\frac{AY140 - AY100}{AY100} \times 100$

of African yam beans and pigeon pea flours at 140 °C rather than 100 °C reduced TAA, TEAA and TNEAA by 30.45 and 21.13%, 33.36 and 24.47%, and 27.62 and 18.82% respectively. On the other hand, extrusion of Bambara groundnut flours at 140 °C rather than 100 °C did not significantly influence TAA and TEAA concentrations, whilst reducing TNEAA concentrations by 8.94%.

4. Discussion and conclusion

Few studies exploring the effect of HTST extrusion cooking and extrusion cooking temperatures on chemical composition of sole African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) flours exist (Okafor et al., 2014; Okpala et al., 2016; Omeire, 2012). The choice of extrusion conditions i.e. moisture and temperature, was based on earlier studies (Adeleye et al., 2020; Al-Rabadi et al., 2011). The chemical composition of raw African yam beans, Bambara groundnut and Pigeon pea flours observed in this study are similar to those published in earlier literature and databases (Heuzé et al., 2016, 2017; Heuzé and Tran, 2016; Nwokolo, 1987; Solomon et al., 2017). Increased dietary fibre content of extrudates as observed in this study, was also observed in wheat and barley flours extruded at temperatures between 150 °C and 200 °C. Conversely, no significant effect of extrusion at 140 °C to 180 °C was observed on total dietary fibre of two cultivars of *Phaseolus vulgaris*, although a significant redistribution of insoluble to more water soluble fibre fractions was

reported (Martin-Cabrejas et al., 1999). A varietal influence on fibre profile of barley was also reported in response to increased extrusion temperature and moisture, with a decline in insoluble fibre reported for CDC-Candle barley and a significant increase in insoluble fibre and resistant starch for Phoenix barley (Vasanthan et al., 2002). Modification in fibre profile of extruded products in response to increasing temperature is explained as due to a combination of: (a) reorganization of dietary fibre fractions with a shift from insoluble to soluble dietary fibre (Martin-Cabrejas et al., 1999; Vasanthan et al., 2002), (b) the formation of retrograded amylose (RS3) which are fermentable and insoluble (Warren et al., 2018), and mimic dietary fibre (Slavin, 2013), and, (c) formation of “enzyme resistant indigestible glucans” by the process of transglycosidation (Vasanthan and Temelli, 2008). The extrusion of blends (50:50) of navy bean-corn and pinto bean-corn in a twin extruder (110 °C, 310rpm) similarly lowered lipid content of extrudates by 47.16% and 75.45% respectively (Vadukapuram et al., 2014) like in this study. The decrease in ether extractable lipids in extrudates could be attributed to lipid binding that occurs under extrusion cooking conditions. The lipid binding abilities of amylose molecules enable the assembly of amylose-lipid complexes (ALCs) and amylose-lipid-protein complexes (ALPCs) under thermomechanical conditions similar to HTST extrusion conditions (Camire, 2000). Lipid molecules bound within these complexes are not extractable by ether extraction. Lipid binding within these complexes also increases with increasing extrusion temperature and feed moisture, evidenced in significant fat loss of extrudates (Gui et al., 2012;

Table 2. Effect of high-temperature, short-time extrusion cooking temperatures on amino acid profile and nitrogen content, N% of Bambara groundnut (*Vigna subterranean*) flours.

	Bambara groundnut			% Variation			Contrast P- value		
	BBR	BB100	BB 140	[1]	[2]	[3]	BBR vs BB100	BBR vs BB140	BB100 vs BB140
Essential amino acids (mg/100mg protein)									
Arginine	1.30 ± 0.00	1.28 ± 0.00	1.16 ± 0.00	-2.17	-11.31	-9.34	***	***	***
Histidine	0.73 ± 0.01	0.71 ± 0.01	0.63 ± 0.00	-3.23	-13.92	-11.04	**	***	***
Isoleucine	0.83 ± 0.01	0.80 ± 0.00	0.73 ± 0.01	-3.24	-12.30	-9.36	***	***	***
Leucine	1.56 ± 0.00	1.49 ± 0.01	1.39 ± 0.01	-4.47	-10.76	-6.58	***	***	***
Lysine	1.27 ± 0.01	1.23 ± 0.01	1.05 ± 0.01	-2.84	-17.22	-14.80	***	***	***
Methionine	0.23 ± 0.01	0.22 ± 0.01	0.18 ± 0.01	-	-23.63	-20.35	NS	***	***
Phenylalanine	0.74 ± 0.01	0.71 ± 0.01	0.65 ± 0.01	-3.08	-11.42	-8.60	***	**	***
Threonine	0.65 ± 0.01	0.59 ± 0.00	0.54 ± 0.00	-9.09	-16.82	-8.50	***	***	***
Tryptophan	0.20 ± 0.00	0.18 ± 0.00	0.19 ± 0.00	-6.88	-3.93	-	***	*	NS
Valine	0.84 ± 0.00	0.81 ± 0.00	0.76 ± 0.01	-4.06	-8.96	-5.11	***	***	***
Non-essential amino acids									
Alanine	0.75 ± 0.00	0.72 ± 0.00	0.73 ± 0.01	-2.92	-1.98	-	***	**	NS
Aspartate	2.15 ± 0.00	2.06 ± 0.01	1.91 ± 0.02	-4.27	-11.24	-7.28	***	***	***
Cysteine	0.23 ± 0.00	0.22 ± 0.00	0.17 ± 0.01	-	-28.03	-25.18	NS	***	***
Glutamate	2.72 ± 0.02	2.70 ± 0.00	2.42 ± 0.01	-	-11.17	-10.30	NS	***	***
Glycine	0.77 ± 0.01	0.74 ± 0.01	0.65 ± 0.01	-4.57	-16.33	-12.33	***	***	***
Proline	0.60 ± 0.01	0.62 ± 0.01	0.54 ± 0.01	-	-10.39	-13.29	NS	***	***
Serine	0.69 ± 0.01	0.69 ± 0.01	0.65 ± 0.00	-	-5.23	-6.21	NS	***	***
Tyrosine	0.63 ± 0.00	0.63 ± 0.01	0.63 ± 0.01	-	-	-	NS	NS	NS
Total amino acids	15.86 ± 1.08	16.39 ± 0.01	14.96 ± 0.03	-	-	-	NS	NS	NS
Total essential amino acids	8.34 ± 0.01	8.02 ± 0.01	7.27 ± 0.01	-3.88	-9.29	-	***	**	NS
Total non-essential amino acids	7.30 ± 0.04	7.13 ± 0.01	6.47 ± 0.00	-1.98	-10.74	-8.94	***	***	***

Values are mean results obtained in triplicates ± standard deviation, and expressed as g/100g sample.

***p < 0.001 **p < 0.01 *p < 0.05.

BBR - unextruded Bambara groundnut flour; BB100 - Bambara groundnut extruded at 100 °C; BB140 - Bambara groundnut extruded at 140 °C.

% variation: [1] = variation due to extrusion at 100 °C, i.e. $\frac{BB100 - BBR}{BBR} \times 100$

% variation: [2] = variation due to extrusion at 140 °C, i.e. $\frac{BB140 - BBR}{BBR} \times 100$

% variation: [3] = variation due to extrusion temperature, i.e. $\frac{BB140 - BB100}{BB100} \times 100$

Gunzman et al., 1992; Nosworthy et al., 2018; De Pilli et al., 2008) and increased bound lipid fractions (Zadernowski et al., 1997). Due to the resistance of amylose-lipid complexes to amylolytic enzyme hydrolysis (Hasjim et al., 2013) and acid resistant characteristics which increase with increasing amylose degree of polymerization, lipid chain length and complexation temperature (Gelders et al., 2005), amylose-lipid complexes also mimic dietary fibre, hence their probable contribution to crude fibre fractions in grain legume extrudates. Similar to observations in our assessment of crude protein in extrudates, increasing the extrusion temperature for sorghum malt-bambara groundnut based extrudates from 100-130 °C (Jiddere and Filli, 2016) and red ginseng from 115-130 °C (Gui et al., 2012) decreased their protein content. However, extrusion of differently processed pigeon pea flours reflected no influence of extrusion cooking temperatures on the protein content of the extrudates (Okpala et al., 2016). On the other hand, significant improvement in *in vitro* protein digestibility observed with African yam beans and pigeon pea extrudates in this study have been reported in similar studies with *Phaseolus vulgaris* (Alonso et al., 2000; Batista et al., 2010) and *Vicia faba* (Alonso et al., 2000). While improved digestibility of proteins of legume extrudates can be attributed to an increase in surface area of proteins resulting from the unfolding of their secondary and tertiary structures, exposing cleavage sites for proteolysis which would have otherwise been inaccessible to protease enzymes (Day and Swanson, 2013), a reduction in the concentration of heat-labile bioactives such as enzyme inhibitors, lectins and cyanogenic glycosides, which are known to interact with proteins, forming complexes that are resistant to proteolytic digestion

(Van der Poel et al., 1992) may also be responsible for improved protein digestibility. However, reasons for the significantly higher protein digestibility in 100 °C extrudates of pigeon pea compared to 140 °C extrudates require further investigations. The application of heat during extrusion in the presence of sugars and amino acids triggers the Maillard's reaction, also termed non-enzymatic browning reaction, which is a complex reaction involving degradation and fragmentation of sugars and amino acid degradation, leading to the formation of Maillard's reaction compounds which could impair protein digestibility (Seiquer et al., 2006). In the current study, these compounds may be responsible for the reduced crude protein of the extrudates, although insufficient to significantly impair their protein digestibility *in vitro*.

Investigations into enzyme inhibitory activities determined in pigeon pea and other legumes corroborate the observations from the current study, with extrusion of pigeon pea at 22% moisture and 120–150 °C significantly reducing trypsin inhibitors by 95% (Okpala et al., 2016), while extrusion of *Phaseolus vulgaris* at 140, 160 and 180 °C amounted to a 45, 89 and 100% reduction in α -amylase inhibitory activity and 55.6, 65.1 and 85.7% reduction in trypsin inhibitory activity (Martin-Cabrejas et al., 1999), confirming that increased extrusion temperatures resulted in further reduction in enzyme inhibitor activity. A total reduction in α -amylase, 52.5 and 100% reduction in chymotrypsin, and 98.9% and 86.1% reduction in chymotrypsin inhibitory activity was also reported for extrudates of *Vicia faba* and *Phaseolus vulgaris* where extrusion was conducted at 152-156 °C (Alonso et al., 2000). Similarly, extrusion cooking in a twin screw extruder (56-184 °C) greatly reduced the activity

Table 3. Effect of high-temperature, short-time extrusion cooking temperatures on amino acid profile and nitrogen content, N% of Pigeon pea (*Cajanus cajan*) flours.

	Pigeon pea			% Variation			Contrast P- value		
	PPR	PP100	PP 140	[1]	[2]	[3]	PPR vs PP100	PPR vs PP140	PP100 vs PP140
Essential amino acids (mg/100mg protein)									
Arginine	1.50 ± 0.01	1.20 ± 0.02	0.93 ± 0.01	-19.81	-38.23	-22.95	***	***	***
Histidine	0.79 ± 0.01	0.67 ± 0.01	0.47 ± 0.02	-15.07	-40.50	-30.32	**	***	***
Isoleucine	0.93 ± 0.01	0.76 ± 0.00	0.60 ± 0.00	-17.47	-34.95	-20.95	***	***	***
Leucine	1.68 ± 0.00	1.39 ± 0.00	1.09 ± 0.01	-17.18	-34.91	21.40	***	***	***
Lysine	1.41 ± 0.00	1.17 ± 0.01	0.85 ± 0.04	-17.16	-39.73	-27.26	***	***	***
Methionine	0.28 ± 0.00	0.21 ± 0.00	0.14 ± 0.01	-25.57	-48.64	-30.99	***	***	***
Phenylalanine	0.87 ± 0.02	0.69 ± 0.01	0.57 ± 0.01	-20.37	-34.39	-17.57	***	**	***
Threonine	0.74 ± 0.02	0.56 ± 0.02	0.47 ± 0.00	-24.41	-36.09	-15.26	***	***	**
Tryptophan	0.23 ± 0.00	0.18 ± 0.00	0.15 ± 0.00	-19.48	-35.80	-20.28	***	***	***
Valine	0.96 ± 0.01	0.78 ± 0.00	0.48 ± 0.00	-18.92	-50.07	-38.42	***	***	***
Non-essential amino acids									
Alanine	0.83 ± 0.01	0.79 ± 0.01	0.58 ± 0.01	-4.85	-30.54	-27.01	**	***	***
Aspartate	2.32 ± 0.01	2.02 ± 0.01	1.65 ± 0.01				***	***	***
Cysteine	0.27 ± 0.01	0.19 ± 0.01	0.13 ± 0.00	-28.43	-51.38	-32.08	***	***	***
Glutamate	2.92 ± 0.01	2.55 ± 0.00	2.16 ± 0.01	-12.56	-25.92	-15.29	***	***	***
Glycine	0.89 ± 0.01	0.69 ± 0.01	0.57 ± 0.00	-22.69	-36.40	-17.73	***	***	***
Proline	0.71 ± 0.00	0.57 ± 0.00	0.47 ± 0.00	-18.94	-33.72	-18.24	***	***	***
Serine	0.80 ± 0.01	0.68 ± 0.01	0.53 ± 0.01	-15.37	-33.81	-21.77	***	***	***
Tyrosine	0.71 ± 0.00	0.61 ± 0.01	0.56 ± 0.00	-14.89	-21.52	-7.78	***	***	***
Total amino acids	18.82 ± 0.08	15.71 ± 0.06	12.39 ± 0.02	-16.49	-34.14	-21.13	***	***	***
Total essential amino acids	9.37 ± 0.07	7.61 ± 0.06	5.75 ± 0.02	-18.80	-38.67	-24.47	***	***	***
Total non-essential amino acids	9.45 ± 0.01	8.11 ± 0.00	6.65 ± 0.00	-14.14	-30.30	-18.82	***	***	***

Values are mean results obtained in triplicates ± standard deviation, and expressed as g/100g sample.

***p < 0.001 **p < 0.01.

PPR - unextruded Pigeon pea flour; PP100 - Pigeon pea extruded at 100 °C; PP140 - Pigeon pea extruded at 140 °C.

% variation: [1] = variation due to extrusion at 100 °C, i.e. $\frac{PP100 - PPR}{PPR} \times 100$

% variation: [2] = variation due to extrusion at 140 °C, i.e. $\frac{PP140 - PPR}{PPR} \times 100$

% variation: [3] = variation due to extrusion temperature, i.e. $\frac{PP140 - PP100}{PP100} \times 100$

of trypsin inhibitors by > 90%, in small white beans (*Phaseolus vulgaris* var. Aurora) with the highest residual inhibitor value reported as 15.3% of trypsin inhibitor in the control (Edwards et al., 1994). The combination of high temperature and pressure plus mechanical shear involved in HTST extrusion have the capacity to physically deform proteins by changing their secondary and tertiary structure (Avilés-Gaxiola et al., 2018), thus deactivating enzyme inhibitors.

Extrusion of African yam beans at 120 °C significantly reduced the concentration of all amino acids with highest and least losses recorded for lysine (62.40%) and leucine (14.4%), respectively (Omeire, 2012). Similarly, significant reduction in essential amino acids for soy flour, sweet potato flour, sweet potato-soy flour blends (80:20) and bambara groundnut-sorghum at extrusion temperature ranging from 100 °C to 130 °C have also been documented (Iwe et al., 2001; Jiddere and Filli, 2015). The role of amino acids and temperature in Maillard's reaction is considered the primary reason for the reduction in amino acid concentration observed in the grain legume extrudates. Worthy of note is the difference between total amino acids (Tables 1, 2, and 3) and crude protein content of the grain legume extrudates determined by the kjeldahl method (Figures 1, 2, and 3), which is deduced to be due to non-nitrogen proteins (NPN) - a group of nitrogenous substances that do not originate from proteins which include urea, small peptides, free amino acids, creatine, creatinine, uric acid, orotic acid, ammonia etc. The proportion of NPN compounds in foods increase under extrusion conditions, especially with barrel temperatures >70 °C (Chaiyakul et al., 2009; Pérez-Conesa et al., 2005).

In conclusion, high-temperature short-time extrusion cooking of African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) at 100 °C and 140 °C varied in their impact on chemical properties, protein digestibility, enzyme inhibitory activity, and amino acid profile. Research should be conducted with the aim of obtaining the best extrusion process conditions to optimize African yam beans (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranean*) and Pigeon pea (*Cajanus cajan*) extrudate quality. Also, the authors propose further research into characterizing and quantifying products such as Millard's reaction compounds, amylose-lipid complexes and indigestible glucans that may have been synthesized during extrusion cooking, exploring their possible impact on nutrient bioavailability in bioassays.

Declarations

Author contribution statement

Oluwafunmilayo O. Adeleye: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Seun T. Awodiran, Atinuke O. Ajayi, Toluwalope F. Ogunmoyela: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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