**Effects of processing moisture on the physical properties and *in vitro* digestibility
of starch and protein in extruded brown rice and pinto bean composite flours**

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13   **ABSTRACT**

14   The influence of pinto bean flour and **processing** moisture on the physical properties
15   and *in vitro* digestibility of rice-bean extrudates has been investigated. Brown rice: pinto
16   bean flour (0%, 15%, 30%, and 45% bean flour) were extruded under 5 moisture
17   conditions (17.2%, 18.1%, 18.3%, 19.5%, and 20.1%). Physical properties [bulk density,
18   unit density, radial expansion, axial expansion, overall expansion, specific volume,
19   hardness, color, water solubility index, and water absorption index] and *in vitro* starch
20   and protein digestibilities were determined. Increasing bean flour and **processing**
21   moisture increased density and hardness while decreasing expansion. Rapidly
22   digestible starch decreased and resistant starch increased as bean substitution and
23   **processing** moisture increased. *In vitro* protein digestibility increased with increasing
24   bean flour or with decreasing **processing** moisture. Incorporating bean flour into
25   extruded snacks can negatively affect physical attributes (hardness, density, and
26   expansion) while positively affecting *in vitro* starch (decrease) and protein (increase)
27   digestibilities.

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30   Keywords: starch; protein; snacks; expansion; health

1. Introduction

Most ready-to-eat snack products on the market are made from refined cereal grains and are generally low in dietary fiber and protein (Anton, Gary Fulcher, & Arntfield, 2009). However the demand for nutritionally improved snacks increased by 33% in 2015 (Mintel, 2015). Therefore, improving the nutritional value of ready-to-eat snacks is desirable.

Whole grains are often used to formulate healthy ready-to-eat snacks. Although whole grain extruded snacks are generally produced from whole wheat or corn flour, brown rice flour is becoming more attractive due to its relatively high starch content and bland taste (Chaiyakul, Jangchud, Jangchud, Wuttijumnong, & Winger, 2009).

Unfortunately, brown rice flour is also lower in dietary fiber (40-50%) and protein (5-90%) compared with wheat and corn (US Department of Agriculture, 2015). Legumes, on the other hand, contain high dietary fiber and the amino acid profile is complimentary to those in cereals (Tharanathan & Mahadevamma, 2003). Thus, production of a ready-to-eat snack made with a blend of brown rice flour and legume flour may produce an extruded product containing both high dietary fiber and complete protein.

The nutritional advantages of a rice-bean extrudate are dependent not only on the increase in dietary fiber and complete protein, but also on the digestibility of protein and starch. Some research has indicated that extrusion cooking increases the *in vitro* protein digestibility of legumes more efficiently than conventional cooking methods (Alonso, Aguirre, & Marzo, 2000). Protein digestibility also varied across types of legumes and within the genotypes (Chitra, Vimala, Singh, & Geervani, 1995). With regards to grains, different processing methods also affect the efficiency of *in vitro*

protein digestibility of rice flour (Sagum & Arcot, 2000). Mertz et al. (1984) indicated that *in vitro* protein digestibility varies across types of cereals grains. Mahasukhonthachat, Sopade, and Gidley (2010) indicated that *in vitro* digestibility starch was enhanced under low moisture conditions when extruding sorghum flour, while another study on sweet potato flour showed that the rate of starch digestion was not significantly affected by moisture content (Warambi, Gidley, & Sopade, 2014). Thus, the digestibility of starch and protein may not be consistently affected when different raw materials are used.

When producing extruded snack products, there are many variables that affect the characteristics of the final product, such as moisture content, feed rate, screw speed, and barrel temperature. Among these variables, however, moisture content is one of the most important aspects in manipulating the physical characteristics of extruded products because it can substantially influence the melting temperature, viscosity, and shear stress experienced by the material inside the extrusion barrel (Thymi, Krokida, Pappa, & Maroulis, 2005). Therefore the aim of this study was to investigate the effects of pinto bean flour and processing moisture on the physical characteristics and *in vitro* starch and protein digestibilities of rice-bean extrudates.

2. Materials and methods

2.1. Brown rice flour and pinto bean flour production and composition

Brown rice flour was obtained from Sage Foods (Los Angeles, California, USA). Pinto beans (cultivar 'Poncho') were obtained from Stateline Bean (Gering, Nebraska, USA). Whole pinto bean seeds were ground in a pilot plant scale hammer mill (C.S. Bell, Tiffin, Ohio, USA) with 1 mm screen size. Brown rice flour: bean flour composites were prepared by using a pilot plant ribbon blender (Wenger, Sabetha, KS, USA) at 4

ratios: 100:0, 85:15, 70:30, and 55:45, respectively. These flours were named based on the percentage of pinto bean flour in the treatment (i.e., 0%, 15%, 30%, and 45%, respectively). Salt was added to the flours at 1% (by total weight of flour) after composites were made. The flours were then stored at 4 °C until extruded.

Proximate composition, including moisture content, total starch, ash, and total dietary fiber, were analyzed on composite flours using approved methods (AACC International, 2015). Total starch was analyzed using a Megazyme total starch assay kit (K-TSTA, Megazyme, Bray, Ireland). Crude fat was determined using an extraction unit (HT 1043, Soxtec, CA, USA) with hexane as the extraction solvent. Crude protein content was analyzed using a nitrogen analyzer unit (Leco FP-528, Leco Corporation 3000, St Joseph, MI, USA) with 6.25 as nitrogen conversion factor. Analyses were performed in triplicate.

2.2. Extrusion process

Ninety kilograms of each composite flour were extruded using a pilot scale twin screw extruder (Model TX-57, Wenger Manufacturing, Inc., Sabetha, KS, USA). The extruder screw diameter was 57 mm. The screw length to diameter ratio was 13.5 to 1. The barrel consisted of three jacketed zones. The temperatures at each zone were 80, 90, and 110 °C (from inlet to die). These temperatures were held constant throughout the trial. The barrel screw speed was maintained at 300 rpm. The extruder die assembly consisted of a two-to-one adapter and a die with six openings, each with an inside diameter of 4.08 mm. The flours were fed into the extrusion barrel at rate of 80 kg/h using a loss-in-weight feed system. Water was injected into the first zone of the barrel (near the inlet) at different rates, resulting with five different processing moisture

contents: 17.2%, 18.1%, 18.3%, 19.5% and 20.1%. Upon exit from the extruder die, materials were cut using a die mounted rotary knife assembly. The knife speed was held constant at 253 rpm throughout the trial. The cut extrudates were pneumatically conveyed to a gas fired belt dryer (Model 4800, Wenger Manufacturing, Inc., Sabetha, Kansas, USA) and dried at 103 °C for 10 min. The samples were stored in plastic bags at room temperature. Moisture content was analyzed within 24 h of production. Texture analysis was conducted within 3 days after production. For chemical analysis, the extrudate was ground with food processor (Waring, New Hartford, CT) and stored at 4 °C until analysis.

2.3. Physical analyses

Bulk density was determined by adding 50 g of extrudate into a 2 L graduated cylinder and inverting the filled cylinder once. The volume was noted and the bulk density was calculated by dividing the exact weight of extrudate (in mg) by the recorded volume (in cm³).

Unit density and true solid density were measured according to Ali, Hanna, and Chinnaswamy (1996), with minor modifications. For unit density, 5 g of extrudate were placed into a cylindrical canister (9.1 cm in diameter X 9.3 cm in height) and then the canister was filled with rapeseed and leveled off. The rapeseed filling the container was then weighed. Unit density was then calculate by the equation:

$$\rho_u = (W_{ex} \times W_{rs}) / (W_{rf} \times V_c)$$

Where ρ_u = unit density (mg/cm³), W_{ex} = mass of extrudate (mg), W_{rs} = mass of rapeseed replaced (mg), W_{rf} = mass of the rapeseed on full container (mg), V_c = Volume of canister (cm³). True solid density was determined by milling the extrudates with a

food processor (Waring, New Hartford, CT) and sieving to pass through a 0.21 mm opening (US standard mesh number 70). The flour was then added to a cylindrical canister (3.6 cm diameter X 6.4 cm height) until the canister was completely filled. The container was tapped 10 times against the benchtop and, if the height decreased, more flour was added to fill the container. This 'tap-and-fill' process was continued until no change in fill height was observed upon tapping 10 times. The density was calculated by dividing the weight of flour (g) by the volume of the canister (cm^3). True solid density is not reported, but data were collected for overall expansion ratio calculation (see below). Unit, bulk, and true solid density were analyzed 5 times for each sample.

Three expansion characteristics were measured: radial expansion ratio, axial expansion ratio, and overall expansion ratio. Radial expansion ratio was the cross-sectional diameter (mm) of each extrudate divided by the diameter of the die opening (mm). Radial expansion was measured 15 times for each sample. The overall expansion was determined as the ratio of true solid density to unit density. Axial expansion was the ratio of overall expansion to radial expansion (Ali et al., 1996). Axial and overall expansions were calculated 5 times for each sample.

Water absorption index (WAI) and water solubility index (WSI) of extrudates were measured three times per sample as reported previously (Anderson, Conway, & Peplinski, 1970). Color was analyzed 5 times for each sample using a colorimeter (Konika Minolta Chroma Co, Osaka, Japan) and expressed as L^* (lightness/darkness), a^* (redness+/greenness-) and b^* (yellowness+/ blueness-).

The texture of extrudates was characterized in terms of hardness (N) and jaggedness (N-s) and was measured 5 times for each sample. The analysis was

performed using a texture analyzer (TA.XT plus, Stable Micro systems, Godalming, UK) with a 5 blade Kramer shear cell as the probe. Extrudates were added to a height of 4 cm in the canister, and were cut with the blades at speed of 2 mm/s and a distance of 48 mm. Hardness was the maximum force (N) achieved during the run. Jaggedness was expressed as the linear distance of the force deformation curve.

2.4. *In vitro digestion*

In vitro starch digestion of starch was determined following Mkandawire, Weier, Weller, Jackson, and Rose (2015). In short, extrudates (containing 120 mg of starch) were dispersed in 2 mL of distilled water and were digested with 4 ml of 3.6% (w/v) pepsin (P-700, Sigma, St. Louis, MO USA) dissolved in 0.05M HCl at 37 °C for 30 min. The intestinal phase was initiated with the addition of 2 mL 0.5 M sodium acetate buffer (pH 5.2, containing 5 mM CaCl₂) followed by addition of 2 mL of 15% (w/v) pancreatin (P-7545, Sigma St. Louis, MO USA). Prior to the addition of the pancreatin, the suspended pancreatin was centrifuged at 4000 g for 10 min and 20 µL of amyloglucosidase (3260 U/mL; Magazyme, Bray, Ireland) were added per mL of recovered supernatant. The starch was digested for 2 h at 37°C with 20 µL aliquots were removed to absolute ethanol after 20 and 120 min. The glucose content in the samples was measured using the glucose oxidase-peroxidase reagent (Megazyme) and converted to starch by multiplying by a factor of 0.9. The fraction of starch digested after 20 min of incubation was expressed as rapidly digestible starch (RDS), after 120 minutes was total digestible starch (TDS), and the digested starch fraction between 20 and 120 minutes was defined as slowly digestible starch (SDS). Resistant starch (RS) was the starch that remained undigested after 120 minutes of digestion.

In vitro protein digestion was conducted according to Genovese & Lalojo (1998) with slight modifications. Flour suspensions (containing 10 mg/mL of protein in 0.15 M NaCl-HCl, pH 2) were first digested with 1 mL of pepsin (3 mg/mL in 0.15 M NaCl-HCl, pH 2, P-7000, Sigma) for 2 h in a horizontal shaking water bath at 37 °C. The pH was then raised by addition of 1 mL phosphate buffer (0.05 M, pH 7) followed by the addition of 2.5 mL of pancreatin (5 mg/mL in 0.05 M phosphate buffer, pH 7, P-7545, Sigma). Samples were then digested for 4 h in a horizontal shaking water bath at 37 °C. The digestion was terminated by heating at 80 °C for 10 min. To analyze the progress of the digestion after the gastric phase only, a separate set of samples were prepared and the pH was raised to 7.0 by the addition of 0.25 mL of NaHCO₃ and the tubes were heated at 80 °C for 10 min directly after the pepsin digestion stage. At the end of digestion, the suspended samples were centrifuged at 4000 g for 20 min and the supernatants were collected and the extent of hydrolysis was determined by reaction of free amino groups with 2,4,6-trinitrobenzenesulfonic acid (TNBS) according Adler-Nissen (1979). The percentage of protein digestibility was expressed as the ratio between the number of free amine groups released (calculated as mM of Leucine equivalents) to the total number of amino acids in a protein (assuming 113 as the average molecular weight of amino acid residues in a protein). The total digestible protein (TDP) was the total protein digested during both the pepsin and pancreatin stages of digestion; gastric digestible protein (GDP) was the protein digested during the gastric phase only. All *in vitro* digestibility analyses were performed 3 times for each sample.

2.5. Data analysis

Data for each treatment combination were averaged and treated as continuous variables for data analysis following a response surface approach using statistical software (version 9.4, SAS Institute, Cary, NC, USA). Regression analysis was first conducted on each factor (percentage of bean flour and processing moisture) and their interaction, allowing main effect terms from linear to cubic and all possible interactions. Next, a new regression model was created using only significant terms ($P < 0.05$) from the initial model, and all non-significant terms were pooled into the error term. Lastly, the significant terms tested were then used in a final model from which prediction equations were generated (Ali et al., 1996). Response surface figures were constructed from the prediction equations with statistical software (R, version 3.2.2, Vienna, Austria). An optimization calculation was performed to maximize bean flour percentage while also maximizing overall expansion ratio, RS, and TDP and minimizing unit density, hardness, and RDS using simultaneous optimization of product responses. This made use of a desirability function (Derringer and Suich, 1980), where the overall desirability was the geometric mean of individual desirabilities (d_i) and was calculated as: $D = (d_1 \cdot d_2 \cdot \dots \cdot d_m)^{1/m}$, where m was the number of responses ($m=6$). The desirability for optimum response based on specific responses was predicted using statistical software (JMP, version 10.0.0, SAS Institute).

3. Results and discussion

3.1. Composition of rice-bean composite flours

Among brown rice: pinto bean composite flours, starch content ranged from 68 to 80%, protein from 9 to 13%, and total dietary fiber from 6 to 15% (Supplementary Table 1), increasing the percentage of pinto bean flour in the composite increased dietary

fiber, protein, ash, and lipid concentrations while decreasing starch, since bean flours have higher protein, fat, ash and dietary fiber and lower starch compared to brown rice (Balasubramanian, Borah, Singh, & Patil, 2012). The compositions reported were similar to those reported for brown rice and wild legume composites (Pastor-Cavada et al., 2011).

3.2. Physical characteristics of rice-bean extrudates

The overall expansion ranged from 3.5 to 4.2 (Table 1). The overall expansion from our study is comparable to other studies: Pastor-Cavada et al. (2013) reported an expansion ratio of 3.2 to 3.4 when extruding rice and wild legume, and Chaiyakul et al. (2009) found an expansion between 2.7 to 3.9 when extruding glutinous rice.

From the regression equations, it was observed that bean flour percentage had a cubic trend on overall expansion (Table 2). Overall expansion was negatively affected by the addition of bean flour, where the lowest overall expansion was reported for 15% and 45% bean flour substitution and the highest at 0% bean flour (Fig. 1A). The higher overall expansion of 30% flour as compared to 15% was likely due to high axial expansion at higher bean flour substitutions (Table 1). Although high axial expansion was also the case for 45% bean flour, the accompanying decrease in radial expansion resulted in a decrease in overall expansion. Elastic forces and viscous forces are the two main factors governing extrusion expansion. While radial expansion is mainly driven by elastic forces, axial expansion is dependent on viscous forces (Pitts, Favaro, Austin, & Day, 2014). The addition of bean flour may have increased the viscous forces inside the extruder to be more dominant than the elastic forces, which caused the product to be more axially expanded. Higher axial expansion may be due to a reduction in flow

properties due to the presence of increased hydrophilic groups from bean flour constituents (such as protein) that compete with starch for water availability (Pitts et al., 2014).

An increase in **processing** moisture resulted in an increase in radial expansion but decreased axial and overall expansion (Table 2). Expansion is usually the highest with lower **processing** moisture (Pastor-Cavada, et al, 2013; Chaiyakul, et al, 2009) as was depicted for overall expansion in our study. The increase in radial expansion at lower **processing** moisture in our study was unexpected. Expansion properties can occur both radially and axially. In our study, axial expansion seemed to have a more predominant effect on the overall expansion properties than radial expansion.

Two methods for measuring density of the extrudates were used: bulk density and unit density. Bulk density was the density of a filled container of extrudates, which took into account the porous nature of the extrudates as well as the shape of the extrudates and how they packed together. Unit density was the density of the extrudates excluding the effect of shape. Bulk density ranged from 33.4 to 60.1 mg/cm³, while unit density ranged from 98.1 to 161 mg/cm³ (Table 1). Density properties of the extrudates were increased as **processing** moisture and percentage of bean flour increased (Fig 1B), as has been reported in others studies (Chaiyakul et al., 2009; Pastor-Cavada et al., 2011). Increases in density due to higher fiber content in the flour have been reported (Anton, Gary Fulcher, & Arntfield, 2009). The addition of fiber can interfere with the formation of the porous structure by disrupting pore walls during the extrusion process and result in a denser product (Pérez-Navarrete, González, Chel-Guerrero, & Betancur-Ancona, 2006). Additionally, a **reduction in** starch content and

increase in protein content upon higher bean substitution may also negatively affect the density properties of extrudates (Obatolu Veronica, Omuetti Olusola, & Adebowale, 2006).

Texture analysis indicated that the hardness of rice bean extrudate ranged from 139 to 188 N (Table 1). The highest hardness was reported on the 45% bean flour extrudates at the highest processing moisture (20.1%). The incorporation of higher concentrations of fiber can rupture the cell walls of expanding extrudates and therefore interfere with the expansion of air bubbles (Pérez-Navarrete et al., 2006). A higher concentration of protein can also result in fluctuations in pressure and temperature as the material moves through the extrusion barrel, which can negatively affect the texture of the extrudates (Obatolu Veronica et al., 2006). Furthermore, the reduced shear of the plasticized mass inside the extrusion barrel due to higher processing moisture can result in less gelatinized starch and therefore may impede bubble growth of the extrudate (Stojceska, Ainsworth, Plunkett, & Ibanoglu, 2009).

The water solubility index (WSI) of the extrudates ranged from 30.6 to 48.5 (Table 1), with a decreasing cubic trend at higher processing moisture and increasing linear trend with addition of more bean flour (Table 2). The increase in WSI at lower processing moisture may be related to higher starch depolymerisation due to higher shear (Stojceska et al., 2009). WSI also increased as the proportion of bean flour increased. In pure starch systems, WSI is a measure of the mechanical depolymerisation of amylopectin released from starch granules during extrusion (Seth, Badwaik, & Ganapathy, 2015). However, samples with higher bean flour content had both lower starch and higher WSI. Thus, the increase in WSI at higher bean substitution

may be related to the increase in hydrophilic groups associated with dietary fiber, protein, or other components in the bean flour. The increase in hydrophilic groups with higher bean flour could further explain the increase in viscous forces inside the extrusion barrel that led to the higher axial expansion mentioned previously. These results were similar to a previous study with brown rice and wild legume flours (Pastor-Cavada et al., 2011).

WAI ranged from 3.6 to 6.1 (Table 1). WAI followed a linear trend for both processing moisture and bean flour substitution, with processing moisture positively affecting WAI and bean percentage negatively affecting WAI. The decrease in WAI as bean percentage increased may be due to the reduction in starch content of composite flour (Supplementary Table 1). Similar results were reported by Lazou and Krokida (2010), who showed a decrease in WAI, despite an increase in protein upon addition of legume flour to yellow corn flour. This could be due to an increase in the interaction between the available hydrophilic groups on protein with starch, hindering the ability of starch to bind water (Fernández-Gutiérrez, Martín-Martínez, Martínez-Bustos, & Cruz-Orea, 2004).

The brightness of the extrudate decreased and redness increased with the addition of more bean flour or less moisture (Table 2). Yellowness of the extrudates was only significantly affected by bean percentage. The color analysis from this study was in agreement with a previous report on extruded corn starch and common bean flour blend (Anton et al., 2009). Changes in color characteristics caused by lowering the processing moisture may be due to the maillard reaction which causes the product to decrease in brightness and increase in yellowness (Bates, Ames, & MacDougall, 1994).

3.3. In vitro digestibility of rice-bean extrudates

Rapidly digestible starch (RDS) ranged from 68 to 75%, while resistant starch (RS) ranged from 1.8 to 12.6 % (Table 3). RDS decreased as bean flour increased (Table 2). From the regression equations, it was observed that bean flour had a cubic trend and processing moisture had a linear trend with RDS (Table 2; Fig 2A). The increase in RDS by lowering the processing moisture in this study is in agreement with a previous study extruding hard-to-cook bean and maize flour (Ruiz-Ruiz et al., 2008). Therefore, the increase in RDS may be due to higher mechanical shear exhibited by lower processing moisture which provided a higher mechanical rupture on the plant cell wall. This would create a larger surface area between starch and digestive enzymes and also an increase in gelatinization of the starch (Mahasukhonthachat, Sopade, & Gidley, 2010).

Slowly digestible starch (SDS) was only significantly affected by bean flour substitution (Table 2). However, the adjusted R^2 (0.49) was considerably low. This may be because the SDS was calculated by subtraction of RDS from total digestible starch. Thus, because the total error remained constant, the relative error was large and could have resulted in a lower coefficient of determination.

RS increased as percentage of bean flour increased and also increased as processing moisture increased (Table 2; Fig 2B). The increase in RS as bean flour increased may be attributed to increasing total dietary fiber in higher levels of bean flour, since dietary fibers can impart viscosity and slow the rate of enzymatic digestion of starch (Regand, Chowdhury, Tosh, Wolever, & Wood, 2011). Alternatively, the increase in RS with increasing bean flour substitution may be due to differences in

starch composition between bean and brown rice. The percentage of amylose in pinto bean starch is 25-30% (Hoover & Ratnayake, 2002), while that of brown rice starch is about 17% (Deng et al., 2010). The higher amylose content may be contributing to an increased in RS as bean flour increased (Shandu & Lim, 2008). The reduction in RS as the processing moisture decreased was in agreement with a previous study that reported a decrease in RS on a higher mechanical degradation of waxy and regular barley flours (Faraj, Vasanathan, & Hoover, 2004).

In vitro protein digestibility consisted of two digestion sequences: GDP, which ranged from 16-32%, and TDP, which ranged from 83-90% (Table 3). The GDP results from this study were similar to pepsin digestion to dry bean protein fractions ranging from 8-25% (Genovese & Lalojo, 1998). TDP results were in agreement with Pérez-Navarrete et al. (2006) and Pastor-Cavada et al. (2011) who reported *in vitro* protein digestibilities of 82% for extruded maize and lima bean flour and 82-85% for extruded rice and wild legumes flours, respectively. The protein digested during the gastric phase was increased by a linear increase in processing moisture and followed a quadratic trend for bean flour percentage (Table 2; Fig. 3A). The TDP was only significantly affected by a linear effect of feed moisture (Fig. 3B).

The increased protein digestibility as bean flour increased during GDP may be because bean protein is more susceptible to pepsin digestion than rice protein. However, when the digestion continued for 4 h with pancreatin, there was no significant effect of bean flour on the total digestible protein. This is in agreement with other studies have found no significant differences in percentage of protein digestibility upon the addition of legume flours on maize and rice flour (Pastor-Cavada et al., 2011; Pérez-

Navarrete et al., 2006). The protein digestibility was significantly increased as processing moisture decrease in both digestion fractions. This may be due to increasing shear with lower processing moisture. In agreement with these reports, Bhattacharya, Das, & Bose (1988) reported a significant increase in protein digestibility in blended wheat and fish extrudates as shear increased.

3.4. Optimum rice and beans extrudates

The optimum combination of feed moisture and bean flour substitution took into account physical characteristics and *in vitro* digestibility profiles. The optimum conditions were selected to maximize bean flour percentage while also maximizing overall expansion ratio, RS, and TDP and minimizing unit density, hardness and RDS. The generated combination was 30% bean flour substitution and 18.3% processing moisture. This resulted in a desirability ratio of 0.62 with predicted hardness of 156 N, overall expansion ratio of 3.7, unit density of 116 mg/cm³, RDS of 68%, RS of 10%, and TDP of 88%).

4. Conclusion

The use of pinto bean flour in extruded brown rice-bean composites can improve the nutritional profiles of the extrudates by improving the *in vitro* protein digestibility and increasing RS, while some physical attributes, namely, hardness, densities, and overall expansion, may be negatively affected. Reducing the processing moisture inside the extrusion barrel increased *in vitro* protein digestibility and partially alleviated the negative effects of bean flour on the physical characteristics, but decreased RS.

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Conflict of interest

We (the authors) attest that there were no interests that competed with objective interpretation and presentation of the results. The funders imposed no restrictions on presentation of results or decision to publish.

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514 Table 1. Physical characteristics of rice-bean extrudates.¹

Moisture/ Flour blend (%)	RER	AER	OER	BD (mg/cm ³)	UD (mg/cm ³)	Hardness (N)	WSI	WAI	L*	a*	b*
17.2											
0	2.54 ± 0.03	1.65 ± 0.01	4.19 ± 0.01	33.4 ± 0.49	98.1 ± 1.75	139 ± 8	39.8 ± 0.1	5.01 ± 0.19	77.6 ± 0.6	2.31 ± 0.13	19.4 ± 0.3
15	2.62 ± 0.02	1.46 ± 0.10	3.84 ± 0.13	39.3 ± 0.71	112 ± 3.01	143 ± 5	40.9 ± 1.5	4.82 ± 0.01	73.8 ± 0.3	3.06 ± 0.11	19.9 ± 0.3
30	2.46 ± 0.03	1.64 ± 0.05	4.03 ± 0.15	34.3 ± 0.41	105 ± 2.67	147 ± 9	45.3 ± 1.6	4.24 ± 0.15	73.6 ± 0.3	3.77 ± 0.08	21.0 ± 0.6
45	2.41 ± 0.03	1.66 ± 0.04	4.00 ± 0.05	33.8 ± 0.48	106 ± 3.77	140 ± 6	48.5 ± 3.0	3.60 ± 0.25	71.1 ± 0.5	4.15 ± 0.17	20.8 ± 0.3
18.1											
0	2.66 ± 0.07	1.55 ± 0.08	4.13 ± 0.12	37.3 ± 0.56	103 ± 7.83	146 ± 8	36.3 ± 0.2	5.38 ± 0.03	77.6 ± 0.2	1.50 ± 0.14	19.3 ± 0.1
15	2.65 ± 0.01	1.42 ± 0.08	3.77 ± 0.09	41.9 ± 1.09	113 ± 6.22	148 ± 8	37.5 ± 0.9	5.17 ± 0.01	74.6 ± 0.4	3.10 ± 0.16	20.5 ± 0.8
30	2.48 ± 0.02	1.61 ± 0.07	3.99 ± 0.17	38.9 ± 0.41	114 ± 5.16	152 ± 8	44.4 ± 0.3	4.25 ± 0.08	74.0 ± 0.3	3.84 ± 0.09	21.1 ± 0.3
45	2.37 ± 0.02	1.67 ± 0.07	3.96 ± 0.10	40.1 ± 0.52	114 ± 5.48	154 ± 4	48.3 ± 0.8	3.63 ± 0.29	71.8 ± 0.3	4.52 ± 0.15	21.5 ± 0.5
18.3											
0	2.67 ± 0.05	1.54 ± 0.08	4.11 ± 0.16	38.2 ± 0.64	103 ± 7.38	148 ± 5	36.0 ± 0.7	5.21 ± 0.03	78.3 ± 0.2	1.13 ± 0.09	19.4 ± 0.3
15	2.69 ± 0.02	1.40 ± 0.03	3.77 ± 0.08	39.8 ± 0.71	113 ± 6.22	150 ± 6	37.6 ± 0.9	5.17 ± 0.04	76.4 ± 0.4	2.75 ± 0.17	19.9 ± 0.3
30	2.52 ± 0.03	1.53 ± 0.02	3.85 ± 0.01	38.4 ± 0.61	119 ± 2.61	155 ± 6	44.0 ± 0.2	4.31 ± 0.03	75.2 ± 0.3	3.65 ± 0.09	20.7 ± 0.3
45	2.39 ± 0.02	1.62 ± 0.07	3.88 ± 0.09	41.1 ± 0.52	115 ± 7.11	157 ± 3	47.1 ± 1.7	3.84 ± 0.22	72.4 ± 0.3	4.34 ± 0.16	21.6 ± 0.5
19.5											
0	2.82 ± 0.02	1.45 ± 0.06	4.08 ± 0.07	46.2 ± 0.88	109 ± 5.71	149 ± 4	33.1 ± 0.2	5.69 ± 0.05	78.4 ± 0.2	0.93 ± 0.002	18.8 ± 0.3
15	2.80 ± 0.02	1.28 ± 0.01	3.59 ± 0.05	48.5 ± 1.37	122 ± 0.63	152 ± 6	35.5 ± 0.7	5.24 ± 0.14	74.2 ± 0.4	3.05 ± 0.21	20.3 ± 0.5
30	2.76 ± 0.02	1.33 ± 0.10	3.67 ± 0.13	48.3 ± 1.91	126 ± 4.21	177 ± 6	39.0 ± 0.6	4.53 ± 0.04	74.0 ± 0.9	3.63 ± 0.29	20.2 ± 1.0
45	2.51 ± 0.03	1.49 ± 0.03	3.73 ± 0.08	55.1 ± 1.48	135 ± 3.79	182 ± 13	41.2 ± 1.8	4.25 ± 0.03	72.5 ± 0.5	4.32 ± 0.16	20.8 ± 0.5
20.1											
0	2.81 ± 0.03	1.45 ± 0.05	4.07 ± 0.14	53.1 ± 1.03	117 ± 3.01	152 ± 11	30.6 ± 1.3	6.06 ± 0.07	77.6 ± 0.3	0.93 ± 0.00	19.4 ± 0.4
15	2.81 ± 0.03	1.24 ± 0.05	3.48 ± 0.13	54.2 ± 0.21	141 ± 4.67	153 ± 5	32.5 ± 1.2	5.49 ± 0.15	76.0 ± 0.5	2.88 ± 0.23	20.0 ± 0.9
30	2.72 ± 0.02	1.37 ± 0.08	3.74 ± 0.08	57.1 ± 2.08	151 ± 7.14	186 ± 5	36.6 ± 1.7	4.63 ± 0.17	74.6 ± 0.1	3.63 ± 0.18	20.1 ± 0.6
45	2.49 ± 0.01	1.44 ± 0.04	3.57 ± 0.01	60.1 ± 0.79	161 ± 9.74	188 ± 8	42.0 ± 1.9	4.30 ± 0.07	74.0 ± 0.3	4.38 ± 0.23	20.8 ± 0.5

515 ¹ Mean ± standard deviation; flour blend represents the percentage of bean flour in the brown rice: bean flour composite;
516 BD, bulk density; UD, unit density; RER, radial expansion ratio; AER, axial expansion ratio; OER, overall expansion ratio;
517 WSI, water solubility index; WAI, water absorption index; L*, lightness/darkness; a, redness/greenness; b*,
518 yellowness/blueness.

519 Table 2. Regression equations of response variables.¹

Responses	Regression Model	Adjusted R ²	√MSE
RER	$-4.97 + 0.32B + 0.72M - 0.0002B^2 - 0.016M^2 - 0.03MB + 0.0008M^2B$	0.89	0.049
AER	$3.15 + 0.029B - 0.087M + 0.002B^2 - 0.00002B^3$	0.88	0.045
OER	$5.24 - 0.02B - 0.06M - 0.002MB + 0.003B^2 - 0.00003B^3$	0.93	0.05
BD	$492 - 1.24B - 54.05M + 1.59M^2 + 0.07MB$	0.94	1.82
UD	$1681 - 4.36B - 174.2M - 0.013B^2 + 4.79 M^2 + 0.288MB$	0.92	4.65
WAI	$1.07 + 0.036B + 0.24M$	0.94	0.16
WSI	$-1457 + 0.23B + 242M - 12.9M^2 + 0.22M^3$	0.94	1.23
Hardness	$107 - 5.88B + 2.09 M + 3391MB$	0.89	4.83
Jaggedness	$304062 - 48324M + 2550M^2 - 44.4M^3$	0.96	77.2
L*	$85 - 0.114B - 0.42M$	0.84	0.87
a*	$3.68 + 0.112B - 1.13M - 0.001B^2$	0.93	0.29
b*	$19.3 + 0.004B$	0.79	0.35
RDS	$94 - 0.002B - 1.13M - 0.01B^2 + 0.0002B^3$	0.95	0.54
SDS	$23.5 - 0.17B + 0.002B^2$	0.49	1.09
RS	$-17.9 + 0.67B + 1.15M - 0.003B^2 - 0.017MB$	0.98	0.45
GDP	$31.7 + 1.32B - 0.07M - 0.06 MB + 0.002B^2$	0.95	0.97
TDP	$85.6 - 1.59B - 0.76M + 0.07MB$	0.78	2.19

520 ¹ MSE, mean square error; B, percent bean flour in composite flour; M, processing moisture content (%); BD, bulk
521 density; UD, unit density; RER, radial expansion ratio; AER, axial expansion ratio; OER, overall expansion ratio; WSI,
522 water solubility index; WAI, water absorption index; GDP, gastric digestible protein; TDP, total digestible protein.

523 Table 3. *In vitro* digestibility of starch (% of total starch) and protein (% of total protein) of
 524 rice-bean extrudates.¹

Moisture/ Flour blend (%)	Starch digestibility (%)			Protein digestibility (%)	
	RDS	SDS	RS	GDP	TDP
17.2					
0	74.8 ± 3.6	23.3 ± 2.0	1.80 ± 2.44	17.6 ± 0.56	89.4 ± 1.07
15	72.7 ± 0.6	20.9 ± 1.1	6.30 ± 0.55	20.6 ± 5.26	89.4 ± 2.68
30	69.5 ± 1.7	20.3 ± 1.2	10.1 ± 0.9	27.7 ± 3.41	89.8 ± 1.61
45	69.1 ± 0.2	24.0 ± 0.3	11.1 ± 0.2	31.7 ± 3.64	88.9 ± 1.42
18.1					
0	73.4 ± 1.3	23.6 ± 0.6	2.90 ± 0.75	17.9 ± 0.51	88.3 ± 2.21
15	71.5 ± 1.3	21.3 ± 1.0	7.10 ± 0.32	19.5 ± 4.60	87.6 ± 4.31
30	69.5 ± 1.1	19.9 ± 0.6	10.5 ± 1.7	25.7 ± 8.18	87.4 ± 1.49
45	68.8 ± 1.0	19.4 ± 1.4	11.8 ± 0.4	28.5 ± 0.83	87.6 ± 1.80
18.3					
0	73.7 ± 2.0	22.4 ± 1.2	3.90 ± 1.52	18.7 ± 0.67	88.2 ± 3.36
15	71.3 ± 0.7	21.4 ± 1.1	7.10 ± 0.92	18.9 ± 0.61	88.1 ± 1.45
30	67.8 ± 0.6	20.9 ± 0.4	11.2 ± 0.3	24.0 ± 1.89	87.5 ± 0.73
45	68.1 ± 1.2	19.3 ± 1.8	12.1 ± 0.6	28.2 ± 0.72	86.9 ± 0.61
19.5					
0	71.9 ± 0.4	24.4 ± 0.6	3.60 ± 0.41	15.8 ± 1.18	86.9 ± 2.65
15	70.4 ± 1.5	21.0 ± 1.8	8.60 ± 0.36	18.9 ± 0.10	87.2 ± 4.19
30	66.9 ± 1.8	21.6 ± 2.2	11.4 ± 0.5	21.1 ± 5.05	86.7 ± 1.53
45	66.4 ± 0.3	21.4 ± 0.5	12.1 ± 0.7	23.7 ± 6.09	86.6 ± 0.27
20.1					
0	70.1 ± 2.6	24.0 ± 2.8	5.80 ± 0.46	15.8 ± 3.63	83.0 ± 3.18
15	69.1 ± 0.6	21.7 ± 1.0	9.10 ± 0.45	15.8 ± 0.83	85.7 ± 1.29
30	67.1 ± 1.7	21.5 ± 1.8	11.3 ± 0.7	17.9 ± 3.81	84.7 ± 0.62
45	67.0 ± 0.2	20.3 ± 0.5	12.6 ± 0.3	21.7 ± 1.38	86.4 ± 0.96

525 ¹ Mean ± standard deviation; flour blend represents the percentage of bean flour in the
 526 brown rice: bean flour composite; RDS, rapidly digestible starch; SDS, slowly digestible
 527 starch; RS, resistant starch; IVPD, *in vitro* protein digestibility; GDP, gastric digestible
 528 protein; TDP, Total digested protein.

529 **Figure captions**

530 Fig. 1. Effect of **processing** moisture (%) and percentage of bean flour in the brown rice:
531 bean flour composite (bean, %) on (A) OER, (B) UD, and (C) hardness of brown rice-
532 bean extrudates; OER= Overall expansion ratio, UD= apparent bulk density.

533 Fig. 2. Effect of **processing** moisture (%) and percentage of bean flour in the brown rice:
534 bean flour composite (bean, %) on **(A)** RDS, rapidly digestible starch (% of total starch)
535 **and (B)** RS, resistant starch (% of total starch);

536 **Fig. 3. Effect of processing moisture (%) and percentage of bean flour in the brown rice:**
537 **bean flour composite (bean, %) on *in vitro* digestibility of protein: (A) gastric digestible**
538 **protein (GDP, %) and (B) total digestible protein (TDP, %)**

Fig 1

Fig 2

A

B

Fig 3

A

B