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Impact of added enzyme-treated bran on the techno-functional properties of puffed-extruded sorghum snack

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ABSTRACT

Dietary fibre intake is crucial for improving human health and reducing the prevalence of diet-related non-communicable diseases. This study determines the effect of the enzyme (Viscozyme®L: a cocktail of cell wall degrading enzymes including arabanase, cellulase, β-glucanase, hemicellulase and xylanase) hydrolyzed fibre on the techno-functional properties of puffed-extruded sorghum snacks made from sorghum flour. Sorghum flour and sorghum flour mixed with untreated bran, water-incubated bran, and enzyme-treated bran were extruded using a twin-screw extruder. The puffed-extruded snacks from sorghum flour with 2-h enzyme-treated bran showed similar expansion ratios to snacks from sorghum flour without bran. The expansion ratios of the without-bran snacks (2.80) and enzyme-treated bran-added snacks (2.77) were significantly ($P < 0.05$) higher than those made with untreated sorghum bran (1.87). A higher expansion ratio usually results in a lighter, crispier texture and a more attractive appearance of puffed-extruded snacks, which consumers often prefer. The enzyme treatment also increased the water solubility index and decreased the water absorption index compared to snacks with untreated bran. This study explores the potential of using an enzyme-treated sorghum bran to manufacture puffed-extruded snacks comparable to those made with sorghum flour.

1. Introduction

The global market for puffed-extruded snacks was valued at USD 51.59 billion in 2019. It is expected to have a 4% growth until 2026 due to increased demand for ready-to-eat meals and changing consumer eating habits (Tyl, 2021). Puffed-extruded and crunchy snacks are typically made from grains like rice, and corn, as well as tubers like potatoes and are consumed between meals. Ready-to-eat snacks are becoming increasingly popular among consumers, especially younger generations, due to their affordability, appealing appearance and availability in various sizes and shapes (Tas and Shah, 2021). Extruded snacks are convenient, shelf-stable foods due to their low moisture content (4–6%) (Tyl, 2021). The versatility of extrusion cooking as a food processing technology enables innovation in the product, allowing for the incorporation of diverse ingredients and nutrient-rich components to create appealing products.

Grain sorghum, ranks as the world's fifth cereal grain, following wheat, maize, rice and barley in terms of quantity (Taylor, 2003). Sorghum has been traditionally used in various dishes and beverages such as whole grain snacks, "rice", semolina, couscous, doughs and dumplings, flatbreads, porridges and gruels, non-alcoholic beverages, and opaque and cloudy beer. Other non-traditional and limited products are ready-to-eat snack foods, gluten-free pasta, noodles and baked products (Taylor and Duodu, 2015). However, whole grain sorghum has not been extensively utilized in the production of ready-to-eat breakfast cereals despite having a similar chemical composition to other grains commonly utilized in ready-to-eat breakfast cereals such as maize, oats, rice, and wheat (Mkandawire, 2015).

Bran, the primary by-product of the sorghum milling process, accounts for 10–11.5% (w/w) of the entire grain (Luna et al., 2022). Several studies have demonstrated that the bioactive compounds found in sorghum grains can benefit gut microbiota and influence factors associated with non-communicable diseases (de Morais Cardoso, 2017). Additionally, these compounds exhibit various biological activities, including anti-inflammatory, antioxidative, antithrombotic, and anti-diabetic properties (Nguyen et al., 2014; Zhang, 2019). The consumption of whole grain products, which are richer in dietary fibre than refined grain products and a balanced nutritional fibre profile (soluble and insoluble fibre), can potentially reduce the risk of developing non-communicable diseases (Delcour and Poutanen, 2013). The term

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"whole grain products" describes products made with an endosperm, germ, and bran content comparable to what would normally be found in intact grains (Barrett et al., 2019).

Even though consumption of whole grain has various health benefits, the fibre from whole grain can cause negative sensory properties and can affect the techno-functional properties of the food products (Menis-Henrique et al., 2020). Fibre in the extrusion cooking to manufacture snacks can affect the product quality. Fibre can prevent bubble formation by disrupting the dynamics of cell formation during expansion, which results in reduced product expansion and harder texture (Robin et al., 2012). The addition of fibre, mainly insoluble dietary fibre (IDF), during extrusion cooking reduces the starch content in the matrix, which can hinder the expansion process (Oliveira et al., 2015). The reduced expansion of bran-enriched snacks can be attributed to several factors. Adding bran decreased the starch content in the formulation, which ideally should be between 60 and 70% for optimal expansion (Kannadhason and Muthukumarappan, 2010). Bran competes with starch and protein for water in the food matrix, limiting water availability for starch gelatinization. This reduced water availability negatively impacts the rate of starch gelatinization, which in turn affects the expansion rate (Stojceska et al., 2010). According to a review by Tyl (2021), the higher fat content in bran-enriched samples may promote the formation of amylose–lipid complexes during extrusion, further restricting starch swelling and gelatinization, which are crucial for expansion. Bioprocessing of bran by enzymatic hydrolysis can convert insoluble dietary fibre into soluble dietary fibre and can reduce sensory defects (Coda et al., 2014). Santala et al. (2014) reported that the modification of wheat bran by xylanase before extrusion led to a decrease in the hardness of the extrudate. Xylanase breaks down wheat bran's non-starch polysaccharides, increasing water-extractable arabinoxylans and decreasing water-holding capacity, resulting in a less dense, softer extrudate. However, enzymatic modification of sorghum bran on the structure, physicochemical, and functional properties is hardly found in the literature. Sorghum comprises non-starch polysaccharides, primarily found in the pericarp and endosperm cell walls. According to Taylor and Emmambux (2010), sorghum grains contain starch, cellulosic and non-cellulosic polysaccharides (primary glucuronoarabinoxylans [GAX]). Thus, this study determines the effects of added pre-processed sorghum bran by enzymatic treatment (Viscozyme®L-cocktail of cell wall degrading enzymes including arabanase, cellulase, β-glucanase, hemicellulase and xylanase) on the techno-functional properties of puffed-extruded sorghum snacks.

2. Experimental

2.1. Materials and sample preparation

White non-tannin sorghum grain (MACIA-white type I non-tannin) was procured from ICRISAT (Matopos, Zimbabwe). The grains were cleaned by dipping them in sodium hypochlorite [250 ppm] for 90 s and then dried at 40 °C in a hot-air oven for 14 h. The sorghum grain was decorticated by removing the outer pericarp, representing 30% of the whole grain, for 7 min with a tangential abrasive dehulling device (TADD) (Norton type R284 Metalite, Saint-Gobain Abrasives, Isando, South Africa) fitted with a 60-grit sandpaper to obtain endosperm. The decorticated grain was then milled below 250 μm using a hammer mill (Perten Instruments AB, Stockholm-Sweden). This is referred to as sorghum flour in the text. The bran was also milled below 250 μm, washed with water to remove excess sorghum flour, and dried at 40 °C.

2.2. Bran treatment

The dried bran was treated with a Viscozyme L® [number:70075747, Novozymes] containing a cocktail of arabanase, cellulase, β-glucanase, hemicellulase and xylanase at a concentration of 1% (w/w) based on the bran weight. The bran and enzyme were mixed with distilled water at a ratio of 3: 7 (w/w). The time for incubation was 2 h and 24 h at 50 °C in a water bath. At the end of the incubation time, the sample was heated in boiling water for 10 min to inactivate the enzymes and then dried at 40 °C. Viscozyme®L is inactivated at temperatures above 60 °C (140 °F) [according to the manufacturer]. Extended exposure to temperatures above this threshold can result in the complete inactivation of the enzyme. Control brans (water-incubated bran) were produced by incubating at similar conditions (2 h and 24 h at 50 °C) without enzyme. The shorter periods allow for limited enzymatic action and biochemical reactions and longer periods facilitate comprehensive breakdowns (Vong et al., 2017).

2.3. Production of puffed-extruded snack

Sorghum flour was mixed with enzyme-treated bran and control bran (i.e., water-incubated bran without enzymes) in a weight ratio of 90:10 (flour to bran). Additionally, a control sample with untreated bran at the same ratio of sorghum flour to bran was prepared, as well as a control sample of sorghum flour without any bran. The raw materials were then subjected to extrusion cooking. The sample was extruded using a pilot scale co-rotating twin screw extruder TX32 (CFAM Technologies, Potchefstroom – South Africa). Extrusion conditions were set at a screw speed of 500 rpm, a feed rate of 10 kg/h with five-barrel temperature zones of 60 °C, 70 °C, 80 °C, 140 °C and 140 °C [the latter temperature was the die temperature] at a moisture feed of 20%. The specific mechanical energy was calculated according to Godavarti and Karwe (1997) using the following inputs: the screw speed (rev/min), total torque, friction torque, and mass flow rate (kg/hr). The specific mechanical energy (SME) values were similar for all the treatments. Extrudates were dried using a hot air oven at 90 °C for 10 min, cooled at room temperature, and kept in a closed air-tight storage container at 4 °C for further analyses.

2.4. Proximate composition

The measurement of moisture (AOAC 930.15), protein (AOAC990.03), ash (AOAC 942.05), and fat content (AOAC 945.16) of the composite ingredients and snacks was conducted using the standard AOAC methods (AOAC, 2000). The protein content (N x 6.25) was determined using the Dumas combustion method (AACC, 2000). The total carbohydrate content of the samples was determined by the difference of the above-mentioned proximate composition from 100. The snacks were ground with mortar and pestle before the compositional analysis.

2.4.1. Total starch and dietary fibre

The method outlined by McCleary et al. (1997) was employed to determine total starch content [assay version (e), determination of starch in samples, which also contain D-glucose and/or maltodextrins]. This involved the use of thermostable α-amylase (E-BSTAA) and amyloglucosidase (E-AMGDF) obtained from Megazyme (K-TSTA 07/11 in Wicklow, Ireland) to convert starch to glucose enzymatically. Glucose levels were measured colourimetrically via the glucose oxidase-peroxidase reaction and then converted to starch.

The total dietary fibre content of raw materials and extruded snacks was determined using the Megazyme Total Dietary Fibre Kit according to AOAC Approved Method 991.43 (AOAC, 2000). The samples were digested by heat-stable α-amylase (E-BLAAM), amyloglucosidase (E-AMGDF) to break down starch and protease (E-BSPRT) to break down proteins. The sample was filtrated to get insoluble dietary fibre (IDF) as residue, dried and weighed. Soluble dietary fibre (SDF) was precipitated by adding 95% ethanol, filtered, washed, and dried in a hot air oven at 105 °C for 14 h. Total dietary fibre (TDF) content was calculated as the sum of IDF and SDF.

2.5. Estimation of reducing sugars of sorghum snack

The concentration of reducing sugars was determined using the 3,5 Dinitrosalicylic Acid (DNSA) method, as detailed by Lam et al. (2021), with some modifications. The snack was milled with a pestle and mortar and used for the analyses. The snack (1 g) was dispersed in 10 mL of distilled water, vortexed for approximately 5 min and centrifuged. The supernatant containing the soluble reducing sugars was added to a prepared DNSA reagent and incubated in boiling water for 10 min. The absorbance was then measured at 540 nm using the D-glucose standard for the construction of a calibration curve.

2.6. Physical properties analysis

2.6.1. Expansion ratio

The expansion ratio of the snack was calculated by dividing the cross-sectional diameter of the snacks by the diameter of the die (3 mm). A vernier calliper was used to measure the snacks' diameter. Ten different snack pieces from each run were chosen randomly for the diameter measurement at three different points. Three separate locations on each snack were used to measure the diameter.

2.6.2. Bulk density

The bulk density of the snacks was determined by measuring the actual dimensions of the snacks. The diameter of the snack was measured with a vernier calliper, and the length of ten snacks was measured with a metric ruler. The weight per unit length of the snacks was calculated by weighing measured lengths. The following equation was used to determine the bulk density:

$$BD\left(kg\middle|m^3\right) = 4\mathrm{m} \,/\, \pi d^2 l$$

Where: m is the mass of the sample; L is the length of the sample; d is the diameter of the sample.

2.6.3. Hardness

The method of Maskus and Arntfield (2015) was modified to determine the snacks' hardness. The force necessary to break the extruded snacks was measured using a three-point bending break mounted in an EZ-L Texture Analyzer (Shimadzu Corporation, Japan). The samples were fractured using a steel blade with a flat edge by penetrating 3 mm at a constant speed of 10 mm/s. The resistance to breaking was calculated as the force (N) at the fracture point, which was the most significant value in the plot. In this study, twenty replications were used for each treatment. The stress was also calculated by force at fracture over the cross-sectional area of the snacks.

2.6.4. Stereomicroscopy

The snacks were longitudinally and cross-sectionally cut using a scalpel and then fixed to the stage plate using Bostik prestik® (a rubber-like temporary adhesive). Images of the sliced samples and their surfaces were captured with a Discovery V20 stereomicroscope (Zeiss, Göttingen, Germany). Additionally, measurements of bubble size were conducted using the stereomicroscope and ImageJ.

2.6.5. Colour

The sample was ground into powder using mortar and pestle. The extrudates' colour profile was determined using a Chroma meter CR-400 (Konica Minolta Sensing, Osaka, Japan). The CIELAB colour system quantifies colour in terms of lightness (L^*), red/green intensity (a^*), and yellow/blue intensity (b^*). Before taking measurements, chroma was initially calibrated using a white tile. Measurements were then randomly conducted at various points along the circumference of the petri dish's surface. For each petri dish, three readings were recorded.

Table 1
Effects of added enzyme-treated bran (for 2 h and 24 h) on the chemical composition of puffed-extruded sorghum snack.

Treatments		Ash (%)		Protein (%)		Moisture (%)		Fat (%)		Carbohydrate (%)		Total starch (%)	
Bran addition	Incubation time (hour)	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Without Bran	N/A	0.8^{a}±0.00	0.8^{a}±0.01	10.7^{d} ± 0.02	10.9^{d} ± 0.15	9.9^{a}±0.44	5.9^{a}±1.77	1.5^{d} ± 0.00	0.1^{d} ± 0.01	86^{b} ± 0.06	88^{a}±0.15	72^{a}±2.01	64^{a}±1.39
Untreated Bran	N/A	1.2^{a}±0.28	1.0^{a}±0.01	11.5^{a}±0.02	11.6^{a}±0.19	9.7^{b} ± 0.93	4.8^{b} ± 2.88	1.6^{c}±0.07	0.8^{a}±0.27	85^{a}±0.23	86^{b} ± 0.20	65^{b} ± 1.29	60^{b} ± 0.50
Water incubated bran	2	1.1^{a}±0.04	0.8^{a}±0.46	11.0^{c}±0.08	11.4^{b} ± 0.24	8.2^{d} ± 0.86	5.2^{b} ± 2.08	1.9^{b} ± 0.05	0.6^{b} ± 0.18	85^{a}±0.06	87^{a}±0.66	60^{c}±5.01	56^{c}±7.34
	24	1.1^{a}±0.01	0.9^{a}±0.36	11.2^{b} ± 0.07	11.3^{c}±0.08	7.7^{c}±0.12	3.9^{d} ± 0.37	2.1^{a}±0.05	0.6^{b} ± 0.14	85^{a}±0.24	87^{a}±0.24	65^{b} ± 0.53	54^{d} ± 0.27
Enzyme-treated Bran	2	1.1^{a}±0.02	0.9^{a}±0.20	11.3^{b} ± 0.08	11.4^{b} ± 0.02	7.9^{d} ± 0.67	4.9^{b} ± 1.20	1.9^{b} ± 0.04	0.9^{a}±0.09	85^{a}±0.06	87^{a}±0.26	67^{b} ± 0.13	56^{c}±1.17
	24	0.9^{a}±0.02	0.9^{a}±0.21	11.4^{a}±0.03	11.4^{b} ± 0.05	8.4^{c}±0.27	4.5^{c}±1.01	2.0^{a}±0.04	0.3^{c}±0.04	$85.^{a}$±0.01	88^{a}±0.30	59^{c}±1.45	50^{e}±1.18

Values are means ± standard deviation. The value within the same column followed by different letters is significantly different ($p < 0.05$). * Bran was added to endosperm at 10% (w/w) (30% level of decortication). Total carbohydrate is the difference from (100–(Ash + Fat + Protein), before and after means before and after extrusion.

Table 2
Effects of added enzyme-treated bran (for 2 h and 24 h) on the soluble dietary fibre (SDF), insoluble dietary fibre (IDF), total dietary fibre (TDF) and reducing sugar of puffed-extruded sorghum snack.

Treatment		SDF (%)	IDF (%)	TDF (%)	Reducing sugar (mg/g)
Bran addition	Incubation time (hour)				
Without bran	N/A	2.26^{a}±0.06	4.03^{d} ± 0.68	6.29^{b} ± 0.74	0.29^{f}±0.01
Untreated bran	N/A	0.67^{c}±0.32	9.34^{a}±0.21	10.01^{a}±0.11	0.47^{e}±0.00
Water-incubated bran	2	0.98^{d} ± 0.08	9.03^{b} ± 0.47	10.00^{a}±0.39	0.59^{d} ± 0.00
	24	1.15^{d} ± 0.28	8.18^{b} ± 0.26	9.34^{a} ±0.02	0.87^{b} ± 0.01
Enzyme-treated bran	2	1.50^{c}±0.11	7.78^{b} ± 0.08	9.28^{a}±0.19	0.72^{c}±0.01
	24	1.65^{b} ± 0.36	7.48^{c}±0.71	9.13^{a}±0.35	1.17^{a}±0.00

Values are means [as dry basis] ±standard deviation. The value within the same column followed by different letters is significantly different ($p < 0.05$). *Bran was added to endosperm at 10% (w/w) (30% level of decortication).

2.7. Functional properties analysis

2.7.1. Water absorption index and water solubility index

The method used by Mapengo et al. (2021) was modified slightly to determine the extrudates' water absorption index (WAI) and water solubility index (WSI). The milled extrudate using a pestle and mortar was dispersed in water (10 % w/v) and incubated at 30 °C for 30 min while vortexing the mixture every 5 min. The mixture was centrifuged (3000×*g* for 10 min), and the supernatant was decanted into a pre-weighed moisture tin and oven-dried at 105 °C overnight for 14 h. The water solubility index was then calculated as the ratio of the weight of the dried supernatant to the weight of the dry soluble starch and expressed as a percentage (%). The pellet obtained after centrifugation was weighed to determine the water absorption index, expressed as the weight of wet residue (g) per gram of dry sample.

2.8. Statistical analysis

The main effects of treatments [independent variables: 6 treatments as sorghum flour without bran snack (reference), untreated bran snack (control),water-incubated bran snacks (2 h&24 h),enzyme-treated bran snacks (2 h&24 h)] on the dependent variables were analyzed using one-way multivariate analysis of variance (MANOVA) with IBM SPSS version 29 (SPSS, Inc., Chicago, IL, USA). Tukey's HSD test at a 95% confidence level was used as a post hoc comparison test. Results are reported as the mean from triplicate experiments ± standard deviation.

3. Results and discussion

3.1. Chemical composition

The proximate composition of raw flours and sorghum snacks is presented in Table 1. The moisture content of the snack varies from 3.9 to 5.9. The moisture content of the snacks was similar to that reported by Dar et al. (2016), who extruded brown rice grits (4.4–5.4%). Low moisture content, around 4–6%, is preferred for shelf stability and crispiness of extruded snacks (Tyl, 2021).

The fat and protein content increased for the snack manufactured with added sorghum bran compared to the snack manufactured from refined sorghum flour (Table 1). This is because sorghum bran contains germ [rich in fat] and has a higher protein content than endosperm flour. The high temperature and pressure during extrusion cooking cause protein denaturation and fat release. This process improves the digestibility and bioavailability of proteins and fats, making them more available in the final product (Li, 2023).

The fat decreased for the extrudates compared to their respective raw flour mixtures. The enzyme-treated bran-added snack (2 h) decreased by about 52 % compared to its flour before extrusion. During extrusion, a starch component, amylose, can form complexes with lipids (fats). Lipids more specifically monoglycerides can form amylose-lipid complexes and be trapped within the starch matrix by forming amylose-lipid complexes (Huang et al., 2020). The latter can reduce lipid extraction during fat analysis with solvents, such as petroleum ether or hexane thereby reducing the measurable fat content in the extruded snack (Alam et al., 2016).

Adding bran enhanced the total dietary fibre content (both soluble and insoluble) of the snacks from 6 to 10% (Table 2). A food product can be considered high fibre if it contains at least 6 g of fibre per 100 g or a source of fibre if it contains 3 g per 100 g (European Commission, 2006). Based on the total dietary content (approximately 9–10%), extrudate snacks with added bran can be claimed as high-fibre puffed-extruded snacks (Table 2). Food products rich in DF were shown to have potentially beneficial effects against diet-related non-communicable diseases, such as heart disease and diabetes (Shimabukuro, 2014).

Enzymatic treatment with a cocktail of cell wall degrading enzymes (Viscozyme®L) did not affect the total amount of DF in the bran-added snacks, i.e., similar values (9.13–10.01%) were observed between snacks with treated or untreated bran. However, the enzyme treatment hydrolyzed the DF by the observed increase of soluble dietary fibre and concomitant decrease of insoluble DF in the enzyme-treated bran extrudates (Table 2). Similar results from literature reported that cellulase and xylanase treatment on potato residue efficiently promoted the transformation from insoluble dietary fibre to soluble ones (Ma et al., 2022). The enzymatic incubation of rye bran with cellulase and xylanase mixture of Depol 740 L breaks down complex carbohydrates and Grindamyl A 1000 to break down starches present in the bran (Nordlund et al., 2013) and rice bran with xylanase and cellulase (Wen et al., 2017) increased the solubility of fibres.

The soluble dietary fibre value slightly increased in enzyme-treated bran-added snacks (2 h&24 h) and water-incubated bran-added snack-without enzyme treatment (2 h&24 h) compared to untreated bran-added snacks (Table 2). The endogenous hydrolytic enzymes (1,3; 1,4 β-glucanase) in the bran's aleurone layer can partially hydrolyze cell wall polysaccharides (Aubert et al., 2018), but the effect was statistically significant ($p < 0.05$) compared to the enzyme-treated bran.

The snacks containing enzyme-treated bran and water-incubated bran had higher amount of reducing sugars compared to the untreated bran-added snacks, irrespective of the incubation time (Table 2). The higher levels of reducing sugars in the snacks may be caused by the enzyme's capacity to convert complex carbohydrates in the bran, for example, non-starch polysaccharides and residual starches, into simpler sugars like glucose and maltose, leading to increased reducing sugar content. Indeed, Nikinmaa et al. (2022) also observed an increase in free glucose (from 0.67 to 2.39 g/100 g) after the treatment of sorghum flour with Viscozyme®L (1% enzyme, 4 h, 50 °C) via the degradation of the cellulose and beta-glucan present in sorghum. The increase of free reducing sugars in the snack containing control bran suggests that the endogenous enzymes could hydrolyze part of the polysaccharides into free sugars, but the change in the soluble and insoluble DF profile was small.

3.2. Physical properties of snacks

The addition of untreated bran caused a reduction in the expansion,

Table 3
Effects of added enzyme-treated bran (for 2 h and 24 h) on the expansion ratio (ER), bulk density (BD), hardness, stress, and bubble size of puffed-extruded sorghum snacks.

Treatment		ER (g/g)	BD (kg/m^3)	Hardness (N)	Stress (N/m^2)	Bubble size (mm)
Bran addition	Incubation time (hour)					
Without bran	N/A	2.80^{a}±0.03	132.13^{f}±0.02	3.11^{d} ± 0.17	5.59^{e}±0.35	1.29^{a}±75.17
Untreated bran	N/A	1.87^{e} ±0.02	296.19^{a}±0.05	4.41^{a}±0.23	17.79^{a}±0.81	0.77^{b} ± 191.29
Water-incubated bran	2	2.54^{b} ± 0.01	142.30^{d} ± 0.13	3.18^{d} ± 0.05	6.98^{d} ± 0.19	0.87^{b} ± 104.54
	24	2.45^{d} ± 0.02	182.17^{b} ± 0.05	4.31^{b} ± 0.13	10.13^{b} ± 0.23	0.87^{b} ± 123.62
Enzyme-treated bran	2	2.77^{a}±0.09	133.40^{e}±0.11	3.12^{d} ± 0.09	5.74^{e}±0.35	1.14^{a}±77.15
	24	2.53^{c}±0.03	143.16^{c}±0.08	4.13^{c}±0.03	9.15^{c}±0.22	1.13^{a}±102.49

Values are means ± standard deviation. The value within the same column followed by different letters is significantly different ($p < 0.05$). *Bran was added to endosperm at 10% (w/w) (30% level of decortication).

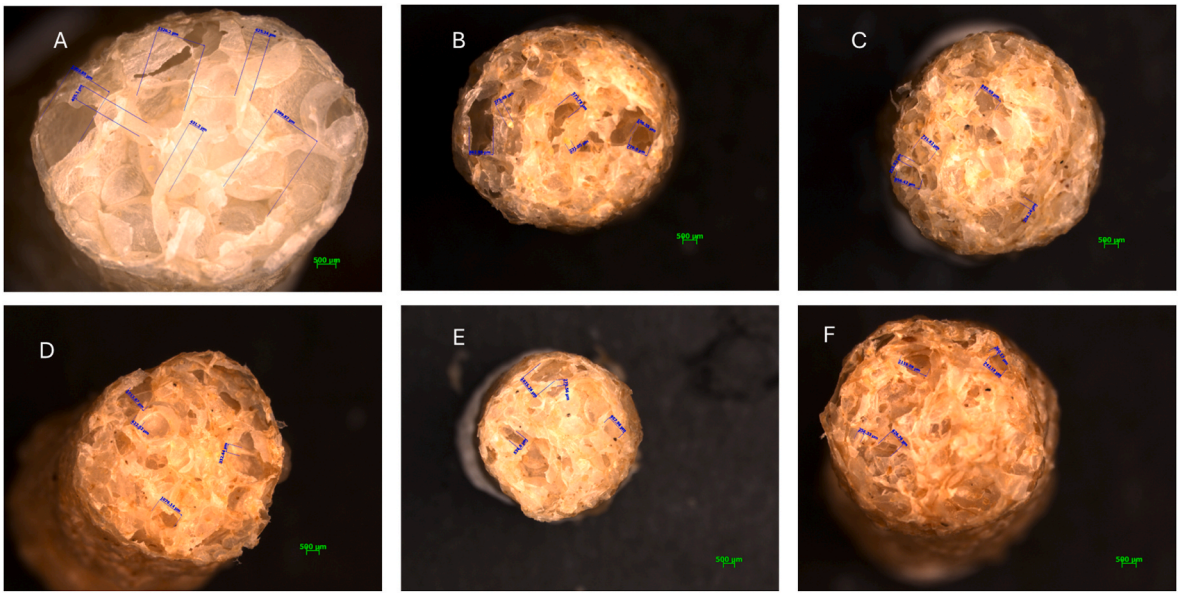

Fig. 1a. A Stereomicroscopy image with a scale bar (500 μm) shows the snack cross-section. **A**: without bran snack; **B**: untreated bran added snack, C: 2 h of water-incubated bran added snack; **D**: 2 h of the enzyme-treated bran added snack; **E**: 24-h of water-incubated bran added snack; **F**: 24-h of enzyme treated bran added snack.

indicated by a decrease in the expansion ratio compared to the other snacks (sorghum flour without bran snacks, enzyme-treated bran-added snacks and water-incubated bran-added snacks) (Table 3). The presence of insoluble dietary fibre caused a decrease in the radial expansion of cereal extrudates and increased their longitudinal expansion (Santala et al., 2014). Dietary fibre can adhere to the structure of air bubbles and puncture their cells, causing an early rupture of the cell walls (Van der Sman and Broeze, 2013). This can reduce the extensibility of the cells and increase the number of small broken cells, which may inhibit air bubbles from expanding (Van der Sman and Broeze, 2013). According to a review by Yadav, 2024, fibres can disrupt the formation of a continuous and uniform matrix, which may hinder the proper expansion of the extrudate.

In the current study, bran particles affected the cellular structure, as shown by the stereomicroscope's cross-sectional and longitudinal section images (Fig. 1a & b). The cells were small in the snacks containing more insoluble dietary fibre (untreated bran-added snack in Fig. 1a and b), and the large bran particles were visible. The extrudates with a high amount of soluble dietary fibre (sorghum flour without bran snack and enzyme-treated bran-added snacks), the cell size distribution was less homogeneous due to the presence of some large cells, and the bran particles were less visible. Similar features in the images of this study were also observed by Santala et al. (2014) on wheat bran-enriched puffed-extruded snacks but did not determine the bubble size. The bran particles in snacks made from untreated bran were more visible with smaller bubble sizes (Table 3) than all the other snacks, probably contributing to a reduced expansion and a higher snack density.

The snacks with water-incubated bran (2 h&24 h) resulted in a higher expansion, lower bulk density, lower hardness, and lower stress for breaking than the untreated bran-added snack (Table 3). The amount of the insoluble dietary fibre for the water-incubated bran-added snacks was significantly ($p > 0.05$) lower than that of the untreated bran-added snacks. The decrease in insoluble DF of treated bran snacks without enzymes could be related to the higher expansion compared to untreated bran-added snacks. This suggests that, after 2 h of bran incubation, bran degraded to produce more soluble fibres through endogenous enzymes from the aleurone layer, as explained earlier. Wheat bran hydration can activate the endogenous enzymes in the aleurone layer, which alters the fibre structure in the bran. This process leads to the solubilization of arabinoxylans and a slight degradation of glucose-containing polymers (Hartikainen and Katina, 2012). It is also possible that endogenous microbes, such as lactic acid bacteria, can grow during incubation for 24 h. This is shown by a lower pH of 4.22 (Supplementary Table 1) for the bran after the 24-h incubation with an enzyme**.** Lactic acid bacteria degrade insoluble dietary fibre into soluble ones (Li et al., 2022). Li et al. (2022) indicated that the fermentation process with lactic acid bacteria on prosomillet bran showed an increased yield of soluble dietary (SDF) from 4.2 % to 7.2 %, which may be caused by the degradation of insoluble dietary fibre (IDF).

The 2-h enzyme-treated bran to refined sorghum flour significantly

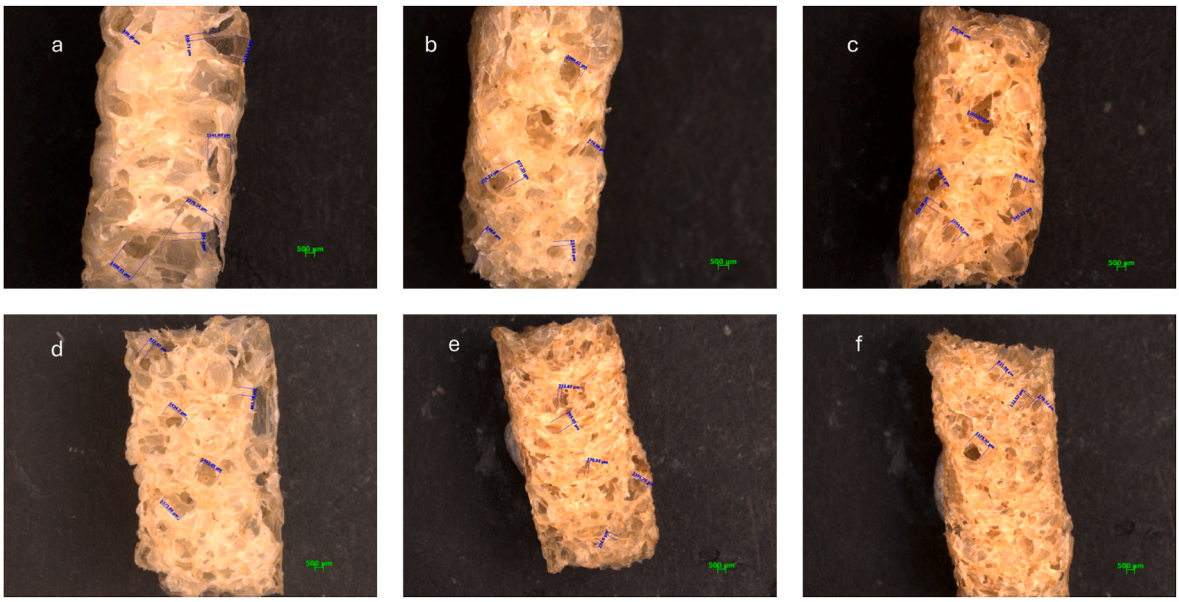

Fig. 1b. A Stereomicroscopy image with a scale bar (500 μm) showing the longitudinal section of the snacks. **a:** without bran snack; **b**: untreated bran-added snack; **c:** 2 h of water-incubated bran added snack; **d**: 2 h of enzyme-treated bran– added snack **e**: 24 h of water-incubated bran– added snack; **f**: 24 h of enzyme treated bran– added snack.

Table 4
Effects of added enzyme-treated bran (2 h and 24 h) on the colour profile of puffed-extruded sorghum snacks.

Treatment		L^*	a^*	b^*
Bran addition	Incubation time (hour)			
Without bran	N/A	79.58^{a}±0.08	0.46^{f}±0.01	20.45^{a}±0.01
Untreated bran	N/A	77.08^{b} ± 0.08	1.31^{e}±0.03	17.53^{e}±0.09
Water-incubated bran	2	75.31^{c}±0.09	2.36^{d} ± 0.11	18.28^{d} ± 0.05
	24	75.26^{c}±0.09	2.74^{c}±0.06	18.31^{d} ± 0.06
Enzyme-treated bran	2	74.26^{d} ± 0.03	2.97^{b} ± 0.02	18.96^{c}±0.09
	24	70.68^{e}±0.07	4.46^{a}±0.05	19.62^{b} ± 0.01

Values are means ± standard deviation. The value within the same column followed by different letters is significantly different ($p < 0.05$). L*: lightness; a*: redness; b*: yellowness. *Bran was added to endosperm at 10% (w/w) to refined sorghum flour (30% level of decortication).

increased the expansion ratio, decreased bulk density, the hardness and stress involved in breaking their snacks compared to the control [sorghum flour with untreated bran] (Table 3). The 2-h enzyme-treated bran-added snack had lower insoluble dietary fibre (7.78 %) than the 2-h water-incubated bran-added snack (9.03%), impacting snacks' bubble size, expansion ratio, bulk density, and hardness. The use of xylanase to modify bran has been shown to enhance crispiness while reducing hardness and piece density in extrudates containing wheat bran (Santala et al., 2014). The 2-h enzyme-treated bran-added snack significantly ($p < 0.05$) increased the bubble size with increased expansion ratio, decreased bulk density, hardness and stress required to break snacks compared to the 24-h enzyme-treated bran-added snack. Statistically, the 2-h enzyme-treated bran-added snack had a similar bubble size and expansion ratio to sorghum flour without bran snacks (Table 3 and Fig. 1a and b). This suggests that increasing soluble dietary fibre during extrusion increased the melt's viscosity, making the matrix more continuous, potentially stabilizing it, and promoting bubble formation to increase expansion. The snacks made with 24-h enzyme-treated bran had a lower expansion despite having higher soluble dietary fibre content than those made with 2-h enzyme-treated bran. The 24-h enzyme-treated bran may have undergone more structural changes or degradation compared to the 2-h enzyme-treated bran, which could potentially reduce its ability to expand as much during the extrusion process. According to Xie et al. (2021), soluble dietary fibre (SDF) may interact with water to alter the viscosity profile of starch melt, maintaining the continuous phase of the melt and facilitating extrudate expansion. A higher expansion ratio usually results in a lighter, crispier texture and a more attractive appearance of puffed-extruded snacks, which consumers often prefer (Kantrong et al., 2022).

The impact of adding enzyme-treated and water-incubated sorghum bran to refined sorghum flour on the colour profile of puffed-extruded sorghum snacks is shown in Table 4. The lightness value of the snacks with bran decreased compared to the sorghum flour without bran snack. This was expected due to the brown colour of the bran showing lower L^* values. The Maillard reaction between the free amino groups of proteins and the carbonyl group of reducing sugars at temperatures between 140 °C and 165 °C (Singh , 2007) can also cause browning and flavour development (Millward, 1999). Additionally, changes in L^* values can be attributed to sorghum bran, which is rich in dietary fibre (Aguiar et al., 2023) and natural pigments such as phenolic compounds (Simnadis et al., 2016) that give it a darker colour compared to refined flour, which lacks colour-imparting compounds. The findings agree with those of Ferreira et al. (2011), who found that wheat bran negatively affected the lightness of puffed-extruded snacks made of corn. Wheat bran layers, including the pericarp and aleurone cells, contain polysaccharides such as cellulose, arabinoxylans, lignin, glucomannans, and β-glucan, which contribute to the brown colour of the flour.

After 2 and 24 h of water-incubated bran, the water-incubated bran-added snacks for 2 h & 24 h snacks were dark brown with higher a^* and b^* values compared to the untreated bran-added snacks. After 2 and 24 h, the enzyme-treated bran-added snacks were less dark brown and had higher a^* and b^* than the water-incubated bran-added snacks. The increase in the redness and yellowness of the water-incubated bran-added snacks for 2 h & 24 h and enzyme-treated bran-added snacks for 2 h &24 h could be caused by non-enzymatic browning between amino acids and reducing sugars, which may have resulted in the formation of melanoidin and, given the final product a reddish-yellow colour (Wani,

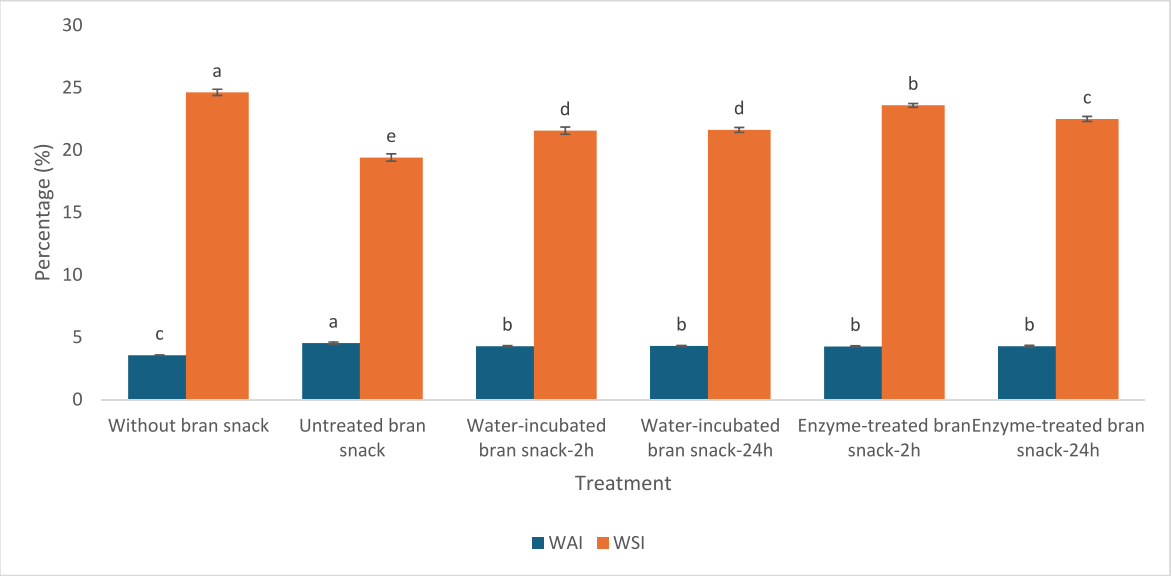

Fig. 2. Effects of added enzyme-treated bran (for 2 h and 24 h) on the water absorption index (WAI) and water solubility index (WSI) of puffed-extruded sorghum snack.

2019).

3.3. Functional properties of extrudates

Water Absorption Index and Water Solubility Index influence the texture of snacks in different ways. The bran-added snacks generally had a higher water absorption index (WAI), ranging from 4.28 to 4.53, than the sorghum flour without bran snack (3.56) in Fig. 2. The increase in WAI for the bran-added snacks might be due to higher water absorption from the bran during extrusion. Extrusion leads to a more open bran structure and allows more water penetration and retention (Qi et al., 2023). The untreated bran-added snacks had the highest value of WAI compared to water-incubated bran-added (2 h&24 h) and enzyme-treated bran-added snacks (2 h&24 h). Water is held together to fibre by surface tension in the pores of the matrix, possibly via hydrogen bonds, ionic bonds, and other physical interactions/entrapment (Yaich, 2015). Additionally, the presence of fibre can significantly absorb water, reducing the amount of free water available for starch gelatinization and expansion. This decrease in available water limits steam formation, which is crucial for puffing (Agarwal and Chauhan, 2019). The decreased water absorption index (WAI) or water binding capacity (WBC) of the water-incubated bran-added snacks (2 h&24 h) and enzyme-treated bran-added snacks (2 h&24 h) could be caused by modified fibre structure, due to partial breakdown of the fibre matrix, as observed by soluble dietary fibre.

The water solubility index (WSI) suggests the severity of insoluble polymer degradation by the enzymatic and extrusion process. A significant ($p < 0.05$) increase in WSI was observed in the sorghum flour without bran snack (about 25% WSI), while all other snacks had lower WSI ranging from 19 to 24 % (Fig. 2). It was found that the solubility of starch in water increased along with expansion in the sorghum flour without bran snacks and enzyme-treated bran-added snacks (2 h&24 h). In addition to snacks made from sorghum flour without bran, snacks with enzyme-treated bran and water-incubated bran with increased soluble dietary fibre (SDF) content (as shown in Table 2) exhibited significantly higher water solubility index (WSI) compared to untreated bran added snack. The increase in WSI for enzyme-treated bran-added snacks (2 h&24 h) than the untreated bran-added snack suggests enzymes decompose complex carbohydrates and fibres into smaller, more soluble molecules, increasing the concentration of water-soluble components in the bran and resulting in higher WSI. According to Yadav et al. (2018), higher WSI typically improves expansion properties. The increased solubility facilitates more efficient steam formation during extrusion, improving puffing and a lighter texture. Also, according to a review by Tyl (2021), water incubation hydrates the bran, softening its structure and increasing the solubility of its components. This process breaks down complex carbohydrates and fibres, leading to a higher concentration of water-soluble materials. Similar findings were reported of increased water solubility index for extrudates produced from barley (Altan et al., 2009) and corn grit-based extrudates (Singha et al., 2018). Snacks with a higher Water Absorption Index (WAI) tend to be softer and break down more easily while chewing, increasing mouthfeel and making swallowing easier. In contrast, snacks with a higher Water Solubility Index (WSI) dissolve quickly in the mouth, minimizing the need for extensive chewing and improving the overall sensory experience (Altaf et al., 2021; Muñoz-Pabon, 2022).

4. Conclusions

Enzymatic treatment of sorghum bran with fibre-degrading enzymes can hydrolyze insoluble dietary fibre, converting it to soluble dietary fibre. This bio-transformation of sorghum bran, which increases the soluble dietary fibre content, allows for the production of a puffed-extruded snack made with enzyme-treated bran. This snack is classified as bran-rich and exhibits bubble size and expansion ratios similar to those of a snack made solely from sorghum flour. Further studies need to be conducted on the sensory and nutritional properties of puffed-extruded sorghum snacks to determine their commercial potential.

CRediT authorship contribution statement

Charles Kwasi Antwi: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Natalia Rosa-Sibakov:** Writing – review & editing, Supervision, Conceptualization. **Mohammad Naushad Emmambux:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2024.104051.

Supplementary data

Table S1
pH results of untreated bran, water-incubated bran (2h&24h) and enzyme-treated bran (2h&24h).

Sample	Treatment	Enzyme concentration % (w/w)	Incubation time (hr)	pH
Sorghum bran	Untreated bran	N/A	0	6.06 ± 0.13
	Water-incubated bran	N/A	2	5.60 ± 0.01
			24	4.33 ± 0.00
	Viscozyme®L	1.0	2	5.42 ± 0.00
	Viscozyme®L	1.0	24	4.22 ± 0.00

Data availability

Data will be made available on request.

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