



Original article

Utilisation of dried shiitake, black ear and silver ear mushrooms into sorghum biscuits manipulates the predictive glycaemic response in relation to variations in biscuit physical characteristics

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Summary The nutritional quality of gluten-free products is important to the health of individuals with coeliac disease. Mushrooms are good sources of vitamins, dietary fibres and proteins, and are a low-calorie option that can be used in gluten-free diets to improve their nutritional value. The effects of incorporating dried mushrooms on the hydration and pasting properties of sorghum flour, as well as the physicochemical characteristics and *in vitro* glycaemic response of sorghum biscuits were studied. Sorghum flour enriched with mushroom powders exhibited higher water absorption capacity and swelling power compared with the control ($P < 0.05$). The addition of shiitake (*Lentinula edodes*) mushroom significantly decreased the pasting viscosities, while the addition of black ear (*Auricularia auricula*) and silver ear (*Tremella fuciformis*) mushroom increased viscosity values ($P < 0.05$). Biscuit diameter, thickness and weight loss were reduced with increasing mushroom powder addition, and the colour parameters of biscuits were affected significantly. Enrichment with shiitake and black ear mushroom increased the hardness of biscuits ($P < 0.05$). Inclusion of mushroom powders significantly reduced the predicted glycaemic response of sorghum biscuits ($P < 0.05$). Correlation analysis was conducted to illustrate that hydration dynamics (such as water absorption capacity and swelling power) were negatively correlated with glycaemic response ($P < 0.001$).

Keywords Glycaemic response, hydration properties, mushroom, physical characteristics, sorghum biscuits.

Introduction

Coeliac disease is an auto-immune disorder that affects around 1% of the world's population where the intake of gluten can cause intestine inflammation. This can further induce acute symptoms and malabsorption of essential minerals and vitamins (Gagneten *et al.*, 2020). The rapid increase in diagnosed cases of coeliac disease has led to increasing demand for gluten-free cereals, such as rice, sorghum, maize, millets and teff, and following a gluten-free diet is the only treatment for this disorder (Barretto *et al.*, 2020). Gluten-free products normally have a poor texture with the absence of

gluten which is a structure-forming protein for bakery products (Nespeca *et al.*, 2020). This has stimulated the utilisation of substances, such as hydrocolloids and starch, to mimic the viscoelastic gluten to improve the quality of the gluten-free products (Zoghi *et al.*, 2020). However, the inclusion of hydrocolloids and dietary fibres can sometimes lead to uncharacteristic food properties and hence poor reception by consumers (Brennan *et al.*, 2012, 2013; Grigor *et al.*, 2016; Hossain *et al.*, 2017). Sorghum is a gluten-free grain and the fifth most produced cereal in the world (de Moraes Cardoso *et al.*, 2017). It has the potential to be a gluten-free material to develop biscuits with acceptable sensory and physicochemical properties (Rao *et al.*, 2016, 2018).

Gluten is important in carbohydrate digestion and absorption, and a long-term gluten-free diet may have

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an association with weight gain and an increase in the risk of obesity and diabetes (Zong *et al.*, 2018; Krupa-Kozak & Lange, 2019). Most gluten-free products have a high glycaemic index because of the extensive use of pure starch and rice flour (Aguilar *et al.*, 2020). Some gluten-free cereal products, such as biscuits, that compose a high amount of sugar are much worse than their equivalent products in elevating blood glucose level. It is easy to use the developed strategy of enriching the gluten-free diet with dietary fibre for lowering the glycaemic index and reducing postprandial glycaemia. Patients with coeliac disease who followed a gluten-free diet might have a lower dietary fibre consumption than the recommended amount (Krupa-Kozak & Lange, 2019). Low dietary fibre intake might predispose coeliac disease patients to persistent symptoms (Laurikka *et al.*, 2019). Most of the coeliac disease patients had a dysbiosis of gut microbiota (Chibbar & Dieleman, 2019), while inclusion of prebiotics, such as inulin and oligosaccharide, into gluten-free diet has the potential to be an adjunctive treatment to stimulate the growth of health-promoting gut microbiota (Ma *et al.*, 2020; Marasco *et al.*, 2020). Edible mushrooms can be a good source of prebiotics because they are rich in dietary fibre, such as chitin, β -glucan, mannans, xylans and galactans (Jayachandran *et al.*, 2017; Lu *et al.*, 2021). Shiitake, black ear and silver ear mushrooms are widely cultivated and used as medicinal ingredients in traditional medicine because of their high dietary fibre content and low-calorie content (Royse *et al.*, 2017; Wu *et al.*, 2019), which makes them an ideal choice as biomedical ingredients in the production of commercial food. However, the largest problem with fibre intake is the formation of gas, and too much fibre consumption may cause some symptoms, such as bloating and flatulence (Algera *et al.*, 2019). Recent studies have suggested that the incorporation of powdered mushrooms into cereal foods enriched their nutritional values and attenuated the predictive glycaemic index during *in vitro* digestion (Lu *et al.*, 2020; Wang *et al.*, 2021). The impact on the lower glycaemic response appears to be due to the enriched dietary fibres that can interact with starch granules, water molecules and digestive enzymes, lowering or decreasing the digestibility of starch. More studies still need to be conducted to illustrate the effects of interactions between starch, water molecules and dietary fibres on the hydration properties of starch in relation to the physical characteristics and glycaemic response of the food matrix.

The objectives of this study were to assess the role mushroom powder substitution played in the hydration and pasting properties of gluten-free sorghum flour, and the physical characteristics and glycaemic response of sorghum biscuits. The Pearson's correlation coefficient was determined to evaluate the

relationships between the biscuits and flour parameters and the glycaemic response.

Materials and methods

Materials

Red sorghum flour (Davis Trading, Palmerston North, New Zealand) and dried shiitake (*Lentinula edodes*), black ear (*Auricularia auricula*) and silver ear (*Tremella fuciformis*) mushrooms (Jade Phoenix, Guangzhou, China) were used.

Flour and biscuits preparation

Dried mushrooms were ground in a Laboratory Mill 3310 (PerkinElmer, Waltham, MA, USA) at position 0 to obtain a particle size <500 μm . All the powders were stored in a sealed bag at room temperature. Sugar (65 g), salt (2.1 g) and sodium bicarbonate (2.5 g) were mixed with RO (reverse osmosis) water (50 mL) using a stand mixer (Breville, Australia) at speed 2 for 5 min. Vegetable shortening (64 g) (Kremelta, Peerless foods, Australia) was added to the mixer and agitated at speed 2 for 3 min with scraping down every 1 min. The sorghum flour or mushroom powder-enriched flour (225 g) was then weighed into the mixer and stirred for another 3 min and scrapped down four times. The biscuit dough was rolled and cut (6 mm thickness and 57 mm diameter) and baked for 15 min at 160 °C in a baking oven (BAKBAR, E311, Moffat Pty Ltd., Sydney, Australia). The biscuits were cooled for 30 min, and packed in airtight polyethylene bags, and then stored at approximate 20 °C for 24 h before experimental evaluation. The biscuits formulations replaced 5%, 10% and 15% of sorghum flour with shiitake, black ear and silver ear powders respectively.

Proximate analysis

The moisture content was determined according to methods of (AACC, 2010), as following 44-15.02. Crude protein content was measured using the Dumas method with the conversion factor of 6.25 for sorghum flour and biscuits, and 4.4 for mushroom powders (Lu *et al.*, 2018). The contents of insoluble dietary fibre (IDF), soluble dietary fibre (SDF), total dietary fibre (TDF), resistant starch and total starch were evaluated using commercial Megazyme assay kits (Megazyme International Ireland Ltd, Wicklow, Ireland) based on the method of (Prosky *et al.*, 1992).

Hydration and pasting properties

The sorghum flour functionalities, including water absorption capacity, swelling power and solubility

index were evaluated following the description from Sulieman *et al.* (2019), and Rao *et al.* (2016). Water absorption capacity was determined as g of water bound per g of flour. To determine swelling power and solubility index, the sample flour (0.5 g, W_F) was vortexed with RO water (10 mL) and then the mixture was heated at 90 °C for 10 min. The slurry was cooled in ice cold water for 10 min, and then centrifuged at 2000 g for 10 min. The supernatant layer was dried at 105 °C for 4 h. The weight of residue (W_R) and dried liquid layer (W_D) were recorded. SP and SI were calculated by the formulations,

$$\text{SP} \left(\frac{\text{g}}{\text{g}} \right) = \frac{W_R}{W_F - W_D},$$

$$\text{Solubility (\%)} = \frac{W_D}{\text{Dried } W_F}$$

The pasting characteristics of sorghum flour with mushrooms were measured by a Rapid Viscosity Analyser (Super 4, Newport Scientific, Warriewood, Australia), according to the method of Kumar *et al.* (2018) using Standard Curve 1.

Physical properties

The thickness (mm) and diameter (mm) of biscuits were determined with callipers (INSIZE Inc., Loganville, GA, USA). The hardness of biscuits was determined via a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK) loaded with a 50 kg load cell and a 3-point bend rig. The operation settings were as follows: pre-test speed (2 mm s⁻¹), test speed (5 mm s⁻¹), post-test speed (10 mm s⁻¹) and distance (5 mm). A colourimeter CR-210 (Minolta, Osaka, Japan) was applied to evaluate the surface colour parameters (lightness- L^* , redness- a^* and yellowness- b^*) of biscuits as reported by Gao *et al.* (2017). The chroma (C_{ab}^*) and hue (h_{ab}) were calculated by the following the equations based on the values of redness (a^*) and yellowness (b^*).

$$C_{ab}^* = \sqrt{a^{*2} + b^{*2}},$$

$$h_{ab} = \tan^{-1}(b^*/a^*)$$

In vitro gastrointestinal digestion

The gastrointestinal digestion was investigated referring to the protocol from Hossain *et al.* (2017), including gastric and intestinal steps to evaluate the release of reducing sugars from biscuits. Biscuits (2 g) were dispersed into simulated digesta and the gastric digestion process (total 32 mL) was conducted by incubation with 1 mL of 10% (g mL⁻¹) pepsin (P/1120/53,

Fisher Scientific, London, UK) for 60 min (37 °C) and intestinal digestion (total 53 mL) added with 5 mL of 2.5% (g mL⁻¹) pancreatin from porcine pancreas (A0585, PanReac AppliChem) for 120 min. The total reducing sugars liberated in digesta at each digestion period (0, 20, 60 and 120 min) was determined, and the total reducing sugars released during the whole digestion process were measured using dinitrosalicylic acid reagent and calculated by trapezoid rule to represent the area under the curve (AUC) (Floch *et al.*, 1990).

Statistical analysis

The experiments were conducted in triplicate and recorded by mean values ± standard deviation. Statistical differences were analysed by the one-way ANOVA and the Tukey test was chosen to assess comparisons between multiple mean values ($P < 0.05$). Principal component analysis and Pearson's correlation coefficient were conducted to assess the effects of incorporation of different mushrooms on the parameters of biscuits and flour, and correlative significances ($P < 0.05$, $P < 0.01$ and $P < 0.001$) between the observed parameters.

Results and discussion

Nutritional composition

The nutritional composition of sorghum biscuits enriched by mushrooms (shiitake, black ear and silver ear) is shown in Table 1. The enrichment with mushrooms significantly increased the moisture content of sorghum biscuits, except for those enriched with 5% black ear and 5% silver ear. Biscuits enriched with mushroom at 10% and 15% all had a significant decrease in the total starch content and an increase in the resistant starch content. The dietary fibre profile of sorghum biscuits was changed and increased significantly by the mushroom enrichment. Biscuits enriched with 15% silver ear had the highest SDF content (3.24 g/100 g dm), whilst those enriched with 15% black ear had the highest IDF (14.37 g/100 g dm). Shiitake mushroom enrichment increased the protein content of the biscuits, whilst enrichment with black ear and silver ear mushrooms did not show any effects on the protein content. Any food product that can provide more than 20% of daily value for a nutrient per serving is claimed by US FDA to high in that nutrient. The daily value for dietary fibre and protein is recommended to be more than 25 g and 50 g per 2000 caloric intake, and this means that a food containing more than 5 g of dietary fibre and 10 g of protein per serving can have a 'high in fibre' and 'high in protein' claim (Makhlouf *et al.*, 2019). The results of nutritional composition indicated that all these mushrooms could be used reliably as a source of dietary

fibre to produce high fibre biscuits, while the shiitake can also be used as a source of protein. The change in the nutritional composition of the sorghum biscuits may be associated with the physical characteristics of the biscuits, and the mushroom enrichment might have effects on the hydration and pasting properties of sorghum flour. Pearson's correlation coefficients were performed to illustrate the correlations between the observed parameters and the composition of biscuits (analysed data are shown in Table 4).

Hydration properties

The hydration properties of mushroom powders and sorghum flour with mushroom powder (5–15%) are shown in Table 2. Compared with sorghum flour, mushroom powders had higher values in hydration properties, including water absorption capacity, swelling power and solubility. The water absorption capacity of mushroom-enriched sorghum flour was significantly higher than the control flour ($P < 0.05$). This could be related to the increase in dietary fibres, as positive correlations ($P < 0.001$) between water absorption capacity and IDF ($r = 0.849$), and SDF ($r = 0.910$) were observed. Black ear mushroom exhibited a significantly stronger water absorption capacity than shiitake and silver ear mushroom, and shiitake-enriched samples showed lower values at each substitution level. The difference in the composition of dietary fibres and their molecular structure may result in a different hydrophilic ability. The water absorption capacity results were in

agreement with a study by Sulieman *et al.* (2017), who reported that the inclusion of white button mushroom into fortified gluten-free flour based on sweet potato and rice flour increased water absorption capacity values compared with the control flour.

The swelling power and the solubility index determination of the flour samples involved a thermal (gelatinisation) process, which is affected by the characteristics of water hydrogen bonding and the degree of the amylose leaching from starch granules (Xiao *et al.*, 2020). Sorghum starch is mainly composed of amylopectin (de Morais Cardoso *et al.*, 2017), and the swelling pattern of starch is dependent on the content of amylopectin whose double helices are disrupted during gelatinisation process, while amylose acts as a swelling inhibitor (Singh *et al.*, 2017). The effects of mushroom varieties and substitution levels on the swelling power and solubility index are presented in Table 2. Substitution of black ear and silver ear mushroom powder into sorghum flour significantly increased swelling power of sorghum flour with the increased addition levels. Shiitake-enriched flour had a significantly higher swelling power at 15% substitution than sorghum flour, with no differences observed at 5% and 10% levels. A higher swelling power means that more water molecules were received by the disrupted starch granules or other macromolecules, such as mushroom dietary fibre. This was supported by positive correlations between swelling power and water absorption capacity ($r = 0.922$; $P < 0.001$), as well as swelling power and TDF ($r = 0.880$; $P < 0.001$). Zhou & Kang (2018)

Table 1 Nutritional composition of sorghum biscuits enriched by mushroom powder

Samples	Moisture (g/100 g)	Protein (g/100 g dm)	Total starch (g/100 g dm)	RS (g/100 g dm)	IDF (g/100 g dm)	SDF (g/100 g dm)	TDF (g/100 g dm)
<i>Materials</i>							
Sorghum flour	10.87 ± 0.04 ^C	11.74 ± 0.05 ^B	70.46 ± 0.01 ^A	2.01 ± 0.01 ^A	9.25 ± 0.13 ^D	2.25 ± 0.16 ^D	11.50 ± 0.21 ^C
Shiitake	10.49 ± 0.10 ^D	24.68 ± 0.24 ^A	0.17 ± 0.01 ^C	0.07 ± 0.01 ^{BC}	34.99 ± 0.02 ^C	3.48 ± 0.23 ^C	38.46 ± 0.35 ^B
Black ear	13.81 ± 0.05 ^A	10.92 ± 0.09 ^C	0.23 ± 0.01 ^C	0.08 ± 0.01 ^B	67.79 ± 0.66 ^A	8.99 ± 0.07 ^B	76.78 ± 0.69 ^A
Silver ear	11.83 ± 0.06 ^B	10.88 ± 0.01 ^C	2.03 ± 0.17 ^B	0.04 ± 0.00 ^C	61.41 ± 0.82 ^B	15.20 ± 0.44 ^A	76.56 ± 0.31 ^A
<i>Biscuits</i>							
Control	5.66 ± 0.04 ^f	7.21 ± 0.02 ^e	43.39 ± 0.45 ^a	2.14 ± 0.05 ^c	6.70 ± 0.05 ^f	1.53 ± 0.02 ^f	8.23 ± 0.03 ^f
5% SB	6.75 ± 0.04 ^a	7.76 ± 0.02 ^c	42.08 ± 0.24 ^{bc}	2.54 ± 0.08 ^b	9.06 ± 0.05 ^e	1.68 ± 0.04 ^f	10.75 ± 0.02 ^e
10% SB	6.76 ± 0.04 ^a	8.25 ± 0.11 ^b	40.77 ± 0.21 ^{de}	2.51 ± 0.07 ^b	10.87 ± 0.29 ^d	1.76 ± 0.03 ^f	12.63 ± 0.32 ^d
15% SB	6.75 ± 0.05 ^a	8.84 ± 0.05 ^a	39.55 ± 0.31 ^f	3.13 ± 0.10 ^a	12.75 ± 0.49 ^{bc}	1.79 ± 0.02 ^f	14.54 ± 0.51 ^c
5% BEB	5.72 ± 0.02 ^{ef}	7.28 ± 0.02 ^{de}	41.81 ± 0.30 ^{cd}	2.18 ± 0.08 ^c	9.59 ± 0.30 ^e	1.84 ± 0.10 ^{ef}	11.42 ± 0.20 ^e
10% BEB	5.94 ± 0.02 ^d	7.29 ± 0.05 ^{de}	38.91 ± 0.26 ^{fg}	2.52 ± 0.07 ^b	12.90 ± 0.01 ^b	2.42 ± 0.11 ^{cd}	15.31 ± 0.12 ^{bc}
15% BEB	6.43 ± 0.07 ^b	7.38 ± 0.07 ^d	37.98 ± 0.43 ^g	2.51 ± 0.06 ^b	14.37 ± 0.32 ^a	2.98 ± 0.03 ^{ab}	17.36 ± 0.34 ^a
5% SEB	5.71 ± 0.03 ^{ef}	7.20 ± 0.01 ^e	42.99 ± 0.49 ^{ab}	2.21 ± 0.11 ^c	9.44 ± 0.25 ^e	2.17 ± 0.04 ^{de}	11.60 ± 0.21 ^{de}
10% SEB	5.82 ± 0.04 ^e	7.31 ± 0.03 ^{de}	41.36 ± 0.47 ^{cd}	2.54 ± 0.01 ^b	11.81 ± 0.19 ^{cd}	2.66 ± 0.16 ^{bc}	14.48 ± 0.35 ^c
15% SEB	6.24 ± 0.04 ^c	7.33 ± 0.05 ^{de}	39.94 ± 0.36 ^{ef}	2.49 ± 0.08 ^b	13.13 ± 0.01 ^b	3.24 ± 0.17 ^a	16.37 ± 0.16 ^{ab}

Abbreviations: BEB, black ear biscuit; IDF, insoluble dietary fibre; RS, resistant starch; SB, shiitake biscuit; SDF, soluble dietary fibre; SEB, silver ear biscuit; TDF, total dietary fibre.

Values are means ± standard deviation of triplicates. Values in the same column for biscuits with different lowercase letters are significantly different ($P < 0.05$). Values in the same column for materials with different uppercase letters are significantly different ($P < 0.05$).

Table 2 Hydration and pasting properties of sorghum flour enriched by mushroom powder

Samples	WAC (g g ⁻¹)	SP (g g ⁻¹)	SI (g g ⁻¹)	PV (cP)	TV (cP)	FV (cP)	BV (cP)	SV (cP)	PT (°C)
<i>Mushrooms</i>									
Shiitake	3.76 ± 0.04 ^C	4.74 ± 0.10 ^C	26.55 ± 0.36 ^A						
Black ear	11.63 ± 0.07 ^A	16.12 ± 0.15 ^A	5.26 ± 0.11 ^C						
Silver ear	9.65 ± 0.08 ^B	10.28 ± 0.09 ^B	8.39 ± 0.09 ^B						
<i>Flour</i>									
Control	2.04 ± 0.02 ⁱ	6.01 ± 0.12 ^f	4.94 ± 0.23 ^{ef}	606.33 ± 4.93 ^g	603.67 ± 5.69 ^f	2080.33 ± 14.36 ^g	2.67 ± 1.16 ^b	1476.67 ± 12.01 ^e	92.28 ± 0.49 ^a
5% SF	2.19 ± 0.05 ^h	6.18 ± 0.05 ^{ef}	6.06 ± 0.00 ^{cd}	503.67 ± 6.51 ^g	498.00 ± 7.55 ^g	1553.70 ± 27.00 ^h	5.67 ± 1.53 ^b	1055.70 ± 19.50 ^f	92.62 ± 0.54 ^a
10% SF	2.38 ± 0.02 ^g	6.53 ± 0.05 ^{def}	7.78 ± 0.26 ^b	202.33 ± 14.29 ^g	510.33 ± 13.32 ^g	1448.00 ± 35.20 ⁱ	10.00 ± 1.00 ^b	937.70 ± 22.90 ^{ab}	92.10 ± 0.00 ^{ab}
15% SF	2.54 ± 0.03 ^f	6.78 ± 0.06 ^{cd}	9.05 ± 0.13 ^a	512.30 ± 17.60 ^g	499.30 ± 17.60 ^g	1281.00 ± 31.20 ⁱ	13.00 ± 0.00 ^b	781.67 ± 13.65 ^{ab}	92.08 ± 0.08 ^{ab}
5% BEF	2.79 ± 0.08 ^e	6.77 ± 0.28 ^{cd}	4.41 ± 0.34 ^f	987.67 ± 12.66 ^d	959.33 ± 11.15 ^{cd}	3188.33 ± 3.21 ^c	28.33 ± 1.53 ^b	2229.00 ± 13.11 ^c	89.63 ± 0.08 ^d
10% BEF	3.25 ± 0.01 ^c	7.62 ± 0.08 ^b	4.79 ± 0.13 ^{ef}	1454.00 ± 19.70 ^b	1406.00 ± 14.80 ^b	4198.67 ± 16.50 ^b	48.00 ± 6.93 ^b	2792.67 ± 10.02 ^b	87.73 ± 0.46 ^e
15% BEF	3.61 ± 0.02 ^a	8.27 ± 0.44 ^a	4.94 ± 0.23 ^{ef}	2003.00 ± 19.30 ^a	1699.70 ± 48.60 ^a	5363.00 ± 45.10 ^a	303.30 ± 63.20 ^a	3663.30 ± 89.40 ^a	79.95 ± 0.05 ^f
5% SEF	2.47 ± 0.07 ^g	6.54 ± 0.05 ^{de}	5.09 ± 0.13 ^{ef}	747.30 ± 21.40 ^f	737.30 ± 22.70 ^g	2438.30 ± 33.80 ^f	10.00 ± 2.65 ^b	1701.00 ± 11.14 ^d	92.33 ± 0.40 ^a
10% SEF	3.04 ± 0.01 ^d	7.02 ± 0.14 ^{cd}	5.98 ± 0.13 ^d	934.33 ± 2.08 ^e	901.67 ± 1.53 ^d	2641.67 ± 12.01 ^e	32.67 ± 0.58 ^b	1740.00 ± 11.14 ^d	91.27 ± 0.03 ^{bc}
15% SEF	3.44 ± 0.05 ^b	7.28 ± 0.10 ^{bc}	6.58 ± 0.26 ^c	1059.70 ± 26.40 ^g	1002.00 ± 25.50 ^c	2755.30 ± 55.20 ^d	57.67 ± 1.16 ^b	1753.30 ± 29.90 ^d	90.73 ± 0.54 ^c

Abbreviations: BEF, black ear-enriched flour; BV, breakdown viscosity; FV, final viscosity; PT, pasting temperature; PV, peak viscosity; SEF, silver ear-enriched flour; SF, shiitake-enriched flour; SI, solubility index; SP, swelling power; SV, setback viscosity; TV, trough viscosity; WAC, water absorption capacity.

Values are means ± standard deviation of triplicates. Values in the same column for flour samples with different lowercase letters are significantly different ($P < 0.05$). Values in the same column for mushrooms with different uppercase letters are significantly different ($P < 0.05$).

illustrated that the addition of *Auricularia auricula-judae* (black ear) polysaccharide into yam starch significantly improved its swelling. However, dietary fibre in the paste system normally competes with starch for water absorption during heating, limiting the swelling of starch granules. Lascombes *et al.* (2017) mentioned that incorporation of carrageenan increased friction between starch granules, leading to an increase in swelling power. The positive impacts of black ear and silver ear on the swelling power of sorghum flour could be that mushroom dietary fibre increased shear forces between swollen starch granules (Luo *et al.*, 2020). Shiitake incorporation dramatically increased the solubility index of sorghum flour at all levels of enrichment. The solubility index of silver ear-enriched samples increased slightly (except the 5% substitution level) compared to the control. This increase in SI could be contributed to the increment of soluble dietary fibre and proteins (Suliman *et al.*, 2019).

Pasting properties

The pasting parameters and curves of sorghum flour with or without mushroom enrichment are presented in Table 2 and shown in Fig. 1a respectively. During the heat-hydrogen treatment, many molecular reactions took place on the starch granules, including unwinding of double helices, breakdown of hydrogen bonds, rupture of crystallites and amylose leaching (Khatun *et al.*, 2019). The viscosity of samples increased with the increment of the pasting temperature until the values reached peak viscosity, and it represented the largest starch swelling degree. The continuous thermal treatment at peak temperature decreased the viscosity to trough viscosity because the starch granules had been disrupted and their integrity had been damaged by heating (95 °C) (Liu *et al.*, 2016; von Borries-Medrano *et al.*, 2019).

Black ear and silver ear enrichment significantly increased the peak viscosity, trough viscosity and final viscosity values of sorghum flour when compared with the control ($P < 0.05$). This result was in agreement with those starch pastes included soluble or insoluble polysaccharides (Liu *et al.*, 2016, 2019; Feng *et al.*, 2019; Ren *et al.*, 2020). Several factors, such as the volume fraction of swollen starch granules, granules rigidity and integrity and interaction on the surface of granules, can be attributed to the changes in viscosities of starch. The dietary fibre content of the black ear and silver ear mushroom-enriched biscuits was much higher than the control and shiitake fortified biscuits (Table 1), and positive correlations ($P < 0.001$) were found between the dietary fibres (TDF, SDF and IDF) of black and silver ear-enriched biscuits and the peak, trough and final viscosities of their enriched flour (Table 5). The interactions between the dietary fibres

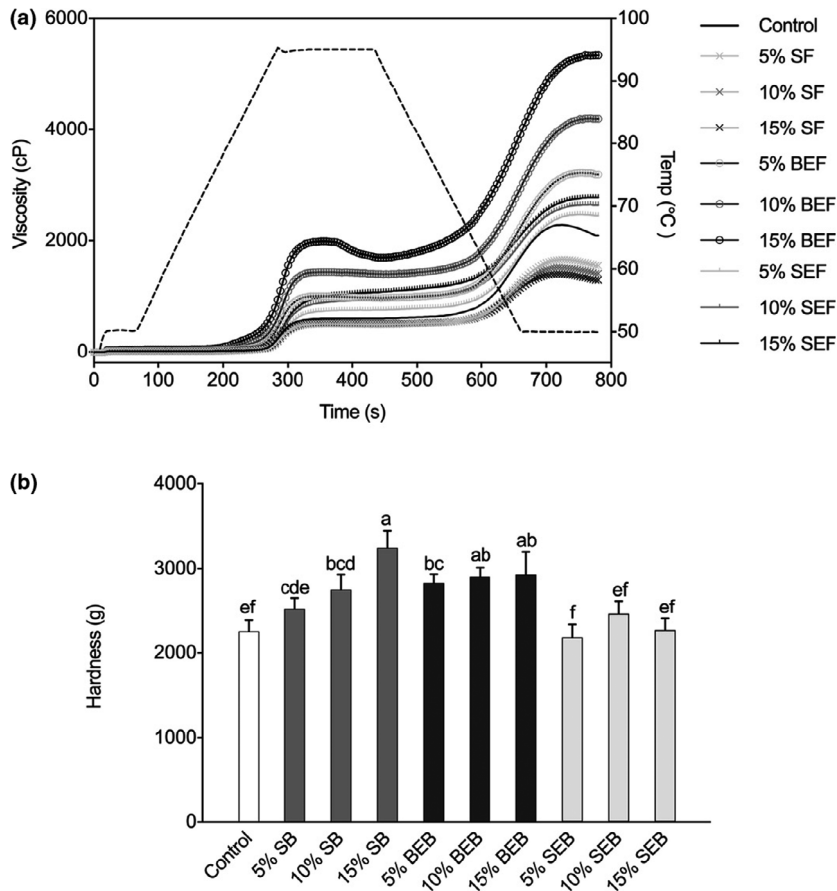


Figure 1 Pasting curves of sorghum flour with the enrichment of shiitake (SF), black ear (BEF) and silver ear (SEF) mushrooms (a). Hardness of sorghum biscuits with the enrichment of shiitake (SB), black ear (BEB) and silver ear (SEB) mushrooms (b). Error bars represent standard deviation of triplicates. Different letters are significantly different between biscuits samples ($P < 0.05$).

and sorghum starch are likely to be the cause of the increased viscosity in samples enriched with black ear and silver ear. The SDF may form a coat around the surface of starch granules during pasting and thus improving starch rigidity and increasing the volume of the granules (Xiao *et al.*, 2020). IDF might increase the work required to move swollen starch in the paste system (Liu *et al.*, 2016).

Conversely, enrichment with shiitake powder significantly decreased the peak viscosity, trough viscosity and final viscosity of sorghum paste ($P < 0.05$), although these samples had an increased swelling power. The decreased viscosity was in agreement with previous studies of Olawuyi & Lee (2019) and Biao *et al.* (2020), showing that the addition of shiitake and oyster mushroom significantly reduced the pasting viscosity of rice flour and wheat flour-based mixtures. These pasting properties were negatively correlated ($P < 0.001$) with dietary fibres (TDF, SDF and IDF) of shiitake-enriched biscuits (Table 5). The reduction in the viscosities could be due to the inhibition of leaching of amylose and granules swelling by shiitake dietary fibre. The pasting viscosity may relate to the

molecular structure of mushroom dietary fibres, for example Feng *et al.* (2019) found that the addition of high molecular weight of *Hericium Erinaceus* β -glucan to wheat starch had higher pasting viscosities than the lower molecular weight β -glucan. Further studies are needed to verify the association between molecular structure of mushroom dietary fibre and starch pasting properties. The high protein content in shiitake mushroom may also have an effect on the pasting properties. Olawuyi & Lee (2019) mentioned that shiitake inclusion increased the protein content of rice paste, and affected the pasting viscosities possibly by protecting the integrity of starch granules and thus inhibiting starch swelling.

The breakdown viscosity represents starch constancy and resistance (Zhang *et al.*, 2019), and a high breakdown viscosity means easier disruption and breakdown degree of starch granules upon thermal treatment. Black ear mushroom significantly increased the breakdown viscosity of the sorghum paste from 2.67 cP to 303.30 cP (15% substitution level) and this value was highest among all the samples, indicating that black ear powder supplementation decreased the stability of the paste.

Setback viscosity, which is the final increase in viscosity during the cooling period, positively relates to starch gelling ability and retrogradation (Abdel-Aal *et al.*, 2020). The increased viscosity of paste during cooling results from the short-term retrogradation and re-aggregation of amylose. Mushroom incorporation markedly affected the values of setback. The substitution of black ear and silver ear mushroom increased setback viscosity values ($P < 0.05$), while an inverse trend was observed in the paste samples replaced with shiitake mushroom. The decrease in setback viscosity with shiitake may be due to the lack of leached amylose caused by the inhibition of swelling of the starch granules during the pasting process, or the inhibitive effect on the short-term retrogradation. On the other hand, the increase in setback viscosity might be due to the hydrogen bonding within amylose (or amylopectin) and dietary fibres presented in black ear and silver ear mushroom, which promoted a positive effect on the network formation of cold paste.

Physical characteristics of biscuits

Diameter, thickness and weight loss

Baking is a process that transforms dough to products by creating physical and structural properties which are influenced by the magnitude of physiochemical and biological transformations, such as water evaporation, starch gelatinisation and gas volume expansion (Martínez & Gómez, 2017). The physical characteristics of sorghum biscuits substituted with three types of mushroom powder are summarised in Table 3. The addition of the three different mushroom powders resulted in a significantly lower weight loss (ranging from 2.79 to 3.14 g) compared to the control biscuit (3.71 g), and a higher moisture content. This could be due to the abundance of dietary fibres in mushroom-enriched biscuits (Table 1), which could have a stronger water-holding ability than the control biscuits (Suliman *et al.*, 2019). The mushroom-enriched biscuits showed significantly ($P < 0.05$) smaller diameters and thickness than the control sample, especially at 15% enrichment. This could be due to the higher moisture retention by the hydrophilic nature of incorporated ingredients from mushroom powders (dietary fibres and proteins) (Okpala *et al.*, 2013) and was illustrated by negative correlations between moisture content and diameter ($r = -0.762$, $P < 0.001$) as well as thickness ($r = -0.869$, $P < 0.001$). Giuberti *et al.* (2018) mentioned that the spread factor was negatively related to the viscosity of dough. A higher protein and IDF content might increase the dough viscosity and thus decrease the spread factor of the biscuits. This agrees with the observation that the protein content was negatively correlated with diameter and thickness respectively ($r = -0.775$ and $r = -0.807$; $P < 0.001$), while the IDF was also negatively correlated with diameter

($r = -0.722$, $P < 0.001$) and thickness ($r = -0.498$, $P < 0.01$).

Hardness and colour values

Hardness is an essential indicator for the quality parameter of biscuits determined by the breaking force, which is associated with the taste and crispness of the biscuits. The textural attributes, such as hardness, easy to chew and crispy, of biscuits generally contribute to the satisfaction and acceptance of consumers (Di Cairano *et al.*, 2021). This parameter is affected by the interaction between ungelatinised starch granules and other nutrients, such as moisture, protein, fibre, sugar and fat (Chevallier *et al.*, 2000). In wheat biscuits, the gluten-starch interaction is the main determinate for the matrix hardness (Pauly *et al.*, 2013). However, gluten-free sorghum flour cannot form a gluten network (de Morais Cardoso *et al.*, 2017). The inclusion of mushroom powder altered the biscuits composition, especially the dietary fibre content, and its effect on the hardness is shown in Fig. 1b. Enrichment with shiitake and black ear powder significantly increased the breaking force of biscuits compared with the control ($P < 0.05$), while silver ear powder had no significant effect on the breaking strength of biscuits. This observation may be associated with the increased IDF, protein content and moisture content of biscuits ($P < 0.05$). As shown in Table 4, there are positive correlations between hardness and IDF, protein content and moisture content ($r = 0.556$, $r = 0.584$, $r = 0.493$ respectively; $P < 0.01$). Gluten-free cookies enriched by egg white protein (or whey protein) had a higher hardness compared to the control, which might be due to the protein-starch interactions via hydrogen bonding (Sarabhai & Prabhasankar, 2015; Sahagun & Gomez, 2018). Dietary fibre, as a macromolecule, can also interact with starch by hydrogen bonding (Ren *et al.*, 2020), and this interaction can affect the starch gelatinisation and biscuit dough structure. The inhibition of starch gelatinisation can increase the proportion of the ungelatinised starch granules. The mushroom-enriched biscuit dough may have a restricted mobility and distribution of free water, resulting in a lower vertical expansion after baking and a denser inner structure of final products (Saric *et al.*, 2019). As demonstrated in Table 4, diameter and thickness values of biscuits were inversely correlated with their hardness values ($r = -0.728$ and $r = -0.666$; $P < 0.001$ respectively).

The colour parameters of biscuits are shown in Table 3. The lightness (L^*) of the sorghum biscuits enriched by shiitake and black ear mushroom was reduced ($P < 0.05$) with the increasing enrichment (except for 5% shiitake-biscuits), that is to say they appeared darker. This trend is more obvious in the black ear-enriched biscuits. The decrease in lightness (L^*) may be influenced by the differences in proteins, dietary fibres and coloured pigments present in the

Table 3 Physical properties and colour parameters of biscuits

Biscuits	Diameter (mm)	Thickness (mm)	Weight of biscuits (g)	Weight loss (g)	Lightness (L*)	Redness (a*)	Yellowness (b*)	Chroma (C _{ab} *)	Hue-angle (h _{ab})
Control	62.67 ± 0.02 ^a	11.42 ± 0.01 ^a	18.71 ± 0.09 cd	3.71 ± 0.03 ^a	55.45 ± 0.07 ^d	9.70 ± 0.09 ^a	14.49 ± 0.22 ^c	17.44 ± 0.23 ^b	0.98 ± 0.00 ^c
5% SB	60.50 ± 0.07 ^c	10.52 ± 0.13 cd	19.83 ± 0.23 ^a	2.92 ± 0.06 cd	56.56 ± 0.35 ^c	8.13 ± 0.08 ^d	14.62 ± 0.01 ^c	16.72 ± 0.05 ^c	1.06 ± 0.00 ^a
10% SB	57.73 ± 0.01 ^f	9.49 ± 0.05 ^e	19.88 ± 0.25 ^a	2.79 ± 0.06 ^{de}	51.32 ± 0.01 ^e	9.12 ± 0.07 ^{bc}	13.53 ± 0.11 ^d	16.31 ± 0.12 ^c	0.98 ± 0.00 ^c
15% SB	56.58 ± 0.09 ^g	9.57 ± 0.13 ^e	19.78 ± 0.18 ^a	2.61 ± 0.10 ^e	51.34 ± 0.43 ^e	9.02 ± 0.13 ^{bc}	13.66 ± 0.16 ^d	16.37 ± 0.18 ^c	0.99 ± 0.01 ^c
5% BEB	60.60 ± 0.01 ^c	10.88 ± 0.18 ^b	18.10 ± 0.15 ^e	3.14 ± 0.10 ^b	51.57 ± 0.10 ^e	6.60 ± 0.03 ^e	10.07 ± 0.28 ^e	12.04 ± 0.22 ^d	0.99 ± 0.01 ^c
10% BEB	59.95 ± 0.27 ^d	10.69 ± 0.20 ^{bc}	18.57 ± 0.16 ^{cde}	2.96 ± 0.02 ^{bcd}	47.51 ± 0.20 ^f	5.75 ± 0.10 ^f	7.65 ± 0.20 ^f	9.57 ± 0.20 ^e	0.93 ± 0.01 ^d
15% BEB	58.78 ± 0.15 ^e	10.34 ± 0.06 ^d	18.49 ± 0.14 ^{de}	2.95 ± 0.09 ^{bcd}	44.32 ± 0.09 ^g	5.30 ± 0.03 ^g	4.98 ± 0.08 ^g	7.28 ± 0.06 ^f	0.75 ± 0.01 ^e
5% SEB	62.31 ± 0.08 ^b	11.55 ± 0.04 ^a	19.08 ± 0.25 ^{bc}	3.05 ± 0.06 ^{bc}	56.98 ± 0.30 ^{bc}	9.06 ± 0.16 ^{bc}	15.12 ± 0.19 ^{bc}	17.63 ± 0.22 ^b	1.03 ± 0.01 ^b
10% SEB	59.88 ± 0.01 ^d	11.33 ± 0.01 ^a	19.45 ± 0.10 ^{ab}	3.07 ± 0.06 ^{bc}	57.99 ± 0.57 ^a	8.94 ± 0.04 ^c	15.64 ± 0.46 ^b	18.02 ± 0.39 ^b	1.05 ± 0.01 ^{ab}
15% SEB	59.01 ± 0.18 ^e	10.29 ± 0.08 ^d	19.54 ± 0.20 ^{ab}	3.07 ± 0.05 ^{bc}	57.75 ± 0.45 ^{ab}	9.26 ± 0.19 ^{bc}	16.72 ± 0.19 ^{ab}	19.11 ± 0.26 ^a	1.07 ± 0.00 ^a

Abbreviations: BEB, black ear biscuit; SB, shiitake biscuit; SEB, silver ear biscuit.

Values are means ± standard deviation of triplicates. Values in the same column with different letters are significantly different ($P < 0.05$).

mushroom powder, as was reported by Sulieman *et al.* (2019). Chemical reactions, such as Maillard reaction and degradation of protein during baking, may also contribute to the increase in darkness of biscuits. Duta & Culetu (2015) found that the higher the protein content in oat bran-enriched cookies the greater the Maillard reaction and thus resulting in a darker sample. This supports the results of increased darkness of shiitake biscuits (10% and 15% enrichment) that had significantly higher protein content than the control biscuit (Table 1). An inverse phenomenon was observed in the biscuit samples supplemented with silver ear mushroom, and the L^* values significantly increased in all enrichment levels, while a similar increase level of lightness was observed at 5% enriched shiitake biscuits. This lightness may be caused by the increased porosity and moisture retention promoted by enriched fibres (Lu *et al.*, 2020). The supplemented biscuits with shiitake and black ear mushroom showed significantly lower values in redness (a^*) and yellowness (b^*) compared to the control. Similarly, enrichment with silver ear mushroom powder significantly reduced a^* values, however, the b^* values had an increasing trend. The observed a^* , and b^* values can readily obtain information regarding the colour attributes of chroma and hue. The chroma values of biscuits was slightly decreased by the enrichment of shiitake but dramatically declined by the addition of black ear mushroom. However, the silver ear-enriched biscuits had higher chroma values compared with the control. Minor changes in the hue values were found in the samples enriched by mushroom powders, except for 15% black ear-biscuits which showed a great decrease in hue value.

In vitro digestion of biscuits

Biscuits are starchy foods composed of a large amount of sugar and starch, and over-intake of reducing sugars can lead to a number of metabolic diseases (Gao *et al.*, 2019). Starch was the main component of sorghum flour (70.46 g/100 g dm). The replacement of sorghum flour by mushroom powders reduced the starch content of the biscuit formulations and decreased the amount of released reducing sugars during *in vitro* analysis (starch content was positively correlated with AUC, $P < 0.001$).

The AUC represented the predicted glycaemic index of biscuits during *in vitro* gastrointestinal digestion over 120 min (Fig. 2). Enrichment of the sorghum biscuits with mushroom powders resulted in a 14.98–33.32% reduction ($P < 0.05$) of reducing sugar released compared to the control biscuit (399.42 mg g⁻¹ dm), and the reduction was greater at higher rate of enrichment. For shiitake mushroom enrichment (5%–15%), there was not a significant difference in AUC values. In addition, no significant

Table 4 Correlations between hydration and pasting properties of sorghum flour and physical characteristics and glycaemic response of mushroom-enriched biscuits

	WAC	SP	SI	PV	TV	BV	FV	SV	PT	T	D	WL	MS	PC	Starch	Hardness	AUC	TDF	SDF
SP	0.922***																		
SI	-0.206	-0.171																	
PV	0.865***	0.903***	-0.507**																
TV	0.872***	0.897***	-0.541**	0.993***															
BV	0.684***	0.778***	-0.271	0.858***	0.792***														
FV	0.798***	0.836***	-0.641***	0.984***	0.987***	0.802***													
SV	0.757***	0.801***	-0.681***	0.971***	0.972***	0.800***	0.997***												
PT	-0.736***	-0.846***	0.386*	-0.941***	-0.904***	-0.941***	-0.911***	-0.908***											
T	-0.100	-0.181	-0.748***	0.071	0.114	-0.135	0.202	0.240	0.095										
D	-0.323	-0.379*	-0.771***	-0.069	-0.031	-0.226	0.080	0.131	0.188	0.896***									
WL	-0.219	-0.291	-0.616***	-0.013	0.012	-0.124	0.096	0.133	0.099	0.715***	0.792***								
MS	-0.062	0.046	0.727***	-0.168	-0.231	0.139	-0.303	-0.333	-0.037	-0.869***	-0.762***	-0.721***							
PC	-0.310	-0.178	0.904***	-0.463*	-0.505**	-0.198	-0.568**	-0.592**	0.261	-0.807***	-0.775***	-0.686***	0.788***						
Starch	-0.766***	-0.825***	-0.223	-0.680***	-0.661***	-0.647***	-0.565**	-0.516**	0.703***	0.588**	0.721***	0.569**	-0.447*	-0.258					
Hardness	0.275	0.437*	0.354	0.283	0.267	0.304	0.215	0.190	-0.429*	-0.666***	-0.728***	-0.580**	0.493**	0.584**	-0.690***				
AUC	-0.833***	-0.729***	-0.087	-0.596**	-0.580**	-0.565**	-0.494**	-0.450*	0.298	0.476*	0.476*	0.523**	-0.231	0.058	0.663***	-0.215			
TDF	0.899***	0.880***	0.195	0.689***	0.677***	0.624***	0.566*	0.510*	-0.626**	-0.409*	-0.643***	-0.552**	0.293	0.091	-0.891***	0.454*	-0.881***		
SDF	0.910***	0.778***	-0.156	0.718***	0.718***	0.595***	0.639***	0.597***	-0.556**	0.027	-0.197	-0.117	-0.084	-0.389*	-0.577***	-0.045	-0.844***	0.832***	
IDF	0.849***	0.859***	0.273	0.645***	0.631***	0.598***	0.518*	0.462*	-0.611***	-0.498**	-0.722***	-0.633***	0.373*	0.207	-0.923***	0.556**	-0.844***	0.990***	0.746***

Abbreviations: AUC, area under the curve; BV, breakdown viscosity; FV, final viscosity; IDF, insoluble dietary fibre; MS, moisture content; PC, protein content; PT, pasting temperature; PV, peak viscosity; SDF, soluble dietary fibre; SI, solubility index; SP, swelling power; SV, setback viscosity; TDF, total dietary fibre; TV, trough viscosity; WAC, water absorption capacity; WL, weight loss.

Significance: *** Significant at $P < 0.001$; ** Significant at $P < 0.01$; * Significant at $P < 0.05$.

differences were observed on each black ear and silver ear mushroom incorporated samples between the 5% and 10% substitution levels. However, the 15% addition level of samples for black ear and silver ear mushroom had significantly lower standardised AUC values, with a 31.53% and 33.32% reduction respectively. A previous study had reported that the biscuits enriched with oyster mushroom powder decreased the *in vitro* starch digestibility and *in vivo* glycaemic index, which was related to the changed starch granule size and integrity (Ng *et al.*, 2017). Mushroom enriched other starchy foods, such as pasta and extrudates, have also shown a reduction in glycaemic response (Lu *et al.*, 2018, 2020). Mushroom dietary fibres (non-digestive polysaccharides) can interact with starch molecules, and have an impact on the digestion due to altering the physicochemical characteristics of the starch-based system (Jia *et al.*, 2020). The decrease in starch digestibility could also be related to the fact that dietary fibre inhibits the contact between pancreatic α -amylase and starch granules by encapsulating amylase molecules, thus reducing enzyme hydrolysis (Zhou & Kang, 2018). Previous studies have reported the inhibitive effects of fruit polysaccharides (or soluble dietary fibre) towards α -amylase and α -glucosidase (Kasipandi *et al.*, 2019). These explanations are supported by the negative correlations ($P < 0.001$) between AUC and SDF ($r = -0.869$), IDF ($r = -0.935$) and TDF ($r = -0.940$). An *in vivo* study indicates that dietary fibre can affect the absorption of nutrients in the gastrointestinal tract by changing the chyme rheology or viscosity of the digesta, which may inhibit the glucose bioaccessibility (Chen *et al.*, 2020). It has been reported that natural mushroom polysaccharides improve insulin sensitivity and lower *in vivo* blood glucose (Yang *et al.*, 2019).

Correlations between AUC and parameters of flour and biscuits

The results of the predicted glycaemic response of the biscuit samples showed an inverse relationship with water absorption capacity, swelling power and pasting viscosities (Table 4), with significant correlations between AUC values and water absorption capacity ($r = -0.833$, $P < 0.001$), and swelling power ($r = -0.729$, $P < 0.001$). Availability of water for enzyme substance reaction plays an important role in starch hydrolysis (Bordoloi *et al.*, 2012). The increased water absorption capacity was associated to the increased content of dietary fibre. They can be released into the digesta during digestion, and absorb water molecules thus increasing the viscosity and decreasing the water mobility of the digesta. The water availability for starch hydrolysis was therefore reduced which might be the mechanism for the reduced AUC values in mushroom-enriched biscuits. Shiitake-enriched biscuits (5%–15%) had no significant difference in AUC values among each other and the values were also close to the AUC values of biscuits enriched by 5% black ear and 5% silver ear. These samples also showed a comparable water absorption capacity, and the reason for similar AUC results could be the close level of water availability during digestion. The analysed results of the correlations between AUC and pasting viscosities for mushroom-enriched samples separately are shown in Table 5. There were positive correlations ($P < 0.001$) for black ear and silver ear-enriched biscuits, and a negative correlation ($P < 0.001$) for shiitake-enriched biscuits. These correlations support the idea that the predicted glycaemic response of the biscuits is associated with starch swelling, breakdown, gelatinisation and retrogradation.

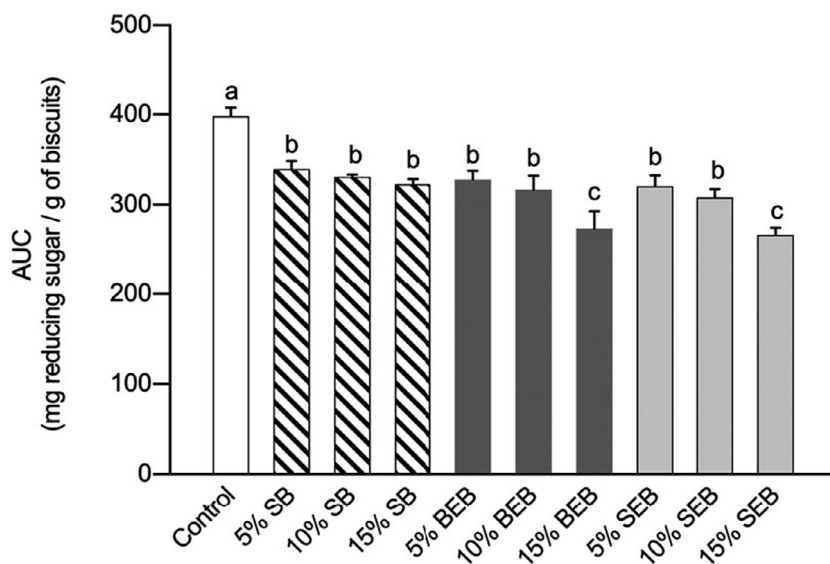


Figure 2 AUC values of sorghum biscuits with the enrichment of shiitake (SB), black ear (BEB) and silver ear (SEB) mushrooms. Error bars represent standard deviation of triplicates. Different letters are significantly different between biscuits samples ($P < 0.05$).

Table 5 Correlations between dietary fibre and AUC of biscuits and pasting viscosities of flour for each type of mushrooms

	Shiitake			Black ear			Silver ear		
	PV	TV	FV	PV	TV	FV	PV	TV	FV
TDF	-0.740**	-0.782**	-0.953***	0.983***	0.998***	0.989***	0.992***	0.994***	0.987***
SDF	-0.836**	-0.867***	-0.974***	0.994***	0.984***	0.988***	0.989***	0.988***	0.970***
IDF	-0.735**	-0.777**	-0.951***	0.976***	0.995***	0.985***	0.989***	0.992***	0.988***
AUC	0.903***	0.924***	0.965***	-0.919***	-0.918***	-0.936***	-0.925***	-0.928***	-0.956***

Abbreviations: FV, final viscosity; IDF, insoluble dietary fibre; PV, peak viscosity; SDF, soluble dietary fibre; TDF, total dietary fibre; TV, trough viscosity.

Significance: ***Significant at $P < 0.001$; **Significant at $P < 0.01$; *Significant at $P < 0.05$.

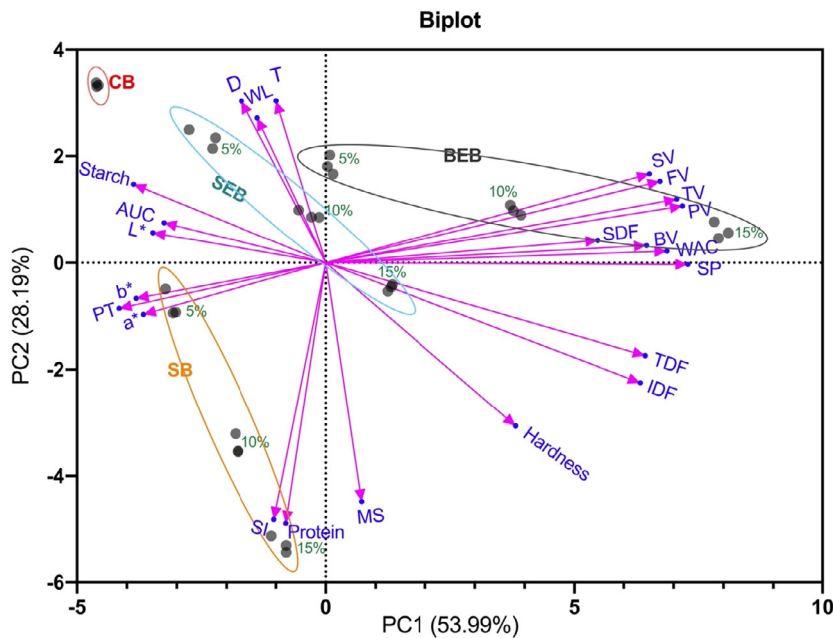


Figure 3 PC scores distribution and component loadings of sorghum flour and biscuits attributes fortified with shiitake, black ear and silver ear mushrooms. Abbreviations: WAC, water absorption capacity; SP, swelling power; SI, solubility index; PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SV, setback viscosity; PT, pasting temperature; WL, weight loss; MS, moisture content; PC, protein content; AUC, area under the curve; TDF, total dietary fibre; SDF, soluble dietary fibre; IDF, insoluble dietary fibre.

Pearson's correlation analysis showed that the physical properties of sorghum biscuits were related to their glycaemic response. It illustrates positive correlations between AUC and diameter ($r = 0.476$, $P < 0.01$), and weight loss ($r = 0.523$, $P < 0.01$). Mushroom-enriched biscuits had a lower diameter, thickness and weight loss, which means these samples had a higher density and a stronger structure network. This might promote more contacts between macromolecules, including proteins, fibres and starch, to improve their intermolecular reactions. These interactions may reduce the accessibility of starch granules to the digestion enzymes.

Principal components analysis

The PCA analysis was performed to provide an outline of the relationships among the observed parameters

(Fig. 3). The PC1 and PC2 explained a total 82.18% of the variation, with PC1 explaining 53.99%. The control samples were plotted on the negative side of PC1 and the positive side of PC2. The control biscuits indicated a high content of starch, and high values of diameter, thickness, weight loss, lightness and AUC. The black ear included samples were positively loaded on the axis of PC1, which means that black ear fortification was more positively correlated with water absorption capacity, swelling power, pasting viscosities and SDF. Similarly, the silver ear fortified samples were moved to the positive side of PC, indicating high values of TDF, IDF, SDF water absorption capacity, swelling power and pasting viscosities. The shiitake-enriched biscuits were negatively correlated with PC2, and they were characterised by high protein, moisture, solubility index and hardness.

Conclusions

Nutritional therapy is crucial in the management of chronic diseases. The mushroom-enriched sorghum biscuits could be developed as a gluten-free product that can simultaneously regulate obesity and diabetes. Enriching gluten-free sorghum biscuits with mushroom powder resulted in decreased diameter, thickness and weight loss, and increased moisture content compared to the control. The sorghum biscuit enriched with shiitake and black ear had a higher hardness. Mushroom enrichment also had a significant effect on the hydration, swelling, gelatinisation and retrogradation of the starch in the sorghum flour due to the interactions with dietary fibres enriched from mushroom powder. The predicted glycaemic response (AUC) of biscuits was decreased with mushroom powder enrichment, especially for black ear and silver ear mushroom which can be a better source of mushroom in regulating glucose level. The reduced glycaemic response was related to the physical characteristics of biscuits, and starch hydration and gelatinisation properties.

Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

Juncai Tu: Data curation (lead); Formal analysis (lead); Investigation (equal); Methodology (equal); Writing – original draft (lead); Writing – review & editing (equal). **Margaret Anne Brennan:** Conceptualization (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal). **Ruibin Wang:** Investigation (equal); Methodology (equal). **Xiaodan Hui:** Investigation (equal); Writing – original draft (equal). **Donatella Peressini:** Conceptualization (supporting); Writing – review & editing (equal). **Weidong Bai:** Funding acquisition (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – original draft (equal). **Ping Cheng:** Conceptualization (equal); Funding acquisition (equal); Project administration (equal); Resources (equal); Writing – original draft (equal). **Charles Stephen Brennan:** Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal).

Peer review

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Data availability statement

Embargo on data due to commercial restrictions.

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