Original article

Physicochemical characterisation and impact of *Gryllus bimaculatus* addition on gluten-free flour blends

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Summary Gryllus bimaculatus (field cricket) powder is a prospect in enriching gluten-free food products' protein and mineral contents. However, the technological impacts of replacing common gluten-free cereal flours with G. bimaculatus powder are still incipient. This study aimed to characterise nutritional composition, particle morphology, and particle size distribution of G. bimaculatus powder and the gluten-free flours (maize, rice, finger millet and proso millet). Additionally, it investigated the effects of substituting these gluten-free flours with 5%, 10%, 15% and 20% G. bimaculatus powder on proximate composition, hydration and colour properties. G. bimaculatus powder showed significantly ($P \le 0.05$) high protein (44.8 g/100 g) content and essential minerals such as zinc (208.5 mg/kg) and iron (132.2 mg/kg). Micrographs of G. bimaculatus powder revealed compact and heterogenous particles while the flours' particles varied in sizes, and the starch granules had different shapes, sizes and arrangements. Flour particles exhibited bi-and trimodal size distributions ranging from 1.5 µm to 1100 µm with minor peaks preceding major peaks. Finger millet flour had a greater span of 4.12, while proso millet flour had a lower span of 1.97. Particle size correlated negatively with water absorption capacity (WAC) (r = -0.938) and oil absorption capacity (OAC) (r = -0.955), respectively. G. bimaculatus powder addition significantly increased (P < 0.05) fat, protein and fibre contents, decreased (P < 0.05) nitrogen-free extracts, but had no effect on ash content. Hydration properties differed significantly ($P \le 0.05$) except for WAC in proso millet blends. Samples exhibited significantly higher (P < 0.05) WAC than OAC in exception of G. bimaculatus powder and showed no emulsifying activities. Addition of G. bimaculatus powder significantly ($P \le 0.05$) increased colour differences, reduced redness (a^*) , yellowness (b^*) and lightness. The use of G. bimaculatus powder demonstrates the potential to enhance the nutritional properties and modify the colour and hydration of gluten-free flours in the development of novel gluten-free foods.

Keywords Colour, edible insects, Gluten-free cereals, hydration properties.

Introduction

Edible insects are now considered as new alternative sustainable source of proteins due to several reasons such as the global population growth (Guiné *et al.*, 2021) and the rising cost and demand for animal protein among others (Berti, 2020; Smetana *et al.*, 2021). Insect consumption has potential

prospects on both environmental and economic benefits (Lange & Nakamura, 2023; Smetana, 2023). The nutritional benefits include highly digestible proteins with a balanced composition of essential amino acids, (Ayieko *et al.*, 2017), fat, fibre in form of chitin, essential minerals and vitamins (Kinyuru, 2009; Kinyuru *et al.*, 2015, Kinyuru *et al.*, 2018). Among the most commonly consumed insects, Orthoptera (grasshoppers, locusts and crickets) is fourth (13%) most consumed class (Cerritos, 2009). In particular, the use of

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field crickets (*Gryllus bimaculatus*) is receiving greater attention in many parts of the world especially in East Africa (Alemu *et al.*, 2017; Okello *et al.*, 2022). Mainly this is due to its ease of rearing under farm conditions, high fecundity and shorter life cycles (van Huis & Oonincx, 2017). In Kenya, *G. bimaculatus* farming is a growing venture poised to help alleviate protein deficiency and improve households' living standards by incorporating the insect flour in common plant-based diets (Ayieko *et al.*, 2017).

Gluten-free cereal market is one of the fast-growing sector that is estimated to expand considerably from 6.7 billion to 14 billion U.S. dollars, between 2022 and 2032 (Wunsch, 2022). This is due to the increasing population of people experiencing different gluten-related health conditions such as celiac disease, non-celiac gluten sensitivity (NCGS), irritable bowel syndrome or wheat allergy (Biesiekierski & Iven, 2015; Taraghikhah et al., 2020). To meet this demand, the food industry is focusing on the production and diversification of gluten-free products through flour formulations and new ingredients (Culetu et al., 2021). The removal of gluten from flours not only leads to nutrient deficiency such as proteins on the other hand, the inclusion of new ingredients in formulations has technological challenges including techno-functional problems. Besides, glutenfree cereal products such as those from maize are low in dietary fibre and low in lysine and tryptophan (Melini & Melini, 2019). Altogether, this offers the potential for exploring edible insects as a suitable ingredient for enriching the gluten-free products.

Cricket flour utilisation as a food ingredient has shown great potential in the development of novel foods due to its comparable techno-functional properties with other cereals (Lucas-González et al., 2019). Several studies have demonstrated that plant-based flours supplemented with cricket flour could develop novel products such as high-protein and antioxidant-rich millet-based pastas (Jakab et al., 2020) and high-protein chapati with a desirable texture containing 5% cricket flour (Khatun et al., 2021). A study on replacing soy flour with cricket flour reported significant (P < 0.05) reductions in water absorption capacity, water solubility of the flour and viscosity, making a suitable blend ingredient for feeding children (Aboge et al., 2022). These studies have evidently shown that the proportion of insect flour inclusion is specific to the species of cereal flour and to the type and properties of the product intended. The use of common/local ingredients offers a chance to further utilisation of edible insect into mainstream diets and their acceptability through consumer acquaintance of a familiar product (Mishyna et al., 2020).

Although few studies have incorporated G. *bimaculatus* powder into food products, there is still knowledge gap in the fundamental characterisation of

the raw materials used. Therefore, this study investigated the morphology and size of particles and nutritional contents of gluten-free flours (maize, rice, finger millet and proso millet flour) and *G. bimaculatus* powder, which help to understand the effects on the flour blend properties, like functional characteristics and colour. The study further analysed the changes in hydration and emulsification properties of the flour blends composed of maize, rice, finger millet and proso millet flour upon the inclusion of 5%, 10%, 15% and 20% (w/w) *G. bimaculatus* powder. The findings could highlight the potential application of *G. bimaculatus* powder in the formulation of gluten-free cereal-based food products in promoting healthier diet and the utilisation of edible insects.

Material and methods

Raw material acquisition and preparation

Gluten-free cereal flours were commercially purchased in two batches (each 400 g) in Wroclaw, Poland as follows: Whole grain rice flour (Oryza sativa) (BioPlanet S.A, Leszno, Poland), whole grain maize flour (Zea mays) (BioPlanet S.A), whole grain finger millet or Raggi (Eleusine coracana) (Rani Foods, Houston, TX, USA) and of proso millet (Panicum miliaceum) flour from hulled millet grain (BioLife Sp, Bielsk, Poland). Adult G. bimaculatus (1 kg total from two harvest seasons) were obtained from Jomo Kenyatta University insect farm, Kenya. They were reared on chicken feeds, starved for 12 h, drowned during washing and blanched at 90 °C for 1 min (Cacchiarelli et al., 2022). Then, they were oven-dried on aluminium trays using a laboratory oven (Memmert UF 110, Memmert, Germany) at 50 °C for 72 h (Kipkoech et al., 2017) and stored in air-tight containers at -20 °C.

Proximate composition analysis

Moisture content was determined by drying in a forced-air oven according to Association of Official Analytical Chemists [AOAC] (2008: method 967.08). Ash content was analysed gravimetrically using incineration method according to AOAC (2008: method 942.05). Fat content was determined using acid hydrolysis Soxtherm Gerhardt - ISO 8262-1 method according to Bench et al. (2005). Protein content was analysed using the Kjeldahl method according to AOAC (2008: method 988.05), and the nitrogento-protein conversion factor kP of 5.00 was used (Ritvanen *et al.*, 2020). The total carbohydrates were calculated by difference as nitrogen-free extracts (NFEs) as described by (WHO/FAO, 2002) as shown below in eqn 1. All the analyses were conducted in triplicates.

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$$NFEs = (100 - X) \tag{1}$$

where X = total weight of protein, fat, water and ash in 100 g of food.

Mineral analysis

The concentrations of Ca, Fe, K, Mn, Na and Zn were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (iCAP O, Thermo Scientific, Waltham, Massachusetts, United States) in line with a modified method of Mazarakioti et al. (2022). A sample flour weighing 0.3 g was microwave-digested in a MWS-1 (Berghof Products + Instruments GmbH, Eningen, Germany) with 5 mL of concentrated HNO₃ (65% v/v) and 2 mL of HCl (30% v/v) and then heated up as follows: At 70 °C and held for 5 min, up to 150 °C and held for 10 min and to 180 °C and held for 20 min. Dilution was done according to the content of the elements in the samples and then measured in 2% (v/v) HNO₃ using collision mode with a dwell time of 10 ms and argon 5.0 (plasma gas) and hydrogen (collision gas) (Linde AG, Dortmund, Germany). ¹⁰³Rhodium was used and a set of digestion blanks with each sample batch (n = 11).

Blend preparation and proximate composition by calculation

Dried whole *G. bimaculatus* were milled using a 1.5 L mill (Model LM-KA 1908, UKA Co., Guangdong, China) at maximum speed for 30 s. *G. bimaculatus* powder and gluten-free flours were mixed in ratios of 5:95, 10:90, 15:85 and 20:80 (w/w), respectively, in a rotary drum mixer (TM100, Vevor, Shanghai, China) for 10 min. The blends were stored in airtight plastic jars at -20 °C. Thereafter, proximate composition of the blends was calculated based on the proximate composition of the ingredients and their respective ratios as per eqn 2.

$$B_{\rm x} = (G_{\rm x} \times G_{\rm w}) + (C_{\rm x} \times C_{\rm w}) \tag{2}$$

where B_x is the proximate composition of a nutrient x in a blend (%), G_x is the proximate composition of the nutrient x in *G. bimaculatus* powder (%), G_w is the weight ratio of the *G. bimaculatus* powder used, C_x is the proximate composition of the nutrient x in cereal flour (%), C_w is the respective weight ratio of the cereal flour used.

Particle size determination

The average particle size and distribution based on volume distribution of cereal flours was determined using a laser diffraction Mastersizer 2000 (Malvern Panalytical Limited, Malvern, UK) in accordance with ISO method 13320 (ISO 13320, 2020). It was equipped

with the wet measurement cell 'Hydro G' and Mastersizer software version 6.00. Briefly, 1 g of the sample was stirred into 10 mL deionised water and then 2 mL was put into the measuring cell. A refractive index (RI) of 1.53 and absorption 0 was chosen as reference for starch (Malvern Analytical Ltd.). Three analyses were done per sample; without ultrasound and with 1and 2-min ultrasounds and in each, nine measurements were done. The mean particle sizes at the 10th (D_{10}), 50th (D_{50}), and 90th (D_{90}) percentiles of the particle size distribution were recorded and the particle size width span was calculated as follows in eqn 3:

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}} \tag{3}$$

where Span is the width of particle size distribution, D_{90} , D_{10} and D_{50} means 90% or 10% or 50% of the volume that is smaller than the size indicated respectively.

Morphological analysis

The particle morphology of all flour samples was visualised by scanning electron microscopy (SEM). A small amount of each flour sample (approximately 1–3 mg) was placed on an aluminium specimen stub (Agar Scientific Ltd, Stansted, UK) with an adhesive support tab (Spectro-Tab, Plano, Wetzlar, Germany). For increasing sample conductivity, the samples were sputter-coated with a thin layer of platinum of ca. 5 nm (Q 150 T ES, Quorum Technologies Ltd, Laughton, UK). Multiple micrographs of the samples were acquired using different magnifications (250× and 2500×).

Hydration properties

Determination of water absorption index (WAI), water solubility index (WSI) and swelling power (SP)

A sample of each 2 g was weighed into three centrifuge Falcon tubes of 50 mL and recorded as TW. Distilled water, 30 mL, was added and mixed in vortex for 30 s at high speed and then immersed in 90 °C water bath for 10 min as illustrated by Harasym *et al.* (2020). Cooling was done before centrifugation in 3000 g for 10 min and the supernatant was decanted pre-weighed steel Petri dishes and recorded as PDW. Drying was done at 110 °C for 24 h while the tubes and content left were weighed as STWS. Finally, weighing of the cooled Petri dishes with solid residue was done and recorded as SRW. Calculations were done as g of water g⁻¹ of dry basis in eqns 4–6:

(14)

$$SP = STWS - TW/(SW - (SRW - PDW))$$
(6)

where WAI is water absorption index, STW is weights of tubes and contents in g, TW is weights of Falcon tubes in g, SW is sample flour in g, PDW is weight of petri dish, and STW is the weight of petri dishes with solid residue in g.

Determination of water and oil absorption capacity (WAC and OAC)

A method by Villanueva *et al.* (2018) was used in determination of WAC and OAC. The weights of Falcon tubes and sample flour (2 g) were recorded as TW and SW, respectively. Distilled water or corn oil each 30 mL was added, vortexed for 30 s and rested for 10 min. The mixing and resting were repeated two more times. Centrifugation for 25 min at 3000 g and the discarding of supernatant was done. The tubes were drained at 45 °C for 5 min followed by (in exception of OAC samples) drying in hot air oven at 45 °C for 25 min. The tubes and contents were weighed as STW and results in g water g^{-1} sample dry basis content were calculated as follows in eqns 7 and 8:

$$WAC = (STW - TW)/SW$$
(7)

$$OAC = (STW - TW)/SW$$
(8)

where WAC/OAC is water/oil absorption capacity, STW is weights of tubes and contents in g, TW is weights of Falcon tubes in g and SW is sample flour in g.

Determination of water holding capacity (WHC)

WHC was determined according to modified method by Heywood *et al.* (2002). Samples weighing 2 g flours were weighed in quadruplicates and recorded as SW in 50 mL. The weight of cylinders was recorded as TW and then 30 mL of distilled water was added. Gentle tapping on tubes was done to facilitate submersion and then left in room temperature to absorb for 24 h. The supernatant was discarded and tube with its content was weighed as STW. Calculations were done in g water/g sample dry basis content as follows in eqn 9:

$$WHC = (STW - TW)/SW$$
(9)

where WHC is Water holding capacity, STW is weights of tubes and contents in g, TW is weights of Falcon tubes in g and SW is sample flour in g.

Determination of emulsion activity (EC) and emulsion stability (ES)

According to the method of Wani et al. (2013), 1 g sample was dispersed in 100 mL distilled water and

15 mL of the dispersion was homogenised with 15 mL of corn oil at for 1 min. The sample was then centrifuged at 3000 g for 5 min, and the resulting volume of layers was recorded. For emulsion stability, the emulsion was heated in a water bath at 80 °C for 5 min, cooled and centrifuged as mentioned. EC and ES were calculated from the formula:

$$EA\% = \frac{V_e}{V} \times 100 \tag{10}$$

$$ES\% = \frac{V_{30}}{V_e} \times 100$$
 (11)

where $V_{\rm e}$ is the emulsified layer in volume, V is total volume of tube contents and V_{30} is volume of the emulsified layer after heating.

Colour analysis

Colour attributes, L^* (lightness), a^* (redness) and b^* (yellowness) indexes were measured according to the CIE lab system (Hardin, 2005) using a Handy Colorimeter, Model No. NR-3000, NIPPON Denshoku, Japan. Hue, chroma and colour difference (ΔE^*) were calculated according to Ozdemir & Devres (2000). Browning index (BI) and yellowing index (YI) were calculated by the method of van Huis & Tomberlin (2016) as follows in eqns 11–16:

$$Hue = Degrees\left(ATAN\left(\frac{b}{a}\right)\right)$$
(12)

$$Chroma = \sqrt{\left(b^2 + a^2\right)} \tag{13}$$

 $\Delta E^* = \sqrt{\left(\left(L_{\rm o} - L_{\rm S}\right)^2 + \left(a_{\rm o} - a_{\rm S}\right)^2 + \left(b_{\rm o} - b_{\rm S}\right)^2\right)}, where$ = subscript o' or s' denotes control and samples

$$BI = ((100 * (x - 0.31))/(0.17)),$$

where $x = \frac{(a + (1.75 * L))}{((5.64 * L) + a - (3.012 * b))}$ (15)

$$YI = (142.86 * b)/L$$
(16)

Statistical data analysis

Data were treated with Microsoft Excel (Microsoft Office, 2019) for calculation of means and standard deviations. Analysis of variance (ANOVA) was conducted for statistical significance, and Tukey's HSD (honestly significant difference) test ($\alpha = 0.05$) was used to identify the significant difference of each sample treatment. The multivariate analysis (MANOVA) was carried to assess second-order relation between the level of insect flour addition and different type flour

types. The correlation between measurements was determined by Pearson's correlation coefficient. Statistical analysis was performed using R Software, version 4.2.2 (R Core Team, 2018).

Results and discussion

Proximate composition and mineral contents

The results of proximate composition and mineral content of the ingredients are presented in Table 1. The proximate values varied significantly ($P \le 0.05$). G. bimaculatus had significantly (P < 0.05) higher fat. protein, chitin/fibre and ash contents compared to cereal flours. Additionally, G. bimaculatus showed significantly ($P \le 0.05$) higher levels of iron (132.2 mg/kg) and zinc (208.5 mg/kg) in comparison to the cereal flours. The protein content of G. bimaculatus was reportedly lower than the reported amounts by Ghosh et al. (2017) (58.3%) and Jeong et al. (2021) (51.3%). This could be attributed to the higher nitrogen conversion factor (kP) of 6.25 compared to the kP value used in this study, which was 5.00. However, according to FAO (Food and Agriculture Organization, 2007), G. bimaculatus powder qualifies as an alternative high-protein source. The fat content was higher than the reported in literature (Udomsil et al., 2019; Jeong et al., 2021). The variation in nutrient contents such as fat in the edible insects has been associated with factors such as diet, stage of development, and environmental conditions (Finke & Oonincx, 2014).

On the other hand, the nitrogen-free extracts which supposedly represent the soluble carbohydrates, such as sugars and starch (FAO, 2003) were significantly $(P \le 0.05)$ higher (>79.5 g/100 g) in cereal flours. Among the cereals, there were significant variations on nutritional contents possibly due to botanical differences and environmental conditions (Culetu et al., 2021). However, the study results were comparable to previous findings (Verma & Srivastav, 2017; Adeniyi & Ariwoola, 2019; Mohapatra et al., 2019). Overall, the results show that G. bimaculatus powder could provide macronutrients and essential micronutrients that are often limiting in unfortified plant-based complementary foods (Parker et al., 2020; Culetu et al., 2021).

Table 2 shows that the addition of G. bimaculatus resulted in a significant and gradual increase $(P \le 0.05)$ in amounts of fat, protein and fibre; however, ash content exhibited no significant increase (P > 0.05). On the other hand, there was a significant gradual decrease ($P \le 0.05$) in nitrogen-free extracts (NFEs) as G. bimaculatus was included in the composition. Studies have shown that blending edible insects with cereal flours during product development significantly improves the protein and fat contents (Osimani et al., 2018; Zielińska et al., 2021; Maiyo et al., 2022).

| | Macronutrients (g/100 g | s (g/100 g) | | | | Micronutrients (mg/kg) | 'kg) | | | | |
|----------------|-------------------------|--------------------|-------------------------------------|---------------------------|---------------------|---|------------------------|------------------------|-------------------------------|-----------------------|------------------------|
| Sample | Fat | Protein | Nitrogen Chitin/Fibre extracts | Nitrogen-free extracts | Ash | Calcium | Iron | Potassium | Manganese Sodium | Sodium | Zinc |
| G. bimaculatus | 26.60 ± 0.21^{a} | 44.88 ± 0.03^{a} | $\textbf{7.06}\pm\textbf{0.19}^{a}$ | $17.18\pm\mathbf{0.26^c}$ | 4.28 ± 0.20^{a} | $ \begin{array}{c} 3. \ bimaculatus \ \ 26.60 \pm 0.21^{a} \ \ 44.88 \pm 0.03^{a} \ \ 7.06 \pm 0.19^{a} \ \ 17.18 \pm 0.26^{c} \\ \end{array} \ 4.28 \pm 0.20^{a} \ \ 1767.00 \pm 143.01^{b} \ \ 132.20 \pm 12.90^{a} \ \ 10946.00 \pm 20.00^{a} \ \ 50.73 \pm 1.91^{b} \\ \end{array} \ 3231.00 \pm 13.01^{a} \ \ 208.50 \pm 11.10^{a} \\ \end{array}$ | 132.20 ± 12.90^{a} | 10946.00 ± 20.00^{a} | $50.73 \pm 1.91^{\mathrm{b}}$ | 3231.00 ± 13.01^{a} | 208.50 ± 11.10^{a} |

Nutritional composition of Gryllus bimaculatus powder and cereal flours on dry basis

Table 1

column are significantly different. * $P \leq 0.05$; *** $P \leq 0.001$. standard error. Values with different superscripts (a^{-c}) on the same /alues are means values \pm

 $\begin{array}{l} 22.59 \pm 0.17^{c} \\ 38.72 \pm 0.24^{b} \end{array}$ $\mathbf{16.09}\pm\mathbf{0.02^{c}}$ 17.51 ± 0.14^{c}

 $\begin{array}{l} {\bf 24.35} \pm 0.24^{b} \\ {\bf 3.27} \pm 0.20^{c} \end{array}$ $\mathbf{26.29}\pm\mathbf{0.23}^{\mathrm{b}}$ $0.97\,\pm\,0.22^c$

 122.30 ± 1.50^{a}

 $\begin{array}{l} 4175.00 \pm 7.01^{b} \\ 2503.00 \pm 25.01^{e} \end{array}$

 $\begin{array}{l} 46.70 \ \pm \ 0.23^b \\ 38.42 \ \pm \ 0.69^{bc} \end{array}$

 $4676.00\,\pm\,72.01^{a}$ $129.60 \pm 10.10^{\circ}$ $\mathbf{159.10}\pm\mathbf{5.70^{c}}$ $86.37 \pm 7.07^{
m dc}$

> $2.33\,\pm\,0.18^{ab}$ 2.30 ± 1.77^{ab}

 $\begin{array}{l} 85.05 \,\pm\, 0.25^{a} \\ 79.50 \,\pm\, 2.52^{b} \end{array}$

 $\begin{array}{l} \textbf{2.66} \pm \textbf{0.22}^{b} \\ \textbf{2.27} \pm \textbf{0.02}^{c} \\ *** \end{array}$

*

**

*

P-value

 84.28 ± 0.25^{a} 84.28 ± 0.29^{a}

 0.81 ± 0.09^{d} $1.94 \pm 0.02^{\circ}$

> $10.20\,\pm\,0.05^{\circ}$ $\mathbf{11.79}\pm\mathbf{0.15}^{\mathrm{b}}$

 $4.37 \pm 0.01^{\rm b}$ $3.12\,\pm\,0.01^{c}$ $\textbf{4.14}\pm\textbf{0.01}^{b}$

Maize

Rice

 $\textbf{8.36}\pm\textbf{0.08}^d$ $7.90\pm0.04^{\mathrm{e}}$

0.01^d

1.60 ± (

Finger millet Proso millet

 $1.58 \pm 0.41^{\rm b}$ 1.59 ± 0.53^{b} **

**

 $\mathbf{16.79}\pm\mathbf{0.07^{c}}$ $\mathbf{10.13}\pm\mathbf{0.04^d}$

 2617.00 ± 14.02^{d} $3528.00 \pm 14.01^{\circ}$

 $29.89 \pm 0.98^{\circ}$ 0.68 ± 0.01^{d}

 $4.78\pm0.05^{\rm e}$

**

| Blends | | Fat | Protein | Chitin/fibre | Nitrogen-free extracts | Ash |
|-------------------------------|----------|--|--|--|---|---|
| Maize: G. bimaculatus | 100 M | $\textbf{4.37}\pm\textbf{0.00}^{f}$ | $\textbf{7.90} \pm \textbf{0.04}^{f}$ | $\textbf{1.94} \pm \textbf{0.02}^{f}$ | $84.28\pm0.29^{\text{a}}$ | 1.58 ± 0.41^{b} |
| | 95 M:5C | $\textbf{5.01} \pm \textbf{0.04}^{e}$ | $\textbf{9.75} \pm \textbf{0.04}^{e}$ | $\textbf{2.19} \pm \textbf{0.02^{e}}$ | $80.92\pm\mathbf{0.26^{b}}$ | $1.72\pm0.39^{\rm b}$ |
| | 90 M:10C | $\textbf{6.09}\pm\textbf{0.05^d}$ | $\textbf{11.60} \pm \textbf{0.03}^{d}$ | $\textbf{2.45} \pm \textbf{0.01}^{d}$ | 77.57 ± 0.23^{c} | 1.85 ± 0.36^{b} |
| | 85 M:15C | $\textbf{7.18} \pm \textbf{0.05^c}$ | $\textbf{13.45}\pm\textbf{0.03^c}$ | $\textbf{2.71} \pm \textbf{0.00^c}$ | $\textbf{74.21} \pm \textbf{0.21}^{d}$ | $\rm 1.99\pm0.34^{b}$ |
| | 80 M:20C | $\textbf{8.26} \pm \textbf{0.06}^{b}$ | $\textbf{15.29} \pm \textbf{0.03}^{b}$ | $\textbf{2.96} \pm \textbf{0.01^{b}}$ | 70.86 ± 0.18^{e} | $\textbf{2.12} \pm \textbf{0.32}^{b}$ |
| | 100C | $\textbf{26.60}\pm\textbf{0.21}^{a}$ | $\textbf{44.88} \pm \textbf{0.03}^{a}$ | 7.06 ± 0.19^{a} | $\textbf{17.18} \pm \textbf{0.26}^{f}$ | $\textbf{4.28} \pm \textbf{0.20}^{a}$ |
| <i>P</i> -value | | *** | *** | *** | *** | *** |
| Rice: G. bimaculatus | 100R | $\textbf{3.12}\pm\textbf{0.01}^{f}$ | $10.20\pm0.05^{\rm f}$ | $0.81\pm0.09^{\rm f}$ | 84.28 ± 0.25^a | $1.59\pm0.52^{\rm b}$ |
| | 95R:5C | $\textbf{3.96} \pm \textbf{0.05}^{e}$ | $\textbf{11.93} \pm \textbf{0.05}^{e}$ | 1.12 ± 0.09^{e} | $80.93\pm0.25^{\mathrm{b}}$ | $1.73\pm0.51^{\rm b}$ |
| | 90R:10C | $\textbf{5.10}\pm\textbf{0.05}^{d}$ | $\textbf{13.66} \pm \textbf{0.05}^{d}$ | $\textbf{1.43} \pm \textbf{0.09}^{d}$ | $\textbf{77.57} \pm \textbf{0.24}^{\rm c}$ | $\textbf{1.86}\pm\textbf{0.49}^{b}$ |
| | 85R:15C | $\textbf{6.24}\pm\textbf{0.044}^{c}$ | $15.40\pm0.04^{\rm c}$ | $1.74\pm0.100^{\rm c}$ | $74.21 \pm \mathbf{0.25^d}$ | $1.99\pm0.48^{\rm b}$ |
| | 80R:20C | $\textbf{7.38} \pm \textbf{0.04}^{b}$ | $\textbf{17.13} \pm \textbf{0.04}^{b}$ | $\textbf{2.06} \pm \textbf{0.10}^{b}$ | $\textbf{70.86} \pm \textbf{0.24}^{e}$ | $\textbf{2.13} \pm \textbf{0.46}^{\rm b}$ |
| | 100C | $\textbf{26.60} \pm \textbf{0.21}^{a}$ | 44.88 ± 0.04^{a} | 7.06 ± 0.19^{a} | $\textbf{17.18} \pm \textbf{0.26}^{f}$ | $\textbf{4.29}\pm\textbf{0.20}^{a}$ |
| <i>P</i> -value | | *** | *** | *** | *** | *** |
| Finger millet: G. bimaculatus | 100F | $\rm 1.60\pm0.00^{f}$ | $\textbf{8.36}\pm\textbf{0.08}^{f}$ | $\textbf{2.66} \pm \textbf{0.22}^{e}$ | 85.05 ± 0.25^{a} | $\textbf{2.33}\pm\textbf{0.18}^{b}$ |
| | 95F:5C | $\textbf{2.62}\pm\textbf{0.00}^{e}$ | 10.18 ± 0.07^{e} | $\textbf{2.88} \pm \textbf{0.22}^{de}$ | $81.65\pm0.22^{\mathrm{b}}$ | $\textbf{2.42} \pm \textbf{0.18}^{b}$ |
| | 90F:10C | $\textbf{3.83} \pm \textbf{0.01}^{d}$ | $\textbf{12.01} \pm \textbf{0.06}^{d}$ | $\textbf{3.10} \pm \textbf{0.21}^{cd}$ | $\textbf{78.26} \pm \textbf{0.19}^{c}$ | $\textbf{2.52} \pm \textbf{0.18}^{b}$ |
| | 85F:15C | $5.04\pm0.02^{\rm c}$ | $\textbf{13.84}\pm\textbf{0.06}^{c}$ | $\textbf{3.32}\pm\textbf{0.21}^{bc}$ | $\textbf{74.86} \pm \textbf{0.17}^{d}$ | $2.62\pm0.18^{\rm b}$ |
| | 80F:20C | $\textbf{6.25}\pm\textbf{0.03}^{b}$ | $\textbf{15.66} \pm \textbf{0.05}^{\rm b}$ | $\textbf{3.54} \pm \textbf{0.20}^{b}$ | 71.47 ± 0.15^{e} | $\textbf{2.72} \pm \textbf{0.18}^{b}$ |
| | 100C | $\textbf{26.60} \pm \textbf{0.21}^{a}$ | 44.88 ± 0.034^{a} | 7.06 ± 0.19^{a} | $\textbf{17.18} \pm \textbf{0.26}^{f}$ | $\textbf{4.28} \pm \textbf{0.20}^{a}$ |
| <i>P</i> -value | | *** | *** | *** | *** | *** |
| Proso millet: G. bimaculatus | 100S | $\rm 4.14\pm0.00^{f}$ | $11.79\pm0.15^{ m f}$ | $\textbf{2.27}\pm\textbf{0.02}^{f}$ | 79.50 ± 2.52^{a} | $\textbf{2.31} \pm \textbf{1.76}^{a}$ |
| | 95S:5C | $\textbf{4.79}\pm\textbf{0.00}^{e}$ | $\textbf{13.45}\pm\textbf{0.13}^{e}$ | $\textbf{2.50} \pm \textbf{0.02}^{e}$ | $\textbf{76.38} \pm \textbf{2.40}^{\texttt{ab}}$ | $\textbf{2.41} \pm \textbf{1.68}^{a}$ |
| | 90S:10C | $\textbf{5.89} \pm \textbf{0.01}^{d}$ | $\textbf{15.10} \pm \textbf{0.13}^{d}$ | $\textbf{2.74} \pm \textbf{0.03}^{d}$ | $\textbf{73.26} \pm \textbf{2.28}^{\texttt{abc}}$ | $\textbf{2.50} \pm \textbf{1.61}^{a}$ |
| | 85S:15C | $6.98\pm\mathbf{0.02^{c}}$ | $\textbf{16.76} \pm \textbf{0.12^c}$ | $\textbf{2.98} \pm \textbf{0.03^c}$ | $\textbf{70.15} \pm \textbf{2.16}^{\textbf{bc}}$ | 2.60 ± 1.53^{a} |
| | 80S:20C | $\textbf{8.08} \pm \textbf{0.03}^{b}$ | $\rm 18.41 \pm 0.12^{b}$ | $\textbf{3.22}\pm\textbf{0.04}^{b}$ | $67.03 \pm \mathbf{2.04^c}$ | $\textbf{2.70} \pm \textbf{1.45}^{a}$ |
| | 100C | $\textbf{26.60} \pm \textbf{0.21}^{a}$ | $44.88\pm0.034^{\text{a}}$ | 7.06 ± 0.19^{a} | $\textbf{17.18} \pm \textbf{0.26}^{d}$ | $\textbf{4.28}\pm\textbf{0.20}^{a}$ |
| <i>P</i> -value | | *** | *** | *** | *** | ns |

Table 2 Proximate composition of Gryllus bimaculatus powder-cereal flour blends (g/100 g dry weight)

C, G. bimaculatus powder; F, finger millet flour; M, maize flour; R, rice flour; S, proso millet flour. Values are means values \pm standard error. values with different superscripts (^{a-c}) on the same column are significantly different. ns, P > 0.05; * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$.

Particle size distribution

The particle size distribution of cereal flour samples is illustrated in Fig. 1, and the corresponding parameters are shown in Table 3. Noteworthy, the G. bimaculatus powder was not analysed due to the large lumps that were not suitable for the instrument's standard method of analysis used on flours. The flours showed a diverse distribution of particle sizes from monomodal, bimodal to trimodal sizes ranging from 1.5 µm to 1100 µm (Fig. 1d). This indicates that the flours consisted of a mixture of varying particle sizes (as also shown in Fig. 2). It was observed that ultrasound treatment had an effect on the particle size distribution of the flours. After 2 min of ultrasound, the modal peaks were more defined, slightly reduced and shifted to smaller particle sizes with minor peaks preceding major peaks. This shift indicating possible particle size reduction is attributed to breakdown of agglomerates through fragmentation and abrasion (Harasym et al., 2020). Additionally, the ultrasound induces microturbulence which can promote better particle dispersion (Kiani et al., 2011).

The flours (D_{50}) had a greater number of small particles, accompanied by a smaller number of larger particles in the order of rice than finger millet, maize and proso millet flours as shown in Table 3. Presumably, the smallest fractions mainly contained finely crumbled protein matrix components $(1-5 \mu m)$ and finer starch granules (15–40 μ m), while the larger one contained larger starch granules, fibre (above 40 µm) and lipid particles (Sivakumar et al., 2022; Guo et al., 2023). The variation in flour fractions could arise from botanical differences or milling/processing methods (Ashogbon, 2014). Finger millet flour had a greater span of 4.12 indicating a broader distribution with more variability, while proso millet flour had a lower span of 1.97 suggesting a more uniform distribution as explained in Zhang et al. (2022). The heterogeneity and homogeneity of flours' particle sizes and distribution of flour components can influence the hydration properties (Aprianita et al., 2009).

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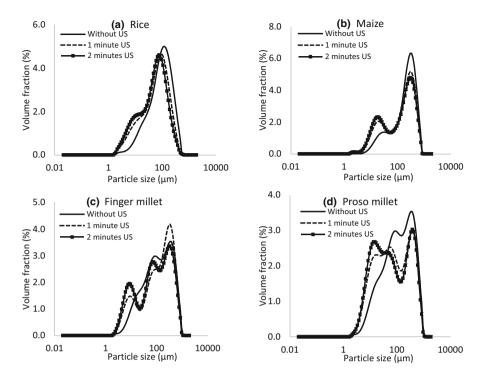


Figure 1 Particle size distribution of the cereal flours (a) rice, (b) maize, (c) finger millet, (d) proso millet with ultrasound and without ultrasound. US, ultrasound.

| Table 3 | Average | particle size | distribution | of the | different | cereal flours |
|---------|---------|---------------|--------------|--------|-----------|---------------|
|---------|---------|---------------|--------------|--------|-----------|---------------|

| | Particle size [µm] | | | | | | | | | |
|---------------------|--|---------------------------------------|---------------------------------------|----------------------------------|------|--|--|--|--|--|
| Sample | D ₅₀ [μm] | <i>D</i> ₉₀ [μm] | D ₁₀ [μm] | D ₉₀ /D ₁₀ | Span | | | | | |
| Maize flour | 228.81 \pm 17.82 ^{ab} | 503.85 ± 16.34^{b} | $\textbf{28.39}\pm\textbf{4.03}^{b}$ | 17.75 | 2.08 | | | | | |
| Rice flour | 93.23 ± 1.71^{d} | $\textbf{261.45} \pm \textbf{3.44^c}$ | $18.71\pm1.06^{\rm c}$ | 0.45 | 2.60 | | | | | |
| Finger millet flour | $117.60 \pm 7.10^{\circ}$ | ${\bf 499.49\pm17.26^{b}}$ | $\textbf{16.30}\pm\textbf{0.46}^{c}$ | 30.64 | 4.12 | | | | | |
| Proso millet flour | $\textbf{265.90} \pm \textbf{42.63}^{a}$ | 571.59 ± 35.06^{a} | $\textbf{45.76}\pm\textbf{16.84}^{a}$ | 12.49 | 1.97 | | | | | |

 D_{10} , 10% of the volume that is smaller than the size indicated; D_{50} , 50% of the volume that is smaller than the size indicated; D_{90} , 90% of the volume that is smaller than the size indicated. Values are means values \pm standard error. Different superscripts (^{a-c}) on the same column signify significant differences ($P \le 0.05$).

Morphology analysis

Figure 2 shows the SEM micrographs for the four different cereal flours and *G. bimaculatus* powder at two magnifications (250× and 2500×). The SEM images showed a diverse distribution of particle shapes and sizes, which are in good agreement with the particle size distribution data obtained from the laser diffraction measurements presented above in Table 3. At 250× magnification, the flour micrographs showed a mixture of small and large particles and aggregates; rice flour has smallest particles of about 20 µm (Fig. 2, 2a), while proso millet had largest particles of up to ~500 µm (Fig. 2, 4a). In addition, the *G. bimaculatus* powder particles were composed of irregular and large flakes and stuck together due to the high fat content $(\sim 26\%)$.

At 2500× magnification, morphologically different constituent particles such as starch granules in the flours and non-starch components such as chitin in the case of insect powder were observed. Also, the starch microstructural features including shape, size and arrangement were revealed. The micrographs showed that maize flour (Fig. 1b) had large polygonal and small spherical-shaped starch granules, similarly reported (Zhang *et al.*, 2018). Rice flour exhibited small polyhedral-shaped granules packed together. Finger millet flour consisted of amorphous shaped granules or different sizes fused together. Proso millet flour exhibited polyhedral granules loosely packed 13652621, 2024, 7, Downloaded from https://ifst.onlinelibrary.wiley.com/doi/10.1111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.1111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.online.ibrary.wiley.com/doi/10.111/ijfs.17185 by INASP Kenya - Masinde Muliro, Wiley Online Library on [12/07/2024]. See the Terms and Conditions (https://ifst.

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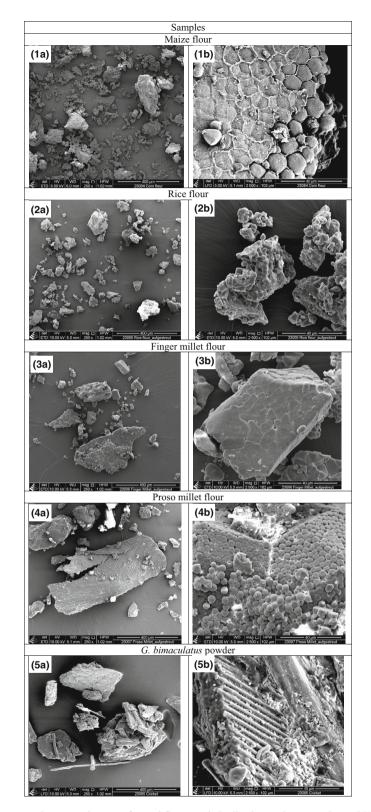


Figure 2 (1-5a, b): Scanning electron microscopy images of cereal flours and *Gryllus bimaculatus* powder at $250 \times$ (a) and $2500 \times$ (b) magnification.

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together with tiny particles on their surface. These differences in the size and morphology of the starch granules are specific for plant species (Bertoft, 2017), and depend upon the structural compactness of amylopectin (AMP) in endosperm during grain development (Singh et al., 2010). Finally, in G. bimaculatus a rough and thick structure composed of heterogenous aggregated particles was observed. In addition, parallel and elongated structures which resembled chitin nanofibers were observed alongside small to large granular particles, presumably protein and fat matrices. Similar compact structures were reported on flour micrographs from six edible insects including crickets (Aguilera et al., 2021) as well as long and orderly chitin nanostructures in Dociostaurus maroccanus and Holotrichia parallela (Erdogan & Kaya, 2016). This physical structure and composition of insect flour can directly affect its physicochemical and techno-functional properties.

Colour analysis

Table 4 shows the colour coordinates L^* , a^* , b^* and the values of the chroma (C^*) , hue (h^*) , browning index (BI), yellowing index (YI) and the total colour difference (ΔE) of blends. G. bimaculatus powder had the lowest L^* values of 45.35 while cereal flours had high L^* values ranging from 96.5 (finger millet) to 107.8 (rice). The G. bimaculatus powder addition in cereal flour significantly ($P \le 0.05$) reduced the lower L* values leading to darker colour; the higher the L^* value, the brighter the sample. Similar results were observed in Acheta domesticus powder addition in corn grits (Igual et al., 2020). This darkening effect was reported to be highly desirable in gluten-free products (Gallagher et al., 2003) and signals healthiness to the consumers (Sandvik et al., 2018). Most adult edible insects can develop a dark brown melanin through non-enzymatic reactions that is predominant on their exoskeleton (Janssen et al., 2017) while cereal flours generally have a white starchy endosperm (Barnes, 1986).

Regarding redness (a^*), insect flour addition to cereal flours resulted in significant reduction ($P \le 0.05$); however, there were no significant (P > 0.05) differences for finger millet blends. On the same, finger millet flour and blends had the highest a^* values of 13.18 to 2.83 and highest redness decrement rate of ~78%. On the contrary, proso millet and maize flours and their respective blends had the lowest a^* values ranging from -3.84 to -1.52 and -5.95 to -3.98, respectively. The high positive values in finger millet were attributed to the high contents of naturally occurring total phenolics in the seed coat such as anthocyanins and tannins compared to light-coloured grain types (Siwela *et al.*, 2010).

The yellowness (b^*) significantly reduced with increasing amounts of *G. bimaculatus* powder. Maize

and proso millet flours and respective blends had the highest b^* values of 27.02 to 16.43 and 20.98 to 11.54, respectively. These could be attributed to the higher levels of total carotenoids in their endosperms (Siwela *et al.*, 2010). Consequently, after *G. bimaculatus* powder addition, finger millet flour exhibited the highest reduction in yellowness by up to ~93%.

Hue angle which is the attribute to which colours are conventionally defined as reddish (0°) , yellowish (90°), greenish (180°) and blueish (270°) (Sant'Anna et al., 2013). Substitution of maize flour with G. bimaculatus powder showed no significant (P < 0.05) differences in the hue values. However, there was a significant increment in hue value for rice flour blends from blue to yellow colour and finger millet flour blends from light blue to purple. Contrary, proso millet blends displayed a significant decrease $(P \le 0.05)$ in hue values from blue-green to purple colour upon insect addition. In general, hue of the cereal blends transitioned towards the darker blue or purple colour of G. bimaculatus (-49.22).

Regarding chroma or the vividness of colour in maize and proso millet blends significantly ($P \le 0.05$) and gradually reduced by $\sim 12\%$ with each level of insect addition. However, in finger millet, there was an initial significant ($P \le 0.05$) reduction of ~90% followed by constant ($P \le 0.05$) chroma values with further insect addition. For rice samples, chroma values reduced gradually by $\sim 12\%$ up to 10% insect addition then shifted to an increase by $\sim 38\%$. Browning and vellowing index reduced on maize and proso millet blends: however, significant (P < 0.05)changes occurred only at 10% insect addition and no further differences were reported. On the other hand, BI and YI increased on the rice and finger millet blends; however, there were no significant differences ($P \le 0.05$) observed in YI except for the initial (5%) insect addition into finger millet.

The colour differences (ΔE) for all cereal flours significantly ($P \leq 0.05$) increased with *G. bimaculatus* powder addition, mainly due to its low L^* values. In general, these results highlight the varied modification of the perceived colour upon insect powder addition to different cereal flours, which is key to consumers' perception of food quality (Sant'Anna *et al.*, 2013).

Hydration properties of *G. bimaculatus* powder-cereal flour blends

The hydration properties of the cereal flours, blends and *G. bimaculatus* powder differed significantly $(P \le 0.05)$ except for water absorption capacity in proso millet blends as shown in Table 5. Water absorption capacity (WAC) and oil absorption capacity (OAC) depict the ability to take up and retain water or oil, respectively which is key to texture and

| Blends | | L* | a* | b * | Hue | Chroma | 8 | ¥ | ΔE |
|-------------------------------|------------------|---|-------------------------------------|---|--|---|---|---------------------------------------|--------------------------------------|
| Maize: G. <i>bimaculatus</i> | 100 M | 105.30 ± 0.10^{a} | $-5.95\pm0.33^{\rm e}$ | 27.02 ± 1.49^{a} | $-77.56\pm0.02^{\rm b}$ | 27.66 ± 1.53^{a} | 24.71 ± 1.57^{a} | 36.63 ± 1.99^{a} | n.d. |
| | 95 M:5C | 101.10 ± 0.62^{b} | -5.78 ± 0.11^{de} | $\textbf{23.63} \pm \textbf{1.52}^{\text{b}}$ | $-76.21 \pm 0.63^{\rm b}$ | $24.33 \pm \mathbf{1.50^{b}}$ | $\textbf{21.75} \pm \textbf{1.94}^{ab}$ | $33.38\pm\mathbf{2.32^ab}$ | 5.53 ± 0.50^{d} |
| | 90 M:10C | $98.01\pm\mathbf{0.82^{c}}$ | -5.28 ± 0.09^{cd} | $\textbf{20.71}\pm\textbf{0.79}^{c}$ | $-75.66 \pm 0.46^{\rm b}$ | $\textbf{21.37}\pm\textbf{0.78}^{c}$ | $19.22\pm\mathbf{1.00^{bc}}$ | $30.18\pm1.21^{\mathrm{bc}}$ | $9.73\pm\mathbf{0.89^c}$ |
| | 85 M:15C | 94.46 ± 0.74^{d} | $-4.84\pm0.12^{\rm c}$ | $19.23\pm\mathbf{0.04^{c}}$ | $-75.85 \pm 0.33^{ m b}$ | $19.83\pm\mathbf{0.050^{c}}$ | $18.47\pm0.25^{\mathrm{bc}}$ | $\textbf{29.08}\pm\textbf{0.26}^{bc}$ | $\textbf{13.44}\pm\textbf{0.27}^{b}$ |
| | 80 M:20C | $90.82 \pm 0.76^{\mathrm{e}}$ | $-3.98\pm0.19^{\rm b}$ | $16.43\pm0.45^{\mathrm{d}}$ | $-76.37 \pm 0.27^{\rm b}$ | $16.90 \pm \mathbf{0.48^d}$ | $16.33\pm\mathbf{0.55^{c}}$ | $25.84\pm\mathbf{0.88^{c}}$ | 18.10 ± 0.38^{a} |
| | 100C | $\textbf{45.35} \pm \textbf{1.05}^{\text{f}}$ | 6.53 ± 0.142^{a} | -7.59 ± 0.60^{e} | -49.22 ± 1.72^{a} | $10.01 \pm \mathbf{0.54^{e}}$ | -5.17 ± 0.99^{d} | -23.92 ± 2.19^{d} | n.d. |
| | P-values | *** | *** | *** | *** | *** | *** | *** | *** |
| Rice: G. bimaculatus | 100R | 107.80 ± 0.10^{a} | $0.80\pm\mathbf{0.04^{b}}$ | $-0.36\pm0.01^{\rm c}$ | $-24.25\pm\mathbf{0.96^d}$ | $0.87\pm\mathbf{0.05^{bc}}$ | $0.37\pm\mathbf{0.02^{b}}$ | -0.47 ± 0.01^{a} | n.d. |
| | 95R:5C | 103.40 ± 0.60^{b} | $0.61 \pm \mathbf{0.08b^{c}}$ | -0.29 ± 0.081^{c} | $-\textbf{25.64}\pm\textbf{3.38}^{d}$ | $0.68\pm0.11^{\mathrm{bc}}$ | $0.31\pm0.03^{\rm b}$ | -0.41 ± 0.11^{a} | $4.35 \pm 0.59^{\mathrm{d}}$ |
| | 90R:10C | 101.70 ± 0.30^{b} | $0.48\pm\mathbf{0.048^c}$ | $0.19\pm0.02^{\mathrm{bc}}$ | $21.97 \pm 1.19^{\circ}$ | $0.53\pm\mathbf{0.05^c}$ | $0.69\pm\mathbf{0.05^{ab}}$ | $0.27\pm\mathbf{0.03^a}$ | $6.11\pm0.01^{\rm c}$ |
| | 85R:15C | $97.21\pm0.93^{\rm c}$ | $0.58\pm\mathbf{0.06b^{c}}$ | $\textbf{0.64}\pm\textbf{0.04}^{ab}$ | $47.66 \pm 0.82^{\rm b}$ | $0.87 \pm 0.08^{\mathrm{bc}}$ | $\textbf{1.24}\pm\textbf{0.09}^{ab}$ | $0.94\pm\mathbf{0.08^a}$ | $10.64 \pm 1.13^{\rm b}$ |
| | 80R:20C | $94.05\pm0.93^{\mathrm{d}}$ | $0.64\pm\mathbf{0.06b^{c}}$ | 1.01 ± 0.11^{a} | $57.45\pm\mathbf{3.23^a}$ | $1.19 \pm 0.11^{\mathrm{b}}$ | $1.70\pm\mathbf{0.12^a}$ | $1.53\pm0.16^{\mathrm{a}}$ | $13.83\pm\mathbf{0.86^a}$ |
| | 100C | $\textbf{45.35} \pm \textbf{1.05}^{\text{e}}$ | $6.53\pm0.14^{\rm a}$ | $-7.59\pm0.60^{\rm d}$ | -49.22 ± 1.72^{a} | $\textbf{10.01}\pm\textbf{0.54}^{\text{e}}$ | -5.17 ± 0.99^{c} | $-23.92\pm2.19^{\rm b}$ | n.d. |
| | P-values | *** | *** | *** | *** | *** | *** | *** | *** |
| Finger millet: G. bimaculatus | 100F | 96.52 ± 0.69^{a} | 13.18 ± 0.05^{a} | -27.63 ± 0.42^{d} | $-64.49 \pm 0.43^{\rm e}$ | $30.61\pm\mathbf{0.36^a}$ | -15.60 ± 0.28^{d} | -42.20 ± 0.35^{c} | n.d. |
| | 95F:5C | $93.45\pm\mathbf{0.66^{b}}$ | $2.81 \pm 0.11^{\mathrm{c}}$ | $0.01 \pm 0.23^{\mathrm{b}}$ | $0.09\pm4.84^{\rm c}$ | $2.82 \pm 0.11^{\mathrm{c}}$ | $\textbf{2.26}\pm\textbf{0.29}^{b}$ | 0.011 ± 0.34^{a} | 2.93 ± 0.78^{d} |
| | 90F:10C | $92.96\pm\mathbf{0.62^{b}}$ | $\textbf{3.06}\pm\textbf{0.06}^{c}$ | $0.68\pm\mathbf{0.18^{ab}}$ | $12.58 \pm 3.25^{\rm b}$ | $\textbf{3.14}\pm\textbf{0.08}^{c}$ | $3.25\pm\mathbf{0.22^{ab}}$ | $1.05\pm0.28^{\mathrm{a}}$ | $\textbf{4.78}\pm\textbf{0.11}^{c}$ |
| | 85F:15C | $90.14\pm\mathbf{0.24^{c}}$ | $\textbf{3.07}\pm\textbf{0.04}^{c}$ | $1.66\pm\mathbf{0.18^a}$ | 28.31 ± 2.40^{a} | $\textbf{3.49}\pm\textbf{0.12}^{c}$ | $\textbf{4.43}\pm\textbf{0.24}^{a}$ | $2.63\pm\mathbf{0.30^{a}}$ | $7.65\pm1.57^{\mathrm{b}}$ |
| | 80F:20C | $88.67\pm\mathbf{0.44^{c}}$ | $2.83 \pm \mathbf{0.18^{c}}$ | $1.30\pm0.34^{\rm a}$ | 24.46 ± 4.99^{a} | $\textbf{3.12}\pm\textbf{0.27}^{c}$ | $3.90\pm\mathbf{0.50^a}$ | $\textbf{2.09}\pm\textbf{0.57}^{a}$ | $14.72\pm\mathbf{0.78^a}$ |
| | 100C | $45.35\pm\mathbf{1.05^d}$ | $6.53\pm\mathbf{0.14^{b}}$ | $-7.59\pm0.60^{\rm c}$ | -49.22 ± 1.72^{a} | $\textbf{10.01}\pm\textbf{0.54}^{\text{e}}$ | -5.17 ± 0.99^{c} | $-23.92\pm2.19^{\rm b}$ | n.d. |
| | <i>P</i> -values | *** | *** | *** | *** | *** | *** | *** | *** |
| Proso millet: G. bimaculatus | 100S | 100.80 ± 1.20^{a} | $-3.84\pm0.35^{\rm d}$ | 20.98 ± 0.52^{a} | $-\textbf{79.62}\pm\textbf{0.74}^{bc}$ | $21.33 \pm \mathbf{0.57^a}$ | $\textbf{20.05}\pm\textbf{0.52}^{a}$ | 29.72 ± 0.73^{a} | n.d. |
| | 95S:5C | $94.99\pm\mathbf{0.80^{b}}$ | -3.53 ± 0.20^{cd} | $18.34\pm\mathbf{0.38^{b}}$ | $-$ 79.10 \pm 0.67 ^{bc} | $18.68\pm0.37^{\mathrm{b}}$ | $18.31\pm0.65^{\mathrm{a}}$ | ${\bf 27.59}\pm{\bf 0.68^{a}}$ | $6.43\pm\mathbf{0.36^d}$ |
| | 90S:10C | $91.41\pm\mathbf{0.64^{c}}$ | $-3.02\pm0.15^{\rm c}$ | $14.73\pm0.37^{ m c}$ | $-78.39 \pm 0.30^{\rm b}$ | $15.04\pm\mathbf{0.39^c}$ | $14.84\pm0.36^{\mathrm{b}}$ | $\textbf{23.03}\pm\textbf{0.60}^{b}$ | $\textbf{11.34}\pm\textbf{0.40}^{c}$ |
| | 85S:15C | 87.39 ± 0.71^{d} | $-1.91 \pm 0.11^{\rm b}$ | 12.73 ± 0.81^{d} | $-81.40\pm\mathbf{0.18^{cd}}$ | 12.87 ± 0.82^{d} | $13.91\pm0.98^{\mathrm{b}}$ | $20.81\pm\mathbf{1.35^{b}}$ | $15.90\pm\mathbf{0.15^{b}}$ |
| | 80S:20C | $83.13\pm0.17^{\rm e}$ | $-1.52\pm0.14^{\mathrm{b}}$ | $\textbf{11.54}\pm\textbf{0.48}^{d}$ | $-\textbf{82.45}\pm\textbf{0.82}^{d}$ | $\textbf{11.64}\pm\textbf{0.48}^{d}$ | $\textbf{13.41}\pm\textbf{0.65}^{b}$ | $19.84\pm0.80^{\mathrm{b}}$ | 20.19 ± 0.504^{a} |
| | 100C | $\textbf{45.35} \pm \textbf{1.05}^{\texttt{f}}$ | $6.53\pm0.14^{\mathrm{a}}$ | $-7.59\pm0.60^{\rm e}$ | -49.22 ± 1.72^{a} | 10.01 ± 0.544^{e} | -5.17 ± 0.99^{c} | -23.92 ± 2.19^{c} | n.d. |
| | P-values | *** | *** | *** | *** | *** | *** | *** | * * |

Table 4 Colorimetric profile of Gryllus bimaculatus powder-cereal flour blends

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Hydration properties

| Hydration properties | | | | | | | | | | | |
|------------------------|-------------------|-----------------|-------|-----------------------------------|--------------------|------------------------------------|------------------|-------------------------------------|---|---|---|
| Blends | | | WAI | | WSI | | SP | | OAC | WHC | WAC |
| Maize: G. bimaculatus | s 100 l | N | 6.79 | 0 \pm 0.020 ^a | 3.45 | 4 ± 0.020^{e} | 7.06 | 1 ± 0.079^{a} | $\textbf{2.097} \pm \textbf{0.036}^{b}$ | $\textbf{2.156} \pm \textbf{0.166}^{b}$ | $\textbf{2.439} \pm \textbf{0.017b}^{c}$ |
| | 95 M | :5C | 6.56 | 1 ± 0.101^{b} | 4.08 | 4 ± 0.037^{d} | 6.84 | 1 ± 0.103^{b} | $\textbf{2.077}\pm\textbf{0.018}^{b}$ | $2.381\pm0.039^{	ext{b}}$ | $\textbf{2.554} \pm \textbf{0.006}^{ab}$ |
| | 90 M | :10C | 6.40 | $5~\pm~0.032^{b}$ | 4.53 | $7~\pm~0.045^{c}$ | 6.71 | $0\pm0.0304^{ m b}$ | $\rm 1.988\pm0.003^{c}$ | $\textbf{2.245} \pm \textbf{0.058}^{b}$ | $\textbf{2.573} \pm \textbf{0.008}^{a}$ |
| | 85 M | :15C | 6.03 | $4~\pm~0.119^{ m c}$ | 4.66 | $6~\pm~0.156^{ m c}$ | 6.32 | $9 \pm 0.115^{\circ}$ | $\rm 1.980\pm0.005^{c}$ | $\textbf{2.381} \pm \textbf{0.055}^{b}$ | 2.561 ± 0.001^{ab} |
| | 80 M | :20C | 5.96 | $4\pm$ 0.058 ^c | 5.37 | $7~\pm~0.119^{ m b}$ | 6.30 | $2\pm0.054^{ m c}$ | $\textbf{2.032} \pm \textbf{0.011}^{\texttt{bc}}$ | $\rm 2.375\pm0.025^{b}$ | $\textbf{2.433} \pm \textbf{0.033}^{c}$ |
| | 100C | | 2.90 | 8 ± 0.030^{d} | 16.1 | $\textbf{20}\pm\textbf{0.058}^{a}$ | 3.46 | 8 ± 0.034^{d} | $\textbf{2.408} \pm \textbf{0.009}^{a}$ | ${\bf 3.527}\pm0.036^{a}$ | $\textbf{2.274} \pm \textbf{0.066}^{d}$ |
| | <i>P</i> -val | ues | *** | | *** | | *** | | *** | *** | *** |
| | | | WAI | | WSI | | SP | | OAC | WHC | WAC |
| Rice: G. bimaculatus | 100R | - | 7.041 | \pm 0.016 ^{bc} | 2.84 | \pm 0.0173 ^d | 7.29 | 5 ± 0.034^{ab} | $\textbf{2.013} \pm \textbf{0.009}^{b}$ | $\textbf{2.548} \pm \textbf{0.062}^{b}$ | 2.26 ± 0.0031^{al} |
| | 95R:50 | | | \pm 0.198 ^a | 3.16 | $7~\pm~0.140^{ m d}$ | | 2 ± 0.208^{a} | 1.827 ± 0.002^{c} | $\textbf{2.108} \pm \textbf{0.040}^{c}$ | $\textbf{2.341} \pm \textbf{0.038}^{a}$ |
| | 90R:10 | | | \pm 0.166 ^{ab} | 3.88 | $7~\pm~0.235^{ m c}$ | 7.52 | $3\pm$ 0.176 ^{ab} | $\rm 1.799\pm0.010^{c}$ | $\textbf{2.114} \pm \textbf{0.025^c}$ | $\textbf{2.328} \pm \textbf{0.041}^{a}$ |
| | 85R:15 | 5C (| 6.756 | \pm 0.307 ^{bc} | 4.69 | $7~\pm~0.251^{ m b}$ | 7.08 | $9\pm0.308^{ m b}$ | $\rm 1.829\pm0.014^{c}$ | $\textbf{2.103}\pm\textbf{0.016}^{c}$ | 2.269 ± 0.025^{al} |
| | 80R:20 |)C (| 6.700 | \pm 0.153 ^c | 4.973 | $3\pm$ 0.237 ^b | 7.05 | $1\pm0.176^{ m b}$ | 1.831 ± 0.017^{c} | $\textbf{2.133}\pm\textbf{0.056}^{c}$ | $2.155\pm0.005^{ m b}$ |
| | 100C | : | 2.908 | \pm 0.030 ^d | 16.1 | $20~\pm~0.058^{a}$ | 3.46 | $8\pm0.034^{ m c}$ | $\rm 2.408\pm0.009^{a}$ | $\textbf{3.527}\pm\textbf{0.036}^{a}$ | $\textbf{2.274} \pm \textbf{0.066}^{\text{al}}$ |
| | <i>P-</i> valu | es ³ | *** | | *** | | *** | | *** | *** | * |
| | | | | WAI | | WSI | | SP | OAC | WHC | WAC |
| Finger millet: G. bima | culatus | 100R | | 6.645 ± 0.0 | 23 ^a | 2.899 ± 0.13 | 9 ^f | 6.918 ± 0.100 | ^b 2.252 ± 0.008 | ^b 2.589 \pm 0.037 ^b | $2.695 \pm 0.054^{\circ}$ |
| | | 95R:5 | 5C | $\textbf{6.803} \pm \textbf{0.0}$ | 07 ^a | $\textbf{3.826} \pm \textbf{0.05}$ | 8 ^e | $\textbf{7.074} \pm \textbf{0.035}$ | a^{a} 1.895 \pm 0.009 | c 2.296 \pm 0.130 b | $2.737 \pm 0.021^{\circ}$ |
| | | 90R:1 | 0C | $\textbf{5.854} \pm \textbf{0.0}$ | 64 ^b | 5.056 ± 0.02 | 7 ^d | $\textbf{6.166} \pm \textbf{0.032}$ | $^{ m c}$ 1.894 \pm 0.013 | c 2.432 \pm 0.019 b | $2.714 \pm 0.011^{\circ}$ |
| | | 85R:1 | 5C | $\textbf{5.630}\pm\textbf{0.1}$ | 22 ^{bc} | $\textbf{5.441} \pm \textbf{0.01}$ | 9 ^c | 5.954 ± 0.072 | $^{\rm d}$ 1.883 \pm 0.008 | c 2.257 \pm 0.154 b | 2.688 ± 0.014^{3} |
| | | 80R:2 | 20C | $\textbf{5.493}\pm\textbf{0.1}$ | 47 ^c | $\textbf{6.125} \pm \textbf{0.18}$ | 4 ^b | $\textbf{5.852} \pm \textbf{0.045}$ | $^{\rm d}$ 1.895 \pm 0.008 | c 2.431 \pm 0.019 b | 2.532 ± 0.053^{11} |
| | | 100C | | $\textbf{2.908} \pm \textbf{0.0}$ | 30 ^d | 16.120 ± 0.0 | 58 ^a | $\textbf{3.468} \pm \textbf{0.034}$ | e 2.097 ± 0.036 | a 3.527 \pm 0.036 a | 2.274 ± 0.066 |
| | | P-valu | ues | *** | | *** | | *** | *** | *** | *** |
| | | | | WAI | | WSI | | SP | OAC | WHC | WAC |
| Proso millet: | 100S | | | 6.862 ± 0 | 0.044 ^c | 1.968 ± 0.0 | 24 ^e | 7.144 ± 0.102 | 2^{c} 2.067 \pm 0.002 | 2^{b} 2.223 \pm 0.178 ^b | 2.149 ± 0.015 |
| G. bimaculatus | 95S:5C | | | 7.099 \pm 0 | 0.045 ^b | $\textbf{2.926} \pm \textbf{0.1}$ | 24 ^d | $\textbf{7.313} \pm \textbf{0.042}$ | 2^{b} 2.057 \pm 0.04 | $1^{ m bc}$ 2.109 \pm 0.023 ^I | 2.223 ± 0.064 |
| | 90S:10 | C | | 7.281 ± 0 | 0.003 ^a | $\textbf{3.100} \pm \textbf{0.2}$ | 38 ^{cd} | 7.514 ± 0.018 | 3^{a} 1.937 \pm 0.002 | $2^{ m d}$ 2.075 \pm 0.006 | 2.191 ± 0.079 |
| | 85S:15 | C | | 6.912 ± 0 | 0.019 ^c | $\textbf{3.541} \pm \textbf{0.1}$ | 92 ^c | 7.165 ± 0.033 | 3^{c} 1.986 \pm 0.018 | 3^{cd} 2.117 \pm 0.003 | 2.162 ± 0.019 |
| | 80S:20 | C | | 6.883 ± 0 | 0.014 ^c | $\textbf{4.110} \pm \textbf{0.3}$ | 41 ^b | 7.178 ± 0.030 | $0^{ m bc}$ 2.036 \pm 0.009 | $5^{ m bc}$ 2.169 \pm 0.044 | 2.153 ± 0.036 |
| | 100C | | | 2.908 ± 0 | 0.030 ^d | 16.120 \pm 0. | 058 ^a | 3.468 ± 0.034 | 1^{d} 2.408 \pm 0.009 | Θ^{a} 3.527 \pm 0.036 | 2.274 ± 0.066 |
| | <i>P</i> -value | s | | *** | | *** | | *** | *** | *** | ns |
| MANOVA | Insect a | amount | t | *** | | *** | | *** | *** | *** | *** |
| | Cereal | type | | *** | | *** | | *** | *** | *** | *** |
| | Insect a Cerea | amount | t: | *** | | *** | | *** | *** | *** | * |

C, *G. bimaculatus* powder; F, finger millet flour; M, maize flour; R, rice flour; S, proso millet flour. Values are means values \pm standard error. Values with different superscripts (^{a-c}) on the same column are significantly different. ns, P > 0.05; * $P \le 0.05$; * $P \le 0.01$; ** $P \le 0.001$; OAC, oil absorption capacity; SP, swelling power; WAC, water absorption capacity; WAI, Water Absorption Index; WHC, water holding capacity; WSI, Water Solubility Index.

flavour characteristics of foods. Among the cereal flours, WAC were higher than the OAC values indicating a greater ability to bind and retain water compared to oil. This implies that the flours can be suitable in dough formations with high moisture retention but possessing a limited ability to form and stabilise oil-inwater emulsions (Li *et al.*, 2016).

In flour samples, the WAC (r = -0.937) and OAC (r = -0.954) values correlated negatively with particle

size distribution: The smaller the particles, the larger the specific surface area and therefore the potential contact surface for absorption or adsorption. A wider span in finger millet was linked to its higher WAC and OAC while the narrower span in proso millet was linked to lower WAC among the cereal flours. It has been found that a composition with a complex fraction or wider distribution of particle size has a higher water absorption (Zykova *et al.*, 2015). *G. bimaculatus*

Table 5 Hydration properties of Gryllus bimaculatus-cereal flour blends (g water or oil/g sample dry weight)

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powder had the highest OAC at 2.40 g oil/g dry sample weight and this was attributed to the high amounts of insect's protein that bind to the lipids. Studies have shown that the amino acid composition, the surface polarity and conformation of proteins influence the OAC and WAC (Akpossan *et al.*, 2015). Substitution of cereal flours with *G. bimaculatus* powder decreased the OAC of blends possibly due to fewer hydrophilic constituents of flour due to substitution. Also, inclusion of *G. bimaculatus* powder had an initial increase in WAC at 5% and further addition caused a decrease. This could be due to formation of starch-lipid complexes and insoluble films around the starch granules thus limiting the absorption capacities of starch (Song & Jane, 2000).

Swelling power (SP) which represents the entrapped water within the network of a starchy gel in water during heating and stirring (Koo et al., 2010) was highest in rice flour with values of 7.29 followed by proso, maize and finger millet flours. Swelling of starches is a property of amylopectin and decreases linearly with amylose content (Agama-Acevedo et al., 2018). Similar to WAC, inclusion of 5%-10% G. bimaculatus powder significantly ($P \le 0.05$) increased SP except in maize blends – which had a significant ($P \le 0.05$) decrease – followed by a decrease at 15%-20% G. bimaculatus powder inclusion. Literature has reported an inhibition of SP in flours by fatty acids (Devi et al., 2020). High lipid contents from the insect can cause an incomplete hydration of starch (as illustrated by lower WAC) causing a low degree of gelatinisation resulting in limited swelling (Li et al., 2016). The decrease in SP with high amounts of G. bimaculatus powder could increase the compactness of the network in dough sheet leading to smaller dough products such as breads or lead to reduced average network length and width of cooked noodles (An et al., 2022).

Water solubility index is defined as the fraction of the sample solubilised in water (Birch *et al.*, 2019). WSI was highest in *G. bimaculatus* powder at 16.12 g/g dry sample weight compared to cereals which ranged between 3.45-1.96 g/g dry sample weight. Consequently, inclusion of *G. bimaculatus* powder resulted in a significant increase ($P \le 0.05$) in WSI in all blend samples. This could be attributed to the high amounts of soluble proteins, water-soluble fibres and other biomolecules (Alcázar-Alay & Meireles, 2015). The increasing WSI in blends upon inclusion of *G. bimaculatus* powder may facilitate a soft and less dense internal structure of food products (Awuchi *et al.*, 2019).

The water absorption index (WAI) describes the absorption of water when the flour undergoes a heating treatment (Kinsella & Melachouris, 1976). In this study, the flours had relatively higher WAI compared to *G. bimaculatus* powder. Rice flour had the highest

WAI followed by proso, maize and finger millet flour. Inclusion of *G. bimaculatus* powder into maize and finger millet flours had a significant decrease ($P \le 0.05$) in WAI, however in rice and proso millet flours the WAI significantly increased ($P \le 0.05$) with 5%–10% *G. bimaculatus* powder addition followed by a decrease. In regards, to WHC, there was no significant difference (P > 0.05) between blends. Although *G. bimaculatus* powder had higher WHC, its inclusion into flours did not influence (P > 0.05) their ability to imbibe and retain water (except a significant decrease ($P \le 0.05$) in 5% *G. bimaculatus* powder as an ingredient interacts differently with cereal starches in their ability to absorb and hold water.

Finally, the samples did not exhibit emulsion activity (EC) and emulsion stability (ES); the emulsion-like layers collapsed immediately. Generally, the proteins are surface active agents responsible for the formation and stabilisation of emulsions depending on their hydrophobicity and conditions such as temperature, pH and ionic strength (Fatima *et al.*, 2014). *G. bimaculatus* has been found to form adequate emulsion but with poor stability (Adebowale *et al.*, 2005). Nevertheless, emulsifying capacities have a correlation with OAC (Gravel & Doyen, 2020), and the low OAC observed especially on the blends could potentially lead to a phase separation and instability of emulsions (Dapčević Hadnadev *et al.*, 2013).

Conclusion

The incorporation of edible insects in commonly consumed gluten-free flours without negatively affecting the final products' characteristics could promote sustainable and novel healthy diets. The study demonstrated that understanding the raw material characteristics is a valuable keyline in predicting the final properties of formulations and possible end products. The morphological features of cereal flours and G. bimaculatus powder differed, notably in terms of particle sizes and aggregation. Cereal flours also exhibited variations in particle sizes and starch granules' shapes, and arrangements. Incorporating sizes, G. bimaculatus powder into gluten-free cereal flours offers significant potential for enhancing their protein, fat, and fibre contents. Increasing amounts of G. bimaculatus powder significantly altered the colour profile of the blends, particularly resulting in a darker coloration. Furthermore, the reduction in water absorption capacity, oil absorption capacity and swelling power of cereal flours upon G. bimaculatus powder inclusion offers opportunities for texture and structure modification, including increased compactness and improved moisture retention. However, the observed increase in water solubility index and lack of emulsion

activity in *G. bimaculatus*-cereal blends may impact the internal structure by facilitating a soft, less dense internal structure and induce emulsion instability, respectively. These findings highlight the potential of *G. bimaculatus* powder in modifying the properties of gluten-free flours; however, further research is necessary to optimise its utilisation across various food applications.

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Conflict of interest statement

The authors declare there are no known competing interests.

Ethical guidelines

Ethics approval was not required for this research.

Peer review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author upon reasonable request.

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