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Research article

Macrotermes *subhylanus* flour inclusion in biscuits: Effects on nutritional, sensorial and microbial characteristics

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A B S T R A C T

As the world's population expands, edible insects have been proposed as a food source that might address issues related to nutrition, health, the environment, and the economy. This study aimed to create a novel biscuit by adding *Macrotermes subhylanus* (*M. Subhylanus*) flour to wheat flour in various concentrations (5,10, 15 and 20 %). The moisture content of the insect composite flours varied between 6.83 % and 7.76 %, whereas the moisture content of the biscuits ranged from 2.86 % to 7.90 %. A significant difference ($p < 0.05$) was noted in the protein content of both the composite flours and biscuits as the concentration of insect flour increased, with values ranging from 15.03 % to 21.52 % for the flours and 17.38 % to 20.63 % for the biscuits. The lightness (L*) of the composite flours significantly decreased ($p < 0.05$) with higher additions of edible insect flour, whereas the redness (a*) and yellowness (b*) attributes did not show any statistical differences ($p > 0.05$). The biscuits were generally darker than the composite flours, as indicated by substantially lower L* values. The water activity of the biscuits was between 0.44 and 0.67. Sensory evaluation revealed that the substitution level (up to 15 %) is ideal for preparing acceptable insect-based biscuits. The panellist perceived no significant differences ($p > 0.05$) in terms of the texture between the insect-enriched biscuits and the control, except for MZ-20. The absence of pathogenic microogranisms in all baked biscuits containing edible insect flour highlights the effectiveness of heat treatment, ensuring that the biscuits meet microbiological safety guidelines. Additionally, *Macrotermes subhylanus* flour shows promise as a novel functional ingredient for the food industry.

1. Introduction

The vital role of edible insects in enhancing food and nutrition security has received considerable attention in policy development, production, research and development and consumer studies. Edible insects have garnered a lot of attention lately because of their potential to address concerns with food safety brought on by the projection that the world's population will increase to 9.5 billion people by 2030 [1,2]. Entomophagy, the practice of consuming insects, has long been established in regions such as Africa, South America, and Southeast Asia, yet it remains relatively unfamiliar to many Western societies [3–5]. According to Van-Huis [6], two Billion people consume insects as part of their diet, which is considered a solution to mitigate food security problems and the increase in food prices worldwide. The increase in food prices, poverty and malnutrition has prompted the search for affordable, alternative, sustainable protein sources. Insects are rich in protein [4,7,8], fat [9,10], vitamins and minerals [11,12], and are emerging as viable alternative sustainable food sources compared to traditional protein sources [7,13]. It was Meyer-Rochow [14] who suggested as early as 1975 that rearing edible insects for food is a viable strategy that may help ensure global food security.

Termites *(Macrotermes subhylanus, M. subhylanus),* also called isusu in Nigeria and madzhulu in Venda, are gregarious insects that are traded in informal marketplaces in South Africa. They are consumed as whole insects and are most prevalent in KwaZulu Natal and Limpopo provinces [2]. However, in certain metropolitan regions in Western and African countries, insects are not considered as a

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Table 1
Product formulations for edible insect-enriched biscuits.

Ingredients	Control	MZ-5	MZ-10	MZ-15	MZ-20
Wheat-flour(g)	100	95	90	85	80
M.subhylanus flour (g)	0	5	10	15	20
Sugar (g)	130.0	130.0	130.0	130.0	130.0
Butter (g)	240.0	240.0	240.0	240.0	240.0
Egg (g)	94.0	94.0	94.0	94.0	94.0
TOTAL	**564 g**	**564 g**	**564 g**	**564 g**	**564 g**

[a] Control = 100% wheat flour, MZ-5 = 5%, MZ-10 = 10%, MZ-15 = 15%, MZ-20= 20%, *M. subhylanus* flour substitution.

source of protein. In addition, insects as food are regarded as a cultural "taboo", with the general attitude of disgust, high price, poor sensory appeal and unfamiliarity [1,15]. Therefore, lower consumer acceptance and attitudes towards edible insects are barriers that the food industry needs to overcome to successfully incorporate edible insects in food products. Research has shown that when edible insects are added to food items in invisible ways, people are ready to eat them [16–18]. This raises the possibility of developing novel foods utilizing insects, especially in areas where traditional methods are unlikely to be embraced due to a lack of sensory appeal. Therefore, incorporating processed insects into food products such as biscuits, sausages, bread, cookies, pasta, muffins, and dressings and researching ways to improve insect flours will ensure the availability of nutritious and healthful food items.

Based on the nutritional value of edible insects, the scientific community has already begun to investigate how to utilise this new food source to create fortified foodstuffs. In a study by Zielińska and Pankiewicz [19], biscuits enriched with mealworm flour (*Tenebrio molitor*) showed increased protein and ash contents. The textural and sensorial properties of muffins were affected significantly by the incorporation of grasshopper (*Locusta Migratoria*) flour [20]. While in bread formulations containing cinereous cockroach (*Nauphoeta cinerea*), de Oliveira [21] reported that bread enriched with 10 % roasted insect flour had acceptable sensorial properties. Moreover, defatted *Macrotermes subhylanus* sorghum composite flour significantly improved the biscuits' protein content, and sensory analysis showed that the biscuits were acceptable up to 25 % DMF incorporation [22]. To ascertain the impact of insect powders on the techno-functional, nutritional, microbiological safety, and organoleptic qualities of baked products prepared with these unique ingredients, a great deal of research is necessary. To our knowledge, however, little is known about the impact of adding *M. subhylanus* (Madzhulu) flour to biscuits. Therefore, the aim of this study was to investigate the effect of edible insect flour incorporation in wheat-based composite flours and novel biscuits. The nutritional, physicochemical, microbiological and sensorial properties were established with the view to produce novel insect-based biscuits.

2. Materials and methods

2.1. Preparation of composite flour and biscuit

Edible insect flour (*M. subhylanus*) was prepared following the protocol described by Vanqa et al. [2] with minor adjustments. Dried *M. subhylanus* edible insects were procured from local vendors (Limpopo province, South Africa). A laboratory blender (Bamix, Cape Town, South Africa) was used to grind and mill the edible insects. The proximate composition of this flour was (Protein = 52,74 %, Ash = 6.41 %, Moisture = 6.40 %, crude fat = 6.36 %). To prepare an insect-wheat composite flour, *M. subhylanus* flour with varying concentrations (0, 5, 10, 15 and 20 %) was mixed with wheat flour. Following the product formulation in Table 1, the novel edible insect-enriched biscuits were prepared. All ingredients were mixed and kneaded at high speed to prepare the insect biscuits in a laboratory mixer (Kenwood, model KM240 series, United Kingdom) for 10 min. After a 20-min resting period, the dough was rolled out to a thickness of 5 mm using a rolling pin with side rings and a diameter of 30 mm. The biscuits were subjected to an oven (Macadams, Johannesburg, South Africa) and baked at 180 °C for 20 min. After baking, the biscuits were cooled immediately for 10 min prior to being packaged in polyethene bags. The prepared samples were sealed and stored at ambient temperature until they underwent chemical analysis and sensory evaluation. Using the same methodology, biscuits prepared exclusively with 100 % wheat flour served as the control.

2.2. Chemical composition analyses

2.2.1. Proximate analysis

The chemical composition of the freshly baked biscuits, including moisture (925.10), crude fat (932.06), and ash content (923.03), was determined using methods outlined by the Association of Official Analytical Chemists International (AOAC, 2006). The total protein content was assessed via the Kjeldahl method (920.87), with a nitrogen-to-protein conversion factor of 5.60, as recommended by Janssen et al. [23]. The moisture content was determined by drying the sample in a vacuum oven at 105 °C for 3 h. After drying, the sample was placed in a desiccator to cool and then weighed, repeating this process until a constant weight was achieved. Crude fat content was measured by extracting fats using a Soxhlet apparatus with petroleum ether, followed by drying. The ash content was quantified by combusting the samples in a silica crucible at 550 °C in a muffle furnace. Total carbohydrate content was determined by difference. The biscuits' energy content was assessed using the calculation method from Farzana & Mahajan [24] using the formula:

$$\text{Energy}\left(\frac{Kcal}{100g}\right) = 4\,(\%\,\text{Carbohydrate} + \%\,\text{Protein}) + (9 \times \%\text{fat})$$

2.2.2. Pasting properties of edible insect-enriched flours

The pasting properties of insect-wheat flour blends were analysed using a Rapid Viscosity Analyzer (Perten Instruments, Tecmaster, Sweden). The study included control wheat flour and wheat flour mixed with *M. subhylanus* powder at concentrations of 5 %, 10 %, 15 %, and 20 %. For each sample, 3 g of flour with a known moisture content were weighed into aluminum RVA canisters, and distilled water was added based on the moisture content of each sample as calculated by the program. The samples were initially heated to 50 °C and held at this temperature for 1 min[25]. Then, the sample was heated up to 95 °C and held at that temperature for 2.5 min and the sample was cooled down to 50 °C. The values obtained from the pasting profile included pasting temperature (the temperature at which starch granules begin to swell), peak viscosity (the maximum viscosity achieved by the paste), breakdown viscosity (the difference between the maximum and minimum viscosities during heating), final viscosity (the viscosity measured at the end of the cooling period), and setback viscosity (the difference between the final viscosity and the minimum viscosity during cooling).

2.3. Physicochemical analysis

2.3.1. Colour analysis

The colour of the composite flouts and biscuits was assessed according to the method described by Krystyjan et al. [26]. The upper surface colour was measured using a Konica MINOLTA CM-3500d spectrophotometer (Konica Minolta Inc., Tokyo, Japan) under illuminant D65 and a 10° viewing angle. The results were recorded in the CIELab colour space, determining the following parameters: L* (lightness, with L* = 0 indicating black and L* = 100 indicating white), a* (indicating the extent of green when a* < 0 or red when a* > 0), and b* (indicating the extent of blue when b* < 0 or yellow when b* > 0). The colour change (ΔE) was calculated, where indices 0 and s represent the measured values of wheat flour, wheat-insect flour composites, and biscuit samples. The measurements were carried out on the day of baking.

$$\Delta \mathrm{E} = \sqrt{(L_0 - L_s)^2 + (a_0 - a_s)^2 + (b_0 - b_s)^2}$$

2.3.2. Determination of water activity

The water activity (a_w) of edible *M. subhylanus* composite flours and biscuits was analysed following the procedure outlined by Vanqa et al. [2] with minor adjustments. Three different salt humidity standards, set at 53 %, 75 %, and 90 % relative humidity, were employed to calibrate the measurement cell. A five gram sample of either insect flour or ground biscuit was placed into a sample dish and inserted into the instrument (Novasina, AW SPRINT 500, Zurich, Switzerland). The cell's measuring protection filter was promptly sealed, and readings were taken after an interval of 60–80 s. Each sample was analysed in triplicate.

2.3.3. Texture profile analysis of biscuits

The textural properties of the insect-enriched biscuits were evaluated using the method reported by Suriya et al. [27]. The hardness of baked biscuits was examined in terms of breaking strength using a Texture analyser (Instron 3340, Grove City, United States of America). The Instron was configured to reset to its initial cycle at a velocity of 1.5 mm.s^{-1} and a displacement of 15 mm. This setup simulates the consumer evaluation of biscuit hardness, where the biscuit is held and broken by bending. The maximum force recorded from the resulting curve represents the biscuit's breaking strength.

2.3.4. Determination of physical properties

The method described by Suriya et al. [27] was employed to analyze the physical attributes of the control biscuits and those enhanced with edible insect. The mass (weight) of the biscuit was assessed utilizing the analytical balance (ELB3000, Shimadzu, Japan), while the dimensions of the biscuit, including thickness (the vertical distance between the upper and lower surfaces) and width (the horizontal span across the biscuit), were measured using a Vernier calliper. The spread ratio of the baked edible insect-enriched biscuits was calculated as the quotient of their width to thickness.

2.3.5. Microbial safety assessment of biscuits

The presence of aerobic mesophilic bacteria was determined using 10-fold serial dilution (Harrigan and McCance, 1990). To one gram of the sample, 10 mL of the saline water was added and thoroughly shaken to displace microorganisms. Successive dilutions were performed in a decadic manner by combining 1 mL of the preceding dilution with 9 mL of freshly prepared diluent solution. Lastly, 1 mL of an appropriate dilution was placed in a Petri dish; the appropriate sterilised agar (Nutrient Agar) was poured into the Petri dish, allowed to cool, solidify and incubated at 37 °C for 48 h. After the incubation period, visible colonies were enumerated and recorded. The presence of total coliforms, *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* was determined analogously to the enumeration of aerobic mesophilic bacteria, albeit utilizing specific culture media: MacConkey agar (MCA), Eosin methylene blue agar (EMB), Salmonella-Shigella agar (SSA), Mannitol salt agar (MSA), Palcam Agar, and Mannitol yolk polymyxin agar (MYP), respectively. These cultures were then incubated at 37 °C for 48 h. In parallel with the enumeration of aerobic mesophilic bacteria, counts of fungi (including yeast and mould) were conducted by employing Potato Dextrose Agar (PDA) and subjected to incubation at 25 °C for 72 h.

Table 2
Proximate composition of *M. subhylanus* composite flours and Biscuits.

Sample	Moisture (%)	Ash (%)	Crude Protein (%)	Fat (%)	Carbohydrates (%)	Energy (KJ)
			M. subhylanus-wheat composite flours			
Control	7.71 ± 0.27^{a}	1.72 ± 0.53^{a}	16.64 ± 2.81^{ab}	1.18 ± 0.21^{ab}	72.75 ± 3.42^{b}	368.19 ± 3.61^{a}
MZ-5	6.83 ± 0.35^{a}	2.61 ± 0.30^{ab}	15.03 ± 0.26^{a}	0.94 ± 0.03^{a}	74.59 ± 0.32^{b}	366.91 ± 2.22^{a}
MZ-10	7.74 ± 0.41^{a}	2.58 ± 0.30^{ab}	16.94 ± 1.10^{ab}	1.73 ± 0.62^{bc}	71.01 ± 1.12^{b}	367.37 ± 5.24^{ab}
MZ-15	7.29 ± 0.70^{a}	5.59 ± 3.25^{b}	18.84 ± 0.28^{b}	2.21 ± 0.49^{c}	66.07 ± 4.21^{a}	359.53 ± 12.98^{a}
MZ-20	7.76 ± 1.07^{a}	2.80 ± 1.31^{ab}	21.52 ± 0.22^{c}	1.83 ± 0.15^{bc}	66.10 ± 1.56^{a}	366.92 ± 4.41^{ab}
			Biscuits enriched with *M. subhylanus* flour			
Control	2.66 ± 0.22^{a}	7.21 ± 0.11^{b}	11.75 ± 2.17^{a}	12.86 ± 0.49^{a}	65.52 ± 2.67^{b}	425.00 ± 4.00^{a}
MZ-5	2.86 ± 0.25^{a}	3.19 ± 0.17^{a}	17.38 ± 0.99^{c}	16.60 ± 7.87^{a}	59.98 ± 8.95^{ab}	458.67 ± 38.08^{c}
MZ-10	6.13 ± 0.17^{b}	6.78 ± 0.23^{b}	14.63 ± 0.52^{b}	15.22 ± 0.49^{a}	57.23 ± 0.54^{a}	424.33 ± 4.16^{ab}
MZ-15	7.90 ± 0.21^{b}	2.84 ± 1.01^{a}	18.36 ± 1.52^{cd}	15.90 ± 0.80^{a}	54.99 ± 1.81^{a}	436.67 ± 6.51^{abc}
MZ-20	6.13 ± 0.23^{b}	3.25 ± 1.64^{a}	20.63 ± 0.73^{d}	15.04 ± 1.63^{a}	54.95 ± 1.47^{a}	437.33 ± 9.29^{abc}

Means in a column followed by different letters are significantly different ($p < 0.05$). Proximate values are expressed in g/100 g Control = 100% wheat flour, MZ-5 = 5%, MZ-10 = 10%, MZ-15 = 15%, MZ-20= 20%, *M. subhylanus* flour substitution.

2.4. Sensory evaluation of biscuits

The organoleptic acceptability analysis was conducted among 50 untrained panellists from a Univerisity of Technology (UoT) in the Western Cape South Africa. Sensory evaluation assessments were conducted within a sensory laboratory containing 14 demarcated booths, maintained at an ambient temperature ranging from 28 to 30 °C, and illuminated with white light. Samples, enclosed in polystyrene packaging and labelled with unique three-digit identifiers, were presented randomly to panellists at 30-min intervals. Panellists received instructions to cleanse their palates with water between each sample evaluation. The evaluation of the insect-enriched biscuits among the panellists was based on the following attributes: appearance, colour, aroma, taste, texture, and overall liking on a 5-point hedonic scale, with 1 representing the least score (dislike very much) and five the highest score (like very much). This study obtained approval from the Ethics Committee of the Faculty at the institution (Approval Number: 215062965/05/2021) and was conducted in compliance with applicable regulations and guidelines. Prior to participating in the sensory evaluation of the novel insect-enriched biscuits, participants provided voluntary consent by completing a consent form.

2.4.1. Statistical analysis

Statistical analyses were carried out using SPSS software version 29.0 (2005) (SPSS Inc., Chicago, IL, USA). All measurements were conducted in triplicate for each sample unless otherwise specified. The means and standard deviations of the results are displayed. The multivariate analysis of variance (MANOVA) was utilized to identify significant differences ($p < 0.05$) between treatments. Duncan's multiple range test was then performed to separate means when differences were found. Principal component analysis (PCA) was applied to evaluate the proximate, physicochemical, and textural properties of the biscuits. Graphs and figures were produced using Origin software version 9.9 (Origin Labs, Northampton, MA, USA).

3. Results and discussion

3.1. Proximate composition of insect-enriched composite flours and biscuits

The proximate composition of *Macrotemes subhylanus*-wheat (MZ) composite flours and biscuits is reported in Table 2. *M. subhylanus* is a valuable source of highly nutritious compounds, especially proteins. Edible insects are recognized for their high protein content, and *M. subhylanus*, in particular, has been found to contain 52.74 % protein [2]. The addition of 20 % (MZ-20) *M. subhylanus* flour significantly increased ($p < 0.05$) the protein content of the composite flour when compared to the control. The moisture content of the insect-wheat flour composite flours is shown in Table 2 and it can be seen that it ranged from 6.83 to 7.76 %. The control (wheat flour) and the composite flours containing *M. subhylanus* edible insects did not differ significantly ($p > 0.05$). In addition, the biscuits enriched with insect flour contained a significantly higher ($p < 0.05$) moisture content compared to the control (2.26 %). A product's nutritional makeup is crucial, and moisture content has significant effects on packing, transportation convenience, and preservation [28]. Higher moisture content may lead to reduced shelf-life due to microbial growth and softer biscuits compared to the control. The crude protein content of *M. subhylanus*-wheat composite flours ranged from 15.03 to 21.52 %. This effect was expected since *M. subhylanus* flour contains the highest protein among all ingredients in the formulation; hence, its content causes a comparative increase in protein content in the end product. The highest protein content was observed in MZ-20, which is significantly higher than the control ($p < 0.05$). A similar trend was observed regarding the biscuits enriched with *M. subhylanus* flour, with MZ-20 exhibiting significantly ($p > 0.05$) higher protein content. The findings of this study align with those documented by Koffi et al. [22], who examined the effect of defatted *M. subhylanus* and sorghum-enriched biscuit, which contained 21.66 ± 0.40 protein for the highest concentration (25 %). No statistically significant ($p < 0.05$) differences were observed in the crude fat content of the biscuits enriched with *M. subhylanus* insect flour. The crude fat content of the composite flour was lower than that of the biscuits since the butter added as an ingredient in the formulation contributed to the high fat content in the final biscuit. In terms of the biscuits' moisture content, there was not a significant difference ($p > 0.05$). The findings of this study align with previous research,

Table 3
Pasting properties of wheat flour enriched with edible insect *M. subhylanus*.

Sample	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final viscosity (cP)	Setback Viscosity (cP)	Time to Peak Viscosity (min)	Pasting Temperature (°C)
Control (wheat flour)	2678.67 ± 8.08^{e}	1021.00 ± 3.46^{e}	1658.33 ± 5.77^{e}	2821.00 ± 1.73^{a}	1805.00 ± 3.46^{e}	8.92 ± 0.01^{a}	83.48 ± 0.04^{a}
MZ-5	1881.33 ± 0.58^{d}	944.33 ± 4.04^{d}	941.00 ± 3.46^{d}	2295.00 ± 3.46^{b}	1356.67 ± 9.81^{d}	9.14 ± 0.11^{a}	88.29 ± 0.06^{b}
MZ-10	1652.33 ± 0.58^{c}	771.00 ± 3.46^{c}	885.00 ± 3.46^{c}	1914.33 ± 5.77^{c}	1143.67 ± 2.89^{c}	9.23 ± 6.35^{c}	88.64 ± 0.01^{c}
MZ-15	1146.00 ± 3.46^{a}	542.67 ± 9.81^{a}	608.00 ± 1.73^{a}	1328.33 ± 5.77^{a}	781.67 ± 10.97^{a}	9.13 ± 0.01^{a}	89.87 ± 0.06^{e}
MZ-20	1383.67 ± 2.31^{b}	693.67 ± 2.89^{b}	695.00 ± 3.46^{b}	1639.33 ± 5.77^{b}	939.67 ± 7.51^{b}	9.12 ± 1.15^{b}	89.52 ± 0.06^{d}

Values are mean ± standard deviation. Values with different superscript letters in the same column are significantly different ($p < 0.05$). Control = 100% wheat flour, MZ-5 = 5%, MZ-10 = 10%, MZ-15 = 15%, MZ-20= 20%, *M. subhylanus* flour substitution.

Table 4
Pasting properties of wheat flour enriched with edible insect *M. subhylanus*.

Sample	Composite flours				Insect flour-enriched biscuits			
	L*	a*	b*	Δ E	L*	a*	b*	Δ E
Control	93.03 ± 0.15^{e}	1.15 ± 0.43^{a}	9.58 ± 0.58^{a}	-	79.70 ± 0.22^{e}	5.97 ± 0.55^{a}	31.27 ± 1.09^{b}	–
MZ-5	89.23 ± 0.08^{d}	1.68 ± 0.08^{ab}	8.71 ± 0.66^{ab}	4.00 ± 0.10^{a}	59.41 ± 0.25^{c}	8.54 ± 0.72^{c}	30.16 ± 0.54	20.49 ± 0.32^{b}
MZ-10	86.22 ± 0.10^{c}	1.81 ± 0.30^{b}	8.47 ± 0.45^{a}	6.90 ± 0.20^{b}	67.97 ± 0.17^{d}	7.46 ± 0.38^{b}	29.52 ± 1.51^{ab}	12.01 ± 0.38^{a}
MZ-15	84.17 ± 0.08^{b}	1.92 ± 0.33^{b}	9.00 ± 0.47^{ab}	8.90 ± 0.10^{c}	54.14 ± 0.21^{b}	9.17 ± 0.37^{c}	27.68 ± 0.21^{b}	37.94 ± 20.47^{c}
MZ-20	81.53 ± 0.17^{a}	1.98 ± 0.42^{b}	9.49 ± 0.41^{b}	11.50 ± 0.20^{d}	45.74 ± 0.23^{a}	8.65 ± 0.54^{c}	24.31 ± 1.50^{a}	34.79 ± 0.10^{c}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different. L* = Lightness, a* = Redness, b* = Yellowness. Control = 100% wheat flour, MZ-5 = 5%, MZ-10 = 10%, MZ-15 = 15%, MZ-20= 20%, *M. subhylanus* flour substitution.

demonstrating that incorporating insect flour as an ingredient enhances or alters the nutritional composition of food products like chocolate chip cookies [29], muffins [30], snack fillings [31], porridge [32], and bread [33]. Therefore, given its nutritional properties, *M. subhylanus* insect flour shows promise to be utilized as an innovative/novel ingredient in various food applications.

3.2. Pasting properties

Pasting property evaluation is a fundamental aspect of assessing the structural and functional characteristics of flour ingredient samples, which is pivotal in determining their potential applications in industrial processing. By simulating real food production processes, the analysis of various pasting parameters becomes crucial for predicting both the sensory attributes and the potential industrial uses of the sample. The pasting properties of insect-enriched flours are shown in Table 3. Peak viscosity (PV) indicates the starch's capacity to absorb water and is commonly used as a measure of its thickening ability [34]. The PV of the wheat flour enriched with *M. subhylanus* insect flour ranged from 1146 to 2678.67 cP. Among the flours containing edible insects, the highest PV was observed on the samples with 5 % edible insect flour. Significantly ($p < 0.05$) higher PV for the control could be attributed to its high starch content. The trough/holding viscosity values ranged from 542.67 to 1021 cP. Notably, the insect-containing flour at low concentration addition (MZ-5) exhibited the highest trough viscosity ($p > 0.05$). Conversely, the trough viscosity of MZ-20 was significantly lower ($p > 0.05$) compared to the control sample. The trough viscosity is influenced by variables such as the rate of amylose exudation, granule swelling, and amylose lipid-complex formation [35]. At low concentrations of insect flour addition (MZ-5), there might be a higher proportion of amylose present relative to the overall flour composition. Amylose molecules tend to leach out of starch granules and form a gel network during heating, contributing to increased viscosity. Thus, the increased trough viscosity noted in MZ-5 may result from the elevated amylose levels and the consequent formation of a gel. Moreover, MZ-15 displayed a significantly lower ($p > 0.05$) breakdown viscosity compared to MZ-5. This disparity could be attributed to the varying amylose content of the starch due to the increase in edible insect flour concentration. Furthermore, setback viscosity, a measure of the retrogradation tendency of flour gel during cooling, varied significantly ($p > 0.05$) from 781.67 to 1805 cP. Retrogradation occurs due to hydrogen bonding among starch molecules with hydroxyl and hydrogen acceptor sites.

Another important parameter, the pasting temperature, which indicates the onset of viscosity rise, ranged from 83.47 to 89.87 °C. Notably, the addition of insect flour significantly ($p > 0.05$) increased the pasting temperature. MZ-15 exhibited the highest pasting temperature among the insect-enriched flours. This phenomenon could be due to the larger particle size and edible insect protein content (Table 2) compared to the control (wheat flour). These results are similar to those Aleman et al. [29] reported in wheat flour chocolate-chip cookies enriched with cricket flour (*Acheta domesticus*). The incorporation of *M. subhylanus* flour resulted in a decrease in peak, final and setback viscosity. Moreover, Khuenpet et al. [36] also reported a decrease in peak and breakdown viscosity of

Fig. 1. Biscuits formulated with different amountsof M. *subhylanus* flours. S1 = 100% wheat flour, MZ 2 = 5%, MZ 3 = 10%, MZ 4 = 15%, MZ 5= 20%, *M. subhylanus* flour substitution.

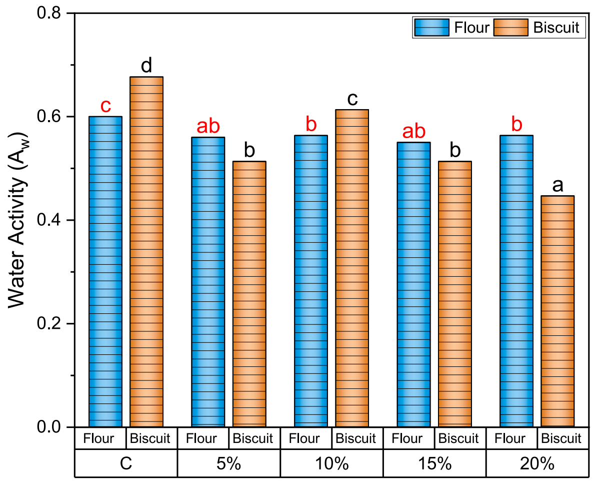

Fig. 2. Water activity of *M. subhylanus* composite flours and biscuits.

mealworm (*Tenebrio molitor*) enriched flours. To our knowledge, this study represents the pioneering exploration into the pasting characteristics of insect-enriched flours derived from *M. subhyalinus* sourced from South Africa and evaluates their viability for utilization in bakery settings.

3.3. Physicochemical properties

3.3.1. Colour

The colour of food is a crucial attribute that greatly impacts how it is perceived and accepted by consumers. This physicochemical property of food products can be easily evaluated, and the results are presented in Table 4. The Lightness (L*) of the composite flours demonstrated a notable decrease ($p < 0.05$) with an increase in the inclusion of edible insect flour. Notably, MZ-20 exhibited a significantly reduced luminance compared to the control sample, indicative of darker composite flours consequent to insect flour addition. Furthermore, the redness (a*) attribute significantly escalated ($p < 0.05$), reaching a peak of 1.98 in the composite flours. Total colour difference (ΔE) signifies the magnitude of colour change between the test and control samples [37], which was more pronounced as the concentration of the insect flour increased. This result confirms that the colour changes in the biscuits were noticeable enough for customers to notice them. Previous investigations have shown that a colour difference is observable when ΔE is greater than 3.0 (Table 4). In general, biscuits had significantly lower L* values, signalling their colour was darker than that of the same composite flours or control samples (Fig. 1). The lower L* values indicate that Maillard browning stemming from reactions between the amino group of proteins and the carbonyl group of sugars within the biscuits due to the heat applied during baking. Statistically significant ($p < 0.05$) higher b* values were found in the control samples compared to the enriched biscuits, indicating the yellowness of the control biscuits. The red colour indicated by the positive a* value was statistically significant ($p < 0.05$) in all biscuits enriched with edible insects, respectively (Table 4). These findings were anticipated as edible insect flours inherently possess darker hues compared to the wheat flour utilized in the formulation of these biscuits. Consequently, the incorporation of edible insect flours is

Table 5
Physical qualities of biscuits enriched with *M. subhylanus* composite flour.

Sample	Hardness (N)	Thickness (mm)	Weight (g)	Diameter (mm)	Spread ratio
Control	38.48 ± 13.16^a	3.58 ± 0.15^a	14.26 ± 2.21^b	38.37 ± 0.78^a	10.73 ± 0.30^a
MZ-5	50.02 ± 10.82^a	3.64 ± 0.23^a	14.26 ± 2.21^{ab}	46.40 ± 1.35^c	12.78 ± 0.51^b
MZ-10	34.81 ± 6.49^a	3.64 ± 0.41^a	14.71 ± 0.90^b	45.10 ± 2.23^c	12.48 ± 1.21^b
MZ-15	45.21 ± 1.18^a	3.61 ± 0.01^a	11.59 ± 0.22^a	42.74 ± 0.64^b	11.85 ± 0.16^{ab}
MZ-20	43.40 ± 6.55^a	4.35 ± 0.17^b	12.20 ± 1.15^{ab}	47.30 ± 0.44^c	10.89 ± 0.51^a

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different.

expected to impart a darker colour to the resulting products. Typically, the colour profile of a baked item is directly influenced by the pigmentation of its constituent raw materials. Analogous findings of colour intensification were noted by researchers in muffins fortified with *T. molitor* and *A. domesticus* powder [38], cookies enriched with *T. molitor* powder [39] and pasta enriched with cricket powder [40]. The highest colour difference in the biscuit samples was seen in sample MZ-20, which contained the most significant amount of edible insect flour. Comparable findings were reported by Zielińska & Pankiewicz [19] in their study on shortcake biscuits supplemented *with T. molitor* flour. The study by Pauter et al. [38] proposed that consumers associate darker bakery products with higher healthier benefits and greater fibre or whole grain content. Consequently, this colour shift could make consumers more interested in this particular variety of biscuits.

3.3.2. Water activity

One of the main elements that could influence microbial growth in food items is water activity. Water activity stands as a paramount factor influencing microbial proliferation within food products. The water activity of composite flours and biscuits is depicted in Fig. 2, and it can be observed that the water activity of the composite flours ranged from 0.55 to 0.60, and they were statistically significant ($p < 0.05$) differences between the control and the flours enriched with edible insect flour. Moreover, the water activity of the biscuits ranged from 0.44 to 0.67. After a 10 % addition of *M. subhylanus* insect flour, the water activity significantly decreased ($p < 0.05$) from 0.613 to 0.44 in MZ-15 and MZ-20, respectively. As stated by Ying et al. [41], the critical water activity threshold for food safety is 0.60. Beyond this point, microorganisms and moulds may proliferate, leading to chemical alterations in the food product. Therefore, the water activity of MZ-5, 15 and 20 ($a_w < 0.60$) is insufficient for the growth of food spoilage or pathogenic microbes like bacteria and fungi due to the inhibition of cell division activities at such low water activity range.

3.3.3. Physical characteristics of biscuits enriched with insect flour

The findings of the physical properties of the biscuits enriched with edible insect flour from *M. subhylanus* are shown in Table 5. Biscuit hardness is a crucial parameter for assessing its textural quality and it is the most vital aspect of biscuit texture. No significant differences ($p > 0.05$) were observed for the hardness and thickness of the biscuit. *M. subhylanus* flour, being rich in protein, can contribute to the formation of a strong protein network during dough formation and baking. This protein network provides structural support to the biscuits, influencing their hardness and thickness. Similarly, González et al. [42] observed that the textural hardness of bread products increased with the addition of insects such as *H. illucens, A. domestica* and *T. molitor* flours. MZ-15 had the lowest weight (11.59 ± 0.22), and it was significantly different ($p < 0.05$) compared to the control (14.26 ± 2.21). These findings indicate that biscuits enriched with *M. subhylanus* flour possess unique properties affecting the dough's consistency and thus, the final weight of the biscuits. All insect biscuits had a significantly higher diameter than the control (100 % wheat flour). In addition, this study evaluated the spread ratio of the insect-based biscuits. The spread ratio is a key parameter for assessing the leavening capability of bakery products. It is influenced by protein and fat content and is closely associated with the texture, bite, and overall mouthfeel of the biscuit. The spread ratio ranged from 10.73 to 12.78, where the control sample had the lowest spread ratio compared to enriched biscuits. In the case of MZ-15, the lower weight may be linked to its spread ratio, suggesting differences in the dough's ability to expand during baking. According to Obeta et al. [43], biscuits exhibiting a higher spread ratio are considered more preferable. A reduced spread ratio signifies heightened hydrophilicity of starch, resulting in increased thickness. The inclusion of insect flour in biscuit formulation has the potential to alter both the microstructure of the dough and the characteristics of the final baked product, warranting further investigation. These microstructural changes can influence the overall texture, hardness, and appearance of the biscuits. Therefore, insect composite flours can be used as novel functional ingredients in bakery applications. To the best of our knowledge, this is the first study to investigate the incorporation of *M. subhylanus* flour into wheat-based biscuits. Novel and innovative food options are preferred by consumers, and biscuits with distinctive physical qualities, such as those enhanced with insect flours from *M. subhylanus,* may meet their needs, making them more appealing to the market.

3.4. Microbial safety assessment of biscuits

A primary concern associated with the consumption of insects as food pertains to microbiological safety [44,45] According to reports, water and food-borne pathogens, chemical hazards and bacteria, including *Salmonella* spp, *Staphylococcus aureus*, and *Campylobacter* spp. are all carried by insects [6,46,47]. In this study, *Bacillus cereus*, total coliforms, *E. coli* and *Salmonella* spp were not detected (Table 6). The absence of Salmonella spp. in all batches of biscuits containing edible insect flour validates their susceptibility to heat treatment, resulting in biscuits that adhere to microbiological standards for food safety. The observed reduction in microbial

Table 6
Microbial properties of biscuits enriched with *M. subhylanus* composite flour.

Media	Microbes	Control (cfu/g)	MZ-5 (cfu/g)	MZ-10 (cfu/g)	MZ-15 (cfu/g)	MZ-20 (cfu/g)
NA	Aerobic mesophilic bacteria	1.0×10^{-2}	1.0×10^{-2}	1.0×10^{-2}	1.0×10^{-2}	1.0×10^{-2}
MCA	Total coliforms	n.d	n.d	n.d	n.d	n.d
EMB	*Escherichia coli*	n.d	n.d	n.d	n.d	n.d
SSA	Salmonella spp	n.d	n.d	n.d	n.d	n.d
MSA	*Staphylococcus aureus*	1.0×10^{-2}	1.0×10^{-2}	1.0×10^{-2}	1.0×10^{-2}	1.0×10^{-2}
Palcam Agar	*Listeria monocytogenes*	Negative	Negative	Negative	Negative	Negative
MYP	*Bacillus cereus*	n.d	n.d	n.d	n.d	n.d
PDA	Yeast and mould	4.0×10^{-2}	0.5×10^{-2}	0.5×10^{-2}	1.0×10^{-2}	1.0×10^{-2}

NA = Nutrient agar, MCA = MacConkey agar, EMB = Eosin methylene blue, SSA = Salmonella-Shigella agar, MSA = Mannitol salt agar, PDA = Potato dextrose agar.

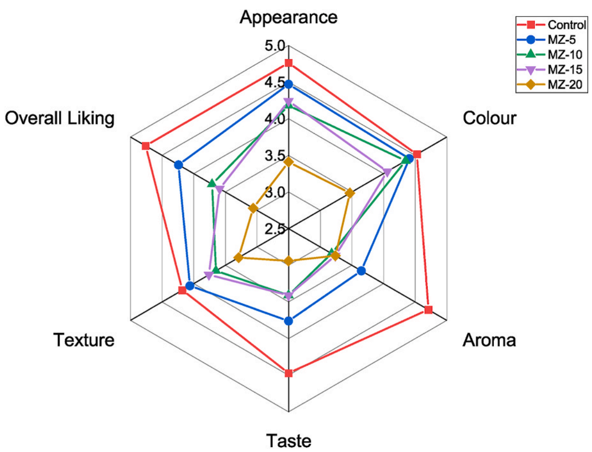

Fig. 3. Sensory evaluation properties of *M. subhylanus* edible insect biscuits.

counts could be attributed to the utilization of pre-treated insect-derived components, alongside meticulous adherence to stringent human and environmental hygiene protocols throughout the formulation of novel insect flour and production of the insect-enriched biscuits. The temperatures of 180 °C used during the 15-minute baking period were likely effective in suppressing most microorganisms. Simultaneously, rigorous compliance with post-baking hygiene guidelines combined with quick packaging into sterile polyethylene bags is expected to have successfully reduced the possibility of contamination from the surrounding air after baking. Furthermore, it has been shown that the biscuit matrix's naturally low pH and water activity levels prevent bacterial development and colonisation.

3.5. Sensory evaluation of biscuits enriched with M. subhylanus flour

The effect of *M. subhylanus* flour incorporation on the organoleptic properties (appearance, colour, aroma, texture and overall liking) of biscuits is exhibited in Fig. 3. The panellist perceived no significant differences ($p > 0.05$) in terms of the texture between the insect-enriched biscuits and the control, except for MZ-20. Moreover, there were no significant differences ($p > 0.05$) in the perceived taste of the samples with the control except for MZ-20. Appearance is an essential parameter in judging sensory attributes of any food due to its influence on acceptability. In terms of appearance, MZ-5 and MZ-15 biscuits were significantly different ($p < 0.05$) from MZ-20, with the latter having lower ratings or liking. MZ-20 might exhibit unique interactions with other ingredients in the formulation, influencing the overall texture. Moreover, the colour of the MZ-20 was rated as the least liked or preferred. MZ-20 was darker in colour, and these observations align with the results reported in section 3.1.2.1 under the discussion of the colour of the composite flours and biscuits. Aleman et al. [29] reported that the overall liking of chocolate chip cookies enriched with cricket (*A. domesticus*) decreased with an increase in concentration to 10 %. While MZ-20 is the highest in terms of protein content, it is the least liked when it comes to overall liking by the panellist. The presence of *M. subhylanus* flour, especially at higher concentrations, could introduce flavours that are unfamiliar or unappealing to the panellists. Unpleasant flavours can significantly impact overall liking, overshadowing the positive attributes related to protein content. This implies that the enrichment of biscuits with *M. subhylanus* flour is only acceptable at levels not exceeding 15 %. There were no significant differences ($p > 0.05$) in the overall acceptability of the biscuits between MZ-15 > MZ-10 and MZ-5, and they were similar to the other sensory attributes.

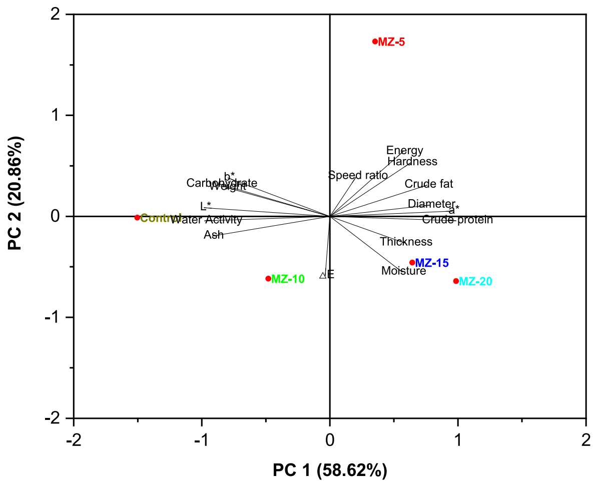

Fig. 4. Principal components analysis plot for nutritional, physicochemical and textural properties of *M. subhylanus* edible insect biscuits.

3.6. Principal component analysis explaining the variability in M. subhylanus proximate and textural properties

In this study, Principal component analysis (PCA) was utilized to reduce the data variability among biscuits enriched with *M. subhylanus* flour. PCA transforms the original data matrix into vectors of loadings and scores (tested parameters), generating new variables known as principal components. This technique was applied to analyze the nutritional (proximate analysis), physicochemical, and textural properties of the enriched biscuits. The results are presented through score and loading plots (Fig. 4).

To determine the principal components' contributions to overall variability, only eigenvalues greater than one were considered. This approach facilitates the interpretation of how *M. subhylanus* flour affects the physicochemical properties of the biscuits. Two main components were selected, explaining 79.48 % of the total variance across the 16 analysed variables. The first principal component (PC1) accounted for 58.62 % of the information about the tested products, while the second principal component (PC2) explained 20.86 % of the variance.

Each vector in the PCA represents a variable, with its size and direction indicating its impact on the principal components. The analysis revealed that MZ-10, MZ-15, and MZ-20 samples were located on opposite sides of PC1, with colour change, thickness, and moisture being the primary factors differentiating the enriched biscuits. A cluster of strongly positively correlated composition parameters (lightness, greenness/redness, water activity, ash, carbohydrates, and weight) had negative loadings on PC1. Conversely, parameters such as spread ratio, total energy, hardness, crude fat, diameter, crude protein, thickness, and moisture exhibited high positive loadings.

Thus, PCA provides valuable insights into the classification and discrimination of edible insect flours, as well as the relationships between the nutritional, colour, and textural properties of the biscuits.

4. Conclusion

This study was undertaken to establish the effect of incorporating edible insect flour from *M. subhylanus* on wheat-based composite flour and novel insect biscuits. The results of this study show that *M. subhylanus* insect flour is a good source of high nutritional value, particularly protein and carbohydrates. The addition of *M. subhylanus* insect flour to wheat flour results in a proportional increase in protein content in the end product, which can enhance the nutritional composition of food products such as biscuits. However, incorporating *M. subhylanus* insect flour may also cause a darker colouration in the composite flours and biscuits. This is attributed to Maillard browning, which occurs during baking due to reactions between protein amino groups and sugar carbonyl groups. The moisture content of the composite flours and biscuits enriched with insect flour can also significantly impact preservation, packaging, and transport convenience. In this study, the enrichment of biscuits with *M. subhylanus* flour is only acceptable at levels not exceeding 15 %. Overall, the results of the study suggest that *M. subhylanus* insect flour can be used as a novel food ingredient for food applications. Further research could investigate the shelf-life, sensory acceptability and structural properties of insect-enriched products and potential food allergenicity concerns associated with using insects as food ingredients.

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The data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Nthabeleng Vanqa: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis. **Vusi Vincent Mshayisa:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Investigation, Formal analysis, Conceptualization. **Moses Basitere:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Z.T. Hlongwane, R. Slotow, T.C. Munyai, Indigenous knowledge about consumption of edible insects in South Africa, Insects 12 (2021) 1–19, https://doi.org/10.3390/insects12010022.
[2] N. Vanqa, V.V. Mshayisa, M. Basitere, Proximate, physicochemical, techno-functional and antioxidant properties of three edible insect (gonimbrasia belina, hermetia illucens and Macrotermes subhylanus) flours, Foods 11 (2022) 976, https://doi.org/10.3390/foods11070976.
[3] V.B. Meyer-Rochow, C. Jung, Insects used as food and feed: isn't that what we all need? Foods 9 (2020) 1003, https://doi.org/10.3390/foods9081003.
[4] J.N. Kinyuru, S.O. Konyole, N. Roos, C.A. Onyango, V.O. Owino, B.O. Owuor, B.B. Estambale, H. Friis, J. Aagaard-Hansen, G.M. Kenji, Nutrient composition of four species of winged termites consumed in western Kenya, J. Food Compos. Anal. 30 (2013) 120–124, https://doi.org/10.1016/j.jfca.2013.02.008.
[5] B.A. Rumpold, O.K. Schlüter, Potential and challenges of insects as an innovative source for food and feed production, Innovat. Food Sci. Emerg. Technol. 17 (2013) 1–11, https://doi.org/10.1016/j.ifset.2012.11.005.
[6] A. Van Huis, Potential of insects as food and feed in assuring food security, Annu. Rev. Entomol. 58 (2013) 563–583, https://doi.org/10.1146/annurev-ento-120811-153704.
[7] V.V. Mshayisa, J. Van Wyk, B. Zozo, Nutritional, techno-functional and structural properties of black soldier fly (hermetia illucens) larvae flours and protein concentrates, Foods 11 (2022) 724, https://doi.org/10.3390/foods11050724.
[8] L. Yi, C.M.M. Lakemond, L.M.C. Sagis, V. Eisner-Schadler, A. Van Huis, M.A.J.S.V. Boekel, Extraction and characterisation of protein fractions from five insect species, Food Chem. 141 (2013) 3341–3348, https://doi.org/10.1016/j.foodchem.2013.05.115.
[9] D.A. Tzompa-Sosa, L. Yi, H.J.F. van Valenberg, M.A.J.S. van Boekel, C.M.M. Lakemond, Insect lipid profile: aqueous versus organic solvent-based extraction methods, Food Res. Int. 62 (2014) 1087–1094, https://doi.org/10.1016/j.foodres.2014.05.052.
[10] S. Costa, S. Pedro, H. Lourenço, I. Batista, B. Teixeira, N.M. Bandarra, D. Murta, R. Nunes, C. Pires, Evaluation of Tenebrio molitor larvae as an alternative food source, NFS J 21 (2020) 57–64, https://doi.org/10.1016/j.nfs.2020.10.001.
[11] S. Ghosh, C. Jung, V.B. Meyer-Rochow, Nutritional value and chemical composition of larvae, pupae, and adults of worker honey bee, Apis mellifera ligustica as a sustainable food source, J. Asia Pac. Entomol. 19 (2016) 487–495, https://doi.org/10.1016/j.aspen.2016.03.008.
[12] E. Zielińska, B. Baraniak, M. Karaś, K. Rybczyńska, A. Jakubczyk, Selected species of edible insects as a source of nutrient composition, Food Res. Int. 77 (2015) 460–466, https://doi.org/10.1016/j.foodres.2015.09.008.
[13] F.G. Hall, O.G. Jones, M.E. O'Haire, A.M. Liceaga, Functional properties of tropical banded cricket (*Gryllodes sigillatus*) protein hydrolysates, Food Chem. 224 (2017) 414–422, https://doi.org/10.1016/j.foodchem.2016.11.138.
[14] V.B. Meyer-Rochow, Meyer-rochow, Can insects help to ease the problem of world food shortage, Search 6 (1975) (1975) 261–262.
[15] M. Shelomi, Why we still don't eat insects: assessing entomophagy promotion through a diffusion of innovations framework, Trends Food Sci. Technol. 45 (2015) 311–318, https://doi.org/10.1016/j.tifs.2015.06.008.
[16] A. Lombardi, R. Vecchio, M. Borrello, F. Caracciolo, L. Cembalo, Willingness to pay for insect-based food: the role of information and carrier, Food Qual. Prefer. 72 (2019) 177–187, https://doi.org/10.1016/j.foodqual.2018.10.001.
[17] A. Gmuer, J. Nuessli Guth, C. Hartmann, M. Siegrist, Effects of the degree of processing of insect ingredients in snacks on expected emotional experiences and willingness to eat, Food Qual. Prefer. 54 (2016) 117–127, https://doi.org/10.1016/j.foodqual.2016.07.003.
[18] K. Emory, C. Liu, E. Woolf, J. Zhao, Y. Zhu, Willingness to consume insect-containing foods: a survey in the United States, Lwt 102 (2018) 100–105, https://doi.org/10.1016/j.lwt.2018.12.010.
[19] E. Zielińska, U. Pankiewicz, Characteristics of shortcake biscuits enriched with, Molecules 25 (2020).
[20] B. Çabuk, Influence of grasshopper (Locusta Migratoria) and mealworm (Tenebrio Molitor) powders on the quality characteristics of protein rich muffins: nutritional, physicochemical, textural and sensory aspects, J. Food Meas. Char. 15 (2021) 3862–3872, https://doi.org/10.1007/s11694-021-00967-x.
[21] L.M. de Oliveira, A.J. da Silva Lucas, C.L. Cadaval, M.S. Mellado, Bread enriched with flour from cinereous cockroach (Nauphoeta cinerea), Innovat. Food Sci. Emerg. Technol. 44 (2017) 30–35, https://doi.org/10.1016/j.ifset.2017.08.015.
[22] N. Koffi, B. Kouakou, G.K. Gildas, A. Thierry, K. N'golo, G. Dago, Quality characteristics of biscuits made from sorghum and defatted Macrotermes subhyalinus, Int. J. Biosci. 3 (2013) 58–69.
[23] R.H. Janssen, J.P. Vincken, L.A.M. Van Den Broek, V. Fogliano, C.M.M. Lakemond, Nitrogen-to-Protein conversion factors for three edible insects: Tenebrio molitor, Alphitobius diaperinus, and hermetia illucens, J. Agric. Food Chem. 65 (2017) 2275–2278, https://doi.org/10.1021/acs.jafc.7b00471.
[24] T. Farzana, S. Mohajan, Effect of incorporation of soy flour to wheat flour on nutritional and sensory quality of biscuits fortified with mushroom, Food Sci. Nutr. 3 (2015) 363–369, https://doi.org/10.1002/fsn3.228.
[25] S.M.M. Duque, S.Y. Leong, D. Agyei, J. Singh, N. Larsen, I. Oey, Understanding the impact of Pulsed Electric Fields treatment on the thermal and pasting properties of raw and thermally processed oat flours, Food Res. Int. 129 (2020) 108839, https://doi.org/10.1016/j.foodres.2019.108839.
[26] M. Krystyjan, D. Gumul, R. Ziobro, A. Korus, The fortification of biscuits with bee pollen and its effect on physicochemical and antioxidant properties in biscuits, LWT (Lebensm.-Wiss. & Technol.) 63 (2015) 640–646, https://doi.org/10.1016/j.lwt.2015.03.075.
[27] M. Suriya, R. Rajput, C.K. Reddy, S. Haripriya, M. Bashir, Functional and physicochemical characteristics of cookies prepared from Amorphophallus paeoniifolius flour, J. Food Sci. Technol. 54 (2017) 2156–2165, https://doi.org/10.1007/s13197-017-2656-y.

[28] M. Kaur, V. Singh, R. Kaur, Effect of partial replacement of wheat flour with varying levels of flaxseed flour on physicochemical, antioxidant and sensory characteristics of cookies, Bioact. Carbohydrates Diet. Fibre. 9 (2017) 14–20, https://doi.org/10.1016/j.bcdf.2016.12.002.

[29] R.S. Aleman, J. Marcia, S.K. Pournaki, I. Borrás-Linares, J. Lozano-Sanchez, I.M. Fernandez, Formulation of protein-rich chocolate chip cookies using cricket (Acheta domesticus) powder, Foods 11 (2022) 3275, https://doi.org/10.3390/foods11203275.

[30] Su-Young Hwang, Choi Soo Keun, Quality characteristics of muffins containing mealworm(Tenebrio molitor), culin, Sci. Hosp. Res. 21 (2015) 104–115, https://doi.org/10.20878/cshr.2015.21.3.009009009.

[31] O.A. Akande, O.O. Falade, A.A. Badejo, I. Adekoya, Assessment of mulberry silkworm pupae and african palm weevil larvae as alternative protein sources in snack fillings, Heliyon 6 (2020) e03754, https://doi.org/10.1016/j.heliyon.2020.e03754.

[32] N.C. Maiyo, F.M. Khamis, M.W. Okoth, G.O. Abong, S. Subramanian, J.P. Egonyu, C. Xavier, S. Ekesi, E.R. Omuse, D. Nakimbugwe, G. Ssepuuya, C.J. Ghemoh, C.M. Tanga, Nutritional quality of four novel porridge products blended with edible cricket (scapsipedus icipe) meal for food, Foods 11 (2022), https://doi.org/10.3390/foods11071047.

[33] A. Mafu, S. Ketnawa, S. Phongthai, R. Schönlechner, S. Rawdkuen, Whole wheat bread enriched with cricket powder as an alternative protein, Foods 11 (2022), https://doi.org/10.3390/foods11142142.

[34] L. Li, M. Zhang, B. Bhandari, Influence of drying methods on some physicochemical, functional and pasting properties of Chinese yam flour, Lwt 111 (2019) 182–189, https://doi.org/10.1016/j.lwt.2019.05.034.

[35] I.A. Wani, D.S. Sogi, A.A. Wani, B.S. Gill, Physico-chemical and functional properties of flours from Indian kidney bean (*Phaseolus vulgaris* L.) cultivars, LWT–Food Sci. Technol. 53 (2013) 278–284, https://doi.org/10.1016/j.lwt.2013.02.006.

[36] K. Khuenpet, C. Pakasap, S. Vatthanakul, S. Kitthawee, Effect of larval-stage mealworm (Tenebrio molitor) powder on qualities of bread, Int. J. Agric. Technol. 16 (2020) 283–296.

[37] O.O. Onipe, D. Beswa, V.A. Jideani, A.I.O. Jideani, Development of a low-fat, high-fibre snack: effect of bran particle sizes and processing conditions, Heliyon 5 (2019) e01364, https://doi.org/10.1016/j.heliyon.2019.e01364.

[38] P. Pauter, M. Różańska, P. Wiza, S. Dworczak, N. Grobelna, P. Sarbak, P. Kowalczewski, Effects of the replacement of wheat flour with cricket powder on the characteristics of muffins, Acta Sci. Pol. Technol. Aliment. 17 (2018) 227–233, https://doi.org/10.17306/J.AFS.0570.

[39] K.-T. Min, M.-S. Kang, M.-J. Kim, S.-H. Lee, J. Han, A.-J. Kim, Manufacture and quality evaluation of cookies prepared with mealworm (Tenebrio molitor) powder, Korean J, Food Nutr. 29 (2016) 12–18, https://doi.org/10.9799/ksfan.2016.29.1.012.

[40] A. Duda, J. Adamczak, P. Chelminska, J. Juszkiewicz, P. Kowalczewski, Quality and nutritional/textural properties of durum wheat pasta enriched with cricket powder, Foods 8 (2019) 1–10, https://doi.org/10.3390/foods8020046.

[41] L.Y. Ying, L.H. Ying, N.S. Sofian-Seng, W.A.W. Mustapha, N.S.M. Razali, Physicochemical characteristics and microbiological quality of silkworm (Bombyx mori) larval and pupae powder: comparative study, Sains Malays. 51 (2022) 547–558, https://doi.org/10.17576/jsm-2022-5102-18.

[42] C.M. González, R. Garzón, C.M. Rosell, Insects as ingredients for bakery goods. A comparison study of H. illucens, A. domestica and T. molitor flours, Innovat. Food Sci. Emerg. Technol. 51 (2019) 205–210, https://doi.org/10.1016/j.ifset.2018.03.021.

[43] N.A. Obeta, G.C. Azike, I.S. Asogwa, Nutritional composition, physical characteristics, and sensory attributes of wheat based biscuits enriched with bullet pear (Canarium schweinfurthii) fruit pulp, J. Food Sci. Technol. 57 (2020) 832–839, https://doi.org/10.1007/s13197-019-04114-9.

[44] R.C. Megido, S. Desmedt, C. Blecker, F. Béra, É. Haubruge, T. Alabi, F. Francis, Microbiological load of edible insects found in Belgium, Insects 8 (2017) 1–8, https://doi.org/10.3390/insects8010012.

[45] D. Vandeweyer, S. Lenaerts, A. Callens, L. Van Campenhout, Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (Tenebrio molitor), Food Control 71 (2017) 311–314, https://doi.org/10.1016/j.foodcont.2016.07.011.

[46] M. Van-der Spiegel, M.Y. Noordam, H.J. van der Fels-Klerx, Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production, Compr. Rev. Food Sci. Food Saf. 12 (2013) 662–678, https://doi.org/10.1111/1541-4337.12032.

[47] B.A. Rumpold, O.K. Schlüter, Nutritional composition and safety aspects of edible insects, Mol. Nutr. Food Res. 57 (2013) 802–823, https://doi.org/10.1002/mnfr.201200735.