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Unravelling the nutritional and health benefits of wheat bread enriched with meat powder from laying hen fed diet with insect (*Hermetia illucens*) meal

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ABSTRACT

Wheat bread is among stable foods that are nutritionally imbalanced, thus enrichment is crucial. We evaluated the nutritional impact of high-valued wheat bread enriched with varying levels of meat powder from hen fed diet with insect (*Hermetia illucens*)-based meal. Crude protein and ash in bread increased with increasing inclusion of meat powder. Limiting amino acids like lysine and threonine in enriched bread products increased by 3.0–4.5 and 1.8–3.1-folds, respectively. Omega 3 fatty acids were significantly enhanced in bread fortified with meat powder. Vitamins (retinol, nicotinic acid, and pantothenic acid) were significantly increased in supplemented bread products. Iron, zinc, and calcium increased by 1.1, 1.2 and 3.0-folds in enriched bread with 30% meat powder. Colour, flavour and overall acceptability of breads prepared with 25 and 30% meat powder were highly ranked. Our findings demonstrate that meat powder (i.e., from hen fed insect-based diets) enrichment would provide added health and nutritional benefits to bread products without having adverse effects on any functional or sensory properties. Thus, this could be a novel strategy and trend for improving bread products, that might generate increasing demand for a healthier consumer-oriented lifestyle.

1. Introduction

Production of traditional feed, especially fish meals and soy meal, have been linked to adverse ecological pressures characterized by overexploitation of natural resources [1]. For instance, massive production of soy bean has been associated with deforestation, high water consumption, extensive pesticides and fertilizer application and introduction of transgenic varieties which are a threat to the local biodiversities [2]. On the other hand, fish meal production coupled with deteriorating marine environment and fish stripping have negatively impacted the abundance of small pelagic forage fish [3]. The prolonged scarcity of the requisite resources for production of the conventional protein has resulted in exhorbitant prices amidst escalating demand further constraining production of

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animal proteins. In this scenario, a sustainable alternative protein sources for animal husbandry is inevitable.

The recent adoption of insects has been a game changer in the poultry industry as the industry has been grappling with a major challenge of obtaining feeds that contain all the essential nutrients necessary for rapid growth to productive maturity within a short period [4]. Utilization of black soldier fly larvae (BSFL) as a sustainable feed with abundant levels of protein, amino acids; lysine, methionine, cysteine, arginine and tryptophan, minerals; calcium and phosphorous and high energy [5,6]. The BSFL can cheaply be reared on organic wastes of agricultural and household origin which underlines their cheap production with significant contribution to environmental depollution. Several researchers have unanimously attested to the satisfactory carcass weight, breast weight, breast meat yield, and dressing percentage from BFL-fed broilers being comparable to those from birds fed on conventional diets [3,4,7]. On the other hand, dietary BSFL inclusion has been reported to enhance the breast meat saturated and monounsaturated fatty acids, amino acids; aspartate, glutamate, alanine, serine, tyrosine, and threonine and minerals; calcium and potassium contents as compared to soya bean diets [4].

Layers fed on BSFL guarantee a short generation time prior to egg laying, express full genetic egg laying potential and increased dressed carcass weights than those fed on fish meal [8]. However, upon achieving their full potential, the birds depreciate in value and are referred as spent hens which comprise the second highest cost in the egg production farms after the cost of feeds. Recently, the layer industry has experienced prodigious growth in the number of spent hens [9] with the farmers increasingly experiencing difficulty in selling the spent hens with reasonable prices resulting into minimal profit margins [10]. Furthermore, these birds have low weight and low carcass yield, less juicy and tough meat with poor functional properties due to increased collagen content and cross linkages, requiring more energy to cook. These properties have further reduced their utilization in whole meat food which have negatively affected their market value. With the widespreading adoption of novel cheap feeds such as BSFL which confer improved meat qualities and nutritional advantages to poultry over conventional sources, discarding such valuable food products will not only amount to economic loss but an untapped opportunity for enhanced food circulation to combat food insecurity. To ensure maximum returns to farmers, there is need for diversification of end use of these hens into more profitable forms at the end of production [11].

Interestingly, the nutritional profile of spent hen meat mirrors that of broilers. It has high unsaturated fats (over 67% of total FA), less saturated fats and less cholesterol which is good for human health [12]. Thus, it is a valuable source of nutrients that cannot remain to be under-exploited amidst escalating need for nutritious food by the spiraling global population. In developed countries, it has been utilized in processing value added/convenience products [13] such as sausages, chicken soup, traditional delicacy recipes and animal feeds to mask the unwanted characteristics featuring in the final product [11]. In developing countries such as Kenya, there is need to integrate the IBM fed-spent hen products into cereal-based products such as bread with inferior nutritional attributes but consumed by a wider populace.

Among wheat products, bread has the highest consumption level [14] regarded as an important staple food worldwide [15]. It is preferred by urban and some rural consumers of all socio-economic classes because it is a ready to eat food, has good taste/eating quality, inexpensive and has no socio-cultural and religious barriers linked to its consumption [16]. In Kenya, the demand, consumption and importance of bread in diets is on the rise [17]. Even though different types of bread exist, white bread is the most consumed bread type due to its appealing sensory characteristics [18]. Its main ingredients is refined hard wheat flour [19] which is low in protein, fibre, vitamins, minerals, fatty acids and essential amino acids particularly threonine, tryptophan and lysine [6] hence, its termed as nutritionally imbalanced food item [20]. Such imbalanced diets have been reported to potentially contribute to malnutrition in the world [21]. Interestingly, white bread has proved to be a good carrier of functional ingredient and it is among the best vehicles for food fortification/enrichment [22] in attempts to gap malnutrition [23] globally.

Previously, wheat bread has been fortified/enriched with legumes [24], fruits and vegetables [25], herbs [26], roots and tubers [27], grains [28], insects [29–32], beef [33], fish [34–37], mushroom [6] and chicken meat [38,39] in attempts to revamp the nutritional status. These studies have reported appreciable levels of proteins and other key nutrients in the end products, critical for human nutritional improvement. In this respect, this study focuses on enriching and formulating bread products with meat powder from hen fed insect-based feed using standard and acceptable methods that can be applied locally and industrially in white bread production. Subsequently, the white bread products were assessed for the first time to unravel their proximate composition, minerals, vitamins, amino acids and fatty acids profiles *vis a vis* the conventionally formulated bread.

2. Materials and methods

2.1. Raw materials and ingredients

Spent hens were acquired and processed into powder according to descriptions by Makokha et al. [40]. Briefly, six spent hens fed on a blend of 50% black fly larvae meal and 50% fish meal for 80 weeks at the Poultry Research Unit in the Non-Ruminant Research Institute of the Kenya Agricultural and Livestock Research Organization (KALRO) located in Naivasha, Kenya (0°43′ 12.9″ S 36°25′ 42.7″ E) were randomly selected and slaughtered. The slaughtered chicken were defeathered, eviscerated and thigh, drum stick and breast isolated. The isolated parts were skinned, deboned, minced and boiled at 15 psi for 30 min. Cooked meat were then oven-dried at 60 °C for 24 h, milled and sieved through 500 μm screen to yield fine powders. The meat powders were packaged into polyethylene sample storage bags and temporarily frozen-stored at −20 °C for future studies. The baking ingredients; all-purpose fortified white wheat flour (brand – EXE), yeast, sugar, shortening and salt were purchased from a local supermarket.

2.2. Bread formulation and baking

Spent hen meat powder (SHMP) was used to substitute wheat flour in the ratios (wheat: SHMP w/w) of 800:200 (B_{20}), 750:250 (B_{25}), 700:300 (B_{30}) and 1000:0 SHMP (B_0) representing the control, resulting into four experimental variants. The straight dough method was used for bread making as illustrated by de Oliveira et al. [41]. Briefly, the dry ingredients: wheat flour for control or wheat flour-chicken meat powder blend (59.0%), yeast (0.9%), sugar (2.4%) and salt (1.2%) were whisked in a mixing bowl, transferred into a kitchen mixer (BJY-BM10, Berjaya, Malaysia) and mixed for 4 min on low speed followed by addition of water (35.4%) and shortening (1.2%). It was then mixed at full speed for 10 min to yield a consistent dough. Afterwards, the doughs (250 g) were molded in greased aluminum bread mold trays, covered in pans and placed in an oven at 30 °C for 95 min to ferment. The doughs were then transferred into a 200 °C preheated oven (BISTROT 665; BestFor®, Ferrara, Italy) and baked for 20 min. This process was repeated thrice for every treatment to give twelve experimental units. The breads (Fig. 1) were allowed to cool at room temperature for 5 min, and apportioned into two batches. First batch was immediately subjected to microbial assesement and the sensory tests while second batch was packed in sterile plastic bags (Zip loc bags, SC Johnson brand) and temporarily stored at −10 °C for later analysis.

2.3. Proximate determinations

Proximate compositions of breads were determined according to Association of Official Analytical Chemists method [42]. Briefly, determination of moisture composition was done by drying the breads in a forced draft air oven (WTB binder, Tuttlingen, Germany) at 135 °C for 2 h. Kjeldahl method was used to determine nitrogen content in an automatic Kjeldahl analyzer (Velp UDK 159, Velp Scientifica, Europe) and a conversion factor of 6.25 applied to get the crude protein content. The ash composition was estimated by sample combustion in a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) at 600 °C for 3h. Crude fat was determined following the Randall Technique (modified Soxhlet extraction method) using petroleum ether as an extraction solvent in a Soxhlet extractor (Velp SER 148, Velp Scientifica, Europe). Carbohydrate content was estimated by subtracting the fat, protein, ash and moisture content from 100%. All parameters were conducted in triplicate and results presented on dry mater basis.

2.4. Amino acids analysis

The amino acids were determined according to methods described by Cheseto et al. [43]. Into a 5 mL micro-reaction vial, 100 mg of bread was mixed with 1.5 mL of 6 N HCl and the contents hydrolyzed at 110 °C for 24 h under nitrogen. The hydrolysates were then dried *in vacuo* and reconstituted in 1 mL of 0.01% formic acid/acetonitrile (95:5) followed by a 30 s-vortexing, 30 min-sonication and 10 min-centrifugation at 14000 rpm. The supernatant was filtered through 0.20 μm membrane filter and analyzed (0.2 μL) using UPLC-MS/MS (Waters XEVO TQ-S, Waters Technologies, USA). Chromatographic separation was performed on a ACQUITY UPLC I-class system (Waters Corp., Milford, MA) fitted with an ACQUITY UPLC BEH C18 column (2.1 mm X 150 mm, 1.7 μm particle size; Waters Corp., Wexford, Ireland, oven temp 45 °C). The autosampler tray was cooled to 5 °C. The mobile phase contained (A) water and (B) methanol (solvent B) both acidified with formic acid (0.01%). MassLynx version 4.1 SCN 712 (Waters) was used to acquire data. The amino acids were identified by comparing mass spectrometric data, retention time, and co-injection of the hydrolysate with those

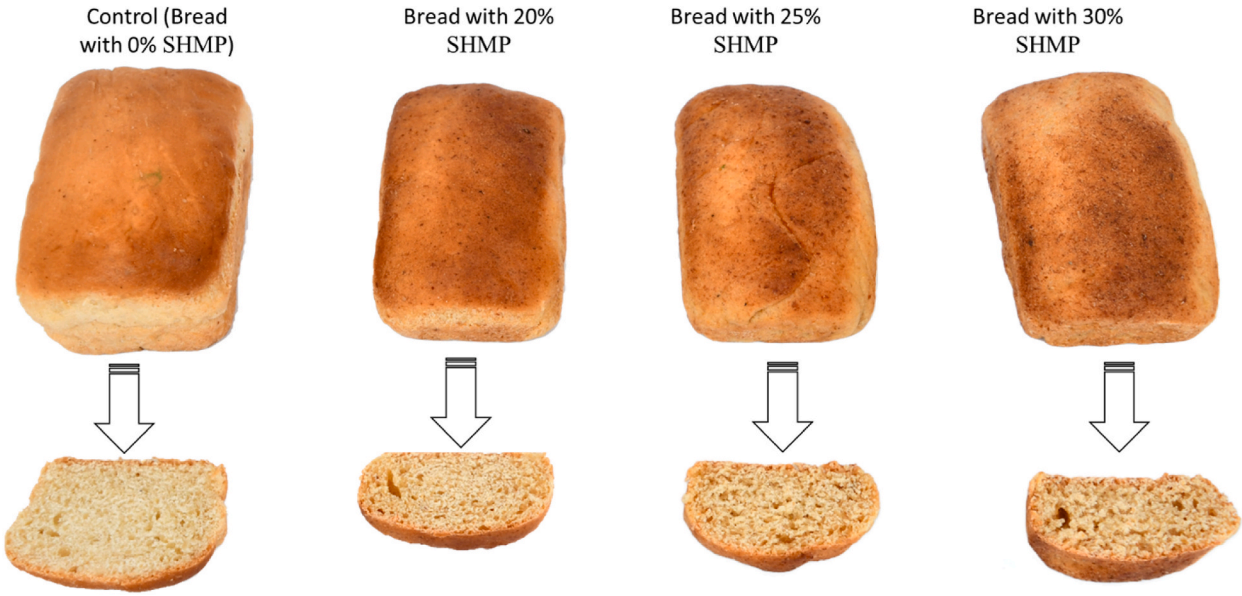

Fig. 1. Wheat bread without and with spent hen meat powder (SHMP) derived from laying hen fed insect-based diets.

of an authentic standard mixture of amino acids. Amino acid standard (Sigma-Aldrich, Chemie GmbH, Munich, Germany) was also analyzed by the UPLC-MS/MS for external quantification of each amino acid present. This was done in triplicates using different samples batch. Asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, during hydrolysis thus, the amounts of these acids were determined as sum of those respective components [44].

2.5. Fatty acid analysis of breads

Bread samples (100 mg) were methylated into different fatty acid methyl esters (FAMEs). Upon quenching the methylation process using 100 μL of distilled water, the resulting FAMEs were extracted using 1 mL of gas chromatography (GC)-grade hexane, centrifuged at 14000 rpm for 20 min and the supernatant dried over anhydrous sodium sulphate. Approximately 1.0 μL of the supernatant was analyzed by GC–MS on a 7890A gas chromatograph linked to a 5975C mass selective detector (Agilent Technologies Inc.,Santa Clara, CA, USA). The GC was fitted with a (5%-phenyl)-methylpolysiloxane (HP5 MS) low bleed capillary column (30 m × 0.25 mm i.d., 0.25 μm; J&W, Folsom, CA, USA). The instrument conditions, quantification of the FAMEs and data acquisition were in consonance with the descriptions by Cheseto et al. [45].

2.6. Determination of water-soluble vitamins

Water-soluble vitamins were determined according to Thermo Fisher Scientific [46]. Briefly, 100 mg of each sample was mixed with 25 mL of distilled water in 50 mL falcon tubes, sonicated for 15 min and the solution filtered through 0.2 μm filters into UPLC vials. The chromatographic analysis was performed on a Liquid chromatographic system with Diode Array Detector (LC-30AC with Nexera column oven CTO-30A, Shimadzu, Tokyo, Japan) equipped with a Phenomenex C18 Column Synergi 100 × 3.00 mm, 2.6 μm polar (Phenomenex, Torrance, CA, USA) at 30 °C. Stock solutions (1.0 mg/mL) of individual vitamins (except for vitamin B2 and vitamin B9 which were dissolved in 5 mM potassium hydroxide and 20 mM potassium hydrogen carbonate respectively) were serially diluted by dissolving in distilled water an later mixed together to yield calibration standards of concentrations 2, 5, 10 and 15 μg/mL. The chromatographic analysis was achieved on a Nexera Liquid chromatograph LC-30AC with Nexera column oven CTO-30A, fitted with Phenomenex Synergi (2.6*u*m polar C18– 100 mm × 3.00 mm) column and a diode array detector. The column oven temperature and sample flowrate was maintained at 30 °C and 0.4 mL/min. The column flushing solution was distilled water and the LC was programmed to run for 12min. The mobile phase comprised of gradient solutions: A (25 mM phosphate buffer) and B (7:3v/v Acetonitrile-Mobile phase A). The analysis was repeated three times.

2.7. Determination of fat-soluble vitamins

Fat soluble vitamins were determined according to the methods described by Hosotani and Kitagawa [47] and Bhatnagar-Panwar et al. [48]. Samples (0.5 g) were mixed with 6 mL ethanol containing 0.1% BHT in 25 mL tubes and homogenized for 1 min. Exactly 120 μL of 80% (w/v) potassium hydroxide was added, then the contents vortexed and incubated in a water bath at 85 °C for 5 min. Upon ice-cooling, 4 mL of deionized water was added into each tube and the contents vortexed. Hexane (5 mL) was then added into each tube to extract fat soluble vitamins and the contents vortexed again, centrifuged at 3000 rpm for 5 min and the supernatants transferred into centrifuge tubes. The pellets were re-extracted twice using hexane into separate test tubes and the upper phases subsequently pooled. The extracts were dried under nitrogen and the residue reconstituted in 1 mL of methanol: tetrahydrofuran (85:15 v/v), vortexed, sonicated for 30 s and transferred (0.8 mL) into HPLC vials. A reverse-phase HPLC (Shimadzu Nexera UPLC system) linked to SPD -M2A detector analyzed the sample analysis (10 μL) at a flow rate of 0.4 mL/min. The mobile phase comprised methanol/*tert*-butyl methyl ether/water (85:12:3, v/v/v, with 1.5% ammonium acetate in the water) and methanol/*tert*-butyl methyl ether/water (8:90:2, v/v/v, with 1% ammonium acetate in the water). The analysis was repeated three times.

2.8. Mineral analysis of breads

Mineral profiling was performed following descriptions by Makokha et al. [40]. Bread samples (0.5 g) were wet-ashed using 8 mL of concentrated nitric acid (VWR Chemicals, Fontenay-sous-Bois, France) and 2 mL of 30% w/w hydrogen peroxide (Sigma- Aldrich, USA) on a block digester (BD50/BD28, Seal Analytical Limited, US) programmed as follows; 75 °C for 30 min, 120 °C for 20 min, 180 °C for 20 min and 200 °C for 10 min. Upon cooling, the digests were carefully transferred to 50 mL Falcon tubes, topped up to the mark with 2% nitric acid and analyzed by Inductively coupled plasma optical emission spectroscopy (ICP-OES) (optima 2100™ DV ICP-OES, PerkinElmer Massachusetts, United states). A standard, CatNo.43843 (Sigma-Aldrich, USA), serially diluted using 2% nitric acid to obtain calibration standards of 400, 800, 2000 and 4000 μg/L, was also analyzed by the ICP-OES to yield standards curves for external quantification of the mineral elements. PerkinElmer Winlab 32 software (PerkinElmer, USA) was used for the external standard calibration and data acquisition. The analysis was done in triplicates.

2.9. Microbial assesment

Ten grams of sample were homogenized in a stomacher bag (Bagmixer 400W, Interscience, St. Nom, France) with 90 mL of sterile peptone water (0.85% (wt/vol) NaCl, 0.1% (wt/vol) (OXOID LP0034) and 8.5 g/L NaCl) for 3 min under aseptic conditions. A 10-fold serial dilution then ensued by transferring 1 mL of the penultimate dilution to 9 mL of sterile diluents. One-milliliter of an appropriate

dilution was placed on sterile media in petri-dishes for enumeration of the different microorganisms. Total viable counts (TVC), *Staphylococcus aureus* (selectively pre-enriched on tetrathionate broth at 35 °C for 24 h) and coliforms were determined on plate count agar (PCA- Oxoid CM0463), Baird Parker agar (Oxoid CM1127) and Violet Red Bile agar (VRBA), respectively incubated at 35 °C for 48 h. Yeast and moulds were cultured on potato dextrose agar (Oxoid Ltd., United Kingdom) and incubated at 25 °C for 5 days. *Salmonella* spp were pre-enriched on sterile tetrathionate broth media (35 °C for 24 h) before streaking on Salmonella-shigella agar and incubating at 35 °C for 24 h. *Eschechia coli* was assessed on Sorbitol MacConkey Agar incubated at 37 °C for 24 h. All the microbial assays were done in triplicates.

2.10. Sensory evaluation

Sensory attributes of the bread samples were performed according to Haber et al. [31] using 60 naive panellists selected randomly among postgraduate students and staff of International Centre of Insect Physiology and Ecology, Nairobi, Kenya. The panelists were given instructions, guidelines and objectives of the study before commencement according to the institution's ethical requirement. Colour, flavour, mouthfeel, texture and overall acceptability were evaluated on a five-point hedonic scale, in which 1 represented 'dislike very much' and 5 'like very much'. The samples were then assigned random codes and served to each panellist in identical containers at individual booths under room temperature conditions. Drinking water was also provided for panellists to clean their mouths before and after tasting each bread sample.

2.11. Statistical analysis

All the statistical analyses were conducted using R Studio version 1.3.1093–1 [49] for windows was used for statistical analysis. The data was subjected to normality test ($p \geq 0.05$ – Data is normal distributed) using Shapiro –Wilk test. One-way Analysis of Variance (ANOVA) was performed to test the effect of substituting white wheat flour with SHMP on proximate, minerals, vitamins, amino acids, fatty acids composition and sensory attribute scores of resultant breads. The differences in the treatment means were identified by Tukey's multiple comparison test at $\alpha = 0.05$.

3. Results and discussion

3.1. Proximate composition of bread products enriched with bread

Moisture, crude protein, crude ash, crude fat and carbohydrate content of breads were significantly different ($p < 0.05$) across the breads (Table 1). Moisture content of bread increased by 1.1 folds between bread without SHMP and bread containing 30% SHMP. The moisture content of bread directly corresponds to the amount of water absorbed during mixing of the dough. The higher moisture content of the chicken powder enriched breads may be attributable to the presence of increased levels of water-binding substances such as protein and fibre mainly consisting of more engaging hydrophilic groups [50–53]. In this study, chicken meat powder-breads expressed significantly higher protein content relative to the control breads, diluting the significant effect of hydrophobicity of >35% amino acids in wheat gluten [54] hence accounting for the disparity in moisture contents. Similar observation was made in other studies involving wheat bread enrichment with spent hen meat powder [55], chicken meat powder [38], cheese [56], grasshopper powder [31] and strip loin beef powder [33]. In contrast, Monteiro et al. [57] reported decrease in moisture content with increasing level of tilapia waste flour in wheat bread, possibly due to the dominating hydrophobic components of the formulation mix.

The crude protein, crude ash and crude fat increased by 2.0–2.4, 1.6–2.0, and 1.3–1.7 folds in chicken meat powder-breads, respectively whereas carbohydrates reduced by 0.7 folds compared to the reference bread (Table 1). Further, a progressive increase in these proximate components correlated with the increasing levels of the incorporated chicken powders. This signifies that chicken meat powder-breads reflected the amounts of protein, ash and fat in the varying levels of chicken meat powder incorporated. A similar phenomenon manifested in breads enriched with house cricket powder [58,59], *Tenebrio molitor* powder [60] and lupin [61], linking the elevated proximate parameters to the added ingredients. In the present study, chemical characterization indicated that chicken powder contains excellent levels of protein (86.5%), ash (7.2%) and fat (8.2%) (Table S1). These findings therefore suggest that enrichment of bread with insect-based meal (IBM)-fed chicken meat powder depicts nutritional relevance pertinent to human

Table 1
Proximate composition (%) of enriched bread on dry mater basis.

Parameter	B_0	B_{20}	B_{25}	B_{30}	$F_{(3,8)}$	P-value
Moisture	36.5 ± 0.50^{a}	38.8 ± 0.29^{b}	40.0 ± 1.00^{b}	40.0 ± 0.50^{b}	20.6	0.001
Crude protein	12.7 ± 0.39^{a}	25.1 ± 0.30^{b}	27.3 ± 0.27^{c}	31.0 ± 0.97^{d}	603.5	0.001
Crude ash	3.5 ± 0.38^{a}	5.7 ± 0.08^{b}	6.1 ± 0.39^{bc}	6.9 ± 0.40^{c}	51.2	0.001
Crude fat	3.9 ± 0.03^{a}	4.9 ± 0.02^{b}	5.8 ± 0.10^{c}	6.7 ± 0.06^{d}	1186.6	0.001
Crude fibre	0.8 ± 0.01	0.8 ± 0.08	0.9 ± 0.04	0.9 ± 0.05	2.3	ns
Carbohydrate	79.0 ± 0.67^{d}	63.5 ± 0.30^{c}	59.9 ± 0.18^{b}	54.4 ± 0.65^{a}	1333.3	0.001

Values are presented as means ± SD of triplicate determinations. Means followed by similar letters are not significantly different at $p < 0.05$. Breads made from B_0: bread 1000g white wheat flour (Control); B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP; ns: not significant.

nutrition. Consumption of these breads can easily contribute to the achievement of protein Recommended Dietary Allowance (RDA) [62] and satisfy the functional body requirements such as growth, health and growth improvement [59]. The low carbohydrate levels in the chicken meat powder-breads is indicative of the dilution effects of the low carbohydrate chicken ingredients since, wheat flour, the key ingredient in bread-baking, comprises 50–80% of carbohydrates [39,63]. Such observation was replicated by other researchers enriching wheat breads with low carbohydrate ingredients such as fish flour [34,37] and mushroom powder [19].

3.2. Amino acid profile

Blending white wheat flour with spent hen meat powder caused significant variations ($p < 0.05$) in the levels of all the amino acids examined (Table 2). The concentration of individual amino acid in bread increased with increasing levels of meat powder inclusion, with histidine, lysine and threonine recording the highest margins of 1.5–3.3, 3.0–4.5 and 1.8–3.1 folds, respectively for the essential amino acids, and glycine, alanine and arginine registering 1.4–1.8, 1.4–2.2- and 2.0–2.7-folds increment, respectively for the non-essential amino acids. This trend demonstrates the amino acids quality and superiority of the substrate relative to the reference breads, purely made of wheat. Such a tendency also emerged in other studies where wheat bread was formulated with quality protein ingredients [32,64]. Wheat flour is deficient in certain essential amino acids necessitating enrichment to revamp its nutritional quality [64]. Leucine and isoleucine were the most dominant essential amino acids whereas proline and glutamic acid were the predominant non-essential amino acids in all the bread types. The amino acids concentrations in the enriched breads appeared to reflect the levels in the chicken meat powder used in the formulations. For instance, escalated leucine levels in the breads may have been derived from chicken powder utilized for enrichment since, it has been reported the most abundant essential amino acid in high protein animal products [65]. The abundance of glutamic acid and proline in wheat flours and breads has previously been reported elsewhere [32]. Notably, despite the significant differences in glutamic and aspartic levels between control breads and those enriched with chicken powder, no significant variations were discernible in the latter. It can therefore be postulated that some of these amino acids are utilized by yeast fermenters or thermo-degraded during baking [36].The marked increase in the levels of certain amino acids especially alanine and serine may also be hypothesized to rise from hydrolytic breakdown of peptides by yeast fermenters and activated flour proteases in the doughs into free amino acids [36,60]. Some of these free amino acids, particularly lysine are reactants in Maillard reactions causing the browning of bread crumps and yielding aromatic compounds to the detriment of their biological value [36]. Therefore, controlled fermentation and cooking parameters tenable to the biological activity of such essential amino acids should be considered. However, in this study, lysine levels rose steadily with increasing levels of chicken powder in the baked product. This may be due to delayed denaturation of lysine as result of hydrophobic proteins interaction in the formulation mix [66]. Further, the supplemental effects of the other amino acids as reactants in the browning reactions may have limited their excessive utilization. In this study, the limiting amino acids in cereals, lysine and threonine, recorded 3.0–4.5 and 1.8–3.1-folds higher levels, respectively, between control and enriched breads, signifying a strong correlation with chicken powder addition. This justifies the aim of this study to develop nutritious breads with well-balanced essential amino acid profiles. Other researchers have also succeeded in correcting the amino acids imbalances in wheat breads through enrichment with nutritionally superior ingredients [32,36,60,64]. The ratio of Essential Amino Acids:Non-essential Amino Acids (EAA/NEAA) followed the order $B_{30} > B_{25} > B_{20} > B_0$ with breads containing chicken

Table 2
Amino acid profile of breads in mg/g of sample.

Amino acid	B_0	B_{20}	B_{25}	B_{30}
Phenylalanine	5.2 ± 0.02^a	8.5 ± 0.04^b	11.4 ± 0.07^c	12.5 ± 0.04^d
Isoleucine	6.8 ± 0.56^a	10.8 ± 0.23^b	13.8 ± 0.23^c	15.9 ± 0.09^d
Leucine	9.0 ± 0.05^a	15.2 ± 0.04^b	20.2 ± 0.03^c	23.2 ± 0.04^d
Methionine	2.6 ± 0.04^a	3.7 ± 0.09^b	4.3 ± 0.21^c	4.8 ± 0.11^d
Valine	4.7 ± 0.10^a	7.7 ± 0.09^b	9.6 ± 0.07^c	10.7 ± 0.04^d
Histidine	2.2 ± 0.11^a	3.4 ± 0.10^b	5.8 ± 0.10^c	7.2 ± 0.10^d
Lysine	2.5 ± 0.09^a	7.5 ± 0.11^b	9.4 ± 0.21^c	11.2 ± 0.27^d
Threonine	3.3 ± 0.28^a	6.0 ± 0.19^b	8.2 ± 0.27^c	10.2 ± 0.09^d
Total EAA	36.3	62.8	82.7	95.7
Tyrosine	4.8 ± 0.19^a	5.8 ± 0.21^b	6.3 ± 0.24^b	7.4 ± 0.07^c
Proline	19.6 ± 0.55^a	24.1 ± 1.03^b	26.8 ± 0.58^c	27.6 ± 0.13^c
Glycine	4.6 ± 0.30^a	6.3 ± 0.35^b	7.5 ± 0.20^c	8.4 ± 0.30^d
Alanine	4.5 ± 0.28^a	6.5 ± 0.11^b	7.8 ± 0.40^c	9.7 ± 0.16^d
Cystine	3.8 ± 0.21^a	4.6 ± 0.52^b	5.4 ± 0.21^c	6.3 ± 0.10^c
Glutamic acid	35.6 ± 1.01^a	41.6 ± 2.01^b	42.6 ± 1.01^b	42.6 ± 2.01^b
Aspartic acid	4.6 ± 0.30^a	7.5 ± 05^b	8.1 ± 0.16^b	8.0 ± 0.21^b
Serine	5.7 ± 0.16^a	6.3 ± 0.20^b	7.1 ± 0.26^c	7.9 ± 0.11^d
Arginine	4.5 ± 0.36^a	8.9 ± 0.22^b	10.5 ± 0.19^c	12.2 ± 0.10^d
Total NEAA	87.7	111.6	122.1	130.1
Total AA	124.0	174.4	204.8	225.8
EAA/AA	29%	36%	40%	42%
EAA/NEAA	0.4	0.6	0.7	0.7

Breads made from B_0: bread 1000g white wheat flour (Control); B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP; means followed with similar letter are not significantly different, AA: amino acid, NEAA: non-essential amino acids, EAA: essential amino acids.

Table 3
Fatty acid profile (μg/g dry matter) of bread.

tR (min)	FAMEs	ω-n (Δn)	B_0	B_{20}	B_{25}	B_{30}
Saturated Fatty Acids (SFAs)						
13.87	Methyl octanoate		1.3 ± 0.36^{a}	3.8 ± 0.73^{b}	4.3 ± 0.43^{b}	4.7 ± 0.48^{b}
15.33	Methyl nonanoate		0.4 ± 0.03^{a}	0.7 ± 0.06^{b}	0.8 ± 0.03^{b}	0.9 ± 0.03^{bc}
16.49	Methyl decanoate		3.7 ± 0.10^{a}	6.5 ± 0.10^{b}	11.8 ± 0.92^{c}	13.8 ± 1.01^{d}
17.77	Methyl undecanoate		–	1.1 ± 0.17	1.9 ± 1.00	1.8 ± 0.43
18.46	Methyl 10-methyl undecanoate		–	3.6 ± 0.58^{a}	3.7 ± 0.53^{a}	4.8 ± 0.40^{b}
18.91	Methyl dodecanoate		122.4 ± 4.90^{a}	205.0 ± 11.76^{b}	220.1 ± 2.95^{bc}	226.9 ± 4.37^{c}
19.70	Methyl 11-methyl-dodecanoate		2.9 ± 0.59^{a}	5.5 ± 0.71^{b}	6.8 ± 1.04^{b}	6.8 ± 0.64^{b}
19.79	Methyl 10-methyl dodecanoate		5.8 ± 0.25^{a}	8.7 ± 0.27^{b}	9.9 ± 0.43^{c}	11.9 ± 0.56^{d}
20.12	Methyl tridecanoate		4.6 ± 0.13^{a}	8.4 ± 0.48^{b}	9.1 ± 0.48^{b}	12.3 ± 1.02^{c}
20.79	Methyl 12-methyl tridecanoate		5.3 ± 0.86^{a}	13.4 ± 0.61^{b}	18.5 ± 4.65^{bc}	21.7 ± 1.52^{c}
21.24	Methyl tetradecanoate		64.6 ± 22.24^{a}	308.7 ± 6.47^{b}	382.4 ± 10.33^{c}	530.1 ± 29.51^{d}
21.76	Methyl 4,8,12-trimethyl tridecanoate		2.8 ± 0.10^{a}	3.8 ± 0.10^{b}	4.6 ± 0.26^{c}	8.7 ± 0.37^{d}
21.89	Methyl 13-methyl tetradecanoate		22.1 ± 4.36	29.4 ± 6.63	32.5 ± 4.52	33.5 ± 4.62
21.98	Methyl 12-methyl tetradecanoate		12.0 ± 4.29^{a}	38.2 ± 2.04^{b}	46.4 ± 4.50^{b}	45.6 ± 3.10^{b}
22.29	Methyl pentadecanoate		28.4 ± 7.81^{a}	76.5 ± 31.07^{b}	100.3 ± 12.38^{b}	104.9 ± 9.57^{b}
23.50	Methyl hexadecanoate		1085.0 ± 12.64^{a}	2184.3 ± 13.42^{b}	2203.0 ± 29.03^{b}	2229.6 ± 4.89^{b}
23.75	Methyl 14-methyl hexadecanoate		7.8 ± 1.82	8.5 ± 0.42	7.8 ± 0.50	8.6 ± 1.02
23.97	Methyl 15-methyl hexadecanoate		26.8 ± 0.92^{a}	41.5 ± 4.33^{b}	44.4 ± 0.64^{b}	47.5 ± 1.33^{b}
24.26	Methyl heptadecanoate		0.3 ± 0.25^{a}	5.6 ± 0.37^{b}	6.2 ± 0.69^{bc}	6.9 ± 0.58^{c}
25.52	Methyl octadecanoate		122.7 ± 10.77^{a}	357.4 ± 4.40^{b}	370.9 ± 7.18^{bc}	379.0 ± 4.50^{c}
26.98	Methyl eicosenoate		38.2 ± 6.81^{a}	79.6 ± 5.24^{b}	79.5 ± 3.96^{b}	92.3 ± 3.90^{b}
27.80	Methyl heneicosanoate		7.9 ± 3.77^{a}	18.3 ± 5.08^{b}	23.9 ± 1.68^{b}	24.2 ± 0.97^{b}
28.59	Methyl docosanoate		12.6 ± 1.73^{a}	25.2 ± 1.24^{b}	30.2 ± 3.01^{b}	31.1 ± 4.26^{b}
29.35	Methyl tricosanoate		6.9 ± 1.73^{a}	15.9 ± 1.68^{b}	16.0 ± 1.85^{b}	18.2 ± 1.48^{b}
30.13	Methyl tetracosanoate		7.8 ± 2.01^{a}	17.5 ± 0.86^{b}	17.0 ± 2.29^{b}	16.7 ± 1.72^{b}
32.04	Methyl hexacosanoate		18.5 ± 1.70	16.9 ± 2.52	16.5 ± 1.95	18.5 ± 0.91
Monounsaturated Fatty Acid (MUFAs)						
20.95	Methyl (11*Z*)-tetradecenoate		14.8 ± 0.97^{a}	15.6 ± 2.21^{ab}	16.1 ± 1.55^{ab}	17.5 ± 0.89^{b}
21.08	Methyl (9*Z*)-tetradecenoate		28.0 ± 1.79^{a}	40.1 ± 10.34^{a}	64.2 ± 3.47^{b}	66.5 ± 4.89^{b}
21.69	Methyl 10-undecenoate		2.8 ± 0.34^{a}	6.1 ± 1.39^{ab}	6.0 ± 1.21^{ab}	5.6 ± 1.64^{b}
22.12	Methyl (9*E*)-dodecenoate		4.3 ± 0.15^{a}	7.1 ± 0.66^{b}	8.4 ± 0.96^{b}	8.5 ± 1.17^{b}
22.14	13-Methyl (9*E*)-tetradecenoate		0.9 ± 0.46^{a}	3.2 ± 0.50^{b}	3.2 ± 0.19^{b}	3.2 ± 0.23^{b}
23.19	Methyl (9*Z*)-hexadecanoate		352.4 ± 10.17^{a}	843.5 ± 9.65^{b}	861.5 ± 5.24^{b}	935.4 ± 16.56^{c}
24.11	Methyl (10*Z*)-heptadecenoate		5.0 ± 0.97^{a}	12.5 ± 1.36^{b}	13.2 ± 13^{b}	14.4 ± 0.85^{b}
24.67	Methyl (9*E*)-Octadecenoate		2.9 ± 0.16^{a}	3.8 ± 0.67^{ab}	3.5 ± 0.40^{ab}	4.2 ± 0.47^{b}
25.14	Methyl (9*Z*)-octadecenoate		393.2 ± 4.49^{a}	428.7 ± 5.08^{b}	431.7 ± 3.05^{b}	447.0 ± 1.65^{c}
25.97	Methyl (10*Z*)-nonadecenoate		33.1 ± 1.77^{a}	71.8 ± 4.49^{b}	73.8 ± 4.00^{bc}	83.1 ± 5.57^{c}
26.15	Methyl (10*Z*)-nonadecenoate		14.4 ± 2.73^{a}	36.3 ± 1.09^{b}	36.9 ± 2.57^{b}	46.9 ± 5.62^{c}
26.82	Methyl (11*Z*)-eicosenoate		102.6 ± 17.52^{a}	202.9 ± 25.73^{b}	206.5 ± 12.02^{b}	226.6 ± 12.47^{b}
28.41	Methyl (13*Z*)-docosenoate		11.0 ± 0.96^{a}	32.0 ± 3.97^{b}	33.0 ± 3.32^{b}	38.3 ± 2.91^{b}
29.95	methyl (15*E*)-tetracosenoate		5.3 ± 1.77^{a}	17.2 ± 2.48^{b}	16.7 ± 1.77^{b}	22.5 ± 1.92^{c}
Polyunsaturated Fatty Acids (PUFAs)						
24.80	Methyl (6*Z*,9*Z*,12*Z*)-octadecatrienoate	C18:3, n-6	11.4 ± 1.36^{a}	23.0 ± 1.27^{b}	23.6 ± 1.50^{b}	27.5 ± 0.72^{c}
25.18	Methyl (9*Z*,12*Z*)-octadecadienoate	C18:2, n-6	2661.5 ± 23.58^{a}	3683.6 ± 7.32^{b}	3843.7 ± 23.45^{c}	4187.8 ± 23.43^{d}
25.83	Methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate	C18:3, n-3	469.0 ± 6.92^{a}	665.9 ± 5.22^{b}	704.6 ± 2.69^{c}	774.3 ± 6.91^{d}
26.21	Methyl (9*Z*,11*E*,13*E*)-octadecatrienoate	C18:3, n-6	35.9 ± 1.69	34.0 ± 2.54	33.4 ± 2.62	35.6 ± 1.50
26.44	Methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate	C20:4, n-6	189.0 ± 79.03^{a}	281.8 ± 22.95^{ab}	327.8 ± 28.50^{b}	334.1 ± 9.97^{b}
26.62	Methyl (8*Z*,11*Z*,14*Z*)-eicosatrienoate	C20:3, n-6	109.5 ± 21.44	113.1 ± 4.25	105.1 ± 12.06	124.6 ± 11.86
28.05	Methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosahexaenoate	C22:6, n-3	111.3 ± 38.11	131.8 ± 26.96	141.4 ± 8.56	185.5 ± 14.26
28.16	Methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoate	C20:5, n-3	43.5 ± 4.93	46.9 ± 4.37	50.9 ± 3.91	52.3 ± 4.07
	$\sum$ SFA		1610.8	3484.0	3668.5	3901
	$\sum$ MUFA		970.7	1720.8	1774.7	1919.7
	$\sum$ PUFA		3631.1	4980.1	5230.5	5721.7
	$\sum$UFA		4601.8	6700.9	7005.2	7641.4
	$\sum$ n-6		3007.3	4135.5	4333.6	4709.6
	$\sum$ n-3		623.8	844.6	896.9	1012.1
	n-6/n-3		4.8	4.9	4.8	4.7
	PUFA/SFA		2.3	1.4	1.4	1.5
	Total FA		6212.6	10184.9	10673.7	11542.4

Breads made from B0: bread 1000g white wheat flour (Control) +0g SHMP; B20: 800g white wheat flour + 200g SHMP; B25: 750g white wheat flour + 250g SHMP; B30: 700g white wheat flour + 300g SHMP, Mean ± SE (standard error) of triplicate determinations; means followed with different letters are significantly different, FA: fatty acid, SFAs; saturated fatty acids, MUFAs; monounsaturated fatty acids, PUFAs; polyunsaturated fatty acids, UFAs; Unsaturated fatty acids, n-6; omega-6 fatty acids, n-3; omega-3 fatty acids.

meat powder attaining ≥0.6, an index indicating good amino acid source [67]. Bread with 25% and 30% chicken meat powder met the FAO/WHO requirements of essential amino acids, accounting for ≥40% of the total amino acids [67].

3.3. Fatty acid profiles

A total of forty-eight fatty acids (FAs) were identified in the experimental breads; saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) contributed 26, 14 and 8 components, respectively (Table 3). These lipids improve the nutritional value, contribute to stability of flours and baked products during storage, forestall bread staling, influence the baking and functional properties of doughs, and release hydroperoxides which improve the aroma and flavour of the baked products [32,58]. The SFAs, MUFAs and PUFAs accounted for 26–34%, 16–17% and 49–54% of the total FAs, respectively in all the breads (Table 3). The amounts of the SFAs, MUFAs and PUFAs in the breads with chicken powder increased 2.2–2.4, 1.8–2.0 and 1.4–1.6-folds relative to the control breads exposing the influence of addition of chicken powder in the formulation mix. Methyl hexadecanoate (palmitic acid), methyl tetradecanoate (myristic acid) and methyl octadecanoate (stearic acid) of the SFA, methyl 9Z-hexadecanoate (myristoleic acid) and methyl (9Z) octadecenoate (oleic acid) of the MUFAs, and methyl (9Z,12Z)-octadecadienoate (linoleic) and methyl (9Z,12Z,15Z)-octadecatrienoate (α-linolenic) of the PUFAs were the most predominant fatty acids. Likewise, Belichovska et al. [68] identified linoleic acid, oleic acid and palmitic acids as the most prevalent FAs in chicken, particularly with regards to drumstick and breast parts, which were considered in this study. Furthermore, these fatty acids were the dominant profiles in the baking ingredients wheat flour [69–71] and chicken meat powder (Table S1) suggesting that the fatty acid of the breads reflected the peculiarities of the FA profile of the ingredients. Such a trend was revealed by other authors who formulated breads integrated with novel ingredients [32,61]. Methyl (9Z,12Z,15Z)-otadecatrienoate (ALA), methyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosahexaenoate (DHA) and methyl (5Z,8Z,11Z,14Z,17Z)-eicosapentaenoate (EPA) were the main omega 3 FAs detected and were significantly ($p < 0.05$) higher in the chicken enriched breads than the control breads. The FA profile is susceptible to influence by the dietary intake as demonstrated by Panda et al. [72]. Chicken used in this study were fed on black soldier fly larvae which is known to possess excellent profiles of unsaturated fatty acids (UFAs) derived from their feeds [73]. Of greater interest are the omega 3 eicosapentaenoic acid (EPA) which is related to cardiovascular health and docosahexaenoic acid (DHA) which is associated with the formation and functionality of the nervous and visual tissues [69]. The UFAs increased by 1.5–1.7-folds between control breads and those enriched with chicken powder, indicating the significant amount of UFA remained stable upon baking, making the enriched bread healthier. Similar observation was demonstrated when fish powder [35] and *kinako*/chia [70] were incorporated in bread. The ratios PUFA/SFA and n-6/n-3 ranged 4.7–4.9 and 1.4–2.3, respectively. The ratio PUFA/SFA is an indicator of food healthiness [69]. The PUFA/SFA ratios of the breads in this study exceeded 0.45, the minimum recommended threshold for a healthy food, associated with blood pressure reduction and prevention of hypertension in human body [69,70]. The notable high n-6/n-3 in the control breads can be linked to the predominance of n-6 fatty acids in cereal grains. That notwithstanding, the n-6/n-3 ratios of all the breads were compliant with the ratios of between 1 and 5, depicting cardio-friendliness, as recommended by food agencies, scientific societies, and national and international organizations [35].

3.4. Vitamins contents of the breads

The levels of all the vitamins examined except ɣ-tocopherol varied significantly ($p < 0.05$) with the increasing levels of chicken powder in the breads (Table 4). The values of retinol, nicotinic acid and pantothenic acid increased by 1.4–4.1, 1.8–2.1 and 1.4–1.5-folds, respectively between the control breads and those with chicken powder added. Additionally, the levels of the vitamins; ascorbic acid, α-tocopherol, ɣ-tocopherol and riboflavin were significantly higher in the control breads than the breads with chicken powder included.

Wheat is naturally scarce in lipid, which negatively affects their content of fat-soluble vitamins such as vitamins A [63] however, other lipophilic vitamins like E and K are known to be less abundant in meat products but abundant in plant-based products [74]. This may explain why retinol, a precursor of vitamin A and the tocopherols, precursors of vitamin E, were relatively lower in the control breads and chicken powder enriched breads, respectively. On the other hand, the progressive increase in retinol, nicotinic acid and pantothenic acids with the rising levels of chicken powder inclusion manifests the contribution of the latter in boosting the levels of

Table 4
Concentration of vitamins (mg/kg dry matter) of breads.

Vitamin	B_0	B_{20}	B_{25}	B_{30}	$F_{(3,8)}$	P-value
Retinol	10.0 ± 0.05^{a}	14.0 ± 0.09^{b}	30.0 ± 0.11^{c}	41.0 ± 0.34^{d}	174.6	0.001
Ascorbic acid	128.9 ± 13.68^{b}	97.6 ± 4.12^{a}	93.3 ± 11.29^{a}	89.2 ± 2.26^{a}	11.6	0.01
α- tocopherol	64.0 ± 0.29^{b}	16.0 ± 0.18^{a}	15.0 ± 0.02^{a}	12.0 ± 0.14^{a}	533.4	0.001
ɣ- tocopherol	13.0 ± 0.10^{b}	12.0 ± 0.08^{b}	11.0 ± 0.05^{b}	8.0 ± 0.07^{a}	16.5	0.001
Riboflavin	6.3 ± 0.59^{b}	5.9 ± 0.38^{b}	5.5 ± 0.80^{ab}	4.5 ± 0.14^{a}	6.3	0.05
Nicotinic acid	66.2 ± 3.79^{a}	113.6 ± 7.50^{b}	137.2 ± 9.97^{c}	138.7 ± 12.02^{c}	52.8	0.001
Pantothenic acid	445.9 ± 39.63^{a}	613.7 ± 39.38^{b}	649.7 ± 41.03^{b}	657.7 ± 34.82^{b}	19.6	0.001

Values are presented as mean ± SE (standard error) of triplicate determinations; means followed by different letters are significantly different at $p < 0.05$. B0: 1000g white wheat flour + 0g SHMP (Control); B20: 800g white wheat flour + 200g SHMP; B25: 750g white wheat flour + 250g SHMP; B30: 700g white wheat flour + 300g SHMP.

such micronutrients deficient in wheat breads. Hydrophilic vitamins such as nicotinic and pantothenic acids are common to animal products like poultry meat and are able to withstand cooking conditions owing to their thermal stability [74], hence their escalated levels in the baked breads formulated with chicken meat powder. Ascorbic acid and riboflavin are also prevalent in cereals than in animal products hence, replacing wheat flour with the chicken meat powder may have diluted their concentrations in the enriched bread. The vitamins nicotinamide, thiamine, pyridoxine and cobalamin were the least abundant evidenced by their non-detection. Their levels may have been affected by cooking time, pH, temperature and mixing process of dough [63]. Due to the paucity in information regarding vitamins contents of breads enriched with chicken powder, we could not compare our data with any other.

3.5. Mineral profile of breads

The incorporation of chicken meat powder into the bread formulation mixes significantly ($p < 0.05$) influenced the content of all minerals identified with the exception of cobalt which was below the detection limit (Table 5). The levels of all the minerals except cobalt positively correlated with increasing levels of chicken meat powder. Spent hen meat powder used in this study had high content of ash (7.2%) (Table S1) which may have translated to the increased minerals levels. This is concurrent with related studies which indicated enhancement in the mineral levels of bread incorporated with pumpkin, mushroom and fish flours [25,37,75]. The predominant minerals in the breads were sodium, potassium, phosphorus, magnesium and calcium especially in the breads enriched with chicken powder. These minerals have previously been reported in black soldier larvae [76] fed to chicken used in this study. Further, the levels of these minerals in animal products depends on their concentrations in the dietary sources [76]. In the current study, iron, phosphorous, zinc, copper and calcium increased 1.0–1.1, 1.6–1.7, 1.1–1.2, 1.2–1.5 and 2.0–3.0-folds between the control breads and the enriched breads, respectively. Iron is crucial in hemoglobin synthesis and co-factor for enzymes [77]. The concentrations of zinc and iron in the breads containing 30% chicken powder can be estimated to contribute 48.2% and 22.5%, respectively of the recommended daily intake of minerals for a person aged between 12 and 18 years [78]. Copper also plays a role in hemoglobin synthesis, redox reaction and cuproenzymes [77]. Their levels in the breads enriched with 30% chicken powder can be estimated to contribute 0.18 mg of 5 mg/day copper daily intake for adults [79].

3.6. Microbial levels in breads enriched with chicken meat powder

Bacteria and fungi were not detected from the freshly baked breads (Table 6). Microbial characteristics of the breads were therefore compliant with the permissible microbial levels as prescribed in the Food and Drug Administration (FDA) circular on microbiological quality of baked products. Elevated baking temperatures which subdues most microorganisms and fungal spores and hygienic post-baking handling largely contribute to products with low microbial counts [80]. The lack of detection of *Salmonella* sp. and *E. coli* suggest no feacal contamination of the breads produced hence safe for consumption.

3.7. Sensory evaluation of the breads

The mean sensory scores of the breads are shown in Table 7. The panellists preferred the dark colour of bread crumbs and crust as depicted in breads enriched with 25% and 30% chicken powders. Similar findings were reported by Umaraw & Chauhan [80] on bread fortified with chicken powders. The darkening in colour may have yielded golden brown colour which is a characteristic colour of bread crusts that consumers are accustomed to. Flavour preference of the breads correlated with the chicken powder inclusion levels. This may be due to fermentation-mediated release of free amino acids from the enriching substrate which may have contributed flavour enhancement of the product compared to the control bread [80]. Over acceptability were highly ($p < 0.05$) rated for the breads enriched with 25% and 30% chicken powder. This reflected the trend observed in the ranking of the breads based on colour and flavour. The panellists may have relied on such attributes to gauge the acceptability of the products.

Table 5
Mineral profile (mg/100g dry matter) of breads.

Mineral	B_0	B_{20}	B_{25}	B_{30}	RDA age 12–18 (mg/day) *	$F_{(3,8)}$	P-value
Iron	2.8 ± 0.19^{a}	2.8 ± 0.12^{a}	3.0 ± 0.18^{a}	3.0 ± 0.09^{a}	13.5	7.1	0.05
Phosphorus	20.1 ± 0.85^{a}	32.5 ± 0.83^{b}	35.0 ± 2.90^{b}	35.1 ± 0.34^{b}	1250	61.9	0.001
Manganese	0.4 ± 0.02^{a}	0.5 ± 0.02^{a}	0.6 ± 0.02^{b}	0.7 ± 0.03^{c}	–	52.5	0.001
Zinc	3.5 ± 0.02^{a}	3.8 ± 0.17^{ab}	3.9 ± 0.08^{bc}	4.1 ± 0.09^{c}	8.5	14.3	0.01
Magnesium	18.7 ± 0.98^{a}	22.3 ± 0.72^{b}	23.0 ± 0.97^{b}	28.0 ± 0.62^{c}	375	63.8	0.001
Molybdenum	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	–	1.0	ns
Potassium	162.1 ± 5.36^{a}	212.2 ± 8.16^{b}	228.2 ± 8.16^{bc}	245.5 ± 5.65^{c}	3500	45.1	0.001
Sodium	215.0 ± 5.80^{a}	232.1 ± 5.62^{b}	286.1 ± 11.85^{c}	318.9 ± 12.38^{d}	2000	139.9	0.001
Aluminum	0.7 ± 0.02^{a}	1.6 ± 0.05^{b}	1.9 ± 0.13^{b}	5.9 ± 0.32^{c}	–	556.9	0.001
Copper	0.1 ± 0.01^{a}	0.1 ± 0.02^{ab}	0.2 ± 0.01^{bc}	0.2 ± 0.00^{c}	–	11.2	0.01
Calcium	16.3 ± 1.06^{a}	32.5 ± 1.77^{b}	38.9 ± 2.51^{c}	48.9 ± 1.10^{d}	1200	191.7	0.001

Values are presented as mean ± SE (standard error) of triplicate determinations; means followed by different letters are significantly different at $p < 0.05$. Breads made from B_0: bread 1000g white wheat flour (Control) + 0g SHMP; B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP.

Table 6
Microbial levels (log cfu/g) in breads enriched with chicken meat powder.

Bread	TVC	*Staphylococcus aureus*	Yeast and mold count	Coliform counts	*Salmonella* spp	Escherichia coli
B_0	<30	ND	ND	ND	ND	ND
B_{20}	<30	ND	ND	ND	ND	ND
B_{25}	<30	ND	ND	ND	ND	ND
B_{30}	<30	ND	ND	ND	ND	ND

B_0**:** Breads made from 1000g white wheat flour (Control) +0g SHMP; B_{20}: Breads made from 800g white wheat flour + 200g SHMP; B_{25}: Breads made from 750g white wheat flour + 250g SHMP; B_{30}: Breads made from 700g white wheat flour + 300g SHMP; TVC: Total Viable Count.

Table 7
Mean sensory scores of breads enriched with chicken meat powder.

Sensory Attribute	B_0	B_{20}	B_{25}	B_{30}
Color	2.4 ± 0.83^{a}	2.8 ± 0.87^{a}	4.2 ± 0.87^{b}	4.1 ± 1.05^{b}
Flavour	3.3 ± 1.86^{a}	4.2 ± 0.74^{b}	4.5 ± 0.60^{bc}	4.7 ± 0.52^{c}
Mouth feel	3.6 ± 1.17^{a}	4.1 ± 0.94^{b}	4.3 ± 0.80^{bc}	4.5 ± 0.65^{c}
Texture	4.5 ± 0.62	4.3 ± 0.78	4.2 ± 0.79	4.2 ± 0.77
Overall acceptability	3.8 ± 0.86^{a}	3.8 ± 0.87^{a}	4.3 ± 0.58^{b}	4.5 ± 0.53^{b}

Values are presented as means ± SD of triplicate determinations. Means followed by similar letters are not significantly different at $p < 0.05$. Breads made from B_0: bread 1000g white wheat flour (Control); B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP; ns: not significant.

4. Conclusion

We conclude that the inclusion of meat powder from hen fed insect-based feeds into wheat bread formulation linearly enhanced the levels of essential nutrients, particularly protein, limiting amino acids (lysine and threonine), fatty acids [Methyl (9Z,12Z,15Z)-octadecatrienoate (ALA), methyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosahexaenoate (DHA) and methyl (5Z,8Z,11Z,14Z,17Z)-eicosapentaenoate (EPA)], vitamins [retinol, nicotinic acid and pantothenic acid] and minerals [iron, phosphorous, zinc, and calcium]. This is an indirect reflection of the dietary benefits of edible insects, which can be tapped through the utilization of meat products derived from animal fed insect-based feeds. This is consistent with the growing consumer health awareness and technological advances of functional foods incorporated with active edible insect ingredients, that is generating significant traction globally. Therefore, functional wheat bread enriched with such meat powder could be a vehicle for scaling novel market-driven food products for consumers with specific healthier diet-oriented lifestyle.

Ethics statements

The authority to conduct the experiment and collect data was in accordance with the animal welfare regulations and granted by the Institutional Animal Care and Use Committee (IACUC) of Kenya Agricultural and Livestock Research Organization (KALRO)-Veterinary Science Research Institute (VSRI); Muguga North upon compliance with all provisions vetted under and coded: KALRO-VSRI/IACUC028/16032022. The sensory study was reviewed and approved by Egerton University and the National Council for Science Technology and Innovation in Kenya (NACOSTI/P/21/8780). Further, the informed consent of the participants was obtained via the statement "I am aware that my responses are confidential, and I agree to participate in this survey as well as affirming that I can withdraw from the survey at any time without giving a reason.

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Author contribution statement

Marcasy P. Makokha, Cheseto Xavier: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Patrick S. Muliro, Peninah N. Ngoda, Changeh J. Ghemoh, Brian O. Ochieng: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sevgan Subramanian, Sunday Ekesi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Chrysantus M. Tanga: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20506.

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