

Effects of utilizing isolated protein from *Hermetia illucens* larvae as partially substituted functional food ingredients for breads fortification

^{1,*}Kipalahi, S.O., ¹Abdel-Aal, M.H., ¹Attia, R.S., ¹Youssef, M.M., ²Analia Sari and ²Elly Misyani

¹Food Science and Technology Department, Faculty of Agriculture, University of Alexandria, El-Shatby, 21545, Alexandria, Egypt

²Elbo Roti Bakery Company, Marga-Kencana, Tulang Bawang Barat, 34691, Lampung, Indonesia

Article history:

Received: 28 February 2026

Received in revised form: 12

March 2026

Accepted: 13 March 2026

Available Online: 22 March 2026

Keywords:

Hermetia illucens larvae,

Isolated protein,

Fortification,

Bread

Abstract

Bread is a popular food product made by baking flour with yeast, and its formulation depends on individual preferences, health goals, or market philosophy. Bread can be much healthier when fortified with good ingredients that improve its functional properties. In this study, the isolated protein from *Hermetia illucens* larvae was used as a food ingredient to improve the health quality of bread by partially substituting wheat flour in the bread formulation. The physicochemical, color, texture profile, and sensory properties of fortified breads with isolated protein were studied. Breads prepared without fortification had the highest carbohydrate content, followed by breads fortified with isolated protein, which was replaced with wheat flour at 2.5%, 5%, and 10%, resulting in $73.89 \pm 4.03\%$, $63.95 \pm 2.09\%$, $51.66 \pm 3.02\%$, and $37.56 \pm 3.56\%$, respectively. This indicated that carbohydrate levels decreased when an isolated protein was added, while protein levels increased as the fortification ratio increased. For the texture profile, the fortification level increased and hardness increased, ranging from 239.80 ± 18.43 to 601.02 ± 23.65 g, equivalent to a force of 2.35 ± 0.18 N to 5.89 ± 0.23 N. The springiness varied across treatments; the breads prepared without fortification had the lowest value, followed by those prepared with fortification at 10%, 2.5%, and 5%, which recorded the highest value. Gumminess and chewiness increased as the fortification percentage increased. The value of lightness (L) of bread crusts of prepared breads ranged from 69.45 ± 1.87 to 47.82 ± 1.02 , while for crumbs ranged from 72.50 ± 6.31 to 37.18 ± 2.71 . The overall acceptability of breads prepared by partially substituting 5% of isolated protein from HIL was high.

OPEN ACCESS

Citation: Authors, A.B. (2026). Effects of utilizing isolated protein from *Hermetia illucens* larvae as partially substituted functional food ingredients for breads fortification. *Letters in Food Research*, 2(3), e26123. <https://doi.org/10.26656/lfr.2.e26123>

1. Introduction

Bread is one of the most common bakery products made from baked flour with yeast. There are various types of breads, widely popular in Middle Eastern countries, including Egypt and Italy. The formulation of bread depends on the individual's desired or market philosophy. Bread can be much healthier when fortified with ingredients that improve its functional properties. Bread formulations can be tailored to meet consumer demand and specifications, aligning with their purpose and preferences. Some consumers need whole toast bread for healthy purposes, while others don't like it because its taste is not as sweet compared to bread prepared by dehulling wheat flour. Some consumers preferred low-calorie breads, while others preferred high-calorie breads because of their taste (Bala *et al.*, 2015). Bread fortification can be done in several ways,

depending on the manufacturer's purpose. Sometimes the government can formulate guidelines for bread fortification to meet public health demands. For example, the government can promote the fortification of certain minerals or vitamins in bread to achieve a target for individual health in the community.

Thakaeng *et al.* (2021) fortified bread with unripe bananas by partially substituting wheat flour with unripe green banana (*Cavendish* spp.) flour, aiming to utilize unripe green bananas obtained from those graded as unacceptable for sale and market. Kure *et al.* (2021) prepared breads with orange flesh and sweet potato as substitutes for wheat flour. Not only can breads be fortified, but many confectionery products can be fortified. IPIFF (2019) fortified loaves of bread and pasta with cricket proteins, and Rashmi (2019) fortified pasta with cricket powder. Cappelli *et al.* (2020) fortified

*Corresponding author.

Email: selemanikipalahi@gmail.com

bakery products with mealworm larvae.

Entomophagy, the eating of insects, is not a new practice, yet many researchers have left it behind. Currently, this field is newly researched; few reviews have examined the use of insects as functional food ingredients in various food products. No review was found reporting the use of non-traditional edible insects as food or food ingredients. In this work, *Hermetia illucens* are non-traditional edible insects also known as black soldier fly are utilized through isolated protein from *Hermetia illucens* larvae (HIL) used as functional food ingredients on bread formulation, this was achieved by characterization of its physicochemical properties, effects of storage, texture profile analysis, color and sensory properties.

2. Materials and methods

2.1 *Hermetia illucens* larvae's flour

One kilogram of HIL flour was prepared by washing and drying the larvae in a hot-air oven, then grinding. The hot-air oven was maintained at 50°C until the larvae were dry. The sieve size used was 297 µm, and safety and hygiene were maintained throughout the whole process.

2.2 Wheat flour

Wheat flour (fine flour extract, 72% free from bran wheat) is the primary component for making breads. Three kilograms of wheat flour were purchased from the local market in Tulang Bawang Barat, Lampung Province, Indonesia. All ingredients for bread making, other materials, and chemical reagents were purchased from the local market in Tulang Bawang Barat, Lampung Province, Indonesia.

2.3 The proximate chemical composition

2.3.1 Crude protein

The crude protein was determined by the Kjeldahl method as described by AOAC (2000). The protein factor used was 6.25, and the sample weight was 2.5 g. The protein content was calculated ($N \times 6.25$), where T was the titer value of the sample (mL), T_0 = titer value of Blank (mL), meq. N_2 = 0.014, and N is the nitrogen content, calculated by using the equation:

$$\%N = \frac{(T - T_0) \times \text{Normality} \times \text{meq. } N_2}{\text{Weight of sample}} \times 100\%$$

2.3.2 Fat content

Fat content was determined using the Soxhlet method, as described by AOAC (2000), which involved isolating lipids from matrices using n-hexane as the solvent.

2.3.3 Moisture content

Percentage of moisture content of the samples were determined by measuring the weight loss after oven drying at a temperature of 105°C and calculated by:

$$\% \text{ Moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%$$

2.3.4 Ash content

Ash content was determined by measuring the total inorganic mineral residue after completely burning the organic matter in a muffle furnace at a temperature of 550°C. The percentage of ash is calculated by dividing the final mass of ash by the initial sample mass and multiplying by 100%.

2.3.5 Carbohydrate and total energy

The amount of carbohydrate was calculated by difference ($\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Protein} + \% \text{ Fat} + \% \text{ Ash})$). The total energy is calculated by following the summation of energy in each component ($\text{Energy (kcal)} = (4 \text{ kcal/g} \times \text{grams of protein}) + (9 \text{ kcal/g} \times \text{grams of fat}) + (4 \text{ kcal/g} \times \text{grams of carbohydrate})$).

2.4 Defatting of HIL flour and protein isolation

The preparation of isolated protein powder from larvae involved several steps, including pre-treatment (flour preparation), defatting (protein solubilization) and recovery (protein purification), and finally drying and re-grinding into powder. Oil was defatted from HIL flour by direct solvent extraction using n-hexane (ratio of 1:10 w/v, HIL flour: n-hexane). The filtration was performed twice: the aqueous solution was removed after stirring and settling for 24 hours, as described by AOAC (2000). The remaining organic solvent (n-hexane) was removed from defatted protein flour by the evaporation process. Further steps in protein purification and isolation were performed using the alkaline solubilization technique, and 0.15 M NaCl was added to increase protein solubility. Insoluble material was removed by centrifugation, and protein precipitation at pH, which corresponds to the isoelectric point (6.5 pH), was performed as described by Kipalahi et al. (2025).

2.5 Physical properties of breads

The physical properties of the breads were determined using various methods. The dimensions, width, height, and length, were determined using a Vernier caliper, as described by Bala et al. (2015). The weight determination was performed using an analytical weighing balance. For loaf volume was determined by multiplication dimensions (width x height x length), while for the Loaf volume index, it was determined by dividing the volume of the loaf sample by the weight of the bread, as follows:

$$\text{Volume index (cm}^3/\text{g)} = \frac{\text{Volume of the loaf}}{\text{Weight of the loaf}}$$

2.6 Texture profile analysis

The texture parameters of bread samples were measured using a texture analyzer (TA-XT-plus, Chapa Techcenter, Bangkok, Thailand) according to the method of Thakaeng *et al.* (2021). The "Two-bite test," with a cylinder probe, was used to quantify sensory characteristics. Bread slices were compressed by 25–50% to simulate mastication, and corresponding data were recorded. Parameters measured included hardness (peak force during the first compression), springiness (ability of the sample to recover height between compressions), cohesiveness (ratio of the area under the second peak to the first), chewiness/gumminess (derived from hardness, cohesiveness, and springiness), and resilience (how the sample recovers from deformation).

2.7 Color analysis

The color of the bread crust and crumbs was analyzed using the Hunter Lab USVIS 1310 (Hunter Laboratory, USA), as demonstrated by Srivastava *et al.* (2010). The (L^* , a^* and b^*) values were recorded where L^* = lightness, a^* = red/green, and b^* = yellow/blue.

Table 1. Experimental designs for recipe formulation for breads.

Component	Isolated protein (%)			
	0	2.5%	5%	10%
Wheat flour (%)	250	243.75	237.5	225
HIL protein (%)	0	6.25	12.5	25
Yeast (g)	2	2	2	2
Sugar (g)	35	35	35	35
Milk (ml)	100	100	100	100
Water (ml)	50	50	50	50
Butter (g)	50	50	50	50
Egg	1	1	1	1
Salt (g)	2	2	2	2

2.8 Preparation of breads

Fortified breads were prepared by using fortified wheat flour with isolated protein powder from *Hermetia illucens* larvae (HIL). The amounts of isolated protein added to wheat flour were 2.5%, 5%, and 10% for fortified bread, and none was added to non-fortified bread, which served as a control. The dough was

prepared using the basic bread recipe, which included yeast, sugar, and fat (Table 1). The dough was prepared using the straight method in a fast-rotating spiral mixer (Diosna, DIOSNA Dierks and Sohne GmbH, Osnabrück, Germany). After fermentation (30 min, 21°C, 60% relative humidity), the dough pieces were placed in metal molds (30 min, 37°C, 90% relative humidity), then the bread was baked in a batch oven (MIWE CONDO - MIWE Michael Wenz GmbH, Arnstein, Germany) at 230°C for 30 min.

2.9 Sensory evaluation

The sensory evaluation of the samples was conducted with 20 panelists from the Elbo Roti Bakery Company in Marga-Kencana, Tulang Bawang Barat, Lampung, Indonesia. A 9-point hedonic scale was used, where 1 indicates "extremely dislike" and 9 indicates "extremely like." To obtain unbiased data on the product's acceptability, individual panelists were oriented and asked to provide verbal consent to participate in the product testing. The samples were coded using three-digit random numbers, and the qualities assessed included color, texture, taste, flavor, crispness, and overall acceptability.

2.10 Statistical analysis

All analyses were performed in triplicate and subjected to one-way Analysis of Variance (ANOVA). Means were compared using Duncan's Multiple Range Test at the 5% significance level ($p \leq 0.05$) in SPSS (Version X; Jones, 2004). Results are presented as mean \pm standard deviation.

3. Results and discussion

3.1 Proximate chemical composition of prepared breads

The proximate chemical composition of breads contained the highest amount of carbohydrate compared to other components. Bread prepared without fortification with isolated protein from HIL had the highest amount of carbohydrate, followed by breads prepared by fortification with HIL isolated protein, which were replaced with wheat flour by 2.5%, 5%, and 10% resulting in $73.89 \pm 4.03\%$, $63.95 \pm 2.09\%$, $51.66 \pm 3.02\%$ and $37.56 \pm 3.56\%$, respectively, as shown in Table 2. The higher the protein fortification ratio, the lower the carbohydrate percentage; the level of

Table 2. The proximate chemical composition of bread prepared with different ratios of isolated protein from HIL.

Content (%)	Breads prepared with isolated protein*			
	0	2.5%	5%	10%
Moisture	6.46 ± 0.71^b	6.78 ± 0.54^{ba}	7.35 ± 0.96^{ab}	10.37 ± 0.85^a
Protein	8.54 ± 0.65^b	14.81 ± 0.39^{ba}	24.03 ± 1.57^{ab}	31.46 ± 1.09^a
Fat	10.18 ± 0.9^b	13.19 ± 0.58^{ba}	15.02 ± 0.30^{ab}	17.79 ± 0.33^a
Ash	0.93 ± 0.00^b	1.27 ± 0.05^{ba}	1.94 ± 0.12^{ab}	2.82 ± 0.07^a
Carbohydrate	73.89 ± 4.03^a	63.95 ± 2.09^{ab}	51.66 ± 3.02^{ba}	37.56 ± 3.56^b

*Mean \pm Standard deviation

Means in each row sharing the difference superscript letter are significantly different ($p \leq 0.05$)

Table 3. Physical properties and texture profile analysis of breads with different levels of fortified protein.

Parameter	Breads prepared with isolated protein*			
	0	2.5%	5%	10%
Length (cm)	21.00 ± 0.95	19.00 ± 1.00	20.00 ± 0.50	20.00 ± 0.85
Width (cm)	11.50 ± 0.34	9.90 ± 0.61	10.00 ± 0.35	6.80 ± 0.22
Thickness (cm)	11.00 ± 0.61	9.90 ± 0.40	7.50 ± 0.65	9.50 ± 0.00
Volume (cm ³)	2656.50 ± 15.74	1862.19 ± 18.76	1500.00 ± 11.88	1292.00 ± 10.00
Weight (g)	468.74 ± 2.40	470.00 ± 2.52	467.72 ± 8.21	468.78 ± 2.45
Specific volume (cm ³ /g)	5.67 ± 0.70	3.96 ± 0.81	3.21 ± 0.61	2.76 ± 0.58
Texture profile analysis of breads				
Hardness (g)	239.80 ± 18.43 ^b	280.61 ± 9.65 ^{ba}	364.29 ± 12.09 ^{ab}	601.02 ± 23.65 ^a
Adhesiveness (g.s)	-0.68 ± 0.01 ^{ba}	-0.66 ± 0.06 ^b	-1.31 ± 0.10 ^a	-1.19 ± 0.16 ^{ab}
Springiness	0.58 ± 0.09 ^b	0.73 ± 0.03 ^{ab}	0.85 ± 0.01 ^a	0.69 ± 0.01 ^{ba}
Cohesiveness	0.51 ± 0.01 ^a	0.44 ± 0.00 ^{ab}	0.38 ± 0.01 ^{ba}	0.32 ± 0.01 ^b
Gumminess	122.30 ± 6.81 ^b	123.47 ± 11.01 ^{ba}	138.43 ± 8.56 ^{ab}	192.33 ± 14.55 ^a
Chewiness	70.93 ± 4.13 ^b	90.13 ± 6.86 ^{ba}	117.67 ± 9.10 ^{ab}	132.71 ± 17.29 ^a

*Mean ± Standard deviation

Means in each row sharing the difference superscript letter are significantly different (p≤0.05)

fortification was significantly different (p≤0.05) and inversely proportional to carbohydrate content. The fortification of breads with isolated protein showed that this technique can be used to produce special food products that aid weight loss by reducing carbohydrate intake. Kipalahi *et al.* (2025) prepared low-calorie biscuits by fortifying them with isolated protein HIL, resulting in a lower calorie and carbohydrate content in the final product (biscuits). Barbarisi *et al.* (2019) showed that bread quality improved by increasing protein content through fortification techniques, ingredients, or preparation methods.

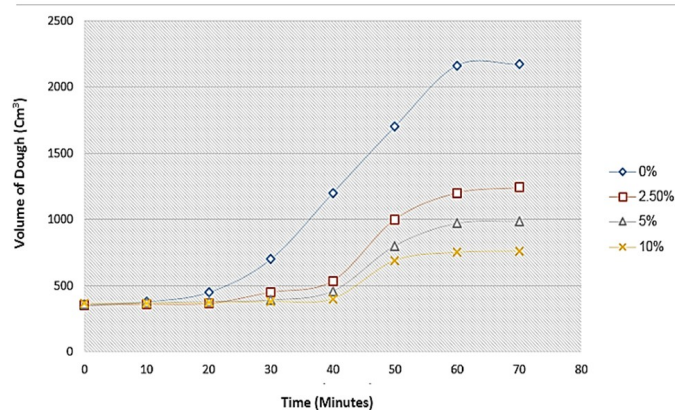
Protein content is a crucial parameter in the fortification process, especially in bakery products. Most fortification in bakery products, aimed at raising protein levels, can also be used to boost vitamin and mineral levels. The protein content of breads prepared increased as the fortification ratio of isolated protein increased. Hence, the final protein content was significantly different (p≤0.05) and was influenced by the percentage of isolated protein added. The limit of adding isolated protein to replace wheat flour must be considered, because it was observed that during bread preparation, the amount of protein added directly affected dough raising during leavening and baking. Hence, for high-quality, acceptable fortified bread products, protein content must be mandatory for establishing the limits.

Moisture content is another parameter that determines the quality and shelf life of processed breads. The moisture content of processed breads fortified by replacing the wheat flour with isolated protein with ratios of 0, 2.5%, 5% and 10% was 6.46 ± 0.71%, 6.78 ± 0.54%, 7.35 ± 0.96%, and 10.37 ± 0.85, respectively, as shown in Table 2. This revealed the addition of fortification ingredients, linked with increased water-holding capacity during the baking process, resulting in an increase in the moisture content. Oluwasegun and Apaa (2024) reported moisture contents of commercial breads ranging from 6.5% to 7.80%, which are within the

values reported for breads prepared.

Fat contents of breads prepared by fortification with different ratios (0%, 2.5%, 5% and 10%) were 10.18 ± 0.9%, 13.19 ± 0.58%, 15.02 ± 0.30% and 17.79 ± 0.33%, respectively (Table 2). The higher amount of isolated protein added results in a greater amount of oil retained in the loaf. Oluwasegun and Apaa (2024) outlined that the fat content of the various fortified breads ranged from 12.92% to 21.36%. This indicated that the amount of fat content retained in breads is also influenced by ingredients, the type of wheat flour used (whole or dehulled), and cooking methods.

Ash content of bread prepared by fortification with 0%, 2.5%, 5% and 10% were 0.93 ± 0.00%, 1.27 ± 0.05%, 1.94 ± 0.12% and 2.82 ± 0.07%, respectively. The ash content values ranged within the recommended amount, from 1.05% to 3.84%, reported by Oluwasegun and Apaa (2024). The fortification of food products had a direct effect on the ash content of final products when other factors remained constant.

Figure 1. Plot of dough volume (cm³) during leavening against time (minutes).

3.2 Effect of fortification on the leavening process

During the leavening process, yeast ferments and converts sugar into carbon dioxide and ethanol, causing the dough to rise (leaven) and develop complex flavors. The carbon dioxide, trapped by gluten, forms airy

pockets, while the alcohol mostly evaporates during baking, resulting in a light, flavorful loaf. The doughs raised at different volume rates; the breads prepared without the addition of isolated protein had the highest rate of dough volume increase compared to other breads, as shown in Figure 1 and Figure 3a. This indicates that an isolated protein added to wheat flour influences the fermentation process by either reducing the capacity for carbon dioxide trapping or altering the interaction between sugar and yeast. Hence, the amount of protein fortified added influences the rate of dough rising, texture, quality, and final volume of bread products.

3.3 Physical properties of breads

The quality of breads was assessed using several parameters: loaf properties, specific volume, and other attributes, including sensory acceptability. Specific volume of a loaf is one of the most crucial parameters that describes the quality of a loaf or bread. In this work, the specific volume of breads was determined after baking, during storage, and after storage, when packaged in plastic bags. After the baking process, the specific volume of breads prepared by fortification with isolated protein at different ratios (0%, 2.5%, 5%, and 10%) was 5.67 ± 0.70 , 3.96 ± 0.81 , 3.21 ± 0.61 , and 2.76 ± 0.58 (cm^3/g), respectively, as shown in Table 3. This result revealed that fortifying breads with isolated protein significantly affected specific volume, with the relationship being inversely proportional. Kure *et al.* (2021) also showed that the specific volume is inversely proportional to the amount of material used for fortification and the nature of ingredients. Prepared breads with wheat and orange-fleshed sweet potato; more fortified ingredients used resulted in a decrease in specific volume, with values ranging from 2.10 to 1.25 cm^3/g .

3.4 Texture profile analysis of breads

The texture profile analysis (TPA) of breads prepared with and without fortification by adding isolated protein from HIL is shown in Table 3. The hardness of breads prepared with the addition of isolated protein was higher compared to bread prepared without fortification. The more the fortification level increased, the more significant the difference ($p \leq 0.05$) appeared in hardness, which ranged from 239.80 ± 18.43 to 601.02 ± 23.65 g, which is equivalent to a force 2.35 ± 0.18 N to 5.89 ± 0.23 N (Gravitational force (G) = 9.8 N). The ability of breads to return to their previous shape (springiness) varies within treatments. The breads prepared without fortification had the lowest value, followed by those prepared with fortification; 10%, 2.5%, and 5% recorded the highest values. For storage purposes, 5% treatment is more preferred because it is the strongest to resist the physical change (volume of

breads) during storage, because of its higher ability of springiness when compressed with other products, it's difficult to change its shape and recover its original shape when compressed products are removed during transportation or storage. The energy required to disintegrate (gumminess) increased when the fortification percentage increased; gumminess had a significant difference ($p \leq 0.05$) in the amount of fortified isolated protein added. For the energy required to chew bread until swallowing (chewiness), the amount of fortified isolated protein had a significant effect, with chewiness increasing directly with the amount of fortified isolated protein. Thakaeng *et al.* (2021) also reported that the texture profile of breads was influenced by the fortification process and the use of unripe green banana (*Cavendish* spp.) flour as a partial substitute for wheat flour during bread preparation.

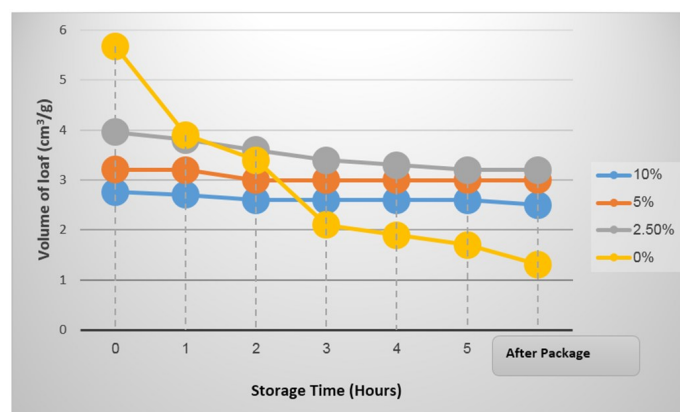


Figure 2. Plot of effect of specific volume of loaf (cm^3/g) against storage time (hours).

3.5 Effect of the specific volume of the loaf during storage

During storage, the loaf's specific volume gradually decreased, and the rate of loaf swelling varied across treatments. The highest rate of swelling was recorded for breads prepared without the addition of isolated protein from HIL, followed by breads prepared with fortified isolated protein at 2.5%, 5%, and 10% as shown in Figure 2. The swelling occurred on breads prepared without the fortification indicates that the fortification process is essential for maintaining the rigid shape of breads during storage and transportation, which will keep the original shape (size) and can be the first priority for consumers' choice who rely on the shape and size of the loaf. For breads prepared by fortification with isolated protein, loaf swelling was very slight. Shalaby *et al.* (2014) reported the effects of storage on the physical and chemical properties of bread. The initial loaf decreased in specific volume and original until it reached the point at which no further change occurred during storage. It is suggested that many factors contributed to the physical and chemical properties, such as ingredients used, fortification, and storage conditions. Hence, the selection

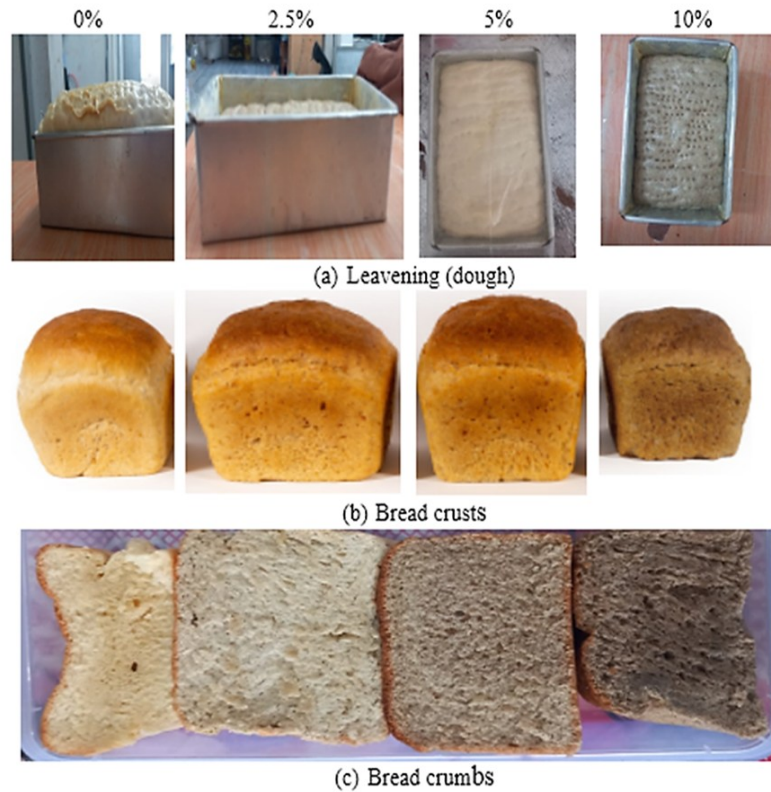


Figure 3. Bread prepared without and with protein fortified by 0%, 2.5%, 5% and 10%. Whereby: (a) Leavening (dough), (b) Bread crusts, and (c) Bread crumbs.

of ingredients during the preparation of breads is pivotal for maintaining the texture, shape, and size of breads during storage, especially for marketing purposes.

3.6 Color of breads

The color of the crusts and crumbs of the prepared breads showed variation between treatments (ΔE). For both bread crusts and bread crumbs, the lightness value of bread decreased with increasing amounts of fortified isolated protein from HIL; hence, the relationship between color lightness is inversely proportional to the percentage of isolated protein added. The value of lightness (L) of bread crusts of prepared breads ranged from 69.45 ± 1.87 to 47.82 ± 1.02 , while for crumbs ranged from 72.50 ± 6.31 to 37.18 ± 2.71 , as shown in Table 4. The more isolated protein added increased the brownness of both crumbs and crusts, as shown in Figure

3(b-c). Khoozani *et al.* (2020) showed the effect of bread fortification with whole green banana flour on its physicochemical properties, including the color. The color of crusts, breads, and crumbs was influenced by the amount of banana flour added, and the range of lightness for bread crusts and crumbs reported were 66.6 to 33.2 and 72.5 to 36.6, respectively.

3.7 Sensory evaluation of prepared bread

The overall acceptability of breads prepared with 5% HIL isolated protein was the highest, followed by 2.5% and 0%, and the least acceptable was breads prepared with 10% fortified isolated protein. Each treatment is superior to another for only certain parameters; there's no single treatment superior to others for all parameters, as shown in Table 5. Appearance of breads, breads prepared by 2.5% of fortification were highly accepted,

Table 4. The color of bread crusts and crumbs of breads prepared with fortified protein with different ratios of isolated protein from HIL.

Breads treatment	Parameters of bread crusts			
	L	a*	b*	ΔE
0%	69.45 ± 1.87^a	0.57 ± 0.01^b	14.01 ± 0.67^b	-
2.5%	63.11 ± 3.98^{ab}	1.29 ± 0.00^{ba}	17.12 ± 0.29^{ba}	6.34
5%	51.55 ± 1.35^{ba}	1.96 ± 0.03^{ab}	20.56 ± 0.89^{ab}	17.90
10%	47.82 ± 1.02^b	2.36 ± 0.01^b	22.79 ± 0.54^a	21.63
	Parameters of bread crumbs			
	L	a*	b*	ΔE
0%	72.50 ± 6.31^a	2.46 ± 0.94^a	14.80 ± 1.38^a	-
2.5%	65.15 ± 3.87^{ab}	0.79 ± 0.18^b	8.65 ± 0.45^{ab}	7.35
5%	47.71 ± 1.91^{ba}	3.69 ± 0.59^{ab}	7.31 ± 0.67^{ba}	24.79
10%	37.18 ± 2.71^b	3.57 ± 0.67^{ba}	4.19 ± 0.17^b	35.32

*Mean \pm Standard deviation

Means in each column sharing the difference superscript letter are significantly different ($p < 0.05$)

Table 5. Sensory evolution of breads prepared by partially substituting wheat flour (fortification) with HIL isolated protein.

Parameter	Breads prepared by fortified*			
	0%	2.5%	5%*	10%
Appearance	7.25 ± 0.96 ^{ba}	8.01 ± 0.50 ^a	7.46 ± 0.68 ^{ab}	6.73 ± 0.19 ^b
Color	7.10 ± 0.44 ^{ba}	7.64 ± 0.81 ^{ab}	7.97 ± 0.87 ^a	6.29 ± 0.33 ^b
Texture	7.04 ± 1.29 ^{ba}	7.39 ± 0.53 ^{ab}	7.89 ± 0.51 ^a	5.68 ± 0.71 ^b
Taste	8.50 ± 0.58 ^a	8.25 ± 0.35 ^{ab}	7.91 ± 0.69 ^{ba}	6.91 ± 0.86 ^b
Odor	8.75 ± 0.5 ^a	8.68 ± 0.41 ^{ab}	8.06 ± 0.28 ^{ba}	7.19 ± 0.84 ^b
Overall acceptability	7.31 ± 0.71 ^{ba}	7.46 ± 0.63 ^{ab}	7.93 ± 0.46 ^a	6.07 ± 0.95 ^b

*Mean ± SD

Means in each row sharing the same superscript letter are not significantly different (p≤0.05)

followed by 5%, 0% and 10% least accepted. The panelists preferred the color and texture of breads treated with 5%, followed by 2.5%, 0%, and 10%, which was the least accepted. The color of bread prepared with isolated protein by 10% was unpleasant and deviated much from normal. Taste and odor of breads: breads prepared without fortification are more accepted, followed by those prepared with fortification at 2.5%, 5%, and 10%; those prepared with fortification at 10% are least accepted. Even though fortified breads with isolated protein from HIL are accepted much more than non-fortified bread, some panelists refused to test because of their eating habits.

Conclusion

The use of isolated protein from *Hermetia illucens* larvae as a food ingredient to improve the health quality of breads by partially substituting wheat flour in the bread formulation is successful. The physicochemical, color, texture profile, and sensory properties of fortified breads with isolated protein were studied. However, fortified breads with isolated protein from HIL are highly accepted over non-fortified breads; still, some panelists refused to test due to their eating habits. More research, education, and promotion are needed to ensure that societies adopt entomophagy or insect food products as part of daily meals, as with other eating habits.

References

Association of Official Analytical Chemistry (AOAC). (2000). Official Methods of Analytical of AOAC International. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

Bala, A., Gul, K. and Riar, S. (2015). Functional and sensory properties of cookies prepared from wheat flour supplemented with cassava and water chestnut flours. *Cogent Food and Agriculture*, 1(1), 1019815. <https://doi.org/10.1080/23311932.2015.1019815>

Barbarisi, C., Vito, V., Pellicano, M., Boscaino, F., Balsamo, S., Laurino, C., Sorrentino, G. and Volpe, M. (2019). Bread chemical and nutritional characteristics as influenced by food grade sea water. *International Journal of Food Properties*, 22(1), 280-289. <https://doi.org/10.1080/10942912.2019.1579837>

Cappelli, A., Oliva, N., Bonaccorsi, G., Lorini, C. and Cini, E. (2020). Assessment of the rheological properties and bread characteristics obtained by innovative protein sources: Novel food or potential improvers for wheat flour. *Food Science and Technology*, 118, 108867-108889. <https://doi.org/10.1016/j.lwt.2019.108867>

Jones, E. (2004). Gelatin: Manufacture and physico-chemical properties. In *Pharmaceutical Capsules*. Podczeczek, F. and Jones, E. (Eds.), London: Pharmaceutical Press, pp. 23-60.

Khoozani, A., Kebede, B., Birch, J. and Bekhit, A. (2020). The effect of bread fortification with whole green banana flour on its physicochemical, nutritional and in vitro digestibility. *Foods*, 9, 152. <https://doi.org/10.3390/foods9020152>

Kipalahi, S., Abdel-Aal, H., Attia, H. and Youssef, M. (2025). Utilization of isolated protein powder from black soldier fly larvae as functional food ingredients for fortifying biscuits. *Alexandria Science Exchange Journal*, 46(4), 953-961. <https://doi.org/10.21608/asejaiqjsae.2025.470177>

Kure, O., Ariaahu, C. and Igbabul, D. (2021). Physico-chemical and sensory properties of bread prepared from wheat and orange-fleshed sweet potato (flour, starch and non-starch residue flour) blends. *Asian Food Science Journal*, 20(3), 1-17. <https://doi.org/10.9734/afsj/2021/v20i330274>

Oluwasegun, O. and Apaa, J. (2024). Bioactive Compounds, Chemicals Properties, Physical and Sensory Properties of Bread Produced from Wheat (*Triticum aestivum* L.), Defatted Sesame Seed (*Sesame indicum*) and Unripe Plantain (*Musa paradisiaca*) Flour Blends. *Asian Journal of Food Research and Nutrition*, 3(3), 526-543. Retrieved from <https://journalajfrn.com/index.php/AJFRN/article/view/153>

Rashmi, A. (2019). Functional Insect Protein Extracts for Food Applications. Master Thesis University of Central Oklahoma, Edmond, Oklahoma, United States of America. Retrieved from <https://hdl.handle.net/11244/325105>

Shalaby, M.T., Abou-Raya, M.A., Rania, E., EL-gammal, H. and Al-Janabi, H. (2014). Effect of storage on some physical and chemical properties of iraky bread. *Journal of Food and Dairy Science, Mansoura University*, 5(12), 891-904. Retrieved from https://jfds.journals.ekb.eg/article_53248_cc56159673c73a2a838e0393d197e5ea.pdf

Srivastava, Y., Tamhane, V. and Meinolf, G. (2010). Effect of virgin coconut meal (VCM) on the textural, thermal and

physicochemical properties of biscuits. *Food and Nutrition Science*, 1, 38-44. <https://doi.org/10.4236/fns.2010.12007>

Thakaeng, P., Boonloom, T. and Rawdkuen, S. (2021). Physicochemical Properties of Bread Partially Substituted with Unripe Green Banana (*Cavendish* spp.) Flour. *Molecules*, 26(7), 2070. <https://doi.org/10.3390/molecules26072070>

The International Platform of Insects for Food and Feed (IPIFF). (2019). The insect sector milestones towards sustainable food supply chains. Brussels, Belgium.