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Research

Influence of pH and salt conditions on extraction efficiency and functional properties of *Macrotermes nigeriensis* protein concentrate for food applications

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Abstract
The search for alternative proteins from non-conventional sources for food processing has become important due to the shortage of conventional protein sources. Protein extractability from *Macrotermes nigeriensis* and its functional properties were investigated under various conditions of pH (2–10) and salt concentration (0.1–1.0 M). Extracted proteins were precipitated through pH adjustment and micellization, respectively. Results showed that maximum protein extractability was 68% and 62.1% at 0.5 M salt concentration and pH10.0, respectively. Salt-extracted protein-rich fraction (SP) had the highest protein composition (68.68 ± 0.41 g/100 g), seconded by alkaline-extracted protein-rich fraction (AP) (62.91 ± 0.53 g/100 g), compared with the raw and defatted fraction (34.36 ± 0.44 and 42.12 ± 0.15 g/100 g), respectively. The highest emulsion capacity (49%) and emulsion stability (35%) were recorded in alkaline pH 10.0. In comparison, the highest foaming capacity (24%) and foaming stability (16%) were recorded in the salt-extracted fraction at 6%. This information could be useful for further studies on the food application potential of proteins isolated from edible *M. nigeriensis*.

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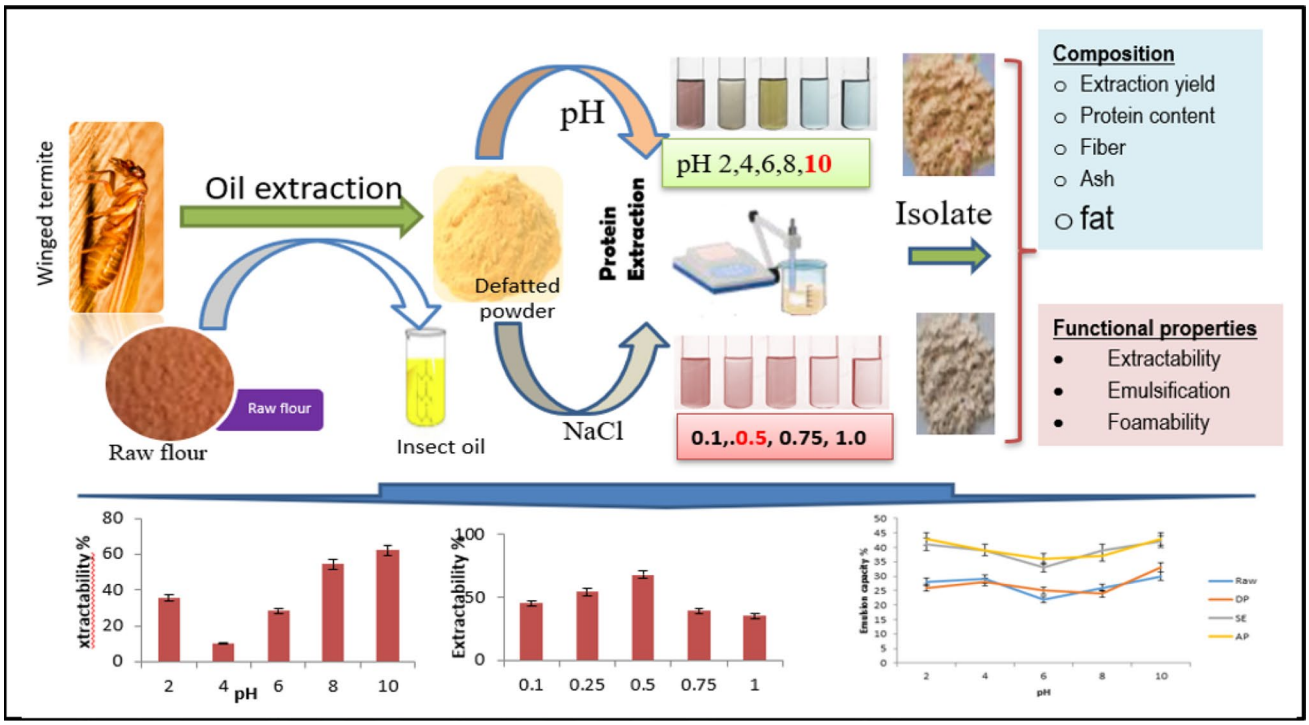
Graphical Abstract



Keywords Alternative proteins · Functional properties · *Macrotermes nigeriensis* · Protein extractability · pH and salt conditions

1 Introduction

Due to inadequate supplies of conventional protein sources and the rising meat consumption per capita [1], research into alternative protein sources for potential food applications has become important. Edible insects have been cited in recent studies [2–5] as a probable alternative source of protein to meet the demand of the world's growing population which is predicted to reach more than 10 billion people in the year 2050 [6]. This is not due to poverty or starvation, but rather because edible insects are rich in protein and micronutrients [3, 4]. When compared with plant and meat protein in most cases, insects have higher quality proteins in terms of their essential amino acid composition and digestibility [7, 8]. Generally, edible insects are sources of valuable protein that contain all essential amino acids that aid in growth synthesis unlike plant and whey proteins [4, 7, 9]. Depending on the species and the metamorphic stage, insects contain between 19 to ~ 70% of proteins on a dry weight basis [8–12]. Bioavailability of proteins and other nutrients (e.g., iron, zinc) in insects may differ from conventional food sources, which may affect their dietary impact. The sustainability of insect farming includes a short cultivation period [11, 13], a high production rate [14, 15], and low release of greenhouse emissions [14], making the edible insect a potential option to address global protein shortage. For example, the period of harvesting for insects is generally 45 days, which is shorter than 4–36 months for domestic farm animals such as chickens, pork, and cow [11].

There are over 2000 species of insects that have been identified as safe for human consumption globally [2, 8]. In Nigeria for instance, more than 30 different species of insects are commonly utilized as food. These include winged termites, locusts, grasshoppers, beetles, and weevils. *Macrotermes nigeriensis* (Winged termite) is among the most popular

insect species which belongs to the family '*Termitidae' and* class "*Insecta*". It is the most common edible insect in Nigeria in terms of consumption and acceptability. *M. nigeriensis* is known locally in many parts of Nigeria with different names. In Ibo, it is called '*aku' while* in Hausa and Yoruba languages, it is known as '*chinge*' and '*Esusu*, respectively. It is usually available every year, especially at the start of the rainy season. Previous studies showed that *Macrotermes nigeriensis* has a high protein composition (~45 g/100 g d.w.b) [16–18].

A study carried out by Oibiokpa et al. [9] investigating four different insects commonly eaten in Nigeria found that *M. nigeriensis* contains relatively high amounts of essential amino acids including lysine and methionine that are lacking in cereal protein. In another study according to Igwe et al. [17], moderate intake of the termite-incorporated meal does not have any harmful effect on the cholesterol level and hematological indices of laboratory animals and hence may not initiate risk of cardiovascular diseases for the consumers. Despite these benefits, the utilization of *M. nigeriensis* and other edible insects remains quite limited. This is largely attributed to the repulsive or disgusting feelings associated with the consumption of insects by some individuals [13, 19], especially when the insects are presented in a recognizable form. Therefore, the extraction of proteins from *M. nigeriensis* for use in food processing may increase consumer acceptability and reduce the strange feelings attached to its consumption as food. In terms of food safety, consuming edible insects has not led to disease transmission in humans [2]. However, allergic reactions to insect proteins (especially among those allergic to shellfish) have been documented, prompting further investigation [2, 8]. Additionally, insects can harbor pathogens, parasites, and accumulate toxins, posing health risks if not properly processed or cooked after harvesting from contaminated areas. Continued research is crucial to understand and mitigate these risks.

Protein extractability is defined as the proportion or number of proteins that are soluble under specific conditions [20]. Proteins are commonly extracted from their natural sources by alkaline and/or salt-assisted extraction followed by precipitation and ultrafiltration [21–24]. The alkaline extraction consists of solubilizing the protein in alkaline conditions followed by precipitation through adjusting the pH to the pH of the isoelectric point. This renders the protein molecules insoluble, hence they form aggregates and precipitate out of the solution [22]. Salt-based extraction involves solubilizing the proteins in salt solution followed by precipitation of the solubilized protein through micellization [21, 25, 26]. The principle behind these separation techniques depends on the biochemical properties of proteins such as their molecular size, charge, adsorption properties, and solubility. The solubility of protein under different conditions is very useful in choosing the optimum conditions for extracting proteins from different natural sources for use in food processing [27].

Protein concentrate is a product that contains high percentage of protein composition (60–80%) relative to other nutrients. It is regarded as the ideal ingredient for the formulation of food products [28]. The functional properties of proteins (such as emulsification and foamability) are regarded as physicochemical indicators that determine the characteristic performance of proteins during food processing [24]. These properties are mainly linked to the structure and composition of amino acids in the protein [29, 30]. They are also influenced by several factors including environmental conditions such as temperature, pH, and ionic strength [21, 24, 31]. Protein functional properties are responsible for many of the factors that affect consumer acceptance of food products [32]. Hence, they play a crucial role in food processing depending on the type of food products to which the protein is to be added.

Protein concentrates and isolates from edible insects have not yet been fully utilized in food processing. This is due to the scarcity of information regarding the condition of extraction and functional properties of individual insect-based proteins. Previous studies have isolated proteins from various species of edible insects across different geographical regions, such *Tenebrio molitor* (Mealworm) [33–36], *Migratory locust* [37], *Acheta domesticus* (Cricket) [15, 25, 38], *Bombyx mori* (Silkworm pupae) [7, 11], *Schistocerca gregaria* (Grasshopper) and *Apis mellifera* (honeybee) [20, 23]. However, most of these reports focused mainly on the quality of the isolated proteins in terms of amino acid characterizations and protein digestibility but did not sufficiently cover the protein functional properties for potential food applications. Moreover, differences in insect type and geographical settings could affect insect protein compositions and functional properties [39]. No such report has been documented for *Macrotermes nigeriensis* in terms of its protein extraction and functional properties as influenced by extraction methods. Therefore, the objective of this study was to evaluate the effect of pH and salt conditions on the extractability and functional properties of proteins from *M. nigeriensis*. Findings from the study can facilitate the successful application of *M. nigeriensis* protein in food processing to improve protein quality and consumer acceptability. This is critically needed, especially in sub-Saharan Africa, where protein malnutrition remains a major health challenge.

2 Materials and methods

2.1 Insect collection

Fresh adult *M. nigeriensis* (winged termites) were collected from residential buildings in the early morning after rainfall the previous day using a traditional method which involves attracting the winged termites with fluorescent lights at night followed by handpicking. The termites were moved to the laboratory inside an ice block, for drying and processing. The choice of using edible winged termites for this study was due to the indigenous people's local preference for the insect. The Research Ethics Committee of the College of Natural Science, Michael Okpara University of Agriculture, Umudike, approved the study protocol.

2.2 Insect processing

The harvested *M. nigeriensis* was sorted out to remove stone and metallic particles. The wings and legs were removed before cleaning the insects three times by washing with clean running tap water to remove debris. The cleaned termites were pre-treated by immersing in hot water (100 °C) for 1 min and draining before drying using an oven set at 45 °C to constant weight. Dried termites were ground into a fine powder with an electric blender to a particle size of < 1 mm, sieved using 200 µm mesh sieve, and packed in an air-tight container, labeled, and stored at −4 °C for further analysis.

2.3 *M. nigeriensis* protein extraction: experimental design

The extraction process was conducted at different pH treatments and salt conditions using a randomized complete block design (RCBD). Each treatment was considered a block, and within each block three independent replicates were performed to ensure robustness and reliability of the results. All analysis were performed in triplicates to understand variability and consistency across treated samples compared with the raw insect powder.

2.3.1 Defatting process

Raw powder (200 g) of *M. nigeriensis* was dispersed in hexane at a ratio of 1:5 (w/v). The mixture was stirred using a magnetic stirrer at 27 °C (room temperature) for 6 h [25]. After sedimentation, the hexane containing the fat was separated and the defatted insect powder was re-extracted again two times by following the same procedure until the hexane became clear. Defatted termite powder was air-dried, labeled (DE), and stored at room temperature (27 °C) for further use (Fig. 1).

2.3.2 Effect of treatment at different pH levels on protein extractability

Defatted *M. nigeriensis* powder (1 g) was suspended in 100 mL of deionized water following the methods described in Adebowale et al. [21]. The pH of the solution was adjusted to different values (2, 4, 6, 8, and 10) using 1 M NaOH and HCl. Each suspension was mixed thoroughly on a magnetic stir plate for 30 min at 30 °C and centrifuged at 2000 g for 10 min. The supernatant was collected and subjected to protein determination using the biuret method. Extractions were performed in triplicate, and the protein extractability from each treatment was calculated using Eq. 1.

$$\text{Protein extractability}\,(\%) = \left[\frac{\textit{amount of protein obtained}\,(g)}{\textit{Amount of protein in starting material}\,(g)}\right] \times 100 \quad (1)$$

2.3.3 Protein precipitation procedure

After the determination of protein extractability from each pH regime, extraction at pH 10 gave the highest protein extractability. Hence, this supernatant was used for the preparation of the alkaline-extracted protein-rich fraction (AP). To prepare the protein-rich fraction, the pH of the supernatant was re-adjusted to a pH of 4.5 at which the protein extractability was lowest using 0.1 M HCl [31]. After an hour of incubation at room temperature, the solution was centrifuged at 15,000 g for 10 min at 4 °C. The resulting supernatant was removed after this centrifugation while the precipitate which

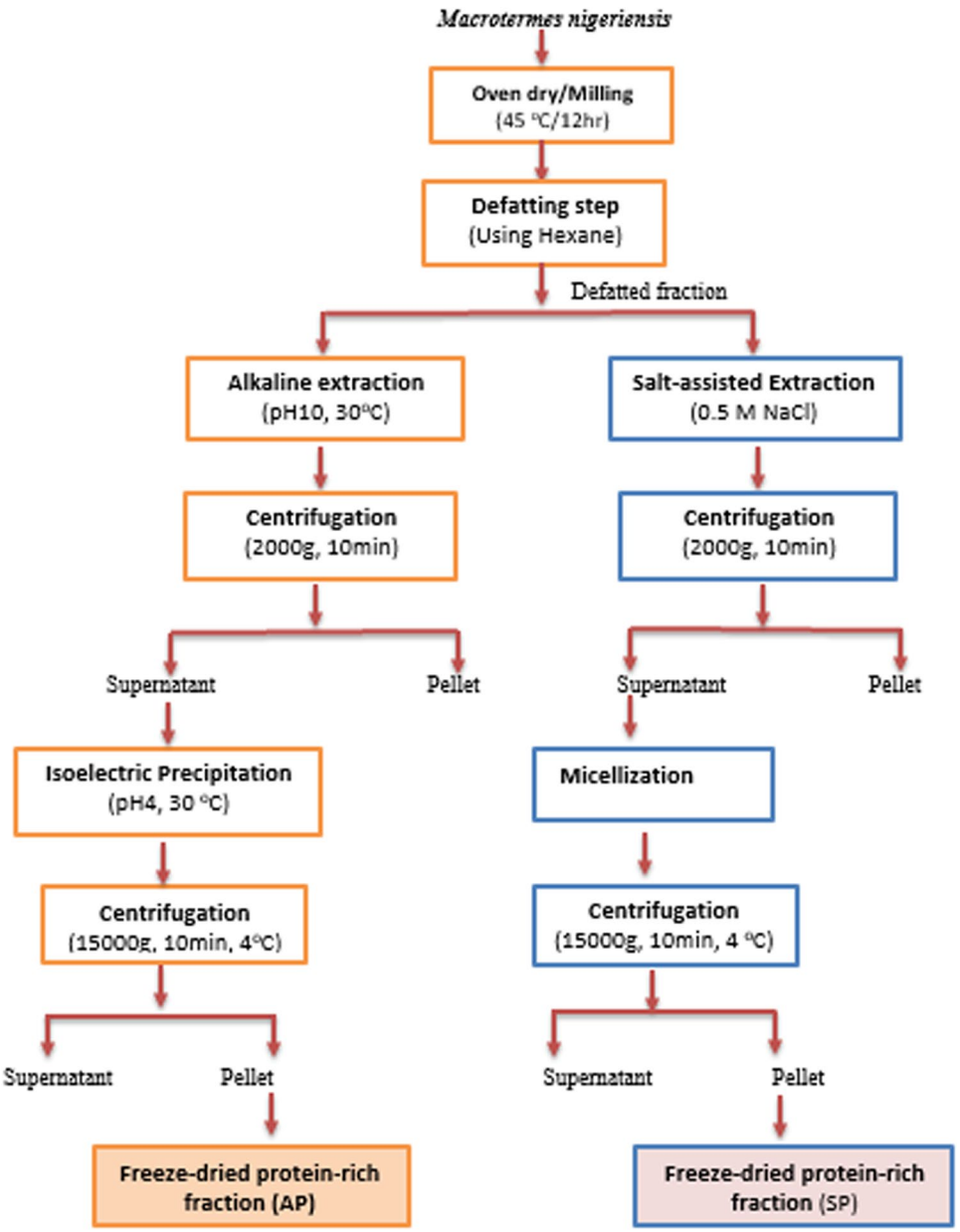


Fig. 1 Schematic process of production of *M. nigeriensis* protein-rich fractions

contains the protein extract was retained, followed by freeze-drying. Freeze-dried protein samples were ground into powder, labeled alkaline-extracted protein-rich fraction (AP), and kept dry for further assay.

2.3.4 Effect of treatments at different salt concentrations

Defatted *M. nigeriensis* powder (DE) was introduced in salt (NaCl) solutions of various concentrations (0.1, 0.25, 0.50, 0.75, and 1.0 M) at a ratio of 1:10 (w/v) following the method described in Illingworth et al. [26]. Each suspension was properly mixed on a magnetic stir plate for 30 min at 30 °C, followed by centrifugation at 2000 g, 4 °C for 10 min. The extractions were also performed in triplicate and the supernatants obtained were analyzed for protein composition using the biuret method. The protein extractability from each treatment was calculated using the same Eq. 1.

2.3.5 Precipitation of protein from salt extraction

Extraction parameters of 0.5 M salt concentration resulted in the highest protein composition compared to other treatments and therefore was used to prepare the salt-extracted protein-rich fraction (SP) by following the Illingworth et al. [26] method. The supernatant was diluted with cold deionized water at the ratio of 1:10 (v/v) and left to stand (4 °C). The micellized protein precipitate was recovered by centrifugation (15,000 g, 10 min), washed with deionized water, and froze followed by freeze-drying at −20 °C for 24 h. Dried protein samples were milled into powder, labeled salt-extracted protein-rich fraction (SP), and stored at room temperature for further analysis.

The percentage protein yield in both treatments was determined with Eq. 2.

$$\text{Protein yield}\,(\%) = \left[\frac{\textit{Weight of dried protein} - \textit{rich fraction}\,(g)}{\textit{Weight of sample before extraction}\,(g)}\right] x100 \tag{2}$$

2.4 Proximate analysis

All analysis for proximate composition was done in both the raw powder, defatted flour, salt-extracted (SP), and alkaline extracted protein-rich fractions (AP). The analysis was performed in triplicates according to the AOAC [40] methods. Kjeldahl method was followed for the determination of protein composition using a nitrogen-to-protein conversion factor of 6.25. The dry ashing method at 550 °C was used for the determination of ash, while the soxhlet method was used for the measurement of fat with petroleum ether. The oven-dry method (drying at 105 °C to constant weight) was adopted for moisture determination, while the crude fiber was evaluated following digestion with hot sulfuric acid (1.25% w/v) and hot sodium hydroxide (1.25% w/v). The residue obtained was oven dried (105 °C) for 2 h, weighed and ashed in muffle furnace. The fiber composition was calculated by weight difference.

2.5 Functional properties

2.5.1 Preparation of reagent

Different salt (NaCl) concentrations were prepared by dissolving 2, 4, 6, 8, and 10 g of sodium chloride in distilled water (100 mL) as described by Ndiritu et al. [25]. The salt solutions were then used for further study of functional properties of protein concentrate from African winged termite. Similarly, solutions of pH 2, 4, 6, 8, and 10 were also prepared using 0.1 M sodium hydroxide (NaOH) and 0.1 M hydrochloric acid (HCl). The emulsifying and foaming properties of raw, defatted, and each protein-rich fraction were evaluated by using these solutions with different pH and salt concentrations.

2.5.2 Emulsifying properties determination

Emulsifying capacity (EC) was determined following the method described in Adebowale et al. [21], with little modification. The dried sample (1 g) was added with 100 ml of each prepared salt and pH solution separately. The solution was mixed thoroughly for 10 min. Corn oil was added after 5 min. The mixture was centrifuged (3000 rpm for 10 min). Emulsion capacity was calculated using Eq. 3.

$$\text{Emulsion Capacity}\,(\%) = \left(\frac{VE}{Vs}\right) \times 100 \tag{3}$$

where; VE; is the emulsified layer volume, Vs; is the volume of the suspension.

The Emulsion stability (ES) was determined by mixing each of the raw, defatted and the dried protein-rich fractions (1 g) with 100 ml of each salt concentration and pH solution by following the method described in Ndiritu et al. [25]. The solution was mixed very well by using a mechanical shaker for about 10 min. Corn oil was added after 5 min and the resulting emulsion was heated (85 °C) for 30 min and allowed to cool to room temperature (27 °C). This was followed by centrifugation (3000 rpm) for 10 min. The volume of the emulsified layer was measured and recorded. The emulsion stability was calculated by using Eq. 4

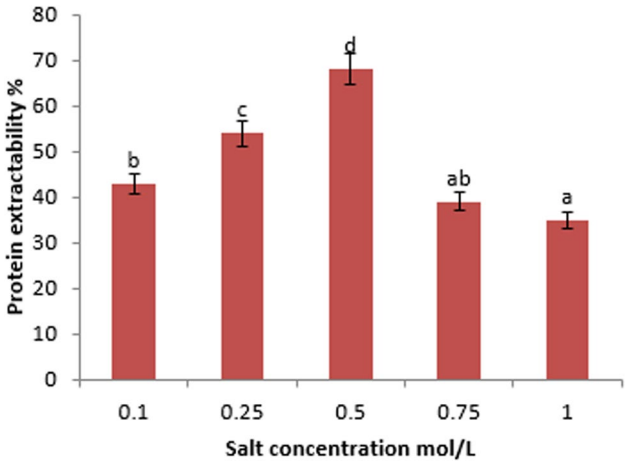


Fig. 2 Effect of salt concentration on *M. nigeriensis* protein extractability

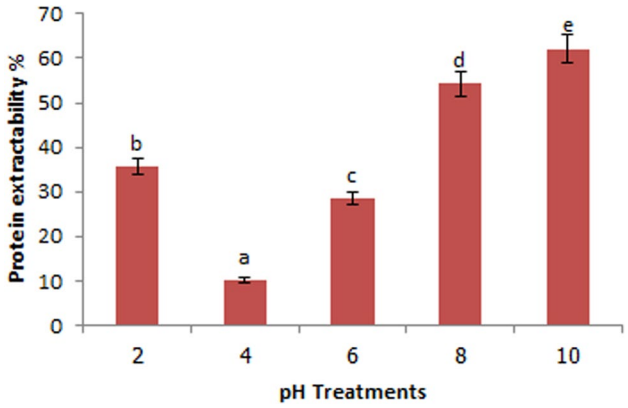


Fig. 3 Effect of pH treatments on protein extractability of *M. nigeriensis*

$$\text{Emulsion Stability}\,(\%) = \left(\frac{Ve}{Vx}\right) \times 100 \tag{4}$$

where: Ve = amount of emulsion layer, Vx = volume of the suspension.

2.6 Foaming properties determination

Foaming capacity (FC) and foaming stability (FS) were determined following the method described by Inyang and Iduh [41] with slight modifications. The raw, defatted and protein-rich fractions (10 g) were introduced into 100 ml of each salt concentration and pH solution and thoroughly mixed. The resulting suspension was blended with a laboratory blender for 2 min. The initial volume (V1) and the final volume after mixing (V2) were measured and recorded. FC was determined by using Eq. 5. The Foaming stability was evaluated as the volume of foam (V3) that remains after about 5 min by using Eq. 6.

$$\text{Foam Capacity}\,(\%) = \frac{(V2 - V1)}{V1} \times 100 \tag{5}$$

$$\text{Foam Stability}\,(\%) = \frac{V3 - V1}{V1} \times 100 \tag{6}$$

2.7 Statistical analysis

The data obtained was analysed by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) version 20. Results obtained were presented as the mean ± SD of triplicate determinations. Comparisons between

Table 1 Proximate composition (g/100 g) and yield of *Macrotermes nigeriensis* protein-rich fraction

Fraction	Moisture	Protein	Fat	Fiber	Ash	% Yield
Raw flour	9.42 ± 0.06^{a}	34.06 ± 0.44^{a}	28.36 ± 0.9^{a}	5.19 ± 0.02^{a}	4.06 ± 0.12^{a}	NA
Defatted	9.02 ± 0.24^{a}	42.12 ± 0.15^{b}	3.98 ± 0.02^{b}	5.60 ± 0.00^{a}	5.81 ± 0.14^{b}	76.20 ± 0.24^{a}
SP	8.68 ± 0.05^{ab}	68.68 ± 0.41^{c}	ND	ND	3.12 ± 0.01^{c}	32.04 ± 0.80^{b}
AP	7.35 ± 0.11^{b}	62.91 ± 0.53^{d}	ND	ND	2.78 ± 0.00^{c}	21.82 ± 1.2^{c}

Data is expressed as mean ± standard deviation of triplicate determinations. *NA* not applicable, Mean ± SD followed by different letters within each column are significantly different ($p \leq 0.05$). ND = not detected. *SP* salt-extracted protein-rich fraction, *AP*alkaline-extracted protein rich fraction

means were performed using the Least Significant Difference (LSD) test. Acceptable significant difference was placed at $P \leq 0.05$. Charts were prepared using the Microsoft® Office Excel 2010.

3 Result

3.1 Effect of different pH levels and salt conditions on protein extraction efficiency

The protein extractability in various salt concentrations and pH is shown in Figs. 2 and 3, respectively. An increase ($p \leq 0.05$) in protein extraction efficiency was noted with increasing salt concentration from 0.1 to 0.5 mol/L (Fig. 2). Beyond 0.5 mol/L, a sharp decline ($P < 0.05$) in protein extractability was observed which indicates that the maximum extraction (68%) was achieved at a salt concentration of 0.5 mol/L. On the other hand, protein extractability was lower (10.16%) at pH 4 (Fig. 3). Beyond pH 4, protein extractability gradually increased again ($p \leq 0.05$) with an increase in pH and was highest (62.10%) at pH10.0

3.2 Proximate composition and percentage yield

The proximate composition of raw, defatted, and protein-rich fractions of *M. nigeriensis* is shown in Table 1. The protein composition of the raw flour increased significantly ($p < 0.05$) from 34.06 ± 0.44 to 42.12 ± 0.15 g/100 g after the defatting (fat removal) process. Salt-extracted protein-rich fraction (SP) gave a higher concentration of protein (68.68 g/100 g) compared to alkaline-extracted protein-rich fraction (AP) (62.91 g/100 g). The moisture content ranged from 9.42 ± 0.06 to 7.35 ± 0.11 g/100 g in raw flour and alkaline- extracted protein-rich fraction, respectively. The ash composition for SP (3.12 ± 0.09 g/100 g) and AP concentrate (2.78 ± 0.10 g/100 g) were significantly lower ($p < 0.05$) than the raw (4.06 ± 0.12 g/100 g) and defatted samples (5.81 ± 0.14 g/100 g) of *M. nigeriensis*. However, the defatted protein fraction had the highest ash composition (5.81 g/100 g) compared with other fractions. Also, low composition in fat ($P < 0.05$) was observed in the defatted sample (3.98 g/100 g) compared with the raw flour (28.36 ± 0.9 g/100 g) following the hexane extraction process. However, no fat was detected for SP and AP protein fractions. In like manner, fiber composition improved (albeit $p > 0.05$) following the defatting process but none was detected in salt-extracted (SP) and alkaline-extracted (AP) protein-rich fractions, respectively, in comparison with the raw powder. The percentage yield of dried protein extract in AP and SP protein-rich fractions was 21.82 and 32.04%, respectively, which was lower ($P < 0.05$) than the yield obtained after the defatting process (76.2 ± 0.24%).

3.3 Effect of treatment at different pH and salt conditions on emulsion properties of *M. nigeriensis*

The emulsion capacity (EC) of all fractions (except AP which decreased up to 6% salt), slightly increased (albeit, $P > 0.05$) with increasing salt concentration up to 6–8% (Table 2). Beyond this concentration, emulsion capacity decreased for salt-extracted protein-rich fraction (SP) and raw sample but increased gradually for alkaline-extracted protein-rich fraction (AP). The highest emulsion capacity was seen in SP (~49 and 48%) and AP (44%) at 6–8% salt and pH 10.0, respectively, compared to the defatted fraction which gave lower values of emulsion capacity under salt influence. The emulsion capacities of the alkaline and salt-extracted protein-rich fractions were superior in the salt solutions than pH conditions.

Table 2 Effect of salt and pH conditions on emulsion capacity of *M. nigeriensis* protein-rich fraction (%)

Treatment	pH treatment					Salt conditions (mol/L)				
	2	4	6	8	10	2.0	4.0	6.0	8.0	10.0
Raw	28.12 ± 0.11^{b}	29.18 ± 0.03^{a}	25.37 ± 0.05^{b}	26.08 ± 0.02^{b}	33.62 ± 0.08^{b}	18.44 ± 0.06^{a}	21.52 ± 0.28^{a}	23.13 ± 0.15^{a}	18.82 ± 0.04^{a}	24.84 ± 0.11^{a}
Defatted	26.03 ± 0.32^{a}	28.88 ± 0.22^{a}	22.07 ± 0.13^{a}	24.17 ± 0.23^{a}	30.06 ± 0.03^{a}	31.22 ± 0.16^{b}	34.14 ± 0.23^{b}	35.69 ± 0.12^{b}	39.48 ± 0.14^{b}	37.19 ± 0.42^{b}
SP	41.21 ± 0.04^{c}	39.08 ± 0.18^{b}	33.74 ± 0.04^{c}	37.11 ± 0.07^{c}	42.62 ± 0.18^{c}	43.25 ± 0.19^{c}	45.61 ± 0.32^{c}	48.86 ± 0.56^{d}	47.74 ± 0.25^{d}	42.58 ± 0.06^{c}
AP	43.16 ± 0.27^{d}	39.21 ± 0.12^{b}	36.18 ± 0.33^{d}	39.96 ± 0.11^{d}	43.86 ± 0.28^{c}	49.22 ± 0.36^{d}	46.12 ± 0.10^{c}	44.14 ± 0.17^{c}	45.06 ± 0.02^{c}	46.28 ± 0.18^{d}

Data is expressed as mean ± standard deviation of triplicate determinations. Mean followed by different letters within each column are significantly different ($p \leq 0.05$). *SP* salt-extracted protein rich fraction, *AP* alkaline-extracted protein-rich fraction

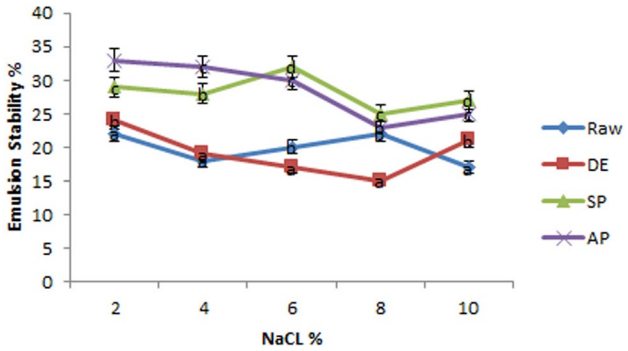


Fig. 4 Effect of different salt connditions on emulsion stability of *M. nigeriensis* protein-rich fractions

A significant ($P < 0.05$) decrease in emulsion capacity was observed from pH 2.0 towards pH 6.0 in both protein-rich fractions (SP and AP) followed by an increase beyond pH 6.0.

3.4 Effect of pH and salt conditions on emulsion stability of *M. nigeriensis* protein-rich fraction

The emulsion stability (ES) varied with each pH and salt treatment, and no consistent pattern was observed across all fractions, making the results somewhat challenging to interpret. However, a decrease in ES was seen for AP and defatted fraction up to 8% salt concentration followed by an increase at 10% NaCl. A significant ($P < 0.05$) increase in emulsion stability was recorded for salt-extracted protein-rich fraction (SP) up to 6% NaCl followed by a decrease at 10% (Fig. 4). Both AP and SP recorded the highest emulsion stability (33 and 32%) at 2 and 6% salt concentration, respectively, compared with the raw and defatted fraction. On the other hand, the pH effect on emulsion stability showed a similar pattern. The emulsion stability of AP and SP decreased with increasing pH up to pH 6.0. This was followed by an increase at pH 8.0 and then remained almost constant ($P > 0.05$) at pH 10 (Fig. 5). The highest emulsion stability was observed at pH 2.0 (35%) and pH 10.0 (32%) for AP and SP protein fractions, respectively. Again, alkaline and salt-extracted protein-rich fractions (AP and SP) were superior in emulsion stability at all pH values compared with the raw and defatted fraction which recorded lower values.

3.5 Effect of salt and pH conditions on foaming properties of *M. nigeriensis* protein-rich fraction

The foaming capacity (FC) of all protein extracts significantly increased ($P < 0.05$) with increasing salt concentration up to 6% (Table 3). Beyond 6% salt concentration, a reduction in FC was observed until 10% salt concentration. The foaming capacity of the protein-rich fractions was ($P < 0.05$) affected by salt concentration with salt-extracted protein-rich fraction (SP) having the highest value at 6% salt concentration. Similarly, foaming capacity in all fractions improved until pH 4.0 followed by a decline at pH 8.0 and then an increase again at pH 10.0. Salt and alkaline-extracted protein fractions were also dominant ($P < 0.05$) in foaming capacity at all pH treatments compared to the raw and defatted protein fractions.

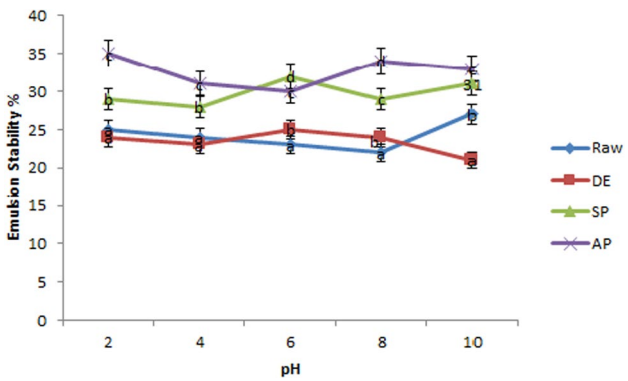


Fig. 5 Effect of different pH levels on emulsion stability of *M. nigeriensis* protein-rich fractions

Table 3 Effect of salt and pH conditions on foaming capacity of *M. nigeriensis* protein-rich fraction

Treatment	pH treatment					Salt conditions (mol/L)				
	2	4	6	8	10	2.0	4.0	6.0	8.0	10.0
Raw	10.83 ± 0.18^{a}	13.66 ± 0.11^{a}	9.33 ± 0.56^{a}	10.19 ± 0.22^{a}	12.16 ± 0.02^{a}	11.95 ± 0.08^{a}	15.63 ± 0.32^{a}	17.04 ± 0.14^{a}	16.44 ± 0.17^{b}	17.25 ± 0.19^{a}
Defatted	12.14 ± 0.06^{ab}	14.26 ± 0.03^{a}	10.58 ± 0.18^{a}	12.78 ± 0.44^{b}	14.86 ± 0.01^{b}	10.11 ± 0.42^{a}	16.11 ± 0.18^{a}	17.68 ± 0.12^{a}	14.39 ± 0.02^{a}	16.76 ± 0.05^{a}
SP	13.16 ± 0.12^{b}	18.06 ± 0.14^{b}	17.43 ± 0.07^{b}	15.45 ± 0.05^{c}	15.28 ± 0.29^{bc}	17.18 ± 0.12^{b}	18.74 ± 0.04^{b}	24.41 ± 0.26^{b}	22.39 ± 0.08^{c}	21.48 ± 0.33^{b}
AP	15.34 ± 0.02^{c}	18.22 ± 0.21^{b}	19.62 ± 0.12^{d}	17.36 ± 0.13^{d}	16.11 ± 0.16^{c}	16.66 ± 0.21^{b}	19.48 ± 0.22^{b}	23.64 ± 0.03^{b}	22.51 ± 0.18^{c}	20.77 ± 0.04^{b}

Data is expressed as mean ± standard deviation of triplicate determinations. Mean with different superscript abc are significantly different at ($P < 0.05$) along the columns. *SE* Salted-extracted protein-rich fraction, *AP* alkaline-extracted protein-rich fraction

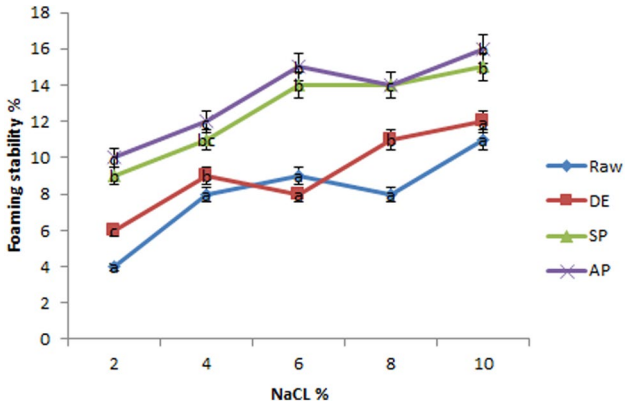


Fig. 6 Effect of salt conditions on foaming stability *M. nigeriensis* protein-rich fractions

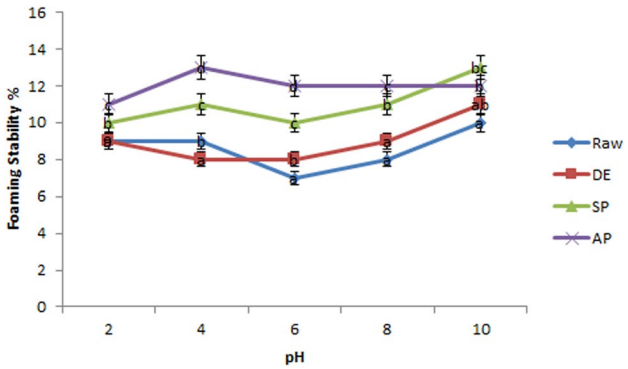


Fig. 7 Effect of different pH levels s on foaming stability of *M. nigeriensis* protein-rich fraction

3.6 Effect of different pH levels and Salt conditions on foaming stability of *M. nigeriensis* protein

The results in Figs. 6 and 7 showed a significant improvement in foaming stability ($P < 0.05$) of SP and AP protein fractions compared with the raw and defatted samples of *M. nigeriensis* under salt and pH treatments. The foaming stability of all fractions (except the defatted fraction) was seen to increase with increasing salt concentration up to 6% (Fig. 6). Beyond this, a reduction in foaming stability was noted at 8% and increased again at 10% salt. Again, AP protein fraction showed the highest foaming stability (16%) followed by SP (15%) at 10% NaCl compared to other fractions. A similar trend was observed at different pH ranges. Foaming stability increased ($P < 0.05$) until pH 4, which was followed by a reduction at pH 6, then increased again at pH 8. The maximum foaming stability was noted for AP (12%) and SP (13%) at pH10. (Fig. 7).

4 Discussion

4.1 Effect of different pH levels on protein extractability from *M. nigeriensis*

The extraction of protein from its natural source depends on the solubility of the protein in the extracting solvent [26, 27]. Protein solubility is also a useful indicator of protein characteristics when incorporated into food products during food processing [27]. The result obtained from this present study showed that the extractability of *M. nigeriensis* protein was dependent on pH and salt concentrations. There was a significant increase ($P < 0.05$) in protein extractability with increasing pH. The highest protein extractability (62.1%) was recorded at alkaline pH 10. This could be attributed to the unfolding of the structure of the protein at alkaline pH thereby exposing the hydrophilic (water-loving) groups of the proteins [22, 25]. According to the reports of Pan et al. [12], non-protein components that interfere with protein extraction are dissolved at alkaline pH, resulting in improvement in protein extraction and recovery after precipitation. Another possible reason could be due to the improved ionization of amino acids at

alkaline pH which leads to an increase in protein solubility [25, 26]. At alkaline pH, the protein–protein interactions reduce while the protein-water interactions increase due to the negative net charges (i.e., COO- group) which are present at the surface of molecules of protein [42]. In this condition, the protein's net charged molecules (positive and negative) interact effectively with water. These interactions could further explain the increase in protein extractability observed in our study at alkaline pH. Similarly, to our result, different authors [11, 23, 36] have all equally reported improved protein extraction from different species of edible insects at alkaline pH (8–12). For instance, in a study conducted by Purschke et al. [37] on *migratory locusts,* the maximum protein extractability (78%) was observed at pH 9 which was the same level as egg-white protein. In like manner, the solubility of Cricket (> 80%), and silkworm pupae (*B. mori*) (~ 70%) protein was highest in alkaline pH 9–11 respectively compared to 5–15% achieved under acidic conditions (pH 4–5) [11, 32, 36]. Also, the solubilization of grasshopper proteins at alkaline pH 12 (without thermal treatment) led to an increase in protein extraction [31]. Contrary to our result, Nahar et al. [42] noticed a sharp decline in protein solubility of broiler chicken meat at alkaline pH 8.0–9.0. It is important to recall that extreme pH conditions (especially > pH 10) could cause amino acid racemization of L to D- isomer thereby making amino acid unavailable [43, 44, 45] . Also, high alkaline pH can initiate the synthesis of undesirable compounds like the nephrotoxic lysine-alanine complexes, which are produced from lysine and dehydro-alanine following serine and cysteine degradation [43, 46]. High alkaline solutions have also been linked with protein discoloration due to the oxidation of polyphenols (found in many insects) to quinones including protein denaturation [24, 46]. Hence, alkaline pH beyond pH 10 was not included in the present study. The decrease in protein extractability (10.16%) recorded at pH 4.0 in this study supports previous studies on mealworm and black soldier fly protein isolates [32, 36]. This could be due to decreased repulsion between the amino acids leading to more coalescence towards the isoelectric point (IP) [26]. Proteins have positive charges at a pH below their IP and negative charges at a pH above the isoelectric point. Therefore, the pH at which the net charge of a protein molecule is zero is referred to as the isoelectric pH. In addition to that, low protein extractability has also been observed in proteins due to denaturation at a low pH [30]. Poor extractability of mealworm, black soldier fly, soybean, and meat proteins at pH 4.0–5.0 (5–15%) has also been reported by other authors [32, 36, 37]

4.2 Effect of salt conditions on protein extractability from *M. nigeriensis*

The extractability of protein from a natural source also relies on its solubility in a salt solution, a key factor influencing protein functional properties [42]. A significant ($P < 0.05$) increase in protein extractability (68%) was observed with increasing salt concentration up to 0.5 mol/L. Beyond this concentration, the protein extraction efficiency of termite protein decreased ($P < 0.05$) which shows that the highest protein extractability (68%) was achieved at a salt concentration of 0.5 mol/L. The association between salt concentration and the solubility of protein could be explained by the salting-in and salting-out phenomena. The different conformational characteristics of proteins in different salt solution concentrations could cause differences in solubility [26, 33]. At low salt concentrations, protein fractions begin to solubilize due to low ionic strength [33]. At this point, the charged amino acids that are found on the protein surface relate with the ions from salt and water molecules, hence increasing protein solubility. This process is known as salting-in. In other words, low ionic strength enhances protein charge which improves protein solubility. When the salt concentration exceeds certain optimum levels, protein solubility decreases due to high ionic strengths [25, 27]. The addition of more salt weakens the electrostatic repulsion by screening the charges, thereby forming protein aggregates and decreasing the solubility (known as salting out). In addition, to charge neutralization, another possible reason for the decrease in protein extractability beyond 0.5 M salt concentration could be attributed to the competition between ions of salt and the charged molecules of protein for binding molecules of water. High concentration of mineral ions in solution could reduce the availability of water molecules in the solution resulting in a decrease in protein hydration and increased hydrophobic interactions [47]. The result is consistent with previous reports in the literature on different insect species [11, 25, 32] and chicken meat [42] at different salt concentrations. This observation indicates the potential of utilizing proteins from *M. nigeriensis* as an alternative ingredient in the preparation of meat-based products.

4.3 Proximate composition of *M. nigeriensis* protein-rich fractions

Proximate composition is regarded as an important determinant of the nutritional values and quality of food. From the result obtained, both salt-extracted and alkaline-extracted protein-rich fractions (i.e., SP and AP) have higher composition in protein (68.6 and 62.9 g/100 g) than the raw (34.06 g/100 g) and defatted samples (42.12 g/100 g). Salt treatment

resulted in significantly higher ($p \leq 0.05$) protein concentration (68.68 g/100 g) than other fractions (alkaline and defatted). This observation is in line with the study of Illingworth et al. [26], showing that salt treatment and micellization resulted in more protein precipitation from *Moringa oleifera* seed than isoelectric precipitation. Alkaline-extracted protein-rich fraction (AP) gave a protein concentration of 62.9 g/100 g which is considerably similar to the result (60–70%) obtained by Brogan et al. [11] from two insect species (Cricket and Locust). A study by Mohan and Mellem [48] also reported similar observations from hyacinth bean protein extract. In their report, salting treatment resulted in higher protein extraction (87.8%) than alkaline extraction (84.4%). The higher protein composition seen in both conditions could be due to increased protein–protein interactions during the extraction process, which caused the greater exclusion of non-protein material from the solution. The protein composition of *M. nigeriensis* recovered in both isolates is similar to the protein extract from soybean (60–90 g/100 g), a commercially available plant-based protein [49]. A significant improvement in protein composition ($P < 0.05$) from 34.06 to 42.12 g/100 g (i.e., ~ 22% increase) was observed following fat removal from termite flour. A similar observation was noted by Choi et al. [10] where the defatting process improved the protein composition of three edible insect species (Mealworm, Cricket, and Silkworm pupae) from 33.46 up to 62%. In like manner, a recent report by Asen et al. [50] and Uddin et al. [51], showed that the removal of lipids from raw peanuts also increased the protein composition from 28.7 to 51.58 and 25 to 53%, respectively. Mintah et al. [52], also reported a similar observation in the black soldier fly (*Hermetia illucens*) where protein composition increased from 42.00 to 55.98 g/100 g following fat removal. This shows that *M. nigeriensis* protein could be a potentially good source of high-quality protein.

The moisture composition of both alkaline (7.35 g/100 g) and salt-extracted protein-rich fraction (8.68 g/100 g) were lower (albeit $p > 0.05$) when compared to the raw (9.42 g/100 g) and defatted samples (9.02 g/100 g). This observation is in line with the report of Illingworth et al. [26] showing lower moisture content from plant-based protein isolates. The low moisture content recorded for the protein isolates was around the safe water activity range (i.e., < 6%), thereby reducing the risk for moisture-related problems, especially microbial proliferation [18]. The growth of bacteria and mold in flour is usually encouraged by high moisture composition. This could reduce the stability and shelf-life capacity of the protein dry extract [8]. High fat composition (28.36 g/100 g) was seen for raw *M. nigeriensis* which was reduced to 3.98% after the defatting process. This is similar to the 24.81 g/100 g obtained by Anyiam et al. [18] on *M. nigeriensis* dried flour before fermentation. The defatting step was necessary due to the high fat composition of *M. nigeriensis* which can interfere with the protein extraction procedure [35]. Moreover, lipid extraction has been shown to improve certain functional characteristics of insect-based protein isolates [10]. The absence of fat and fiber on the protein extract could be due to the defatting processing method which removed most of the fat and fiber observed in the raw sample. This finding supports the work of Adebowale et al. [21] and Illingworth et al. [26], who reported the absence of fat in *Moringa oleifera* and Bambara groundnut protein isolates, respectively using similar extraction methods as we used in this study. The presence of fiber in the raw and defatted protein fraction could be explained by the composition of chitin, a non-digestible polysaccharide that makes up the exoskeleton of insects. Chitin provides structural support and protection, similar to how cellulose and hemicellulose function in plants.

The presence of ash is an indication that *M. nigeriensis* protein may contain some level of minerals. The lower ash composition recorded for the SP and AP protein-rich fractions (3.12 and 2.78 g/100 g) could be due to the loss of minerals during the extraction process. The same reasoning could also be applied to the significantly ($P < 0.05$) low protein recovery yield observed in both methods (32.04 and 21.82%) compared to the defatted sample with a 76.20% yield. This could also be explained by the removal of impurities and other nutrients during the protein extraction process. The extraction yields of protein obtained in this study (21.82–32.04%) are in the same range as Miron et al. [32], who obtained an extraction yield of 37.22 and 24.30% from BSF larvae. However, the extraction yields are lower compared to yields (41–61%) reported by Mintah et al. [52], from *Hermetia illucens* protein isolate.

4.4 Effect of different salt and pH conditions on emulsion properties of *M. nigeriensis* protein

Proteins play a vital role in the functionality of food as well as in pharmaceutical products [42]. Emulsion capacity is defined as a measure of the number of oils per gram of protein that can be emulsified, while emulsion stability refers to the ability of an emulsion to minimize or resist phase separation over a specific time [25]. The net charge of the lipophilic-hydrophilic interphase determines the emulsifying properties of the protein [21]. Variations in salt concentration and pH significantly ($P < 0.05$) affected the emulsion capacity and stability of the protein isolates of *M. nigeriensis*. The alkaline and salt-extracted protein-rich fractions possess better emulsion capacity (EC) and emulsion stability (ES) than the raw and defatted flour at all pH and salt concentrations used in this study. The highest EC (49, 42%) and ES (35, 32%) were found for AP and SP protein fractions at pH 10.0 and 2% salt concentration, respectively. Similarly, the protein extraction

of Crickets (*Acheta domesticus*) recorded 41.7% emulsion capacity and 33.6% emulsion stability [25]. However, our values are lower than the EC of 70 and 80% reported in *T. molitor and* Grasshopper, respectively [31, 43]. Differences in insect species and extraction methods could account for the differences observed in emulsification properties observed. Also, like our report, proteins that were extracted from defatted mealworm powder through alkaline method showed improved emulsifying properties at alkaline pH, and temperature stability [53]. This could be attributed to the higher protein concentration in the protein isolates compared with raw and defatted flour which have lower protein composition. An increase in protein concentration has been linked with the enhancement of emulsion activities [54]. This could be due to the combination of factors which includes the amphiphilic nature of proteins, increased surface activity and viscosity. Higher protein concentrations reduce interfacial tension and also increase the viscosity of the continuous phase [54]. This prevents droplet movement and coalescence. The lower emulsion properties seen in defatted flour compared to the isolates could be due to the effect of hexane used in fat extraction. Previous reports have shown that emulsion capacity and stability might reduce after defatting step as the process might increase surface hydrophobicity and further protein aggregation [55].

With increasing salt concentration, the emulsion capacity (EC) of the alkaline-extracted protein-rich fraction decreased ($P < 0.05$) up to 6%. However, the emulsion capacity of salt-extracted protein-rich fraction increased (albeit $P > 0.05$) at 6% and then decreased at 10%. The highest EC (49%) was seen at 6% salt concentration. Similar observation was also recorded for the emulsion stability (ES) of all protein isolates from *M. nigeriensis* where the isolates are superior to the defatted flour. This could be attributed to the improved amphiphilic characteristic of the protein isolate which supports the formation as well as the stabilization of emulsions through the reduction of surface tension at the fat–water interface. Our finding is not in isolation but supports previous studies in the literature for cricket, locust and African palm weevil protein isolates [11, 54]. Proteins can function as an emulsifier due to the presence of both the hydrophilic and hydrophobic groups that can interact with water and oil in food systems respectively. The improved emulsion properties suggest that *M. nigeriensis* protein extract could comprise both polar and non-polar amino acids which can increase both oil and water molecules interactions.

Variations in pH also affected the emulsion properties of the protein in the study. Both protein isolates showed a similar trend in emulsion capacity and stability under pH influence. For example, emulsion stability for both isolates was highest (33%) at alkaline pH 10. Similarly, Purschke et al. [37] demonstrated an increase in emulsifying activity of *migratory locust* at alkaline pH 8.0. The higher values of emulsion capacity in protein fraction at a pH 10 might be due to the greater protein composition, enhanced protein solubility, and/or due to the presence of hydrophobic groups in the protein extract of the insect [56]. The lower emulsion activity observed at acidic $pH > 2.0$ could be attributed to the coalescing and precipitation of protein due to lower electrostatic repulsion between the protein molecules and reduction of oil–protein interaction [57]. The stability of emulsion is preferable in food processing applications for new product development. Despite the low emulsifying properties observed in our study, *M. nigeriensis* protein can still be effectively used in applications where emulsification is less critical, such as in baking and confectionery (e.g., in bread making) to enhance texture and increase protein composition. However, for broader applications and improved functional stability, additional purification of *M. nigeriensis* protein may be necessary, highlighting the need for further research in this area.

4.5 Effect of different salt and pH conditions on foaming properties of *M. nigeriensis*

As emulsifying properties, the foamability is also influenced by intrinsic and extrinsic factors (such as (hydrophobic groups, pH, and ionic strength). Foamability is very important in maintaining the organoleptic properties of food such as constituency and appearance. The foaming capacity (FC) and foaming stability (FS) of *M. nigeriensis* protein isolates were significantly ($P < 0.05$) affected by salt concentrations and pH. The highest FC (19, 24%) and FS (13, 16%) were noted at pH10.0 and 6% salt concentration respectively for protein isolates. Both protein isolates were dominant in foaming capacity at alkaline pH and low salt concentration compared to raw and defatted which recorded lower values. This could be explained by the improved protein flexibility at alkaline pH, which results in increased net charge on the molecules of the protein that reduces the strength of its hydrophobic interactions [47]. The foaming stability of *M. nigeriensis* protein isolate from our study (13–16%) was lower than those reported previously for Lupin proteins (30%) [23], *T. molitor* larvae salt-assisted protein isolate (65.59%) [33] and Cricket powder (86–155%) [35, 58]. However, our result is higher than the foaming stability of 8.57% reported by Chatsuwan et al. [59] for grasshopper species (*Patanga succincta)* while adult mealworm protein isolate was identified as non-foaming. From these observations, it means that *M. nigeriensis* protein isolates may need additional purification to further improve and stabilize the foaming properties at

different processing conditions. The greater foaming property seen in the protein isolates towards alkaline pH could be attributed to the higher protein composition of the protein isolates. According to Ogunyika et al. [60], increased protein composition increases viscosity which in turn encourages the formation of cohesive protein layers hence enhancing protein foamability. The initial increase in foaming activity to 6% salt concentration could be caused by the improved protein solubility. The decrease in foaming capacity towards 10% salt could be due to the salting-out effect of NaCl [22, 33]. Similarly to our report, Nafisa et al. [61] observed that the addition of salt up to 0.6 M concentration improved the foaming properties of tree locusts followed by a decrease in foaming properties beyond this salt concentration. This shows the potential of *M. nigeriensis* proteins as an alternative ingredient in food processing. However, due to of their limited foaming properties, proteins derived from *M. nigeriensis* can be incorporated into baked foods, cookies, and snacks to increase protein composition and nutritional value without affecting the texture or foaming characteristics of these products. The differences in functional properties between salt-extracted and alkaline-extracted protein fractions in our study are likely due to the distinct extraction methods used. Salt extraction affects proteins with ionic interactions [26, 33], while alkaline extraction disrupts protein structures by breaking ionic bonds and altering charge distribution.

5 Conclusion

The study found that protein extractability from *M. nigeriensis* was more effective using an alkaline solution (pH 10.0) or a salt concentration of 0.5 M. Protein fractions obtained under these conditions exhibited higher protein composition and better emulsifying and foaming properties compared to the raw and defatted fractions. Additionally, the salt-extracted protein-rich fraction outperformed the alkaline-extracted protein-rich fraction in terms of protein yield and functional properties.This suggests that *M. nigeriensis* could serve as a promising alternative protein source for food formulations. However, the protein fractions may not be ideal for all food processing applications due to their limited emulsion and foaming properties. They are suitable for specific uses where these properties are less critical, such as in snacks, cookies, and bread. The variations in functional properties between salt-extracted and alkaline-extracted protein fractions indicate that extraction methods may also influence the protein profile, underscoring the need for further investigation.

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Data availability The data that supports the findings of this study are available on request.

Declarations

Competing interests The authors declare no competing interests.

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