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NUTRITIONAL ENHANCEMENT OF SPROUTED CEREAL FLOURS WITH *Macrotermes subhyalinus* and *Cirina butyrospermi*: A STRATEGY FOR COMBATING MALNUTRITION

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ABSTRACT

Malnutrition, a significant global health concern, necessitates innovative solutions to enhance food nutritional profiles sustainably. This study focused on augmenting the nutrient content of traditional cereal flours, specifically corn (Ma) and millet (Mi), by incorporating edible insects, *Macrotermes subhyalinus* (MS) and *Cirina butyrospermi* (CB) known for their high protein, vitamin and mineral levels. The research aimed to create fortified flour formulations by adding these insects at various proportions (20%, 22.5% and 25%), thus addressing nutritional deficiencies common in staple diets. The study employed a comprehensive methodological approach incorporating biochemical analyses to measure protein, lipid content, pH and vitamin C levels in 16 distinct flour blends. These measures provided a basis for evaluating the nutritional enhancement achieved through insect incorporation. Additionally, nutrient density calculations and statistical analyses including analysis of variance (ANOVA) and *post-hoc* tests were conducted to assess the overall nutritional value and identify significant differences among formulations. Results demonstrated a marked improvement in the nutritional quality of cereal flours with insect addition. Formulations MiMS25 (75% Millet + 25% MS) and MiCB25 (75% Millet + 25% CB) exhibited the highest mineral content, with ash values of 3.69 ± 1.00% and 2.60 ± 0.28%, respectively. Protein levels were significantly increased in MaCB22.5 (77.5% Corn + 22.5% CB) and MiCB25 showing concentrations of 18.78 ± 0.20% and 18.66 ± 0.10%. Furthermore, the inclusion of MS notably enhanced lipid content, particularly in MiMS25 which displayed an energy value of 451.19 kcal. Among the tested blends, MaCB22.5 was identified as the most nutritionally promising with a nutrient density score of 0.255 indicating a balanced and improved nutritional profile. This study underscores the potential of integrating edible insects into cereal flours as a sustainable strategy to combat malnutrition. It highlights the need for further research into optimizing these formulations for consumer acceptance, taste, and shelf-life aiming to incorporate them into mainstream diets to achieve global nutritional well-being.

Key words: Edible insects, Nutrient Density, Malnutrition, Sprouted cereals, Biochemical analyses, *Macrotermes subhyalinus*, *Cirina butyrospermi*

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INTRODUCTION

Malnutrition remains a critical and urgent global public health issue, affecting an estimated 9.2% or 735 million people worldwide [1]. This complex problem is particularly acute in African nations, where nearly one in five individuals contends with food insecurity, leading to severe repercussions on both human development and overall quality of life [2,3]. In such geographically and economically disadvantaged regions, the primary staple foods are often derived from cereal-based flours, such as corn and millet. Regrettably, these staple flours frequently lack the essential nutrients vital for human health, including proteins, lipids and a range of critical micronutrients [4]. This nutritional inadequacy can result in a wide array of diseases, including but not limited to beriberi, scurvy, rickets, xerophthalmia, kwashiorkor and marasmus [5]. The situation highlights an urgent need for interventions that can provide balanced nutrition to vulnerable populations.

One innovative approach that has gained increasing attention in scientific communities is the fortification of these cereal-based flours to enhance their nutritional value [6]. Among the most noteworthy strategies in this domain is the incorporation of edible insects such as *Macrotermes subhyalinus* (winged termite) and *Cirina butyrospermi* (shea caterpillar) into flour formulations. These insect species are rich in essential nutrients, including high-quality proteins, beneficial lipids and essential micronutrients [7,8]. It is noteworthy to mention that the nutrient compositions in these insects can differ substantially, as influenced by a multitude of factors such as the insect's order, species, developmental stage and even their dietary habits [9]. Yet, despite growing interest and emerging evidence supporting the nutritional benefits of insect-fortified flours [10], the field still lacks comprehensive, systematic and comparative analyses that evaluate the nutritional attributes of such flours. There are limited studies that incorporate nutrient density scores in their methodology, posing a substantial challenge for policymakers who rely on evidence-based strategies to formulate effective public nutrition programs [11]. Faced with this observation, the present study aims to provide a robust assessment, comparing the total nutritional density of corn and millet flours enriched with the aforementioned insect species, *Cirina butyrospermi* and *Macrotermes subhyalinus*. The results will help to influence public health policies, while providing valuable information to consumers concerned about their nutrition.

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MATERIALS AND METHODS

Biological material

The biological material included germinated maize and millet, as well as winged termites of the species *Macrotermes subhyalinus* (Termitidae) and larvae of the species *Cirina butyrospermi* (Saturniidae). The corn and millet came from the primary market in Daloa, Côte d'Ivoire, and were then germinated under controlled laboratory conditions for analysis. On the other hand, specimens of *Macrotermes subhyalinus* and *Cirina butyrospermi* were collected in the town of Korhogo and identified by a qualified entomologist before undergoing a drying process (45-50°C for 4 days) to prepare them for incorporation into flours of cereals.

Production of sprouted cereal flours (Corn and Millet)

Flour production followed the methodology outlined by Angaman *et al.* [2]. Initially, corn and millet grains underwent a sprouting process, which entailed a 24-hour soaking period in water. Following this, the grains were drained, rinsed and evenly spread over a cloth on trays, where they were left to sprout at room temperature for three days. Subsequently, these sprouted grains were transferred to an oven and dried at 50-55°C for an additional three-day period. Post-drying, the grains were manually de-germinated and pulverized using a Moulinex BLENFORCE1 600W blender. The resultant material was then sifted through a 500 µm mesh sieve, and the collected flours were securely stored in airtight containers. To ensure optimal preservation, all flour samples were stored at a room temperature until further analysis, in accordance with established best practices for food sample preservation [12].

Production of insect powders

Insect (*Macrotermes subhyalinus* and *Cirina butyrospermi*) powders were obtained using the same method described by Angaman *et al.* [2]. The insects were crushed using a blender (Moulinex BLENFORCE1 600W), and the powders obtained were kept in separate bowls and conditioned at room temperature until analysis.

Enrichment of local flours

The enrichment process of sprouted corn and millet flours was carried out, strictly adhering to the scientific methodology outlined by Niaba *et al*. [13]. In this procedure, powders derived from *Macrotermes subhyalinus* (MS) and *Cirina butyrospermi* (CB) were incorporated into the base flours. The inclusion levels were selected, and the powders were added at varying proportions to create distinct blends. Specifically, the insect powders were integrated at three different concentration levels: 20%, 22.5% and 25% by weight of the total flour content, as detailed in Table 1. These proportions were chosen to optimize nutritional benefits while maintaining palatability.

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Biochemical analysis methods
Physicochemical parameters were ascertained using the standard methodologies described by Angaman *et al*. [2]. Both pH and moisture content were measured in accordance with the association of official analytical chemistry (AOAC) methods [14]. Dry matter content was calculated by subtracting the moisture percentage from 100 [15]. Total ash content was evaluated using a muffle furnace set at 550 °C, also following AOAC guidelines [15]. Titratable acidity was assessed through titration using a 0.1 N sodium hydroxide solution, with phenolphthalein as the indicator [14]. Additionally, the Brix degree was established based on the method by Dadzie and Orchard [16], employing an Atago N-1α, Model N refractometer (McCormick Fruit Tech).

In terms of nutritional potential, total lipid content was determined through solvent extraction using hexane as the solvent in a Soxhlet apparatus [17]. Vitamin C content in the formulated flours was measured following the methodology proposed by Feszterová *et al.* [18]. Crude protein content was ascertained from nitrogen levels using the Kjeldahl method [19]; the obtained nitrogen content (N) was then multiplied by a factor of 6.25 to calculate the crude protein content (P). Total carbohydrates (TC) were computed by deducting the sum of moisture, proteins, lipids and ash percentages from 100. Finally, the energy values were calculated using the Atwater and Benedict factors [20], including 4 for proteins, 9 for lipids and 4 for carbohydrates.

Methods for calculating Nutrient Density Score
Nutrient density refers to the ratio of nutrient content to energy content in a given food item, facilitating a balanced comparison across different flour types. This metric was calculated by aggregating the normalized values for each nutrient on a scale from 0 to 1, as per the methodology outlined by Drewnowski [21]. The formula employed for this calculation is: Nutrient Density = Nutrient Content / Energy Value.

The criteria chosen for ranking included protein content, lipid content, vitamin C content, carbohydrate content, and energy value. Upon calculation, the composite score for each flour type was derived by averaging their respective nutrient densities.

Statistical analysis of data
The initial data for the study were generated using Microsoft Excel and subsequently imported into R software, version 4.2.0 [22], for further analysis. A multivariate analysis of variance (MANOVA) was conducted to compare the mean values and to assess the presence of statistically significant differences among the samples under investigation. Additionally, radar charts were employed to visualize

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the nutrient density results, a technique specifically endorsed for multi-dimensional representation in the field of nutrition [23].

RESULTS AND DISCUSSION

Nutritional potential of formulated flours

The physicochemical properties of the various flour formulations are meticulously detailed in Table 2. The integration of *Macrotermes subhyalinus* and *Cirina butyrospermi* powders into the formulations led to perceptible compositional changes in both sprouted corn and millet flours. However, it is essential to note that the magnitude and nature of these modifications were highly dependent on the specific types of insects and grains involved in the formulation. For example, adding powders of both insect species to millet flours generally resulted in a decrease in pH. Notable exceptions to this trend were the MaCB22.5 (4.26±0.01) and MiCB25 (4.93±0.02) samples, which behaved differently. This variation in pH of formulated flours is significantly influenced by the acidic properties of insect powders and the chemical reactions occurring during processing and storage [2]. Additionally, enriching millet flours with these insect powders led to a significant increase in titratable acidity of millet-based flours, with values ranging from 19.82±3.9 mEq/100g in the pure millet sample (Mi100) to as high as 55.26±4.05 mEq/100g in the MiCB22.5 sample. This observed increase in acidity aligns well with previous findings reported by Angaman *et al*. [2]. According to these authors, increased acidity levels, when coupled with low moisture content, can significantly enhance the preservation qualities of flour. This is primarily because the acidic environment offers added protection against microbial degradation [13,24], thereby ensuring an extended shelf-life and better product stability. On the flip side, the incorporation of these insect powders into corn flours led to a decrease in titratable acidity, which dropped from 73.01±5.59 mEq/100g in pure corn (Ma100) to 53.04±2.1 mEq/100g in MaMs20. The reduction in acidity may enhance the taste and also suggest improved digestibility of grain flour products. This could be particularly beneficial for individuals with sensitive stomachs or digestive issues [25]. Furthermore, a comparative analysis between native corn flours and their insect-enriched counterparts reveals noteworthy elevations in protein, lipid and vitamin C content. Concurrently, there was a marked decrease in carbohydrate levels (Figure 1). This is attributable to the observed carbohydrate content in insects being lower than that found in cereal matrices [2]. Similar physicochemical trends were also observed for millet flours, including a significant increase in ash content (Figure 2). These findings collectively substantiate the pivotal role that insects can play as valuable, locally available sources of essential nutrients. Hence, food fortification with insects emerges as an effective strategy for mitigating nutrient deficiencies in staple foods [2, 26]. Lastly, the observed decrease in vitamin C content in enriched millet

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samples could potentially be attributed to the specific cereal varieties utilized in the study.

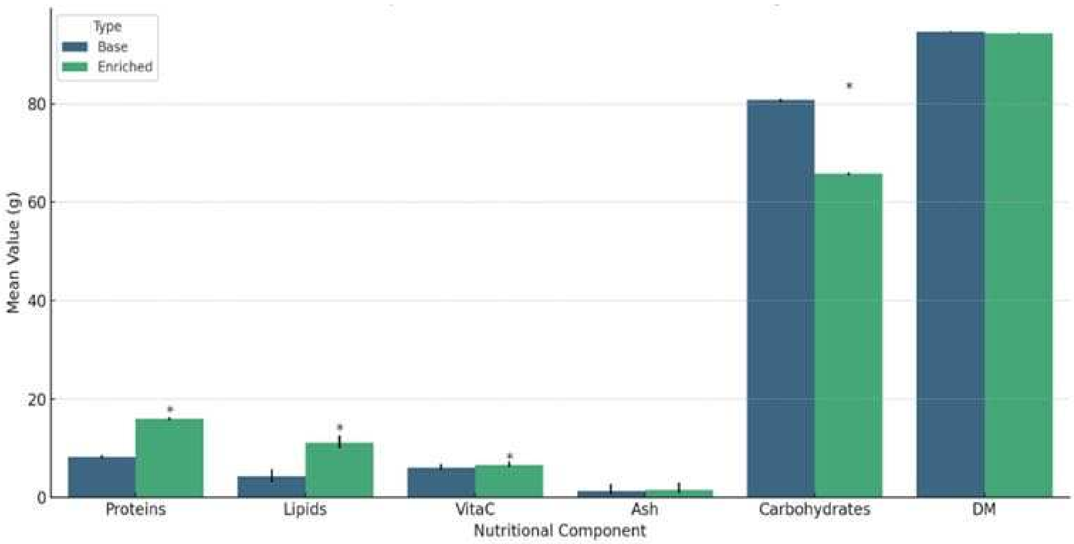

Figure 1: Comparison of the nutritional composition (Proteins, Lipids, Vitamin C, Ash, Carbohydrates and Dry matter) of basic sprouted corn flours versus insect-enriched flours

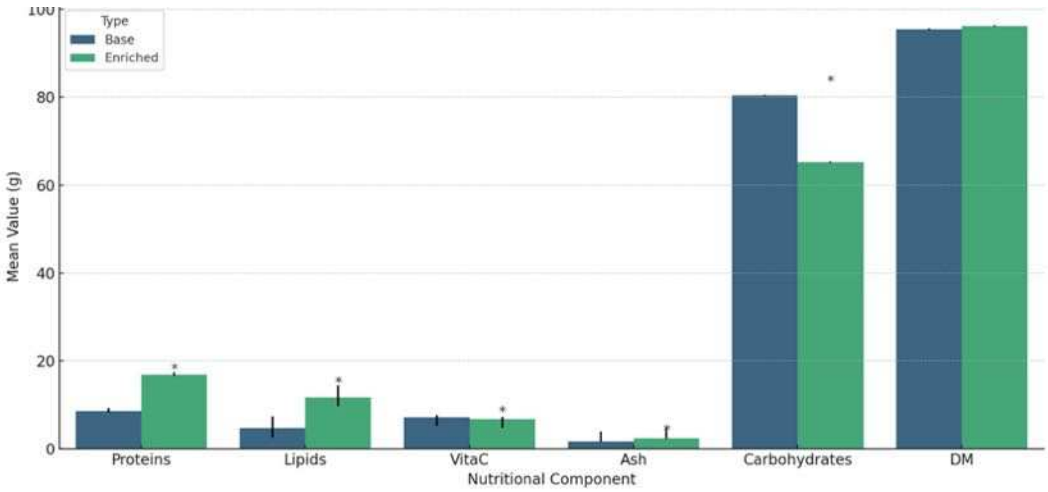

Figure 2: Comparison of the nutritional composition (Proteins, Lipids, Vitamin C, Ash, Carbohydrates and Dry matter) of basic sprouted millet flours versus insect-enriched flours

Ash content:

The addition of insect powder to the enriched flours significantly impacted the ash content, with variations dependent on both the rate of incorporation and the insect species used. For example, samples MiCB25 (2.60±0.28%) and MiMS25 (3.69±1.00%) exhibited increased ash content, potentially beneficial for addressing mineral deficiencies, especially in growing children and lactating mothers. In contrast, samples MaMS20 (0.99±0.52%) and MaMS22.5 (1.06±0.41%) showed reduced ash content. Notably, *Macrotermes subhyalinus* (6.78±0.80%) had a higher ash content compared to *Cirina butyrospermi* (4.87±0.01%). A decline in carbohydrate levels in enriched flours was observed, primarily due to the low carbohydrate content in the insect species used, aligning with previous research findings [2,13,27]. However, insects are known to contain substantial amounts of immune-boosting polysaccharides [28,29], which also serve functional roles in the food industry by altering food texture and consistency. It is important to note that some of these polysaccharides, such as chitins, may cause allergies and food intolerances [30].

Proteins:
Proteins, essential for various physiological processes, including growth and immunity, were abundant in the formulated products, making them promising nutritional sources [31]. These enriched flours could be instrumental in alleviating protein deficiencies, a prevalent form of malnutrition in Africa [32]. For instance, flours enriched with *Cirina butyrospermi* demonstrated notably high protein levels (Table 2), although lower than those of maize flour enriched with *Oryctes owariensis* [2]. The variance in protein levels underscores the diverse nutritional potential inherent to different protein sources, which can differ significantly depending on regional factors [33].

Lipids:
The lipid content showed a significant increase ($p<0.05$) in enriched flours (Table 2), more so with the incorporation of *Macrotermes subhyalinus* powder, particularly in samples MaMS25 (15.12±0.64%) and MiMS25 (16.65±0.54%). These lipid levels are essential for the absorption of fat-soluble vitamins and carotenoids and are rich in omega-6 and 3 fatty acids, crucial for cognitive health [7,34]. While these lipids serve as energy sources, evidenced by their high caloric values (Table 2), excessive consumption of saturated fatty acids could be health-detrimental, particularly in relation to cardiovascular diseases [35].

Correlations between the nutritional parameters of prepared flours
Figure 3 elucidated the interrelationships among the various components of the formulated flours. A notably strong positive correlation of 0.95 exists between lipid content and the estimated energy value. This high correlation is attributable to the energy-dense nature of lipids, their effectiveness as an energy source, and their significant contribution to the overall caloric content of the flours. Conversely, carbohydrates exhibit a negative correlation with several other components: lipid content (-0.80), energy value (-0.68), protein content (-0.65) and ash content (-0.50). According to existing literature, these inverse relationships among the nutritional elements are influenced by several factors: the initial lipid, protein, and

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carbohydrate levels in the ingredients, the processing methods employed, and the proportions in which these ingredients are combined [36,37]. In essence, these correlations are not arbitrary but are the outcome of deliberate manipulations in the nutritional composition. These adjustments aim to meet specific nutritional or functional goals while ensuring the final product remains balanced and nutritionally effective.

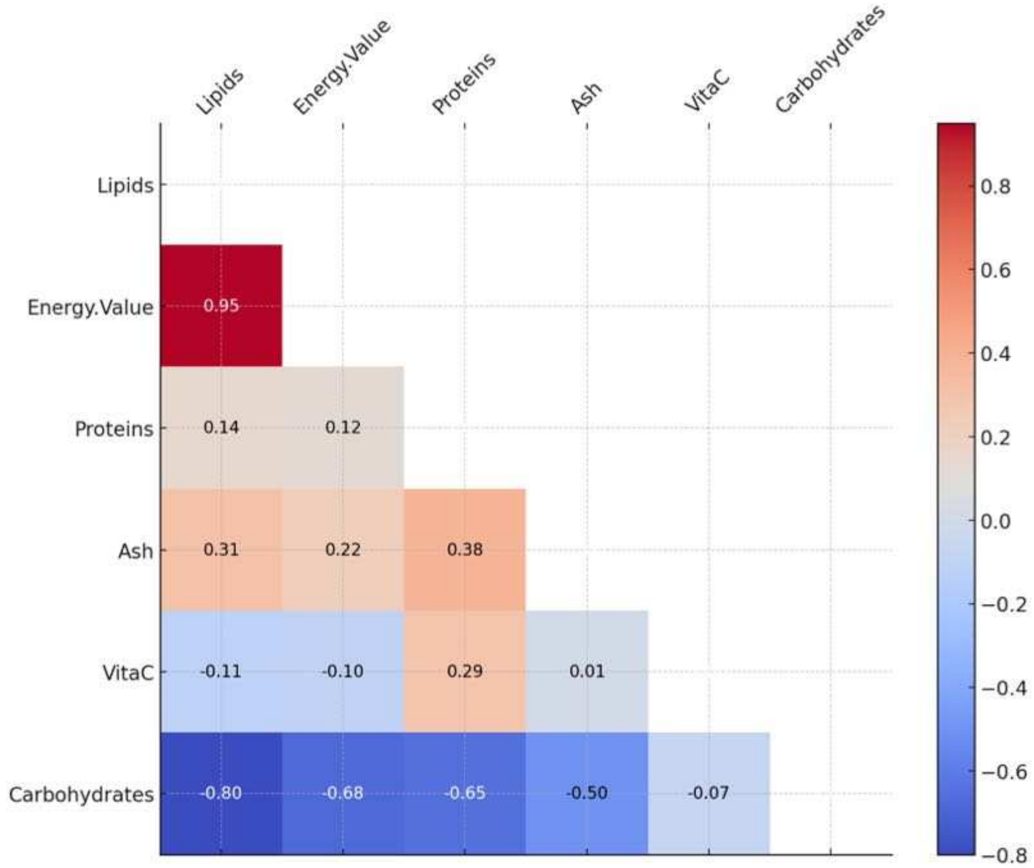

Figure 3: Correlation matrix of physicochemical parameters (Carbohydrates, Vitamin C, Ash, Proteins, Energy value, Lipids) of the twelve formulated flours

Classification of flours based on Nutritional Density score

The Nutrient Density Score serves as a pivotal metric that plays a critical role in distinguishing the most nutritionally advantageous flour options. This metric is particularly crucial as it also considers potential health risks such as obesity (Figure 4). The score quantifies the concentration of essential nutrients like vitamins, minerals and proteins in a given food item or diet, providing a numerical value that can guide dietary choices. This scoring system has emerged as an invaluable tool for facilitating educated and informed dietary decisions, thereby promoting healthier eating habits and lifestyles [38]. In the context of this study, the Nutrient Density Scores for the various flours analyzed exhibited a considerable range,

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varying from a low of 0.244 to a high of 0.255 (Figure 4). Among the multiple flours evaluated, "MaCB22.5" distinguished itself as the standout option, boasting the highest normalized nutrient density score of 0.255. Correspondingly, its Nutrient-Rich Food (NRF) index was 49.74, and its SAIN/LIM ratio was calculated to be 0.34, marking it as the most nutrient-dense choice among the flours assessed. As indicated by Drewnowski and Fulgoni [38], a higher Nutrient Density Score suggests that the food item provides a more substantial amount of essential nutrients per calorie. This is a crucial factor in making a food choice healthier. Opting for MaCB22.5 flour over other enriched flours allows individuals to maintain a balanced diet that supports healthy weight management by providing essential nutrients without contributing to caloric overload [39]. This strategy bears significant relevance, especially in the context of obesity prevention and management. Obesity is a major global public health issue and is closely correlated with a range of other serious health problems, including cardiovascular diseases, diabetes, and certain types of cancer [40,41]. Aside from MaCB22.5, other flours such as MiCB25, MaCB20 and MiCB20 also performed well in terms of nutrient density, with respective scores of 0.248, 0.243 and 0.243, indicating them as viable alternatives for health-conscious consumers.

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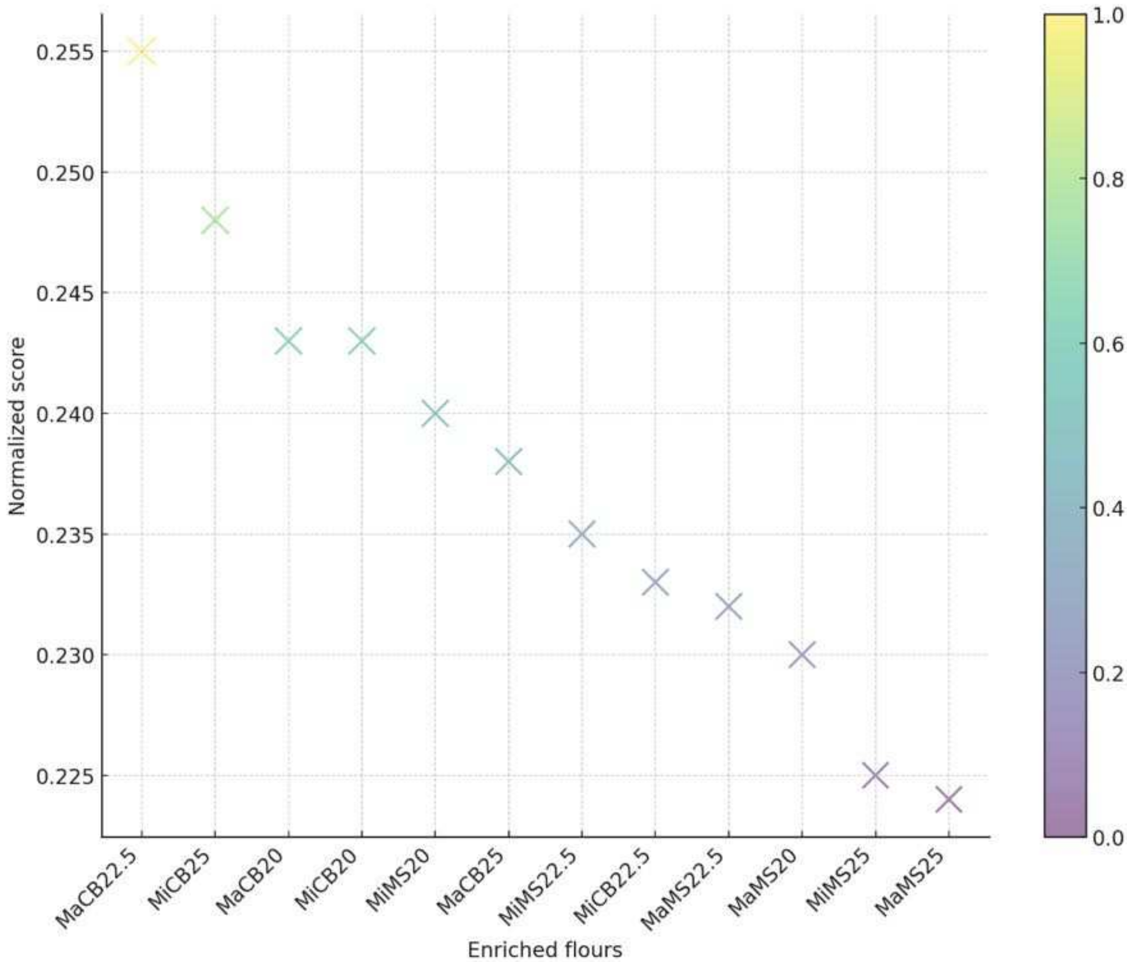

Figure 4: Scatter plot of the normalized nutrient density of different types of enriched flour

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

This study highlights the substantial benefits of incorporating insect powders into cereal flours, helping to improve the nutritional profile. Biochemical analyses indicate marked elevations in lipid content (17.93±0.17% and 46.06±8.31%) and protein content (49.29±0.25% and 34.70±0.50%) for flours enriched with *Macrotermes subhyalinus* and *Cirina butyrospermi*, respectively. Intriguingly, the enrichment process also resulted in reduced carbohydrate levels across the enriched flours. The standout among these is MaCB22.5, which exhibits the highest nutrient density score of 0.255, coupled with a high protein content of 18.78% and an energy value of 399.33 kcal. This enriched flour presents a promising dietary inclusion for populations enduring persistent protein deficiencies. While this research is constrained by a limited sample size and does not delve into the long-term health implications, it sets a methodological benchmark for subsequent inquiries. Overall, this investigation serves as a meaningful addition to

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the extant literature on flour enrichment and nutrition, providing actionable insights for targeted initiatives aimed at alleviating malnutrition.

Ethical approval and consent to participate
Consent for participation was not necessary for the Biochemical analyses on flours. No Human Subjects data gathering was
employed in this study.

Consent for publication
Not applicable

Competing interest
The authors declare no competing interests

Authors' contributions
Conception: Angaman Djédoux Maxime and Koko Anauma Casimir.
Design: Boko Adjoua Christiane Eunice, Koko Anauma Casmir, Blei Sika Hortense, Yao Dago liliane
Analysis and interpretation of data: Boko Adjoua Christiane, Koko Anauma casimir, Blei Sika Hortense, Yao Dago Liliane and Angaman Djédoux Maxime.
Drafting of the article: Boko Adjoua Christiane Eunice and Angaman Djédoux Maxime.
Critical revision for important intellectual content: Koko Anauma Casimir, Blei Sika Hortense and Angaman Djédoux Maxime.
Final approval of the version to be published: Boko Adjoua Christiane Eunice and Angaman Djédoux Maxime

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Table 1: Enrichment of composite flours made from millet and corn incorporated with *Macrotermes subhyalinus* (MS) and *Cirina butyrospermi* (CB) powders

Sample code	Corn flour (Ma %)	Millet flour (Mi%)	MS powder (%)	CB powder (%)
MaMS20	80	0	20	0
MaMS22.5	77.5	0	22.5	0
MaMS25	75	0	25	0
MiMS20	0	80	20	0
MiMS22.5	0	77.5	22.5	0
MiMS25	0	75	25	0
MaCB20	80	0	0	20
MaCB22,5	77.5	0	0	22.5
MaCB25	75	0	0	25
MiCB20	0	80	0	20
MiCB22.5	0	77.5	0	22.5
MiCB25	0	75	0	25
Ma100	100	0	0	0
Mi100	0	100	0	0
MS100	0	0	100	0
CB100	0	0	0	100

MaMS20: Sprouted corn flour (80%) and *Macrotermes subhyalinus* (20%);
MaMS22.5: Sprouted corn flour (77.5%) and *Macrotermes subhyalinus* (22.5%);
MaMS25: Sprouted corn flour (75%) and *Macrotermes subhyalinus* (25%);
MiMS20: Sprouted millet flour (80%) and *Macrotermes subhyalinus* (20%);
MiMS22.5: Sprouted millet flour (77.5%) and Macrotermes subhyalinus (22.5%);
MiMS25: Sprouted millet flour (75%) and *Macrotermes subhyalinus* (25%);
MaCB20: Sprouted corn flour (80%) and *Cirina butyrospermi* (20%);
MaCB22.5: Sprouted corn flour (77.5%) and *Cirina butyrospermi* (22.5%);
MaCB25: Sprouted corn flour (75%) and *Cirina butyrospermi* (25%);
MiCB20: Sprouted millet flour (80%) and *Cirina butyrospermi* (20%);
MiCB22.5: Sprouted millet flour (77.5%) and *Cirina butyrospermi* (22.5%);
MiCB25: Sprouted millet flour (75%) and *Cirina butyrospermi* (25%)

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Table 2 : Physicochemical composition of flours

Flours	Titratable acidity (mEq/100g)	pH	Brix degree	Vitamin C (mg/g DM)	Dry matter (%)	Ash (%)	Lipids (%)	Proteins (%)	Carbohydrates (%)	Energy value (kcal)
CB100	43.59±4.0cd	5.78±0.10h	1.16±0.05def	7.43±0.04 fg	96.47±1.16ac	4.87±0.01d	17.93±0.17f	49.29±0.25j	24.37±1.34a	456.10±3.98b
MS100	18.57±4.61a	5.78±0.01h	0.00±0.00a	7.75±0.16 h	95.37±0.0acd	6.78±0.80e	46.06±8.31g	34.70±0.50i	7.82±8.18a	584.71±43.95c
Ma100	73.01±5.59g	4.12±0.01bd	1.40±0.10g	6.04±0.02ab	94.60±0.37ce	1.30 ±0.28ab	4.29 ±0.63a	8.22±0.30a	80.77± 1.19e	394.66±1.93a
MaCB20	62.26±6.2eg	4.06 ±0.01ad	1.16±0.05def	7.62±0.13gh	93.59±0.40de	1.74± 0.19ab	9.95±3.18ade	17.38±0.20f	64.51±2.98cd	417.14±16.32ab
MaCB22.5	69.82±4.5ef	4.26±0.01de	1.23±0.05cd	6.55±0.08cd	95.12±0.17ace	1.85±0.12ab	5.24±0.04abc	18.78±0.20h	69.24±0.12d	399.33±1.05a
MaCB25	63.42±7.0eg	4.04±0.02abc	1.26±0.05eg	6.46±0.08cd	92.80 ±0.51e	1.98±0.35ab	10.84±0.07adf	18.14±0.10gh	61.83±0.64cd	417.50±2.04ab
MaMS20	53.04±2.1de	3.91±0.01a	1.96±0.05h	6.07±0.08ab	95.59±0.70acd	0.99±0.52a	12.71±0.29cdf	13.00±0.20b	68.87± 1.46d	441.99±3.48b
MaMS22.5	67.28±3.9eg	3.91±0.01a	1.90±0.00h	6.79±0.06de	94.26±0.99ce	1.06±0.41a	12.62±1.02bdf	13.24±0.10bc	67.33±0.88cd	435.96±6.88ab
MaMS25	66.31±6.0eg	3.92±0.01ab	1.23±0.05eg	5.74±0.05 a	94.8±0.96bce	1.65±0.29ab	15.12 ±0.64df	15.28±0.20d	62.73±0.61cd	448.23±6.98b
Mi100	19.82±3.9ab	4.61±0.24f	0.10±0.00a	7.18±0.10 ef	95.4±1.92acd	1.72±0.30ab	4.71±0.10ab	8.63 ± 0.20a	80.41±2.03e	398.58±7.47a
MiCB20	34.62±4.6bc	4.44±0.01ef	0.36±0.15b	6.90±0.15de	97.2±0.68a	1.92±0.30ab	9.64±3.60ade	16.50± 0.20e	69.15±4.45d	429.46±14.22ab
MiCB22.5	55.26 ±4.05def	3.98±0.00ab	1.96±0.05h	7.34±0.13 fg	97.13±0.16ab	2.05±0.11ab	13.58 ±3.31df	18.25±0.20gh	63.24±3.49cd	448.27±16.72b
MiCB25	31.96 ±4.05ac	4.93±0.02g	0.83±0.05c	6.68 ±0.13 d	97.23±0.37a	2.60±0.28bc	7.99±0.80ad	18.66±0.10h	67.96 ±1.06d	418.44±4.66ab
MiMS20	29.10 ±2.40ac	4.00±0.00abc	1.33±0.05fg	7.55±0.06fh	94.92±0.92ace	2.25±0.23ac	11.15±1.03adf	13.88±0.20c	67.63±0.91cd	426.47±8.50ab
MiMS22.5	22.66 ±8.32ab	4.01±0.00abc	1.13±0.05de	6.24±0.11bc	94.72±0.31ce	1.90 ±1.13ab	11.51±1.94adf	17.61±0.10fg	63.68±2.76cd	428.80±6.30ab
MiMS25	29.17±2.2ac	4.18±0.01cd	1.00±0.00cd	5.99±0.34ab	95.67±0.00acd	3.69±1.00cd	16.65±0.54ef	16.04±0.10e	59.29±1.23c	451.19±4.66b

Values sharing different letters within the same column are statistically distinct at the $p < 0.05$ level

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