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A Comparative Analysis of the Nutritional Values of Two Differently Preserved Caterpillar Species (*Gynanisa maja* and *Gonimbrasia zambesina*) in Chitambo District, Zambia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Climate change due to natural causes and anthropogenic causes edible insects have become more and more popular as alternative food sources. Poses a potential to seriously contribute to food security and poverty alleviation in many developing nations. The study compared the nutritional value of two different methods of preserving edible caterpillars in Chitambo district. The proximate composition and minerals of the caterpillars were determined using standard methods. Data was analysed by an analysis tool Statistical Package for Social Sciences. The moisture content of the caterpillar samples ranged from 8.08% to 13.44% respectively. The study found significant variations in crude fat Chipumi (*G. maja*) boiled and pressed with a content of 26.62% per 20g and the Mumpa (*G. zambesina)* roasted and un-pressed with a content of 12.71 % per 20g. For crude fat was recorded higher in treatment with pressed caterpillars and *G. maja* retain more crude fat than *G. zambesina.* The species Mumpa (*G. zambesina)* recorded the highest crude protein content of 30.38% of other treatments included in this study. The highest amount of energy of 26833.43j/g was recorded in Chipumi (*G. maja)*. The mineral content of Mumpa (*G. zambesina)* was appreciably higher than Chipumi (*G. maja)*. However, the preservation methods had little effect on crude protein and the boiled and pressed caterpillars tend to retain more proteins. The treatment with roasted caterpillars had higher mineral and energy content than the treatment with boiled caterpillars, except for sodium which was higher in boiled treatments due to the addition of the seasoning sodium chloride (salt). The findings suggest that preservation methods affected the nutritional composition of edible caterpillars.

Keywords: Edible caterpillars; preservation methods; moisture; sodium chloride; nutritional composition.

1. INTRODUCTION

Many smallholder farmers in the developing world struggle to survive in adverse poverty and rely heavily on agriculture as their primary source of income and household food [1,2,3]. However, climate change has had a devastating impact on their agricultural production [4], leading to a significant reduction in productivity at the farm level. This reduction in turn exacerbates food insecurity and nutritional insecurity, particularly in vulnerable developing countries like Zambia, where agriculture is a vital part of the economy and food systems [5]. As a result, rural communities are left without access to nutritious food, perpetuating the cycle of poverty. To address this critical situation, nongovernmental organizations, government departments, and community-based organizations in Zambia have launched initiatives to promote alternative livelihood activities, such as collecting edible wild fruits, mushrooms, and insects, in an effort to eradicate food insecurity and nutritional insecurity in rural areas [6]. By diversifying their income sources and improving their food systems, these efforts aim to enhance the resilience of smallholder farmers and their communities.

According to the World Food Summit of 1996, food security is achieved when all individuals have consistent access to sufficient, safe, and nutrient-dense food that meets their dietary needs and preferences, regardless of their circumstances [7,8]. Food security is supported by three pillars: availability, access, and utilization. Invertebrates, such as caterpillars, are a crucial supplement to the human diet, providing essential protein and nutrients [9,10]. In Zambia and other developing countries, caterpillars are a vital and preferred food source, rich in protein, fat, minerals, and vitamins [11,12]. Some edible caterpillars have nutritional profiles comparable to meat and fish, while others have higher protein, fat, and energy content [13,14]. The species Chipumi (*Gynanisa maja*) and Mumpa (*Gonimbrasia zambesina*) are abundant forest resources in rural Zambia [15,11]. Locals in Zambia preserve caterpillars through roasting, boiling in salt water, and sun drying. These caterpillars play a vital role in sustaining rural livelihoods and hold significant nutritional and economic importance in many Zambian households [15]. In addition, caterpillar consumption has cultural significance, with different species associated with specific seasons and celebrations. By promoting the sustainable harvesting and consumption of caterpillars, Zambia can enhance food security, support local livelihoods, and preserve cultural heritage.

In Zambia, several studies have explored the ecological and socioeconomic significance of edible caterpillars, including those by Siulapwa et al. [10], Chanda et al. [15], Mwanza [11], and Mutungi et al. [16]. However, these studies have largely overlooked the impact of preservation methods employed by communities that collect caterpillars for household consumption and market on the nutritional composition of these insects. Furthermore, there is a dearth of information on the nutritional composition levels of edible caterpillars preserved in Zambia and the effects of preservation methods on these levels. To address these research gaps, this study investigates the effects of preservation methods on the nutritional composition of two commonly consumed edible caterpillar species, Chipumi (*G. maja*) and Mumpa (*G. zambesina*). The study not only examines the influence of preservation methods on the caterpillars but also compares the effects on the nutritional value of the two species. This comprehensive approach aims to provide valuable insights into the preservation methods that can best maintain the nutritional integrity of edible caterpillars in Zambia.

2. MATERIALS AND METHODS

2.1 Study Area Description

Chitambo District, with a total size of 23, 351 square kilometers, is one of the eleven districts that make up Zambia's Central Province. The district is located between 29˚45" and 31˚00" east of the Greenwich Meridian and between latitudes 12˚00" and 13˚15" south of the equator. The district is located 1120 meters above sea level. The district is located around 357 kilometers north of Kabwe, the provincial headquarters, along the Great North route. It has boundaries with Lavushimanda to the north, Serenje to the south and east, Milenge to the west, and Samfya to the northwest.

2.2 Climate, Topography and Soil

Chitambo District receives an average annual rainfall of 1,161 mm, primarily between November and April. The mean annual temperature is 20^0 C. October is the hottest month, with an average maximum temperature of 34.2^0 C, while July is the coldest month, with an average minimum temperature of 5^0 C and extremes reaching 3^0 C. The district experiences an average annual humidity of 66.1%. Humidity peaks during the rainy season (November to March), ranging between 75 to 80 %, and drops to between 45 and 50 % during the hottest months (September and October).

The landscape is dominated by the Muchinga Escarpment in the southeast, with scattered hills and a soft undulating valley in the southern part. Sandbelt soils, which support better tree growth, are prevalent in the north and west. Chitambo enjoys a mean annual sunshine duration of 8 hours per day. During the dry season (April to October), sunshine lasts between 8.6 and 10.5 hours per day. This is similar to the average daily sunshine hours in Central Province and Zambia. Winds during the dry season blow from 120^0 southeast at 3 knots, and during the rainy season, they blow from 270^0 west and 360^0 north at the same speed.

2.3 Site Selection

An exploratory survey was conducted prior to the actual sample collection to gather essential information about the study area. This preliminary investigation aimed to establish a comprehensive understanding of the local context. The data collected included details such as the distance between villages and the nearby forest, the number of households per village, contact information for caterpillar collectors, and the identification of central collection points for the samples. The rationale behind selecting this particular study area was its abundance of edible caterpillars, which presented a valuable opportunity for the research.

2.4 Sample Collection

In November when the caterpillars were in season, the two species were handpicked from the forest in Chitambo District. They were then exposed to two preservation techniques salting and roasting (ukupupusha). The preservations were done as follows:

Boiled in salted water: After the caterpillars were collected some were gutted and others were not. They were then boiled in salt water and left to dry in the sun until they were bone dry.

Roasted: The caterpillars were placed in hot ashes and left to roast briefly, when removed they were allowed to further dry in the sun for two days.

The preservation methods described were carried out in collaboration with the local people of Chitambo to maintain the authenticity of their traditional preservation processes. After

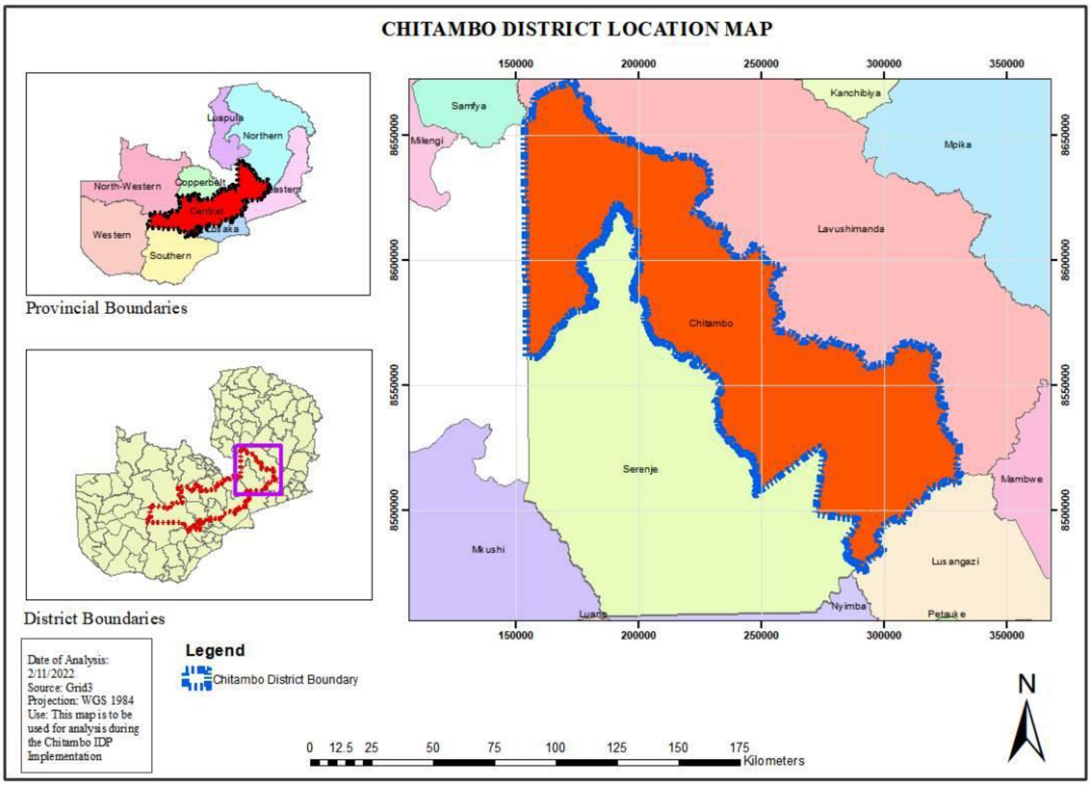

Fig. 1. Map of the study area
Source: Chitambo Town Council 2022

preservation, samples were collected and transported to Kitwe for laboratory nutritional analysis. At least one kilogram of each preservation type was gathered and subjected to various laboratory tests. Each sample underwent analysis for crude proteins, moisture content, crude fat, and mineral elements (specifically potassium, iron, and sodium) using standard laboratory procedures.

2.5 Laboratory Analysis

The proximate compositions of the caterpillars were determined as follows: the moisture content was determined by using the oven drying method as described by the Association of Official Analytical Chemists (AOAC) [17,18]. Fat content was determined using the procedure of AOAC and n-hexane as solvent. The crude protein content was determined using the macro Kjeldahl method. The gram of nitrogen obtained was multiplied by 6.25 to obtain the crude protein content. The wet digestion method described by AOAC in 2005 was used for mineral analysis [19]. The data was collected through direct recording and observation of the experiment process. The readings were recorded from the experimental process for each nutrient in all the samples. The proportion of the readings from the experimental process and the weight of the samples were expressed in percentages.

2.6 Data Analysis

To assess the impact of the two preservation methods on the nutritional composition of caterpillars, mean comparisons and descriptive statistics were employed. The analysis was conducted using SPSS for statistical calculations and MS Excel for creating graphs and tables to visualize the results. This comprehensive approach allowed for a detailed examination of the effects of the preservation methods on the crude proteins, crude fat, and moisture content of the caterpillars.

3. RESULTS

3.1 Effects of Treatment on Fat, Protein, Moisture, and Energy Composition in Caterpillars

Crude fat was higher in Chipumi (*G. maja*) boiled and pressed with a fat content of 26.62% per 20g than in Mumpa (*G. zambesina)* roasted and un-pressed recorded fat content of 12.71 % per 20g (Fig. 2A). In contrast, crude protein was high in Mumpa (*G. zambesina)* boiled and pressed (MBP) with a protein content of 30.38% per 1g than in Chipumi (*G. maja*) boiled un-pressed (CBU) which had a protein content of 21.05 % (Fig. 2B). Similarly, boiled Mumpa (*G. zambesina)* recorded higher moisture content of 13.44% than was recorded in Chipumi (*G. maja*) roasted and pressed with a moisture content of only 8.08% (Fig. 2C). Energy was higher in Chipumi (*G. maja*) roasted un-pressed with an energy level of 26833.4j/g than in Mumpa (*G. zambesina)* boiled pressed with a value of 14038.8j/g (Fig. 2D).

Table 1. Shows the protein composition of common animal meats

Component (g/100g)	Beef meat	Chicken meat
Crude Proteins	21.35	19.40

3.2 Effects of Treatment on Mineral Elements in Caterpillars

Potassium was higher in Mumpa *(G. zambesina)* boiled un-pressed with content of 3.07% per 2.5g than in Mumpa (*G. zambesina)* boiled pressed which had a content of 1.29% per 2.5g (Fig. 3A). In contrast, sodium was higher in Chipumi (*G. maja*) boiled pressed (CBP) with a sodium content of 2.54% per 2.5g than in Mumpa (*G. zambesina)* roasted pressed (MRP) which had a Sodium content of 0.29% (Fig. 3B). Mumpa (*G. zambesina)* again recorded higher Iron content of 0.041% than was recorded in Chipumi (*G. maja*) boiled and pressed with iron content of only 0.002% (Fig. 3C).

3.3 Relationships Between Nutrients

The scatter plots (Fig. 4) provide insights into the relationships between various nutrients in the food source, likely the caterpillars discussed earlier. There is a clear negative linear

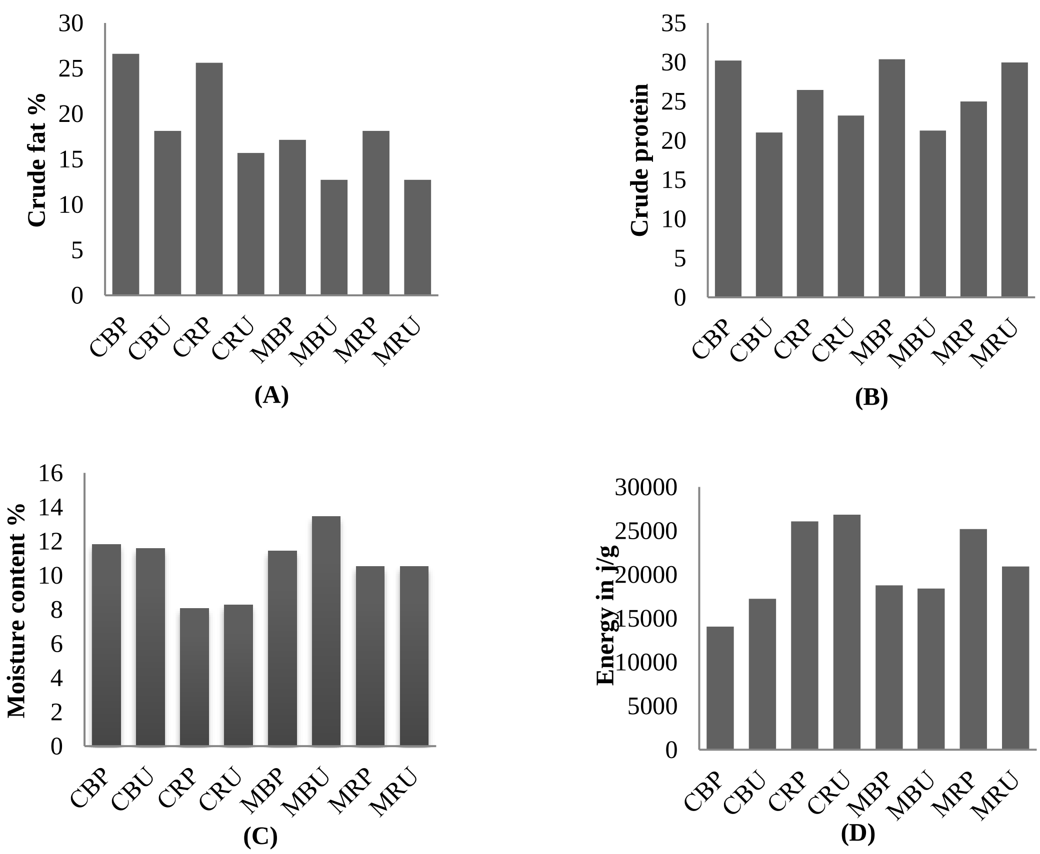

Fig. 2. shows the effect of different treatments on crude fat (A), crude protein (B), moisture content (C), and energy (D) in the two species of caterpillars

Where, C=Chipumi (G. maja), M=Mumpa (G. zambesina), B= Boiled, R=Roasted, U=Unpressed, and P=Pressed

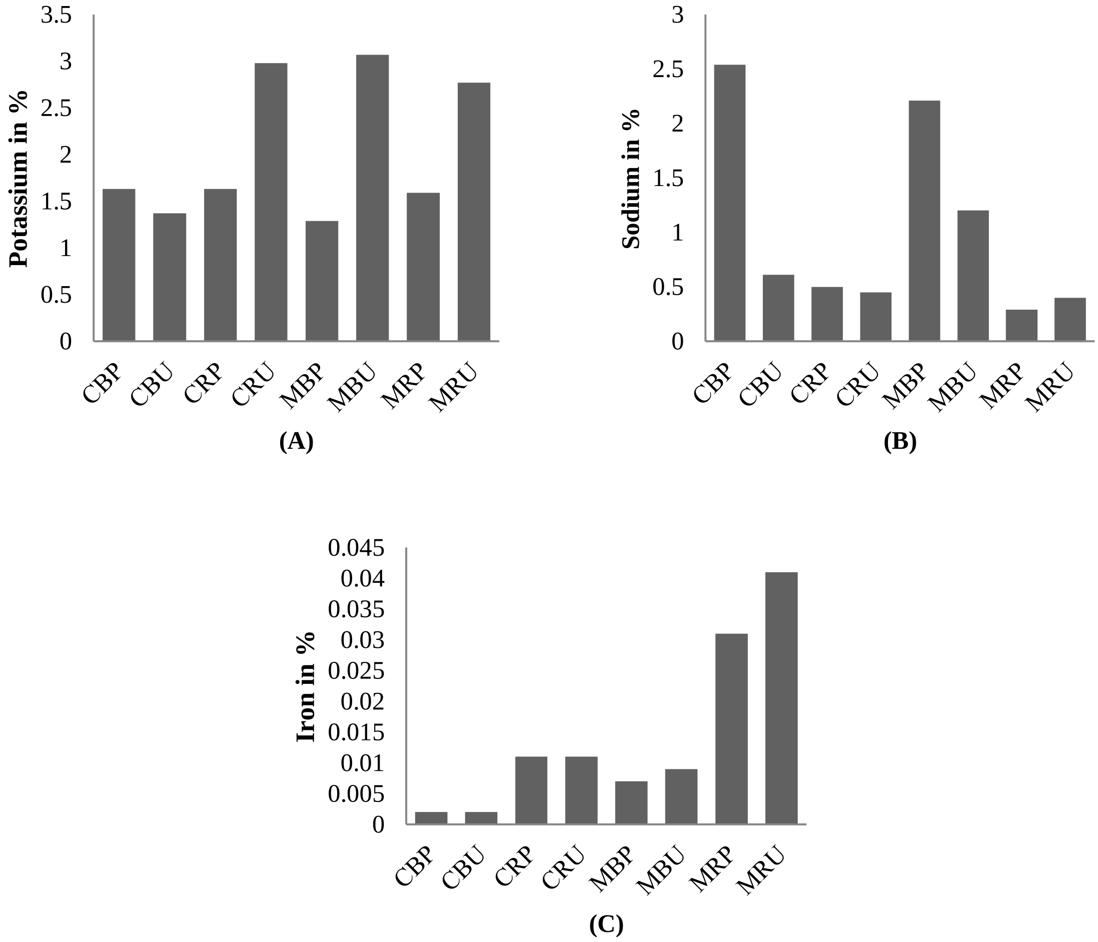

Fig. 3. Shows the effect of different treatments on potassium (A), Sodium (B), and Iron (C) in the two species of caterpillars

Where; C= Chipumi (G. maja), M= Mumpa (G. zambesina), B=Boiled, R= Roasted, P=Pressed and U=Un-pressed

relationship between moisture content and energy, indicating that as moisture increases, the energy content decreases. This is logical, as higher water content typically dilutes energy-providing nutrients like fats, proteins, and carbohydrates. A similar negative relationship is observed between sodium content and energy, though it appears less pronounced than the moisture-energy correlation. The data suggests that higher sodium levels are associated with lower energy content, but there is more variability in this relationship. The third plot reveals a negative correlation between potassium content and crude fat percentage, with increasing potassium levels corresponding to decreasing fat content.

'Energy negatively correlated with moisture content ($F_{1:6}$ = 11.86; P = 0.014; Fig. 4A**)** and sodium composition ($F_{1:6}$ = 7.11; P = 0.037; Fig. 4B**)**. Crude fat also had a marginally negative correlation with potassium content ($F_{1:6}$ = 3.97; P = 0.094; Fig. 4C). Otherwise, no other nutrients which were analyzed corresponded when plotted against each other.'

However, this relationship shows the most scatter, suggesting it may be influenced by additional factors not captured in the graph. Overall, these inverse correlations between nutrients provide valuable information about the nutritional profile of the food source and how different components interact. Understanding these relationships could be crucial for optimizing processing methods, storage conditions, or dietary applications of the caterpillars to maintain or enhance their nutritional value.

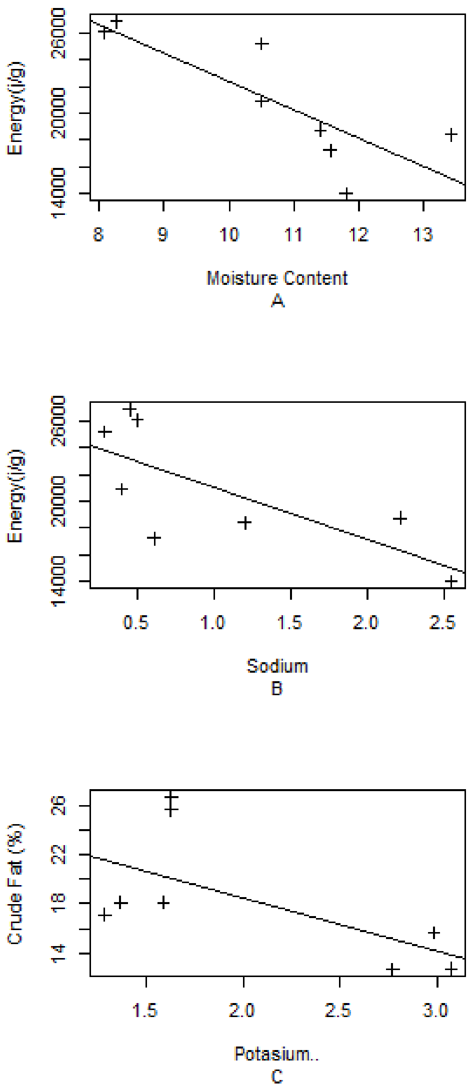

Fig. 4. Relationships between nutrients

Table 2 presents a comprehensive analysis of proximate and mineral content, as well as energy values for different preparations of Chipumi (CB) and Mumpa (M) caterpillars. In terms of protein content, Chipumi shows a range from 21.05% in the boiled un-pressed form (CBU) to 30.38% in the boiled pressed variant (CBP), highlighting its potential as a significant protein source across different preparations. Mumpa, on the other hand, exhibits protein levels ranging from 23.19% (CRU, roasted un-pressed) to 29.96% (MRU, roasted un-pressed). Fat content varies notably between the treatments, with Chipumi ranging from 12.73% (MBU, boiled un-pressed) to 26.62% (CBP), and Mumpa from 12.71% (MRU) to 17.14% (MBP, boiled pressed), reflecting differences in lipid composition influenced by preparation methods.

Moisture content remains relatively consistent across most treatments, with values ranging from 8.08% (CRP, roasted pressed Chipumi) to 13.44% (MBU). Mineral analysis reveals higher potassium levels in Mumpa preparations, reaching up to 3.07%, compared to Chipumi's maximum of 2.98%. Sodium content is generally lower, with Chipumi showing a maximum of 2.54% (CBP) and Mumpa at 1.2% (MBU). Iron content varies between 0.002% and 0.041%, with most treatments falling between 0.002% and 0.011%, highlighting a consistent but relatively low iron presence across all samples.

Table 2. Proximate and Mineral analysis results

Treatment	Crude protein %	Crude fat %	Moisture content %	Potassium %	Sodium %	Iron %	Energy j/g
CBP	30.21	26.62	11.81	1.63	2.54	0.002	14038.801
CBU	21.05	18.1	11.57	1.37	0.61	0.002	17244.226
CRP	26.44	25.62	8.08	1.63	0.5	0.011	26069.718
CRU	23.19	15.68	8.26	2.98	0.45	0.011	26833.425
MBP	30.38	17.14	11.42	1.29	2.21	0.007	18757.428
MBU	21.28	12.73	13.44	3.07	1.2	0.009	18400.547
MRP	24.97	18.1	10.51	1.59	0.29	0.031	25199.849
MRU	29.96	12.71	10.51	2.77	0.4	0.041	20921.964

Where; CRU=Chipumi Roasted Un-pressed, CBU=Chipumi Boiled Un-pressed, CBP= Chipumi Boiled Pressed, MRP=Mumpa Roasted Pressed, MRU=Mumpa Roasted Un-pressed, MBU=Mumpa Boiled Un-pressed, MBP=Mumpa Boiled Pressed, Chipumi (G. maja), Mumpa (G. zambesina)

Energy content varies significantly, with Chipumi treatments ranging from 14038.801 J/g (CBP) to 26069.718 J/g (CRP), and Mumpa from 18400.547 J/g (MBU) to 26833.425 J/g (CRU). These values underscore the considerable energy density of both caterpillar species, influenced by their protein and fat contents. Overall, the data indicates that both Chipumi and Mumpa caterpillars offer substantial nutritional benefits, with variations in composition influenced by preparation methods, making them versatile options in diets or food formulations aimed at maximizing protein intake and energy efficiency.

4. DISCUSSION

In Zambia, caterpillars play a crucial role in the diets of many cultures and communities, where they are incorporated as both a planned part of meals and as popular snacks. This widespread consumption highlights the cultural and nutritional significance of these insects in the region. Moreover, the ability to process caterpillar products for storage extends their shelf life to 2-3 months, significantly increasing their availability for consumption and potential for income generation throughout the year.

The proximate composition of caterpillars analyzed in this study closely aligns with observations reported by other researchers in the field [20]. Interestingly, the species *G. zambesina* was found to have a higher moisture content compared to *G. maja.* This higher moisture content, while potentially affecting taste and texture, presents a challenge for long-term preservation. It increases the risk of microbial deterioration and spoilage, potentially shortening the storage period of these insects [21,22]. In contrast, *G. maja* exhibited a higher fat content than *G. zambesina.* This difference in fat composition likely accounts for the higher gross energy observed in *G. maja*, as fat contributes more than twice the calories per gram compared to carbohydrates and proteins [23]. This finding has important implications for the nutritional value and energy density of these caterpillar species when used as a food source. The study also examined the effects of different processing methods on caterpillar composition. Notably, caterpillars that underwent roasting, boiling, and pressing treatments showed consistently high-fat content with only slight variations between methods. The crude fat content obtained in this research aligns well with previous studies, which reported a mean crude fat content of 10-12% for pressed caterpillars on a dry weight basis. This consistency across studies reinforces the reliability of these findings and underscores the potential of caterpillars as a valuable source of dietary fat.

The result for fat content demonstrates that caterpillars can offer a high-fat content for the human diet among the communities practicing entomophagy [20,24,22]. Fat is essential in human diets because it increases the palatability of foods by absorbing and retaining their flavours. However, one implication of the high-fat content in the insects is that it may increase the susceptibility of insects with high-fat content to storage deterioration via lipid oxidation. Malnutrition in developing countries is also a problem of calorie deficiency. According to Bomolo et al. [25], caterpillars rank high in fat content with little or no cholesterol, especially leaf eater ones, and their fatty acid profile is similar to those of fish and poultry. Caterpillars have the advantage of converting plant materials that may not often be consumed by humans into highly palatable food [25,26]. Consumption of caterpillars would, therefore, not pose any danger or risk of cardiovascular diseases.

The crude protein content recorded showed slight variation between species and among the treatments studied [27]. The species *G. zambesina* with the treatment MBP had the highest crude protein content than the other treatments included in this study. The crude protein content of caterpillars studied is appreciably lower in comparison with the reported mean crude protein content of caterpillars on a dry weight basis. Furthermore, the two species of caterpillars with the treatment boiled and pressed in this study showed higher protein content in comparison to the common lean red meat of different sources (*i.e.* 23.2% for beef, 24.8% for veal and 21.5% for mutton) [28,29]. The results for protein content in the two species of caterpillars with the treatment boiled and pressed were higher than in beef and chicken meat as shown in Table 2. The protein content exhibited by the caterpillars was significantly higher than the conventional animal meats and therefore caterpillars may offer an affordable source of protein to counteract the protein inadequacy commonly faced by humans in Africa. The high protein content in caterpillars suggests that a small amount of the delicacy needs to be consumed daily, especially for children, to meet the required minimum daily protein need [30-33]. Protein is an important component of antibodies as it bolsters the body's immunity function [29]. Insect proteins are highly digestible (between 77% and 98%). This, in turn, is determined to a great extent, by the amino acid composition [34,35].

The mineral content of *G. zambesina* was appreciably higher than *G. maja*. Generally, the mineral content of the two insects studied indicates that they all had a fair source of mineral elements supported by other studies [36]. The species *G. zambesina* had higher mineral content with the major macro-mineral potassium being the highest as seen in the treatment having un-pressed samples, and sodium which is another macro-mineral showed slight variation in the two species [37]. The highest sodium content was observed in *G. maja* with the treatment CBP which is the boiled and pressed treatment. Only one micro-mineral iron was studied, and it was observed that the species *G. zambesina* recorded higher iron content in all the treatments with the highest being in the roasted and un-pressed treatment [38]. The high content of sodium observed could be attributed to the addition of the seasoning salt, sodium chloride (NaCl), or table salt during processing. The high content of iron in *G. zambesina* is of particular interest to combat the deficiencies experienced by households. Micronutrient deficiency, which is referred to as hidden hunger, is of great concern, particularly among pregnant women and children in poor urban and rural dwellers, which often manifests as anaemia [31]. The mineral content of caterpillars suggests they are excellent sources of essential nutrients for young children, pregnant women, and lactating mothers [39-41]. Iron deficiency, a common issue in developing countries, particularly affects children and women of reproductive age, leading to anemia, reduced physical activity, and increased maternal morbidity and mortality [42].

In many third-world countries, cereal-based diets for infants and young children could be significantly improved by incorporating nutrient-rich insects like caterpillars [43]. Minerals play crucial metabolic and physiological roles in living systems [44]. For instance, iron, zinc, copper, and manganese act as antioxidant enzyme cofactors, strengthening the immune system. Furthermore, these minerals are vital in preventing various health issues, including cardiomyopathy, muscle degeneration, growth retardation, impaired spermatogenesis, immunological dysfunction [45], and bleeding disorders [41]. By integrating caterpillars into diets, communities can address mineral deficiencies and their associated health problems more effectively. Therefore, edible caterpillars can supply necessary nutritive elements for human body functions and could be consumed along with other food and animals rich in other essential minerals to further complement the diet [46,47]. Findings from the study by Babarinde et al. [36] revealed that the average cost of the insects (caterpillars inclusive) per kilogram was cheaper than the cost of common meats (beef and chicken). However, in terms of nutrient content, insects were far superior to beef and chicken meat [40].

4.1 Effect of the Preservation Methods on Caterpillars

The increase in the proximate composition of the boiled and roasted pressed caterpillars can be explained by the larger edible portion made available after the pressing process. This process effectively removes inedible parts, concentrating the nutritional content in the remaining edible portion. The reduction in mineral composition observed in these two preservation methods (boiling and roasting) is primarily due to the discarded guts, which contain a significant amount of minerals [48]. In contrast, unpressed caterpillars demonstrated a higher mineral composition. This can be attributed to the fact that the retained guts contain various minerals, which are not lost during processing. Interestingly, roasted caterpillars were observed to retain more potassium compared to other preparation methods. This increased potassium content is likely due to the accumulation of potash from the ashes produced during the roasting process [49]. Furthermore, a notable correlation was observed between moisture content and energy levels. Treatments that resulted in lower moisture content were associated with higher energy levels in the caterpillars. This finding has important implications for maximizing the energy content of caterpillars as a food source. For individuals seeking to obtain high energy from caterpillars, it is crucial to keep the moisture content as low as possible [39,40,41]. This can be effectively achieved through thorough drying processes. By reducing moisture content, not only is the energy density increased, but there is also a significant added benefit of prolonging the shelf life of the caterpillars [39,24,41]. In areas where caterpillars constitute a staple meal, protecting these species is essential to preserving biodiversity and promoting food security. An extended shelf life of preserved caterpillars is especially advantageous for distribution and storage, guaranteeing a consistent food supply all year round [50,51]. This preservation effort can be strengthened by putting into practice sustainable management techniques like mulching Turyasingura *et al.* [52]. Mulching can help maintain soil moisture and decrease surface runoff, both of which are important for caterpillar habitat. Mulching helps caterpillar populations grow and develop even in the midst of seasonal changes by stabilizing the environment. This method ultimately contributes to increased food security and sustainable resource use in caterpillar-dependent countries by supporting the preservation of caterpillar species and supporting improved agricultural management practices.

5. CONCLUSION

This study provides an overview of the nutrient composition of the caterpillars (*G.Gonimbrasia zambesina and G.Gynanisa maja*, comparing the nutritional values of these two species. The results indicate that pressed (gutted) samples have significantly higher percentages of crude protein and crude fat than unpressed samples. This suggests that pressing caterpillars enhances their crude fat and protein content. Additionally, these caterpillars were found to contain substantially higher proportions of proteins, fats, and other nutrients compared to common meats like beef and chicken. Thus, consuming these insects can significantly contribute to the recommended daily intake of iron, sodium, and potassium. Based on these findings, boiling and pressing caterpillars is recommended as a preservation method to retain more nutrients. The moisture content of caterpillars preserved in this way needs to be kept as low as possible as this increases the concentration of nutrients. Moreover, the cost of these insects is lower as compared to the cost of beef and chicken meat. This knowledge therefore creates a justification that edible caterpillars are important food items that need industrial application and commercialisation and ought to be cultivated with modern techniques to increase their commercial value and availability. There is a need for further research to determine the shelf life, especially in the context of nutrient retention over time for edible caterpillars. the government and NGOs to recognize the role of edible caterpillars in the lives of the people in areas where caterpillars occur. Commercialize and cultivate these caterpillars with modern techniques to increase their market value and seasonal availability.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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