Inclusion of African winged termites (Macrotermes nigeriensis) improves the nutrients and quality of fermented cassava mahewu

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Received 18 December, 2021; Accepted 31 January, 2022

Cassava mahewu is a non-alcoholic fermented beverage used by school children, but it has low contents of protein and micronutrients. This study examined the effect of inclusion of African winged-termite (AWT) on the nutritional quality and acceptability of cassava mahewu. Cassava flour was replaced with AWT at varying proportions of 100:0 (as control), 90:10, 80:20, 70:30 and 60:40% and fermented to obtain cassava mahewu. After freeze drying, proximate and micronutrients were determined using standard methods. Sensory attributes were assessed using 9-point Hedonic scale. The proximate compositions showed an improvement (p<0.05) in crude protein (1.35 to 32.65), fibre (1.26 to 4.0), fat (1.56 to 19.15) and ash (1.47 to 4.04 g/100 g) following the addition of AWT at the highest ratio compared with the control. A decrease (P<0.05) in carbohydrate (84.90 to 15.65 g/100 g) was recorded. The iron (1.53-31.65), zinc (0.70-4.60), vitamin C (4.90-13.90) and riboflavin (0.40- 2.09 mg/100 g) increased with the addition of AWT. In terms of overall acceptability, inclusion at 70:30 was significantly (P<0.05) rated higher than other ratios. Inclusion of termite flour prior to fermentation, improved the nutritional and sensorial qualities of cassava mahewu and hence could be utilized to manage the widespread nutritional deficiency in developing countries.

Key words: Cassava mahewu, edible insects, termites, enrichment, fermentation, nutrient, sensory quality.

INTRODUCTION

Protein-energy malnutrition is still highly prevalent in sub-Saharan Africa including Nigeria (Okwu et al., 2010; FAO, *Corresponding author. E-mail: paul.anyiam@uem.ac.mz.

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as the main staple at the expense of nutrient-rich foods. Currently, one out of five children under the age of six suffers from protein energy malnutrition and nearly half of all deaths in children under the age of five have been attributed to undernutrition (FAO, 2015). The world population is estimated to reach 10 billion people in 2050 (UN, 2019), consequently, conventional protein sources such as meat and fish may be insufficient and thus may not be within the reach of low-income households (Godfray et al., 2010; Agrodk, 2015). There is therefore need for alternative and cheaper sources of protein to meet the rising nutrient demands. One of the non-conventional nutrient-rich sources that have recently gained attention is edible insects.

As the world’s population continues to grow rapidly, there is a renewed interest in the use of insects as human food. This is so because, edible insects are rich in protein with good amino acid, fatty acid profiles and high contents of a variety of micronutrients including vitamins, minerals and useful bioactive substances as compared to traditional sources of protein such as meat (Finke and Oonincx, 2014; Inje et al., 2018). The nutritional value of edible insects is largely variable due to thousands of species, the stage of metamorphosis and methods of processing (Finke and Oonincx, 2014; Kourimsksa and Adamkova, 2016; Mutungi et al., 2017). In Nigeria, over 30 species of insects are commonly used as food amongst which are locust, winged termites, grasshoppers, weevils and beetles (Ekop et al., 2010; Adeoye et al., 2014). While every measure is currently being taken to improve food production through conventional agriculture to meet the global food demands, almost zero interest has been shown to the consumption of edible insects and studies on the same is done very low as consumption is sometimes characterized by repulsive feelings and regarded as primitive behavior.

Winged termite (Macrotermes natalensis) is one of the most common species of insects belonging to the family, termitidae. It is the most represented insect in Nigeria in terms of acceptability and consumption (Adeoye et al., 2014). Winged termite is known locally in various parts of Nigeria by different names such as ‘aku’ in Ibo, ‘chinge’ in Hausa and ‘Esusu’ in Yoruba and present every year at the onset of the rainy season. The termites contain high-quality nutrients including highly digestible proteins (Kinyoru et al., 2010) as well as minerals which are more bioavailable than minerals from plant foods (Oja et al., 2021) with good flavor and texture, which makes it suitable to be added to a specific staple food to improve its nutrient quality. Though the nutrient composition of various insects species in Nigeria has been studied by several researchers (Ekop et al., 2010; Igwe et al., 2011a; Solomon and Prisca, 2012; Oibiekpa et al., 2017), very few studies are available on the possibility of enriching local staple foods such as cassava with nutrient-dense edible insect.

Cassava (Manihot esculenta) is a perennial woody shrub that belongs to the family Euphorbiaceae. It is cultivated as an annual crop in tropical and subtropical areas of the world (FAO, 2015). Cassava is the cheapest food and one of the most utilized staple food crops in developing countries in Africa after maize, with Nigeria being the largest producer of cassava in the world (FAO, 2021). Cassava is widely consumed in diverse ways in the country as excellent source of carbohydrate. However, its protein and micronutrient quality is relatively poor (Montagnac et al., 2009). As a result, micronutrient deficiency diseases and lack of dietary diversity becomes a particularly severe problem in various communities where diets are based predominantly on cassava as main staple. Owing to the food importance of cassava, the improvement of nutritional quality of cassava-based food products has received considerable attention and significant efforts have been made to improve its nutritional value (Mesfin and Shimelis, 2013; Salvador et al., 2016; Boyiako et al., 2020), so that consumers can still derive essential nutrients other than carbohydrate from cassava.

Fermented foods, which form a considerable part of diet in Nigeria, are products obtained from the enzymatic modification of food by microorganisms to bring about desired biochemical changes, improving the nutritional value of foods, extending shelf-life and serves as potential sources of probiotics (Fadahunsi and Soremekun, 2017; Anyiam et al., 2020). Cassava mahewu is a novel cassava-based non-alcoholic fermented beverage widely used as complementary drink for school children in Mozambique and other parts of Southern African countries (Salvador et al., 2016). However, being a direct product of cassava, it is low in proteins and micronutrients (Boyiako et al., 2020). Hence, cassava mahewu drink alone cannot cater for the daily nutrients requirement of the school children. Therefore, this study was aimed to determine the suitability of blending African winged termite flour with cassava flour for cassava mahewu production and to evaluate the sensory and the nutritional contents of the final product. The findings from this work may encourage the use of edible insects in addressing the problem of protein-energy malnutrition and food insecurity especially in poor rural settings.

MATERIALS AND METHODS

Sample collection

Selection of edible winged termites (Macrotermes nigeriensis) for this study was based on the perceived nutritional value of the specie, its availability and local preference in the community. The adult winged termites were freshly collected in early morning hours during raining season (May to June) from residential buildings in Umudike community using traditional methods of attraction to light at night and handpicking. Harvested insects were transported under ice blocks to the laboratory at the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike for processing.
Cassava roots used for mahewu preparation were sourced from the Natural Root Crops Research Institute, Umudike, Nigeria and transported in a container to the laboratory for processing into flour. The ethical guidelines provided by the ethical committee of College of Natural Science, Micheal Okpara University of Agriculture Umudike were strictly followed. All chemicals used in the study were of analytical grade.

Sample processing

The winged termites were washed three times with clean tap water to remove soil and dirt, de-winged, de-legged and blanched by soaking in boiled water for 2 min and drained before subjecting to oven drying at 45°C to constant weight for 12 h. The dried insects were milled into fine powder using an electric mill (BN-2001-62WC-Germany). The processed termite was sieved using a 250 μm mesh sieve to fine brown flour, packaged in labeled dry glass jar and stored in an airtight container until used for mahewu preparation. The collected cassava roots were cleaned, peeled and washed again with tap water to remove sand particles. They were cut into smaller pieces and oven dried at 50°C to constant weight before subjecting to milling. The milled white powder was sieved to produce smooth fine dried flour which was stored in an airtight container until used.

Composite blend formulation

Five experimental diets were formulated (Table 1). The milled cassava flour and winged termite flour were thoroughly mixed together with the aid of a laboratory sized mixer to make 100 g at different ratios and stored in well labeled plastic containers prior to fermentation. The formulation was designed in different proportions so as to obtain the most acceptable product that has the highest nutritional value.

| Sample | Cassava flour (%) | Termite flour (%) | Label |
|--------|-------------------|-------------------|-------|
| A      | 100               | 0                 | Negative control |
| B      | 90                | 10                | Test diet 1 |
| C      | 80                | 20                | Test diet 2 |
| D      | 70                | 30                | Test diet 3 |
| E      | 60                | 40                | Test diet 4 |

Changes in pH and titratable acidity during fermentation

Titratable acidity (TTA) and pH were measured at the beginning and after every 12 h during fermentation according to the AOAC (2000) and Salvador et al. (2016). The pH values of the complementary beverage were determined in triplicate by a pH meter. For TTA, 5 g of dried sample was mixed with 25 ml distilled water, the mixture was allowed to stand for 15 min, shaken at 5 min intervals and centrifuged at 3000 rpm for 15 min. 10 ml of the filtrate were titrated against 0.1 M NaOH using 1% phenolphthalein (3 drops) as indicator. The acidity was calculated as % (W/W) lactic acid equivalent using the formula described by Salvador et al. (2016).

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\text{% lactic acid} = \frac{\text{ml of } 0.1\,\text{M NaOH (end point)}}{\text{ml of sample taken}} \times 100
\]

Proximate analysis

Proximate parameters such as % crude protein, crude fibre, ash, fat, carbohydrate and moisture contents were determined on each sample using the standard procedures described by AOAC (2000). Percentage moisture was calculated by drying the sample in an oven at 105°C for 3 h. The dried sample was put into a desiccator, allowed to cool and the reweighed. Crude protein was determined by Kjeldahl method using a nitrogen-protein conversion factor of 6.25, crude fibre by acid and alkaline hydrolysis, fat was determined by Soxhlet extraction method with petroleum ether. Percentage ash was evaluated by combusting the samples in silica crucible placed in a muffle furnace at a high temperature of 600°C. The percentage of carbohydrate present was determined by subtracting all of the components assayed (crude protein, crude fat, moisture, fibre and ash) from 100 (AOAC, 2000; Kambabazi et al., 2021).

Analysis for minerals

Samples were analyzed for selected minerals following the method described by Okalebo et al. (2002). A portion (2 g) of the ash obtained was digested with nitric and hydrochloric acid (1:3) and allowed to stand for 30 min. It was later filtered using Whatman filter paper (0.45 μm). The resulting extract was used for the determination of calcium, iron, manganese, zinc, and copper by the use of an atomic absorption spectrophotometer (ICP-OES 720 series Agilent). Standard solutions (Sigma Chemicals, USA) of each of the minerals were also prepared and co-analyzed with the sample extract preparations.

Analysis for vitamins

The B-complex vitamins (Thiamine, Riboflavin and Niacin) were determined following the methods described by Okwu and Josiah.
(2006), while vitamin C content of the enriched fermented cassava mahewu was determined by the methods described by Desai and Desai (2019) using the spectrophotometric methods.

Sensory evaluation

The sensorial analysis was carried out following the methods described by Boyiako et al. (2020). A semi-trained panel of thirty members who were familiar with the standard product evaluated the sensory properties of the enriched product. The panelists were asked to rate each sensory attribute of all samples including the standard cassava mahewu. The fermented samples were coded with letters and served to the panelists at random to guard against any bias. The basis for evaluation included appearance/thickness, taste, flavour, colour and overall acceptability. The rating was done on a 9-point hedonic scale (9. Like extremely; 8. Like very much; 7. Like moderately; 6. Like slightly; 5. Neither like nor dislike; 4. Dislike slightly; 3. Dislike moderately; 2. Dislike very much; 1. Dislike extremely). Water was provided to rinse the mouth after each testing.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data generated using the Statistical Package for Social Sciences (SPSS) version 17.0. Least significant difference (LSD) test (P<0.05) for means separation was done using Statistix 10. Results were expressed as mean ± standard deviation of triplicate determinations

RESULTS

Changes in pH and titratable acidity during fermentation

The result of changes in pH and titratable acidity (TTA) during the production of the improved cassava mahewu is shown in Table 2. It was observed that the highest pH (6.55) was recorded at 0 h which decreased to the lowest pH of 4.30 for 40% at 36 h. Titratable acidity (TTA) increased from minimum of 0.07% at 0 h to a maximum of 1.56% at the end of the fermentation in all the enriched samples.

Proximate composition of improved cassava mahewu

The quantitative estimations (in percentages) of the proximate compositions of M. nigeriensis-enriched mahewu are shown in Table 3. A significant (p<0.05) improvement in % protein (from 2.87 to 32.65), fat (0.65 to 29.15), fibre (1.26 to 4.04) and ash (1.55 to 4.14) was observed with the inclusion of M. nigeriensis at different blend ratios. Cassava mahewu with 40% M. nigeriensis inclusion had the highest amount of protein, fat and ash content when compared with the control mahewu. No significant difference (p>0.05) was recorded in water content of the mahewu with the inclusion of M. nigeriensis flour. However, a significant decrease (p<0.05) in carbohydrate content was recorded in the mahewu following the inclusion of M. nigeriensis flour at all levels.

Mineral composition of termite-improved cassava mahewu

Table 4 shows the selected mineral composition of termite flour incorporated cassava mahewu at different blend levels. The mineral contents of all the composite blends increased significantly compared with the control mahewu (p<0.05). It was observed that the sample having 30% termite flour inclusion had a significant (p<0.05) higher improvement in calcium, zinc and manganese. However, iron content was higher in 40% termite flour inclusion when compared with the control mahewu which recorded the least in all minerals assayed.

Effect of M. nigeriensis flour inclusion on vitamin content of cassava mahewu

Results of the vitamins assay of M. nigeriensis-improved cassava mahewu are shown in Table 5. It was observed that inclusion of termite flour at different proportions significantly improved the amount of vitamin C (from 42.4 to 55.8 mg/100 g), riboflavin (0.15 to 1.80 mg/100 g), thiamine (0.67 to 2.30 mg/100 g) and niacin (0.85 to 1.98 mg/100 g). Inclusion at 30% had higher vitamin C and niacin content than other proportions and the control. Riboflavin and thiamine were higher in 40% inclusion.
Effects of winged termite inclusion on the sensory attributes of cassava mahewu

The sensory evaluation of the fermented beverage prepared from various percentage inclusion of termite flour (Table 6) showed that the sample with 30% termite inclusion was rated significantly higher (P<0.05) for taste (7.56) than other samples. Sample with 10% termite inclusion had the least score for taste (5.80). Cassava mahewu with 40% termite flour was rated significantly higher (P<0.05) for flavour (7.80) than other samples. The control sample was more preferred in terms of appearance/thickness (7.90) and colour (7.20) followed by 10% inclusion. Inclusion at 40% recorded the least (5.60) in terms of colour.

In terms of the overall acceptability (Figure 1) of the product, termite flour inclusion at 30% was significantly (P<0.05) rated higher than other samples. Inclusion at 10 and 20% showed no significant difference (P<0.05) in terms of acceptability when compared with the standard cassava mahewu. Cassava mahewu with 40% winged termite inclusion had the least score for overall acceptability.

**DISCUSSION**

Providing information about the benefits of entomophagy and incorporating same in traditional foods can increase the willingness to eat this kind of food due to their high nutritional profile (Finke and Oonincx, 2014). In the present study, the fermentation of winged termite-incorporated cassava mahewu was characterized by a decrease in pH and a corresponding increase in TTA. This observed inverse proportional trend between pH and TTA was previously reported by Salvador et al. (2016) and Fadahunsi and Soremekun (2017). This might probably be caused by the microbial activities during fermentation which degraded some of the carbohydrates content into lactic acids and other organic acids, consequently causing the fall in pH and increase in TTA. According to Agarry et al. (2010), a decrease in pH and corresponding increase in TTA is a key factor for growth of microorganisms during fermentation which signified a good rate of fermentation. Moreover, cassava flour had been reported as being a good substrate for lactic acid production through fermentation (Salvador et al., 2016). In addition, the occurrence of un-dissociated forms of organic acids at low pH could inhibit a broad spectrum of pathogens thus improving the microbiological stability of the final product (Blandino et al., 2003). This is because most food pathogens are not able to survive at low pH. Therefore, the decrease in pH observed in this study is advantageous to the final product in terms of prolonging its shelf life and microbial stability.

The need for improving the nutritional quality of cassava mahewu with edible insect becomes evident with the observed lower values of protein, ash, and crude fibre contents of standard cassava mahewu used as control which could be due to losses during cassava processing (Montagnac et al., 2009) or complete absence of the nutrient. Following the inclusion of winged termite flour at
different proportions, a significant increase (p<0.05) in protein content was observed when compared with the control standard mahewu. The result indicated that the winged-termite is a good source of protein for man and animals which is in agreement with the report of Kinyuru et al. (2010) and Igwe et al. (2011a). Since an adult male with 70 kg average body weight requires 35 g of protein daily, therefore only about 164 g (on dry weight) of the termite-enriched cassava mahewu beverage would be required to provide an average adult minimum daily protein requirement, with an allowance of 25% made for protein indigestibility and the limiting sulphur amino acid content (Igwe et al., 2011b; Inje et al., 2018). That means a lower amount (<164 g on dry weight) of the fermented beverage needs to be consumed per day by children in order to meet their daily protein requirement.

The carbohydrate content of the fermented cassava mahewu decreased significantly (p<0.05) with the addition of edible winged-termite flour compared with the control. This could be attributed to the effect of fermentation on the carbohydrate content. The indigenous microflora associated with the natural fermentation of cassava mahewu has been reported to be amylolytic (Omenu et al., 2007) thereby causing high hydrolysis of carbohydrate to simple molecules. The fermenters utilize carbohydrates as an energy source during fermentation and produce carbon dioxide as a by-product. This is in agreement with the reports of Fadahunsi and Soremekun (2017) and Boyiako et al. (2020) who revealed that fermentation of starchy foods generally leads to a decrease in the level of carbohydrates content.

A significant improvement (p<0.05) in fat content of the cassava mahewu was observed following the inclusion of African winged-termite flour at different proportions. This indicates that the termite used for enrichment is an important source of fat and oil. Winged-termites have been reported to contain appreciable higher amounts of good fats which are clearly superior when compared with conventional foods of animal origin (Igwe et al., 2011b). Over 60% of the fatty acids present in edible insects generally are in the form of highly desirable mono- and polyunsaturated fatty acids (MUFA and PUFA) (Igwe et al., 2011a; Rumpold and Schluter, 2013). Saturated fatty acids are not good for human consumption because they have been implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging due to their greatest negative effect on LDL cholesterol. In contrast, MUFA and PUFA present in insects have been shown to decrease plasma cholesterol concentrations (Igwe et al., 2011b). Therefore, the increase in fat content observed may not necessarily portend risk for its consumers. Moreover, the termite is prepared by the use of de-fating process that reduces the fat content before consumption.

The crude fibre of the enriched fermented cassava mahewu also increased significantly compared with the 100% cassava mahewu. This could be accredited to the addition of the edible termite flour which has been reported to be high in dietary fibre (Igwe et al., 2011; Akullo et al., 2018). Fiber in the diet is desirable because

| Component       | Vitamin C | Thiamine | Riboflavin | Niacin |
|-----------------|-----------|----------|------------|--------|
| Control mahewu  | 42.4±1.07a| 0.67±0.04a| 0.15±0.03a| 0.85±0.02a|
| 10% Inclusion   | 42.8±2.14a| 0.75±0.07ab| 0.89±0.20ab| 0.88±0.14ab|
| 20% Inclusion   | 53.4±3.2b | 1.30±0.14b | 1.22±0.07bc| 1.60±0.08bc|
| 30% Inclusion   | 55.8±5.2b | 1.56±0.07bc| 1.64±0.14c | 2.08±0.12c |
| 40% Inclusion   | 52.6±2.3b | 2.30±0.14c | 1.80±0.12c | 1.98±0.28c |

Mean with different superscript (a-b-c) are significantly different at (P<0.05) along the columns.

| Sample          | Flavour  | Taste    | Appearance/thickness | Colour  |
|-----------------|----------|----------|----------------------|---------|
| Control         | 6.11±1.2a| 6.80±0.4ac| 7.90±0.4a           | 7.20±1.2a|
| 10% inclusion   | 6.12±0.8a| 5.80±0.3a | 7.10±1.0a           | 6.82±0.8ab|
| 20% inclusion   | 5.80±1.0a| 6.30±0.5a | 6.56±2.1b           | 5.80±0.0ab|
| 30% inclusion   | 6.22±0.6a| 7.56±1.7b | 6.22±0.3a           | 5.78±0.9b|
| 40% inclusion   | 7.80±0.6a| 6.50±0.7c | 5.78±1.6c           | 5.60±1.0b|

Mean with different superscript (a-b-c) are significantly different at (P<0.05) along the columns.
it lowers the serum cholesterol and enhances the excretion of fecal steroids thereby reducing the risk of atherosclerosis. Significant increase (p<0.05) of the ash content seen in this study implied that the improved product had considerable amount of minerals because the level of ash content is a reflection of the total available minerals in any food sample. As observed, the termite-enriched cassava beverage has a fair content of iron, zinc, manganese and copper. These minerals are known to play important metabolic and physiologic roles in the living system. Iron is an indispensable nutrient that is involved in oxygen transport and plays a vital role in brain and cognitive development (Akeredolu et al., 2011). It is well known that young children are vulnerable to the effects of iron deficiency because of rapid growth and development of their organs. Iron deficiency is the most common cause of anemia (Miller, 2013) and the most prevalent important nutritional problem in children. It threatens over 60% of women and children in most developing countries (WHO, 2010). Low intake of iron especially in children, can contribute to anemia which indicates the vulnerability of this age group to the iron deficiencies.

Zinc is another essential element and it is apparently deficient in the diets of many people in developing countries. Zinc and manganese for example, strengthen the immune system and act as cofactors for many enzymes participating in the metabolism of carbohydrates, proteins, fats and nucleic acids. Low zinc status in children has been associated with retarded growth, poor appetite and impaired sense of taste (WHO, 2010). Assuming good bioavailability of the iron contents, the improved fermented cassava mahewu can contribute significant amount of iron to children and women especially during pregnancy in attempts to mitigate the risk of iron deficient anemia and undernutrition of micronutrient that is wide spread in developing countries. Similarly, calcium is an essential mineral which plays vital roles by virtue of its phosphate salts in neuromuscular function in many enzyme-mediated processes like blood clotting, tooth and bone formation (Igwe et al., 2011). Children need calcium for strong bone formation. Apart from the nutritional point of view, calcium has also received substantial interest in the medical field for its role in osteoporosis and several other chronic diseases including hypertension and colon cancer (Thacher et al., 2012). An increase in the amount of calcium was observed in the present study following the addition of termite flour. The present findings is in agreement with Boyiako et al. (2020) who reported increase in calcium and other mineral content of cassava mahewu when enriched with beetroot juice.

The vitamin contents of winged termite-improved cassava mahewu showed that it contained appreciably high amounts of vitamins C, riboflavin (B2), niacin (B3) and thiamine (B1) which increased appreciably with the inclusion of termite flour at different levels. Vitamin C maintains blood vessels flexibility and improves blood circulation in the arteries (Miller, 2013; Desai and Desai, 2019). One of most important benefit derivable from vitamins C from food is their role as antioxidants while that of the B-vitamins is their role as co-enzymes in several enzyme systems of the body. The high vitamins and minerals contents present the developed product enriched as a highly potential good source of vitamins for malnourished children especially in poor-resource
settings.

The sensory evaluation of the samples showed that the enriched cassava product with 30% termite inclusion was rated highest for taste and overall acceptability. Previous report has shown that the taste and flavor of food have the most pronounced effect on consumer acceptance of the food (Kourimska and Adamkova, 2016). Dietary fat in food increases the palatability of food by absorbing and retaining the flavor. Hence, the increased fat content of the winged-termite could have contributed to the taste, flavor and acceptability of the termite-improved cassava mahewu by the consumers. Nevertheless, the sensory evaluation proved that our product gained a level of consumer acceptance comparable with the standard.

Conclusion

The study has revealed African winged termite (M. nigeriensis) as a very good source of proteins and micronutrients necessary for combating protein-energy malnutrition and micronutrient deficiencies rampant in various developing world today. The inclusion of edible M. nigeriensis flour at different levels improved the nutritional quality of fermented cassava mahewu. With regards to the sensory properties and consumer acceptability, cassava mahewu having 70:30% termite flours recorded the highest preference. Therefore, the enriched cassava mahewu could be utilized to manage the widespread nutrient deficiency in developing countries where cassava is regarded as the main staple. However, more research is needed on the technological and safety aspects of such product in order to promote the use of insects as food and mainstream acceptance of such products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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