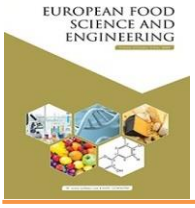

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Fermentation and diet diversity: Some biochemical and techno-functional properties of fermented mango (*Mangifera indica*) mesocarp flour

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ABSTRACT

Indigenous food fermentation is one of the oldest 'food biotechnological processes' dependent on the biological activity of microorganisms from which the development of fermented foods is achieved. Mango (*Mangifera indica*) is a tropical fruit plant that contains high levels of fibre, macronutrients, micronutrients, and minerals as well as abundant bioactive compounds such as antioxidants and polyphenols reported to be an alternative to enhance body immunity. This study aimed to process fermented mango mesocarp flour and the effects of fermentation time on the chemical composition and selected techno-functional properties of mango mesocarp flours were examined. The fermentation time ranged from 0-72 hrs with flour of the unfermented (0 hr) mango serving as control. Fermentation for 24 to 72 hrs significantly increased ($p<0.05$) crude protein and total ash in the flours. Ether extract and carbohydrates significantly decreased ($p<0.05$) in the flours with an increase in fermentation time. Significant ($p<0.05$) reduction in bulk density, swelling index, and water absorption capacity occurred in the flour with fermentation. There was a significant ($P<0.05$) increase in vitamin C and beta–carotene content of the fermented samples and this was constant as fermentation progressed across all the samples. Therefore, fermented mango flours have great potential to be developed into healthy foods since antioxidants have the ability to improve immunity and anti-inflammatory response.

1. Introduction

Malnutrition in all its manifestations, including underweight, micronutrient deficiencies, overweight, obesity, and related non-communicable diseases, is a worldwide health concern, with the majority of cases occurring in low- and middle-income nations (LMICs) (Bhutta & Salam, 2012). The "nutrition transition," in which populations switch from traditional diets high in fibre and micronutrients to more highly processed diets high in sugar, fat, and salt, low in fibre, and less nutrient-dense, is largely responsible for this malnutrition, especially shifts towards an increased prevalence of overweight and obesity (Walls et al., 2018). These dietary changes are also accompanied by changes in eating habits and physical activity patterns (Popkin et al., 1994; Walls et al., 2018). With the UN Sustainable Development Goal (SDG) of Zero Hunger by 2030 still in view, the adoption of a diversified healthy diet, with emphasis on affordable nutrient-rich plant-based foods such as fruits, vegetables, whole grains, and legumes can not only contribute to the achievement of SDG2 but also have an impact on food and nutrition security (Elechi et al., 2022; FAO et al., 2020; EAT, 2019). Dietary fibres, micronutrients and prebiotic compounds, present in fruits and vegetables, considerably improve human health through mechanisms, such as microbiota modulation, reduction of postprandial glycemic response, normalizing the cholesterol level, preventing constipation, transporting the phenolic compounds, and so on (Pop et al., 2021; Delcour et al., 2016; Holscher, 2017). Reutilization of fruits and vegetable wastes and by-products (Ng et al., 2020), or taking the maximum out of them by extracting valuable compounds that can be further used in functional foods or in nutraceuticals may be the most promising approaches with a positive impact on the environment, the food industry and finally, on consumer's health (Dueñas and Garcia-Estevez, 2020; Galanakis, 2020).

Processing of plant foods holds a promising potential for optimization of product quality, such as improvement in flavour, texture, nutrient density, and bioavailability as well as a reduction in viscosity, bulkiness, and antinutritional factors/toxins or for improvement of functional properties for use in other food systems. Indigenous food fermentation is one

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of the oldest 'food biotechnological processes' dependent on the biological activity of microorganisms (Ross et al., 2002) from which the development of fermented foods is achieved in the cultural history of human beings (Geisen & Holzapfel, 1996). During the process, locally available ingredient(s) either plant or animal origin are converted biochemically and organoleptically into upgraded edible products called fermented foods (Campbell, 1994; Steinkraus, 1996). Hence, fermentation is a common traditional practice of food processing and preservation to hence bioavailability of essential nutrients in nutrition deficiency communities in the global south. Several researchers have reported that fermentation modifies some physical characteristics of food matrix such as reduction in bulk density, increases the level of some nutrients such as vitamins and minerals, and decreases the levels of anti-nutrients while improving digestibility as well as bioavailability of essential micronutrients (Sengev et al., 2021; Marina et al., 2013; Ojokoh & Babatunde, 2014; Nkhata et al., 2018). The fermentation process is known to increase the levels of antioxidants or vitamin C in processed food products (Selibata et al., 2017). Recent research has shown that fermented fruit is higher in antioxidants and health benefits (Gagnon et al., 2015) and that increasing the intake of foods high in antioxidants and polyphenols such as Vitamin C, beta-carotene, quercetin and kaempferol can increase the body immunity against viral infection (Levy et al., 2020; Pitsillou et al., 2020; Suhail et al., 2020). Fermentation, therefore, holds promise as a processing method that can be used to diversify the food uses of some under-exploited plant foods. Mango trees grow in home gardens, roadsides, and forests all over Nigeria. During the fruiting season, there is always spoilage because of the lack of adequate storage facilities (Badifu et al., 2000). Drying and fermenting the mesocarp would help to reduce the in-season wastage of the fruit and diversify its uses in food. Incorporation of fermented mango mesocarp flour into food blends such as bread, cookies, complementary foods, and other flour mixture might enrich the provitamin A content, increase the bioavailability of other essential micronutrients and enhance the overall digestibility of foods.

Mangos are the most important tropical fruit crop after bananas and plantains (FAO, 2011). The mango fruit is a large fleshy drupe, highly variable in size, shape, colour, and taste, weighing up to 1 kg in some cultivars. The mesocarp of mango fruit is a good source of provitamin A, with reported concentrations of 2,400 mg/100 g (West et al., 1988 as cited in Badifu et al., 2000). Mango (*Mangifera indica*) is a tropical fruit plant that contains high levels of nutrients, fibre, macronutrients, micronutrients, and minerals as well as abundant bioactive compounds (Maldonado-Celis et al., 2019). The well-known among them include vitamin C, beta-carotene, and polyphenol types of quercetin, and kaempferol (Mantik et al., 2021). A wide variety of processed products derived from mango fruits include canned whole or sliced mango mesocarp in brine or in syrup, mango juice, nectar, jam, sauce, chutney, and pickle. Tapping into the planetary wealth of diverse fruits, vegetables, pulses, and grains, particularly nutrient-dense varieties among these food groups, holds the potential to generate the desired win-win scenario for people and the planet (Gina et al., 2017). Dietary guidelines around the world recommend a varied diet rich in fruits, vegetables, whole grains, nuts, seeds, and legumes for optimal health (Fischer & Garnett, 2015). Consequently, enriching the micronutrient and macronutrient content of any widely consumed traditional food using readily available food commodities and adaptive processing methods would help to reduce the prevalence of nutritional deficiency in the most vulnerable group (Badifu et al., 2000).

Mango mesocarp flour's potential application as a techno-functional food component depends on its physical, chemical, and techno-functional characteristics. Techno-functional properties of food ingredients are the fundamental physicochemical characteristics excluding their nutritional value that take into account the intricate relationship between the composition, structure, molecular conformation, and physico-chemical characteristics of food components as well as the environment in which these factors are related and measured (Kinsella, 1976; Kostic et al., 2015; Chandra & Samsher, 2013). Ingredients' functional qualities have a direct or indirect impact on how they are processed, how well food turns out, and how widely they are used in food and food formulation (Mahajan & Dua, 2002). The majority of processed food items are multicomponent colloidal systems that comprise a variety of particles, including lipid crystals, starch granules, gas bubbles, protein and polysaccharides, and food biopolymers (Kostic et al., 2015). As a result, the kind and strength of these interactions dictate the overall system attributes (Dickinson, 2013). Solubility, the power to create and maintain emulsions and foams, and the ability to absorb water and oil are the techno-functional attributes most relevant to the food processing industry (Kostic et al., 2015). However, to date, a detailed composition of the flour produced from the mesocarp of Mango fruits as well as the accompanying biochemical changes that may affect the quality and acceptability of mango flours especially as influenced by fermentation has not yet been reported. This information is essential for determining the potential uses of this product in food formulations and will add value to this important crop. The result of this study will provide a recipe in an acceptable form that will increase the utilization of Mango fruits vis a vis reducing flour importation. Therefore, the purposes of this study were to assess the influence of traditional fermentation systems on the biochemical composition and techno-functional properties of mango mesocarp flour with the view of enhancing the utilization of this climacteric fruit.

2. Materials and methods

2.1. Collection of raw materials

Fresh mango (*Mangifera indica L.)* of local varieties (Ogbomosho/Enugu type-the most widely acceptable, sweet and aromatic) were purchased from local fruits market in Lafia Metropolis, Nasarawa State during the April 2022 fruiting season.

2.2. Preparation of flours

The mango flour was prepared following the method described by Elechi et al. (2022). The maturity stages were classified as fully mature, according to the morphological development of the fruit shoulder. Ripening was observed at ambient temperature (28±2 °C) for 2-3 days according to the maturity stage until fruits reached full yellowing of peel. Mangoes were sorted (only fruits that did not show any visual signs of bruises, cuts, or infestation were selected for the study), weighed, and washed under running water. After that, they were wiped, peeled by hand, and the seeds were removed. The acquired mesocarp was sliced for size reduction and to speed up the drying and milling processes, and the sliced mesocarp was steam blanched for 10 minutes at 60 °C to deactivate enzymes that may induce browning reactions. The slices were

distributed uniformly on a perforated stainless-steel pan after blanching and dried in a 65 °C oven for 24 hours. The disks were crushed into flour using a tabletop hammer mill (Brook Crompton, Series 2000, England) after drying and passing through a 0.2 mm particle size filter. The flours were then packed in dark-coloured Ziploc low-density polyethylene bags, kept at ambient temperature in a 500 mL plastic container with airtight lids for 24 hours, and utilized for product design and analysis.

2.2.1. Preparation of fermented mango mesocarp flour

Fermented mango mesocarp doughs were obtained by natural fermentation using the method described by as reported by Gernah et al., (2012). In this process, 120 g of each of the mango mesocarp flours were mixed with 80 mL of distilled water and subjected to natural fermentation in a covered 500 mL glass beaker at ambient temperature for 0 hrs, 24 hrs, 48 hrs, 72 hrs. At the end of each period, the fermented concentrates were withdrawn and dried at 80 ºC in a fan-driven electric oven to constant weight and milled in a disc attrition mill to a particle size of 0.2 mm. The resultant flours were then packaged in low-density dark-coloured Ziploc polyethylene bags, stored in 500 mL plastic containers with air-tight lids at ambient temperature, and utilized for analysis within 24 hours.

2.3. Analyses

2.3.1. Proximate analysis

Moisture, crude protein, ash, crude fat, and crude fibre contents of fermented mango mesocarp flour were determined according to the AACC (2000) method. Protein content (%N x 5.7) was determined by the Kjeldahl method. Moisture was determined by oven drying for 4 hours at 100-105 ºC. Ash was measured by dry combustion [AACC, 2000 (Method 08-01)]. Free lipids were measured by petroleum ether extraction, followed by evaporation to constant weight [AACC, 2000 (Method 30-25). The crude fibre was determined according to the procedure of AACC, 2000 (Method 32-07). Available carbohydrate was calculated as 100% - (% moisture + % ash + % fat + % protein + % crude fibre). The pH of the samples was determined using the method described by Akpapunam & Sefa-Dedeh (1995) using a pH meter (Labtech digital pH meter Model 152R). All samples were done in triplicate.

2.3.2. Techno-functional properties

Bulk density (BD)

Bulk density of the flour was determined according to the method of Danbaba et al. (2014) with modifications. The sample (100.00±0.05 g) was weighed and gently filled in a 250 mL graduated cylinder. The bottom of the cylinder was gently tapped ten times until there was no further diminution of the sample level. Bulk density was expressed as the weight of the sample per unit volume of the sample (g/mL). Measurements were made in triplicate.

Water absorption capacity

Water absorption capacity was determined using the method described by Mustapha et al. (2015). Distilled water (40 mL) was added to 2.00±0.05 g of mango mesocarp flour in a pre-weighed centrifuge tube. The dispersions were stirred occasionally, held for 30 min, followed by centrifugation for 15 min at 1000 × *g*. The supernatant was decanted, excess moisture was removed by draining for 24 h at ambient temperature (30 ± 2°C), and the sample was reweighed. The amount of water bound by the flour was determined by difference and expressed as the weight of water bound by dry flour (100 g).

Swelling index

1 g of the sample was weighed into a conical flask. It was hydrated with 15 mL distilled water and shaken for 5 min with mechanical shaker at low speed. Heating was done for 40 min at 80-85 ºC with constant stirring in a water bath the content was transferred into a clean, dried, and pre-weighted tube. 7.5 mL of distilled water was added and centrifuged at 2200 rpm for 20 min. The supernatant was decanted into a pre-weighted can and dried at 100 ºC to a constant weight. The sediment was weighted in the centrifuged swelling index was calculated as follows:

$$Swelling\ (\%) = \frac{(W_2 - W_1)}{W_1} x100$$

W_1= weight of the polymer (before swelling) W_2= weight of the polymer (sfter swelling)

Reconstitution Index (RI)

The reconstitution index was determined using the method described by Banigo & Akpapunam (1987) as reported by Gernah et al., (2011).

2.4. Determination of biochemical properties

2.4.1. Determination of beta-carotene

The method described by Akpapunam & Ibiama (1985) was used. Five grams of the sample was transferred to a separating funnel and a solution containing 40 mL hexane, 60 mL ethanol, and 2% NaCl solution was poured into the funnel to allow extraction. The mixture was allowed to settle and the lower layer was run off. The top layer, which contained carotenoid was collected and the optical density (OD) was determined using a colorimeter filter at 450-470nm. The total carotenoid was calculated thus;

$$Total\ carotenoids\ (mg/100\ g) = \frac{Absorbance}{Specific\ extinction\ coefficient\ x\ path\ length\ of\ cell}$$

Where, molar extinction coefficient (ε) of β -carotene at 460nm = 15^{-4}, specific extinction coefficient (a^{1cm}) =ε x Molar mass of β –carotene, molar mass of β –Carotene = 536.88g/mol. β –carotene

2.4.2. Determination of vitamin C

The vitamin C analysis of the flour samples was carried out using the Titration Iodometric Method, to determine the amount of Vitamin C (mg/100g). Two grams of the samples were weighed and made into a paste. Then 100 mL of distilled water was added to the paste in a volumetric flask. It was then filtered to a clear solution. Then 2 mL of the solution was pipette into small flasks. It was then titrated with indophenols solution (2,6 dichlorophenolindophenol) until a faint pink colour persisted for 15 seconds.

$$Vitamin\ C = \frac{mL\ of\ Titrant\ x\ Volume\ of\ indophenol\ used}{Volume\ of\ sample} x100$$

2.5. Microbiological analysis

2.5.1. Total bacterial count

The method reported by Adegoke (2004) was adopted. About 9 mL of diluent (distilled water) was measured into test tubes. Then 7.5 g of nutrient agar was weighed into a 250 mL conical flask and diluted to mark. Both the diluent and the nutrient agar were put into an autoclave and heated until they reached a temperature of 121ºC. After which 1g of the sample was weighed and poured into the first test tube and serial dilution was prepared. Then 0.1 mL of the dilution was transferred to sterile petri dishes after which the cool nutrient agar was poured and swirled gently and then allowed to solidify. It was then transferred to an incubator in an inverted manner and incubated at 37 ºC for 24 hours. The colonies developed after incubation was counted using a colony counter and recorded in CFU/g.

2.5.2. Total fungal count

The method described by Adegoke (2004) for total yeast and mould counts was used. Distilled water was pipetted into ten test tubes and sterilized in an autoclave along with prepared potatoes dextrose agar (PDA) of 10 g, dissolved in 250 mL of distilled water. After which 10% of tartaric acid with a pH of 3.5 ± 0.1 was used to acidify the sterilized potatoes dextrose agar before it was used. It was poured, swirled, allowed to solidify, and then incubated at a temperature of 25 ºC for 72 hours in an inverted form. The colonies developed after incubation was counted using a colony counter and recorded in CFU/g.

2.6. Statistical analysis

Data were analyzed with SPSS version 11.0 (Illinois, U.S.A) using one-way Analyses of variance (ANOVA). Significant differences were tested using the Duncan Multiple Range test. Statistical significance was accepted at ($p<0.05$).

3. Results and Discussion

3.1. Techno-functional properties of fermented mango mesocarp flour

In this study, techno-functional properties were used to determine or describe the behaviour of the flours during preparation and cooking and also predict how they will affect the finished products in terms of appearance, taste and texture Figure 1 shows the techno-functional properties of mango mesocarp flour. The results indicate that the 0 hrs (unfermented mango) mesocarp flour had the highest value of bulk density (1.20%) while the fermented mango mesocarp flour at 72 hrs had the lowest bulk density, this could be due to the reduction of the total soluble solids during fermentation. The low bulk density of the blends could be an advantage in the formulation of baby foods where high nutrient density to low bulk is desired as observed by Mepba et al., (2007). Nutritionally, low bulk density promotes easy digestibility of food products, particularly among children with weak digestive system (Chandra et al., 2014). Also, the lower bulk density values of fermented mango mesocarp flour implies that less quantity these flour samples would be packaged in constant volume thereby ensuring an economical packaging (Osundahunsi & Aworh, 2002).

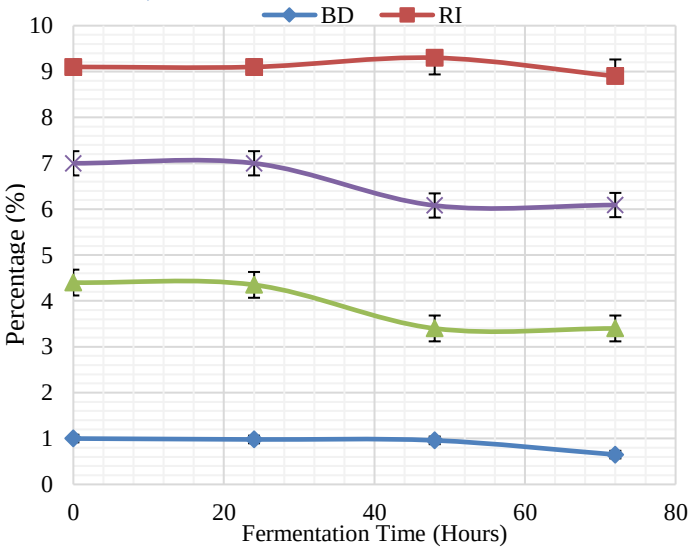

Figure 1. Techno-functional properties of fermented mesocarp flour. Key: BD = Bulk density, RI = Reconstitution index, SI = Swelling index, WAC = Water absorption capacity

The reconstitution index describes the ease of dispersibility of the flours. The reconstitution index of the unfermented sample was 9.25%, which is similar to the fermented sample at 24 hrs. The value of the fermented sample at 72 hrs was 8.85%. Fermentation reduced the reconstitution index significantly ($p<0.05$) in 48 hrs and 72 hrs fermented flours when compared with the controls (0 hrs). Fermentation may have induced a change in the texture of the hydrophilic components of the flour to have influenced the ease of dispersibility of the flour. Similar result trends and observations were reported by Onweluzo & Nwabugwu (2009). Ease of dispersibility is an important flour property in food formulation (Igene et al., 2005).

The water absorption capacity of the samples was high for the unfermented sample but low for the fermented samples. The water absorption capacity decreases with an increase in fermentation time. The rate of instantaneous water absorption appears to correlate with the total surface area while the absorption capacity correlates more with the porosity of the flour. This is because fermentation influenced the ability of the flour macromolecules to absorb water. Elkhalifa et al. (2006) and Onweluzo & Nwabugwu (2009) reported similar observations in fermented sorghum, and fermented millet (*Pennisetum americanum*) and pigeon pea (*Cajanus cajan*) seeds flour respectively. Low water absorption is a desirable functional property required to produce thin gruels that can be used in infant formulations. Reports have shown that flours with high WAC may not be able to keep well because WAC is an indication of the maximal water that foods can absorb and retain (Iwe & Agiriga, 2015). So proper storage of such flours should be ensured (Aburime et al., 2020). Also, flours with higher WAC and lower solubility value indicates that care must be taken when dissolving it in water to avoid the formation of lumps since WAC and solubility of flours are associated with the ability of flours to disperse in aqueous solution and the vicious load that is likely to be encountered during the mixing of the flours (Aburime et al., 2020; Iwe et al., 2017). So, there will be no formation of lumps in reconstituting the fermented mango mesocarp flour in water.

The swelling index of the unfermented sample (4.46%) was higher than that of the fermented samples (3.53%) which indicates that the flour will combine well with other flours and can be used for the formation of variable products, because the SI of flour suggests the level of crystalline packing of the starch

granules, reflecting the extent of the association forces within the granules (Amiri et al., 2009). A similar observation was reported by Aburime et al. (2020).

3.2. Proximate analysis

The proximate composition of fermented and unfermented mango mesocarp flour is shown in Figure 2. The results show that there was an increase in the moisture content of the sample with a significant difference ($p<0.05$). This could be a result of the release of bound water during fermentation as microorganisms break down the cell walls of the food sample; it could also be due to the hydrolysis reaction that takes place during the fermentation. The ash content increased with a significant difference ($p<0.05$) as the fermentation time increased from 2.41% to 3.88%. This increase may be due to the contribution of fermentation microorganisms. The same trend was observed by Eka (1980) on the effect of fermentation on the nutrient status of locust beans where an increase of about 30% in ash content was recorded after fermentation. It also agrees with the observation of Amoo (2003) on the effect of fermentation on the nutrient and mineral content of *Bauhinia reticulata*. The ash trend of this study differs from that of Onweluzo & Nwabugwu (2009) who reported a marginal decreased ($p>0.05$) in total ash with the increase in fermentation time in both millet and pigeon pea flours. This the authors attributed to possible losses of dry matter and volatiles which normally occur during fermentation as reported by Quinn et al. (1975) and Nnam & Obiakor (2003).

The fat content decreased marginally ($p>0.05$) during the first hours of fermentation, but during the last hours of fermentation, it decreased with no significant difference ($p>0.05$). This may be due to the utilization of lipids by fermentation microbes to obtain energy for their activity when sugars were in short supply. This is contrary to the result and observations reported by Achinewhu & Isichei (1990), Obizoba & Atii (1991) and Onweluzo & Nwabugwu (2009) in fluted pumpkin, sorghum seeds and fermented millet and pigeon pea flour respectively. The authors reported an increase in crude ether extract as a result of fermentation which they attributed to the increased activities of lipolytic enzymes which hydrolysed fat to glycerol and fatty acids and the release of some non-lipid ether extractable materials released by fermenting micro-organisms may also have contributed to the observed increase in the crude ether extract of the samples.

The crude fibre increased with a significant difference ($p>0.05$) at 72 hrs of fermentation. This could be a result of the reduction of total soluble solids, and also fermentable sugars. The reason for the unexpected increase in fibre content for the fermented samples may be due to the activities of microorganisms. The fermentation process involves the conversion of materials to the peculiar needs of the microorganisms, which include the bacterial cell wall. The bacterial cell wall is made of peptidoglycan or murein, which is a polysaccharide like cellulose. As the microorganisms were not separated from the biomass, the increase in fibre could be due to such conversion of materials to peptidoglycan by the microorganisms (Eze & Ibe, 2005).

The crude protein values ranged from 2.97 to 6.96% on the basis of fermentation time. There were no intermediary decreases in crude protein with an increase in fermentation time as reported by Onweluzo & Nwabugwu (2009). Hence, the crude protein increased with a significant difference ($p<0.05$). This could be due to the dead cells during fermentation and also the contribution of protein from micro-organisms since the biomass of micro-organisms contains about 50% protein (Sukhikh et al., 2022; Jach et al., 2022; Linder, 2019). During fermentation, microorganisms hydrolyze proteins and it complexes to release free amino acids, which can be used for the synthesis of new proteins (Frazier & Westhoff, 1978). Because of the microorganism's rapid growth in its own biomass, it was hypothesised that it contributed to an increase in protein content (Sukhikh et al., 2022). The results, which showed an increase in protein content of 2.97 to 6.96% supported this hypothesis and correlate with the findings of Sukhikh et al. (2022) and Sharawy et al. (2016) who found an increase in protein content with fermentation time as the result of activities of a consortium of microorganism. These results also provide evidence that fermentation by a consortium of microorganisms is more efficient than by isolated strains (Sukhikh et al., 2022). However, this result disagrees with the report of Wang & Hesseltine (1981), Ene-Obong & Obizoba (1996) and Onweluzo & Nwabugwu (2009) who stated that the fermentation process did not significantly change the total protein content and amino acid composition of substrates.

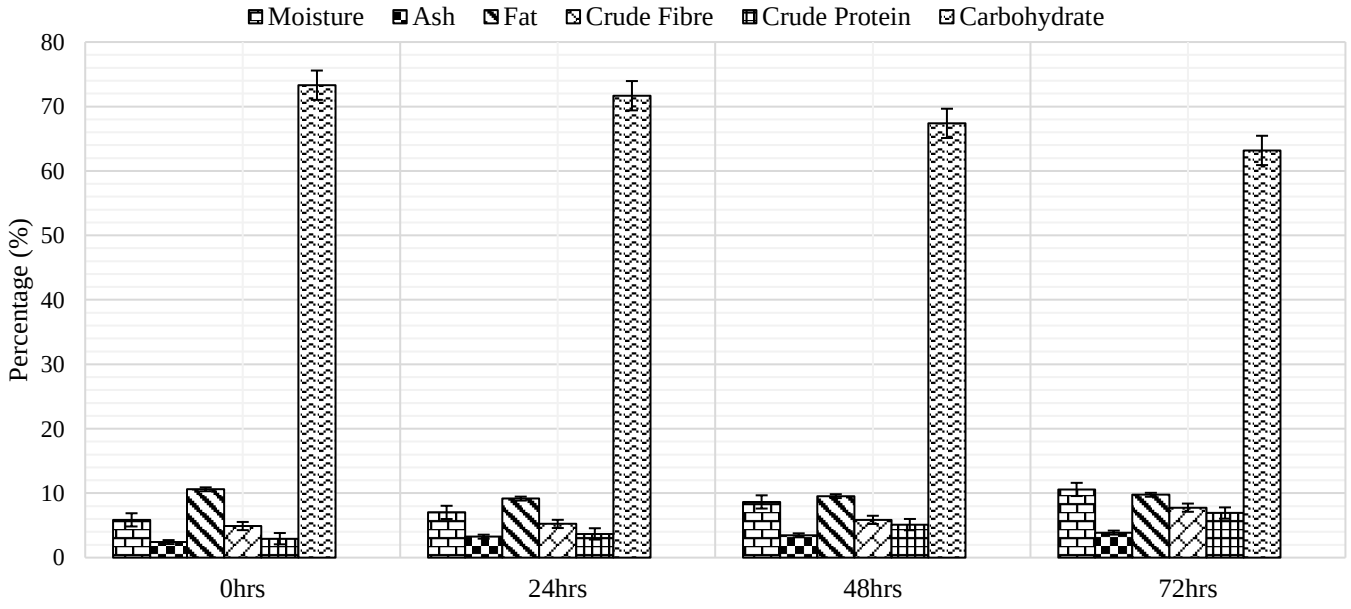

Figure 2. Chemical composition of fermented mango mesocarp

Fermentation significantly ($p<0.05$) decreased carbohydrates in mango mesocarp flour. The observed changes in carbohydrate with fermentation agrees with the report of Achinewhu & Isichei (1990) and Nnam (1995), Onweluzo & Nwabugwu (2009) on fermented fluted pumpkin seeds, fermented cowpea and fermented millet and pigeon peas respectively. The apparent decreases were attributed to the increased activity of amylolytic enzymes, which hydrolyze starch and other complex carbohydrates to simpler sugars. The simpler sugars then provide energy for the fermenting micro-organism and carbon skeleton for the possible synthesis of other compounds (Kazanas & Fields, 1981). Evidently, such enzymatic activities would also increase the availability of nutrients in fermented samples.

The pH level decreased as fermentation progressed. Rambault & Tewe (2001) indicated that the pH of a culture may change due to the metabolic activities of micro-organisms during fermentation, the acidity level of the fruit is increased and the pH is lowered, this inhibits the growth of food spoilage or poisoning bacteria and destroys certain pathogens (Hammes et al., 1994). However, the most obvious reason in agreement with (Ojokoh, 2007) is the secretion of organic acids such as citric, acetic, or lactic, which causes pH to decrease. The decrease in pH of the fermented products could be attributed to acid production by microorganisms during fermentation. This agreed with the findings of Sengev et al. (2021), and Singh et al. (2012) who reported that the pH of fermented sorghum flour was reduced from 5.20 to 3.73 due to the production of organic acids by hetero-fermentors that convert glucose to an equimolar mixture of lactic acid, ethanol, and carbon dioxide.

3.3. Provitamin and vitamin C levels of fermented mango mesocarp flour

Figure 3 shows that the beta–carotene content of the unfermented mango mesocarp flour was low, while that of the fermented samples was a bit higher and constant as fermentation progressed. This is contrary to Sengev et al. (2021) who observed a decrease in beta-carotene in the fermented sorghum flour samples, which the authors ascribed to the loss of some absorbable substances during fermentation. However, the marginal increase in beta-carotene in this study could be attributed to the difference between legumes and fruits macromolecule matrix as reported by Badifu et al. (2000) who observed a significant reduction in beta-carotene in legumes and cereals as compared to mango mesocarp flour. During processing, carotenoids are very prone to degradation with a consequent alteration in bioavailability and biological activity (Badifu & Ilochi, 2004). The vitamin C level was low for all periods of fermentation and this could be due to the fact that they are highly unstable and oxidize easily during processing (Baker, 2000).

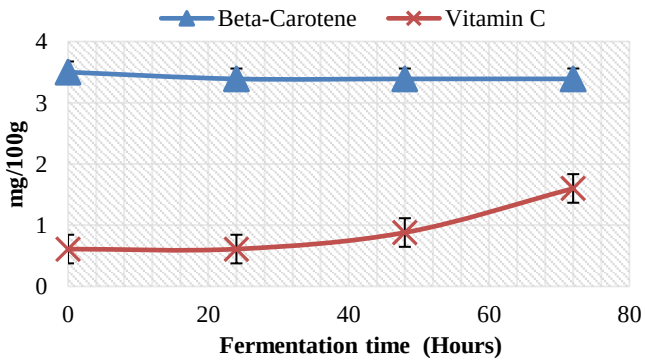

Figure 3. Provitamin and vitamin C levels of fermented mango mesocarp flour

Vitamin A promotes good vision, immune system integrity, growth, cellular differentiation, and proliferation. Deficiency of vitamin A mostly occurs in global south countries and occurs mainly in children under the age of 5 years, which is responsible for most cases of blindness in children and adults. This explains why vitamin A fortification of food is very important. Vitamin C is involved in protein metabolism, and collagen synthesis and is an important physiological antioxidant.

3.4. Microbial safety of fermented mango mesocarp flour

The number of colony-forming units counted during fermentation is shown in Table 1. The total microbial count of the mango flour increased with increased fermentation time until it got to 72 hours where it remained constant. This is because the culture was conducive to the growth of micro-organisms from the beginning of the fermentation but became toxic for the micro-organisms due to the release of waste. The total bacterial count is a quality indicator, not a safety indicator, and while it cannot directly contribute to a safety evaluation of ready-to-eat meals, it may be used as part of a general quality assessment, which includes foods with an extended shelf life (Eke & Elechi, 2021). The microbiological requirements are regulated differently in each nation. TBC in the range of 0-10^3 cfu/g, 10^4-10^5 cfu/g, and > 10^6 cfu/g is considered acceptable, moderately acceptable (tolerable), and unsatisfactory respectively, according to ICMSF (1996) and WHO (1994). While HPA (2009) and FSANZ (2001) have a higher microbial regulatory standard for Ready-To-Eat meals with a TVC of more than 10^5 cfu/g tagged unsatisfactory quality and hence unfit for ingestion. The result of this study agrees with the finding of Ogori (2013) for mango mesocarp flour stored at ambient temperature.

Table 1. Microbial safety of fermented mango mesocarp flour

Fermentation Time (hours)	0	24	48	72
TBC (CFU/g)	4.13×10^2	1.60×10^3	2.75×10^4	3.16×10^5
TFC (CFU/g)	1.44×10^2	2.27×10^2	2.43×10^3	2.76×10^3

KEY: TVC: Total viable count, TFC: Total fungal count

4. Conclusions

It was evident from the study that fermenting Mango Mesocarp for 72 h increased the nutritional value of fermented flour significantly ($p<0.05$). Fermented mango mesocarp flours have improved techno-functional characteristics that make them good base ingredients in infant food formulation. Fermenting for 72 h offers some advantages over other periods evaluated. The results of this study show that mango mesocarp can be fermented to produce fermented mango mesocarp flour which is rich in nutrients and can therefore be used as a supplement in other food products. Fermented mango flours have great potential to be developed into healthy food products such as complementary, and snack cookies. The vitamin C and antioxidant content from the fermentation of mango flour may be a great substitute for food formulation during the pandemic since antioxidants and vitamin C have the ability to improve immunity and anti-inflammatory response. These fermented mango flours are also good prebiotics for the gut microbiome which plays a good role in the immune system. It is recommended that mango mesocarp should be fermented, so as to preserve them and improve their keeping quality and also to avoid waste since they are perishable. It is also recommended that when producing them, fermentation should be stopped at 72 hours because the product is highly nutritive at this stage of

fermentation. This product is also recommended to be used as a supplement in the diet of growing children. It needs clinical trials in humans to find out more about its effects on human health and the authors are very open to joint research collaborations.

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