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Microbiological, proximate, and bioactive nutrient analyses of 'Muzha': A novel traditional Nigerian condiment of the 'Marghi' tribe in Adamawa and Borno States

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ABSTRACT

'Muzha' is a Nigerian traditional, spontaneous-fermented condiment made from a blend of seeds of *Parkia biglobosa* and *Hibiscus cannabinus*. This work assessed the microbiological safety, nutritional value, and bioactive properties of the condiment. Five samples were purchased from hawkers in Madagali Local Government Area of Adamawa State. Microbiological and nutritional analyses were done using standard methods. *Bacillus* and *Candida* species were the fermenting microorganisms. Proximate nutritional analysis showed that protein was most abundant (31±0.2), followed by crude fibre (7.0±0.3), fat (5.9±0.8), and carbohydrates (4.7±0.9). The most abundant mineral ion was potassium (2637±.5 mg/100g), followed by phosphorus (1345.2±1.1 mg/100g), then magnesium (200.4±1.5 mg/100g) and sodium (31.2±0.7 mg/100g). The least were recorded for zinc (6.0±0.03 mg/100g) and manganese (2.7±0.0 mg/100g). Vitamin A was most abundant at 44.8±0.1 mg/100g, vitamin C at 34.1±0.4 mg/100g, and vitamin E at 1.8±0.0 mg/100g. The contents of vitamins B1 and B2 were negligible. Twelve bioactive nutrients that were mostly unsaturated fatty acids were detected and characterised. Oleic acid methyl ester, palmitic acid methyl ester, and 9-octadecenoic acid, -12-hydroxy were the most abundant. Vitamins A and C, and the bioactive compounds could provide needed nutrients against cardiovascular, cancer, and other physiological and metabolic problems among adults. Our findings showed that 'Muzha' is microbiologically safe, nutritionally rich, and can be used to ameliorate malnutrition observed among insurgency-ravaged children of the north-eastern part of Nigeria. Further work is underway to determine the best starter culture for 'Muzha' production. To our knowledge, this condiment has not been reported previously.

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Introduction

African condiments are mostly fermented from legume seeds. These plants contain some carbohydrates and anti-nutrients that are not digestible by humans. Hence, the subjectivity of these legumes to fermentation, which enhances their digestion by humans [1], and nutrient availability, as well as removal of anti-nutritional entities [2]. Production of African condiments has been carried out either spontaneously by resident microbes or by inoculating the new condiment with a previously fermented condiment. This process improves organoleptic properties, increases digestibility, and extends the product's shelf life [3]. Traditionally produced fermented condiments are mostly made from leguminous plant seeds, which are rich in protein [4].

Fermentation is traditionally performed without specified starter cultures, posing a risk of contamination by pathogenic bacteria, fungi, and yeasts, as well as the production of unhygienic metabolites such as amines. Public health concerns are a major issue regarding the consumption of traditionally produced condiments [4]. Africans are known for their diverse fermented foods, which have economic and social impacts and provide health benefits. Fermented foods help ensure food security due to their preservative properties [5]. Unknown

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microorganisms that may be pathogenic or produce toxic or unpalatable substances are often associated with locally produced condiments. There are two methods for producing condiments. It is either by traditional means or by the science-technology method [6]. Condiments are products obtained from plants for direct use as an adjunct in food. They are also used in food flavouring and improve the nutritional value of our traditional foods [6, 7]. They are cheaper sources of nutrients the body needs, such as vitamins, proteins, and mineral salts [6].

During food preparation, condiments are usually added to enhance the aroma and flavour of the food [8]. Some other nutrients, such as proteins, mineral salts, vitamins, fibres, carbohydrates, and fats and oils, are added to the prepared food. Condiments are known to improve texture and supplement the protein content of food [8]. Nigerians are known for their delicacies, and most of the time, synthetic seasonings and spices are used in cooking. Some industrially produced condiments currently used in Nigeria have been suspected of containing high levels of sodium chloride and possible cancer-causing agents [9].

The north-eastern States of Nigeria, which include Borno, Yobe and Adamawa, are yet to recover from the effects of the *Boko haram* insurgency that has killed and displaced many people. The level of malnutrition in Sub-Saharan Africa, including Nigeria, especially in States affected by insurgency, is high, with deficiency diseases noted among the children. These children are malnourished and poorly developed both physically and intellectually [10]. *Parkia biglobosa,* popularly known as locust beans (known as *Iru* in the southwest and *Ogiri* in the Southeast, and *Dawa dawa* or *Daddawa* in the north region) and *Hibiscus cannabinus,* known as Kenaf tree (known as *Rama* in the north and *Idi Orisha* in the southwest, not known in an Igbo word), seeds are planted in the north-eastern part of Nigeria, making 'Muzha' a sustainable product, providing food security for the people. Food security is the state in which food is nutritious, affordable, and consistently available [5].

Hibiscus cannabinus is a dicot plant, grown annually in Asia and Africa, mainly for its fibre, though almost all its parts are used for one thing or another. It is rich in cellulosic fibre, serves as a protein source for human and animal consumption, and provides beneficial healthy metabolites. It can be an alternative to soybean meal and fishmeal [11, 12]. *Hibiscus cannabinus* has been cultivated for several years as a source of fibre for the paper and textile industries, and for biomass energy and seed oil. It has applications as an industrial absorbent and in filtration and is medicinally important [11, 13]. Kenaf has a rapid growth rate, is tolerant to diverse environmental conditions, and is rich in food nutrients, making it a suitable plant candidate for food security [12].

There are about 34 species of *Parkia biglobosa*, commonly known as locust bean, distributed across Asia and Africa [14]. They are widely used in local condiment production, where a traditional method is used. The species available are seven, with fermented *P. biglobosa* seeds as a reputed delicacy and condiment in sub-Saharan Africa. These *Parkia* species produce secondary metabolites that confer their pharmacological properties [14].

'Muzha' in the *Marghi* language, means seasoning or condiment, and it is traditionally produced by fermenting seeds of *P. biglobosa* and *H. cannabinus* for 14 days. Academically, nothing is known about this condiment yet. It has not been documented or evaluated for its microbial safety or its nutritional health benefits to humans. Therefore, this is the first time that this condiment would be characterised. *Marghi* is one of the major tribes that occupies the Northern Senatorial District of Adamawa State and the Southern part of Borno State. This study aimed to assess the microbiological quality, proximate composition, and bioactive nutrient content of 'Muzha', a product traditionally produced and sold at the Madagali main market in Madagali Local Government Area of Adamawa State, Nigeria.

Materials and methods

Sample collection

Five (5) 'Muzha' samples were bought from different local vendors at the Madagali main market in the Southern Senatorial District of Adamawa State. The samples were kept on ice in sterile containers and transported to the Biotechnology Department at Modibbo Adama University for analysis within 6 hours of purchase. Madagali is bordered by Borno State and the Republic of Cameroon. It is situated at longitude 10°.88'28''661 N and latitude 13°63'61''E.

Microbiological assay

Tenfold serial dilution was carried out using sterile peptone water, and a hundred microlitre (100 µL) was taken from diluents 10^{-4} and 10^{-5} and cultured on nutrient agar for general bacteria isolation, potato dextrose agar for fungi and yeasts, and MacConkey agar for enterobacteriaceae. The pour-plate method

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was used for the isolation. Incubation was at 37 degree Celsius and lasted for 18-24 h, while fungi and yeast were isolated at 28-30 °C for 72-96 h [15]. Pure cultures of the isolates were obtained and kept on slants of nutrient agar and potato dextrose agar. All isolates were kept in a fridge (Hair Thermocool) at 4 °C till when needed.

Isolates identification

The bacterial and fungal isolates obtained were subjected to different cultural and biochemical characterisations, such as surface appearance, colour, shape, edge, and sugar and citrate utilisation, hemolysis, urease, catalase, indole and oxidase production, as well as the TSI test for their identification following the description of Fawole and Oso[16].

Proximate analysis

Proximate composition of the samples was determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2005), as described by Al Dhaheri et al. [17]. The following contents were analysed: the ash, fibre, moisture, crude fat, protein, and carbohydrates.

Mineral and vitamin content determination

Mineral nutrients such as copper, magnesium, manganese, phosphorus, and zinc were quantified using atomic absorption spectroscopy (AAS; Model Buck Scientific, VPG230). About 2 g of 'Muzha' was placed in a crucible and completely burnt to ashes using a Bunsen burner in a fume cupboard. The charred remnant was then burnt in a furnace till all the carbon content was removed. The crucible with its content was then cooled in a desiccator. Twenty millilitres of 0.1 M hydrochloric acid was then added and then filtered. The volume was made up to 100 mL in a volumetric flask. A suitable standard was prepared and used for each metal assessed. Each sample and its corresponding standard were separately introduced into the flame of a cathode lamp, mixed with an oxyacetylene flame. The concentration of each metal in the samples was measured in milligrams per hundred grams (mg/100 g) [18].

Sodium and potassium contents of the samples were determined using a Flame Photometer (Jenway Model PFP7). Vitamins such as A, B1, B2, C, D, and E were evaluated using a UV-Vis. Briefly described here. Each sample was extracted with acetonitrile, followed by the addition of potassium hydroxide to remove esters. The reaction lasted for half an hour. An oxidant was then added. The saponified sample was then extracted with acetonitrile in a separating funnel. The extract was then washed several times with distilled water. Vitamin standards and the blank were then prepared. The absorbance was measured using a UV-Vis spectrophotometer at 620 nm. A standard curve was constructed from the vitamin concentrations, and the concentrations were extrapolated. All readings were taken in triplicate [19].

GC-MS analysis

A Gas Chromatography of 7890 series (7890B) joined with an Agilent 5977A (GC-MS, Agilent Technologies Incorporated, CA, USA) was used in determining the bioactive compounds present in the sample. A capillary column, HP-5ms ultra-inert 30m × 0.25 mm × 0.25 μm (Agilent, CA, USA), was used for separation, with helium serving as the carrier gas at a flow rate of 1 mL/min. A sample volume of 1.0 μL was used. The injector temperature remained constant at 310 °C, while the GC temperature remained at 60 °C for 60 seconds, then was raised to 170 °C at a rate of 5 °C per min. It was further raised to 250 °C at a rate of 10 °C/min. The interface temperature was 250 °C. The ion was obtained by EI at 200 °C. An electron energy source of 70 eV was used to achieve positive ionisation, with a detection voltage of 1000 V and an emission current of 100 μA. The spectral analysis was performed by comparing spectra against the NIST 14.0 database. The peak area was normalised, and the percentage area of each bioactive compound in the condiment was determined. This method was adopted from Li et al. [20].

Statistical analysis

The data obtained in triplicate were subjected to one-way analysis of variance (ANOVA) and reported as mean values and standard deviations. The comparison of means was performed using the Duncan Multiple Range Test in SPSS for Windows version 20. A significant difference was observed at the 5% level.

Results

Microbial isolates

Nineteen bacterial isolates were obtained from the five (5) 'Muzha' samples. The cultural features showed that all isolates were creamy, yellowish to

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cloudy whitish, with shiny, smooth surfaces, and seven (7) yeast isolates were recovered based on these characteristics (Tables 1 and 2). The bacterial and yeast isolates were presumptively identified as *Bacillus* and *Candida* species

Table 1: Cultural characteristics of bacteria

S/N	Isolates	Growth pattern	Tentative Identification
1.	Y1A	Creamy and yellowish	*Bacillus* sp.
2.	Y1B	Whitish cloudy	*Bacillus* sp.
3.	Y1C	Creamy and yellowish	*Bacillus* sp.
4.	Y1D	creamy	*Bacillus* sp.
5.	Y2A	Creamy and shiny	*Bacillus* sp.
6.	Y2B	Creamy and shiny	*Bacillus* sp.
7.	Y2C	Creamy with a brown centre	*Bacillus* sp.
8.	Y4A	Whitish crenate	*Bacillus* sp.
9.	Y4A	Irregular in shape/ whitish	*Bacillus* sp.
10.	Y4B	Creamy and smooth	*Bacillus* sp.
11.	Y5A	Creamy and cloudy	*Bacillus* sp.
12.	Y5B	Creamy and cloudy	*Bacillus* sp.
13.	Y5C	Creamy and smooth surface	*Bacillus* sp.
14.	Y5D	Whitish cloudy	*Bacillus* sp.
15.	L1A	Whitish cloudy	*Bacillus* sp.
16.	L1B	Creamy with brown center	*Bacillus* sp.
17.	L1C	Whitish cloudy	*Bacillus* sp.
18.	L1D	Creamy and dry	*Bacillus* sp.
19.	L1E	Creamy and shiny	*Bacillus* sp.

Table 2: Cultural characteristics of yeasts

S/N	Isolates	Growth Pattern	Tentative Identification
1.	SAP1A	Brownish-cream and shiny	*Candida* sp.
2.	SAP1B	Creamy-pinkish with lumps	*Candida* sp.
3.	SAP1C	Dry and creamy	*Candida* sp.
4.	SAP1D	Creamy, whitish, and shiny	*Candida* sp.
5.	SAP1E	Creamy and shiny	*Candida* sp.
6.	SAP1F	Creamy-whitish, shiny	*Candida* sp.
7.	SAP1G	Creamy and shiny	*Candida* sp.

Biochemical characteristics and sugar fermentation

All suspected bacteria were *Bacillus* species, and the yeasts were *Candida* species obtained from the 'Muzha' samples, which were subjected to various biochemical tests. Some *Bacillus* and *Candida* species were positive or negative for oxidase, catalase, citrate, indole, and triple-salt iron tests. All were Gram-positive. The hemolytic reaction consisted of alpha, beta, and gamma. Additionally, sugar fermentation also differed from one species to another. The most utilised sugars were arabinose, fructose, sucrose, and maltose, while D-mannitol and xylose were the least fermented by only a few isolates (Tables 3a and 3b).

Proximate analysis

The proximate composition of the vended 'Muzha' showed that it had the highest moisture content, followed by protein, fibre, fat, and carbohydrates, with ash content the lowest. The results are presented in Table 4. The condiment 'Muzha' was found to contain abundant mineral ions, such as potassium, phosphorus, and magnesium, followed by sodium and manganese. It was, however, low in copper ions. Similarly, vitamin A was the most abundant, followed by vitamins C and E. But it has low content of vitamin B1, B2, and D (Table 5).

GC-MS profile of bioactive nutrients

Thirteen different bioactive nutrients were identified and characterised via GC-MS analysis. The most abundant bioactive compound was oleic acid, methyl ester, followed by 9-octadecenoic acid and palmitic acid, methyl ester. Linoleic acid ethyl ester was also identified in a reasonable amount. However, the least abundant were eicosanoic acid methyl ester and cholestanol, as presented in Table 6. These were

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Table 3a: Bacterial biochemical characteristics and sugar fermentation

S/N	Isolates	D-man	Gly	Xyl	Ara	Ino	Fruc	Suc	Lac	Mal	Gal	Glu	Man	Oxi	Cat	Cit	Indol	TSI	GR	Hem
1.	Y1A	-	-	-	+	-	+	+	+	+	+	-	-	+	+	+	+	-	+	Beta
2.	YIB	-	-	-	+	+	+	+	-	+	+	-	-	-	-	+	-	-	+	Gamma
3.	YIC	-	+	-	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+	Beta
4.	YID	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	Alpha
5.	Y2A	-	-	-	+	-	+	+	-	+	-	-	-	+	+	-	+	-	+	Beta
6.	Y2B	-	-	-	+	+	+	+	-	+	-	-	-	+	-	-	+	-	+	Beta
7.	Y2C	-	+	-	+	-	+	+	-	+	+	-	+	+	-	-	+	-	+	Alpha
8.	Y3A	+	-	-	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	Alpha
9.	Y4A	+	+	-	+	-	+	+	-	+	-	-	-	+	+	+	+	-	+	Alpha
10.	Y4B	-	+	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	+	Alpha
11.	Y5A	+	-	+	+	+	+	+	-	+	-	-	-	+	-	+	-	-	+	Alpha
12.	Y5B	-	+	-	+	+	+	+	-	+	-	-	-	-	+	+	-	+	+	Alpha
13.	Y5C	-	+	+	+	+	+	+	-	+	+	-	-	+	+	+	-	-	+	Alpha
14.	Y5D	-	+	-	+	+	+	+	-	+	-	-	-	+	+	-	+	-	+	Gamma
15.	LIA	+	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	Beta
16.	LIB	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	Alpha
17.	LIC	-	-	-	+	+	+	+	-	+	-	-	-	+	+	-	+	+	+	Alpha
18.	LID	-	-	-	+	-	+	+	+	+	-	+	-	-	-	+	-	-	+	Alpha
19.	LIE	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	Alpha

Keys: D-man = D-mannitol, Gly = Glycerol, Xyl = Xylose, Ara = Arabinose, Ino = Inositol, Fruc = Fructose, Suc = Sucrose, Lac = Lactose, Mal = Maltose, Gal = Galactose, Glu = Glucose, Man = Mannose, Oxi = Oxidase, Cat = Catalase, Cit = Citrate, Indole = Indole, GR = Gram Reaction, Hemo = Hemolysis Negative (-), Positive (+)

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Table 3b: Biochemical characteristics and sugar fermentation of yeasts

S/N	Isolates	D-man	Gly	Xyl	Ara	Ino	Fruc	Suc	Lac	Mal	Gal	Glu	Man	Oxi	Cat	Cit	Indo	TSI	GR	Hem
1.	SAP1A	-	+	-	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	Beta
2.	SAP1B	-	-	-	+	-	+	+	-	+	-	-	-	+	+	+	+	-	+	Alpha
3.	SAP1C	+	-	-	+	-	+	+	-	+	-	-	-	+	+	-	+	-	+	Beta
4.	SAP1D	+	-	+	+	-	+	+	-	+	+	-	-	+	+	-	+	-	+	Beta
5.	SAP1E	-	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	-	+	Alpha
6.	SAP1F	+	+	+	+	-	+	+	-	+	-	-	-	+	+	-	+	-	+	Alpha
7.	SAP1G	+	+	-	+	+	+	+	-	+	-	-	-	+	+	+	+	-	+	Alpha

Keys: D-man = D-mannitol, Gly = Glycerol, Xyl = Xylose, Ara = Arabinose, Ino = Inositol, Fruc = Fructose, Suc = Sucrose, Lac = Lactose, Mal = Maltose, Gal = Galactose, Glu = Glucose, Man = Mannose, Oxi = Oxidase, Cat = Catalase, Cit = Citrate, Indole = Indole, GR = Gram Reaction, Hem = Hemolysis Negative (-), Positive (+)

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Table 4: Proximate analysis of the hawked 'Muzha' (M±SD)

Analysis	(g/100g)
% Moisture	49.7±0.8
% Ash	2.3±0.0.
% Fiber	7.0±0.3
% Protein	31.0±0.2
% carbohydrate	4.71±0.9
% Fat	5.88±0.8

Table 5: Minerals and vitamins (mg/100g)

Sample	Na^+	K^+	Mg^{2+}	Mn^{2+}	Cu^{2+}	Zn^{2+}	Pho	Vit A	Vit B1	Vit B2	Vit C	Vit D
S1	31.2±0.9	2637±0.9	200±0.4	2.7±0.4	1.3±1.5	6.0±0.9	1345±0.7	44.8±0.7	0.1±0.3	0.1±0.5	34.1±0.2	0.03±0.1
S2	31.5±0.4	2600±1.2	198±0.6	2.7±0.7	1.2±1.0	5.8±0.1	1300±0.4	43.7±1.3	0.1±0.5	0.1±0.4	33.9±0.4	0.01±0.3
S3	30.8±0.2	2608±0.6	202±0.2	2.6±0.3	1.3±.9	6.2±0.3	1360±0.2	44.2±0.6	0.1±0.5	0.1±0.6	34.5±1.2	0.04±0.5
S4	32.2±0.3	2645±1.0	201±0.5	2.7±1.1	1.2±0.8	6.2±0.4	1355±0.3	45.3±0.4	0.2±0.2	0.2±1.0	34.7±0.7	0.03±0.2
S5	31.9±0.5	2640±0.4	202±0.4	2.7±0.8	1.2±0.3	5.9±0.4	1367±0.4	44.2±0.8	0.2±0.6	0.1±0.7	33.6±0.5	0.04±0.6

Keys: Na^+ = Sodium, K^+ = Potassium, Mg^{2+} = Magnesium, Mn^{2+} = Manganese, Cu^{2+} = Copper, Zn^{2+} = Zinc, Pho = Phosphorus, Vit A = Vitamin A, Vit B1 – Vitamin B1, Vit B2 = Vitamin B2, Vit C = Vitamin C, Vit D = vitamin D, Vit E = Vitamin E

Table 6: **Profile of bioactive nutrients in hawked 'Muzha' and their nutritional functions**

SN	RT	Bioactive nutrients	% Area	Molecular formula	M. wt (g/mol)	Reported nutritional functions and references
1.	20.4	Cholestanol	1.52	$C_{27}H_{48}O$	388	Functional food, anticancer, treatment of cardiovascular and cholesterol disorders [21]
2.	21.0	Palmitic acid, methyl ester	42.44	$C_{17}H_{34}O_2$	270	Ameliorating cardiovascular disorders, neuroprotective agent, regulation of human mesenchymal stem cells [22-24]
3.	22.5	Hexadecanoic acid, ethyl ester	2.64	$C_{18}H_{36}O_2$	284	Antioxidant, hepatoprotective, and anticancer [25, 26]
4.	26.2	Oleic acid, methyl ester	85.25	$C_{19}H_{36}O_2$	296	Maintenance of cholesterol levels, anti-inflammatory, antimicrobial, antidiabetic, antioxidant, and antithrombotic [27, 28]
5.	26.9	Methyl stearate	25.86	$C_{19}H_{38}O_2$	298	Inhibit cardiac arrest, neuroprotection, antitumour [29, 30]
6.	28.5	Linoleic acid ethyl ester	3.9	$C_{20}H_{36}O_2$	308	Improve skin and cardiovascular health [31]
7.	28.7	Ethyl Oleate	4.79	$C_{20}H_{38}O_2$	310	Weight loss, antimicrobial, and antioxidant [32]
8.	29.6	Octadecanoic acid, ethyl ester	1.00	$C_{20}H_{40}O_2$	312	Anti-inflammatory, antimicrobial, antioxidant, and antiviral [33]
9.	35.6	9-Octadecenoic acid, 12-hydroxy-,	48.84	$C_{18}H_{34}O_3$	298	Anti-inflammatory, antioxidant [34, 35]
10.	35.7	Methyl ester, [R-(Z)]-	8.56	Nil	Nil	No known health benefit
11.	37.2	Eicosanoic acid, methyl ester	2.7	$C_{21}H_{42}O_2$	326	Antimicrobial, antioxidant, anticancer [36]
12.	39.5	Ricinoleic acid	2.68	$C_{18}H_{34}O_3$	298	Antibacterial, anti-inflammatory, anti-herpetic [37]

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identified based on their cultural and biochemical characteristics, including sugar fermentation. Previous studies by Kpikpi et al. [38] showed that various *Bacillus* species, including *B. subtilis*, *B. safensis*, and *B. amyloliquefaciens*, were isolated and identified from a Ghanaian spontaneous local fermented condiment, 'Kantong'.

Discussion

In this study, we assessed the microbiological, proximate, and bioactive nutrient profiles of the condiment. A total of nineteen (19) bacteria and seven (7) yeasts were isolated and characterised. All the bacterial isolates are of the *Bacillus* species, and all the fungal isolates belong to the *Candida* species. Lactobacilli and yeasts such as *Saccharomyces, Candida, Rhodotorula, and Torulopsis* are prominent in traditionally produced African condiments [1, 4, 39-41]. An array of microorganisms is responsible for breaking down indigestible compounds and antinutrients present in the plant used to make the condiment. The aroma or flavour associated with condiments is produced by these microbes during fermentation [1]. Besides these, antimicrobial peptides such as bacteriocins and improved vitamin and mineral content are factors influencing the microbial population in condiments [42, 43]. However, due to unhygienic preparation at times, microbial contaminants such as *Staphylococcus aureus, S. saprophyticus, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis*, and filamentous fungi such as *Aspergillus niger, Mucor* sp., and *Paecilomyces* sp. can contaminate condiments [40, 44, 45]. This contamination could originate from processing materials, seeds, water, or basins, and is undesirable in the product because it can cause food poisoning.

There were varied responses to enzyme production among different species of *Bacillus* and yeasts isolated in this study. It was observed that the majority of the *Bacillus* strains were able to ferment fructose, sucrose, arabinose, and maltose, and to use citrate as a carbon and energy source, while a few utilised inositol, glucose, D-mannitol, and D-xylose. Similarly, catalase and oxidase synthesis were observed in most isolates, whereas indole and TSI tests were positive in a few species. Different authors have previously reported varied responses in enzyme production and sugar utilisation among *Bacillus* species [45, 46].

The crude composition of the condiment showed a relatively high protein content (31±0.2) compared to reports of Ogunlade [51] who mentioned a range of 31.76 to 40.28 % for okpei, 54.06 % for a blend of locust bean and soybean seeds condiment by Tersoo-Abiem et al. [46], 19.0 % by Balogun et al. [47] and Ejimofor and Nwakuche [7] who had 20.2, 23.7 and 28.1 per cent for Ogiri-igbo, Ogiri okpei and Dawa dawa, respectively. The differences in crude protein content could be attributed to the proteolytic activity of various *Bacillus* species and other microorganisms during fermentation. Similarly, different leguminous seeds used for condiment production have varied protein content. Usually, protease activity results in the release of proteinous contents of the seeds. *Bacillus* species are well known as protease producers and are major sources of proteases for industrial processes [48-50].

Furthermore, the proximate quantity of carbohydrates in this study was found to be low (4.7±0.9 per cent) compared to 41.6 to 51.6 % reported by Fowoyo [45], 17.9 to 25.3 % mentioned by Ogunlade [51], and a range of 23.6 to 40.1 % by Ejimofor and Nwakuche [7]. The differences observed in our results and the earlier values reported by different authors could be due to the fermentation conditions and the microbial community, which contributed significantly to the breakdown of the seeds' carbohydrate content using their amylases. *Bacillus* species are known to be prolific producers of amylase. The high protein content of ‘Muzha’, relative to its carbohydrate content, makes it a suitable source of protein for malnourished children in insurgency-ravaged North-eastern Nigeria.

The fat content of ‘Muzha’ was recorded to be 5.8±0.8 per cent, which could be relative when compared to a range of 5.8 to 10.6 % stated by Ogunlade [51], 5.5 % by Balogun et al. [47] and Fowoyo [45], who had fat content between 7.3 and 11.5 per cent. The lipid content of different seeds, as well as the lipolytic activity of fermenting microorganisms, could be responsible for the variation in crude fat values reported by different researchers. Rancidity is undesirable in a condiment and can be caused by oxidation of high lipid content. Hence, a condiment with low lipid content is preferred [45].

In this study, the crude fibre content was 7.0±0.3, which was higher than 3.2 to 4.8 % reported by Fowoyo [45], 1.1 to 1.9 % for three different spontaneously fermented condiments, and 5.1 % by Balogun *et al.* [47], while our result for crude fibre is comparable with

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7.9 to 10.4 % reported by Ogunlade [51]. The crude fibre content noted in 'Muzha' could contribute to the health of the consumer's gut, as it aids digestion.

Additionally, the moisture content observed in our study (49.9±0.8 %) was higher than 31.3-36.1 reported by Ogunlade [51], 20.1% by Balogun et al. [47], and 20.0% reported by Ejimofor and Nwakuche [7]. The production process may be responsible for the variation in moisture content. The ash content is higher (2.3±0.4) per cent compared to earlier reports by Mustapha et al. [52], who had between 1.7 to 2.2 %, Ogunlade [51], who had between 3.5 to 4.6 %, 5.3 to 6.2 % by Fowoyo [45], and 6.2 % by Tersoo-Abiem et al. [46]. The observed differences could be due to different condiment seeds, the fermentation period, and leaching. It takes fourteen (14) days for 'Muzha' to be completely ready for consumption. This might be responsible for its low ash content.

The mineral content of the spontaneously fermented vended 'Muzha' showed that potassium was the most abundant, followed by phosphorus and then magnesium. Sodium was present in considerable amounts, followed by zinc and manganese, while copper was present in trace amounts. Our findings are in agreement with earlier reports by Ejimofor and Nwakuche, Fowoyo, Tersoo-Abiem et al., Ogunlade, and Aremu et al. [7, 45, 46, 51, 53], who reported a similar pattern of abundance, though 'Muzha' had higher quantities of the minerals than those reported by these authors. Nigerian condiments have been reported to be nutritionally rich and potentially improve consumers' health. Minerals are essential and play critical roles in metabolic and physiological activity in the body [54]. For instance, the high potassium-to-sodium ratio could help in assessing cardiovascular health, as it regulates heart function, prevents calcification of the arterial wall, and thereby lowers blood pressure [55]. Potassium is also needed for amino acid and protein synthesis and acts as a cofactor in proteins that help prevent ageing syndromes and metabolic disorders, and improve bone metabolism [56]. Phosphorus and magnesium are essential for renal function, cell and bone formation, energy metabolism, physiological responses, muscle relaxation, and numerous enzymatic activities as cofactors, helping prevent conditions such as diabetes, asthma, migraine, and cardiovascular diseases [57, 58]. Copper and zinc were found in the traditional fermented condiment under study; these two micronutrients are necessary for normal brain function and DNA synthesis [59].

Furthermore, vitamins A and C were the most abundant, with 43.7-45.3 mg/100g and 33.9-34.7 mg/100g, respectively. However, vitamins E, D, B1, and B2 were negligibly present. Low levels of vitamins A, B1, and B2 were previously reported by Tersoo-Abiem et al. [46], who studied a blend of locust and soybean condiment. Our findings are quite in variance with the report by Fowoyo [45], who reported a very low presence of vitamins A and E and a complete absence of vitamin C from okpehe. Differences in the seeds used, the fermentation process, and the microorganisms involved could account for the variation observed. It is possible that the vitamins have also leached. Vitamins are needed for the normal functioning of the body's systems. Vitamin A observed to be high in abundance in 'Muzha' for example is highly needed in the proper functioning of the eyes preventing night blindness, immune system as well as red blood cells and bone formation [59], while vitamin C is an antioxidant needed in neutralizing free radicals that cause damage to vital organs such as liver and kidney, preventing cancer, and stopping renal failure. Also, vitamin E, which was detected sparingly in 'Muzha', plays important roles as an antioxidant and in individuals' fertility status [60]. Thirteen different bioactive compounds were identified in 'Muzha'. They are primarily fatty acids with numerous health benefits (Table 7).

Conclusions

'Muzha' is produced from the seeds of the locust bean and the Kenaf tree and is consumed by the 'Marghi' people of Adamawa, Borno, and parts of Cameroon. It is spontaneously fermented for 14 days using a mixture of locust bean and kenaf seeds. This study revealed two main groups of fermenting microbes: *Bacillus* and *Candida* species. 'Muzha' is rich in protein, followed by fibre and fat, with moderate carbohydrates. It is also rich in potassium and phosphorus, with magnesium and sodium following. The most abundant vitamins were vitamins A and C, with low levels of vitamin E, but deficient in Vitamins B1 and B2. The bioactive nutrients were mostly unsaturated fatty acids such as oleic acid methyl ester, palmitic acid methyl ester, 9-octadecenoic acid, 12-hydroxy, and methyl stearate. The mineral ions, vitamins, and bioactive compounds reported in this study have a variety of health benefits for humans, such as improvements in eye function and cardiovascular health, anti-diabetic and antimicrobial activities, and antioxidant properties, thereby serving

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Table 7: Profile of bioactive nutrients in hawked *Muzha* and their nutritional functions

S/No	RT	Bioactive nutrient	% Area	Molecular Formula	M. wt (g/mol)	Reported nutritional benefits and reference
1.	20.4	Cholestanol	1.52	$C_{27}H_{48}O$	388	Functional food, anticancer, treatment of cardiovascular and cholesterol disorders [21]
2.	20.7	Palmitic acid, methyl ester	12.21	$C_{17}H_{34}O_2$	270	Ameliorating cardiovascular disorders, neuroprotective agent, regulation of human mesenchymal stem cells [22-24]
3.	21.0	Palmitic acid, methyl ester	42.44	$C_{17}H_{34}O_2$	270	Same as in S/No 2 above
4.	22.5	Hexadecanoic acid, ethyl ester	2.64	$C_{18}H_{36}O_2$	284	Antioxidant, hepatoprotective, and anticancer [25, 26]
5.	26.2	Oleic acid, methyl ester	85.25	$C_{19}H_{36}O_2$	296	Maintenance of cholesterol levels, anti-inflammatory, antimicrobial, antidiabetic, antioxidant, and antithrombotic [27, 28]
6.	26.9	Oleic acid, methyl ester	100	$C_{19}H_{36}O_2$	296	Same as in S/No 5 above
7.	26.9	Methyl stearate	3.12	$C_{19}H_{38}O_2$	298	Inhibit cardiac arrest, neuroprotection, antitumour [29, 30]
8.	27.2	Methyl stearate	25.86	$C_{19}H_{38}O_2$	298	Same as in S/No7 above
9.	28.5	Linoleic acid ethyl ester	3.9	$C_{20}H_{36}O_2$	308	Improve skin and cardiovascular health [31]
10.	28.7	Ethyl Oleate	4.79	$C_{20}H_{38}O_2$	310	Weight loss, antimicrobial, and antioxidant [32]
11.	29.6	Octadecanoic acid, ethyl ester	1.00	$C_{20}H_{40}O_2$	312	Anti-inflammatory, antimicrobial, antioxidant, and antiviral [33]
12.	35.6	9-Octadecenoic acid, 12-hydroxy-,	48.84	$C_{18}H_{34}O_3$	298	Anti-inflammatory, antioxidant [34, 35]
13.	35.7	methyl ester, [R-(Z)]-	8.56	Nil	Nil	No known health benefit
14.	35.8	9-Octadecenoic acid, 12-hydroxy-,	2.43	$C_{18}H_{34}O_3$	298	Same as S/No 12 above
15.	35.8	methyl ester, [R-(Z)]-	3.42	Nil	Nil	No known health benefit
16.	36.1	9-Octadecenoic acid, 12-hydroxy-,	34.31	$C_{18}H_{34}O_3$	298	Same as S/No 12 above
17.	37.2	Eicosanoic acid, methyl ester	2.7	$C_{21}H_{42}O_2$	326	Antimicrobial, antioxidant, anticancer [36]
18.	39.5	Ricinoleic acid	2.68	$C_{18}H_{34}O_3$	298	Antibacterial, anti-inflammatory, anti-herpetic [37]

as anticancer agents. This condiment is rich in nutrients that can serve as a flavouring agent, check malnutrition and protein deficiency among children in the north-eastern part of Nigeria who have suffered and are still suffering from the effects of insurgency. 'Muzha' will be an alternative to other costly sources of protein, such as fish and beef and will always be available to rural dwellers. Some other aspects of the work, such as the use of starter cultures for controlled fermentation, comprehensive molecular study of the isolates, and optimisation/industrialisation, are ongoing.

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