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RESEARCH ARTICLE

Effects of fermentation on the proximate, anti-nutrients, minerals, fatty acids, and amino acids profiles of jujube (*Ziziphus mauritiana* Lam) seeds

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ABSTRACT

Background: Fermentation has been recognized as one of the oldest ways of food processing that increase food quality by increasing nutrient bio-availability through the reduction in anti-nutrient compositions. The present study evaluated the effects of fermentation on nutrients and anti-nutrients composition of jujube (*Ziziphus mauritiana* Lam) seed. **Methods:** The seed of *Z. mauritiana* was fermented for 24 hr. Standard analytical procedures were used to analyse the proximate, minerals, amino acid, fatty acid and anti-nutrient compositions of the seed sample at 0, 6, 12, 18 and 24 hr of fermentation. **Results:** The seed has high amounts of proteins, minerals, amino acids and low levels of anti-nutrients. The seed also has higher unsaturated than saturated fatty acids. Fermentation significantly increased the minerals compositions, decreases anti-nutrients and some amino acid levels but had no plausible effects ($p>0.05$) on proximate contents of the seed. Fermentation for 6 and 12 hr had no significant ($p<0.05$) effect on the fatty acids, however, at 18 and 24 hr a significant ($p<0.05$) reduction in fatty acid were recorded. On the basis of nutrient retention, the most plausible and positive effects of the fermentation on *Z. mauritiana* were observed at ≤ 12 of fermentation. **Conclusion:** *Ziziphus mauritiana* seed could be employed as an alternative source of nutrients for humans and animals. However, fermentation of *Z. mauritiana* should be done for a period of ≤ 12 hr if necessary.

Keywords: *Ziziphus mauritiana*; fermentation; proximate; fatty acid; anti-nutrients; minerals; amino acid

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1.0 Introduction

In spite of the exigent effort to meet the nutritional requirements of the ever-increasing populations, many cheap sources of protein are available but not generally acceptable to the consumers due to undesirable characteristics, presence of anti-nutritive factors and lack of knowledge on their nutritional qualities and thus remain relatively under-utilized [1].

Research interest geared towards unravelling the chemical composition of some wild fruits in developing countries like Nigeria is increasing rapidly, owing to their significant roles in the diet of people of the developing world [2]. These wild fruits may offer better or comparable nutritional and phytoconstituents than the cultivated fruits [3]. However, the anti-nutritional contents of some of these fruits may interfere with the metabolic process and thus limit nutrient bioavailability by the body when consumed [4]. Fermentation has been recognized as one of the oldest ways of food processing that increase food quality by increasing nutrient bio-availability through the reduction in anti-nutrient compositions [5]. Thus, fermented foods constitute an important part of the diet in many communities in the world. The functional microorganisms in food fermentations include bacteria, yeasts and moulds.

Ziziphus mauritiana Lam. belongs to the Rhamnaceae family it is an important tropical fruit tree commonly known as Jujube or Magarya by English and Hausa speaking of northern Nigeria [6]. The tree is native to tropical Africa and grows in arid and semiarid regions [7]. *Z. mauritiana* have been reportedly used in traditional medicine for the treatment of sexual disability, cough, digestive disorders, urinary troubles, loss of appetite, convulsion, epilepsy, insomnia

obesity, burning sensations, fever, skin disease, wound, ulcers, diarrhoea and stomatitis [8]. Pharmacologically, *Z. mauritiana* has been reported to contain bioactive metabolites including flavonoids, glycosides, saponins and volatile oil [9], with different pharmacological effects; immune stimulation [10], activities against alcohol-induced oxidative stress [11], anticancer [12], antidiarrhoeal [9], hypoglycemic and hypolipidemic [13], antiulcer [14], and antimicrobial [15] activities.

The fruit of *Z. mauritiana* enclosed a single or two seeds which are often discarded as waste. Seeds are dispersed by human and other mammalian vectors [16]. Despite the well-known medicinal properties of *Z. mauritiana*, the seed is less explored and underutilized and could serve as a rich source of nutrients and phytochemicals for the general benefits of the population [17].

To date, there had not been detailed scientific documentation on the chemical (nutritional, and antinutritional) composition of *Z. mauritiana* seeds. The available literature is limited to only its proximate compositions [17]. Therefore, it becomes relevant to investigate the chemical composition of *Z. mauritiana* seed and also the effect of fermentation on these compositions. This would go a long way to supplement the nutrients needs and demands of humans and animals in Nigeria and Africa at large.

2.0 Materials and Methods

2. 1 Sample collections

Matured fruits of *Z. mauritiana* fruits were picked directly from the trees in March 2017 from the Barnawa area of Kaduna, Kaduna State. The sample was identified and authenticated at the herbarium unit of the Department of Biological Sciences Ahmadu Bello University where the existing voucher number of the specimen (No. 7072) was given

2.2 Sample preparation and fermentation

The fruits were macerated in water to remove the pulp and the seeds were rinsed in clean water. Thereafter the seeds were spread out, sun-dried. The dried seeds were grounded and sieved through a mesh to obtain a fine powder which was stored in an airtight container. The powdered seed (100 g) was fermented with 500 cm3 of distilled water with the aid of yeast (saccharomyces), samples were taken out of the fermentation medium at 6 hr' intervals (0, 6, 12, 18 and 24 hr) for analysis of chemical composition. After each fermentation period, the substrates were dried at 50°C in the oven and kept refrigerated pending analysis

2.3 Mineral Analysis

The concentration of sodium, potassium, calcium, magnesium, copper, manganese, iron zinc and phosphorus in *Z. mauritiana* seeds were analysed according to AOAC [18]. Ten (10g) grams of each of the samples was heated over a flame and ashed in muffle furnace at 450°C for 8 hr. Ten (10 cm^3) of diluted HCl was added to the ash and boiled for 5 minutes. The boiled ash was made up to 100 cm^3 with distilled water. Analysis were performed in triplicate using atomic absorption spectrophotometer according to the standard methods of AOAC [18].

2.4 Proximate analysis:

The proximate indices of *Z. mauritiana* seeds including; crude proteins, crude fibre, moisture content, ash content, crude fat and carbohydrate were determined using standard procedures [18].

2.5 Anti-nutritional analysis

Tannin content was determined by the method of Makkaret al. [19]. Phytate was determined using the procedure of Oboh [20]. Oxalates were determined by the standard method [18]. Saponins were quantified by the method of Doss et al. [21] while Alkaline Titration Method [18] was used for hydrogen cyanide

2.6 Amino acids (AA) analysis

Ten (10 µL) of defatted seed samples were analyzed for the amino acid profile using Amino Acid Analyser (TSM), (Technicon Instruments Corporation, New York). The analysis period was 76 min with a column flow rate of 0.50 cm3 /min at 60oC with reproducibility consistent within ±3%. The net height of each peak produced by the chart record of TSM was measured and calculated for the amino acid it represented.

2.7 Fatty acid analysis

Fatty acid methyl esters (FAMEs) FAMEs were prepared using the methods reported by Yurchenko et al. [22] with slight modification.

FAME standards were used for both qualitative and quantitative analysis of the fatty acid composition of seed oil. Standards used for this analysis were caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), linolenic acid (C18:3), behenic acid (C22:0), erucic acid (C22:1), and lignoceric acid (C24:0). For quantitative analysis, 6 concentrations (1, 10, 20, 40, 60, and 100 mg/mL) of the mixed standards were analyzed, to allow construction of a standard curve of each fatty acid. All data were acquired using the Agilent ChemStation software.

2.8 Statistical Analysis

Data were analyzed using Statistical Analysis System (SAS) and presented as means ± SD. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$

3.0 Results

3.1 Effect of fermentation on fatty acid profiles of *Z. mauritiana* seeds

The Unsaturated and saturated fatty acid compositions of *Z. mauritiana* seeds are presented in Tables 1. Unsaturated fatty acids including palmitoleic, oleic, erucic, linoleic and linolenic acid of *Z. mauritiana* seeds oil were not significantly ($p>0.05$) altered by fermentation for 6 and 12 hr. However, at 18 and 24 hr of fermentation a significant ($p<0.05$) reduction in these fatty acids were recorded. The decrease in nutrients was more pronounced ($p<0.05$) at 18 hr of fermentation than at 24 hr (Table 1).

Saturated fatty acids including caproic, caprylic, capric lignoceric, and margaric acid significantly ($p<0.05$) decreases with all fermentation periods tested. Lauric, myristic, palmitic, stearic, arachidonic and behenic acid concentrations in seed fermented for 6 and 12 hr were not significantly ($p>0.05$) different from the unfermented seed samples. These fatty acid concentrations were significantly ($p<0.05$) lowered in seed fermented for 18 and 24 hr (Table 2)

Table 1: Effect of fermentation on the un saturated fatty acid compositions of *Ziziphus mauritiana* Seeds

Fatty Acids	Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr
Palmitoleic (C16:1)	0.06±0.01^{b}	0.07±0.01^{b}	0.09±0.01^{b}	0.01±0.01^{a}	0.02±0.01^{a}
Oleic (C18:1)	14.01±0.06^{b}	14.11±0.02^{b}	14.15±0.02^{b}	9.71±0.02^{a}	12.80±0.03^{ab}
Erucic (C22:1)	0.19±0.03^{c}	0.22±0.04^{c}	0.26±0.02^{c}	0.05±0.01^{a}	0.11±0.02^{b}
Linoleic (C18:2)	48.71±0.02^{b}	48.73±0.01^{b}	48.76±0.02^{b}	34.52±0.01^{a}	43.81±0.04^{b}
Linolenic (C18:5)	0.56±0.02^{c}	0.59± 0.01c	0.62±0.01^{c}	0.26±0.01^{a}	0.34±0.03^{b}
O/L ratio	1/3.47	1/3.45	1/3.44	1/3.55	1/3.42

Values are mean ± SD of 3 determinations. Values with different superscript alphabet along a column are significantly different at $p < 0.05$: Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

Table 2: Saturated Fatty Acid Compositions of Oils Extracted from Fermented and Unfermented *Ziziphus mauritiana* Seeds

Fatty Acids		Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr
Caproic	C6:0	0.09±0.01^{d}	0.07±0.00^{c}	0.07±0.01^{c}	0.01±0.00^{a}	0.04±0.02^{b}
Caprylic	C8:0	0.12±0.01^{e}	0.10±0.01^{d}	0.07±0.03^{c}	0.01±0.00^{a}	0.05±0.03^{b}
Capric	C10:0	0.11±0.02^{c}	0.07±0.01^{b}	0.06±0.02^{b}	0.01±0.00^{a}	0.02±0.01^{a}
Lauric	C12:0	1.76±0.01^{b}	1.71±0.04^{b}	1.62±0.01^{b}	1.12±0.01^{a}	1.23±0.03^{a}
Myristic	C14:0	1.24±0.02^{c}	1.20±0.02^{c}	1.13±0.02^{bc}	0.58±0.01^{a}	1.00±0.04^{b}
Palmitic	C16:0	13.60±0.04^{b}	13.52±0.05^{b}	13.51±0.04^{b}	10.11±0.02^{a}	10.24±0.01^{a}
Margaric	C17:O	0.17±0.02^{c}	0.12±0.01^{b}	0.10±0.01^{b}	0.07±0.01^{a}	0.06±0.01^{a}
Stearic	C18:0	11.81±0.03^{b}	11.85±0.05^{b}	11.91±0.02^{b}	9.03±0.01^{a}	10.36±0.01^{ab}
Arachidonic	C20:0	1.27±0.01^{b}	1.25±0.00^{b}	1.24±0.02^{b}	0.60±0.03^{a}	1.07±0.01^{b}
Behinic	C22:0	0.18±0.01^{c}	0.17± 0.01^{c}	0.15±0.02^{c}	0.01±0.01^{a}	0.03±0.01^{b}
Lignoceric	C24:0	0.14±0.01^{e}	0.12±0.01^{d}	0.07±0.01^{c}	0.01±0.01^{a}	0.03±0.01^{b}

Values are mean ± SD of 3 determinations. Values with different superscript alphabet along a column are significantly different at $p < 0.05$: Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

3.2 Effect of fermentation on anti-nutrient compositions of *Z. mauritiana* seeds

The Effect of fermentation on anti-nutrient compositions of *Z. mauritiana* seeds is shoen in table 3. The concentrations of cyanide, tannin and oxalate in all fermented *Z. mauritiana* seed decreases ($p<0.05$) significantly when compared with the unfermented sample. Saponins content was lowered only in seed fermented for 24 hr. However, the phytate concentration increases in seed fermented for 18 hr when compared with the unfermented sample (Table 3).

3.3 Effect of fermentation on micro-nutrient compositions of *Z. mauritiana* seeds

The effect of fermentation on micro-nutrient compositions of *Z. mauritiana* seeds is presented in table 4. The concentrations of sodium, potassium, calcium, magnesium, copper, manganese, iron zinc and phosphorus in *Ziziphus mauritiana* seeds significantly ($p<0.05$) increase with increase fermentation period from 6 hr to 24 hr.

3.4 Effect of fermentation on proximate compositions of *Z. mauritiana* seeds

The proximate compositions of both fermented and non-fermented *Ziziphus mauritiana* seeds is presented in table 5. Moisture contents of the fermented seed increase significantly ($p<0.05$) while the fibre content decreases with an increase in fermentation period from 6 hr to 24 hr. Ash, crude fat, protein and carbohydrate concentration of all fermented samples were not significantly altered ($p>0.05$) when compared with the unfermented seed

Table 3: Effect of fermentation on anti-nutrient compositions of *Z. mauritiana* seeds

Antinutrient (mg/100g)	Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr
Cyanide	8.54 ± 0.18^{c}	4.39 ± 0.16^{b}	3.34 ± 0.12^{b}	2.80 ± 0.12^{a}	2.56 ± 0.06^{a}
Oxalate	0.28 ± 0.00^{d}	0.22 ± 0.01^{c}	0.19 ± 0.01^{b}	0.18 ± 0.00^{b}	0.14 ± 0.01^{a}
Phytate	0.32 ± 0.00^{b}	0.27 ± 0.01^{b}	0.17 ± 0.01^{a}	0.54 ± 0.02^{c}	0.37 ± 0.04^{b}
Saponins	55.54 ± 0.17^{b}	51.63 ± 0.73^{b}	51.04 ± 1.03^{b}	50.62 ± 0.85^{b}	45.95 ± 1.03^{a}
Tannins	90.86±0.89c	72.54 ± 0.10^{b}	43.46 ± 0.38^{a}	37.49 ± 0.38^{a}	30.37 ± 3.06^{a}

Values are mean ± SD of 3 determinations. Values with different superscript alphabet along a column are significantly different at $p < 0.05$: Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

Table 4: Effect of fermentation on micro-nutrient compositions of *Z. mauritiana* seeds

Minerals (mg/kg)	Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr
Sodium	5.60 ± 0.20^{a}	9.12 ± 2.83^{b}	12.61 ± 2.26^{c}	20.91 ± 1.41^{d}	21.41 ± 1.70^{d}
Potassium	21.10 ± 3.00^{a}	21.11 ± 1.55^{a}	22.00 ± 4.00^{a}	23.31 ± 0.14^{a}	27.61 ± 1.70^{b}
Calcium	8.10 ± 0.20^{a}	7.90 ± 0.00^{a}	8.63 ± 0.71^{a}	10.5 ± 1.01^{b}	11.30 ± 0.00^{c}
Magnesium	12.75 ± 1.25^{a}	13.62 ± 2.26^{a}	17.26 ± 0.28^{b}	19.05 ± 0.66^{b}	20.12 ± 0.82^{b}
Copper	2.20 ± 0.00^{a}	2.21 ± 0.57^{a}	2.65 ± 0.56^{a}	3.31 ± 0.14^{b}	3.52 ± 0.71^{b}
Manganese	1.30 ± 0.20^{a}	1.30 ± 0.57^{a}	1.54 ± 0.42^{a}	1.62 ± 0.28^{a}	2.00 ± 0.00^{b}
Iron	0.70 ± 0.30^{a}	0.70 ± 0.14^{a}	1.00 ± 0.00^{b}	1.04 ± 0.42^{b}	1.03 ± 0.28^{b}
Zinc	1.20 ± 0.42^{a}	1.20 ± 0.00^{a}	1.65 ± 0.56^{a}	1.74 ± 0.42^{a}	1.91 ± 0.14^{a}
Phosphorus	1.10 ± 0.14^{a}	1.10 ± 0.42^{a}	1.35 ± 0.56^{a}	1.40 ± 0.00^{a}	1.700.14[a]

Values are mean ± SD of 3 determinations. Values with different superscript alphabet along a column are significantly different at $p < 0.05$: Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

Table 5: Effect of fermentation on proximate compositions of *Z. mauritiana* seeds

Proximate (%)	Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr
Moisture	4.15 ± 0.41^{a}	4.70 ± 0.10^{a}	5.59 ± 0.08^{a}b	6.93 ± 1.41^{b}	9.98 ± 1.13^{c}
Ash	3.26 ± 0.25^{a}	3.30 ± 0.10^{a}	3.31 ± 0.14^{a}	3.32 ± 0.40^{a}	3.43 ± 0.34^{a}
Fat	26.46±0.72a	25.33 ± 0.19^{a}	25.27 ± 1.48^{a}	24.18 ± 2.62^{a}	24.26 ± 2.08^{a}
Fiber	4.93 ± 0.35^{c}	4.85 ± 0.35^{c}	4.14 ± 0.50^{b}	3.34 ± 0.06^{a}	3.28 ± 0.25^{a}
Proteins	41.18 ± 3.36^{a}	42.84 ± 2.08^{a}	42.88 ± 1.51^{a}	44.02 ± 0.52^{a}	43.07 ± 5.47^{a}
Carbohydrates	19.73 ± 3.22^{a}	19.00 ± 2.64^{a}	19.05 ± 2.67^{a}	18.74 ± 2.12^{a}	18.01 ± 5.01^{a}
Energy	478.22 ± 0.00^{a}	478.10 ± 0.00^{a}	485.70 ± 0.00^{a}	490.53 ± 0.00^{b}	460.32 ± 0.00^{a}

Values are mean ± SD of 3 determinations. Values with different superscript alphabet along a column are significantly different at $p < 0.05$: Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

3.5 Effect of fermentation on amino acid profiles of *Z. mauritiana* seeds

The total essential amino acid, conditional amino acid and non-essential amino acid composition of fermented and unfermented *Z. mauritiana* seed are presented in tables 6, 7 and 8 respectively. The total essential amino acids composition of the fermented and non-fermented *Z. mauritiana* seed ranged between 25.02±0.36 g/100 g and 34.83±0.32 g/100g. The highest concentration of essential amino acids of the samples was tryptophan (27.18±0.08 g/100g), while the least concentration (7.43±0.06g/100g) was histidine (Table 6). Fermentation for 12, 18 and 12 hr significantly decreases isoleucine, threonine, phenylalanine and histidine however, leucine, valine, methionine and tryptophan were not altered by fermentation (Table 6).

The total conditional-essential amino acids composition of the fermented and non-fermented *Z. mauritiana* seed ranged between 10.44±0.10g/100 g and 14.20±0.10 g/100g. The highest concentrated conditionally essential amino acids of the samples was arginine (19.15±0.25 g/100g), while the least concentrated (1.97±0.01 g/100g) was cysteine. Conditional amino acids including arginine, cysteine and tyrosine were not altered by fermentation, while, proline and glycine decreases during the fermentation (Table 7).

The total non-essential amino acids composition of the fermented and non-fermented *Z. mauritiana* seed ranged between 21.27±0.25 g/100 g and 23.62±0.23 g/100g. The highest concentrated of non-essential amino acids of the samples was glutamic acid (46.19±0.23 g/100g), while the least concentrated (0.47±0.09 g/100g) was ornithine (Table 8). The concentrations of non-essential amino acids including glutamic acid, aspartic acid, alanine were not altered by fermentation, while serine and ornithine decrease during fermentation.

Table 6: Effect of fermentation on the essential amino acid compositions of *Z. mauritiana* seeds

Amino Acids	Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr	Total
Lycine	4.04±0.02	4.04±0.08	3.84±0.02	3.90±0.03	3.92±0.01	19.73±0.07
leucine	4.47±0.02	4.51±0.04	4.30±0.05	4.38±0.01	4.51±0.02	22.15±0.14
Valine	2.54±0.02	2.57±0.04	2.38±0.01	2.43±0.02	2.58±0.01	12.49±0.06
isoleucine	5.76±0.02	5.72±0.03	1.73±0.02	1.78±0.01	1.87±0.02	16.85±0.02
Threonine	3.48±0.02	3.50±0.02	2.37±0.03	2.44±0.02	2.50±0.02	14.27±0.1
phenylalanine	4.56±0.02	4.38±0.23	2.53±0.02	2.61±0.03	2.65±0.02	16.72±0.23
methionine	1.90±0.04	1.66±0.02	1.58±0.01	1.67±0.01	1.66±0.02	8.46±0.03
Histidine	2.17±0.02	1.97±0.06	1.06±0.01	1.11±0.02	1.13±0.01	7.43±0.06
Tryptophan	5.91±0.03	5.37±0.03	5.23±0.02	5.31±0.02	5.37±0.03	27.18±0.08
Total	34.83±0.32	33.79±0.32	25.02±0.36	25.63±0.37	26.19±0.33	

Values are mean ± SD of 3 determinations. Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

Table 7: Effect of fermentation on the conditional-essential amino acid compositions of *Z. mauritiana* seeds

Amino Acids	Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr	Total
Arginine	3.92±0.04	3.91±0.11	3.71±0.05	3.75±0.03	3.87±0.03	19.15±0.25
Cysteine	0.52±0.01	0.40±0.02	0.31±0.03	0.35±0.01	0.40±0.02	1.97±0.01
Tyrosine	2.27±0.01	2.13±0.03	2.04±0.01	2.10±0.02	2.13±0.03	10.67±0.08
Proline	3.94±0.03	3.71±0.04	1.90±0.03	2.01±0.04	2.04±0.01	13.60±0.06
Glycine	3.55±0.03	3.55±0.09	2.48±0.01	2.54±0.01	2.57±0.01	14.69±0.08
Total	14.20±0.10	13.70±0.12	10.44±0.10	10.75±0.11	11.01±0.10	

Values are mean ± SD of 3 determinations. Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

Table 8: Effect of fermentation on the non-essential amino acid compositions of *Z. mauritiana* seeds

Amino Acids	Fmt_0 hr	Fmt_6 hr	Fmt_12 hr	Fmt_18 hr	Fmt_24 hr	Total
Glutamic acid	9.76±0.02	9.43±0.15	8.91±0.03	9.00±0.04	9.10±0.01	46.19±0.23
Aspartic acid	7.04±0.01	7.07±0.04	6.81±0.04	6.92±0.04	7.06±0.02	34.89±0.15
Alanine	3.02±0.07	3.04±0.06	2.87±0.02	2.92±0.01	3.07±0.01	14.91±0.03
Serine	3.69±0.03	3.65±0.31	2.61±0.04	2.70±0.03	2.74±0.02	15.38±0.35
Ornithine	0.11±0.02	0.11±0.02	0.07±0.01	0.08±0.01	0.11±0.02	0.47±0.09
Total	23.62±0.23	23.30±0.21	21.27±0.25	21.62±0.25	21.86±0.19	

Values are mean ± SD of 3 determinations. Fmt_0 hr= unfermented sample; Fmt_6 hr= fermented for 6 hr; Fmt_12 hr= fermented for 12 hr; Fmt_18 hr= fermented for 18 hr; Fmt_24 hr= fermented for 24 hr.

4.0 Discussion

The moisture contents of both fermented and unfermented *Z. mauritiana*, fall within the recommended range of 0–13% as reported by James [23]. These low moisture content will prevent or delay microbial deterioration thus prolong the shelf life of the seed. The increase in the moisture content with an increase in fermentation time was also observed by Okechukwu et al. [24] and Adegbehingbe [25] while fermenting African locust bean, melon seeds and Lima Bean (*P. lunatus*) seeds respectively. This increase could be attributed to increasing soaking in water as fermentation time increases.

A number of studies have documented increases in crude protein during seed fermentation [25, 26], this is, however, contrary to the present study as no fermentation related changes in protein contents of *Z. mauritiana* seeds was observed, this differences could be attributed to the differences in the organism employed in the fermentation. Bacteria fermenting organisms such as Bacillus species are producers of extracellular proteases which could hydrolyze complex proteins in the seed to peptide and amino acids, thus increasing the total nitrogen content [27], this is, however, contrary to the yeast which was used as the fermenting organism in the present study. The reduction observed in crude fibre content was due to the action of cellulolytic micro-organism present in the fermenting substrate [28]

Ash crude fat, protein and carbohydrate concentration of all fermented samples were not significantly altered compared with the unfermented seed. The protein content of *Z. mauritiana* seed reported in this study is higher than 33.64 reported for locust bean [29], 10.77 ± 0.66 g/100g for wheat [30], and 17.37% for Ugba [28]. This is, however, contrary to report of previous studies [31-33] which reported fermentation related changes in ash, crude fat, protein and carbohydrate contents while studying the effect of fermentation on nutrient and anti-nutrient composition of seed flours. Olusey iet al., [34] also reported that crude fibre, fat and protein contents of *T. indica* seed increases as fermentation progressed, while carbohydrate and ash reduced.

Ash is the measure of mineral content. The concentrations of calcium, sodium, magnesium, manganese, zinc, potassium, phosphorus and copper content of *Z. mauritiana* seeds were appreciably higher than those previously reported for Bambara groundnut [35] soybean [36] and *S. stenocarpa* seeds [37] and other legumes such as winged bean, cowpea and peanut [38] which suggested that *Z. mauritiana* could be a better source and/or alternative of these minerals. Though, fermentation had no effect on the ash content of *Z. mauritiana* seeds.

The increase in sodium, potassium, calcium, magnesium, copper, manganese, iron zinc and phosphorus concentration with increase fermentation period in *Z. mauritiana* seeds from 6 hr to 24 hr is an indication that these minerals were increasingly released from chelated complex compounds as the activities of fermenting organism increase with time [39]. Animals required these micronutrients for proper body function including eggshell formation, nervous coordination, muscle and heart activities and blood coagulation among many others [24]. Thus fermentation of *Z. mauritiana* seeds will ensure better mineral supply for the production of healthy animals.

Antinutrients including phytate, oxalate, tannins and cyanide are generally toxic and may negatively affect the nutrient value of seeds by hampering protein digestibility and mineral bioavailability [1]. The oxalate and phytate contents of *Z. mauritiana* seed were lower than those reported for tiger nut seed [40]. The levels of saponins in both fermented and non-fermented *Z. mauritiana* seed are within the recommended safe level (below 22.4mg/g) and adequate for exhibiting hypoglycemic, hypolipidemic and antioxidants effects. Tannins have been reported to enhance good health by preventing heart diseases, diarrhoea, cavities and tooth decay in addition to having antimicrobial properties. Therefore, the presence of a low level of tannins would confer some health benefits to the seed.

Oxalates are known for their inhibitory effect on metabolism and the bioavailability of magnesium, calcium, and protein in man. Oxalate also enhanced the formation of kidney stones via the formation of a complex within human subjects [41]. According to Enwere [42] hydrogen cyanide is considered not poisonous, moderately poisonous and

highly poisonous when concentrations are less than 50 mg/kg BW, 50 to 100mg/kg BW and over 100 mg/kg BW respectively. It can be therefore seen that the levels of hydrogen cyanide observed in both fermented and unfermented *Z. mauritiana* seed were far below the levels that can induce any toxic effect when consumed. Furthermore, the reduction in tannin, oxalate and cyanide contents of *Z. mauritiana* seed during fermentation is an added advantage and could be attributed to their solubility in water. This observation correlates with the reports of other researchers on fermented legumes, sorghum, millet, African locust bean, kidney bean and chickpea flours [1, 43-44]. By implication, the result showed that fermentation had a significant effect in reducing the levels of inherent anti-nutrient factors in *Z. mauritiana* seed.

The digestibility and quality of edible seeds are determined by the type and quantity of unsaturated fatty acids it contains. In the present study, polyunsaturated fats (Linoleic), had the highest concentrations in all the samples obtained. Intake of linoleic acid and linolenic acid are of great medicinal importance for humans; linolenic acid has hypocholesterolemic, anti-inflamatory, immunostimulatory effects and assists in body defence. Linolenic acid on the other hand has been associated with hypoglycemic, anti-inflammatory, decrease the risk of cardiovascular diseases.it also aids proper formations of the visual and central nervous, through the formation and regeneration of neurons [45]. It is also noteworthy that the high levels of unsaturated fatty acid than the saturated fat for both fermented and non-fermented *Z. mauritiana* seed will be beneficial in decreasing the plasma LDL cholesterol concentration by increasing the hepatic uptake of LDL particles from the circulation [46]

Although most unsaturated and saturated fatty acids can be synthesized by animal tissues, linoleic series cannot be synthesized and thus must be provided in the diet. If provided in the diet, arachidonic acid can be synthesized from it. This acid, alone or together with linoleic acid, is essential for the maintenance of the normal skin structure [47]. Oleic/linoleic acid ratio (Ole / Lin) has been accepted as a means of assessing edible seed oil quality. The higher the amount of linoleic acid in relation to oleic acid, the better the quality of vegetable oil in avoiding the formation of bad cholesterol. In the present study, the Ole / Lin ratio for both fermented and non-fermented *Z. mauritiana* seed oil was in the range 1/3.42 and 1/3.47, demonstrating the high oil quality.

The observed effect of fermentation on fatty acid contents of *Z. mauritiana* seed is similar to the report of Ijarotimi and Keshinro [29] observed decreases in unsaturated fatty acid and saturated fatty acid composition of African locust bean during fermentation. Achinewhu [48], also observed that fermentation did not have much effect on the fatty acid composition of *P. macrophylla* except for the slight reduction in the total saturated fatty acids. The decreases in the fatty acid composition of *Z. mauritiana* seed was however seen only after 18 and 24 hr of fermentation, this suggests that fermentation of *Z. mauritiana* seed should be done below 18 hr to ensure retention of the fatty acid composition.

With the current emphasis on the consumption of low levels of saturated fats, eliminating trans fatty acid, and increasing intake of poly and monounsaturated fatty acid, the consumption of *Z. mauritiana* seed should be encouraged as it's a good source of beneficial fatty acid for improving the integrity of cardiovascular system thus decreasing the risk of cardiovascular impairment and other nutritional associated disorders.

Previous studies reported that fermentation of oilseed significantly increases EAA content including lysine, histidine, arginine, serine, glycine, alanine, valine, isoleucine, tyrosine and phenylalanine [49]. This is, however, contrary to the loss of isoleucine, threonine, phenylalanine and histidine contents during fermentation of *Z. mauritiana*. This loss could be attributed to the solubility of these amino acids in the fermentation medium. These could be nutritionally detrimental since these EAA cannot be synthesized by animal's tissue. This is, however, not of nutritional concern as arginine and histidine content recorded for both fermented and non-fermented *Z. mauritiana* seed were higher than the arginine (2.0 mg/day and histidine (1.9 mg/day) recommended for infants by FAO/WHO [50]. These amino acids play crucial roles in the proper growth and development of infants, thus incorporation of *Z. mauritiana* into infant diet would enhance their growth and

development particularly in developing countries where animal-based foods are expensive.

The loss of essential, conditional and non-essential amino acids of *Z. mauritiana* seed were however, not evident at fermentation period of below 12 hr. Summing up this fact and other findings from this study it is reasonable to assumed that the nutritional quality of *Z. mauritiana* seed is best obtained without fermentation or at fermentation period of below 12 hr

5.0 Conclusion

Ziziphus mauritiana has high amount of proteins, amino acid, minerals, unsaturated fatty acid and low levels of saturated fatty acid. The seed also contains antinutrient below the recommended safe limit. Fermentation reduced the anti-nutrient levels, some amino acid contents and significantly affect its fatty acid composition. The study suggests that *Z. mauritiana* seed is an alternative protein, unsaturated fatty acid and other nutrient sources. The seed could be employed as alternative source of protein and food supplement for human nutrition. The most plausible and positive effect of the fermentation with high nutrient retention occur ≤ 12 hr of fermentation, thus fermentation of this seed is encouraged below this period.

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Author's contributions:

The work was conducted in collaboration of all authors. Author ARA conducted the study and wrote the manuscript. Authors AYI: supervised the work and revised the manuscript. Authors JCA and MMN co-supervised the work and revised the manuscript. All authors read and approved the final version of the manuscript.

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