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Changes in the enzymes, amino acids, metal ions and flavor profile during fermentation of African locust bean seeds *'Parkia biglobosa'* to Daddawa

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Abstract

Daddawa/iru, a product of African locust bean (*Parkia biglobosa*) seeds, is widely used as food condiment by many tribes in West Africa. This study aimed to determine some of the changes which occur during the microbial fermentation of African locust bean seed. The daily changes in pH, amylase, lipase and protease secretion were monitored. Changes in amino acid concentration, mineral ion composition were also monitored; the presence of hydrocarbons and volatile flavor compounds were also determined. The pH increased throughout fermentation and was above 8.5 by 96 h fermentation. Enzyme production was highest after 48 h fermentation. The highest increase in amino acid concentration was observed after 72 h fermentation. The essential amino acids which increased after 72 h fermentation include arginine (48.8%), threonine (25.2%), valine (24.8%), and methionine (20.0%), histidine (10.3%), isoleucine (19.2%), leucine (15.9%), lysine (15.24%), phenylalanine (14.9%), and tryptophan (12.5%). A higher metal ion concentration was observed before fermentation (0 h) than during or after fermentation of the locust bean seeds. Various hydrocarbons, fatty acids, esters and alcohols were observed at different stages of the fermentation.

Keywords: African locust bean seeds, Fermentation, Amino acids, Metal content, Flavour compounds, Chemical composition, Daddawa

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INTRODUCTION

Legume seeds such as African locust bean seeds, African oil bean seeds, melon seeds, castor oil seeds, mesquite bean seed, and soybean seeds are fermented to value-added products with increased flavour and taste (Nwagu et al*.,* 2010; Ndamitso et al., 2020). In their natural forms, these seeds possess anti-nutritional substances and toxic elements. Therefore, the process of fermentation enables detoxification, making them edible. Fermented products of these seeds are used as condiments in soups, stews, and porridges by people of different cultures in West Africa (Nwagu et al*.,* 2011; Omafuvbe et al*.,* 2002). Given the socio-economic status of most consumers, the inclusion of these foods as components of the diet provides a major source of essential nutrients during each meal. Microorganisms mostly responsible for these fermentations belong to the genus Bacillus, with *B. subtilis*, *B. megaterium*, *B. cereus* mostly implicated (Omafuvbe et al*.,* 2004). These microorganisms act on the food polymers to release various organic acids, alcohols, esters, aldehydes, and gases that confer characteristic aroma and flavour. Factors that influence the rate at which these products are formed include the pH, temperature, and moisture contents of the seeds. There are numerous advantages of food fermentation; these include improved health benefits due to the detoxification and removal of anti-nutrients in the food, the increase in the nutritional composition, and improvement of the functional properties of the food. Other benefits of food fermentation are enhanced aroma and flavour, increased shelf life (depending on the type of food), and increased food variety (Verardo et al*.,* 2020).

Daddawa is obtained from the traditional solid-state fermentation of African locust bean seeds (*Parkia biglobosa*) by *Bacillus* species (Nwagu et al., 2020). Daddawa also called 'iru' or 'soumbala' by some tribes in West Africa, is widely used as condiment or seasoning in soups, stews, and many other traditional dishes. (Omafuvbe et al*.,* 2004). Though *Bacillus* species are the major drivers of this fermentation, other are also observed during the early stages of the fermentation (Achi, 1992; Ezeokoli et al., 2018; Farinde et al., 2021; Yves et al*.,* 2019).

The optimization and realization of daddawa potentials require an understanding of the biochemical changes during its fermentation. The current study considered the changes in the chemical composition of the seeds as fermentation proceeded to derive the widely accepted food condiment, daddawa. There is a need to understand these changes and how they are influenced/controlled by the duration of fermentation. This knowledge will help gain a fuller understanding of the production process and enable proper control for a better product, especially regarding its nutritional quality.

In this study, an array of biochemical transformations during the fermentation of African locust bean seeds was investigated. These include changes in pH, the concentration of hydrolytic enzymes, essential amino acid, mineral concentration and volatile compounds present.

MATERIALS AND METHODS

Processing of African locust bean (Parkia biglobosa) seeds into daddawa

Africa locust bean seeds were purchased from Taraba State, Nigeria. Daddawa was prepared in the laboratory using the traditional method (Edema and Fawole, 2006). Raw African locust bean (1kg) was boiled for 12 h to soften the firmly attached seed coats and further soaked in the boiling water for another 12 h. Excess water was drained off, and the seeds were dehulled by slightly pounding the seeds with a large wooden mortar and pestle. Further removal of the seed coat was achieved by rubbing the cotyledons between the palms and washing with tap water. About 425 g of the cotyledons were recovered and washed. The cotyledons were cooked again for 6 h, the excess water was drained off, and the cotyledons wrapped with jute sacks to keep the system warm and fermented for four days to produce 'daddawa'. A large stone was placed on the wrapped seed to further drain off excess water and reduce moisture.

pH determination

The pH of both the controlled and traditional fermentation was determined every 24 h throughout fermentation. One gram (1 g) of each sample was ground and homogenized in 9 mL of sterile distilled water and filtered using Whatman

No.1 filter paper. The pH of the suspension was taken using a pH meter.

Enzyme assays

Samples of *daddawa* were collected after 0, 24, 48, 72 and 96 h of fermentation, ground into a paste in a mortar, and extracted in 0.2 M phosphate buffer, pH 7.0 (Odunfa,1985). Extracts were stored in a deep freezer until used. Amylase activity was determined using a slight modification of the method by Awodi et al. (2021). by incubating 0.5 ml of the suitably diluted extract with 0.5 ml of soluble starch 1% w/v in 0.1M pH 7, phosphate buffer at 40^{o}C for 10 minutes. The reaction was stopped using 2,4-dinitrosalicyclic acid (Bernfeld, 1955) and absorbance read at 540 nm. Absorbance values were converted to maltose concentration using the maltose standard curve. One unit of amylase activity was defined as the amount of enzyme, which liberated one µg of maltose per minute under the assay conditions. Protease assay was determined by modifying the method described by Nwagu and Amadi (2013). The reaction mixture consisted of 1 mL of 1% (w/v) casein in 0.2 M phosphate buffer, pH 7.0, and 1 mL of enzyme solution. Absorbance was read at 660 nm, and obtained values were read off a tyrosine standard curve. One unit of protease activity was defined as the amount of enzyme, which released 1 µg of tyrosine from casein per minute under the experimental conditions. Lipase was assayed using a slight modification of the method described by Nwagu and Amadi (2013). The reaction mixture consisted of 2 mL of 0.2 M phosphate buffer, pH 7.0, 1 mL of 0.1 M $CaCl_2$, 0.5 mL of olive oil, 2 mL distilled water, and 1 mL of extract. An oleic acid standard curve was used and one unit of lipase activity was defined as the amount of enzyme, which liberated 1 µg of oleic acid per minute under the prevailing experimental conditions.

Amino acid analysis

The Amino Acid profile in the known sample was determined using the methods of Benitez (1989). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer. The sample was defatted using a chloroform/methanol mixture of ratio 2:1. About 4 g of the sample was put in an extraction thimble and extracted for 15 h in the soxhlet extraction apparatus (AOAC, 2006). A known weight of the defatted sample was weighed into a glass ampoule. Exactly 7 mL of 6N HCL was added, and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 ^{o}C± 5 ^{o}C for 22 h. The ampoule was allowed to cool before broken open at the tip, and the content was filtered. The filtrate was then evaporated to dryness using a rotary evaporator. The residue was dissolved in 5 mL acetate buffer (pH 2.0) and stored in plastic specimen bottles kept in the freezer. The amount loaded was 60 µl. The tryptophan in the known sample was hydrolyzed with 4.2 M sodium hydroxide (Robel, 1967). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Analysis of mineral ions

Samples were digested by the wet-digestion method using a combination of perchloric acid, nitric acid and sulfuric acid. Mineral compositions were determined using the Pye Unicam SP9 atomic absorption spectrophotometer and other methods described by the AOAC (2006).

Gas chromatography mass spectrometry (GC-MS)

GC-MS analysis was performed on an Agilent Technologies AutoSystem XL GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 ^{o}C). The transfer line temperature was 280 ^{o}C. Helium was used as carrier gas (1.3 mL min^{-1}) and the capillary columns used were an HP 5 MS (30 x 0.25 mm; film thickness 0.25 mm) and an HP Innowax (30 x 0.32 mm i.d., film thickness 0.50 mm). The temperature programme was the same as that used for the GC analyses. The identification and interpretation of mass-spectrum GC-MS were conducted using the database of mass spectra library search (NIST).

Statistical analysis

Statistical significance between groups was evaluated using analysis of variance (ANOVA). All experiments were performed at least in triplicate, and the results presented as their mean

value. The error bars represent the standard deviation.

RESULTS AND DISCUSSION

Changes in pH during African locust bean fermentation

Before microbial fermentation, the pH of the cooked African locust bean seed was slightly acidic (pH 5.86). There was a progressive increase in the pH throughout the fermentation period, as shown in Fig. 1. The pH turned alkaline (8.56) after 96 h fermentation; a similar pH change was reported by Adelekan and Nwadiuto (2012). An increase in pH during fermentation is attributed to the gradual release of ammonia during protein degradation (Nwokeleme and Ugwuanyi, 2014).

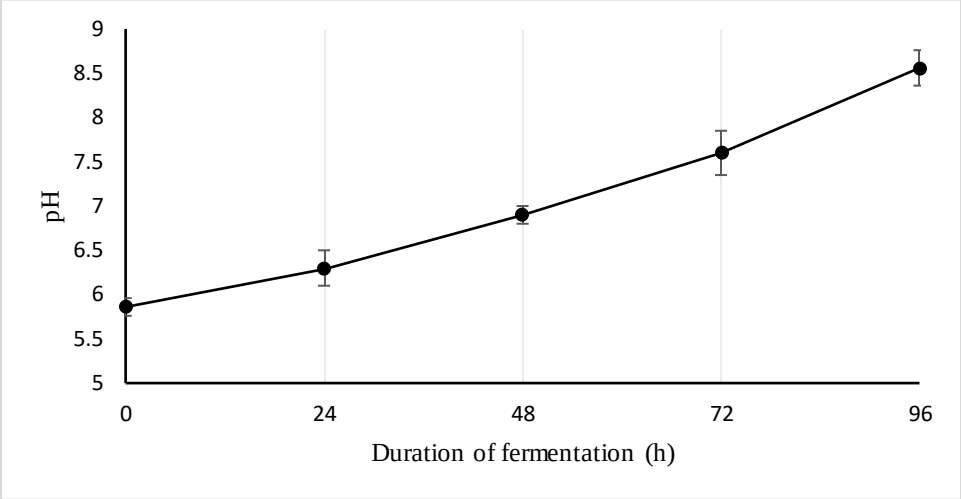

Fig 1. Changes in pH during daddawa fermentation

Presence of amylolytic, proteolytic and lipolytic enzymes during daddawa production

Amylolytic activity was observed during the fermentation of African locust bean seeds (Fig. 2). The presence of amylase during the fermentation of African locust bean seed was previously reported (Omafuvbe et al., 2004). There was a minute concentration of amylase in the fermenting seeds at zero hours, this concentration rapidly increased reaching a peak after 48 h of fermentation. Fig. 2 shows that the hydrolytic enzymes protease and amylase were present at the onset of fermentation and continuously increased until they reached their peak after 48 h. Subsequently, a drop in the synthesis of both hydrolytic enzymes was observed. Omafuvbe et al. (2002) also reported that protease and amylase activities attained their peak after 48 h fermentation of the locust bean seeds. The fermenting microorganisms synthesize these proteolytic and amylolytic enzymes for the hydrolysis of the proteins and carbohydrates in the African locust bean seeds to simpler peptides/amino acids and sugars that they can easily assimilate, for their growth and metabolism. Microorganisms predominantly present during the fermentation of African locust bean seeds are members of the genus Bacillus and Staphylococcus (Amao et al., 2018; Sarkar et al., 2002, Azokpota et al., 2006; Ezeokoli et al., 2018; Nwagu et al., 2020).

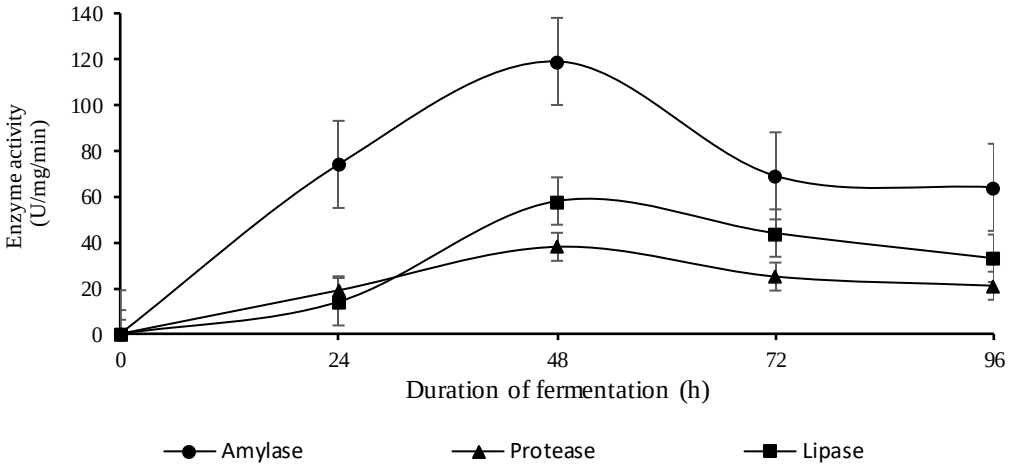

Figure 2. Changes in the activities of amylase (diamond), protease (triangle) and lipase (square) during the fermentation of daddawa

The production of large quantities of proteases is not surprising considering that *Bacillus* species secrete copious amounts of alkaline proteases which are highly stable in adverse environmental conditions such as extremely alkaline environments (Contesini *et al.,* 2018). The enzyme industries have exploited and continues to exploit this ability of *Bacillus species* to secret stable proteases (Contesini *et al.,* 2018; Bhunia *et al.,* 2012). The proteases catalyze the hydrolysis of the abundant protein polymer to peptides and amino acids, contributing to the flavor of the final fermentation product daddawa. Considering that African locust bean seeds are legumes consisting of predominantly proteins (±32 %), the activity of proteases play a major role in providing the necessary nitrogen for microbial growth and proliferation, microbial modification of the cooked dehulled bean seeds and the overall texture and flavor development of the daddawa. Therefore, proteolytic enzymes contribute to developing the organoleptic and rheological properties of the food (Odibo *et al.,* 2008).

The proteolytic activity during the fermentation will release ammonia, thereby increasing the pH of the environment as fermentation progresses. This explains the customary ammoniacal odour of the fermented legumes and the alkaline nature of the food (Azokpota et al., 2006; Odibo et al., 2008).

Lipase activity was also detected during the fermentation of the African locust bean seeds (Fig. 2). Odunfa (1985) observed the increase in the quantity of free fatty acids found in the fermented beans compared to the cooked, dehulled beans and this was attributed to the lipolytic activity during fermentation. Fats and oil constitute 30-31% of locust bean seeds (Odunfa, 1985), and lipases catalyze the hydrolysis of this polymer to fatty acids. *Bacillus* sp. and *Staphylococcus* sp. have been reported as lipase producers (Contesini et al., 2018). Similar to what was observed for amylase and protease, lipase concentration during the daddawa production peaked after 48 h of fermentation. Compared to the African oil bean seed with large quantities of the lipolytic enzyme (Odibo et al., 2008), the lipase yield in this study was low. This may be explained by the higher content of lipids (±50%) contained in the African oil bean seeds. Fatty acids provide energy for the metabolism of microorganisms and serve as effector molecules that help regulate metabolism (Diomandé et al., 2015). Linoleic acid, palmitic acid, and stearic acid are the major fatty acids found in raw and fermented African locust bean seed (Fapojuwo et al., 1986). Fatty acids in their exogenous forms serve as either growth inhibitors or boosters for vegetative *Bacillus* sp. depending on the environmental factors. For example, linoleic acid (the predominant fatty acid found in African locust bean seeds) exerts a decrease in the intracellular ATP concentration of *Bacillus cereus* cells leading to growth inhibition (Diomandé et al., 2015). On the other hand, palmitic acids and stearic acid were reported to serve as growth inducers for

vegetative cells of *B. megaterium* and *B. subtilis* while helping the cells to resist protonophore (Hou, 2009). Moreover, it has been documented that linoleic acid and oleic acid, when consumed, are used to synthesize omega-3 and omega-6 fatty acids, which help the cardiovascular, reproductive, immune and nervous system of the body (Odibo et al*.,* 2008).

Amino acid composition of African locust bean seed 'daddawa'

Analyses of fermented African locust bean seeds for amino acids revealed the presence of a wide range of free amino acids before and during the process of daddawa production (Table 1). This is due to the hydrolyzes of the proteins in the African locust bean seed to amino acids by the secreted proteases. From our results, it is evident that the cooked unfermented locust bean seeds had a high concentration of the following amino acids in g/100g protein; glutamate, 1.43; aspartate, 1.21; leucine, 0.55; lysine, 0.53; alanine, 0.48; tyrosine, 0.45; proline, 0.42; arginine, 0.42; valine, 0.40; serine, 0.40; phenylalanine, 0.36 and isoleucine, 0.31. Amino acids are used as aromatic precursors to synthesize esters, higher alcohols, and aldehydes (Hayek and Ibrahim, 2013). Microorganisms utilize them for the synthesis of precursor metabolites required for cell growth and development. This may explain the reduction in amino acid concentration observed after 24h of fermentation for all the amino acids except for arginine. The drop in amino acid concentration was probably due to its utilization by the growing and multiplying microbial cells in the fermenting bean seeds.

Proliferation of microorganisms will lead to an increase the synthesis of proteases and protein hydrolysis. These actions result in the generation of higher levels of amino acids, as can be seen after 48 h fermentation. Recall that the highest amounts of amylase, lipase and protease enzymes were observed after 48 h fermentation of the dehulled African locust bean seeds. The amino acid concentration detected after 72 h of fermentation implies that the protease concentration led to increased hydrolysis and amino acid generation. It is worthy to note that the highest concentration of the various amino acids was observed after 72 h fermentation. Amino acid concentration decreased over the next 24 h of fermentation. After 72h fermentation, the highest increase in amino acid concentration was observed for arginine (48.8%) and three essential amino acids, including threonine (25.2%), valine (24.8%), and methionine (20.0%).

Fermentation led to an increase in the concentration of the other essential amino acids, amongst which are histidine (10.3%), isoleucine (19.2%), leucine (15.9%), lysine (15.24%), phenylalanine (14.9%), and tryptophan (12.5%). The finding is contrary to Ijarotimi and Keshinro (2012), who observed a decrease in the concentration of essential amino acids such as lysine, threonine, valine, methionine, isoleucine, leucine, phenylalanine after fermentation of locust bean seeds. The variation in the processing methods may have contributed to the decrease in the quantity of amino acid reported by the authors. Ijarotimi and Keshinro (2012) processed the seed by soaking for seven days before dehulling and oven drying at 60 ^{o}C. The long steeping regimen may lead to loss of proteins. The amino acids found in the lowest concentration within the boiled and fermented seeds were tryptophan, methionine, histidine, and cystine; fermentation for 72 h led to a 12.5%, 20%, 10.3% and 11.4% increase in their concentrations, respectively. Ijarotimi and Keshinro (2012) did not detect any tryptophan in the raw and fermented African locust bean flour. Arginine and cysteine were absent during the production of daddawa. However, serine and methionine initially absent from the cooked seeds were produced during fermentation (Odunfa, 1985).

There is a large variation in the chemical quality of the fermented African oil bean seed reported by different authors (Omafuvbe et al*.,* 2002; Yakubu et al., 2022b). The variation in the composition of amino acids between the cooked and fermented African locust beans seeds may result from differences in plant composition based on climatic conditions and soil type. It can also be due to variation in the fermenting microorganisms. When spontaneous fermentation is adopted as is obtainable in most African fermented foods, the exact population and strains of *Bacillus* sp. and other organisms present vary. This variation leads to inconsistency in product quality regarding nutritional composition, flavour and odorant properties, etc.

Table 1. Changes in Amino Acid Composition during the Fermentation of Daddawa

	Duration of Fermentation (h)				
Amino Acid g/100g protein	0	24	48	72	96
Leucine	0.55	0.49	0.58	0.64	0.50
Lysine	0.53	0.50	0.58	0.61	0.51
Isoleucine	0.31	0.28	0.34	0.37	0.30
Phenylalanine	0.36	0.34	0.3	0.41	0.36
Tryptophan	0.08	0.08	0.09	0.12	0.08
Valine	0.40	0.39	0.45	0.50	0.39
Methionine	0.08	0.08	0.09	0.10	0.08
Proline	0.41	0.39	0.47	0.48	0.40
Arginine	0.41	0.42	0.54	0.62	0.43
Tyrosine	0.45	0.23	0.34	0.36	0.26
Histidine	0.19	0.18	0.19	0.22	0.18
Cystine	0.19	0.16	0.19	0.21	0.17
Alanine	0.48	0.41	0.52	0.55	0.43
Glutamic Acid	1.43	1.35	1.48	1.53	1.39
Glycine	0.43	0.34	0.40	0.47	0.35
Threonine	0.27	0.22	0.30	0.33	0.24
Serine	0.40	0.36	0.45	0.49	0.38
Aspartic Acid	1.22	1.13	1.36	1.40	1.16

Mineral ion composition of the fermented African locust bean seed 'daddawa'

Table 2 shows that African oil bean seeds contain a high concentration of potassium (1939.69 mg/kg) and calcium (2562.1 mg/kg). Moderate quantity of magnesium (347.70 mg/kg) and smaller quantities of copper (6.26 mg/kg), zinc (13.44 mg/kg) and sodium (27.31 mg/kg) were also observed. After 72 h and 96 h fermentation, the potassium ion concentration had reduced to 1510.51 mg/kg and 1623.24 mg/kg, respectively. Also, fermentation for 72h and 96 h led to a drop in calcium ion concentration (mg/kg) from 2562.1 to 1885.8 and 1869.0, respectively. A similar trend was noted in all the other cases, with a higher concentration of metal ion observed before fermentation (0 h) than during or after fermentation. Ijarotimi and Keshinro (2012), while investigating the mineral composition of raw, germinated and fermented African locust bean flour, also observed that fermentation for one day led to phosphorus reduction, potassium, sodium, magnesium, zinc and copper content of the food. However, the authors noted a slight increase in the concentration of calcium, iron, and manganese after fermentation. Oluwaniyi and Bazaambo (2016) reported a reduction in iron, magnesium, zinc, sodium potassium and calcium composition of the African locust beans fermentation. A slight increase in manganese and Cu^{2+} ion concentration was observed.

Microorganisms use metals ions for numerous metabolic processes such as co-factors in enzyme function, in membrane transport, the formation of molecules and complexes, pH homeostasis and ATP synthesis (Bhunia et al*.,* 2012). Also, the growth of *Bacillus* species is driven by a sodium gradient, making sodium ions very vital for their growth (Bhunia et al*.,* 2012). The high mineral ion concentration of the food condiment is necessary in the human diet because these minerals serve as co-factors for enzymes that drive the numerous metabolic processes in the human system. Some others, such as calcium ions, serve as structural compounds for bone formation, while copper and ferric ions play a role in haemoglobin formation. Though a reduction in mineral nutrient was observed due to microbiological activities,

sufficient quantity remained to provide the trace quantities needed for body metabolism. An important factor to note here is that the microbial activities also increase the availability of the nutrients and metal ions in the fermented foods. This is made possible by producing microbial phytases that break down the phytic acids contained in legumes. Phytic acids are antinutrients that chelate metal ions preventing their absorption and leading to mineral deficiencies in the body.

Investigation of chemical changes in daddawa as a factor of fermentation time is very important for proper studies (Yakubu et al., 2022a). Based on the available literature, it is obvious that the duration of fermentation of the cooked dehulled seed varies from author to author. Our results show the daily changes, depicting the duration which permits the formation of the highest quality of daddawa in terms of amino acids, metal ions, and other volatile compounds. The amino acids, e.g. glutamate, peptides, fatty acids, and volatile aroma constituents, all contribute to the aroma of the fermented foods (Ohenhen et al*.,* 2008). The current findings imply that though fermentation triggers proteolysis and the release of free amino acid, excessive fermentation, in this case, up to 96 h, led to the deterioration in the nutritive quality of fermented daddawa.

Table 2. Changes in Mineral Composition during the Fermentation of Daddawa

Minerals (mg/kg)	Time (h)				
	0	24	48	72	96
Potassium	1939.69	2222.57	1741.39	1510.51	1623.24
Manganese	16.13	14.18	14.17	13.48	12.11
Copper	6.26	5.30	6.18	4.48	3.45
Calcium	2562.12	2497.08	2349.73	1885.75	1869
Zinc	13.89	12.31	11.83	13.44	12.25
Iron	94.66	73.55	40.09	67.82	58.76
Magnesium	347.70	337.40	326.14	339.71	326.72
Sodium	27.31	41.55	39.56	42.26	22.79

GC-MS Analysis of the fermented locust bean seed 'daddawa'

The GC-MS analysis of the cooked un-fermented and fermented locust bean seed (Table 3) showed that the highest amount of volatile compounds was present after 48 h, a slight drop was observed after 72 h and the least amount obtained after 96 h. The compounds found in the boiled unfermented bean seed were predominantly hydrocarbons comprising aliphatic and branched-chain alkanes and alkenes. Though pentadecane, 3-methyldecane, 4-methyldecane, and 2,6-trimethyldecane, 1-octadeciene, 1,2-diphenyl-1-isocyanoethane, and trans-1,2-diphenyl cyclobutane were present in the non-fermented boiled seeds, only 4-methyldecane was detected after 96 h fermentation. Pentadecane, 3-methyl decane, 7-ethyl-1,3,5-cycloheptatriene, 1-octadecyne were not present after 48 h fermentation, while 2,6 – dimethylundecane was not seen after 72 h fermentation. Hydrocarbons abound in nature, and it is not surprising that a large quantity is present considering that they are derived from lipids contained in high quantities in African locust bean seed. According to Zviely and Hong (Zviely and Hong, 2009), hydrocarbons serve as the main starter materials for developing important aroma compounds. 1-octadecyne is a component of the essential oil of plant extracts (Akhbari et al*.,* 2012). Phytol (diterpenoid alkene alcohol), n-hexadecanoic, linolenic acid, and sulfoxide were also present in the boiled unfermented seeds.

Table 3. Volatile compounds during the traditional fermentation of African locust bean seeds

S/No	*Compounds*	Area(%)			
		0 h	48 h	72 h	96 h
	Hydrocarbons (Alkanes/Alkenes)				
1	Undecane	9.40	9.58	10.283.	14.82
2	2,6-Dimethylundecane	2.36	4.06	1.91	-
3	1,2-Diphenyl-1-isocyanoethane	3.13	-	-	-
4	trans-1,2-Diphenylcyclobutane (styrene dimer,impurity)	3.68	-	-	-
5	Propylcyclohexane	16.56	-	16.94	
6	Cyclopentylcyclohexane	-	-	2.57	-
7	3-Ethyl-2-methylheptane	-	6.98	-	-
8	4-Methylnonane	-	11.18	-	
9	2,3,7-Trimethyloctane	3.90	4.83	-	6.46
10	Decane	10.61	6.39	6.77	22.57
11	4-Methyldecane	9.21	6.96	9.54	
12	3-Methyldecane	4.13	-	-	-
13	3,7-Dimethyldecane	1.62	2.68	1.28	1.93
14	2,9-Dimethyldecane	-	-	7.34	11.29
15	Tridecane	5.19	6.74	4.31	6.58
16	Tetradecane	-	0.70	4.19	
17	Hexadecane	4.33	7.08	0.89	1.61
18	2,6-Dimethylheptadecane	-	2.48	-	-
19	2-Methylheptadecane	-	-	15.08	-
20	Pentadecane	2.33	-	-	-
21	Nonane	-		9.66	14.12
22	2,6,10-Trimethylpentadecane	-	1.93	-	-
23	n-Dodecane	6.82	8.11	2.05	6.32
24	2,6,11-Trimethyldodecane (branched alkane)	2.26	2.92	3.28	3.45
25	1-Octadecyne	1.97	-	-	
26	2,6-Octadiene,2,4-dimethyl- (alkadienes)	-	0.97	-	-
27	Octacosane (acyclic alkanes)	-	0.63	-	-
	Sequisterpene				
28	cis-alpha-Bisabolene (sequisterpenes)	2.32	-	-	-
	Fatty acids				
29	n-Hexadecanoicacid /palmitic acid(saturated fatty acid)	1.34	1.24	0.48	-
30	Linolenicacid (unsaturated fatty acid)	1.70	-	-	-
	Alcohols				
31	Phytol (alcohol)	1.10	1.35	-	-
32	1-Heptadecanol (alcohol)	-	2.38	-	-
33	2-Pentadecyn-1-ol	-	-	-	11.16
	Other compounds				
34	7-Ethyl-1,3,5-cycloheptatriene	3.83	-		
35	(2,3-Diphenylcyclopropyl)methylphenylsulfoxide,trans-	2.22	-	-	-
36	cis-11-TetradecenylAcetate	-	4.32	-	-
37	Linoeolic acidchloride	-	2.06	-	-
38	2,2-Dicyclohexylmalononitrile	-	1.46	-	-
39	Oxalic acid, cyclohexyl dodecyl ester	-	1.46	-	-
40	trans-Decalin,2-methyl-	-	-	2.84	-
41	E-9-Tetradecenal (myristic aldehyde)	-	-	0.5	-

The phytol persisted after 48 h fermentation but not at 72 h fermentation. Bisabolene (2-methyl-6-(4-methylcyclohex-3-en-1-ylidene) hept-2-ene was present in non-fermented seeds but not in fermented seeds, while trans 2-methyl-decahydronaphthalene was not evident after 72 h fermentation. Bisabolene and decahydronaphthalene are sesquiterpenes, constituents of the essential oil of plants; its absence after 48 h fermentation and beyond implies microbial degradation or conversion of the compound to other related substances. Though often reported as pharmacological agents, sesquiterpenes are known flavour and aroma agents (Sharon-Asa et al*.,* 2003), used as natural flavouring agents in the food industries (Caputi and Aprea, 2011). The alpha-linolenic acid was only present in the unfermented seeds, while hexadecanoic acid was present in the properly fermented daddawa (after 72 h) fermentation. The rapid disappearance of linoleic acid can be explained by its polyunsaturated nature, making it a highly reactive compound. Compared to the unfermented oil bean seeds, compounds that developed after 48 h fermentation include hydrocarbons (tetradecane, 3-ethyl-2-methylheptane, 4-methylnonane, 2,6-dimethylheptadecane, 2,6,10-trimethylpentadecane, 2,6-octadiene, 2,4-dimethyl-, octacosane), alcohols (1-heptadecanol), ester (oxalic acid, cyclohexyl dodecyl ester), organic acid (cis-11-tetradecenyl acetate) and fatty acid chloride (lineolic acid chloride). Adebiyi et al. (2021) reported that the major compounds (with their peak area percentages) identified in dehulled *dawadawa* made from bambara groundnut were palmitic acid, ethyl ester (17.7%), lauric acid, ethyl ester (10.2%), and carbonic acid, 2-dimethylaminoethyl 2-methoxyethyl ester (7.3) while for undehulled *dawadawa*, it was indoline, 2-(hydroxydiphenylmethyl) (26.1%), benzoic acid, and 4-amino-4-hydroximino-2,2,6,6-tetramethyl-1-piperidinyl ester (8.2%).

The nitrogen-containing compounds, 1,2-diphenyl-1-isocyanoethane and 2,2-di-cyclohexyl malononitrile, were found in the boiled unfermented seeds and 48 h fermented seeds respectively. Their presence corresponds with the reports of the presence of cyanide containing and related toxic compounds in African oil bean seeds (Isichei and Achinewhu, 1988). Glucosinolates which are found as metabolic products in plants, are readily hydrolyzed by plant or microbial enzymes to give rise to isothiocyanates and the more stable nitriles (Kolodziejski et al*.,* 2019). This probably explains why the malononitrile compound was found in 48 h fermented daddawa but not after 72 h and 96 h fermentation.

Phenolic compounds such as trans-1,2-diphenyl cyclobutane were present in the unfermented African locust bean seeds but were not seen after 48 h fermentation. Trans 1,2-diphenyl cyclobutane is a stilbenoid belonging to a group of naturally occurring phenolic compounds found in various plant species. They are antimicrobial substances produced de novo in plants to help prevent/combat fungal infections and toxins (Akinwumi et al*.,* 2018). It appears these phenolic compounds were degraded during microbial fermentation. Many of the volatile compounds present during 48 h and 72 h fermentation were not present in the samples taken after 96 h of fermentation; except for the hydrocarbons, only 2-Pentadecyn-1-ol was present at this stage. Though pyrazine was the predominant compound found in soumbala produced from fermented African locust bean, it was not among the detected substances during this study. Jelen and co-authors (2013) investigating volatile compounds released during soybean fermentation observed that pyrazines were mainly formed due to the thermal reaction during frying.

CONCLUSION

The spontaneous fermentation of African locust bean seeds is facilitated by microbial synthesis of hydrolytic enzymes lipase, amylase, and proteases. The activities of these microorganisms lead to changes in the concentration of amino acids, fatty acids, and other flavour-enhancing and volatile compounds during fermentation. The pH of the fermenting legume and the concentrations of some essential amino acids including arginine, threonine, valine, methionine, histidine, isoleucine, leucine, lysine, phenylalanine, and tryptophan were increased. A higher metal ion concentration was observed before fermentation (0 h) than during or after fermentation of the locust bean seeds. Various hydrocarbons, fatty acids, esters and alcohols were observed at different stages of the fermentation. Our finding also confirm the need for process control and monitoring to ensure that good quality product is obtained, as variations in

plant type, microbial composition, process conditions, etc., all lead to differences in product quality.

Conflict of interest

Authors have no conflict of interest.

Author contributions

NTN and OCO designed the experiment. ZNE carried out the laboratory experiments and was assisted by NTN and OCO. NTN, OCO and ZNE analyzed the data. ZNE wrote the original draft of the manuscript while NTN, OCO and AOC participated in writing the manuscript and editing the final draft. All authors read and approved the final manuscript.

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