



Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (*Tamarindus indica* L.) seed in the production of daddawa-type condiment

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ABSTRACT

Tamarind seed is an underutilized legume which can potentially be used as a food condiment. In this study, tamarind seed was fermented using the conventional traditional method of *iru* (daddawa) production. Samples withdrawn at intervals of 24 h were tested for proximate composition, antinutrients, mineral profile, and other physicochemical properties using standard methods. Protein, fat and carbohydrates were the major nutrients in the tamarind seed before and after fermentation. Phosphorus was the major mineral element of the raw (0.97 mg/100 g) and fermented (0.96–1.06 mg/100 g) tamarind seed. Fermentation substantially decreased the phytic acid contents (approx. 50%), tannin contents (approx. 75%) and trypsin inhibitor activity (approx. 86%). *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus* were isolated as the organisms fermenting the tamarind seed. Fermented tamarind seed can be potentially used as a condiment for seasoning foods. However, future studies such as consumer acceptability are required to determine the acceptability of the fermented tamarind seed by potential consumers.

1. Introduction

Tamarind (*Tamarindus indica* L.) is an indigenous tropical African plant belonging to the dicotyledonous family Fabaceae (Hiwale, 2015). It is popularly known as tsamiya or okpa in Nigeria, tamarinde in South Africa and tamarind or chinchin in India. Tamarind is a multipurpose tree of which every part (root, body, fruit and leaves) finds some use, either nutritional, medicinal, or industrial. The seeds, often obtained as waste products from the industrial processing of the fruit, are used in the production of TKP (tamarind kernel powder) which is used as a thickener in textile sizing and printing (Hiwale, 2015). The tamarind seed is a good source of protein and contains high amounts of essential amino acids, relatively higher than soybeans and some other legumes. In addition, it is a good source of calcium, phosphorus, magnesium and potassium (Bagul, Sonawane, & Arya, 2015). The presence of tannins, phytic acid and other antinutrients in the seed makes it unsuitable for direct consumption. Hence, it is discarded as waste material. However, the antinutrients are considerably decreased after soaking and boiling

in water. Soaking and cooking legumes are effective in reducing or removing antinutrients (Fabbri & Crosby, 2016).

Fermentation of legumes has great potential as key protein and micronutrient source. It usually occurs in moist solid state and is by chance inoculation; involving members of the genus *Bacillus* and *Staphylococcus*. Fermentation leads to flavor enhancement, reduction of antinutrients and complex molecules (Olasupo, Okorie, & Oguntoyinbo, 2016). Improved protein digestibility and bioavailability of mineral elements such as calcium and phosphorus occur during fermentation of leguminous seed. Quite often, seeds that are used for fermentation are inedible in their raw, unfermented or uncooked state (Balogun, Oyeyiola, & Kolawole, 2014). In Nigeria, *iru* is a popularly produced fermented condiment obtained from the seed of *Parkia biglobosa*, it is usually used as flavoring agent in soups and vegetable dishes as it produces a strong pleasant aroma during cooking and serves as nutritious non-meat protein substitutes. Other leguminous seeds have been used to produce fermented condiments and they include *ogiri* (*Citrus vulgaris*), *ugba* (*Pentacletra macrophylla*), *ogiri-igbo* (*Ricinus*

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communis), *daddawa* (*Glycine max*), *owoh* (*Gossypium hirsutum*) and *ok-pehe* (*Prosopis Africana*) (Oguntoyinbo, 2014).

The protein and mineral content of the tamarind seed portends its adoption as an inexpensive alternative source to alleviate protein malnutrition and micronutrient deficiencies among traditional people living in developing countries. Previous research on tamarind seed and pulp found that the protein content of the seed (approx. 13%) is higher (7 times) than that in the pulp (Khairunnuur et al., 2009). The seed was reportedly high in calcium and potassium with relatively small quantities of phosphorus and magnesium (Khairunnuur et al., 2009). Other studies on tamarind seed focused primarily on roasting and germination of the seeds (Oluseyi & Temitayo, 2015). To the best of authors' knowledge, there is no report on the mineral profile, consumer acceptability and the isolation of bacteria involved in the fermentation of tamarind seed. This study therefore aims to produce a food condiment from tamarind seed using the natural fermentation method of producing *iru*, analyze the nutritional composition and identify the organisms involved in the fermentation process.

2. Materials and methods

2.1. Materials

Harvested and dried tamarind fruits were obtained from an open market, Bode in Ibadan, Nigeria, West Africa.

2.2. Preparation of condiment from tamarind

Fruits were sun dried for 4–5 h and cracked manually. Cleaned uninfected seeds were sorted, washed in potable water and drained, and then boiled in excess potable water for 10–12 h. The seeds were cooled by washing in 2–3 rinses of potable water and then manually dehulled by pressing between the fingers to obtain whole cotyledons which were further cooked for 1 h in distilled water, drained and allowed to cool in air. Cooked (Fig. 1) cotyledons were placed in calabash lined with banana leaves previously washed in distilled water then covered with more banana leaves and left to ferment over a period of 72 h. The fermented mash was dark brown in appearance with creamy mucilaginous slime molding the fermented cotyledons together (Fig. 1). A strong ammonia-like aroma characteristic of legume-based fermented condiment was produced from the mash.

2.3. Microbiological analysis

Stock solution was made from 1 g of fermenting seed and serially diluted. One (1) ml of solution (10^{-6}) was plated on quadruplicate plates of Nutrient Agar, Plate Count Agar, MacConkey Agar; and incubated anaerobically for 24 h at 37 °C using an incubator (Gallencamp/Sanyo, UK). Pure cultures of the bacterial isolates obtained from repeated sub-culturing were streaked for identification on Nutrient Agar.

2.3.1. Biochemical identification of isolates

Representative colonies from the isolation medium were inoculated on Nutrient Agar slants and subjected to Gram's staining. Catalase, sugar fermentation, citrate utilization, starch hydrolysis and gelatin liquefaction tests were carried out on Gram positive rods according to standard methods (Cruickshank, Duguid, Marmion, & Swain, 1975; Harrigan & McCance, 1976).

2.3.2. Characterization of isolates

Taxonomic studies were carried out on the purified isolates on the basis of their cultural, morphological, biochemical and physiological characteristics, identification was carried out using Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974, pp. 529–545).

2.4. Analyses

Samples withdrawn at intervals of 24 h were oven dried at 70–80 °C for 20 h, ground to powder using mortar and pestle and stored at 4 °C until analysis.

2.4.1. pH

Five (5) g of the sample was mixed uniformly with 50 ml distilled water in a beaker and acidity measured using Jenway 3505 pH meter. pH meter was initially calibrated using buffers 4 and 7.

2.4.2. Titratable acidity

Two (2) g of ground sample was mixed with 20 ml distilled water in a 250 ml beaker. The mixture was filtered on Whatman filter paper (no 1) and filtrate collected in a conical flask. Two (2) drops of 1% phenolphthalein indicator was added to 10 ml of the filtrate and titrated against 0.1 N NaOH solution to pH 8.1. The average titre value was recorded and the titratable acidity calculated as the percentage of



Fig. 1. Raw and processed tamarind fruit and seed.

A: Tamarind fruit; B: Tamarind seed; C: Dehulled cooked seed; D: Fermented seed.

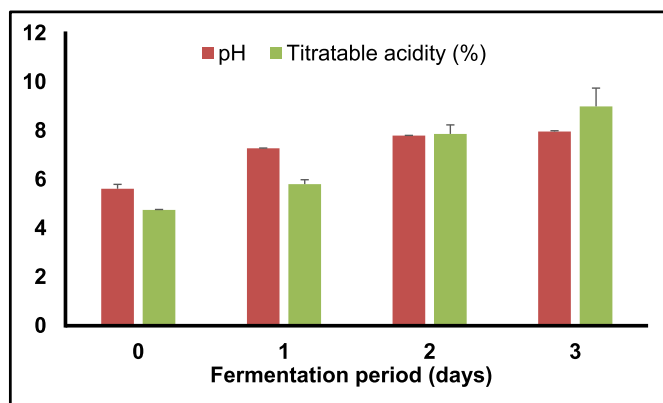


Fig. 2. pH and TTA of fermented tamarind seed. Error bars indicate standard deviation (N = 3).

tartaric acid in the sample (AOAC, 2005).

2.4.3. Tannin content

Samples were extracted with 1% HCL in methanol for 20 min at 30 °C and centrifuged. Assays were performed on supernatants at 30 °C. Vanillin reagent (5 ml) was added to 1 ml of the extract, the reaction mixture left for 20 min at 30 °C and the absorbance read at 500 nm against a blank (1% HCL in methanol) using a UV spectrophotometer (Jenway, 7305 Bibby Scientific UK). Catechin standard curve from 0.00 to 0.10 mg/ml was used in calculating tannin levels (Price, Scoyoc, & Butler, 1978).

2.4.4. Phytic acid content

Phytate contents of the samples were determined as previously reported (Young, 1936). Briefly, 200 ml of 2% HCL was added to 8 g of finely ground sample. After vortexing for 3 h, it was filtered and 10 ml of 0.3% NH_4SCN was added to 50 ml of filtrate. Thereafter, distilled water (107 ml) was added to give proper acidity. The solution was titrated against 0.00195 g/ml ferric chloride solution to give a persistent brownish yellow coloration and the phytic acid content estimated.

2.4.5. Trypsin inhibitor activity

Inhibitor extract (1 ml) was pipetted into triplicate set of tubes and 1 ml of trypsin solution was added to each tube. The tubes were placed in the water bath at 37 °C. Casein solution (2 ml), previously warmed at 37 °C was added to each tube. The tubes were held at this temperature for 20 min. The reaction was halted by adding 6 ml of 5% trichloroacetic acid to the tubes. The suspension was allowed to stand for 1 h at room temperature, filtered and the absorbance of the filtrate read at 280 nm using a UV spectrophotometer (Jenway, 7305 Bibby Scientific UK). The blank contained 6 ml of 5% TCA and 4 ml buffer (Kakade, Rackis, McGhee, & Puski, 1974).

2.4.6. Proximate composition

Proximate analysis was carried out on samples withdrawn at 0, 24, 48 and 72 h of fermentation using standard methods. Moisture content was determined by drying initially for 20 h at 70 °C and then at 105 °C for 2–3 h, total fat was determined by extracting with petroleum ether, ash content was determined after heating in a muffle furnace at 600 °C and crude fibre content was determined by heating a mixture of 1 g of the defatted sample and 100 ml of digestion reagent under reflux for 45 min at 60–70 °C. The crude protein ($\text{N} \times 6.25$) was determined by the micro-Kjeldahl method (AOAC, 2005). Carbohydrate content was calculated by difference.

2.4.7. Mineral contents

The contents of mineral elements was determined according to official method of AOAC (2005). Each sample was placed in weighed

Table 1

Effect of fermentation on antinutrient contents of tamarind seed.

Fermentation period (days)	Phytate (mg/100 g)	Trypsin inhibitor activity (TIU/g)	Tannin (mg/100 g)
0	4.17 ^a ± 0.04	3.50 ^a ± 0.01	4.02 ^a ± 1.41
1	3.93 ^b ± 0.03	2.10 ^b ± 0.10	3.01 ^b ± 1.41
2	3.64 ^c ± 0.13	1.20 ^c ± 0.01	2.00 ^c ± 0.02
3	2.12 ^d ± 0.10	0.50 ^d ± 0.01	1.02 ^d ± 0.01

Mean ± SD. Mean with different superscript along the row are significantly different ($p \leq .05$).

porcelain crucible and heated, first in a stove and then in a furnace at 600 °C for 5 h. The resulting white ash was weighed and refluxed with 20% hydrochloric acid. This was filtered and the filtrate diluted with de-ionized water in a 100 ml calibrated flask to the mark. Sodium and potassium levels were determined using a flame emission photometer with NaCl and KCl as standards. All other metals were determined by atomic absorption spectrometry.

2.5. Statistical analysis

All analyses were performed in triplicate. Data was analysed using analysis of variance (ANOVA) and means were compared using the Fisher Least Significant Difference (LSD) test ($p < .05$).

3. Results and discussion

3.1. Effect of fermentation on pH and titratable acidity

The pH and titratable acidity (TTA) values of the fermenting seeds increased significantly ($p \leq .05$) with increase in fermentation period (Fig. 2). Increase in pH could be attributed to ammonia production by protease deaminase enzyme produced by *Bacillus* spp. Protein of the raw materials in alkaline fermented foods is broken down into amino acids and peptides. Ammonia is released during the fermentation, raising the pH of the final products and giving the food a strong ammoniacal or pungent smell, which tends to mellow during cooking applications (Ogunshe, Jayeola, & Ogundimu, 2008). Previous researchers similarly reported increase in pH and TTA during fermentation of soybeans and tamarind seed (Dakwa, Sakyi-Dawson, Diako, Annan, & Amoah-Awua, 2005; Oluseyi & Temitayo, 2015). However, the reason for the increase in TTA values with a corresponding increase in pH remains unclear. This is because pH and TTA are most times inversely related. According to previous research, fermentation of protein-rich seeds such as tamarind seed resulted in proteolytic degradation of proteins into amino acids and amylolytic breakdown of carbohydrate into sugars and organic acids (Oluseyi & Temitayo, 2015). These changes, possibly occur simultaneously and may explain the increase in both pH and TTA values of the tamarind seed during fermentation.

3.2. Effect of fermentation on antinutrients

Three antinutrients including phytate, trypsin inhibitor and tannin commonly associated with leguminous seeds were determined in the fermented tamarind seed. Fermentation significantly ($p \leq .05$) decreased the concentration of these antinutrients (Table 1). Phytate contents of the tamarind seed decreased to approximately 50%, while higher reductions were observed in tannins (approx. 75%) and trypsin inhibitor (approx. 86%) at the 3rd day of fermentation. Oluseyi and Temitayo (2015) reported 12.9% reduction in phytate and 57.1% reduction in tannin after 72 h of fermentation of tamarind seed. The significant reduction in the anti-nutrients of the fermented tamarind seed may be attributed to various enzymatic activity. For instance, the

Table 2
Effect of fermentation on proximate composition of tamarind seed (g/100 g)*.

Fermentation period (days)	Moisture	Protein	Fat	Ash	Fibre	Carbohydrate
0	9.66 ^{bc} ± 0.15	48.07 ^b ± 0.32	19.00 ^d ± 0.03	3.60 ^c ± 0.16	5.69 ^d ± 0.01	23.62 ^a ± 0.01
1	9.70 ^c ± 0.10	48.67 ^{ab} ± 0.45	21.16 ^b ± 0.02	3.42 ^d ± 0.05	5.93 ^b ± 0.01	20.81 ^c ± 0.02
2	10.79 ^a ± 0.15	49.48 ^a ± 0.38	19.57 ^c ± 0.02	3.96 ^b ± 0.05	5.83 ^c ± 0.01	21.17 ^b ± 0.02
3	10.74 ^a ± 0.15	48.55 ^b ± 0.29	25.49 ^a ± 0.02	4.24 ^a ± 0.05	8.02 ^a ± 0.01	13.69 ^d ± 0.01

Mean ± SD. Mean with different superscript along the row are significantly different ($p \leq .05$).

*Data are reported on dry weight basis.

Table 3
Effect of fermentation on mineral contents of tamarind seed.

Fermentation period (days)	*Ca	*Mg	*K	*Na	**K/Ca + Mg	**P	**Zn	**Cu	**Mn	**Fe
0	0.43 ^c ± 0.01	0.39 ^c ± 0.01	0.12 ^b ± 0.01	0.02 ^b ± 0.01	0.06 ^b ± 0.01	0.97 ^c ± 0.01	88 ^b ± 0.01	20 ^b ± 0.01	50 ^c ± 0.01	33 ^c ± 0.01
1	0.43 ^c ± 0.01	0.39 ^c ± 0.01	0.11 ^b ± 0.01	0.02 ^b ± 0.01	0.05 ^b ± 0.01	0.96 ^c ± 0.01	87 ^b ± 0.01	21 ^b ± 0.01	52 ^b ± 0.01	37 ^c ± 0.01
2	0.46 ^b ± 0.01	0.42 ^b ± 0.01	0.11 ^b ± 0.01	0.02 ^b ± 0.01	0.05 ^b ± 0.01	1.03 ^b ± 0.01	95 ^a ± 0.01	24 ^a ± 0.01	58 ^a ± 0.01	40 ^b ± 0.01
3	0.48 ^a ± 0.01	0.43 ^b ± 0.01	0.11 ^b ± 0.01	0.02 ^b ± 0.01	0.05 ^b ± 0.01	1.06 ^a ± 0.01	95 ^a ± 0.01	24 ^a ± 0.01	58 ^a ± 0.01	44 ^a ± 0.01

Mean ± SD. Mean with different superscript along the row are significantly different ($p \leq .05$).

*mg/100 g; **mg/Kg.

Table 4
Microscopic, biochemical and morphological characteristics of *Bacillus* isolates from fermenting seed of tamarind.

Isolate	Grams' status	Catalase test	Citrate utilization	Starch hydrolysis	Gelatin liquefaction	Glucose	Xylose	Colonial morphology	Probable identity
A	+ rod	+	+	–	+	+	+	Dull surface, thick, opaque	<i>Bacillus licheniformis</i>
B	+ rod	+	+	–	–	+	–	Smooth, low convex	<i>Bacillus pumilus</i>
C	+ rod	+	+	–	+	+	–	Smooth, slightly yellow, low convex	<i>Bacillus pumilus</i>
D	+ rod	+	+	–	+	+	–	Smooth, low convex	<i>Bacillus pumilus</i>
E	+ rod	+	+	+	+	+	+	Dull surface, thick, opaque	<i>Bacillus subtilis</i>
F	+ rod	+	+	+	–	+	+	Dull surface, thick, firmly attached	<i>Bacillus licheniformis</i>
G	+ rod	+	+	+	+	+	+	Opaque, dull, flat	<i>Bacillus subtilis</i>
H	+ rod	+	+	–	+	+	+	Opaque, flat, dull surface	<i>Bacillus licheniformis</i>
I	+ rod	+	+	+	+	+	+	Round, dull surface, thick	<i>Bacillus subtilis</i>
J	+ rod	+	+	+	+	+	+	Opaque, dull, firmly attached	<i>Bacillus licheniformis</i>
K	+ rod	+	+	–	–	+	+	Dull, thick, opaque, firmly attached	<i>Bacillus licheniformis</i>
L	+ rod	+	+	–	–	+	–	Smooth, translucent, slightly yellow	<i>Bacillus pumilus</i>

enzymes tannase and phytase are well known to breakdown tannins and phytic acid respectively during fermentation (Omer, Mohamed, Ahmed, Yagoub, & Babiker, 2016). Antinutrients are known to adversely affect the bioavailability and utilization of nutrients, resulting in neurological disorders and even death. Phytates can bind some essential minerals, for example phosphorus in the digestive tract and lead to mineral deficiencies (Soetan, Akinrinde, & Adisa, 2014). Hence, the significant reduction in the anti-nutrients of the fermented tamarind seed will enhance its safety for consumption.

3.3. Effect of fermentation on proximate composition

Protein, followed by fat and carbohydrate were the major components in the unfermented and fermented tamarind seed (Table 2). The protein content of fermented tamarind seed in this study is higher than the value (32.40%) reported in fermented seed of *Parkia biglobosa* (Ogunyinka, Oyinloye, Osunsanmi, Kappo, & Opoku, 2017) but lower than the value (61.80%) reported for fermented tamarind seed flour (Oluseyi & Temitayo, 2015). Fermented tamarind seeds had slightly higher protein content (approx. 50 g/100 g) compared to the unfermented seeds (approx. 48 g/100 g). The apparent increase in protein content of the tamarind seed after fermentation may be attributed to synthesis of enzymes and a degradation of the substrate during fermentation. Furthermore, the slight increase in protein content may also

be due to a decrease of carbohydrate ratio in the total mass, resulting in redistribution of nutrient percentages (Onyango, Noetzold, Bley, & Henle, 2004). Previous research found that *Bacillus* spp. produce several enzymes including amylase, fructofuranosidase, glucosidase and galactanase which degrade carbohydrates into simple sugars used as energy source by the microorganisms (Boateng, Okai, Frimpong, & Asabere, 2014). The moisture (8.81–9.74 g/100 g), ash (3.13–3.83 g/100 g) and fibre (5.19–7.24 g/100 g) contents were generally low (Table 2). These minor components of the tamarind seed significantly ($p \leq .05$) increased after fermentation. An increase in moisture content during fermentation has been reported in partially and completely fermented seeds of *Parkia biglobosa* (Oluwaniyi & Bazambo, 2016). The moisture content reported in the raw and fermented tamarind seed is slightly higher than the values reported in other leguminous seeds (Aremu, Awala, Opaluwa, Odoh, & Bamidele, 2015; Ogunyinka et al., 2017).

3.4. Effect of fermentation on mineral profile

The mineral contents of the fermented tamarind seed are presented in Table 3. Unfermented and fermented tamarind seed are good sources of phosphorus, calcium and magnesium. These three minerals were the most abundant elements in the tamarind seed. High calcium content is desirable for the prevention of bone diseases and maintaining the

biological role of nerve transmission, muscle contraction, glandular secretion as well as mediating vascular contraction and vasodilation (Itankar, Tauqeer, & Chunoti, 2016). With the exception of potassium and sodium, the mineral contents of tamarind seed increased significantly ($p \leq .05$) after fermentation (Table 3). Eka (1980) also reported increase in the mineral content of fermented locust beans. The iron content of the tamarind seed increased by approximately 33% after the 3rd day of fermentation. Previous research found that fermentation increased the availability of iron and zinc (Greffeuille et al., 2011). However, it is also possible that the increase in iron may be due to the mineral content of the water used for fermentation procedure.

3.5. Isolation and characterization

Gram staining tests, starch hydrolysis, citrate utilization, gelatin liquefaction and glucose fermentation conducted on the bacteria isolates showed their probable identities as *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis* and *Staphylococcus* spp (Table 4). The fermenting organisms found in fermented tamarind seed are similar to those reported for fermented condiments such as African locust bean (Achi, 2005; Boateng et al., 2014).

4. Conclusions

Fermentation is a promising processing method to increase the utilization of tamarind seed. Anti-nutrients decreased after fermentation, which made more minerals such as phosphorus and iron more available. Fermentation did not significantly change the protein content of the tamarind seed. Fermented tamarind seed contain appreciable amounts of phosphorus, calcium and magnesium. *Bacillus licheniformis*, *Bacillus pumilus* and *Bacillus subtilis* were the major fermenting microorganisms. These organisms are safe and have been associated with traditional leguminous condiments. Salting is suggested to improve taste. Tamarind seed condiment, like *iru*, can be used as meat substitutes and flavorings in soups and vegetable dishes.

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