# **Physico-Chemical and Microbiological Properties of Tamarind Seed Flour and Protein Isolate**

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#### *Abstract*

*The physicochemical, functional and microbiological properties of Tamarind full fat flour, defatted flour and protein isolates were evaluated. Tamarind seeds were fermented for four days and samples taken out daily for sampling. The samples were analyzed for proximate composition, pH, titratable acidity, and for their microbial loads. Moisture content, crude protein, fat, ash, pH, increased significantly (p<0.05) as fermentation progressed. Protein, fat, ash and moisture content ranged from 18.54 to 20.89%, 6.07 to 7.17%, 2.44 to 2.94% and 24.38 to 25.90% respectively. After fermentation, the fermented seeds were dried, milled and processed into flour. The flour was defatted and protein isolate was extracted from the defatted flour using isoelectric precipitation. The full fat flour, defatted flour and protein isolate were analyzed for proximate, functional and microbial analysis. Results for proximate analysis were as follows, 5.46, 7.46, 27.18, 3.77, 8.54, and 39.43% for full fat flour; 6.96, 0.31, 29.58, 4.62, 8.86 and 42.71% for defatted flour and 5.56, 0.14, 82.78, 0.87, 0.93 and 9.54% for protein isolates for moisture, fat, protein, ash, fibre and carbohydrate respectively. Defatting of the flour showed significant (p<0.05) influence on the chemical properties of tamarind flour. Protein precipitation had an influence on the protein content(82.78%).The protein isolates exhibited high values of water absorption capacity (475%), oil absorption capacity (230%), high foam capacity (41%) and stability (36%), good protein solubility capacity (80.72%). Knowledge of the chemical and functional properties of Tamarind seed flour and protein would enhance their utilization in various food formulations, thereby contributing to food security in Africa.* 

*Key words: Tamarind seed flour; Protein isolates; Chemical/functional property; Isoelectric precipitation; Fermentation.*

#### **Introduction**

With increasing population, lack of adequate technology, scarcity and high cost of animal protein, food insecurity remains a major threat in developing countries. Identification and utilization of neglected indigenous protein-rich plant materials could be a practical solution to combat this challenge (Falade and Akeem, 2020). Tamarind (*Tamarindus indica* L.) is a multipurpose tropical fruit tree used primarily for its fruits. The fruits are eaten fresh or processed into juices and drinks (El-Siddig *et al.,* 2006). The fruit has a fleshy, juicy and acidulous pulp. It is best described as sweet and sour in taste, and is high in acid, sugar, B vitamins and calcium. The common names for tamarind in Nigeria include tsamiya (Hausa), jetami (Fulani), ajagbon (Yoruba), and Ichekunoyibo (Igbo) (Keay *et al, .*1989). Tamarind thrives in practically all soil types and could be found virtually everywhere growing wild. Numerous national programs have recognized tamarind as an under-utilized crop with wider potential (Sammantray *et al.,* 2013).

Tamarind seed is an abundant and cheaply available by-product of tamarind pulp industry which is often wasted after the extraction of the pulp; over 90 percent of the tamarind seeds are reported as waste annually, while less than 10 percent is being utilized by the non-food industry. Tamarind seed can be valuable for use as a protein supplement in food due to its high protein content (El-Siddig *et al*., 2006). Tamarind seeds are rich in some essential amino acids, essential fatty acids, minerals especially calcium, phosphorus and potassium. The seed protein has a favorable amino acid balance; hence it could be used to supplement legumes with lower methionine and cystine contents. Many of these desirable attributes qualify tamarind seeds as suitable material for protein isolation and an adoption as an inexpensive alternative protein source to alleviate protein malnutrition. However the successful application of tamarind flours and proteins in food formulations depends on their intrinsic properties such as chemical and functional properties.

Legume protein production and utilization have attracted the attention of most food industries both in developing and developed countries owing to their functionality, nutritional enrichment potentials and usefulness as functional food ingredients. Plant materials whose proteins have been isolated and characterized include bambara, peanut, moringa seeds, among others (Falade and Akeem, 2020). Out of the various documented isolation techniques, isoelectric precipitation (alkaline extraction) appears to be the most commonly applied method (Falade and Akeem, 2020). Protein isolation through isoelectric precipitation is based on the ability of proteins to solubilise at high pH (between pH 8 and 11) and precipitate at low pH values (between Ph 4-5) usually referred to as the isoelectric point of such proteins. The aim of this study was to determine and investigate the influence of defatting and protein precipitation on chemical and functional properties of tamarind flour and protein isolate.

# **Materials and methods**

#### **Materials**

Tamarind pods (*Tamarindusindica* L*.*) were purchased from Baga Road Market, Maiduguri, Borno State of Nigeria.

# **Methods**

**Tamarind seed processing**: The preparation of tamarind seed flour was carried out by modifying the method of Odunfa and Oyewole (1988) for fermentation of locust bean. The tamarind pods were manually de-podded by carefully opening the pods to obtain the seeds. Whole un-infested seeds were sorted out for use. Cleaned tamarind seeds were boiled in water for about two hours. After boiling, the water was carefully discarded and the seeds were soaked in distilled water overnight. The seed coat was removed manually by pressing between fingers to obtain the cotyledons. The cotyledons were tightly wrapped with banana leaves (*Musa sapientium*) in different packets and the packets were placed in calabash trays and covered with jute bags and allowed to ferment for four days. Samples were removed daily for analysis. The samples were oven dried at  $60^{\circ}$ C and then milled into flour using an attrition mill. The milled flour was sifted through a 1 mm mesh size sieve and packaged in an airtight container and stored at room temperature until analyzed..

**Extraction of protein isolate:** Modified alkaline extraction method as described by El-Adawy *et al*, (2001) and Adebowale and Lawal (2002) were used for the extraction of protein isolate. One (1.0) kg of flour was suspended in 10 L distilled water containing 0.25% Na<sub>2</sub>SO<sub>3</sub>, and the pH was adjusted to 9.0 using 1N NaOH. The suspension was stirred for about 1 hour at room temperature, and then centrifuged at 3000rpm for 30minutes. In order to obtain higher yields, the extraction and centrifugation was repeated on the residue obtained. The extracts were combined and acidified to pH 4.5. The precipitate recovered by centrifugation at 3000rpm for 30minutes was neutralized using 1N NaOH to pH 7 and washed with distilled water several times. The neutralized precipitate was freeze-dried, milled and sieved through 60 mesh.

#### **Proximate analysis:**

The samples were evaluated for their moisture content, ash, fat, fiber, protein and carbohydrate content using the method as described by AOAC (2010).

### **Chemical properties:**

**pH**: The pH of the fermenting slurry was measured in a 10 % (w/v) dispersion of the sample in distilled water. Each suspension was mixed thoroughly. A standard pH meter (Hanna meter model (H196107) was used for pH determination. The pH electrode was dipped into the solution and after a few minutes of equilibration, the pH of the samples was taken.

# **Functional properties**

**Bulk density**: This was determined by the method of Nyarayana and Narasinga-Rao (1984). Each sample (50 g) was filled into a graduated cylinder and their weight noted. The cylinder was tapped continuously until there was no further change in volume. The weight and final volume of the flour in the cylinder were noted and the difference in weight and volume determined. The bulk density was computed as grams per milliliter (g/mL) of the sample.

**Water and oil absorption capacities (WAC/OAC):** Water absorption capacity (WAC) and oil adsorption capacity (OAC) of tamarind full fat flour, defatted flour and protein isolate were determined following the method of Adebowale *et al*, (2005). One gram of sample was mixed with 10 mL of distilled water or refined vegetable oil (specific gravity 0.9047). The mixture was allowed to stand at  $30 \pm 2$ °C for 30 minutes and centrifuged (SorvallRC-6; Kendro laboratory products, Newtown, CT, USA) at 2000g for 30 minutes. Water absorption capacity and oil absorption capacity was expressed as grams of water or Oil bound per gram of dry isolate.

**Protein solubility:** Protein solubility of tamarind protein isolates was evaluated according to Bera and Mukherjee (1989) with slight modification. A 200 mg of protein isolate was suspended in 20 ml of distilled water followed by stirring and adjustment of pH with 0.1 N HCl or 0.1 N NaOH to 7.0. The mixture was stirred with a magnetic stirrer at  $27\pm2^{\circ}$  C for 1 h during which the pH was monitored and maintained. Protein solubility was expressed as the percentage ratio of protein content in the mixture prior to centrifugation and in the supernatant after centrifugation at 4800x g for 30 min as examined by Kjeldahl method (AOAC, 2005).

**Foaming capacity and stability:** Foaming capacity and foam stability were determined by the modified method of Lin *et al.* (1974). The isolate sample (3%w/w dispersion in distilled water) was homogenized in a homogenizer at a setting of 6 (approximately 10,000 rpm) for 5 minutes. The mixture was immediately transferred into a 500mL graduated cylinder and the foam volume measured. The foaming capacity was expressed as the percentage of foam volume at 0 mins. Foam stability was expressed as foam volume (%) remaining after 20, 40, 60, and 120 minutes.

**Emulsion capacity:** The method described by Aluko and Yada (1995) was adopted. One gram (1g) of sample was mixed with 10ml of distilled water in a test tube and shaken for 30seconds. Ten ml of refined oil was also added and shaken continuously until properly mixed. The test tube was left to stand for 30minutes. The height of oil separated from the sample was measured. The emulsion capacity is expressed as the amount of oil emulsified and held per gram of sample. It was calculated as shown below;

emulsion heightemulsion height 100100 Emulsion capacity = % crude fat = water height water height  $X$  1 1 VI-V2VI-V2  $\mathbf v$  $\overline{\mathbf{V}}$ OR Where:  $V_1$  = Total volume of oil added

 $V_2$  = Volume of oil left after formation of emulsion

# **Microbial analysis**

**Determination of mould count:** This was done using the method described by Prescott *et al.* (2015)**.** Samples were serially diluted in duplicates using surface pour method. One millilitre (1ml) of each dilution was pipette into duplicate Petri dish pouring 15 ml of SDA (Sabauroud Dextrose Agar), tempered to  $45^{\circ}$ C into each dish. It was further mixed thoroughly and spread by swirling the plate. It was allowed to solidify. The Petri dish was incubated upside down for 48 hours at room temperature. The mould count was expressed as cfu/g of sample. Mould count  $(cfu/g) = no of colonies x reciprocal of dilution.$ 

# **Determination of total viable count (TVC)**

The total viable count test was carried out using the method described by Prescott *et al.* (2015). One ringer table was dissolved in distilled water (500 ml). The clear solution formed was sterilized by autoclaving for 15 minutes at  $121^{\circ}$ C and 15 lb pressure. The ringer solution was allowed to cool completely for a temperature of  $28 \pm 2$ °C. Using a sample and sterilized quarter strength ringer solution as diluents, 1 ml of the sample and 9 ml ringer solution was made serial dilution  $(10^{-3})$ . The diluted sample was pipetted into a marked Petri dish, swirling to mix and incubated at the temperature of about  $37^{\circ}$ C for 24 hours. After incubation, the number of colonies was counted and represented as a colony forming unit per grammes.

#### **Experimental design and data analysis**

All experiments were conducted in a completely randomized design (CRD). Data generated was subjected to the analysis of variance (ANOVA) at 0.05 probability level. Duncan multiple range tests were used to compare means using the statistical package for social sciences (SPSS) version 20.0 and significance was accepted at p≤0.05.





Values are mean  $\pm$  standard deviation of duplicate readings. Means with the same superscripts are not significantly (p>0.05) different.

Table 1 shows the effect of fermentation period on the proximate composition (%) of Tamarind seed.

Moisture content: Moisture content of the fermenting slurry ranged from 24.38 to 25.90% with zero day having the lowest moisture content (24.38%) and the fourth day of fermentation having the highest moisture content (25.9%). The moisture contents of the fermented tamarind seed increased as the fermentation period increased. The increase in moisture content as fermentation progressed may be attributed to the activity of the fermenting organisms on the substrate.

The protein content ranged from 18.54 to 25.89%. Protein content increased significantly with increase in fermentation time. Giami (2004) reported that protein fractions (albumin and globulin) increase during fermentation. This increase in protein value with fermentation time could be attributed to net synthesis of protein by fermenting seeds which might have resulted in the production of some amino acids during protein synthesis (Uwagbute *et al.,* 2000). The increased protein content of the fermented seeds may also be due to synthesis of enzymes or a compositional change following the degradation of other constituents. Protein content of oil beans has been reported to increase during fermentation (Olanipekun, 2004).

The fat content of the tamarind seed ranged from 6.07 (zero fermentation) to 10.17% (day four of fermentation). Fermentation increased the fat content with increase in period of fermentation. The increase may be attributed to the increased activity of lipolytic enzymes which produced more fatty acids during fermentation. .

As fermentation progressed, carbohydrate content reduced significantly. The carbohydrate ranged from 39.47 to 27.05%. The reduction in total carbohydrate content of fermented samples could be attributed to the ability of the fermenting microflora to hydrolyze and metabolize them as carbon source (substrate) to synthesize cell biomass (Madigan *et al.,* 2002). Previous research found that *Bacillus spp*. produce several enzymes including amylase, glucosidase and galactanse which degrade carbohydrates into simple sugars used as energy source by the microorganisms (Boateng *et al.,* 2014).





Values are mean  $\pm$  standard deviation of duplicate readings. Means with the same superscripts are not significantly (p>0.05) different.

Effects of fermentation on the pH and titratable acidity of tamarind seed is shown in Table 2. The pH and titratable acidity (TTA) values of the fermenting seeds increased significantly  $(p<0.05)$  with increase in fermentation period as shown in Table 2. The pH and TTA values of the raw seeds were  $5.2 \pm 0.01$  and  $0.2 \pm 0.00$  respectively. It was observed that fermentation increased the pH and TTA values of tamarind seeds. The highest increase was observed at the end of fermentation. The observed pH trend is in accordance with the reports of Omafuvbe et al. (2000) during the fermentation of soybeans. Increase in pH during fermentation has been attributed to proteolytic activities and the release of ammonia by microorganisms involved in the fermentation (Surkar and Deshande, 1993). The concurrent increase in pH and TTA is an indication that both acid and alkaline producing activities were at work during fermentation. This may be due to the fact that while proteolytic activities were taking place on the protein components of the seeds, the carbohydrate components were also being hydrolyzed to simple sugars and organic acids (Surkar and Deshande, 1993).

Dakwa et al. (2005) and Oluseyi and Temitayo (2015) similarly reported an in pH and TTA values during fermentation of soybeans and tamarind seed respectively.





Values are means  $\pm$  standard deviation of 2 determinations. Means within a column with different superscript are significantly (p<0.05) different KEYS: Sample 1: Tamarind seed fermented on the 1st day, Sample 2: second day of fermentation, Sample 3: third day of fermentation, Sample 4: fourth day of fermentation, TVC: total viable count, cfu: colony forming unit.

Table 3 shows the Total viable count and mould count of tamarind seed fermenting slurry.

The total viable count ranged from 1.7x  $10^4$  to 2.4 x  $10^4$ . It was observed that the fourth day of fermentation had the highest value 2.4 x  $10^4$  cfu/g. As fermentation progressed, the TVC increased, this may be attributed to the consumption of the substrate by the microorganism which led to an increase in their growth. Mould count ranged from 2.0 x  $10<sup>1</sup>$  to 1.0 x  $10<sup>1</sup>$ . Mould was detected on the first and second day of fermentation. There was no growth on the third day and fourth day.

<b>Sample</b>	<b>Moisture</b>	Fat	<b>Protein</b>	Ash	<b>Fibre</b>	Carbohyd
						rate
Full	fat $5.46^{\circ} \pm 0.30$	7.46 <sup>a</sup> ±0.15 27.18 <sup>c</sup> ±1.3 3.77 <sup>b</sup> ±0.35 8.54 <sup>b</sup> ±0.12				$39.43^{b}+1.0$
flour						
Defatted	$6.96^a \pm 0.23$	$0.31^b \pm 0.84$ $29.58^b \pm 0.6$ $4.62^a \pm 0.13$ $8.86^a \pm 0.01$				$42.71^a \pm 0.5$
flour			h			6
Protein	$5.56^{\circ} \pm 0.53$ $0.14^{\circ} \pm 0.04$		$82.78^{\circ}$ ± 2.0	$0.87^{\circ}$ ± 0.12 $0.93^{\circ}$ ± 0.10		$9.54^{\circ}$ ± 1.88
isolate						

**Table 4: Proximate composition (%) of Tamarind full fat flour, defatted flour and protein isolate**

Values are means ±standard deviation of triplicate determination. Values bearing different superscript in the same column are significantly different  $(p<0.05)$ 

The proximate composition (%) of tamarind full fat flour, defatted flour and protein isolate is shown in Table 4.

It was observed that, defatting evidently showed significant  $(p<0.05)$  influence on the chemical properties of tamarind flour.

Moisture content ranged from 5.46% to 6.96%. The defatted flour had the highest moisture content (6.96%). Drying methods, duration and conditions are important factors that could influence moisture contents of food products. The drying method (air drying) used for the defatted flour after defatting could be attributed to the increase in moisture content.

Tamarind Full fat flour (8.54%) and defatted flour (8.86%) showed higher fibre content than the protein isolates (Table 4). The defatting process significantly  $(p<0.05)$  increased the crude fibre of tamarind flour. This increment could be attributed to the concentration of fibre in the defatted flour after fat removal. Fibre content of full fat flour and defatted flour of tamarind were observed to be particularly higher than those reported for whole and defatted cowpea flours and bambara groundnut flours (Adebowale *et al.,* 2011). Hence, tamarind flours are rich in fibre and could be explored as functional ingredients in the formulation of high fibre foods.

The defatting process increased the protein content of tamarind flour from 27.18 to 29.58%; this could be attributed to the concentration of protein after defatting. Comparable observations were reported for cowpea and fenugreek flours. Protein isolation influenced protein levels in tamarind protein isolates. Falade and Akeem (2020), observed increased protein to 81.34% when protein was isolated from *P.africana .*

Fat content of the samples ranged from 0.14 (Protein isolate) to 7.46% (Full fat flour). The trace amounts of fat obtained for the protein isolates and defatted flour is due to the removal of fat from the flour sample.

The carbohydrate content of the samples ranged from 9.54 % (Protein isolate) to 42.71% (defatted flour). The carbohydrate content showed a decreasing trend with increasing protein content. The high value of carbohydrate in tamarind full fat flour and defatted flour, could make the flours useful as food ingredients for tackling protein-energy malnutrition in Africa.





Each value is a mean of three determinations±standard deviation. Means within the same row having different superscripts differ significantly  $(p<0.05)$  from each other

**Keys:** WAC/OAC: water/oil absorption capacity, PS: Protein solubility, FC: foaming capacity, FS: foam stability, EC: emulsion capacity, ND: Not determined

Table 5 shows the functional properties of tamarind protein isolate, full fat flour and defatted flour.

The general qualities and industrial applications of food materials depend largely on their functional properties (Adeleke and Odedeji, 2010).

Bulk density of a food material depends on its individual particle mass, property, density and geometry (Kolawole *et al.,* 2016). Knowledge of bulk densities of powdered food material is essential in designing packages and for effective handling. The bulk density of the samples varied between 0.35 g/ml for tamarind protein isolate and 0.68 g/ml for tamarind defatted flour. Low carbohydrate contents could be responsible for the observable low bulk density of the protein isolates.

The oil absorption capacity (OAC) of the samples ranged from 92 to 230 % for tamarind flours and protein isolates, respectively. The values (92 and 130%) obtained for the oil absorption capacity of tamarind flours fell within the range of 0.93 -1.38 g of oil/g of flour reported for some whole legume flours (Du *et al.,* 2014). The defatting process enhanced the oil capacities of tamarind flour. The oil binding capacity of protein materials is an important factor which determines how well the materials will perform as meat extender or analogue. Tamarind protein isolate showed higher OAC and could be used in the formulation of foods with enhanced texture and mouthfeel.

The water absorption capacity obtained ranged from 240 to 475% for tamarind full fat flour and protein isolates respectively. Water binding of food protein could be influenced by protein conformation, surface polarity or hydrophobicity and amino acids composition. High proportion of hydrophilic amino acids and non-protein components could be accountable for the high water-holding capacity of tamarind protein isolate. Mundi and Aluko (2012) have recommended the utilization of ingredients with water absorption capacity ranging from 149 to 472% in viscous food. Tamarind protein isolates could therefore serve as suitable ingredients in processed cheese, soups, baked foods etc that require good oil and water retention capacities.

Two phase systems consisting of air bubbles enclosed by a continuous liquid lamellar phase are referred to as foams. Foaming capacity and stability of tamarind protein isolate were 41 and 36% respectively. The results gotten are higher than that obtained by Marimuthu *et al*., (2013). They reported foam capacity and stability for jack bean protein isolate to be 18.16 and 8.21% respectively.

Proteins function as emulsifiers through formation of a film around oil droplets dispersed in an aqueous medium in order to prevent structural changes such as creaming, sedimentation, flocculation and coalescence (Boye *et al.,* 2010). The defatted flour had the lowest (25.12%) emulsion capacity while the highest (48.33%) was recorded for tamarind protein isolate. Defatting had significant  $(p<0.05)$  effects on the emulsion capacity of tamarind flour. Emulsion capacity of tamarind flour decreased after defatting and then increased in the isolated proteins. Falade and Akeem (2020) had reported similar decreasing effects of defatting on the emulsion capacity of *P. africana* flours.

Protein functionality in food processing and application depends mainly on its solubility in water (Karaca *et al.,* 2011). Tamarind protein isolate had a high (80.72%) value of protein solubility. The high value obtained might be due to the presence of high hydrophilic amino acids.

<b>Sample</b>	TVC(cfu/g)	Mould count $(cfu/g)$
<b>FFF</b>	$1.4 \times 10^4$	ND
DF	$1.7 \times 10^4$	ND
PI	$1.2 \times 10^4$	ND

**Table 6: Total viable and mould count of tamarind full fat flour, defatted flour and protein isolate.**

**Key:** TVC: Total viable count, ND: Not detected, FFF: Full fat flour, DF: Defatted flour, PI: Protein isolate.

Total viable and mould count of tamarind full fat flour, defatted flour and protein isolate is shown in Table 6 above. The total viable count ranged from  $1.2 \times 10^4$  cfu/g (protein isolate) to 1.7 x  $10^4$  cfu/g (Defatted flour). There was no variation in the microbial load among the samples. The microbial loads were generally low. There was no mould growth in the samples which could be attributed to the low moisture content of the flour and isolate. This implies that the protein isolate could be kept for a long period of time without deteriorating.

# **Conclusion**

In conclusion, this research shows that Tamarind seed flours are good sources of proteins, carbohydrates and fibres. Defatting enhanced protein and fibre content. Isoelectric precipitation of tamarind flour gave protein isolate with higher protein content. The high foaming capacity of tamarind protein isolate can be used in applications like making desserts and ice cream, cakes, batters and mayonnaise. It can also find relevance in bakeries, infant foods, and dairy industries due to its ability to absorb moisture, oil and good emulsion capacities.

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The preparation of tamarind seed flour was carried out by modifying the method of Odunfa and Oyewole (1988) for fermentation of locust bean. Samples were removed daily for analysis. The samples were oven dried at  $60^{\circ}$ C and then milled into flour using attrition mill. The milled flour was sifted through a 1 mm mesh size sieve and packaged in an air tight container.

Modified alkaline extraction method as described by El-Adawy *et al*, (2001) was used for the extraction of protein isolate.

Proximate composition was determined using AOAC (2010). Water/oil absorption capacity and least gelation concentration were determined using Adebowale et al. (2005) methods, foaming capacity and stability were determined according to the method of Lin et al. (1974) and emulsion capacity was determined according to the method of Aluko & Yada (1995). Data analysis was carried out using one-way analysis of variance (ANOVA) based on Completely Randomized Design (CRD). Mean separation was done by Duncan's New Multiple Range Test using SPSS Version 17 computer software