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(RESEARCH ARTICLE)

Amino acid profile, microbiological and farinographic properties of African locust bean pulp flour

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Abstract

Flour was prepared from African locust bean (*Parkia biglobosa*) fruit pulp and evaluated for the amino acid composition, microbiological and farinographic characteristics. The locust bean pulp flour contained 4.8% total crude protein and 17 amino acids. The major amino acids in the locust bean pulp flour protein were glutamin acid, aspartate acid, leucine and arginine. Leucine, lysine and phenylalanine were the major essential amino acids. Methionine had the lowest concentration and tryptophan was the second limiting amino acid in the locust bean pulp flour protein. The bacteria isolated from the pulp included *Bacillus cereus, Leuconostoc* and *Streptococcus* species .The yeasts and moulds found in the pulp were *Rhizopus* and *Saccharomyces* species. Colifoms were not detected in the pulp. The total bacteria count was 4.6 x 10^4 cfu/g. The *Bacillus cereus, Streptococcus aureus*, yeasts and moulds counts were 0.5 x 10^4, 3. 2 x 10^4 and 2.4 x 10^4cfu/g, respectively. The farinographic water absorptions were 60 % and 68% for wheat flour and the African locust bean pulp flour, respectively. The water absorption increased with increase in the level of locust bean pulp flour in the blends. The dough development time was higher for the locust bean pulp flour (3.0 min) than for wheat flour (1.7 min).The dough development time increased steadily with increase in the level of African locust bean pulp flour addition. The mixing tolerance index decreased from 79 BU (Brabender unit) in wheat flour to 42 BU in the blend containing 90 % locust bean pulp flour. The dough stability also decreased with increased level of the locust bean pulp flour in the blends. African locust bean pulp flour and wheat flour blends have the potential for use in baked food products.

Keywords: Locust bean; Amino acid; Microbial; Farinograph; Flour; Rheology

1. Introduction

African locust bean (*Parkia biglobosa*) tree grows widely in many parts of the Sahel, particularly, the drier parts of West Africa [1]. In Nigeria, the tree grows in the wild throughout the savanna from Guinea through Sudan to Sahel [2]. The tree produces 25-52 kg pods [2]. In Northern Nigeria alone, about 200,000 tons of locust bean pods are produced yearly [2]. A mature locust bean pod contains yellow dry and powdery pulp in which dark brown seeds are embedded. The pulp is rich in carbohydrates, minerals, vitamins and essential phytochemicals such as flavonoids, carotenoids, polyphenols, saponins etc [3-4]. The pulp is usually licked for its sweet taste but only to a small extent [4]. The pulp is usually washed away when the seeds are processed into condiment called *dawadawa* or *iru.* There is growing interest in the utilization of locust bean pulp due to its nutraceutical potential [4] and this has led to extensive studies on the pulp [5]. The chemical composition and functional properties of locust bean pulp flour have been determined [1, 4]. The effects of pH and NaCl concentration on the functional properties of the pulp flour were recently reported by Akubor and Adedeji [6]. The presence of flavonoids, carotenoids and vitamin C in the locust bean pulp would exert health

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promoting effect in addition to those of the dietary fiber [3, 7]. Akubor [8] reported the changes in the physical, chemical, sensory and microbiological qualities of syrup and jam prepared from locust bean pulp during storage.

Information on the microbiological safety and baking properties of locust bean pulp flour are lacking in the literature. Locust bean pulp, like other fruit pulps has the potential for supporting the growth of both pathogens and spoilage microorganisms [9]. The locust bean pods are picked after they have fallen from the tree to the ground from where they are packed in baskets for the processing seeds. The microorganisms can be introduced into the pods directly from the handlers, the environment, during transportation and storage [10]. Ikuomola and Eniola [11] reported that several factors including water availability, pH, temperature etc. encourage or limit the growth of microorganisms in foods .Consumer preference for safe and quality food product is increasing. Microbial food safety is an increasing public health concern worldwide [12]. Microbial quality is the degree of acceptability of the total number of microbes present in a given food [13]. There is little or nothing in the literature on the microbial safety of locust bean pulp. Therefore, microbiological analysis needs to be carried out to determine the safety of the pulp. The prevention of microbial spoilage is an important element of storage stability.

Wheat flour quality is assessed by physical and chemical tests as well as baking tests. The instruments that are used to test gluten quality include Siman extensimer, Chopin alveograph, Brabender extensograph and Brabender farinograph [14]. The first three measure wheat gluten protein resistance to stretch without breaking (extensibility) [14]. Farinograph is the most widely used to understand the rheological behavior during dough mixing [15]. The farinograph records in graphical format a complex rheological changes taking place in wheat dough during mixing and also facilitates the accurate calculation of the water required to achieve a given consistency [15]. Farinograph is used to evaluate various rheological parameters such as ability of flour to absorb water ,time it takes the dough to develop, strength of dough , dough stability etc. [16].

Therefore, the objective of the present study was to determine the amino acid composition, microbiological and farinographic properties of locust bean pulp flour.

2. Material and methods

Mature and ripe African locust bean (*Parkia biglobosa*) fruit pods were plucked from locust bean trees in a local farm in Ugwaka –Ollah Township, Kogi State, Nigeria. Commercial wheat flour was purchased from a local shop in Idah Township, sieved through 60 mesh sieve (0.05 mm) (British standard) and stored in high density polyethylene bag in a refrigerator prior (10 °C) to use. The nutrient agar (Merk), potato dextrose agar (Oxoid) etc. were purchased from a chemical store in Idah Township.

2.1. Determination of amino acid composition

The total crude protein content (Kjeldahl method) and the amino acid composition of the African locust bean pulp flour were determined using the method described by the AOAC [17] methods. Five gramme of the sample was dried until the weight became constant, defatted in Soxhlet apparatus using hexane, hydrolyzed, concentrated in a rotary evaporator. Then, the sample was placed into the Technicon sequential Amino acid analyzer Union Carbide Corp, New York, NY (TSM) for the amino acid analysis. Amino acid values were calculated from the chromatograph as: Value of amino acid in locust bean pulp flour (g/100 g protein)/FAO amino acid reference value x 100.

2.2. Evaluation of microbiological properties

The bacteria, yeast and mould counts were determined by the methods described by Ray and Bhunia [18]. One gram of the flour was mixed thoroughly with 9 ml of 0.1% w/v peptone water (pH 7.2). One milliliter of the dilution (10^{-1} – 10^{-5}) was plated with on nutrient agar (Merek) for bacterial count and potato dextrose agar (Oxoid) for mould and yeast counts using the pour plate technique. The plates were incubated at 37 °C and 30 °C, respectively for 24 h for bacteria, yeast and mould counts. Counts of microorganisms carried out using colony counter (Gallenkamp, UK) and the results were expressed as colony forming unit (cfu/g).

2.2.1. Identification of isolates

Each of the cultured plates was sub cultured and purer cultures were obtained. The bacterial isolates were identified by their cultural and morphological tests which included gram staining reactions, biochemical tests and oxidative / fermentative utilization of sugars [19]. The yeasts and moulds were identified based their cultural and morphological characteristic [19]. The biochemical analyses were catalase, coagulase, indole production tests and sugar utilization test. For the cultural and morphological identification, 0.5 g of the pure colonies was smeared on a slide after which

methylene blue was added for staining [19]. After staining, the slide was mounted on a microscope for identification of microorganisms.

2.2.2. Bacillus and Staphylococcus spp counts

For the *Bacillus* counts, a 10-fold dilution was prepared from flour sample and plated unto a nutrient agar. Following incubation for 24- 48 h, a confirmatory staining procedure was made on suspected colonies [18]. Colonies confirmed were counted using colony counter and the results reported as cfu/g. For the Staphylococcus counts, the flour was mixed with water from which a 10- fold dilution series was prepared. Aliquots from range of dilutions were transferred into a mixture of nutrient agar and sodium chloride and the plates were incubated under aerobic condition at 35 °C [18]. The number of colonies of *Staphylococcus* spp were counted using colony counter and the level of the pathogen in the flour was expressed as cfu/g.

2.3. Evaluation of the farinographic properties

The farinographic characteristics of the flour were determined as described by the AOAC [17] method using Brabender farinogragh (Brabender, Germany) was used for the study. From the farinogragh, the water absorption, time it takes the dough to develop, mixing tolerance index, stability of dough and the dough softening determined.

2.4. Experimental design and statistical analysis

The experiments were carried out in completely randomized design in three replications. The data generated were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 17, 2007). Means where significantly different were separated using Duncan Multiple range test (DMRT).

3. Results and discussion

3.1. Amino acid composition

Table 1 Amino acid composition of African locust bean pulp flour and chemical scores of the essential amino acids

Amino acid (g/100g protein)	FAO Reference value x	Locust bean pulp flour	Chemical score (%) xx
Lysine	4.20	3.30	78.57
Histidine		1.90	
Threonine	2.80	2.29	81.79
Tryptohan	1.40	0.70	50.00[b]
Serine		3.30	
Aspartate acid		6.50	
Glutamin acid		11.0	
Glycine		3.98	
Proline		2.30	
Alanine		2.60	
Cysteine	2.00	1.50	75.00
Isoluecine	4.20	3.10	73.80
Phenylalanine	2.60	3.29	126.54
Tyrosine	2.50	1.81	72.40
Valine	4.20	2.60	61.90
Leucine	4.20	6.02	143.33
Methionine	2.20	0.47	21.36[a]
Arginine		4.00	
Protein(N x 6.25)		4.80	

a= first limiting amino acid, b=second limiting amino acid, x=FAO [23], xx=chemical score was calculated as value of amino acid in the pulp flour (g/100g, protein) x100/FAO reference value

The amino acid composition of African locust bean pulp flour is presented in Table 1. The pulp flour contained 4.8 % total protein and 17 amino acids. The major amino acids in the locust bean pulp flour protein were glutamin acid, aspartic acid, leucine and arginine. All the amino acids were present in varying amounts with leucine, lysine and phenylalanine constituting the major essential amino acids. Of particular interest was the high level of lysine, leucine and threonine in the pulp. Lysine and threonine are present at very low concentrations in wheat, rice, cassava and diets containing maize that are commonly consumed in developing countries including Nigeria [20]. The presence of high

levels of these amino acids makes locust bean pulp flour a good supplement for these staple food stuffs [21]. The content of the sulphur amino acid cysteine (1.5 g/100g) was comparable to the FAO reference value of 2.0 g/100g [16]. However, methionine was the first limiting amino acid and tryptophan the second limiting amino acid in the locust bean pulp flour protein. Similar amino acid pattern was reported for the locust bean pulp seeds [22]. The role of amino acids in the normal functioning of the human body cannot be over stressed. Leucine and histidine which are substantial in the African locust bean pulp flour are reported to enhance the growth of infants and young children [14]. Histidine plays an important role in the catalytic activity of many enzymes [16]. The threonine, serine, tyrosine contain polar hydroxyl groups which enable them to participate in hydrogen bonding [20]. Alanine is suited for diffusing from muscle cells into the blood to be transported by the blood to the liver for utilization in gluconeogenesis. Valine, leucine and isoleucine are also utilized for the synthesis of substrates for gluconeogenesis [23]. For ketogenesis, phenylalanine is useful for the synthesis of melanin, a pigment that contributes to the color of the eye, hair and skin. Tryptophan is used for the synthesis of serotonin. Aspartate and glutamate transport ammonia to the liver and kidney for the production of urea.

3.2. Microbiological properties

The cultural, morphological and biochemical characteristics of bacteria, yeasts and moulds isolated from the locust bean pulp flour are presented in Tables 2-4. The bacteria isolated from the pulp include *Bacillus cereus*, *Leuconostoc* and *Streptococcus* species (Tables 2 and 3). The yeasts and moulds found in the pulp were *Rhizopus* and *Saccharomyces* species (Table 4). Colifoms were not detected in the pulp. Arora [19] reported that similar spectra of microorganisms as surface flora of fruits and vegetables. Uzugegbu and Emifonige [24] also made similar observation on local fruits and vegetables in Nigeria.

Table 2 Cultural, morphological and biochemical characteristics of bacteria isolates from locust bean pulp flour

Test	**Results**		
Cultural	Large crenated creamy colonies with raised edges	Small round creamy colonies	Whitish raised round colonies
Morphological	Rods in chain	Cocci in pairs	Cocci in clusters
Biochemical reaction			
Grams reaction	+	+	+
Catalase test	+	-	+
Coagulase test	-	-	+
Indole test	-	-	-
Suspected organism	*Bacillus* spp	*Leuconostoc*	*Staphylococcus* spp

Table 3 Sugar utilization by microorganisms isolated from locust bean pulp flour

Sugar	**Pulp**	**Samples**	
	1	2	3
Lactose	A	A	A
Glucose	A	A	A
Fructose	A	A	A
Maltose	A	A	A
Suspected organism	*Lactobacillus* spp	*Lactobacillus* spp	*Lactobacillus* spp

A= Acid production.

The counts for the microorganisms in locust bean pulp flour are shown in Table 5. The total bacteria count was 4.6 x 10^4 cfu/g. The *Bacillus cereus, Streptococcus aureus,* yeasts and moulds counts were 0.5 x 10^4, 3. 2 x 10^4 and 2.4 x 10^4cfu/g, respectively. These values are considered high when compared to the recommended standard counts of 10 x 10^5 to 10 x10^4 [25] for foods.

However, when compared to the other standards, the levels of microorganisms in the locust bean pulp flour were within the tolerable levels [25]. The international microbiological standards recommended limits for bacterial contamination of foods were in the range of $10^1$to 10^2 cfu/g for coliform organisms and less than 10^5 cfu/g of food for total plate count [25]. At levels of 10^4 cfu/g or above, production of heat stable toxin may occur [26]. Some of the organisms isolated from locust bean pulp flour such as *Staphylococcus aureus* and *Bacillus cereus* are pathogenic while others are not [27]. If the count of *Bacillus* reaches high level in food, it produces toxin [26]. Most of the microorganisms in locust bean pulp flour are found on body surfaces or soil [28]. The high level of contamination of locust bean pulp flour was probably due to poor storage and handling practices [29].

Table 4 Cultural and morphological characteristics of yeasts and moulds isolated from locust bean pulp flour

Cultural characteristics	Morphological characteristics	Suspected organism
Black round colony	Non syptate hayphae	*Rhizopus* spp
Moist creamy colony	Round cells with budding	*Saccharomyces* spp

The other sources of contamination of the pulp might have included the soil on which the fruit pods were produced and harvested [30]. Also, the harvested pods were packed in jute bags stored on the floor of the laboratory, which was probably charged with pores of microorganisms. Storage of the pods for a length of time under these conditions at high ambient temperature probably encouraged the proliferation of bacteria [10]. The type of fertilizer used to improve the soil quality and yield as well as the environment were sources of contamination of garden egg [31]. The *Staphylococcus aureus* is generally found in the skin, nose, throat, palms, hairs and mucus membrane of humans [32].The presence of *Staphylococcus aureus* in locust bean pulp flour is of health significance they are capable of causing gastrointestinal problems especially to consumer who eat the raw pulp.

Fungi are well spread in nature, being found as spores on fruits and vegetables [32]. The presence of penicillum species on LBPF was in agreement with the report of Adebolu and Ifsan [31] that isolated *Aspergillus* and *Penicillium* species from vegetables used in preparing salad that were bought from various food shops in Akure, Ondo State, Nigeria. The presence of toxigenic *penicillium* species inlocust bean pulp flour should be viewed with concern. Inyang et al. [30] reported that foods contaminated with *Penicillium* spp are capable of causing liver cancer when consumed. *Apergillus* and *Penicillium* have been reported to produce aflatoxins which have carcinogenic, hemorrhagic, heptaotoxic, neurotoxic and uterotrophic properties [30].

Table 5 Microbial counts of locust bean pulp flour

Type of microorganism	Count (cfu /g)
Bacteria	4.6×10^4
Bacillus cereus	0.5×10^4
Staphylococcus aureus	3.2×10^4
Yeasts and moulds	2.4×10^4

Values are means of three replications.

3.3. Farinographic properties

The farinographic characteristics of the locust bean pulp flour, wheat flour and their blends are shown in Table 6. The farinographic water absorptions were 60% and 68% for wheat flour and locust bean pulp flour, respectively. The water absorption increased with increase in the level of locust bean pulp flour in the blends probably due to addition effect [16, 32]. The water absorption increased from 60% in the wheat flour to 67.5% for the blend containing 90 % locust bean pulp flour. This means that at fixed water absorption, the locust bean pulp/ wheat flour blend showed maximum consistency above the 500 BU (Brabender units) line and in such cases, water has to be increased to center the farinograph curve on 500 BU. Similar results were reported for plantain/wheat flour blends [33].

Table 6 Farinographic characteristics of locust bean pulp flour (LBPF), wheat flour (WF) and the blends

LBPF:WF	Water absorption (%)	Dough development time(min)	Mixing tolerance index (BU)	Dough stability (min)	Dough softening (BU)
100:0	68[a]	3.0[b]	20[k]	1.5c	60[a]
0:100	60[f]	1.7[c]	79[a]	3.0[a]	116[i]
10:90	61[e]	2.0[c]	78[b]	2.9[a]	117[h]
20:80	61.8[de]	2.1[c]	75[c]	2.8[a]	119[g]
30:70	62.5[d]	2.2[c]	74[d]	2.6[a]	120.5[f]
40:60	63[d]	3.0[b]	71[e]	2.5[ab]	122[e]
50;50	63.5[cd]	3.1[b]	70[f]	2.3[b]	131.0[d]
60:40	64[c]	3.4[ab]	68[g]	2.1[b]	132[c]
70:30	64.75[bc]	3.5[a]	67[h]	2.0[b]	133[b]
80:20	64.9[bc]	3.6[a]	65[i]	1.8[b]	133.5[b]
90:10	65[b]	3.7[a]	63[j]	1.6b[c]	132[b]

Values are means of three replications. Means within a column with the same superscript were not significantly different (p> 0.05)

The dough development time, which is defined as the time to the nearest half minute from the first addition of water to the development of maximum consistency of the dough was higher for the locust bean pulp flour (3.0 min) than for wheat flour (1.7 min).The dough development time increased steadily with increase in the level of African locust bean pulp flour addition. The mixing tolerance index defined as the difference in the BU from the top of the farinograph at the peak to the top of the curve measured at 5 min after the peak is reached decreased with increase in the level of African locust bean pulp flour in the blends. The mixing tolerance index decreased from79BU in wheat flour to 42 BU in the blend containing 90% African locust bean pulp flour. The dough stability also decreased with increased level of African locust bean pulp flour in the blends. Bamidele et al. [34] reported a continuous increase in dough stability with increase in the level of plantain flour in the blends. This was contrary to the results of the present study. The differences can be attributed to the variation in the chemical nature of the flours [14]. The longer the stability, the better the dough from plantain/ wheat flour blends [34]. The dough softening increased substantially with increase in the level LBPF in the blends. Bemidele et al. [34] made similar observations for wheat flour and breadfruit flour blends.

4. Conclusion

African locust bean pulp flour contained 17 essential and non-essential amino acids with methionine and tryptophan as the limiting amino acids. The raw African locust bean pulp flour is microbiological safe for human consumption. The blends of African locust bean pulp flour and wheat flour have the potential for use in baked food products.

Compliance with ethical standards

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Disclosure of conflict of interest

The author has declared no conflict of interest in the present study.

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