

**PJN**

ISSN 1680-5194

PAKISTAN JOURNAL OF  
**NUTRITION**

**ANSI***net*

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## Microbial Studies on *Aisa*: A Potential Indigenous Laboratory Fermented Food Condiment from *Albizia saman* (Jacq.) F. Mull

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**Abstract:** A total of 134 bacterial isolates characterized as *Bacillus cereus* var. *mycoides*, *B. coagulans*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis*, *Staphylococcus cereus* and *S. saprophyticus* were isolated from fermenting *Albizia saman* seeds during the laboratory production of *aisa*, a potential food seasoning condiment. *Bacillus* species were the most predominant species and produced the highest ammoniacal smell characteristic of typical indigenous fermented food condiments. There was a general increase in the microbial population throughout the fermentation period. The pH of the fermenting mash was between 6.5-8.2. The physical observation of the fermented mash was dark brown in appearance with creamish mucilaginous slime, moulding the fermented cotyledons together. Process optimization of the fermenting *aisa* mash indicated optimal fermentation temperature of 45°-50°C, optimal pH of 6.9-8.2, while the fermented mash with pawpaw leaves gave the most accepted product as compared to banana leaves, local leaves and almond leaves. Consumers gave 74.0%-96.0% preference to *aisa* as an alternative to *iru* and *ogiri*, the most popular indigenous fermented food condiments in Nigeria. In comparison with the laboratory fermented samples, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Proteus mirabilis* were isolated in addition to the *Bacillus* and *Staphylococcus* species in the traditionally fermented *aisa* samples. Fermentation of *Albizia saman* seeds for 5-7 days gave the best organoleptic parameters of *aisa* even after 3 months of storage at ambient temperature and 6 months storage at 4°C in the refrigerator.

**Key words:** *Aisa*, *albizia saman*, condiment, fermentation, microbial flora, process optimization

### Introduction

Fermented foodstuffs and condiments remain key constituents of diets throughout many parts of Asia and Africa. In cases where the process of fermentation evolved for the development of taste or aroma, it often resulted in enhanced nutrition, stabilization of the original raw materials, and detoxification of anti-nutrient factors. Food condiments in Nigeria and many other countries of west and central Africa are popular strong-smelling fermented food culinary products that give pleasant aroma to soups, sauces and other prepared dishes. They also have great potential as key protein (Umoh and Oke, 1974), fatty acid and good sources of gross energy, therefore, condiments are basic ingredients for food supplementation and their socio-economic importance cannot be over emphasized in many countries especially in Africa and India where protein calorie malnutrition is a major problem.

Some of the most important food condiments are *ogiri* which is produced from melon seeds *Citrullus vulgaris*; *iru* or *dawadawa* or *dadawa*, produced from African locust bean *Parkia biglobosa* (Odunfa, 1981); *ugba*, produced from oil bean seeds (*Pentacletra*

*macrophylla*) (Obeta, 1985; Oyeyiola, 1981). *Ogiri-igbo*, produced from castor oil seeds (*Ricinus communis*) (Odunfa, 1986), *dawadawa*, from soya-beans (Ogbadu and Okagbue, 1988), *owoh* from cotton seeds (*Gossypium hirsutum*), *okpehe* from mesquite (*Prosopis africana*) (Ogunshe, 1989). *Ogiri* is also made from (*Parkia filicoidea*). Most of the fermented vegetable-proteins reported are from leguminous seeds, and of the thousands known legumes, less than twenty are used extensively today. Other leguminous seeds in common use include peanuts, soya beans, locust beans, oil beans, cowpeas, lentils, *alfalfa* (luceme) etc. The remaining species are little used as fermented condiments yet e.g. *Prosopis* spp. and many of them are almost unknown to science in the area of food fermentation such as in the case of *Albizia saman*.

*Albizia saman* *Samanean saman* (Jacq.) Merr. Synonymy: *Albizia saman* (Jacq.) F. Mull., *Pithecellobium saman* (Jacq.) Benth. is also commonly known as monkey pod, rain tree, 5' O'clock tree, cow tamarind, rain tree, saman tree (English); *acacia* (Filipino); *gouannegoul*, *saman* (French); *carreto negro/cenicero*, *delmonte*, *guannegoul*, *samán*

(Spanish); *cham cha*, *kam kram* (Thai); *cồng* (Vietnamese). In French it is also known as *arbre-de-pluie*, *arbre-a-confiture* (WordNet, 2003). The fruits from *Albizia saman* trees are elongated pods which are usually green when unripe but brown on maturing. The pods contain black/brown seeds embedded in brown edible pulp. The pulps are opened up by removing fibrous strands within the pods. About 15 – 21 seeds in each pod can be removed by pounding in a wooden mortar and pestle followed by removal of the brownish pericarp which surrounds the seeds. *Albizia saman* is considered a multipurpose tree (Ayodele *et al.*, 2003). It contains minerals and is considered suitable for feeding cattle and poultry with a complimentary cereal while the pods are considered unfaultable for animal feeds because of high ash content.

The main objective of this study therefore, is to determine the possibility of fermenting boiled cotyledons of *Albizia saman* seeds under controlled and traditionally-based conditions into a fermented indigenous food seasoning condiment. Microbial profiles of the fermentation were also studied to determine the possibility of developing starter cultures in the fermentation of *aisa* for domestic and industrial purposes.

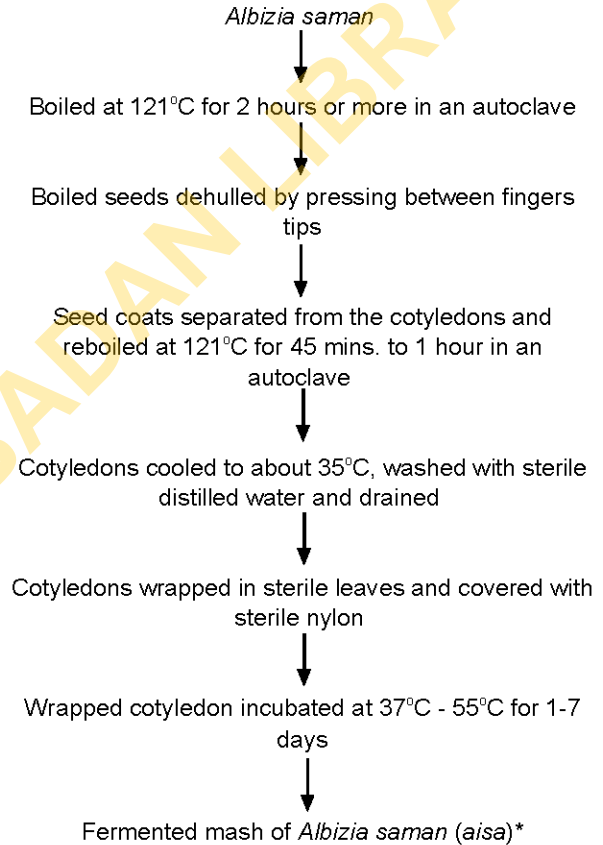
## Materials and Methods

**Sample collection:** The harvested pods of *Albizia saman* were picked within the University of Ibadan campus. The seeds were removed from the pods manually by pounding in a mortar or by cutting the pods along the axis with a knife before direct removal of the seeds from the pods with hands.

**Laboratory preparation:** Dried, clean seeds of *Albizia saman* were weighed and the required quantities i.e. 1000g per fermentation period were washed in tap water followed by rinsing with sterile distilled water. The seeds were boiled at 121°C for 2 hours in an autoclave/pressure cooker and later dehulled manually by pressing in between palms after thorough boiling. The seeds coats were decanted along with the washing water, leaving the *Albizia saman* cotyledons. The cotyledons were cleaned in sterile cold water and later boiled for another 45 mins to 1 hour in an autoclave/pressure cooker (Ogunshe, 1989). The boiled seeds were separately wrapped in clean and surface sterilized (with 75% ethanol) banana, paw-paw and almond leaves. Bundles of the wrapped cotyledons were tied with sterile nylons and then incubated at 37°C – 55°C respectively for 1-7 days.

**Traditional preparation:** Dried, clean seeds of *Albizia saman* were weighed and the required quantities i.e. 1000g per fermentation period were washed in tap water. The seeds were boiled at 100°C for 18-20 hours using a stove and later dehulled manually by pressing

in between palms after thorough boiling. The seeds coats were decanted along with the washing water, leaving the *Albizia saman* cotyledons. The cotyledons were later boiled for another 1 to 2 hours followed by rinsing with water samples obtained from local producers of *iru*. The boiled seeds were separately wrapped in clean banana, paw-paw and almond leaves. Bundles of the wrapped cotyledons were tied with nylons and then incubated at 37°C-55°C respectively for 1-7 days in local calabashes.



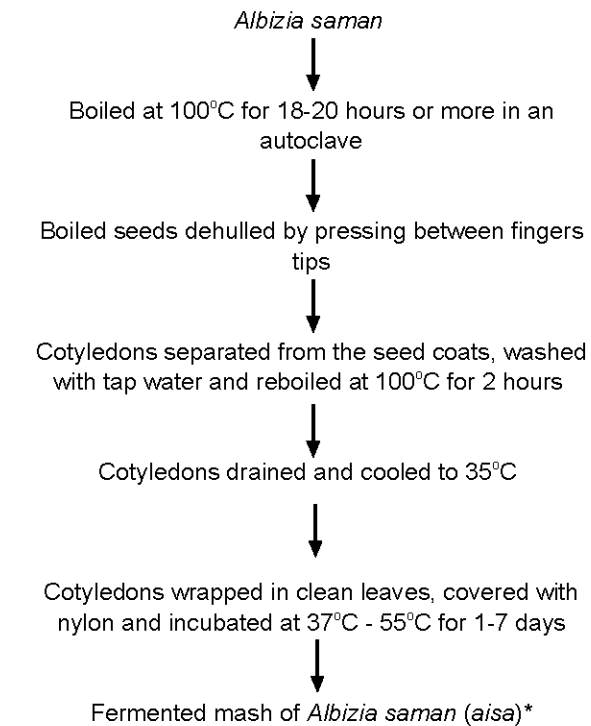
\* Stickily mucilaginous brown strong smelling mash.

Fig. 1: Flow chart for laboratory preparation of *aisa*

**pH determination:** The pH of the fermenting *aisa* samples were determined using a pH meter equipped with a glass electrode. Determinations were done in triplicates.

**Determination of viable counts:** Viable counts of the fermenting *aisa* mesh were determined using the modified pour-plate method of Harrigan and McCance (1976).

**Isolation media:** The isolation media used in the isolation of the microorganisms involved in the fermentation of *Albizia saman* into *aisa* condiment were



\* Stickily mucilaginous brown strong smelling mash.

Fig. 2: Flow chart for traditional preparation of *aisa*

nutrient agar (NA), plate count agar (PCA), De Mann, Rogosa and Sharpe agar (MRS), MacConkey agar (MCC), Sabouraud dextrose agar (SDA) and cystein lactose electrolyte deficient agar (CLED).

**Isolation method:** Isolation of microbial flora of the fermenting mash was carried out 24 hourly at days 1 – 7 and the sampling technique was the pour-plate method of Harrigan and McCance (1976).

**Purification and preservation of isolates:** Representatives of each different bacterial colony types were randomly picked from the primary plates of each fermented sample and sub-cultured onto sterile plates by the streaking method. The isolates were sub-cultured by repeated streaking to obtain pure cultures. All the pure cultures were kept in triplicates on Brain Heart Infusion (BHI) agar slants as working and stock cultures.

**Characterization of isolates:** Taxonomic studies were carried out on the purified isolates from the differently fermented samples on the basis of their cultural, morphological, biochemical and physiological characteristics. The cultural characteristics of the purified isolates were as described by Prescott *et al.* (2005). Gram reactions and shape of cells were determined in 18-24h old cultures of the isolates (Seeley

and Van Denmark, 1972). Biochemical characteristics of the isolates were as earlier described by Edwards and Ewing (1972); Holding and Collee (1972); Seeley and Van Denmark (1972); Harrigan and McCance (1976); Paick (1980); Vera and Power (1980); Bailey and Scott (1974); Cruickshank *et al.* (1975).

## Results

The general flow charts for *aisa* production are shown in Fig. 1 and 2. The main steps of the production process involve extensive boiling and dehulling of the seeds, followed by a second boiling to soften the cotyledons. The boiled cotyledons were then wrapped in different leaves, which provided the moist environment necessary for the fermentation of the cotyledons for about 5-7 days to produce *aisa*.

The pH level of 6.5 at the start of fermentation increased to 8.2 at the end of fermentation (Fig. 3). The viable counts of microbial flora of the fermenting mash showed an increase in population throughout the period of fermentation, but the aerobic viable counts were more than the anaerobic viable counts (Table 1).

The highest growth rates of the associated bacterial isolates at 35°C, 40°C, 45°C and 55°C occurred between the third day and seventh day of fermentation of the *aisa* samples (Table 2). The optimal pH for the isolated bacterial species from the fermenting *Albizia saman* into *aisa* was between 7.8-8.0, while the optimal temperature for the associated bacterial isolates were between 40°C and 45°C, however, the optimal temperature for fermentation of *Albizia saman* into *aisa* was between 45-50°C.

*Bacillus* species occurred most consistently and predominated the fermentation of *Albizia saman* into *aisa*, with the production of the highest ammonia-like aroma, characteristic of leguminous-based fermented condiments. Of the 134 bacterial strains isolated from the fermenting cotyledons of *Albizia saman*, *Bacillus cereus* var. *mycoides*, *B. coagulans*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *Staphylococcus cereus* and *S. saprophyticus* were the characterized bacterial species (Table 3). Among the *Bacillus* species isolated from the *aisa* samples, 89.2%, 78.6% and 94.5% were proteolytic, lipolytic and amylolytic respectively.

*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Proteus mirabilis* were isolated in addition to the *Bacillus* and *Staphylococcus* species in the traditionally fermented *aisa* samples (Table 4).

Fermentation of *Albizia saman* seeds for 5-7 days gave the best organoleptic parameters of *aisa* while the fermented mesh with pawpaw leaves gave the best *aisa* product as compared to banana leaves, local leaves and almond leaves. Consumers' preference for *aisa* as an alternative to *iru* and *ogiri* was between 74.0 % - 96.0 %.

## Discussion

In Africa, many proteinaceous oily seeds are fermented

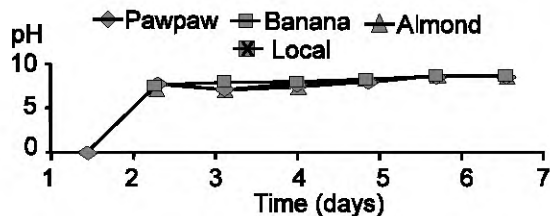


Fig. 3: pH of the fermenting *Albizia saman* samples using various leaves

Table 1: Total viable counts and physical changes during the fermentation of *aisa*

Day	Viable plate counts (cfu ml <sup>-1</sup> )		
	Total aerobic	Total anaerobic	Type of leaf
1	2.1 x 10 <sup>8</sup>	3.6 x 10 <sup>6</sup>	pawpaw
	2.3 x 10 <sup>7</sup>	2.7 x 10 <sup>6</sup>	banana
	3.1 x 10 <sup>6</sup>	3.8 x 10 <sup>6</sup>	almond
2	1.9 x 10 <sup>8</sup>	5.2 x 10 <sup>6</sup>	pawpaw
	2.5 x 10 <sup>7</sup>	3.2 x 10 <sup>6</sup>	banana
	1.7 x 10 <sup>7</sup>	4.1 x 10 <sup>6</sup>	almond
3	2.6 x 10 <sup>10</sup>	2.5 x 10 <sup>9</sup>	pawpaw
	1.2 x 10 <sup>9</sup>	5.6 x 10 <sup>7</sup>	banana
	6.7 x 10 <sup>7</sup>	3.4 x 10 <sup>7</sup>	almond
4	5.8 x 10 <sup>10</sup>	2.9 x 10 <sup>9</sup>	pawpaw
	5.1 x 10 <sup>9</sup>	3.1 x 10 <sup>9</sup>	banana
	2.6 x 10 <sup>8</sup>	7.4 x 10 <sup>7</sup>	almond
5	3.5 x 10 <sup>11</sup>	5.1 x 10 <sup>10</sup>	pawpaw
	1.7 x 10 <sup>10</sup>	6.4 x 10 <sup>10</sup>	banana
	2.7 x 10 <sup>8</sup>	5.2 x 10 <sup>8</sup>	almond
6	4.4 x 10 <sup>11</sup>	2.1 x 10 <sup>10</sup>	pawpaw
	3.5 x 10 <sup>10</sup>	7.9 x 10 <sup>10</sup>	banana
	5.6 x 10 <sup>9</sup>	6.5 x 10 <sup>9</sup>	almond
7	5.3 x 10 <sup>11</sup>	3.7 x 10 <sup>10</sup>	pawpaw
	4.6 x 10 <sup>10</sup>	8.5 x 10 <sup>10</sup>	banana
	6.0 x 10 <sup>9</sup>	7.2 x 10 <sup>9</sup>	almond

to produce food condiments. *Aisa* is a laboratory fermented culinary product of *Albizia saman* (Jacq.) F.V. Muell, *Plihecetlobium saman* (Jacq.) Benth. by *Bacillus* and *Staphylococcus* species. It is an example of an alkaline fermentation process.

The increase in pH of the fermenting mash of *aisa* may be due to the abundant increase of NH<sub>3</sub> during the later stages of fermentation. Achinewhu (1987) and Ogbadu and Okagbue (1988) in their studies involving other legumes, also observed a steady increase in the pH with fermentation period. The initial lower pH observed at the commencement of the fermentation can be caused by the fermenting microbes which might have started fermentation by hydrolyzing available carbohydrate to acid before embarking on extensive proteolysis. Thus, the acid produced initially lowers the pH of the fermenting mash and later leads to alkalinity by

the hydrolysis of protein as illustrated by Whitaker (1978) or due to the protease and deaminase enzymes produced by the *Bacillus* isolates (Heesseltine, 1979). The gradual development of ammonical odour is in agreement with the observed pH changes from acidic range to the reported alkaline pH range by Barimala (1994) in similar experiment.

The list of food produced by microbial fermentation is very long and considering the variety of natural food substances and the methods by which each is processed and preserved, it is apparent that all kinds of microorganisms are potential fermenters. The microorganisms that produce the changes may be the natural flora on the material to be fermented or may be inocula added as a starter cultures (Pelczar, 1996). Several fermented products rely on the participation of various *Bacillus* species. Often, the finished products are of a very local character and exhibit sensory properties resulting from unique flora and processing technologies applied in small scale, home-based fermentations.

Fermentation with *B. natto* and *B. subtilis* can produce very characteristic aromas in fermented products such as *natto* and *dawadawa* (also referred to as *iruldaddawa*). Other African fermentations in which *Bacillus spp.* play a role include the production of Nigerian *ugba* (fermented African oil bean), Nigerian *ogiri* (fermented melon seeds) and *ogiri-saro* (sesame seed, pumpkin or castor oil seed fermentation) from Sierra Leone. Several workers have also characterized *iru* fermentation and the microorganisms associated with it. Organisms reported to be isolated and/or characterized from *iru* include *Bacillus subtilis* (Ogunfa, 1981; Ogbadu and Okagbue, 1988; Ikenebomeh, 1989), *B. pumilus* (Ogbadu and Okagbue, 1988), *B. licheniformis* (Ogbadu *et al.*, 1990) and *Staphylococcus saprophyticus* (Ogunfa, 1981). The isolation of *Bacillus* species as the most predominant bacterial flora in the fermenting *Albizia saman* in the production of *aisa* is therefore in accordance with previous workers on fermented condiments from leguminous seeds.

*Bacillus subtilis*, *B. licheniformis* and *B. pumilus* isolated by Oyewole and Ogunfa (1990) from *iru* were all said to be proteolytic, 40 were lipolytic and 31 were amylolytic. In this study 92.2%, 78.6% and 94.5% of the *Bacillus* species were proteolytic, lipolytic and amylolytic respectively.

The traditional preparation of *aisa* in this study is the same process of locust beans seeds preparation to *iru* which is known to be a traditional family art practiced in homes in a crude manner like other traditional fermented foods. The recovery of some pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Proteus mirabilis* from the fermenting mash is suspected to originate from the water samples used in the traditional preparation. The water samples were obtained from the

Table 2: Growth rates of the associated bacterial isolates in the fermenting *aisa* samples at different temperatures

Fermentation period (Days)	Temperature of fermentation			
	35°C	40°C	45°C	50°C
<b>Pawpaw leaf</b>				
1	1.2 x 10 <sup>5</sup>	1.6 x 10 <sup>5</sup>	1.8 x 10 <sup>4</sup>	2.1 x 10 <sup>2</sup>
2	1.9 x 10 <sup>5</sup>	2.3 x 10 <sup>5</sup>	1.3 x 10 <sup>4</sup>	3.2 x 10 <sup>2</sup>
3	2.4 x 10 <sup>6</sup>	3.2 x 10 <sup>6</sup>	2.6 x 10 <sup>5</sup>	6.7 x 10 <sup>2</sup>
4	3.6 x 10 <sup>6</sup>	3.2 x 10 <sup>7</sup>	3.7 x 10 <sup>6</sup>	1.6 x 10 <sup>3</sup>
5	4.2 x 10 <sup>7</sup>	3.6 x 10 <sup>8</sup>	2.5 x 10 <sup>7</sup>	2.7 x 10 <sup>3</sup>
6	3.6 x 10 <sup>8</sup>	4.5 x 10 <sup>8</sup>	3.8 x 10 <sup>8</sup>	2.4 x 10 <sup>4</sup>
7	5.2 x 10 <sup>8</sup>	6.1 x 10 <sup>10</sup>	4.3 x 10 <sup>10</sup>	3.7 x 10 <sup>4</sup>
<b>Banana leaf</b>				
1	2.2 x 10 <sup>3</sup>	4.0 x 10 <sup>3</sup>	3.8 x 10 <sup>3</sup>	1.1 x 10 <sup>2</sup>
2	3.5 x 10 <sup>3</sup>	2.7 x 10 <sup>4</sup>	2.5 x 10 <sup>3</sup>	1.6 x 10 <sup>2</sup>
3	3.6 x 10 <sup>4</sup>	3.5 x 10 <sup>4</sup>	4.5 x 10 <sup>4</sup>	3.1 x 10 <sup>3</sup>
4	2.4 x 10 <sup>5</sup>	2.7 x 10 <sup>5</sup>	4.1 x 10 <sup>5</sup>	1.8 x 10 <sup>3</sup>
5	3.8 x 10 <sup>6</sup>	2.4 x 10 <sup>6</sup>	2.3 x 10 <sup>6</sup>	2.6 x 10 <sup>4</sup>
6	4.6 x 10 <sup>6</sup>	2.6 x 10 <sup>8</sup>	3.3 x 10 <sup>8</sup>	3.8 x 10 <sup>4</sup>
7	3.1 x 10 <sup>7</sup>	3.3 x 10 <sup>9</sup>	2.6 x 10 <sup>9</sup>	3.4 x 10 <sup>5</sup>
<b>Almond leaf</b>				
1	2.0 x 10 <sup>3</sup>	2.3 x 10 <sup>3</sup>	2.4 x 10 <sup>3</sup>	2.4 x 10 <sup>2</sup>
2	2.3 x 10 <sup>3</sup>	2.6 x 10 <sup>3</sup>	3.5 x 10 <sup>3</sup>	2.9 x 10 <sup>2</sup>
3	3.6 x 10 <sup>3</sup>	3.8 x 10 <sup>4</sup>	2.8 x 10 <sup>4</sup>	3.5 x 10 <sup>3</sup>
4	2.1 x 10 <sup>4</sup>	2.7 x 10 <sup>4</sup>	3.1 x 10 <sup>5</sup>	2.5 x 10 <sup>3</sup>
5	4.2 x 10 <sup>4</sup>	3.1 x 10 <sup>5</sup>	3.3 x 10 <sup>5</sup>	3.3 x 10 <sup>3</sup>
6	3.1 x 10 <sup>5</sup>	2.5 x 10 <sup>5</sup>	3.7 x 10 <sup>6</sup>	2.4 x 10 <sup>4</sup>
7	4.6 x 10 <sup>6</sup>	4.1 x 10 <sup>7</sup>	5.1 x 10 <sup>7</sup>	2.7 x 10 <sup>5</sup>

local producers of *iru* and on questioning it was found that they obtained their water samples from various sources such as streams, rivers, ponds, shallow or deep wells and from rain storage.

A comparison was made between traditional method and laboratory method of production of *aisa* which showed that, except for the faster fermentation of beans/cotyledons in the laboratory method as indicated by the softness of the seeds after the second boiling and after the third day of fermentation, no other remarkable differences especially in organoleptic properties were observed.

Although no formal sensory descriptive analysis of *dawadawa* was found in literature search, those familiar with the product instantly recognize an unmistakable unique ammoniacal or pungent aroma (Beaumont, 2002) however, the unmistakable unique ammoniacal or pungent aroma of *aisa* was found to be consistently stronger than that of *iru* even, after 3 months of storage at ambient temperature in a dry place. The colour was also consistent after 3 months of storage at ambient temperature in a dry place and after 6 months of storage at 4°C in the refrigerator. This study did not identify any comprehensive analysis on the flavour of *aisa*; however, the flavour properties are most likely due to its amino acid content, in particular glutamate.

Evidence for the participation of indigenous enzymes

and flora in the development of the flavour of *iruldawadawa* was presented by Ikenebomeh *et al.* (1986).

These authors demonstrated that both autoclaved (sterile) and gamma irradiated (destroyed indigenous flora) beans were unable to develop the characteristic aroma of *dawadawa*. This report is quite contrary to the observation noted in this study in which the autoclaved (sterile) cotyledons were able to develop very strong characteristic *dawadawa*-like aroma even after 3 months of storage at ambient temperature. Ikenebomeh *et al.* (1986) also indicted additionally that the typical pH increase observed in *dawadawa* fermentation was absent; implicating that active microbial metabolism is required in order to bring about the changes observed in locust beans during fermentation, however, it was observed in this study that autoclaving of the *Albizia saman* cotyledons did not affect the typical pH increase observed in alkaline fermentations of leguminous seeds into condiments.

According to Beaumont (2002), the preservative and flavour characteristics of alkaline fermentations are derived in part from the liberation of ammonia and increased pH, thus, the high pH values and ammoniacal odour recorded in the fermented *aisa* may account for the post fermentation characteristics considered by the respondents in the sensory evaluations of the fresh and

Table 3: Microscopic, biochemical and physiological characteristics of *Bacillus* and *Staphylococcus* species isolated from aisa

Characterization Tests	Bacterial strains					
	BL	BS1	BM	BP	BS2	SA
Gram' reaction	+	+	+	+	+	+
Catalase	+	+	+	+	+	-
Casein hydrolysis	+	+	+	+	+	-
Starch hydrolysis	+	+	+	-	+	-
Gelatin liquefaction	+	+	+	+	+	-
Methyl red	+	+	-	-	-	-
Voges Proskaeuer	+	+	-	-	-	-
Citrate utilization	+	+	+	+	+	+
Nitrate reduction	+	+	-	-	+	-
Lipolytic activity	+	+	+	+	+	+
Motility	+	+	+	+	+	-
Oxygen relationship	FA	FA	FA	FA	FA	FA
Oxidative-fermetative	O	O	O	O	O	O/F
Anaerobic growth	+	-	-	-	+	-
Growth in 5 % NaCl	+	+	+	+	+	+
Growth in 10 % NaCl	+	-	+	+	+	-
Sugar fermentation Fructose	A	A	A	A	A	A
Galactose	A	A	A	A	A	A
Glucose	A	A	A	A	A	A
Lactose	A	A	A	A	A	A
Maltose	A	A	A	A	A	A
Mannitol	A	A	A	A	A	A
Sucrose	A	A	A	A	A	A
Xylose	A	A	A	A	A	A
Growth at different temperatures						
10 <sup>o</sup> C	-	-	-	-	+	+
15 <sup>o</sup> C	+	+	+	+	+	+
25 <sup>o</sup> C	+	+	+	+	+	+
30 <sup>o</sup> C	3+	3+	3+	3+	3+	3+
35 <sup>o</sup> C	3+	3+	3+	3+	3+	3+
40 <sup>o</sup> C	2+	2+	2+	2+	2+	2+
45 <sup>o</sup> C	2+	2+	2+	2+	2+	2+
50 <sup>o</sup> C	+	+	+	+	+	+
55 <sup>o</sup> C	-	-	-	-	-	-
Total no of strains						

Keys: - = negative; + = positive; A = acid production; FA = facultative anaerobe; O = oxidative reaction; O/F = oxidative and fermentative reaction; + = slightly positive; 2+ = positive; 3+ = highly positive. BL = *B. licheniformis*; BS1 = *B. subtilis*; BM = *B. megaterium*; BP = *B. pumilus*; BS2 = *B. subtilis*; SA = *Staphylococcus aureus*

stored product.

Alkaline-fermented foods constitute a group of less-known food products that are widely consumed in Southeast Asia and African countries. The fermentation process (including bean preparation and post-fermentation handling) has been reported to be associated with several benefits by converting otherwise tough, inedible seeds into a valuable, flavour-enhancing condiment (Odunfa, 1986). These benefits include: enhanced digestibility due to degradation of nondigestible oligosaccharides (e.g., stachyose, raffinose); decreased flatulence potential; increased

vitamin content in the form of thiamine and riboflavin; reduction or elimination of phytic and oxalic acids; extended shelf life due to drying, alkaline pH and post-fermentation additions (e.g., salt); protein hydrolysis to peptides and amino acids, providing free glutamate, as well as other amino acids that could function directly in taste, or eventually serve as precursors for aroma active molecules. Therefore, the production of a fermented condiment from *Albizia saman* is an innovative work in Nigeria which will add to the indigenous protein sources of Nigerian populace in order to enrich the diet of an average Nigerian and also to aid in combating the

Table 4: Microscopic, biochemical and physiological characteristics of the Gram-negative bacterial species isolated from *aisa*

Characterization Tests	Bacterial strains			
	EC	KP	EA	PM
Gram <sup>+</sup> reaction	-	-	-	-
Catalase	+	+	-	+
Casein hydrolysis	+	+	+	+
Starch hydrolysis	+	+	+	-
Gelatin liquefaction	-	+	-	+
Methyl red	-	+	+	+
Voges Proskauer	-	+	-	-
Citrate utilization	+	+	-	+
Nitrate reduction	+	+	-	+
Lipolytic activity	+	+	+	+
Indole	-	-	+	-
Oxidase	-	-	-	-
Motility	-	-	+	+
H <sub>2</sub> S production	-	-	-	-
KCN production	-	-	+	+
Sugar fermentation				
Arabinose	A	A	A	A
Fructose	A	A	A	A
Galactose	-	-	A	A
Glucose	A/G	A/G	A/G	A/G
Lactose	A	A	A	-
Maltose	A	A	A	-
Mannitol	A	A	A	-
Raffinose	A	A	A	-
Salicin	A	A	A	A
Sucrose	A	A	A	-
Sorbitol	-	A	A	-
Xylose	A	A	A	A
Total no of strains				

Keys: - = negative; + = positive; A = acid production; A/G = acid and gas production. EC = *Escherichia coli*, KP = *Klebsiella pneumoniae*, EA = *Enterobacter aerogenes*, PM = *Proteus mirabilis*

problem of malnutrition which is still rampant in the country.

The present production of *aisa* did not include a formal inoculation of the fermenting mash but further work to develop starter cultures for domestic and industrial production of *aisa* is currently under investigation in our laboratories. Opportunities for industrial development from traditional fermentations, safer, more effective and consistent traditional fermentations of indigenous fermented food condiments can thus be achieved through the development of starter cultures for the production of *aisa*.

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