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Influence of Fermentation Temperature on Microbial Diversity and Amino Acid Content of African Locust Bean

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

This work is aimed at production of the local spice from the African locust bean, determining the microbial diversity and amino acid contents of spice after fermentation at different temperatures. The raw seeds were boiled for about 12hrs and dehauled. Then the dehauled seeds were boiled again for 2hrs to soften them. They are then washed and wrapped in different banana leaves. The different wraps were allowed to ferment at different temperatures (25°c, 32°c and 40°c) for 3-5 days. After the fermentation period has reached, isolation processes were carried out and identification was also carried out to determine the organisms present in the samples fermented at different temperatures. The thin layer chromatography test was used to determine amino acid contents of the samples fermented at different temperatures. The analysis identified *Bacillus* sp, *Micrococcus* sp,*Staphylococcusaureus*, *Lactobacillus sp,Flavobacterium*sp,*Erwiniasp* and *Shigellasp* to be the microorganisms found but their presence vary at different temperatures. The amino acid composition of the sample includes Arginine (which is found in all temperatures), proline (found at only 25°c) and cystine (found at only 32°c). From this analysis it implies that different fermentation temperature can indeed affect the diversity of microorganisms and amino acid content of the African locust bean.

INTRODUCTION

Fermented foods are those which have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause Fermented vegetable protein seeds for condiment production in West Africa includes; African locust bean (*Parkiabiglobosa),* castor oil seeds (*Ricinuscommunis*), flutted pumpkin seed (*Telfariaoccidentale*), African yam bean seeds (*Stenophylisstenocarpa*), Roselle seeds (*Hibiscus sabdarilfa*), cotton seeds (*Gossypiumhisitum*), and baobab seeds (*Adasoniadigitata*) [1].

Spices are parts of plants that due to their properties are used as colorants, preservatives or medicine. Theuse of spices has been known for a long time due to its chemical composition and some of it beneficial purposes such as antioxidant and antimicrobial purpose [2].FAO reported that spices in food minimize the rate of rancidity, improve colour and flavor intensity of food products [3].

*Parkiabiglobosa*is a popular food condiment in Nigeria and other West African countries. It is called 'locust bean' in English and 'iru' in Yoruba language. Locust bean is a legume seed which is highly nutritious and constitute significant amount of dietary proteins and amino acids [4]. It has high contents of proteins. It can serve as tasty, nutritious, non animal protein

substitute that can be used to compliment sauce and soups [5].Soups are main source of protein and minerals and one of the ways to improve the nutrients in the soups through the use of condiments such as African locust bean [5].

The nutritional adequacy of African locust bean with a proximate composition of 30.00% proteins, 15.00% of fat, 4.00% of crude fibre, 2.00% of ash and 49.00% of carbohydrates [6].

Proteolysis is the main metabolic activity during fermentation of African locust bean which also contribute in the development of the texture and flavours of fermented products [7, 8].The desired state of fermentation of condiment is indicated by breakdown of amino acids during fermentation. Amino acids are known to play a major role in taste and flavour development of foods [9].

Alkaline fermentation causes hydrolysis of protein to amino acids and peptides releasing ammonia which increases the alkalinity of the *Bacillus* species such as *Bacillus subtlis* (dominant species), *Bacillus licheniformis* and *Bacillus pumilus*[10, 11]. It is also reported that slight free amino acids increase during fermentation but higher fermentation temperature in losses of lysine and other amino acids. Fermentation may not increase the content of protein or amino acids unless ammonia or urea is added as a nitrogen source to the fermentation media [12].The preservative and flavour characteristics of alkaline fermentation are derived in part from liberating of ammonia and increased p^{H}[13].

Different bacteria can tolerate different temperatures which provide enormous scope range of fermentation [14].While most bacteria have temperatures optimum of between 20ºc -30ºc, there are some (the thermophiles) prefer higher temperatures and those of colder temperature optimal (15ºc -20ºc). Most lactic acid bacteria work best at a temperature of 18ºc-22ºc. The *Leuconostoc* species that initiates fermentation survive an optimum of 18ºc -22ºc. Temperature above 22ºc favours certain *Lactobacillus* species [15].Every bacterial species have specific growth temperature requirement which is largely determined by the temperature requirement of its enzymes and their survival is also based on that [16].Though many members of micro flora contribute to ecology of locust bean but not all of them survive until the end of the fermentation presumably because of modified environment which developed at different stages [5, 17]. The co dominance of *Staphylococcus* and *Bacillus* species are survivals of fermentation of African locust bean [5].There is usually a general increase in microbial population throughout the fermentation period [18].The multiplication of microorganisms is foods are greatly influenced by intrinsic factors and environmental characteristics of foods [19].

The bacteria responsible for the fermentation in African locust bean have been identified to be *Bacillus* and *Staphylococcus* species [20]. *Bacillus* species were the most predominant species and they produced the highest ammonical smell characteristics of typical indigenous food condiments. *Bacillus* species are responsible for alkaline fermented foods condiments [21,22].Such *Bacillus* includes *Bacillussubtilis*, *Bacillus pumilus,Bacillusbrevis,Bacillusmacrena,Bacilluspoly myxa* and *Bacillus licheniformis*[23-25].It has been reported the presence of lactic acid in traditionally fermented African locust bean [26]. *Lactobacillus mesenteriodes* has been isolated [27]. Lactic acid bacteria from fermented African locust bean may play preservation role in the final product [27].

Aims

- Production of the locust bean spice by fermentation at different temperatures
- To determine the microbial diversity in African locust bean when exposed to different fermentation temperatures.
- To determine the effect of different fermentation temperatures on the amino acid content of African locust bean.

MATERIALS AND METHODS

Sample collection:

African locust bean samples used in this work were bought from Ogbete main market in Enugu

state. The samples were taken to laboratory for further tests to be carried on them.

Fermentation process involved in African locust bean

Traditionally, fermentation of African locust bean involves boiling the beans for 12hours in excess water until they are very soft to allow for hand dehulling after which the separated cotyledons is boiled for another 2hours to soften it. The cotyledons is then wrapped with enough banana leaves (*Musa saplentum)* and packed with cover to ferment at different temperatures (32ºc, 37ºc and 40ºc) for 3-5days [5].The various samples were appropriately labelled based on the temperature of exposure.

Preparation of samples

The samples of the fermented African locust beans from different temperatures were taken aseptically. 1g of each of the samples collected was thoroughly mashed with laboratory pestle and mortar and then mixed in 9mls of sterile distilled water as diluents in a test tubes and contents shaken. After homogenization, subsequent serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) were made from solutions by adding serially 1ml of solutions from preceding concentration to 9mls of diluents using sterile syringes into testubes. This was done to reduce the microbial population sufficiently to obtain separate colonies while platting [28].

Isolation techniques

The technique used in the isolation of the microorganisms present in the mashed sample is the pour plate method of which is used to determine the microbes in a sample.

Nutrient agar is prepared according to the manufacturer's instructions. The plates were labelled appropriately according to the dilutions made. 1ml of innoculum was poured in an empty sterile plate. Then 10mls of sterile warm (45ºc) nutrient agar was poured into the plates containing the innoculum and mixed gently. The plates were allowed to solidify and then inverted and incubated at 37ºc for 24hrs in an incubator.

Colonies obtained after incubation is sub cultured on the fresh nutrient media using the streak method to obtain pure and discrete colonies for easy identification. Each similar colonies of the pour plate culture was transferred to the edge of an agar plate (with inoculating wire loops containing nutrient agar). It is called the 'point of inoculation' at this point. Then with the wire loop, it was streaked out over the surface. After the first sector is streaked, the inoculating loop was sterilized and an innoculum for the second sector is obtained from the first sector. It is then incubated at 37ºc for 24hrs. The pure culture of organisms isolated was preserved on agar slants at refrigeration temperature (4ºc) [28].Pure culture isolated was each transferred into agar slants for future purpose and subsequent biochemical test for identification [28].

Identification of isolates

Morphological, physiological, microscopic, colour atlas and biochemical examinations are all used to identify and distinguish the isolates [28].

BACTERIAL IDENTIFICATION

Biochemical tests

1. **Gram staining:**

The test was used to distinguish gram negative and gram positive organisms [29].Gram staining is not a simple stain but rather it is known as a differential technique. A differential technique is a process that distinguishes between varieties of microbial organisms based on their ability of their cell wall to hold certain dyes. The gram staining technique depends upon the ability of a microbial cell wall to resist decolourisation. The gram stain consists of a primary stain, normally crystal violet, applied to a heat fixed or alcohol fixed smear. The substance that increase the reaction between the stain and cells (mordant) is then applied, normally Gram's iodine. This is then followed with quick water rinse and the application of a decolouriser (usually 95% ethyl alcohol). This will remove the colour from the

cells. Gram negative cells should decolourise when a gram positive organisms will not. The application of decolourisation is then followed by another quick rinse. A counter stain, usually, Safranine, is then applied. Excess safranine is often rinsed off but it should be noted that water is itself a mild decolourizes and long, excessive rinsing can alter the results of the staining procedure.

Staining procedure

- Thin smear is made on a clean grease free slide. The smear was air dried, after air drying; it was fixed by rapidly passing the smear over the flame of Bunsen burner for 3 times.
- The fixed smear was covered with crystal violet for 60 seconds.
- Excess of the stain is rinsed off briefly with water.
- The slide was flooded with dilute gram's iodine solution and leave for 60 seconds.
- After 60 seconds, it is rinsed off with water.
- Alcohol (decolouriser) is used to decolourise the smear to remove extra stain, it is left for 1-2 seconds
- The smear is flooded with clean water
- Safranine stain is used or applied and allowed for 60 seconds.
- Excess safranine stains is rinsed off with water and allow the slides to dry.
- The slides are observed under microscope using oil immersion and 100x objectives [30].

2. Motility staining

The motility test is often used to distinguish certain bacteria. The motility determines the presence of flagella, external appendages used by bacteria for movement. Bacteria with flagella such as *Proteus mirabilis*, are said to be motile, while bacteria without flagella such as *Staphylococcus epidermidis* are said to be non motile [30].The method of choice is stab method.

The medium used is semi solid because of the low concentration of agar, allowing movement of motile organisms through the medium. An isolate is inoculated into a tube with a sterile transfer needle. The needle is inserted and withdrawn in a straight line in the centre of the medium. The tube is inoculated at 37s°c for 24-48 hrs before examining the growth along the line.

Procedure

- A testube of prepared motility media is brought to a room temperature.
- The testube is labelled accordingly.
- The needle is sterilized by flaming, and a small bit of growth is obtained from the stock culture and is stuck straight down into the appropriate media going straight in to the media and back out
- It is then incubated at 37°c for 48hrs.
- Examine tube for growth pattern.

Bacteria with flagella spread away from the line of inoculation when the tube is held up to the light; growth is seen macroscopically as turbidity extending through the semi solid medium. Growth away from the line of inoculation indicates that the organism is motile. Bacteria without flagella do not spread away from the line of inoculation, so their growth does not extend into the medium. Growths along the line of inoculation only indicates that the organisms is non motile [30].

3. Catalase **activity**:

Catalase is the name of an enzyme found in most bacteria that initiates the breakdown of hydrogen peroxide (H_2O_2) into water (H_2O) and free oxygen (O_2). The test is done to check if the isolate produces the enzyme catalase that can break down hydrogen peroxide into water and oxygen.

Method

Few drops of hydrogen peroxide were dropped on a clean glass slide and pure colony was also transferred and emulsified with hydrogen peroxide. Thereaction is observed immediately for gas production indicated by production of gas bubbles which confirms the presence of catalase.

4. Citrate utilization test:

Different groups of microorganisms have different sources of carbon which they use for metabolism. This test is used to isolate and differentiate organisms in Enterobactericeae and most other genera that use citrate as sole carbon for metabolism [29].

The medium under this test is the Simon's citrate. Slant tubes of Simon's citrate were inoculated with young culture of isolates. The inoculation was done by stabbing the medium in the testube using straight inoculating wire loop containing the culture. The tubes were then incubated at 37ºc for about 24hrs of incubation. Change of colour from green to blue after 24hrs of incubation indicates positive results.

5. Coagulase test:

The test is used to identify *Staphylococcus aureus*which produces the enzyme Coagulase that converts fibrinogen into fibrin in plasma.

Two drops of distilled water was dropped on each end of a clean slide. A sterile loop is used to collect a colony of the test organisms and emulsify on the drops to make thick suspensions and mix gently. Clumping of the organisms was looked out for within 10seconds. No plasma was added to the second suspension to differentiate any granular appearance from the true clumping [31].

6. Sugar fermentation:

This test is carried out to determine the organisms that metabolize sugar with production of a gas. The following sugars are used; glucose, maltose and sucrose are used to carry out the test. 0.2g of these sugars is used to carry out the test. 0.2g of these sugars is dissolved in 20mls of peptone water solution. A pinch of bromocresol purple is added as indicator and 5mls of the aliquots is dispensed into bijou bottles containing Durham's tubes and autoclaved. It is allowed to cool and then inoculated with test organisms using sterile wire loops and afterwards incubated at 30ºcfor 48hrs.

A change of colour from purple to yellow indicates positive acid production and gas production was shown by downwards displacement of liquids in the Durham's tube[31].

7. Indole test:

Indole is one of the products produced when the amino acid tryptophan is hydrolysed. The two other products are ammonia and pyruvic acid. Some coliforms are able to break tryptophan down because they contain tryptophanase (the enzyme responsible for hydrolysing tryptophan). Indole can often be degraded by certain bacteria and therefore should never sit longer 4-5 days. Following incubation for 24-24hrs, kovac's reagent is added to the peptone water. If the reagent forms a red ring, indole has been produced and thus the organisms contain tryptophan. This test was used to determine which of the isolate has the ability to split indole from tryptophan present in buffered peptone water. It is used to differentiate members of Enterobactericeae.

The tubes of peptone water were inoculated with young cultures of the isolates. The tubes were incubated at 37ºc for 24hrs. About few drops of kovac's reagent was added to 1ml of the culture tubes. Positive test was indicated by a red coloured ring that occurs immediately at the upper part of the tube[31].

Thin layer chromatography (TLC):

Chromatography is used to separate mixtures of substances into their components. Different compounds travel at different rates. It is used to determine the amino acid content of a mixture.

The thin layer chromatography is done as it says- using a thin, uniform layer of silica gel or alumina coated on a piece of glass, metal or rigid plastic.

Procedure:

A pencil line is drawn near the bottom of the plate and a spot or a small drop of the mixture is placed or spotted on the baseline of the thin layer plate and similar small spots of known amino acids are placed alongside and appropriately labelled (NB: only 5 amino acids can be used on one TLC plate). The plate is allowed to stand in a chromatography tank containing solvent (ethanol and water at 70:30 ratios also the tank should

contain a filter paper which helps to saturate the tank). The tank is sealed using a Vaseline jelly. Then the plate is allowed to stand and develop and the mixture or sample along with the amino acids. The solvent is allowed to rise until it almost reaches the top of the plate. That will give result to maximum separation of the dye components for this particular combination of solvent afterwards the TLC plate is removed. If the spots are not visible the chromatogram is allowed to dry and is sprayed with a solution of ninhydrin. Ninhydrin reacts with amino acids to give coloured compounds mainly brown or purple. Then afterwards, R_f can now be determined. After determining the $R_f$of both the mixture and that of the amino acids, a comparison will be made to find out amino acids which their R_f values are very close to that of the mixtures and those that their values are closely related to the mixtures are said to be amino acids contained in the mixture. Fig 2 shows the thin layer chromatography apparatus.

Measurement of R_f:

This R_f measures the distance travelled by the solvent divided by the distance travelled by individual spots. When the solvent gets close to the top of the plate, the plate is removed from the beaker and position of the solvent is marked with another line using a pencil before it has a chance to evaporate.

R_f= distance travelled by individual spots / distance travelled by solvent front.

RESULTS

At the end of the fermentation of 'dawadawa' from African locust beans (*Parkiabiglobosa*) together with the culture and identification test carried out, different species of microorganisms were isolated and identified using various identification test for different temperatures. Microorganisms isolated include *Bacillus* sp, *Staphylococcus aureus, Micrococcus* sp, *Shigella*sp, *Flavobacterium*sp,*Erwinia*sp and *Lactobacillus* sp. These organisms produced or isolated vary in their diversity in different temperatures as in tables 1 and 2. Their cultural, morphological and biochemical properties are shown in tables 1 and 2 also their diversity and occurrence at different temperatures (25ºc, 32ºc, and 40 º c) were shown on tables 3, 4, and 5 . The amino acid content of these samples at different temperatures is shown in table 6.

At 25ºc, 6 different species of organisms were identified (*Bacillus sp, Staphylococcus aureus, Micrococcus* sp, *Shigella*sp, *Flavobacterium*sp,*Erwinia*sp); at 32ºc, the population reduced to 5 different species of organisms with the emergence of *Lactobacillus* sp. The organisms isolated identified at this 32ºc includes *Bacillus* sp,*Staphylococcusaureus, Micrococcus* sp, *Flavobacterium*sp *and Lactobacillus* sp. At 40ºc, the organisms reduced to only 2 i.e. the population of the organisms identified reduced to 2 which include *Lactobacillus* spand*Bacillus* sp.

The results shows that *Bacillus* sp were isolated at all the temperature with both 25ºc, 32ºc and 40ºc. The *Staphylococcus aureus, Micrococcus* spand*Flavobacterium*sp present in 25ºc and 32ºc but not found at 40ºc. *Lactobacillus* spwas identified in 32ºc and 40ºc but was not present in 25ºc. The amino acid content of the samples at different temperature varies also with different temperatures. At 25ºc, 2 amino acids were found which includes Arginine and Proline while at 32ºc, Arginine and Cystine were detected but at 40ºc only Arginine was detected.

Table 1: Morphological properties of the bacteria isolates

Temperature	Isolation code	Colour	Shape	size	elevation	Edge	Surface colony	opacity	Gram stain	Probable organism
25°c	ALB 1	Creamy	Round	large	Flat	Irregular	Rough	opaque	Gram+ve rod	*Bacillus* spp
	ALB 2	Creamy	Round	small	Slightly raised	Irregular	Smooth	translucent	Gram +vecocci	*Staphylococcus* spp
	ALB 3	Yellowish	Round	small	Slightly raised	Entire edge	Smooth	opaque	Gram +vecocci	*Micrococcus* spp
	ALB 4	Creamy	Round	small	Raised	Entire edge	Smooth	opaque	Gram –ve rod	*Shigella* spp
	ALB 5	Creamy	Round	small	flat	Irregular	Smooth	translucent	Gram –ve rod	*Flavobacterium* spp
	ALB 6	White	Round	small	Slightly raised	Entire edge	Smooth	translucent	Gram-ve rod	*Erwinia* spp
32°c	ALB 1	Creamy	Round	large	Flat	Irregular	Rough	opaque	Gram+ve rod	*Bacillus* spp
	ALB 2	Creamy	Round	small	Slightly raised	Irregular	Smooth	translucent	Gram +vecocci	*Staphylococcus* spp
	ALB 3	Yellowish	Round	small	Slightly raised	Entire edge	Smooth	opaque	Gram +vecocci	*Micrococcus* spp
	ALB 4	Creamy	Round	small	Slightly raised	Round	Smooth	opaque	Gram+ve rod	*Lactobacillus* spp
	ALB 5	Creamy	Round	small	flat	Irregular	Smooth	translucent	Gram –ve rod	*Flavobacterium* spp
40°c	ALB 1	Creamy	Round	small	Slightly raised	Round	Smooth	opaque	Gram+ve rod	*Lactobacillus* spp
	ALB 2	Creamy	Round	large	Flat	Irregular	Rough	opaque	Gram+ve rod	*Bacillus* spp

Key : ALB= African Locust Bean

Table 2: Biochemical properties of the bacteria isolates

Temperature	Isolates	Catalase	Coagulase	Indole	Citrate	Motility	Sugar fermentation: Maltose	Sugar fermentation: Sucrose	Sugar fermentation: Glucose	Organisms
25°c	ALB 1	+	_	_	+	+	A	A	A	*Bacillus* sp
	ALB 2	+	+	_	_	+	A	A	A	*Staphylococcusaureus*
	ALB 3	+	_	_	_	_	A	A	A	*Micrococcus* sp
	ALB 4	+	_	_	_	_	A	A	A	*Shigella*sp
	ALB 5	+	_	+	+	+	A	A	A	*Flavobacteriumsp*
	ALB 6	+	_	_	+	+	AG	A	A	*Erwinia*sp
32°c	ALB 1	+	_	_	+	+	A	A	A	*Bacillus* sp
	ALB 2	+	+	_	_	+	A	A	A	*Staphylococcus aureus*
	ALB 3	+	_	_	_	_	A	A	A	*Micrococcus* sp
	ALB 4	_	_	_	_	_	A	A	A	*Lactobacillus*sp
	ALB 5	+	+	+	+	+	A	A	A	*Flavobacterium*sp
40°c	ALB 1	_	_	_	_	_	A	A	A	*Lactobacillus*sp
	ALB 2	+	+	_	+	+	A	A	A	*Bacillus* sp

Key: += positive; - = negative; A= acid only; AG= acid and gas; ALB= African Locust Bean

Table 3: Occurrence of the bacteria isolates at 25°c

Isolates	Number of isolate	Percentage of occurrence
Bacillus sp	3	30%
Staphylococcus aureus	2	20%
*Micrococcus*sp	2	20%
*Shigella*sp	1	10%
*Flavobacterium*sp	1	10%
*Erwinia*sp	1	10%
Total	10	100%.

Table 4: Occurrence of the bacteria isolates at 32°c

Isolates	Number of isolates	Percentage Occurance
Bacillus sp	2	29%
Staphylococcus aureus	2	29%
*Micrococcus*sp	1	14%
*Flavobacterium*sp	1	14%
*Lactobacillus*sp	1	14%
Total	7	100%

Table 5: Occurrence of the bacteria isolates at 32°c

Isolates	Number of isolate	Percentage Occurrence
*Lactobacillus*sp	3	60%
*Bacillus*sp	2	40%
Total	5	100%

Table 4: Amino acid content of the samples at different temperatures.

Amino acids	Different temperatures 25°c	32°c	40°c
Alanine	–	–	–
Serine	–	–	–
Asparagine	–	–	–
Arginine	+	+	+
Glycine	–	–	–
Aspartic acid	–	–	–
Valine	–	–	–
Leucine	–	–	–
Methionine	–	–	-
Isoleucine	–	–	–
Proline	+	–	–
Phenyl alanine	–	–	–
Tyrosine	–	–	–
Cystine	–	+	–
Threonine	–	–	–
Lysine	–	–	–
Histidine	–	–	–
Tryptophan	–	–	–
Glutamic acid	–	–	–
Glutamine	–	–	–

Key: += Positive (hence contain the amino acid); _=negative (hence do not contain the amino acid).

DISCUSSION

Fermented foods constitute a major portion of the traditional Nigerian diet [32].'Dawadawa' is a smelly product from the fermentation of African locust beans (*Parkiabiglobosa*) and is a seasoning agent used in small quantities in local soups, porridge yam e.t.c. by Igbos of eastern Nigeria.

The microorganisms involved in the fermentations of African locust beans includes, *Bacillus* sp, *Staphylococcus aureus, Micrococcus* sp,*Shigella*sp, *Flavobacterium*sp,*Erwinia*sp and *Lactobacillus* sp. This work is similar to the work of Achi (2005)[5] who isolated *Bacillussp*and *Staphylococcus aureus*. Also it is related to the work of Mulyoridarso (1999)[17] who isolated members of Enterobactericeae such as*Shigellasp* but they could not survive until the end of the fermentation presumably because of the modified environment which has developed at later stage. At the temperature of 25°c which is usually a very favourable temperature for the growth of microorganisms, there were abundance of microorganisms isolated at this temperature, this includes; *Bacillus* sp, *Staphylococcus aureus, Micrococcus* sp,*Shigella*sp, *Flavobacterium*sp, *Erwinia*sp. As the temperature increases, it widens the diversity of bacteria present as well as decrease the amount of organisms found hence only *Bacillus* sp, *Staphylococcus aureus, Micrococcus* sp*andFlavobacterium*sp and also there is an emergence of *Lactobacillus* sp at this temperature (which is 32°c). At the temperature of 40°c, the population of the bacteria identified decreased to only *Bacillus* spand*Lactobacillus* sp. The survival of *Bacillus* sppat this temperaturemust be due to its ability to produce spores that can survive at high temperature. Atai and Ibrahim (1996) isolated similar organisms (*Bacillus* spand*Lactobacillus* sp) during theirfermentation at 40°c[42]. Also Uzoigwe and Ekwealor (2002)[34] also isolated similar organisms during their study at 40°c. The disappearance or decrease in microbial population can be attributed to decrease in oxygen tension as temperature increases [35]. The isolation of *Lactobacillus*sp in African locust bean fermentation has been reported by Uaboi-Egbeni*etal*(2009)[27]. The presence of lactic acid bacteria in fermented African locust bean may play a preservative role in the final products [27].

The isolation of *Erwinia specie andFlavobacterium specie* has never been reported from fermenting oil seeds before, however, they are known to be common contaminants of fresh vegetable matter. Nwokedi and Adichie (1997)[34] isolated *Staphylococcus aureus* and *Micrococcus* sp in their fermentation of a related castor oil seeds (*Ricinuscommunis*) to 'Ogiri' ('Ogiri' is the Igbo name for fermented castor oil seeds.). In this study, the isolation of *Shigellasp*agreed with the findings of Uzoigwe and Ekwealor (2002)[33] that in addition to *Shigellasp* isolated *Escherichia coli* and *Klebsiellasp*from 'dawadawa' production.

The isolation of Coagulase positive *Staphylococcus aureus* from fermenting the African locust bean seeds is a health concern as the organisms is to cause food poisoning [35].

The process of 'dawadawa' production is a nutrifactive one involving end products like amino acids, ammonia, hydrogen sulphidee.t.c which also results to the characteristic offensive odour of fermented 'dawadawa'.

Bacillus spp and other microorganisms aided in the production of 'dawadawa' and this observation is also in consonance with the report of Obeta (1998)[36].*Bacillus* spp are known to be important sources of proteases and amylases (Forgarty and Griffin, 2003) [37]and this may be the reason why the seeds and soften and easily fermented.

Soluble low molecular weight peptides and amino acids that contribute to flavour are produced through enzymatic breakdown of proteins[8, 38].However, there is slight increase in amino acids at 25°c and 32°c but higher temperature led to loss of amino acids. This relates to the work of Wiarno and Reddy (2001)[39] which says that free amino acids increase but higher fermentation temperature results to losses of lysine and other essential amino acids. However, the results of this work is

contrary to the work of Eka (1980) [40]who studied effect of fermentation on the nutrient content of locust beans and reported that increased level of amino acids were produced except Arginine.

There was predominance of Arginine and this is similar or this result is similar to the result from Aidoo (1996)[41]. The presence of Arginine in the samples leads to increased ammonia production and hence a stronger characteristic offensive odour. The improved nutritive values were attributed to the increase in amino acid profile due to fermentation, but higher fermentation temperature and periods resulting to loss of essential amino[40].It should be noted that the organisms isolated in this work might not be the only ones involved in the fermentation of African locust beans. The organisms present may also vary depending on various factors such as air, water, utensil, used in the processing e.t.c. but to an extent, an asceptical environment is ensured.

In conclusion it can be said that the organisms vary with increase in temperature, the higher the temperature, the lower the population of organisms present in the fermentation. Arginine is found to be most predominant of all the amino acids present in the samples at different temperatures. This implies that indeed different temperatures affect the diversity of microorganisms present in African locust bean as well as the type of amino acids present in the samples. Therefore the aim of this research was carried out successfully.

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