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Soy-Dawadawa Production by Traditional Spontaneous Fermentation inside Pieces of Jute Sack

Efunwoye O.O.[1*], Oluwole O.R.[2], Alagbe V.O.[3], Olawale K.S.[4], Ajibola O.O.[5], Omotoyinbo B.I.[6]

[1,2,3,4,5,6]Basic Sciences unit, Department of General Studies, Federal College of Agriculture, Akure, Ondo State, Nigeria

[*]*Corresponding Author: majorbode@yahoo.com, Tel.: +234-8054723221*

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Abstract— Soybean is a highly nutritious legume that is easy to cultivate and process, which can provide a suitable substitute for African locust bean in the production of dawadawa, a widely consumed condiment in the West African region. Traditional spontaneous fermentation of soy-dawadawa using jute sack was carried out to study the effect on the microorganisms implicated and the activities of enzymes involved in the process. The total microbial count of the microorganisms involved in the fermentation increased with fermentation time (72 hr). The only microorganism isolated was *Bacillus subtilis*, without identifying any lactic acid bacteria nor fungi. The pH of the fermenting soybean rose from 6.60 to 8.04, in an alkaline fermentation process. Moisture content in the fermenting bean exhibited significant increase until 48 hr of fermentation, after which it declined. The crude fibre content displayed an irregular pattern over the fermentation period. Crude protein in the soy-dawadawa significantly increased with fermentation time, which increased digestibility of the soybean. The ash content showed no regular pattern across fermentation period, while the crude lipids content reduced significantly with fermentation period. The carbohydrates content showed no significant change from 0 hr to 24 hr but increased significantly at that point to the end of fermentation. Protease activity increased from 0.006297 mg/ml/min (0 hr) to 0.013524 mg/ml/min (72 hr) to explain the observed increase in the protein content. Lipase activity rose from initial 0.262674 mg/ml/min and ended at 1.759916 mg/ml/min which shows direct relation to the reduction in the crude lipid content. Trypsin inhibitors in the soybean dropped with fermentation time with the values at 32.31 % (0 hr) and 11.58 % (72 hr). Fermentation time had influence on the fermentation parameters. Fermentation of soy-dawadawa in jute sack led to an improvement in the overall nutrition and digestibility of the soybean.

Keywords— fermentation, soybean, dawadawa, proximate, enzymatic

I. INTRODUCTION

Soybean, botanically known as *Glycine max*, is a leguminous plant. It originated from northeast Asia, specifically China [1]. Legumes are important sources of protein, carbohydrates, dietary fibre and minerals [2]. The soybean, in terms of economic value, is the most important legume in the world. It is a common source of vegetable protein for millions of people. Soybean proffers the richest and cheapest source of protein in the main diets of people and animals in numerous parts of the world [3]. Low income earning families often use it generously as an easily affordable source of protein and meat substitute [4]. [5] reported that the quality of protein in soybean can be comparable to animal proteins such as meat and milk. Soybeans produce significantly more protein on an agrarian acre than most other land uses [6]. Soybean contains approximately 40-45% protein and 18-22% oil [7] and is a rich source of vitamins and minerals. Raw soybean contains a high quantity of anti-nutritional factors such as trypsin inhibitors, phytic acid and saponins, which reduce the nutritive value of grain legumes and cause health detriments to both human and the animals when taken in large amounts [2]. In order to improve the nutritional quality and increase the organoleptic appeal of soybean, these anti-nutrition factors should be removed from the bean. Several processing methods such as roasting, cooking, extruding, salt treatment, fermentation, germination pressure cooking, cooking, soaking, urea treatment help to improve the nutritive value of soybean and reduce the quantities of these anti-nutritive factors [8, 9, 10].

Dawadawa is a food condiment widely consumed in West Africa. It is traditionally produced from locust beans (Parkia biglobosa) in Nigeria. Dawadawa, as a condiment, enhances the taste of soups and provides a cheap protein source in the diets of low income families [11]. Soybean is gradually used as a substitute to locust beans for dawadawa production across West Africa, and especially in Nigeria. Soybean is easier to grow, can be cultivated all year round and has softer seed coat which allows for easier dehulling during dawadawa production, while the cotyledon is softer, requiring shorter boiling time and energy [4]. Soy-dawadawa is widely consumed in Benue, Kogi and Plateau states (North central Nigeria) and has spread to the southern parts in recent years. Dawadawa production involves pre-boiling the soybean, dehulling, cooking the cotyledons, fermentation and packaging [4, 10, and 11]. The traditional fermentation involves wrapping the cooked

cotyledons in banana leaves and placing in calabashes as fermentors. The resulting soy-dawadawa product is usually brownish in colour, sticky, with strong ammoniacal aroma [9].

Reported microbiological studies of soy-dawadawa production discovered the predominance of the *Bacillus* spp and lactic acid-producing bacteria as the main fermentative organisms [4, 10 and 11]. Fungi (yeasts) were not commonly isolated from the fermenting soybean. Other reported isolated microorganisms like *Streptococcus faecium* and *Staphylococcus aureus*, were traced to the utensils, water and surfaces involved in the production [11].

Most literature available on the traditional production of soy-dawadawa evaluated the effect of using banana leaves to ferment soybean on the microbiology of the soy-dawadawa and the nutritional composition. However, in Benue and Kogi states, there is a growing trend of wrapping fermenting soybeans with jute sack, with scanty literature investigating the effect on the final products. Also, few works have been done to profile the effect of fermentation on the anti-nutrition compounds in the fermenting soybean. This study was carried out to determine the effect of wrapping the fermenting soybean in pieces of jute sack, using plastic bowls as fermentors. In addition, the work focussed on the enzymatic activities occurring in the fermenting soybean and the effect of fermentation on the anti-nutritional constituents of soybean.

II. RELATED WORK

The effect of roasting and boiling on soybean fermentation was investigated by [4]. The objective of the study was to determine the effect of these treatments on the microbial profile and nutritional composition of the soy-dawadawa. Roasting and boiling methods are heat-dependent processes which may have significant effects on the overall fermentation process. The roasting and boiling showed slight effect on the microflora but a significant effect on the nutritional composition of the soy-dawadawa. The microbial profile of both the soy-dawadawa made from the roasted and boiled soybean were discovered to be similar, as proteolytic Bacillus species were the dominant fermenting microorganisms. The microbial profile of soy-dawadawa using starter culture of *Bacillus subtilis* and *Leuconostoc mesenteroides* was investigated by [9] with the biochemical changes and sensory evaluation further studied by [10]. Traditional method of soy-dawadawa production is spontaneous, which precludes the use of starter cultures. However, backslopping and the use of materials already impregnated with fermenting microorganisms is a common occurrence in the process, which help to initiate fermentation in freshly prepared substrates. Combination of the *Bacillus subtilis* and *Leuconostoc mesenteroides* provided the highest protein value when compared to fermentation runs using the isolates singly. That combination also produced the highest biochemical activities in the fermenting substrates with respect to protease and amylase activities. The presence of anti-nutrition factors like phytic acids, saponins and trypsin inhibitors in soybean was studied and established by [2] and [7], while effects of processing on these anti-nutrition factors in soybean was investigated by [8]. Legumes are known to possess substances which make the nutritional constituents unavailable for use when consumed. The activities of the anti-nutrition factors have remained a major contributor to malnourishment in populations that have legumes as part of their staple diets.These nutrition inhibiting compounds were found to be located in the testa and cotyledons of soybean. The presence of the anti-nutrition factors in soybean pose health challenges to both human and animals when soybean is consumed.

III. METHODOLOGY

Sample Collection

Soybean (*Glycine max*) was purchased from Oba Adesida market, in Ondo State, Southwest Nigeria. The beans were manually cleaned by removing foreign materials, and sorted by selecting the bad seeds.

Location of the work

Most of the laboratory work were conducted in the Biology laboratory of the Federal College of Agriculture, Akure (FECA). The enzymatic and trypsin inhibitory activity analyses were carried out in the Biotechnology laboratory of Federal University of Technology, Akure (FUTA).

Laboratory fermentation of Soybean

1 kg of cleaned and sorted soybean was washed and pre-boiled in a pressure cooker over a gas burner flame for 30 mins. The pre-boiled soybeans were allowed to cool and dehulled manually under running tap water. The exposed cotyledons were cooked for 1 hr 30 mins and boiling water allowed to dry on the beans before turning off the fire. This method ensures that nutrients in the soybeans were retained [4]. The cooked soybeans were allowed to cool to about 40 ^{0}C. 50 g were weighed out aseptically on a top-loading balance and wrapped in sterilized pieces of jute sack. The wraps were tied up with pieces of twine ropes to ensure that the soybean was not exposed to contamination by air. The wraps were placed in clean, disinfected plastic bowls that served as fermenters, and covered. The bowls were labelled according to hour of fermentation of the soybean, with each bowl containing three wraps. The fermenters were placed in an incubator at 28 ^{0}C for 72 hr, with samples taken out at 12-hr intervals for microbiological, proximate and enzymatic studies.

Isolation and characterization of Microorganisms

1 g of the fermenting soybean was carefully transferred into a test tube containing 9 ml of 1 % sterile peptone water. The soybean was mashed into bits inside the tube with the aid of a sterile glass rod until a good distribution was obtained. 1 ml of the aliquot was then taken and was serially diluted in tubes holding 9 ml of 1 % sterile peptone water to the factor of 10^{-10}. 1 ml aliquot was pipetted from

each tube containing 10^{-6}, 10^{-8} and 10^{-10} dilution factors, and transferred into sterile petri dishes which had been accordingly labelled. Molten forms of the sterilized growth media were transferred to the inoculums by pour plate method. Nutrient Agar (NA) was used for the total viable count, Man deRogosa Sharpe Agar (MRS) for lactic acid bacteria count and Potato Dextrose Agar (PDA) for the fungal growth count. For each growth medium, the petri dishes were labelled in duplicates. The inoculated NA and MRS petri dishes were incubated at 30 ^{0}C for 48 hr, inside an incubator. The inoculated PDA petri dishes were incubated at room temperature for 72 hr.

Gram staining of isolates and biochemical characterization were carried out. The biochemical tests include catalase, coagulase, indole utilization, oxidase production, methyl-red and cirate production tests, sugar utilization test, and spore staining. Identification was achieved using Bergey's Manual of Determinative Microbiology. Microbial count was carried out manually.

Proximate studies

Moisture**:**

The moisture content of the sample was determined using hot air oven [12].The petri dishes were washed and dried in hot air oven at 60^{0}C. The dishes were transferred into the desiccator and left to cool. Weights of the petri dishes were determined. 3 g of sample was weighed in to a dry petri dish and transferred into an oven, maintaining a temperature of 105 ^{0}C. The content were dried at this temperature for 6hrs. The petri dishes with their contents were removed from the oven and placed in the desiccator. After cooling, the content was dried to constant weight, and the final weight recorded. The percentage of moisture was determined from the following equation:

$$\text{Moisture \%} = \frac{\text{Original sample weight (g)} - \text{Dried sample weight (g)}}{\text{Original sampleweight (g)}} \times 100$$

Ash:

Clean and dry crucibles were ignited at 350 ^{0}C for about 15mins, cooled in a desiccator and weighed. 1 g of sample was transferred into each of the appropriately labeled crucibles and then reweighed. After, the crucibles with their contents were transferred into the muffle furnace at 550 ^{0}C for about 5hrs. After complete ashing, the crucibles were cooled in a desiccator and the weight retaken. The percentage of ash was then calculated [12].

$$\text{Ash content (\%)} = \frac{\text{Weight of crucible with ash (g)} - \text{Weight of empty crucible (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude protein:

Crude protein of the samples was estimated by using Kjeldahl. A sample of 0.5 g and a blank was estimated in the digestion tube. The sample was digested at a high temperature, therefore, 10 ml of concentrated sulfuric acid and 1.1 g digestion mixture were added in the tube. Then the digestion tubes were set in digestion chamber fixing at 420^{0}C for 45 minutes ensuring water supply, easier gas outlets etc. After digestion the tubes were allowed to cool and 5 ml of sodium thio-sulphate ($Na_2S_2O_3$, 33 %) and 30 ml sodium hydroxide (NaOH) solution was added in each tube. Then the distilled extraction was collected with 25 ml of Boric acid (4 %) and titrated with standard hydrochloric acid (0.2 N). The nitrogen values obtained was converted into percentage of crude protein by multiplying with a factor of 6.25 assuming that protein contains 16 % nitrogen [12].

$$\text{\% Nitrogen} = \frac{\text{Milliequivalent of nitrogen (0.014)} \times \text{titrant value (ml)} \times \text{strength of HCL}}{\text{Sample weight (g)}} \times 100$$

$$\text{\% Crude protein} = \text{\% Nitrogen} \times 6.25$$

Crude lipid:

Crude lipid was determined by extracting a weighed quantity (3 g) of samples with analytical grade acetone in ground joint Soxhlet apparatus. Extraction was carried out by heating in the electric heater at the temperature of 70 ^{0}C until clear acetone (without oil) was seen in siphon, in a process that took about 3 hours. After, the round bottom flask of the apparatus was detached and the extract was transferred to a pre-weighed beaker and left for evaporation of acetone. After the evaporation of acetone, only the lipid was left in the beaker which was later calculated in percentage [12].

$$\text{\% Crude lipid} = \frac{\text{Weight of beaker with lipid} - \text{Weight of empty beaker}}{\text{Weight of sample (g)}} \times 100$$

Crude fibre:

A small amount of finely ground sample (2 g) was taken into a filter crucible and was inserted into the hot extraction unit (Hot Extractor, Model-1017). Sufficient amount of pre-heated 0.128M H_2SO_4 was added into the reagent heating system and few drops of octanol were added through the valves. The mixture was digested for 30 minutes. Acid was then removed from it by filtering and washing with boiling water. The residue in the flask was boiled with required amount of 0.223 M potassium hydroxide solution (KOH) for 30 minutes and then filtered with subsequent washing in boiling water and acetone. The residual content was then dried in an oven at 105 °C for a few hours and then ignited in muffle furnace at 550 °C for 3 hours. The loss of weight represented the crude fibre [12]. Then percent crude fibre was calculated by the following formula:

$$\text{Crude fibre (\%)} = \frac{\text{Oven dried weight of sample (g)} - \text{Ash weight of sample (g)}}{\text{Weight of sample (g)}} \times 100$$

Carbohydrate:
Carbohydrate (CHO), a soluble carbohydrate was calculated by subtracting the sum of the percentage contents of moisture, crude protein, lipid, ash and crude fibre from 100.
CHO % = {100- (moisture + crude protein + crude lipid + ash + crude fibre)} [12].

pH
1 g of the sample was mashed in sterile distilled water (100 ml) with the aid of a sterile glass rod. The digital table-top pH meter was calibrated at two-point calibration with buffer solutions 4.88 and 10.8 respectively. The electrode was dipped in the sample to take the pH when the display stabilized.

Enzymatic studies
Protease assay:
1 % casein was prepared in 0.05 citrate phosphate buffer of pH 7.5. The solution was heat- denatured at 100 ^{0}C for 15minutes in water bath.

1ml of 1 % casein was pipetted into test tubes. It was incubated at 37 ^{0}C for 15 mins. 0.2 ml of the enzyme solution was added and allowed for one hour inside the water bath. 3 ml of 10 % Trichloroacetic acid (TCA) was added to terminate the reaction. The tubes were centrifuged at 3000 rpm to remove the undigested protein and the supernatant was read at 280 nm for precipitated protein hydrolysates using UV spectrophotometer [13].

Lipase determination:
Lipase activity was determined with p-nitrophenyl palmitate (pNPP) by the method reported by [12]. The substrate for this reaction was composed of solution A and solution B. Solution A contained 40 mg of pNPP dissolved in 12 ml isopropanol. Solution B contained 0.1 g of gum arabic and 0.4 ml of triton X-100 dissolved in 90 ml of water. The substrate solution was prepared by adding 1 ml of solution A to 19 ml of solution B drop wise with constant stirring to obtain an emulsion that remains stable for 2 hr. The assay mixture contained 1 ml of the substrate, 0.5 ml of buffer (glycine-NaOH, pH 11, 0.5 M), 0.1 ml of enzyme (the filtrate) and the volume was made up to 3 ml with distilled water. This was incubated at 40 ºC for 45 min. The enzyme activity was stopped by adding 0.2 ml of isopropanol. The absorbance was measured at 410 nm against substrate free blank. The standard graph was prepared by using para-nitrophenol (0.4 to 4 µmoles). One lipase unit (U) is defined as the amount of enzyme that liberated 1 µmol p nitrophenol per min under the assay conditions described [14].

Determination of trypsin inhibitor:
Trypsin inhibitor was extracted by mixing 1g of the sample with 50ml of 0.01N sodium hydroxide solution (NaOH) at pH of between 8.4-10.0 and allowing the mixture to stand for 3h, while stirring at intervals. 2ml of diluted extract was then dispensed into test tubes to which 2ml of cold trypsin solution (4mg in 200ml of 0.001M HCl) was added, and the tubes were placed in water bath at 37 ^{0}C. Benzoyl-DL-arginine-P-nitro anilide hydrochloride (BAPNA) was prepared by dissolving 40 mg in 1ml of dimethyl sulfoxide and diluted to 100ml with tris buffer 0.05M (pH 8) and diluted to 100ml with tris buffer 0.05M (pH 8.2), and pre warmed to 37 ^{0}C . 5 ml of BAPNA was added as substrate to each tubes. After 10 min the reaction was terminated by adding 30 % acetic acid and the content of each tube were thoroughly mixed. Thereafter the content of each tube was centrifuged at 3000 rpm and the absorbance of the filtrate was measured at 410 nm against reagent blank. The reference was prepared in the same way as the sample except that 2ml of distilled water was added in place of extract. [15].

Statistical analysis
All mean values were calculated and the analyses of variance (ANOVA) was carried out using SPSS 20 version.

IV. RESULTS AND DISCUSSION

Table I shows the total microbial count of microorganisms in the soy-dawadawa fermentation. The microbial count of the microorganisms in the fermenting soy-iru increased progressively from 0 hr of fermentation with initial load of 1.2×10^7, and the highest total viable count was recorded at 48hr with a load of 3.98×10^8. After 48 hr, the count gradually declined until 72 hr of fermentation at which it dropped to 98×10^7. The result revealed that active fermentation occurred from the 12 hr and the peak of fermentation was during the 48 hr of fermentation having the highest microbial count. This trend in microbial count agrees with the work of [4, 9, 16], where microbial count increased with prolonged fermentation of soybean. Boiling process must have reduced the microbial population in the raw soybean. The increase in the total microbial count at the early hours of fermentation could be as a result of availability of high nutrient content, and the observed decrease at the latter hours could probably be due to combination of factors such as depletion of available nutrients and the accumulation of inhibitory metabolites in the fermenting soybean. As the nutrient profile in the substrate decreases, less of the microorganisms will be supported and some will die off. In addition, accumulation of metabolites from the activities of the fermenting microorganisms lead to inhibition of microbial growth.

Table 1: Total microbial count in fermenting soy-dawadawa

PERIOD OF FERMENTATION	**0 HR**	**12 HR**	**24 HR**	**36 HR**	**48 HR**	**60 HR**	**72 HR**
TOTAL VIABLE COUNT (cfu/ml)	1.2×10^7	1.24×10^8	2.08×10^8	1.75×10^8	3.92×10^8	1.62×10^8	9.8×10^7

The spontaneous fermentation of soybean revealed that *Bacillus subtilis* was the only fermenting organism implicated. This finding is at variance with [4] that

reported other species of Bacillus in addition to B. subtilis in the fermentation of soy-dawadawa. [16] used *B. subtilis* as the only starter culture in the fermentation of soy-dawadawa, while *B. subtilis* was used in mixture with other bacteria as starter cultures in the works of [9, 10]. [10] stated that *Bacillus* spp are the most dominant naturally fermenting agent in soybeans, which are also known to reduce activity of anti – nutrients that hinder availability of proteins and phytochemicals present in soybeans. In this study, lactic acid-producing bacteria were not detected, which is in contrast with the findings of [4, 11], which may probably be due to the absence of banana leaves and plastic sack lining in the fermenting container as was used by [4, 11] respectively; only jute sack was used to wrap the soybean which were placed in plastic bowls as fermentor, which is a novel method used in Benue and Kogi states in Nigeria, in soy-dawadawa production. The use of jute sack may also account for the absence of other enteric bacteria. The jute sack is not a natural surface which can promote the growth of wide variety of microflora in the environment. *Bacillus subtilis* has been found to exhibit protease activity that helps to increase the rate of hydrolysis of protein, which leads to the release of ammonia gas [17]. This mechanism produces an alkaline condition and soybean products are often termed "alkaline fermented soybeans" in which soy-dawadawa is inclusive, and these foods are generally regarded as safe for consumption [5]. No fungus was isolated in the fermentation study which agrees with the reports of [9, 11]. Lack of fungal growth may be due to the short period of incubation because fungi require longer period of incubation to grow.

The results of the proximate analysis of the iru samples are presented in Table 2**.**

Table 2: Nutritional compositon of soy-dawadawa with fermentation period

Nutrition composition/fermentation period	M.C. (%) Mean± SD	C.F. (%) Mean± SD	C.P. (%) Mean± SD	Ash (%) Mean± SD	C.L. (%) Mean± SD	CHO Mean±SD
0 HR	20.490^{d}	5.950^{ab}	12.580^{g}	2.670^{bc}	35.540^{a}	13.770^{ef}
12 HR	20.350^{d} ±0.127	4.750^{c}± 0.141	16.845^{f} ±0.417	3.880^{a}± 0.268	34.660^{b} ±0.268	13.015^{f}± 0.233
24 HR	24.030^{c} ±1.739	4.720^{c}± 0.183	17.525^{e} ±0.516	2.595^{bc} ±0.063	25.730^{c} ±0.254	14.150^{e}± 0.169
36 HR	28.495^{b} ±1.195	3.125^{d}± 0.021	18.845^{d} ±0.035	3.575^{a}± 0.190	24.590^{d} ±0.056	18.120^{d}± 1.088
48 HR	32.175^{a} ±0.332	5.055^{c}± 0.007	22.690^{c} ±0.169	2.465^{bc} ±0.219	23.580^{e} ±0.565	22.365^{c}± 0.275
60 HR	30.870^{a} ±0.848	5.560^{b}± 0.410	23.415^{b} ±0.247	2.335^{c}± 0.063	16.690^{g} ±0.169	24.605^{b}± 0.190
72 HR	30.705^{a} ±0.615	6.170^{a}± 0.141	25.500^{a} ±0.084	2.915^{b}± 0.247	17.770^{f} ±0.070	30.440^{a}± 0.311
P-value	0.000	0.000	0.000	0.000	0.000	0.000

Note: All mean values in each column with same superscript shows no significant difference at P ≤0.05. The mean values were calculated with the standard deviation
KEYS: M.C. – Moisture Content, C.F. –Crude Fibre, C.L.-Crude Lipids
CHO-Carbohydrates.

From Table 2, the moisture content in the fermented soy-dawadawa showed significant difference in values up to the 48 hr, after which it showed no significant difference till the end of fermentation. Moisture content value increased from the 0 hr to 48 hr and declined from 60 hr to the end of fermentation, exhibiting a similar trend with the report of [9]. Fermentative activities of microorganisms often lead to release of water in legumes. This observed trend could be due to the liberation of moisture by active fermentation from 0 hr to 48 hr, and the dip followed due to the microbes utilizing the moisture as fermentative rate declined. The condiment has a high moisture content which favours microbial growth, therefore, must be stored in a dry environment to elongate the shelf-life. The crude fibre content indicated an irregular pattern. The mean value initially dipped from 5.95 % to 3.125 % at 36 hr before attaining its peak with 6.17 % at 72 hr (Table II). Similarly, the soy-dawadawa fermented with only *Bacillus subtilis* as starter culture by [9] showed fluctuating trend. Fermentative activities of the bacterium liberates the insoluble fibre and converts it to soluble fibre. Crude protein showed significant difference in the mean values with the fermentation period. There was a progressive increase in the crude protein content of the samples from 0 hr to 72 hr (Table 3). Similar trend was observed in [5, 9, 11, 18]. Fermentation of legumes has been widely reported to lead to increase in protein content by breaking down fibre protein into soluble protein which can easily be assimilated in the body, thereby improving the digestibility of legumes. In addition, *Bacillus* spp are known to be proteolytic in activity leading to the release of free amino acids in fermented legumes, which improves the protein content [10].

The ash content exhibited an irregular trend, in a similar pattern as reported by [18]. [9] revealed an irregular pattern in the ash content of fermenting soy-dawadawa with different inocula and fermentor, which may indicate that effect of fermentation on the ash content of soy-dawadawa could be dependent on the fermenting organism.

The crude lipid content showed significant decline with fermentation time, ending with a slight increase at 72 hr (Table 2). This is in consonance with the report of [4]. Only a few reports on the trend in crude lipid content of fermented soy-dawadawa are available. The fermentation of soybean indicated a progressive breakdown of the fats content. This could be a desirable factor due to reduction in the chance of spoilage of the soy-dawadawa by rancidity. Carbohydrates content showed no significant difference from 0 hr to 12 hr but exhibited a significant increase from 24 hr with fermentation period, which is in agreement with the report of [9]. Also, [10, 16] reported increased total sugars with fermentation hours as a result of active amylase activities recorded. These evidences explained that fermentation of soy-dawadawa improves the carbohydrates content by breaking down the inaccessible polysaccharides into utilizable monosaccharides.

Table 3: Change in pH of soy-dawadawa with fermentation time.

Fermentation period (hr)	0	12	24	36	48	60	72
pH	6.60	7.23	7.30	7.64	7.58	7.80	8.04

From table 3, there was a progressive increase in the pH to alkaline region. This falls within the pH range reported for soy-dawadawa fermentation by [10, 16]. The increase of pH to alkaline region is probably due to the release of ammonia gas by the proteolytic activity of *Bacillus subtilis*, which is characteristic of legume fermentation.

Protease enzyme activity was measured in the samples and the trend showed a steady increase in the protease activity with fermentation (Fig.1).Similar trend in protease activity was observed by [4, 10]. Protease activity in fermenting soybean can be attributed to the secretion of exogeneous enzymes by the microbial flora responsible for the fermentation process. The progressive increase in protease activity explains the observed continuous increase in crude protein, which indicates that the protease enzymes actively hydrolysed complex proteins in the soybean into simpler and more abundantly detectable forms. Protein hydrolysis is reported to be the major biochemical change which occurs during iru fermentation of African locust bean [19]. *Bacillus subtilis* has been reported to produce about seven types of protease enzymes [4].

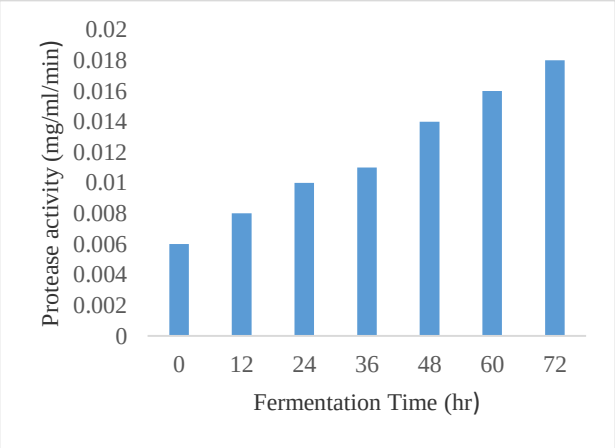

Figure 1: Trend of protease activity in soy-dawadawa with fermentation time.

From Fig. 2, the lipase activity in this work showed a gradual increase with fermentation time, which agrees with [10]. The progressive increase in the lipase activity indicates a continuous breakdown of lipids in the soybean to fatty acids which was observed in the decrease in crude lipids content as fermentation progressed (Table II). This activity reveals that lipid hydrolysis is one of the most important biochemical activities in soy-dawadawa fermentation. The hydrolysis of lipids in soybean is a desirable process due to the high content of oil in soybean.

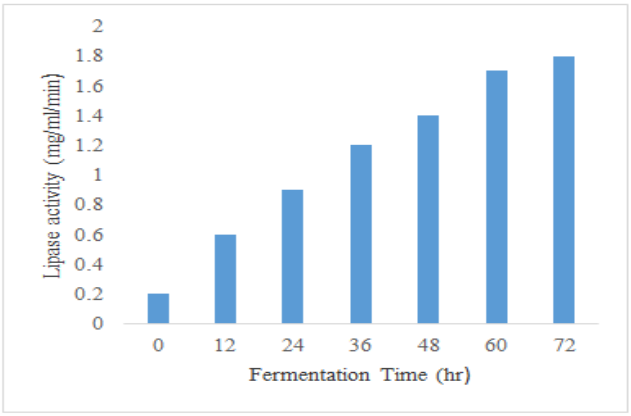

Fig. 2: Trend of lipase activity in soy-dawadawa with fermentation time.

Results revealed a general decrease in the percentage quantity of trypsin inhibitors present in the samples from 0 hr to 72 hr (Fig. 3). In the first 12 hr, there was about 9% decrease in the quantity of trypsin inhibitor (the highest rate) which continued through-out the fermentation period. Similar result was reported by other workers on African locust beans [20].The decrease in trypsin inhibitor of legumes by fermentation may be attributed to the leaching during soaking, heat treatment during boiling and also by the action of microorganisms during fermentation. A trypsin inhibitor is a protein and a type of serine protease inhibitor (serpin) which impedes the biological activity of trypsin by controlling the release and catalytic properties of proteins [21]. The unprocessed forms of legumes contain anti-nutrients such as phytic acid, tannins, trypsin inhibitors, gluten, oxalates, etc. These anti-nutrients decrease the bioavailability of nutrients in foods by creating complexes with the nutrients or inhibiting the activities of certain enzymes resulting in the indigestibility of foods, decreasing the nutritional value of the foods [22]. Trypsin is an enzyme involved in the breakdown of many different proteins, primarily as part of digestion in humans and other animals such as mono-gastric and young ruminants. When trypsin inhibitor is consumed it acts as an irreversible and competitive substrate. The process of soy-dawadawa production resulted in reduction in the trypsin inhibitor component, which improves the digestibility of the soybean by humans and animals and increases the bioavailability of nutrients in the soy-dawadawa.

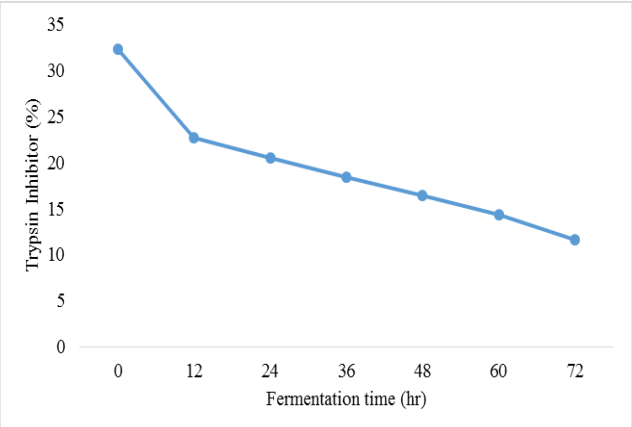

Fig. 3: Change in Trypsin Inhibitors content of soy-dawadawa with fermentation time.

V. CONCLUSION AND FUTURE SCOPE

Fermentation of soy-dawadawa inside jute sack influenced the microflora implicated in the process. Jute sack, being a synthetic material, did not support the growth of diverse microorganisms often associated with soy-dawadawa. This factor perhaps could have an effect on the sensory properties. The proximate characteristics generally increased with fermentation period showing that fermentation led to an improvement in the nutritional components of the soy-dawadawa condiment. More protein and fibre were available in the soy-dawadawa than in the boiled soybean. Also, fermentation increased the digestibility of soybean and reduces the risk of allergy to soybean constituents such as phytic acid and tannins. The trypsin inhibitory composition of soybean greatly reduced in the end product, which makes the soy-dawadawa more easily digestible for both human and animals. Fermentation time influenced the fermentation of soybean. The organoleptic test and palatability comparison of the end product with the popular Africa locust bean (iru) condiment can be examined in future studies.

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AUTHORS PROFILE

Mr Olabode Olufemi, Efunwoye bagged B.Sc. in Microbiology and M. Sc. In Industrial Microbiology option from the prestigious University of Ibadan, Nigeria, West Africa, in the year 2002 & 2016 respectively. He started his career as a quality assurance manager in food processing and packaging industry before venturing into teaching and research. He is currently working as a lecturer and researcher in the basic science unit of department of General Studies, Federal College of Agriculture, Akure, Ondo state, Nigeria. He is a member of American Society of Microbiology since 2007. He has published 4 research articles in reputable local and international journals and 6 resarch articles in notable scientific conferences, including the ASM Microbe 2018 and 2020 meetings. His main research interest areas include, postharvest microbiology, food fermentation and bioconversion of organic waste. He has over 12 years working experience in the food processing and packaging industry, and 5 years teaching and research experience.

Mrs Rita Omowunmi, Oluwole obtained B. Sc.in Microbiology from Federal University of Agriculture, Abeokuta (2008), and M.Sc. in Applied Microbiology and Biotechnology from Wolverhampton University (2010), UK. She is currently a lecturer and researcher in the basic science unit of the department of General Studies, Federal College of Agriculture, Akure, Nigeria. She has 4 publcations in reputable journals and conferences. Her area of research focus is in the application of microbiology in agriculture.

Oluseyi Valerie, Alagbe obtained B.Sc. in Biochemistry (2016) from Salem University, Kogi state, Nigeria. Presently an assistant lecturer in department of General Studies, Federal College of Agriculture, Akure, Nigeria, she has 3 publications in reputable journals and 2 conference presentations. Her area of research include toxicology, enzymology, food biochemistry and microbiology.

Mr Kolapo Solomon, Olawale has B. Sc. Microbiology (2016) and M.Sc. Medical Microbiology (2018) from the University of Ilorin, Kwara state, and University of Lagos, Nigeria. Presently, he is an assistant lecturer at the department of General Studies, Federal College of Agriculture, Akure, Nigeria. Current research involvements include mycological quality assessment of water distribution systems, histoplasmosis, cryptococcossis and ocular diseases of microbial origin. He has 4 publications in reputable journals and 3 publications in conference proceedings.

Olufunmilola O. Ajibola has a B.Sc. degree in Microbiology (2015) from the University of Abuja, Nigeria. She currently is an assistant lecturer at the department of General Studies, Akure, Nigeria. Research interest includes, food fermentation and sanitation quality management. She has contributed to 2 conference publications.

Mrs Blessing Ebiloma Omotoyinbo bagged B.Sc. Biochemistry (2008) from the University of Jos and M.Tech Applied Biochemistry (2014) from the Federal University of Technology, Akure. She is a lecturer at the Federal College of Agriculture, Akure, Nigeria, in the General Studies department. Her research interest covers agricultural biotechnology. She has 4 publications in both local international journals and has participated in 1 conference.

