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MICROBIOLOGICAL, PHYSICAL AND NUTRITIVE CHANGES OCCURRING DURING THE NATURAL FERMENTATION OF AFRICAN YAM BEAN (*SPHENOSTYLIS STENOCARPA* HARMS) INTO DAWA DAWA

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ABSTRACT

Dawa dawa condiments were produced from soybean and African yam bean seeds using traditional methods of fermentation. Changes in the microbial load and physical conditions of the processed substrates were observed throughout a 72-hr fermentation period. Bacterial species isolated during the fermentation of the seeds were *Bacillus, Staphylococcus, Lactobacillus, Micrococcus* and *Alcaligenes.* Fungal species isolated included *Saccharromyces ,Fusarium, Cladobotryrium* and *Aspergillus. Bacillus* and *Staphylococcus* were present throughout the fermentation period. The dawa dawa types differed in their microbial load but no pathogenic staphylococci and coliforms were present. Aerobic and anaerobic counts increased during fermentation while the fungal count declined. The pH and temperature also increased during fermentation. Proximate analyses of dry legume seeds and fermented products revealed an increase in crude protein, lipid, ash, and crude fiber while there was a decrease in the carbohydrate content after fermentation. These observations indicate that fermentation of the African yam bean was an exothermic process that increased the digestibility and nutritive value of the bean.

Key words: Fermentation, African yam bean, soybean, dawa dawa, microorganisms, pH, temperature, and nutritive properties.

INTRODUCTION

The African yam bean (*Sphenostylis stenocarpa*) is a legume crop that is adapted to the lowland tropical agro-ecosytem. It is cultivated especially in Southern Nigeria. The seeds are hard to cook (HTC) which limits its utilization and consumption. HTC is a common condition of beans stored under tropical climatic conditions (Parades-Lopez *et al.* 1990). Plant proteins such as those found in legumes are of economic importance in developing countries since they are largely used as supplements in replacements for expensive proteins of animal origin. For example, in Nigeria, tropical legumes and protein-rich oilseeds with HTC characteristics are often fermented into palatable products, some of which include ogiri, ugba, and dawa dawa. Scientific studies have shown that solid state fermentation of tropical legumes improves the nutritional quality of the product besides reducing the annual losses attributed to infestation and decay of seeds during storage (Parades-Lopez *et al.* 1990, Ataga and Umechuruba 1998).

The chemical composition of dawa dawa obtained from naturally fermented African locust bean (*Parkia biglobosa*) and soybean (*Glycine max*) has been previously investigated (Ikenebomoh *et al* 1986, Onyejegbu and Oguntunde 1993). During natural fermentation of African locust bean, soybean, groundnut and cowpea, significant reductions occur in the antinutritional factors and carbohydrate contents (Onyejegbu and Oguntunde 1993). But when African yam bean was fermented to produce tempeh, Njoku *et al.* (1991) observed an improvement in the nutritional quality.

A number of microorganisms have also been isolated during the natural fermentation of African locust bean and soybean into dawa dawa, with *Bacillus* and *Staphylococcus* being the most predominant and frequently isolated species (Popoola and Akueshi 1984). Studies on the population dynamics reveal a positive correlation of the population of *Bacillus* and *Staphylococcus* with a reduction in carbohydrate content (Popoola and Akueshi 1984, Odunfa 1986).

Dawa dawa condiment has been produced by natural fermentation of African yam bean (Aziagba 1996). Its taste and acceptability was comparable to the conventional African locust bean dawa dawa and soybean dawa dawa. A major concern about traditionally fermented African foods is the microbial load and nutritive value of the fermented products. This study was initiated to investigate the microbiological, physical, and nutritive changes occurring during the natural fermentation of African yam bean and soybean.

MATERIALS AND METHODS

Legume source

Dry seeds of African yam bean var. light gray and soybean var. Malayan were purchased at a local market, packaged in polyethylene bags and stored at room temperature.

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Dawa dawa production

The procedure for dawa dawa production was as described by Aziagba (1986). Two batches of soybean seeds weighing 25 g were hand sorted and either boiled for 15 min after washing or toasted for 15 min. After the heat treatment, seeds were de-hulled and boiled for one and a half-hour in water, drained, and allowed cooling. Cooled processed substrates were placed in clean earthen pots lined with freshly harvested and washed banana (*Musa sapientum*) leaves. Layers of washed leaves were used to cover processed substrates after which earthen lids were placed over the pots. The soybean substrate, which was boiled before de-hulling, was labeled BEND while the latter, which was toasted was labeled KAFD.

African yam bean substrate was processed as follows. Twenty-five grams of the dried seeds were hand sorted, washed and boiled in water containing one gram of potash (*kaun*). After draining, seeds were placed in a wooden mortar and lightly mashed with a wooden pestle. Seeds were not de-hulled. The processed yam bean substrate was also placed in a clean earthen pot similarly prepared for fermentation as described above. The processed yam bean substrate was labeled AYBD. All pots containing processed substrates were incubated at room temperature (30±2° C) for 3 days (72 hr). Each treatment was replicated three times.

Microbiological studies

Microorganisms involved in the fermentation process were enumerated, isolated and identified as described by Speck (1976). At onset and ~ 24 hr interval duplicate, samples were collected from each replicate into sterile glass bottles, and transferred in an ice bath to the laboratory. A gram of each sample was homogenized in nine ml of 0.1 % peptone water, after which tenfold dilutions were prepared. An 0.1 ml aliquot of each preparation was spread plated onto NA for enumeration of bacteria and APDA or Corn Meal Agar for enumeration of moulds and yeasts. Bacteria were isolated by streaking a loopful of each sample onto triplicate Nutrient Agar (NA) plates while fungi were isolated on Potato Dextrose Agar acidified with lactic acid (APDA). Plates were incubated at 37° C for 24 to 48 hr for bacteria and 30° C for 7 days for fungi. Samples were analyzed for total viable aerobic and anaerobic bacteria, spore formers, moulds and yeast. Anaerobic counts were carried out under anaerobic conditions using Gas Pak disposable system. Plates were incubated as described above. Spore counts were carried out by heat treating samples at 80° C for 20 min, spread plating onto Tryptone Soy Agar and incubating at 37° C. Staphylococci were also enumerated on Tripticase Soy Agar (Speck 1976).

Purified colonies were grouped according to their morphology and cell characteristics. Yeast and moulds were identified after staining with cotton blue lactophenol. The biochemical tests used to characterize the isolates were as described by Buchanan and Gibbons (1974), Cruickshank *et al.* (1975), and Harrigan and McCance (1976).

Physical analysis and proximate composition

The pH and temperature measurements were carried out simultaneously using a temperature pH meter (Mettler, model 340). Moisture content was determined according to the method of Osborne and Voogt (1978).

Total Kjeldahl nitrogen, and ash was determined as stated by Osborne and Voogt (1978). Total available carbohydrate expressed as glucose was determined by the method of Cleggy (1956). Extractable fat was determined by the Soxhlet method, while crude fibre was determined by difference.

All fermentation experiments were repeated with similar results. Samples from each pot were analysed in duplicates for microorganisms, pH, temperature, moisture content and proximate composition.

RESULTS AND DISCUSSION

A number of aerobic and facultative anaerobic bacteria were isolated from the fermenting mashes. Based on morphology and biochemical characteristics, they were identified as *Bacillus, Staphylococcus, Lactobacillus*, *Micrococcus*, and *Alcaligenes* (Table 1). Pathogenic staphylococci and coliforms were absent during and after fermentation. The absence of pathogenic bacteria indicated that adequate heat treatment was given during processing and that there was no post-processing contamination. The different dawa dawa samples differed in their microbial quality. *Bacillus* and *Staphylococcus*, were predominant in the BEND samples while *Lactobacillus* was least in number. Only *Bacillus* and *Staphylococcus* were isolated from the KAFD samples. In the AYBD samples, *Bacillus* and *Staphylococcus* were also predominant although *Micrococcus* and *Alcaligenes* were also isolated. *Bacillus* and *Staphylococcus* were consistently isolated from all the samples during the fermentation process.

The findings of this study agree with earlier reports (Popoola and Akueshi 1984, Omafuvbe 1994) on the involvement of *Bacillus* and *Staphylococcus* in the fermentation of African locust bean and soybean to produce dawa dawa. These groups of bacteria have also been implicated in the fermentation African oil bean (*Penthaclethra macrophylla*) and melon (*Citrullus vulgaris*) seeds (Njoku *et al.* 1990, Barber and Achinewhu 1992).

The genera of fungi isolated were identified as *Saccharomyces*, *Mucor*, *Fusarium*, *Cladobotryrium*, and *Aspergillus*. *Saccharomyces* sp. was predominant in all the samples. The population dynamics of microorganisms isolated during fermentation are presented in Figures 1, 2, and 3. In all the samples, the population of the aerobic bacteria consistently and rapidly increased throughout the fermentation period (Figure 1) while there was only a slight increase in the growth of anaerobes *Bacillus* and *Staphylococcus* (Figure 2). On the contrary, the population of fungi declined as fermentation continued (Figure 3).

With the exception of the BEND samples which had an initial pH slightly above neutral (7.3), an increase from acidity to alkalinity was observed during fermentation (Figure 4). In all the samples, the pH increased as fermentation progressed, with KAFD samples having the highest change from 6.2 to 7.8. The temperature of the fermenting substrates also rose from an initial 28° C to a maximum of 45° C, which was recorded for KAFD samples after 72-hr incubation period (Figure 5). Nevertheless, the temperature of the AYBD dropped to 38.5° C after an initial rise to 42° C while that of the BEND sample steadily rose to 41° C after 72 hr. Parades-Lopez (1990) observed similar changes in both pH and temperature during fermentation of legume seeds. Ikenebomoh *et al* (1986) associated increase in pH during fermentation of African locust bean seeds to high proteolytic bacterial counts, particularly *Bacillus* and *Staphylococcus* which grow well at pH 7.0 to 8.0 and Odunfa (1986) noted that fermented beans have a strong proteolytic and slightly ammonia-like smell. Thus, an increase in the pH could be associated with the production of ammonia by proteolytic bacteria during fermentation.

Table 2 shows the proximate composition of raw seeds, processed substrates and fermented products. The proximate composition of dry yam bean and soybean seeds obtained in this study are similar to those earlier reported (Ezueh 1984, Nwokolo 1987, Njoku *et al.* 1991, Ofuya *et al.* 1991).

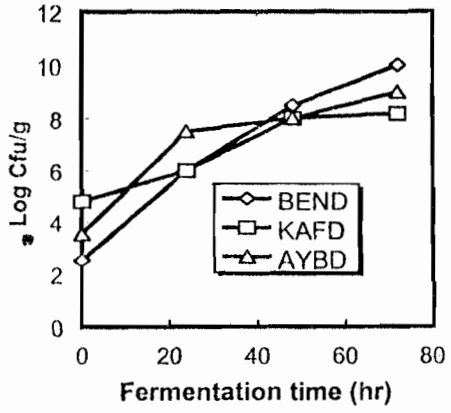

Figure 1. Changes in aerobic count during the fermentation of soybean and Africa.. yam bean seeds for dawa dawa production.

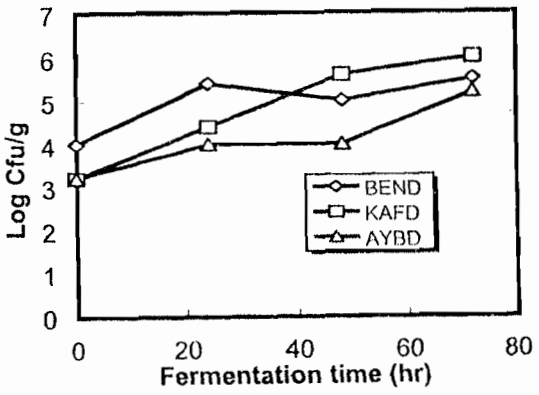

Figure 2. Changes in anaerobic count during the fermentation of soybean and African yam bean seeds for dawa dawa production.

Table 1. Biochemical characterization of bacteria isolated during the fermentation of soybean and African yam bean seeds.

Biochemical tests	Sample Description								
	BEND[A]			KAFD[A]		AYBD[B]			
	Isolate 1	Isolate 2	Isolate 3	Isolate 1	Isolate 2	Isolate1	Isolate 2	Isolate 3	Isolate 4
Cell shape	Rod	Cocci	Rod	Rod	Cocci	Rod	Cocci	Cocci	Rod
Gram's reaction	+	+	+	+	+	+	+	+	-
Spore stain	+	-	-	+	-	+	-	-	+
Motility	+	-	-	+	-	+	-	-	-
Methyl red	-	+	-	-	+	-	+	+	-
Voges-Proskauer	+	-	-	+	-	+	-	-	-
Indole production	-	-	-	-	-	-	-	-	-
Citrate utilisation	+	-	+	+	-	+	-	-	+
H_2S production	-	-	-	-	-	-	-	-	+
Oxidase	-	-	-	-	-	-	-	-	-
Catalase	+	+	-	+	+	+	+	-	+
Coagulase	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	-	-	+	-	+	-	-	-
Lactose	AG	AG	AG	AG	AG	AG	AG	AG	A
Glucose	AG	AG	AG		AG	AG	AG	AG	A
Sucrose	A	-	A	AG	-	A	-	-	-
Mannitol	AG	A	AG	A	A	AG	A	A	-
Identity	Bacillus	Staphylo coccus	Lactobac illus	Bacillus	Staphylo coccus	Bacillus	Staphylo coccus	Micrococcus	Alcaligenes

Key: + positive; - negative; A acid; G gas; [A] Soybean dawa dawa; [B]African yam bean dawa dawa;

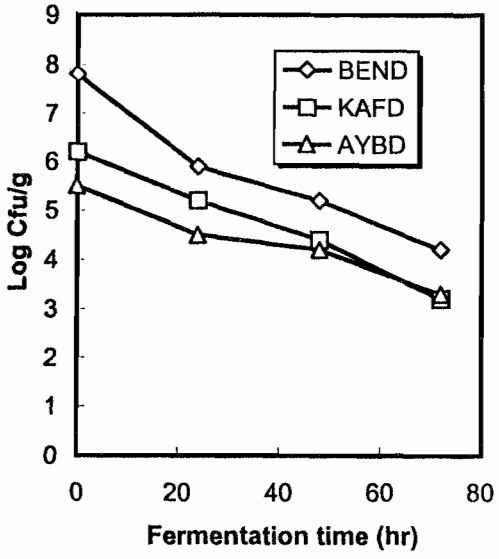

Figure 3. Changes in mould count during the fermentation of soyabean and African yam bean seeds for dawa dawa production.

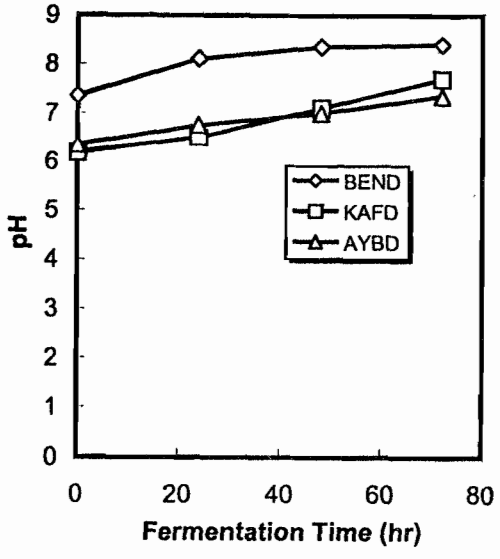

Figure 4. Changes in pH during the fermentation of soybean and African yam bean seeds for dawa dawa production.

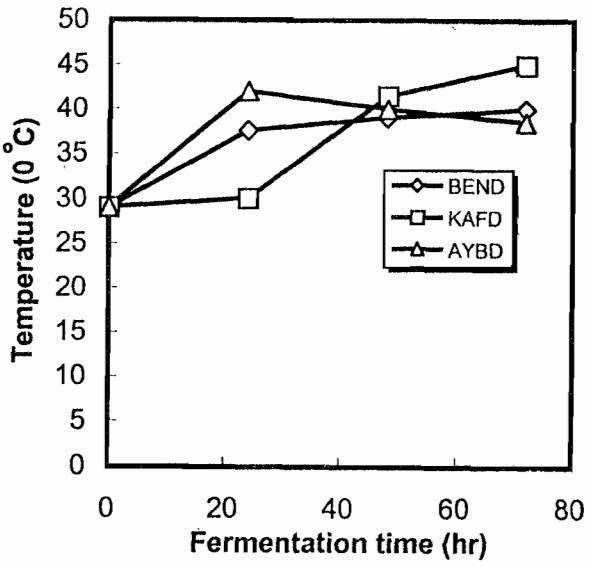

Figure 5. Changes in temperature during the fermentation of soybean and African yam bean seeds for dawa dawa production.

The protein digestibility of the yam bean is known to be low Ene-Obong and Obizoba 1996). The fermentation of the yam bean into dawa dawa increased the digestibility of the beans as evident in the relative increase in the crude protein and crude fibre values. This is evident by the rise in pH during fermentation, which could be associated with the accumulation of ammonia resulting from the breakdown of protein by the proteolytic bacteria. The fat content of soybean and yam bean did not change during and after fermentation indicating that the microorganisms did not readily utilize the oils.

However, carbohydrate significantly reduced, during fermentation. This reduction was partially due to the bioconversion of the substrate by the microorganisms involved in the fermentation process, which derive their energy from carbohydrate metabolism. The bioconversion of the substrates was accompanied by

Table 2. Percent proximate composition of dry legumes seeds, processed substrate, and dawa dawa produced from soybean and African yam bean (AYB).

Sample Description[a]	Crude Protein	Lipid	Available carbohydrate	Ash	Crude fiber	Moisture Content
Soybean						
Dry soybean seeds	42.26	23.09	30.41	4.22	5.6	6.40
[b]BEND processed substrate	41.01	24.09	25.30	4.36	5.67	56.30
BEND dawa dawa	48.12	28.90	10.87	4.50	14.24	68.20
[c]KAFD processed substrate	41.6	24.20	27.40	4.43	7.8	59.30
KAFD dawa dawa	49.38	26.50	11.18	4.70	12.24	65.20
African yam bean						
Dry AYB seeds	22.0	1.20	74.20	3.20	5.70	8.70
[d]AYBD processed substrate	21.2	1.28	66.10	3.61	6.34	44.20
AYBD dawa dawa	32.8	1.38	57.21	4.60	7.77	50.00

[a]Mean of three replications.

[b]BEND processed substrate was produced from soybeans seeds boiled for 15 min before de-hulling.

[c]KAFD processed substrate was produced from soybeans seeds toasted for 15 min before de-hulling.

[d]AYBD processed substrate was produced from AYB seeds boiled in water containing potash, then mashed with a wooden pestle.

the release of heat, which also accounts for the rise in temperature during fermentation. The natural fermentation of soybean and African yam bean therefore occurred as an exothermic process.

Studies on the toxic components of yam bean revealed the presence of alkaloids, flavonoids and saponins (Potter 1992, Ene-Obong and Obizoba 1996). A minimum of 12 to 14 hr cooking period is recommended for the complete destruction of the toxic components. Apart from improving the nutritive value and flavor enhancement, which is evident in the present study, reduction of toxic components such as phytate can occur during natural fermentation of African yam bean (Ene-Obong and Obizoba 1996). The fermentation of yam bean to produce dawa dawa condiment also improved the digestibility. The organoleptic evaluation the AYB dawa dawa (Aziagba 1996) and meat pies prepared with yam bean tempeh (Njoku *et al.* 1991) indicate that food products of the yam bean may readily gain acceptance. More recent studies on African yam bean utilization further show that the yam bean can be used to make moimoi, a popular Nigerian dish (N. O. Frank-Peterside, University of Port Harcourt, personal communication).

CONCLUSION

African yam bean seeds were converted into dawa dawa by natural fermentation, which occurs as an exothermic process. A rise in pH, an appreciable decrease in the carbohydrate content, an increase in the bacterial load, and a decrease in the fungal load also accompanied the bioconversion to fermented beans. *Bacillus* and *Staphylococcus* were consistently isolated from the different samples of the fermented dawa dawa product. The absence of pathogenic bacteria indicated that adequate heat treatment and handling during processing prevented post-processing contamination.

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