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RESEARCH ARTICLE

BACILLUS SUBTILIS ISOLATED FROM LOCALLY FERMENTED SOYABEAN SEEDS (SOY-DAWADAWA) FOR PRODUCTION OF COOKING CONDIMENT

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ABSTRACT

In this research work, fresh locally fermented soy-dawadawa was investigated for the presence of bacteria. Samples of fresh locally fermented soy-dawadawa each from four (4) different materials (banana leaves, pawpaw leaves, gmalina leaves and polythene sacks) used as fermentation media for soy-dawadawa production were collected from local women vendors in Makurdi town, Benue state, Nigeria and analysed for the presence of bacteria. The bacteria isolated were *Bacillus* spp., *E.coli, Salmonell* spp., *Streptococcus* spp., *Lactobacillus* spp. and *Staphylococcus aureus*. The predominant bacteria species isolated (*Bacillus subtilis)* was used to ferment a laboratory prepared soy-dawadawa under controlled conditions. The pH, temperature and the population of the fermenting organisms were measured after every 10hrs during the fermentation of the laboratory prepared dawdawa that lasted for 72hrs. The sensory evaluation was conducted on both the locally fermented and laboratory fermented soy-dawadawa and the result showed that laboratory fermented soy-dawadawa was the most acceptable.

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INTRODUCTION

Soy-dawadawa is a food flavouring condiments prepared by fermenting whole soyabean. It is a product of alkaline fermentation of soyabean (*Glycine max)* used as food condiment in much the same way as African locust bean (*Parkia biglobosa)* dawadawa. It is evident that these products have played a major role in the food habits of communities in the rural regions serving not only as a nutritious non–meat proteins substitute but also as a condiment and flavouring agent in soups (Achi, 2005). Fermentation of soyabean (*Glycine max)* into condiments, that is dawadawa is normally carried out in a moist solid state involving contact with inoculate of different microorganisms and is accomplished by natural temperature. The weight of the microorganisms in the food is usually small, but their influences on the nature of the food, especially in terms of flavour and other organoleptic properties are profound (Okafor, 2009). Currently, soyabean is an alternative raw material for dawadawa production in some localities in Nigeria since Africa locust bean is seasonal with a dwindling supply. Soy-dawadawa and African locust bean are organoleptically similar (Omafuvbe *et al*., 2002) and their fermentation is accomplished by *Bacillus* species especially *Bacillus subtilis* (Ogbadu and Okagbue 1988; Omafuvbe *et al*., 2000; Dike and Odunfa, 2003).

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Bacillus subtilis is the dominant functional bacterium in all naturally fermented soybean foods (Tamang, 2015). The gram-positive, spore forming bacterium *Bacillus subtilis* produces and secretes proteases, esterases, and other kinds of exoenzymes at the end of the exponential phase of growth (Priest, 1977). The principal extracellular proteolytic enzymes, the alkaline (subtilisin) and neutral (metallo-) proteases, are encoded by the *apr* and *npr* genes, respectively (Sloma *et al*., 1988). The production process in local dawadawa is not reproducible because the microorganisms normally associated with the fermentation process are mix flora and consequently some may produce undesirable metabolites. Thus, this may result in the inconsistency of the quality of the product. This work attempt to solve this problem by isolating the organisms from the already locally fermented soy-dawadawa, growing the predominant species in pure culture and using it to produce soy-dawadawa in the laboratory under controlled scientific conditions.

MATERIALS AND METHODS

Sample collection: A total of four (4) samples of freshly prepared soy-dawadawa fermented from four different fermentation media (banana leaves, gmalina leaves, pawpaw leaves and polythene sacks) and soyabeans seeds were collected from local vendors at Wurukum market in Makurdi, Benue State, Nigeria. All the samples were quickly taken to the microbiology laboratory of the University of Agriculture, Makurdi for the appropriate analysis.

Isolation and identification of bacteria on soy-dawadawa: The appropriate serial dilution was made from all the four samples and were inoculated on Nutrient agar, Mackonkey agar and Salmonella/Shigella agar aseptically and incubated at 37^0 for 24 hours. Colonies that appeared on the agar plates were counted using colony counter (model 6399, Staurt Scientific Co. Ltd Great Britain) and the result recorded as colony forming unit per gram (cfu/g) of sample. Pure bacteria isolates were characterized based on colony morphology, cell morphology and biochemical tests. The isolates were identified using the scheme of Cheesebrough (1987). The predominant isolate was then used for the preparation of laboratory soy-dawadawa.

Identification of the predominant bacteria isolates (*Bacillus subtilis*): *Bacillus subtilis* was fully identified based on the method described by Fall *et al*, (2004); Amin *et al*, (2015).

Laboratory preparation of soy-dawadawa: The soyabean seeds were screened by sorting out the clean seeds and discarding the bad seeds. The clean seeds were then fried at 100^0 for 10 minutes. The fried seeds were allowed to cool and then dehulled and winnowed to remove the hulls. The dehulled seeds at this stage were separated into cotyledons and were then washed. 300g of these cotyledons were boiled in 2litre of distilled water at 100^0 for 3hours after which it was cooled and the remaining water poured away.

Preparation of Starter Cultures: This was done using the method of Omodara and Aderibigbe, (2013). The inoculum was prepared by growing *Bacillus subtilis* earlier isolated in 50ml Nutrient Broth (NB) in 250ml conical flasks for 24 hours under agitation (200rpm) at 35^oC. The turbid cultures were centrifuged at 10,000rpm, 4^oC for 10mins. The supernatant was decanted and the cell pellets were re-suspended in 5ml of sterile distilled water. The cell population was determined by measuring the optical densities of broth cultures at 540nm with Pye Unicam SP6-250 visible spectrophotometer. The volume of the inoculum required to inoculate 300g of prepared soyabean seeds to give a final inoculation ratio of 10^4 cells per gram of prepared soyabean seeds was calculated.

Fermentation of the boiled soyabean seeds: The inoculum prepared above was uniformly mixed with the boiled soyabean cotyledons in a sterile container and aseptically covered with aluminium foil paper. This was incubated for 3 days under room temperature so as to allow the set up to undergo fermentation to produce the condiment (dawadawa). The pH, temperature and the number of cells were monitored at the interval of 10hours during the course of fermentation.

Sensory evaluation: A panel of five judges of consumers of soy-dawadawa were used in the sensory evaluation of the locally and laboratory fermented soy- dawadawa using a five-point hedonic scale as described by Tyokusa and Owuama, (2018).

RESULTS

Occurrence of bacteria on different materials locally used as fermentation media for preparing soya-dawadawa: A total of six (6) different Genera of bacteria were isolated from the four (4) samples. These were *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Lactobacillus* spp. and *Streptococcus* spp. as recorded in table 1 below

Table 1. Bacteria species isolated from fresh soya-dawadawa from different fermentation media

S/N	Fermentation Mediun	Bacteria Isolates
1	Banana leaves	*Bacillus* spp., *S.aureus*, *E. coli* and *Salmonella* spp.
2	Pawpaw leaves	*Bacillus* spp., *S.aureus*, *E. coli* and *Streptococcus* Spp.
3	Gmalina leaves	*Bacillus* spp., *E. coli*, *Salmonella*, and *Streptococcus* Spp.
4	Polythene sack	*Bacillus* spp., *S.aureus*, *E. coli*, *Salmonella*, and *Lactobacillus* spp.

Bacillus spp. was present in all the samples and has the highest % occurrence as reported in the figures below.

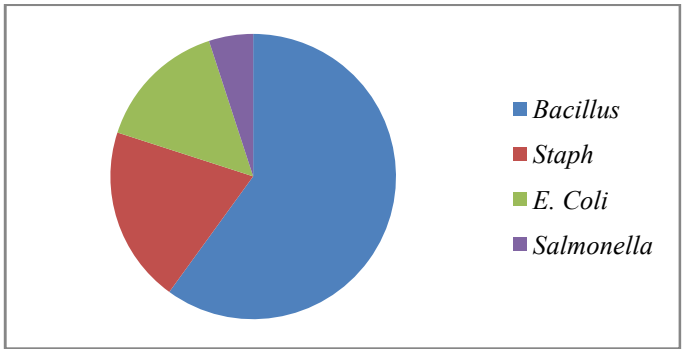

Figure 1. % occurrence of bacteria isolated from soya-dawadawa fermented from banana leaves

The % occurrence on banana leaves was; 60% *Bacillus* spp., 20% *Staphylococcus aureus*, 15% *E. coli* and 5% *Salmonella* spp.

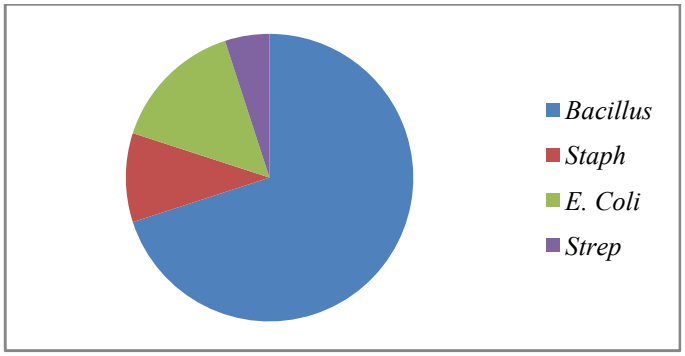

Figure 2. % occurrence of bacteria isolated from soya-dawadawa fermented from pawpaw leaves

The % occurrence on pawpaw leaves was; 70% *Bacillus* spp., 10% *Staphylococcus aureus*, 15% *E. coli* and 5% *Streptococcus* spp.

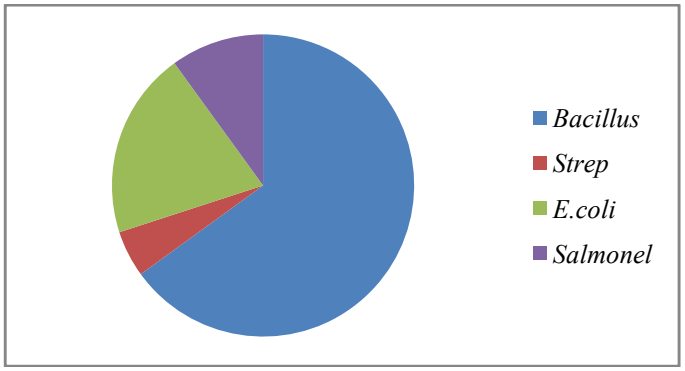

Figure 3. % occurrence of bacteria isolated from soya-dawadawa fermented from gmalina leaves

The % occurrence on gmalina leaves was; 65% *Bacillus* spp., 5% *Streptococcus* spp. 20% *E. coli* and 10% *Salmonella* spp.

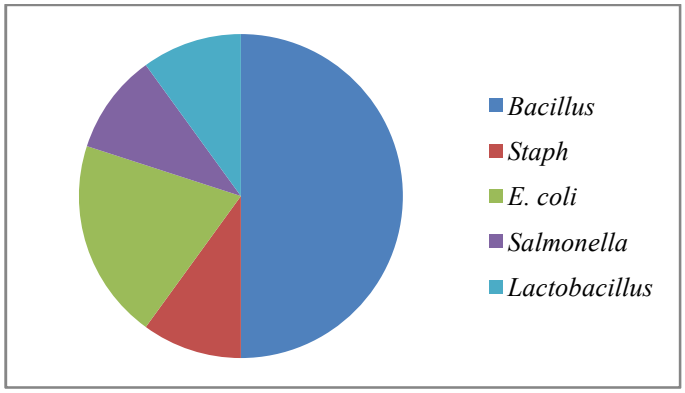

Figure 4. % occurrence of bacteria isolated from soya- dawadawa fermented from leather sacks

The % occurrence on leather sacks was; 50% *Bacillus* spp., 10% *Staphylococcus aureus*, 20% *E. coli*, 10% *Salmonella* spp..and10% *Lactobacillus* spp.

Changes in pH, Temperature and Population of fermenting organisms during labolatory fermentation of the soya- dawadawa.

The pH, temperature and bacteria populations were measured at 10 hr interval during fermentation of soyabeans seeds for a period of 50 days as seen in Fig.5 below.

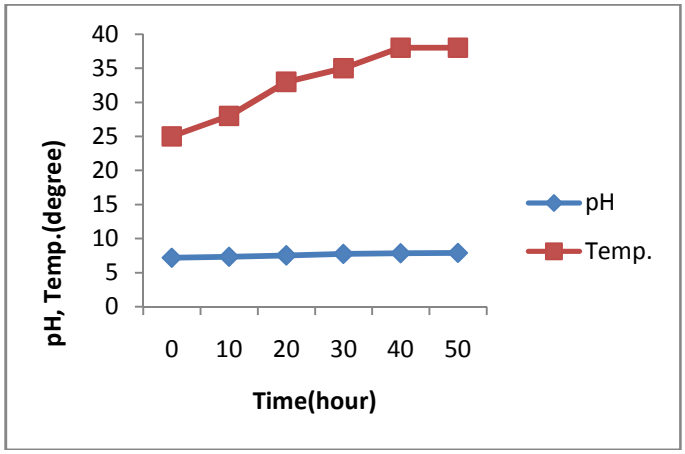

Figure 5. Changes in pH and temperature during fermentation of soyabeans seeds for cooking condiment production

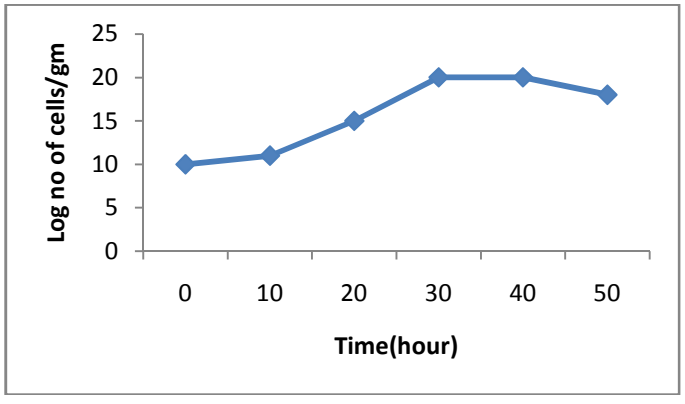

Figure 6. Change in bacteria population with time during fermentation of the soyabeans seeds for cooking condiment production

Table 2. pH of the locally fermented soy-dawadawa and that of labolatory Soy-dawadawa

Fermentation medium	pH
Banana leaves	7.52
Pawpaw leaves	7.26
Gmalina leaves	7.48
Polythene sack	7.53
Laboratory Aluminium foil	7.90

Table 2 above indicate the pH of soyabean dawadawa from different fermenting media and that of the laboratory prepared soy-dawadawa.

DISCUSSION

The isolation of bacteria from locally fermented soy-dawadawa agreed with Afolabi *et al*, (2016), who also reported the presence of bacteria from fermented locust bean and soy-dawadawa. The bacteria isolated were *Bacillus* spp., *Streptococcus* spp., *E.coli, Staphylococcus aureus, Salmonella* spp. and *Lactobacillus* spp. (Table 1). The percentage occurrence of bacteria from each sample (Fig.1-4) showed that *Bacillus* species has the highest percentage occurrence from all the samples. This conformed with the work of Afolabi *et al*, (2016); Darkwa *et al.* (2005) who both reported the dominance of *Bacillus* in fermented soy- dawadawa. Similary, (Tamang, 2015) reported the presence of *Bacillus subtilis* in naturally fermented soyabean foods. Ogbagu and Okagbue (1988); Omufuvbe *et al*, (2000) reported that *Bacillus subtilis, Bacillus pumulus*, and *Bacillus licheniformis* are the predominant microorganisms in the fermentation of soyabean dawadawa production in Nigeria. In most fermented high protein food, the extent of protein hydrolysis is one of the important factors in the change in texture and flavour (Whitaker, 1978). During the fermentation of the soyabean seeds, microbial enzymes break down the legume proteins (Song *et al*., 2007). Fig. 5 indicated that the pH increased during the course of fermentation. This was so because, during the process, the fermenting organisms (*Bacillus subtilis*) secrete enzymes which breakdown the proteins in the soyabean seeds into amino acids and then ammonia, resulting in a more alkaline environment. This agreed with Priest, (1977) who stated that the gram-positive, spore forming bacterium *Bacillus subtilis* produces and secretes proteases, esterases, and other kinds of exoenzymes at the end of the exponential phase of growth. According to Sloma *et al*, (1988), the principal extracellular proteolytic enzymes, the alkaline (subtilisin) and neutral (metallo-) proteases, are encoded by the *apr* and *npr* genes respectively. As seen in fig. 5 also, the temperature at inoculation time was 25^0c and it gradually increased to 38^0 c at 40hrs and then dropped to 37^0c at the end of fermentation of 50hrs. The rise in temperature was as a result of the fermenting bacteria which produced heat during the process. The gradual rise in temperature coincided with the typical microbial growth curve. At the inoculation time, the temperature of 25^0c was the room temperature. At this stage, the organisms were adjusting to the new environment and there were no serious activities that could generate heat. But as the organisms began to multiply and the activities became vigorous, much heat was generated and that resulted in the rise in temperature. Towards the end of fermentation at 40-50hrs, the organisms reach their stationary phase and consequently the death phase, thus their activities slowed down and less heat was produced which resulted in the dropped in temperature. The pattern of change in population of fermenting organisms during the fermentation

of the soyabean seeds for dawadawa production (fig. 6) is typical of a normal microbial growth curve (Skarstad *et al*., 1983). In the first 10hrs after inoculation, the organisms were adjusting to the environment and there was no remarkable increase in population. But from 10-30hrs, the organisms were at their log phase of growth, multiplying actively and increasing in number as seen in the graph (fig. 6). From30-40hrs, the organisms were at their stationary phase of growth and their population showed no significant change. Finally, from 40-50hrs, the organisms were at their death phase and that explain the decreased in population. The pH of all the locally fermented dawadawa range between 7.26 and 7.55, while that of laboratory dawadawa was 7.92 (Table 2). Both pH agreed with the pH of soy – dawadawa reported by Omufevbe *et al.* (2000). The laboratory dawadawa had higher pH because there was more break down of proteins and amino acids into ammonia by pure culture of *Bacillus subtilis* unlike the locally fermented dawadawa that has mixed flora of microorganisms that resulted in the limited break down of these compounds. The Sensory evaluation of the laboratory soy- dawadawa produced by activities of *Bacillus subtilis* showed it was better in terms of taste, appearance, texture, and general acceptability. However, there was no much difference observed in the odour. In all the organoleptic attributes studied, there was preference for the soy-dawadawa produced from the laboratory.

Conclusion

This work confirmed that bacteria are associated with locally fermented soy-dawadawa and are responsible for the fermentation of soyabean seeds to produce local dawadawa. That the dominant bacteria species present was *Bacillus subtilis* and that it can be isolated and grown in pure culture to produce soy-dawadawa with better sensory qualities compare to the one produced by local women in some parts of Nigeria.

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