



# Isolation and Identification of Microorganisms Encountered in Traditional Fermentation of Soya Beans and Roselle seeds for the production of Food Tasty condiment (Dawadawa)

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## ABSTRACT

Study on isolation and identification of microorganisms encountered in traditional fermentation of Soya Beans and Roselle seeds for the production of Dawadawa was carried out, using standard microbiological and biochemical methods. The organisms identified are from genera *Bacillus*, *Kurthia*, *Staphylococcus*, *Listeria* and *Micrococcus*. Yeast and fungi were also isolated. Hazard and critical control point (HACCP) at each stage during fermentation was determined, washed seeds and water used for washing was found to have high number of microorganisms. Microbial fermentation increased the bioavailability of nutrients in the condiments produced from the seeds of *G. max* and *H. sabdariffa*.

**Keywords:** Microorganisms;

Soybeans; Roselle seeds;

Dawadawa; Traditional

fermentation

## INTRODUCTION

Fermentations are enzyme-induced chemical alterations in food or substrate. The enzyme involved may be produced by microorganisms or they may be indigenous to the food or substrate (Ihekoronye and Ngoddy, 1985). Generally, fermentation results in the breakdown of complex organic substances into smaller ones through the action of catalysis. Fermentation is one of the oldest methods of food preservation known to man. In Africa, the art of fermentation is wide spread including the processing of fruit and other carbohydrate source to yield alcoholic and non-alcoholic beverages, the production of sour tasting Ogi - the fermented cereal product which provide instant energy in breakfast and convalescent diets (Adewusi *et al.*, 1991; 1992) oil seed such as African locust bean, melon seed, castor oil seed mesquite bean and soybean are also fermented to give condiment.

Production of fermented tasty food condiment has remained a traditional family art in homes with rudimentary utensils (Audu *et al.*, 2004). Adewumi and Olalusi (1995) reported that women mainly do processing of Soya Beans and Roselle seeds locally. Methods used vary from one locality to another depending on the culture of the people, their beliefs, taste and practice of the fore-parents who were involved in the same vocation. These variations in processing techniques in turn bring about variations in the quality of iru (Sadiku, 2010).

A substantial literature documents the successful fermentation of vegetable proteins for condiment production. Both microbial and biochemical changes involved therein have received much attention. A further understanding about the interactions of the microorganisms and plant materials is necessary to improve the quality of fermented condiments. Such understanding will aid in further development and control of the fermentation process (Dakwa *et al.*, 2005)

## MATERIALS AND METHODS

Dried sample of soybean (*Glycine max*) Roselle seeds (*Hibiscus sabdariffa*.) were obtained from Sokoto central market located in Sokoto metropolis. All the materials used in dawadawa production (Basket, Calabash, Sieve ,pawpaw /banana leaves, Wood ash/Potash, jute bags, plastic buckets) were purchased from Sokoto central market. The samples were taken to laboratory for analyses.

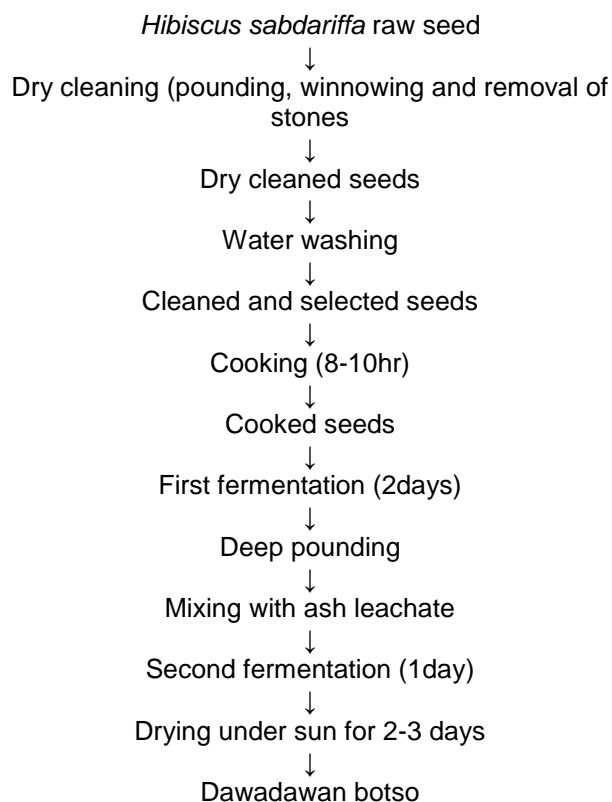
### Traditional fermentation of dawadawa from Soya bean

To make traditional soy –dawadawa, soybean are cleaned, soaked overnight in tap water, dehulled manually and cooked by boiling for two hours (Omafuvbe *et al.*, 2000). The dehulled cooked beans are then placed in calabashes or bamboo baskets lined

with banana or plantain leaves and left to ferment spontaneously in a worm place for up to 72 hours

### Traditional fermentation of daddawan botso from Rosselle seed

Seeds fermentation was done in two phase, the cooked seeds were allowed in the pot to ferment naturally for two days by closing the pot tightly to prevent aeration. After the first fermentation, the cooked seeds were pounded nearly to paste in a mortar with the addition of ash leachate and mixed. This was returned back to the pot for second fermentation which lasted for one day and the pot was tightly closed. At the end of the second fermentation, the ammonia – like flavor condiment was sun dried by repeated turning to form balls and to enable good drying for 2 – 3 days according to the intensity of sunshine and packaged in polythene bags (Ibrahim *et al.*, 2011).



**Fig. 1: Flow chart for traditional fermentation processing of *Hibiscus sabdariffa* raw seed (Ibrahim *et al.*, 2011)**

### Isolation of the fermenting microorganisms

One gram [1g] of the fermented Soya bean Roselle seeds were taken and diluted serially. One (1ml) from different dilutions was streaked onto nutrient agar. Isolates from plate count agar (PCA) was picked and sub-cultured in nutrient agar and *Bacillus* species was characterized using morphological examination and biochemical test comprising of colony and cell

morphology. The biochemical tests that were carried out are: anaerobic growth, acid production from D-glucose, hydrolysis of casein and starch, growth at pH 5.7, in 6.5% (w/v) NaCl and 10% (W/V) NaCl, at 37°C and 65°C (Claus and Berkeley, 1986).

### Characterization of fermenting isolates from fermented Locust and Soya bean

#### Anaerobic Growth

The organism was isolated using method described by Claus and Berkeley, (1986) using standard isolation medium. 0.1ml of the test organism was spread out on the solid standard medium plates. The plates were incubated in anaerobic jar for 2 days at 35°C.

#### Acid production from D-glucose

Isolates were inoculated into nutrient broth sugars and examine daily for seven days for acid and gas production (Barrow and Feltham, 1993)

#### Hydrolysis of Casein

Plates of Casein agar were inoculated at intervals and examined for up to 14days for clearing of the medium around the bacterial growth (Barrow and Feltham, 1993).

### Starch Hydrolysis

Isolates were inoculated onto nutrient agar plates containing 0.2% soluble starch and incubated at 30°C for 5 days. Plates were flooded with lugols iodine solution, the medium turned blue for negative hydrolysis. A clear colourless zone indicates hydrolysis (Barrow and Feltham, 1993).

### Growth in Media with Increased NaCl Concentration

Required amount of sodium chloride 6.5% (wv) and 10% (wv) was added to broth and inoculated with organism to be tested. The tubes were incubated at 37°C for 24hrs (Barrow and Feltham, 1993)

### Determination of Hazard Analysis and Critical Control Point during the production of condiments

At each different stage of Daddawa production, sample was collected from raw seeds, fermented seeds, washed seeds, floor swab, mortar and pestle to determine critical control point, according to the method of the Food and Agricultural Organization (FAO, 1979).

## RESULTS

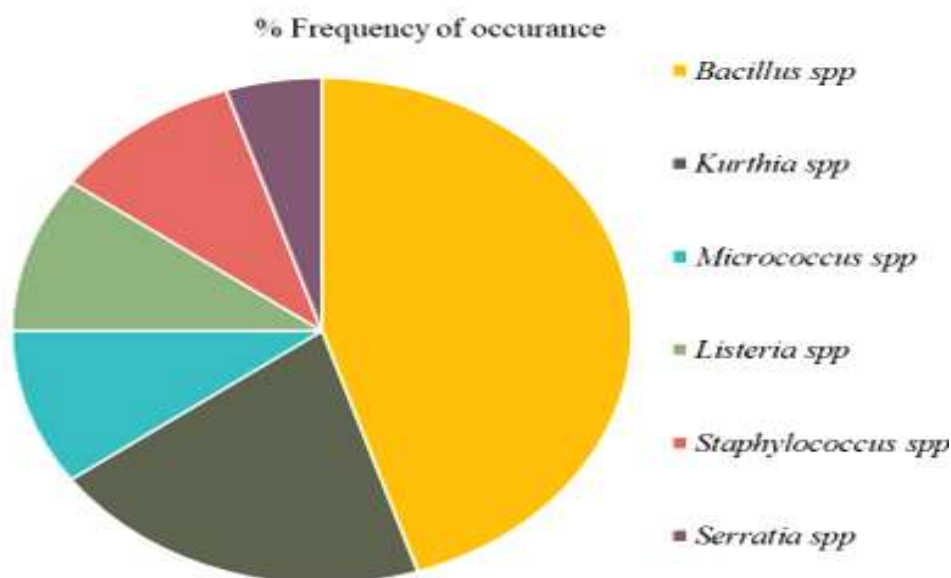


Figure 2: Percentage frequency of occurrence of bacteria isolated from Dawadawa production

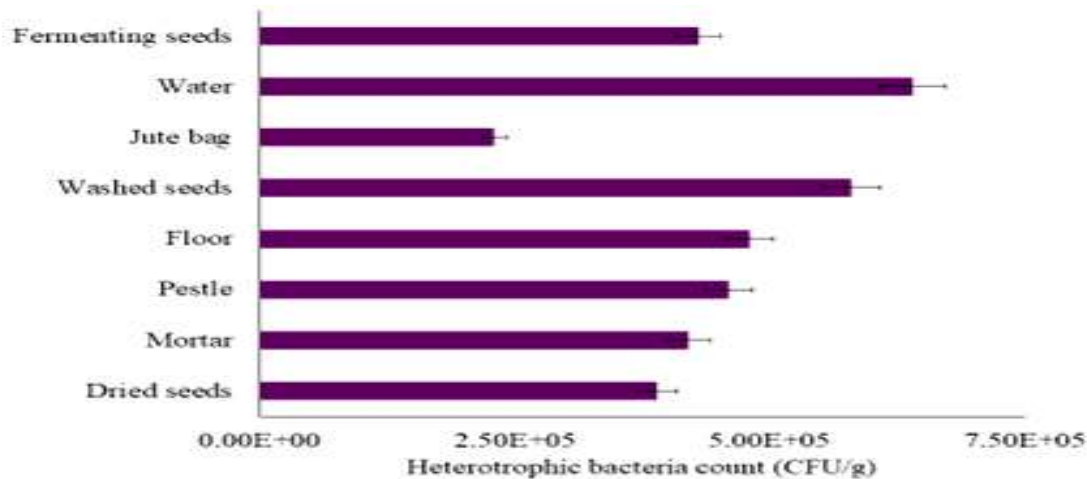


Figure 3: Viable colony count from Soya beans at each stage of production

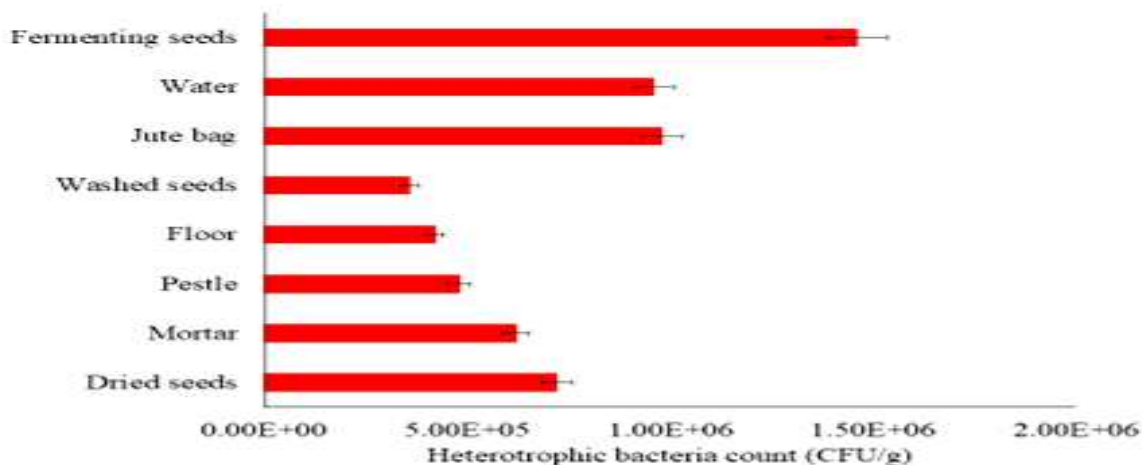


Figure 4: Viable colony count from *Habiscus sabdariffa* at each stage of production

## DISCUSSION

The microorganisms identified are from *Bacillus* genera which are the dominant organisms, other microorganisms isolated are from the genera of *Staphylococcus*, *Kurthia*, *Listeria*, *Micrococcus* and *Serratia*. Similar result was also reported by Achi (1992) who investigated the microorganisms associated with the natural fermentation of *Prosopis africana* seed. A wide array of microorganisms including *B. subtilis*, *B. megaterium*, *B. licheniformis*, *Staphylococcus* spp, *Micrococcus* spp, *Klebsiella* spp, *Enterobacter* spp and *Lactobacillus* spp. The involvement of a variety of microorganisms in spontaneous food fermentation is normal and does not render the product unsafe for human consumption, especially when none of the microorganisms is pathogenic to man (Oyeyiola, 2002). The growth of microorganisms during the fermentation of daddawa is likely to have a significant influence on the quality and flavor of the final product.

*Bacillus* species were frequently isolated from traditional daddawa and this explain the reason why the

organism was used as starter culture in the laboratory fermentation of daddawa. The presence of *Bacillus* species is expected since they have been found to be associated with fermenting legume seeds during the production of Okpehe (Achi, 1992, Ogunshe et al, 2007), Ugba (Obeta, 1983) Dawadawa (Odunfa, 1981, Antai and Ibrahim, 1986), Iru (Oyeyiola, 2002) and dawadawa botso (Ibrahim et al., 2011a) and soumbala (Ouoba et al, 2007). The presence of these organisms which are proteolytic, am ylolytic and lipolytic may lead to an increased degradation of the major nutritional components of the seeds. *Bacillus* have been implicated in the fermentation of most vegetable oil protein seeds like African locust bean seeds, soybean seeds, African oil bean seeds e.t.c. (Odunfa, 1981, Antai and Ibrahim, 1986).

The presence of *Staphylococcus* species in the samples was typical of the micro flora of fermenting beans (Obeta, 1983, Antai and Ibrahim 1986). *Staphylococcus* species have been associated with fermenting foods of plant origin especially vegetable proteins (Odunfa and Komolafe, 1989). The presence of

Staphylococcus may also be linked to the hands of the handlers/producers of the condiments.

The coagulase-negative staphylococcus species are non-pathogenic and safe organisms on vegetable proteins. These organisms may contribute to the flavor of Okpehe because of their lipolytic activity.

*Kurthia zopfil* was also isolated from traditional fermented daddawa, this result agrees with the work of Chotwanawirach (1980) who reported the presence of some strains of lactic acid bacteria in Kapi (a traditional fermented shrimp paste that serves as a flavoring/seasoning for many decades) are *Staphylococcus*, *Micrococcus*, *Kurthia* and *Bacillus*. *Listeria monocytogenes* isolated in this work, has also been detected by other researchers, Dajanta et al; (2012) also isolated Gram positive that include *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus* and *L. monocytogenes* by fermenting soybean in traditional production of thua nao.

*Saccharomyces cerevisiae* was found only in one sample of traditional daddawa produced from locust beans. Similar result was obtained by Oyeyiola (2002) *S. Cerevisiae* is known to be able to grow well in the complete absence or presence of very little oxygen as was likely to prevail during the fermentation. The reduced sugar released following the breakdown of the carbohydrates may also influence the presence of yeast in this dawadawa sample.

Based on the critical control point decision tree (NACMCF, 1997) a step is considered as a critical control point if it involves a hazard of sufficiently likelihood of occurrence and severity to warrant its control, is necessary in order to eliminate or reduce the hazard to an acceptable level. Based on this there are two steps that need to be controlled (washed seed and water used for the washing) since there was high bacteria count of ( $1.7 \times 10^6$ ) which was higher than acceptable limit of ( $10^5$  cfu/g) provided by the food and Agriculture organization (FAO, 1979) of the United Nations. The high count recorded from the washed seeds was due to the water used in washing the seeds. Edema and Fawole (2006) reported water used for washing the dehulled seeds may remove some of the contaminating microorganisms introduced during dehulling, but it may also add new ones. Another reason for not having high count in other steps during production of traditional daddawa was the daddawa was carefully done by the researcher at home taking consideration of all sources of contamination and thereby producing the condiment under hygienic conditions.

The raw materials, (that is soya bean seeds and Roselle seeds) usually comes with a substantial microbial load comprising spores of aerobic spore-forming bacteria and mould spores, most of which are drastically reduced during the prolonged boiling to soften the testa as obtainable in related fermentation (Feng et al, 2005) potential pathogens like *E. Coli*, *Clostridium spp* were not isolated in this research, but higher counts of these organisms were obtained in similar production

of daddawa by fermenting African locust bean seeds (Rabi et al., 2013).

## CONCLUSION

The predominant microorganisms in the fermentation process of both samples were found to be *Bacillus species* of which *Bacillus subtilis*, *B. pumilus* and *B. licheniformis* were most involved. The study has shown that a daddawa analogue, comparable to well accepted locust bean daddawa in terms of microbiological characteristics, could be produced from soya bean and roselle seed an under-utilized legumes. There is need for clean environment and materials to be used during traditional fermentation, most especially the use of clean water as it contains high count of bacteria from the research.

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