Effect of Inoculum Sizes in Laboratory Fermentation of Daddawa Condiment from Glycine max (Soya Bean) and Hibiscus sabdariffa (Roselle Seeds)

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Effect of Inoculums size in laboratory fermentation of daddawa from Glycine max (soya bean) and Hibiscus sabdariffa (roselle seeds) was carried out. Samples of soya bean and roselle seeds were fermented under laboratory conditions to produce condiment by using cultures of B. subtilis, B. licheniformis and M. varians that were previously isolated from locally fermented daddawa. The isolate was prepared for various concentrations of 0.2, 0.5 and 0.8 respectively. The pH and temperature was recorded at twelve hour intervals. Fermentation has occurred in all the concentration of inoculum used as starter culture. Best fermentation with right organoleptic properties (aroma/flavor) was achieved in all the concentration used in the fermentation of Roselle seeds. The consortium of three isolates yielded best result in the laboratory fermentation of soya beans and roselle seeds.

Key words: Fermentation, soya bean, roselle seeds, B. subtilis, B. licheniformis, M. varians

INTRODUCTION

A condiment is a seasoning that one adds to food in order to increase its flavour like salt and pepper, Webster’s Reference Library (WRL) (2009). Indigenous fermented condiments have recently assumed a greater importance of Nigeria since the majority of the populace can no longer afford the high cost of edible plant and animal proteins (Ogbadu et al., 1990). The fermentation process of condiment production is still being carried out by the traditional village-art method. There is needed to apply modern biotechnological techniques like the use of starter cultures in improving traditional food processing technologies (Achi, 2005). It has been suggested that even though hitherto most fermentation processes used in developing countries do not use inoculants or extrinsic cultures, these processes could be improved by using starter cultures, and also by back-slopping, which entails application of brine from prior fermentation cycles (Holzapfel, 2002; Na’iba, 2003).

Appropriate starter cultures are widely applied as inoculants across the fermented food sector, from the household to industrial level in low-income and lower-middle-income economies. These starter cultures are generally produced using a back slopping process which makes use of samples of a previous batch of a fermented product as inoculants (Holzapfel, 2002). Appropriate starter cultures are widely applied to the production of fermented fish sauces and fermented vegetables in Asia and in cereal or grain fermentations in African and Latin American countries. The inoculation belt (Holzapfel, 2002) used in traditional fermentations in West Africa serves as a carrier of undefined fermenting micro-organisms, and is one example of an appropriate starter culture. It generally consists of a woven fibre or mat or a piece of wood or woven sponge, saturated with “high” quality product of a previously fermented batch. It is immersed into a new

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batch, in order to serve as an inoculant. The inoculation belt is used in the production of the indigenous fermented porridges, “ujii” and “mawe”, as well as in the production of the Ghanaian beer, “pito”. Iku, also referred to as iru, is yet another example of an “appropriate” starter culture produced by back slopping. This starter culture is produced from concentrated fermented dawadawa (a fermented legume product), mixed with ground unfermented legumes, vegetables such as pepper, and cereals, such as ground maize. It is stored in a dried form and is used as an inoculant in dawadawa fermentations in West Africa (Holzapfel, 2002). Therefore, this study aimed at investigating the Effect of Inoculums size of laboratory fermentation of daddawa from Glycine max (soya bean) and Hibiscus sabdariffa (roselle seeds).

MATERIALS AND METHODS

Sample Collection

Glycine max (soya bean) and Hibiscus sabdariffa (roselle seeds) were obtained from Sokoto Central market. The seeds were authenticated at Herbarium of Biological Science of Usman Danfodiyo University, Sokoto and assigned the Number: UDUH/ANS/0185 and UDUH/ANS/0186. The samples were taken to Postgraduate laboratory, Department of Microbiology, Usman Danfodiyo University, Sokoto for Analysis.

Laboratory Fermentation of Soya Dawadawa (Glycine max)

Soybeans was cleaned and soaked overnight until they doubled in weight. The soaked soybeans was steamed at 115°C for 60min in an autoclave and cooled to room temperature. The cooled soybeans were inoculated with starter cultures (both singly and in consortium) and incubated at 30°C for 24hours (Lee et al, 2007).

Laboratory fermentation of daddawanbotso (Hibiscus sabdariffa)

The same method used in traditional fermentation was also employed in laboratory production of some modification according to the method of Ibrahim et al (2011). Roselle seeds were cooked in pressure cooker for 4hrs at temperature of over 100°C. The seeds were allowed to cool and inoculated with starter culture (both singly and in consortium) and incubated at 300C. After the first fermentation ash leachate was added and incubated again for 24 hours at 30°C.

Preparation of Inoculum

The organisms used as starter cultures were Bacillus subtilis, Bacillus licheniformis and Micrococcus varians. The selected microorganisms were grown in nutrient broth for 24hours. After incubation, 0.1ml each of broth culture was placed on appropriate agar plates using the pour plate technique to determine cell concentrations.

Broth cultures containing approximately the same concentration of viable cells in the range of 10^5 were centrifuged at 4000rpm for 10 mins, washed in sterile distilled water and centrifuged again. The turbidity of the cells was compared with Mcfarland standard of 0.2, 0.5 and 0.8 concentration.

The cells were then used as inoculum, singly and as mixed combinations in the laboratory fermentation of soya beans and roselle seeds.

RESULTS

The effect of inoculums size in laboratory fermentation of daddawa from Glycine max (soya bean) and Hibiscus sabdariffa (roselle seeds) shows that temperature changes during the fermentation of the various seeds using the bacteria isolated previously as inoculums (Figure 1 and 2) the temperature increased during the fermentation period with an initial temperature of 27°C to 32°C for Glycine max and 27.2°C to 32°C for Hibiscus sabdariffa seeds.

![Figure 1: Temperature changes during fermentation of Glycine max](image)

Key : F= Bacillus subtilis, 4 = Bacillus licheniformis, 15 = Micrococcus varians
Effect of Inoculum Sizes in Laboratory Fermentation of Daddawa Condiment from *Glycine max* (Soya Bean) and *Hibiscus sabdariffa* (Roselle Seeds)

The pH profile during the fermentation of *Glycine max* and *Hibiscus sabdariffa* using bacteria isolated previously as inoculums (Figure 3 and 4) the pH value move slightly acidic to neutrality during the fermentation period with *Glycine max*, while that of *Hibiscus sabdariffa* seeds move from slightly acidic to slightly alkaline pH.
DISCUSSION

Daddawa was produced from the laboratory fermentation of Glycine max and Hibiscus Sabdariffa seeds. After 72 hours of fermentation, the mash became soft and dark with a strong ammonialdour. The organism growing in the fermenting daddawa produced a whitish mucilaginous substance that covered and linked the individual cotyledons during fermentation. The increase in temperature during fermentation agrees with report of Jonathan et al., (2011), which showed significant increase in the temperature of the fermenting mash from an initial temperature of 28°C to around 30°C at the 96th during the spontaneous fermentation of bambara nut to produce Iru. The change in pH in the fermenting daddawa varied. This result is in agreement with result of Odunfa (1986) who reported an increase in pH from the beginning to the end of fermentation. This result also agree with the result of Omafuvbe et al., (2002) who reported an initial pH of 6.50 – 6.55 which increased to between 7.50 – 8.00 after 72 hours of fermentation, but the result disagree with that of Jonathan et al., (2011) who reported that the pH decreased from 6.7 at oh to 4.4 at the end of fermentation of bambara nut for the production of Iru. The result disagree with that of Jonathan (2011) who reported that pH decreases from 6.7 at oh to 4.4 at the end of fermentation of bambara nuts for the production of Iru, the result of Ibrahim et al., (2011) who reported that pH decreased from 6.7 at oh to 4.4 at the end of production of daddawan botso. The result of inoculum size used in laboratory fermentation of the seeds shows that in all the concentration of the microorganisms (inoculum size) used (both singly and consortium), fermentation has taken place. But the best fermentation with right/best organoleptic properties (aroma/flavor) was achieved in all the concentration/organisms used in the fermentation of Hibiscus sabdariffa, this also explain the reason why daddawan botso was used as starter culture in local fermentation of parkia biglobosa seeds and Glycine max. Another reason is B. spp spores are resistant to high temperature and pressure even after long cooking to soften the seeds, and autoclaving to sterilize the seeds. And also the consortium of three (3) organisms yielded best result in the fermentation of Parkia biglobosa and Glycine max.

CONCLUSION

Daddawa was produced in the laboratory by using single and mixed cultures of B. subtilis, B. licheniformis and M. varians. Increased in pH and temperature throughout the fermentation was observed. Best fermentation was achieved in laboratory fermented daddawa from roselle seeds using both single and mixed cultures of isolates. Its recommended that appropriate starter culture for the production of daddawa should be developed, this will help the guarantee of microbiological safety and nutritional standard of the condiment.

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