

EFFECT OF LEVELS OF BACILLUS SPP AS INOCULUM ON PH AND TITRATABLE ACIDITY DURING CONTROLLED FERMENTATION OF SOYBEAN TO DAWADAWA.

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ABSTRACT

Effect of levels of *Bacillus subtilis* and *Bacillus licheniformis* used singly or in combination as inoculum on pH and titratable acidity (TA) development during soybean fermentation for dawadawa production were investigated. Soybean seeds were processed and then fermented with 0.01%, 0.025%, 0.05%, 0.075% and 10% levels of *B. subtilis* and *B. licheniformis* inoculum in singles or in combination. The results showed that there was a significant increase ($p < 0.05$) in titratable acidity values at all the levels of *B. subtilis* and *B. licheniformis* used singly or in combination as inoculum. Fermentation time and inoculum levels as titratable acidity changes resulted in significant increase in titratable acidity. Fermentation of soybean with *B. licheniformis* showed that inoculum levels (0.01%, 0.025%, 0.05%, 0.075%, 0.10%) used as titratable acidity changes were not significant ($p < 0.05$). pH values increased significant ($p < 0.05$) at all levels of *Bacillus* spp used singly or in combination. Fermentation time and inoculum levels (0.01%, 0.025%, 0.05%, 0.075%, 0.10%) used for *B. subtilis* were significant ($p < 0.05$). There was no significant difference in the product quality attributes based on inoculum type and levels used as evaluated by sensory panelists. Therefore, dawadawa could be produced using either *B. subtilis* or *B. licheniformis*.

Keywords:

INTRODUCTION

Legumes are important foods in many developing countries. Most of the legumes are fermented to enhance the acceptability of the end products. Hesseltine (1965) reported that fermentation improves flavours, nutritive values, increases digestibility and destroys some natural toxins which may occur in the legumes. Perhaps, these advantages have contributed to the popularity and acceptability of fermented soybean products such as miso, tempeh and shogun in the Orient. Soybean, which has been described as a miracle crop and a good source of body building protein (Simmons, 1970) has gained wide acceptability in Nigeria probably because of the high cost of animal protein in Nigeria. Dawadawa, traditionally fermented from locust bean, is now being produced from soybean (Mebrahtu and Hahn, 1986) as a result of the advantages which soybean has over locust bean (i.e. low cost and ease of cultivation). However, dawadawa production from soybean is still a family art, produced using chance fermentation. This has therefore limited the acceptability of the product due to variability in quality and flavour as well as the unhygienic nature of the production environment. While various workers have studied the nutritional quality and the microorganisms

associated with the fermentation of soybean for dawadawa production (Popoola and Akueshi, 1984; Ogbadu and Okagbue, 1988; Onyejebu and Oguntunde, 1993), no information is available on the effect of using pure isolates of *B. subtilis* and *B. licheniformis* (which have been identified as the predominant microorganisms involved in soybean fermentation into dawadawa) to inoculate soybean for dawadawa production. The present study was aimed at providing such information as well as establish the benefit or otherwise of the combined use of the isolates for soybean fermentation into dawadawa.

MATERIAL AND METHODS

Culture cultivation and inoculum preparation

A loopful each of *Bacillus subtilis* and *Bacillus licheniformis* cultures on agar slant isolated from traditionally fermented soybean in the laboratory were inoculated separately into 50 ml nutrient broth contained in a 250 ml conical flasks covered with cotton wool and aluminium foil. A ratio of 1:5 was maintained between the medium and the flask volume for maximum aeration. The flasks were shaken for 24h on a continuous shaker at low speed. Each isolate was

cultivated in triplicates and a control (sterile nutrient broth) was also shaken.

Processing of dawadawa using pure isolates as inoculum

About 100g of soybean was roasted for 10 min, and cook under aseptic conditions for 1h using sterile distilled water which was afterwards drained off. The seeds (100g) were then aseptically transferred into sterile plates (15x 150m) diameter and inoculated with 0.01%, 0.025%, 0.05%, 0.075%, 0.10% w/v of the appropriate cell suspension (*Bacillus subtilis* or *Bacillus licheniformis*) as a single inoculum or in combination with the two isolates in approximately 1: 1 ratio. The 100g seeds were thoroughly mixed to ensure even and proper distribution of the inoculum. The samples were subsequently fermented for 48h at 37°C. Samples were harvested at 12h intervals and two duplicate fermentations were conducted.

Analysis

The method of Ikenebomeh et al (1986) was used for determining the titratable acidity (TA) and PH of the samples. Ten (10g) of fermented bean was ground in a mortar and 90 ml of distilled water added. The mixture was stirred for 2 min, and the resultant pH read with a single electrode (model PHM 82). For the titratable acidity, distilled water was boiled to expel carbon dioxide, cooled to room temperature and then used to prepare a 10⁻¹ homogenate as described above. A 10 ml aliquot was titrated with 0.1N NaOH and phenolphthalein (1%) used as end point indicator. A 10 ml of decarbonated distilled water was also titrated and the water titre value was subtracted from the sample titre. Titratable acidity was calculated as lactic acid per g sample (mg lactic acid/g).

Oranoleptic evaluation

Sample of dawadawa produced from the inoculum of soybean with different levels of *B.*

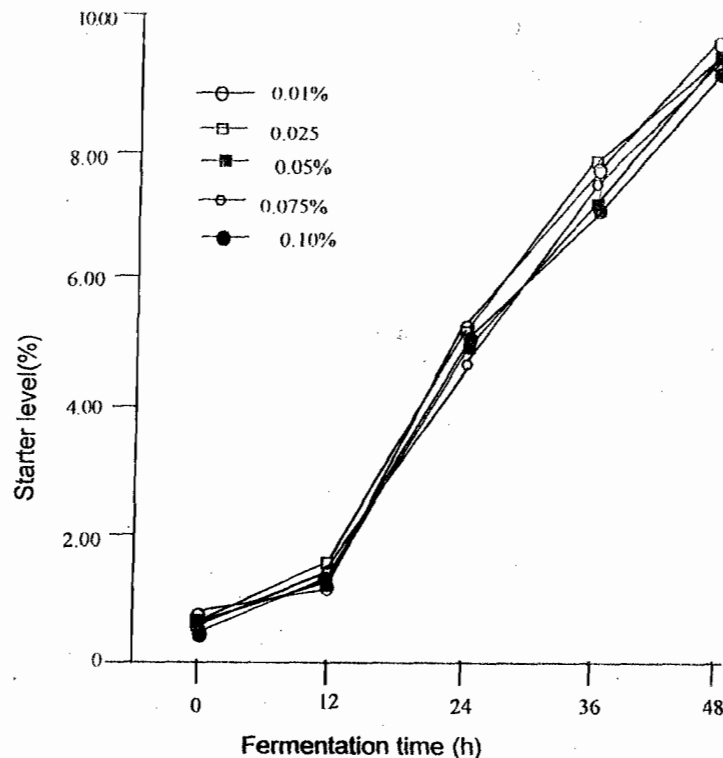


Fig 1: Effect of levels of *B. subtilis* as starter on titratable acidity during controlled fermentation of soybean

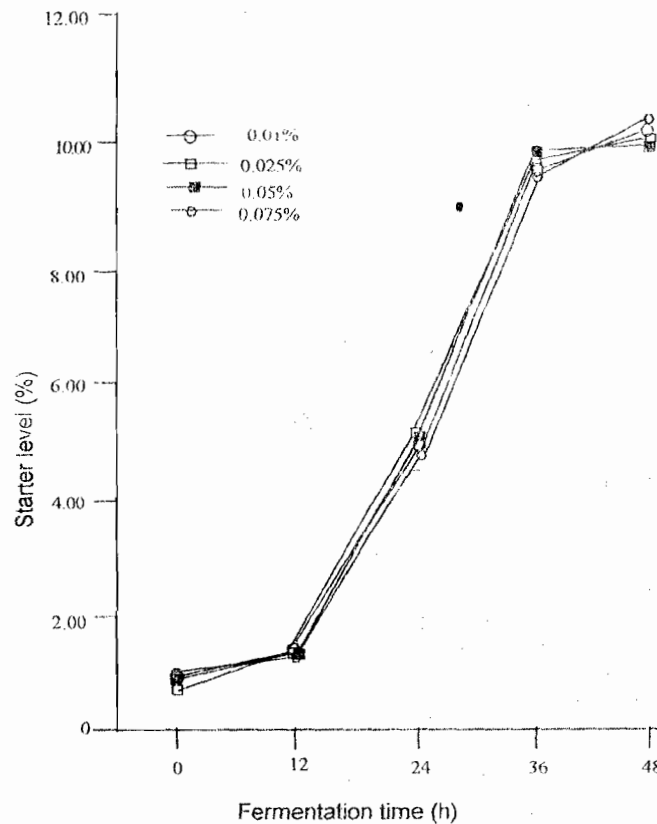


Fig. 2: Effect of levels of *B. licheniformis* as starter on titratable acidity during controlled fermentation of soybean

subtilis and *B. licheniformis* (0.01%, 0.025%, 0.05%, 0.075%, 0.10%) were subjected to sensory panelists. Data were evaluated using multiple comparison analysis (Larmond, 1979). Panelists were asked to smell and rate the sample twice using soybean dawadawa purchased from a local market as a reference. A 9-point hedonic scale was used, where 1= like extremely and 9= extremely inferior. The samples were presented to the assessors in a random order.

Data analysis

Data obtained were subjected to analysis of variance using SAS (1985) package.

RESULTS AND DISCUSSION

Figure 1 shows the trend in titratable acidity (TA) of fermenting soybean during the use of *B. subtilis* as single starter. The TA values during the fermentation using *B. subtilis* as starter (inoculum) ranged from 0.87 mg lactic acid/g to 9.24 mg lactic acid/g across all the levels used. The increases in the TA levels in the various levels used were as follows; 0.88 – 9.11 mg lactic acid/g (0.01%), 0.92 – 9.18 mg lactic acid/g

(0.025%), 0.91 – 9.24 mg lactic acid/g (0.05%), 0.89 – 9.16 mg lactic acid/g (0.075%) and 0.87 – 9.16 mg lactic acid/g (0.10%). There was sharp significant ($p < 0.05$) increase in TA from 12 – 48 h of fermentation for all the levels of inoculum used. Statistical analysis of fermentation time and levels of inoculum, as well as the interaction between the factors were all significant ($p < 0.05$).

There was also a significant increase ($p < 0.05$) in the amount of TA generated in all the levels in samples inoculated with *B. licheniformis* as shown in Fig. 2. TA values during fermentation of soybean using *B. licheniformis* as starter ranged from 0.88 mg lactic acid/g to 10.20 mg lactic acid/g across all the levels used. Individual increase of TA in the various levels of inoculum used were as follows: 0.91 – 10.20 mg lactic acid/g (0.01%), 0.88 – 1.18 mg lactic acid/g (0.025%), 0.89 – 10.15 mg lactic acid/g (0.05%), 0.90 – 10.2 mg lactic acid/g (0.075%) and 0.92 – 10.10 mg lactic acid/g (0.10%). Fig. 3 shows the trend in TA of fermenting soybean during combined use of *B. subtilis* and *B. licheniformis* as inoculum. The TA values obtained using combination of *B. subtilis* and *B. licheniformis* as inoculum ranged from 0.89 – 10.25 mg lactic acid/g across all the levels used. The increase in

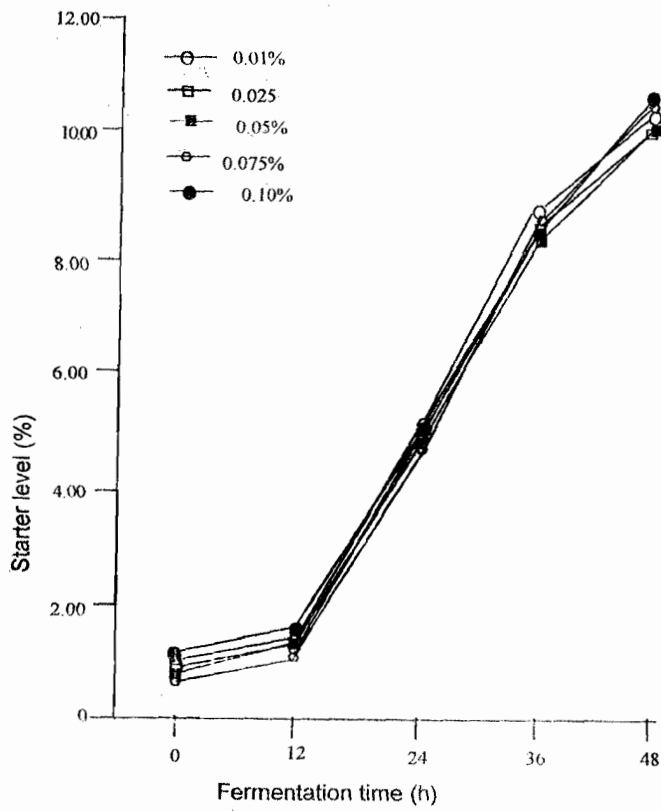


FIG 3: Effect of levels of *B. subtilis* and *B. licheniformis* as starter ratio (1:1) on titratable acidity during controlled fermentation of soybean

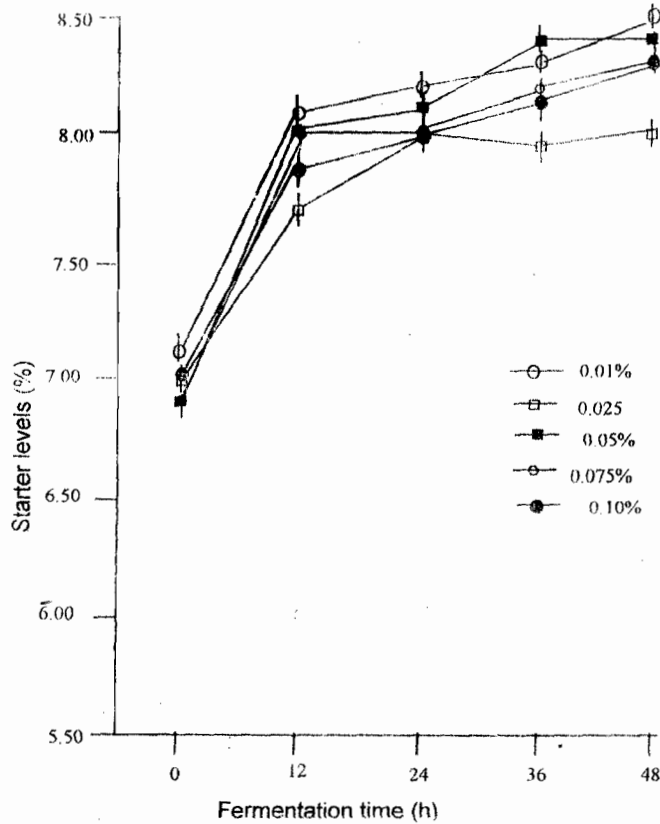


FIG 4: Effect of levels of *B. subtilis* on pH during controlled fermentation of soybean

the TA values obtained from the various levels of the combined inoculum were 0.92 – 0.23 mg lactic acid/g (0.01%), 0.96 – 10.0mg lactic acid/g, (0.025%) < 0.89 – 10. 18mg lactic acid/g (0.05%), 0.89-10.24 mg lactic acid / g (0.075%), 0. 91 – 10.25 mg lactic acid /g (0.0.1%).

There was a significant increase ($p < 0.05$) in the amount of TA for all the levels of combined inoculum used. Statistical analysis of fermentation time and inoculum levels as well as the interaction between fermentation time and inoculum levels were all significant ($p < 0.05$). The result is consistent with reports of increases in the

TA of fermented seeds of melon (Achinewhu, 1986a), oilbean (Achinewhu, 1986b) and locust bean (Ikenebomeh et al, 1986).

Changes in pH values as affected by levels of *B. subtilis* are shown in Fig. 4. The pH values during the fermentation using *B. subtilis* as starter ranged from 6.90 - 8.50 across the levels used. The increases in the PH values obtained from the various levels used were; 7.10 – 8.50 (0.01%), 7.0 – 8.0 (0.025%), 6.9 – 8.40 (0.05%), 6.9 – 8.45 (0.075%), 7.0 – 8.40 (0.1%). There was an abrupt increase in pH value in all the levels at 12h, thereafter the increases were

gradual. Increase in the pH values of values of various levels of *B. subtilis* except for 0.05% level were significant ($p < 0.05$). Statistical analysis of fermentation time and inoculum levels as two factors in pH changes, showed that fermentation time and inoculum level were both significant ($p < 0.05$).

Figure 5 shows the changes in pH during inoculation with various levels of *B. licheniformis* as starter. PH values during fermentation of soybean using *B. licheniformis* as starter ranged from 6.90 – 8.35 across all the levels used. Individual increases of pH in the various levels of inoculum used were 7.0 – 8.35 (0.01%), 7.0 – 8.15 (0.025%), 6.90 – 830 (0.05%), 6.90 – 8.20 (0.075%), 7.0 – 8,35 (0.1%). Samples inoculated with various levels of *B. licheniformis* had significant increase in pH ($p < 0.05$). Statistical analysis of fermentation time and inoculum levels as two factors showed that fermentation time, and inoculum level were significant ($p < 0.05$) for pH changes.

Figure 6 shows pH changes during the substrate fermentation with a combination of starters as inoculum. The pH values for the combined used of *B. subtilis* and *B. licheniformis*

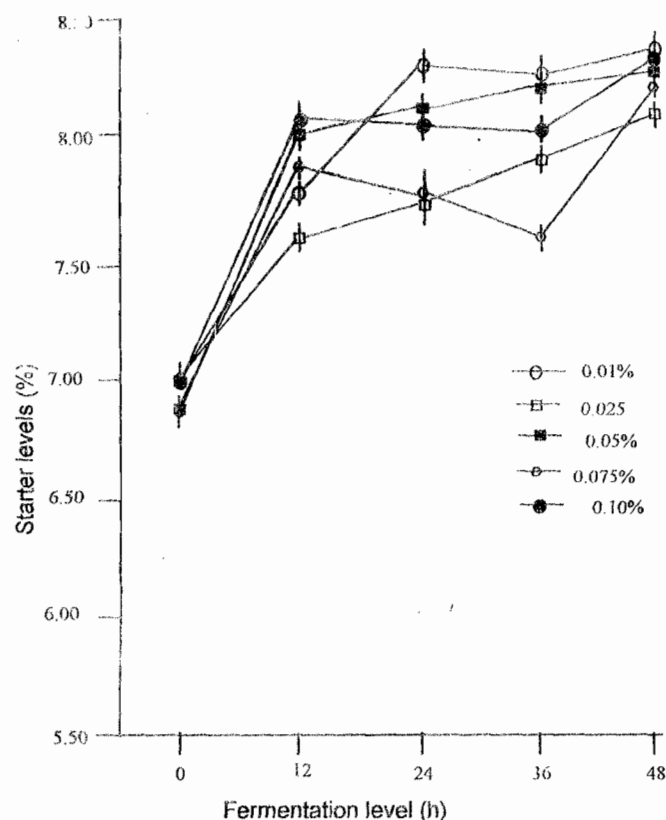


FIG 5: Effect of levels of *B. licheniformis* as starter on pH during controlled fermentation of soybean

Table 1: Sensory Evaluation Result on the Use of the Different Levels of Isolates to Ferment Soybean into Dawadawa

Source of variation	Sq	Sum of Sq	Mean Sq	F- value	Prob -f
Panelist	8	158.80	19.85	7.33	0.83
Isolate	14	20.93	1.50		
Error	112	303.20	2.71	0.55	0.89
Total	134	482.93	3.61		

as starters ranged from 7.0 – 8.50 across all the levels. The increases in the pH values in the various levels used were as follows; 7.10 – 8.30 (0.01%), 7.0 – 8.40 (0.025%), 6.90 – 8.40 (0.05%), 6.90 – 8.40 (0.075%), 7.0 – 8.50 (0.1%). The changes of pH in various levels of inoculum used increased significantly ($p < 0.05$). Statistical analysis of fermentation time and inoculum level as two factors showed that fermentation time and inoculum level were significant ($p < 0.05$) for pH changes.

Increases in pH during the fermentation of oil seeds have been attributed to the production of proteases by the fermenting organisms, which degrade the proteins of the substrates to amino

acids, peptides and free ammonia. It is the free ammonia that causes the pH increases in the substrates. Increase in pH during fermentation of protein rich oil seeds have also been observed by various workers (Odunfa, 1981; Achinwhu, 1986b; Ikenebomeh et al, 1986; Barber et al, 1988). This simultaneous increases in pH and TA in the fermenting substrate inoculated with the starter were not unusual. Simultaneous increases in both pH and TA values were observed during a study of burong dalag, a fish and moist rice fermented product. It was suggested that liberated ammonia or other basic end products of protein decomposition were the cause of this. A similar trend has also been reported to occur

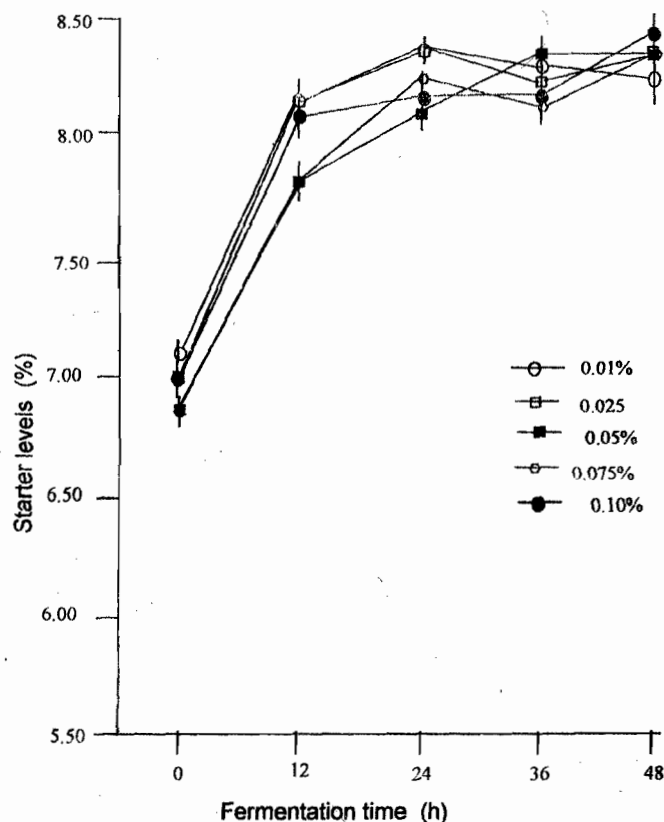


FIG 6: Effect of levels of *B. subtilis* and *B. licheniformis* as starter (1:1) ratio on pH during controlled fermentation of soybean

during the natural fermentation of soybean (Onyejebu and Oguntunde, 1993), in which pH increased despite the relatively large amount of acid that was liberated. Organoleptic evaluation showed that there was no significant difference ($p < 0.05$) between isolate type and isolate levels used (Table 1).

This study shows that soybean fermented with pure isolates produced the characteristic desired dawadawa odour. It is thus possible to commercialize the production of soybean dawadawa using controlled fermentation. However, further investigation might be needed to develop the starter in a convenient form that can be easily used as has been done for temph-mould.

CONCLUSION

Dawadawa being a highly desired food ingredient in Nigeria especially in the South-West, South-East regions, because of its dual qualities of characteristic smell and high nutritional quality, can be commercially produced using controlled fermentation as against the current practice of chance fermentation. Soybean dawadawa has a great potential as a leading food ingredient with the possibility of attracting international acceptance, as is the case with Asian soybean based foods e.g. Tofu, if dawadawa is produced hygienically using controlled fermentation and then adequately packaged.

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