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Effect of Soaking and Roasting Dehulling Methods of Soybean on *Bacillus* Fermentation of Soy-Daddawa

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Abstract: Soy-daddawa was prepared by fermenting soaked dehulled and roasted dehulled soybeans by a starter culture of *Bacillus subtilis* SDA3 (isolated previously from traditional fermented soy-daddawa) for 72 h. The viable cell counts of *B. subtilis*; accompanying biochemical changes as well as the products were evaluated. The viable cell count increased from an initial value of 10^4 to 10^9 cfu/g wet wt. at the end of fermentation. The pH of the fermentation of soybeans dehulled by the two methods rose from 6.7 to 8.4 with a concomitant increase in proteolytic activity, free amino acids and ammonia concentration. Alpha amylase and beta fructofuranosidase activities exhibited a rapid increase in activity in the first 24 h. Reducing sugars increased in the first 24 h and dropped in the fermentations of soaked dehulled and roasted dehulled soybeans. Soybean dehulled by the two methods showed similar biochemical and viable cell count profile during fermentation with *B. subtilis* SDA3. The two types of soy-daddawa differ significantly ($p<0.05$) in color, texture and general acceptability while there was no significant difference in aroma, stickiness and taste. In all the organoleptic attributes scored, there was preference for soy-daddawa produced from roasted dehulled soybean.

Key words: Soybean, soaking, roasting, daddawa, *Bacillus subtilis*, fermentation

INTRODUCTION

Soy-daddawa, a product of alkaline fermentation of soybean (*Glycine max*) is used as a food condiment in much the same way as African locust bean (*Parkia biglobosa*) daddawa. Currently, soybeans is used as an alternative raw material for daddawa production in some localities in Nigeria since African locust bean is seasonal with a dwindling supply (since the locust bean tree has been neglected in agricultural/forestry research and extension) making the seed very expensive. In addition, the processing of African locust bean into daddawa is tedious, fuel and time consuming (the entire production time requiring about 5-6 days). African locust bean involve first boiling the seeds for 12-24 h, dehulling of seeds, second boiling for 1-2 h before fermentation for 72 h under ambient temperature (Odunfa, 1981: Ouoba *et al*., 2003; Achi, 2005). Soy-daddawa and African locust bean daddawa are organoleptically similar (Omafuvbe *et al*., 2002) and their fermentation is accomplished by *Bacillus* species especially *Bacillus subtilis* (Ogbadu and Okagbue 1988a; 1988b; Omafuvbe *et al*., 2000; Dike and Odunfa, 2003).

To make traditional soy-daddawa, soybeans are dehulled using either of two methods prior to fermentation. In the first method, soybeans are soaked overnight (about 12 h) in cold tap water and then dehulled manually. In the second method, soybeans are roasted over a fire in a frying pan and dehulled manually. The dehulled soybeans are washed with water, boiled for 1-2 h and then fermented (Popoola and Akueshi, 1985; Omafuvbe *et al*., 2000).

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Optimization of the traditional process conditions of soy-daddawa is necessary to reduce the production time, guarantee improved and consistent flavour, increased shelf life and allow scale up of the process. In line with this, *Bacillus subtilis* as a monoculture starter has been reported to produce soy-daddawa of same organoleptic quality with the traditional fermented soy-daddawa (Omafuvbe *et al*., 2002; Terlabie *et al*., 2006). Furthermore, 1% salt improved the organoleptic properties of fermented soy-daddawa (Omafuvbe, 1994; 2006).

One of the aims of this study was to investigate the effect of dehulling soybean by soaking and roasting on some biochemical changes during starter culture fermentation with *B. subtilis* (SDA3) previously isolated from natural fermented soy-daddawa. The second aim was to compare the organoleptic attributes of soy-daddawa produced by the two dehulling methods.

MATERIALS AND METHODS

Seeds

Soybean seeds were purchased from a local market in Ile-Ife, Osun State, Nigeria.

Organism

Bacillus subtilis SDA3 used as starter culture was previously isolated from natural fermenting soy-daddawa (Omafuvbe *et al*., 2000) and reported to produce daddawa of the same organoleptic quality with the traditional soy-daddawa (Omafuvbe *et al*., 2002). The organism was maintained on nutrient agar (Oxoid CM3) slope in the refrigerator. The morphological and biochemical characteristic of the *B.subtilis* SDA3 is shown on Table 1.

Production of Soy-Daddawa

Soy-daddawa samples were prepared in the laboratory using the two traditional methods for dehulling soybeans. In the first method, 1.0 kg of soybeans was roasted for 4 min in a frying pan over a gas cooker flame. The roasted seeds were grind to remove the hulls, which were blown-off. The

Table 1: Morphological and biochemical characteristics of *Bacillus subtilis* SDA3 used as starter culture

Characteristics	*Bacillus subtilis* SDA3*
Colony shape on nutrient agar	Irregular
Catalase	Positive
Gram reaction	Positive rod
Spore stain	Positive (C)
Slime production **	Positive
Starch hydrolysis	Positive
Gelatin liquefaction	Positive
Nitrate reduction	Positive
Lipolytic activity	Positive
Citrate utilization	Positive
Acetoin production	Positive
Indole	Negative
Anaerobic growth	Negative
O/F test (Hugh and Leifson`s medium)	Fermentative
Acid from:	
Glucose	Positive
Mannitol	Positive
Sucrose	Positive
Xylose	Positive
Growth at:	
45°C	Positive
50°C	Positive
60°C	Negative

* Isolated from natural fermented soydaddawa (Omafuvbe *et al*., 2000); O/F, Oxidative/Fermentative test; ** Slime production on sucrose-glutamate medium (Aumayr *et al*., 1981); C: Central

roasted dehulled cotyledons were then washed with warm water (60°C). In the second method, 1.0 kg of soybeans were soaked in tap water overnight (12 h), dehulled manually by rubbing between the palms of the hand and the hulls were separated from the cotyledons by floating in cold water. In both cases, the dehulled beans were dispensed in 50 g amounts into 250 mL conical flasks. The contents of the flasks were steamed at 121°C for 20 min to obtain sterile cooked soybeans.

Suspension of predominantly vegetative cells of *B. subtilis* (SDA3) was prepared in Maximum Recovery Diluent (MRD, Oxoid CM733) as previously described by Omafuvbe (2006). Sterile cooked soybeans held in 250 mL flasks were inoculated with 500 µL of the cell suspension of *B. subtilis* (this gave approximately 10^4 cells/g wet wt. of soybeans). Inoculated soybeans were incubated at 30°C for 72 h. Duplicate flasks were removed for analysis at selected times from each of the two sets of fermentation.

Viable Cell Counts

Fermenting soybeans (5.0 g) were homogenized with 45 mL of sterile MRD in stomacher bags by stomaching for 2 min (Colworth Stomacher 400). Further dilutions were made in MRD and 1.0 mL of appropriate dilutions was plated in duplicate nutrient agar using the pour plate method. Inoculated plates were incubated at 30°C for 48 h after which the bacteria colonies were counted and expressed as colony forming units (cfu) per gram sample.

pH and Titratable Acidity Determination

Fermenting soybeans (10.0 g wet wt.) were homogenized with 90 mL of distilled water and the homogenate filtrate was titrated with 0.1 M NaOH to pH 8.3, using a pH meter (Hanna instruments 8250). Titratable acidity was expressed as mg lactic acid/g sample. The pH of the fermenting soybeans was determined as previously described (Omafuvbe, 2006).

Estimation of Ammonia

The ammonia content of the fermenting soybeans (2.0 g wet wt.) was extracted as previously described by Sarkar *et al*. (1993) and Omafuvbe (2006). The extracted ammonia was assayed enzymatically (Boehringer Kit 11 112 732 035, Boehringer-Mannheim/R-Biopharm, Germany).

Determination of Reducing Sugar and Free Amino Acids

The soluble sugars and free amino acid content of the fermenting soybean were extracted with 80% ethanol (v/v) as previously described by Odibo *et al*. (1990). The ethanolic extract was appropriately diluted for the determinations. The total free amino acid content was determined by the ninhydrin colorimetric method (Rosen, 1957). The free amino acid concentration was calculated from a standard curve of known concentration of glycine. The reducing sugar was estimated by the colorimetric method (Somogyi, 1945) using glucose as standard solution.

Determination of α-amylase, β-fructofuranosidase and Proteolytic Activities

α-amylase enzyme in the fermenting soybeans was extracted with 0.1 M phosphate buffer (pH 6.0) as previously described by Omafuvbe *et al*. (2000). The blue value assay method (Fuwa, 1954) was followed for the determination of α-amylase activity. One unit of activity was defined as the amount of enzyme that produced a 10% reduction in the intensity of blue colour under the experimental conditions.

β-fructofuranosidase enzyme in the fermenting soybeans was extracted with 0.05 M sodium citrate buffer (pH 6.5) as previously described by Omafuvbe *et al*. (2000). The assay mixture comprised of 500 µL sucrose (5.0% in extracting buffer) and 500 µL of appropriately diluted enzyme solution incubated at 30°C for 30 min. The amount of reducing sugar produced was estimated by the

colorimetric method (Somogyi, 1945) using glucose as standard solution. One unit of enzyme activity was defined as the amount that produced 100 μg of glucose under the assay conditions.

Proteolytic enzyme in the fermenting soybeans (3.0 g wet wt.) was extracted with 0.05 M phosphate buffer (pH 7.0) as previously described by Omafuvbe (2006). The assay for proteolytic activity was based on the method described by Sarkar *et al*. (1993) using azocasein (2.5 g L^{-1}; Sigma A2765) as substrate. One unit of proteolytic activity is defined as the amount that produced an absorbance increase of 0.01 units under the assay conditions.

Sensory Analysis

Soy-daddawa samples produced by starter culture fermentation of soybean dehulled using the two methods (roasted and soaked dehulled) were evaluated organoleptically by a panel of 20 judges made up of regular consumers of daddawa using a score scale of 1 (dislike extremely) to 5 (like extremely) as previously described (Omafuvbe *et al*., 2002).

The data obtained were subjected to one way analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test (Primer for Biostatistics software package version 3.01 by Glantz (1992). Statistical significance was accepted at p-value equal to or less than 0.05.

RESULTS AND DISCUSSION

During fermentation, the growth rate pattern of viable cells of *Bacillus subtilis* SDA3 in both soaked and roasted dehulled soybeans were similar (Table 2). In both fermentations, there was an initial rapid increase in viable cell count in the first 24 h of fermentation followed by a gradual increase with a final microbial population reaching 10^9 c.f.u/g wet wt. of sample. Similar growth pattern has been reported in *Bacillus* fermentation of soybean for kinema production (Sarkar *et al*., 1993). The viable cell count in fermenting soaked dehulled soybeans was slightly higher between the 24th to 72nd h of fermentation when compared with values obtained for roasted dehulled soybeans. The data on viable cell count is an indication that the nutrients in soybean (dehulled either after soaking or roasting) supported the growth of *Bacillus subtilis* SDA3.

Table 2: Changes in viable cell count, pH and titratable acidity in soybean inoculated with *Bacillus subtilis* SDA3 during fermentation into daddawa

	Viable cell count (log_{10} c.f.u. g^{-1} wet wt.)		pH		Titratable acidity (mg lactic acid/g wet wt.)	
Fermentation time (h)	Soaked dehulled beans	Roasted dehulled beans	Soaked dehulled beans	Roasted dehulled beans	Soaked dehulled beans	Roasted dehulled beans
0	4.85	4.86	6.67	6.71	0.72	0.63
24	9.51	9.00	6.86	6.90	3.87	3.87
48	9.70	9.36	8.30	8.25	1.89	1.53
72	10.18	10.04	8.37	8.35	1.17	1.26

Values are means of determinations on duplicate fermentations

Table 3: Concentrations of ammonia, reducing sugars and total free amino acids in soybean inoculated with *Bacillus subtilis*SDA3 during fermentation into daddawa

	Ammonia (g kg^{-1} wet wt.)		Reducing sugars (mg glucose g^{-1} dry wt.)		Total free amino acids (mg glycine g^{-1} dry wt.)	
Fermentation time (h)	Soaked dehulled beans	Roasted dehulled beans	Soaked dehulled beans	Roasted dehulled beans	Soaked dehulled beans	Roasted Dehulled beans
0	0.04	0.04	1.67	2.83	12.70	9.63
24	0.40	0.37	14.16	11.06	86.00	85.00
48	0.91	0.85	10.03	10.24	179.50	178.80
72	0.86	0.85	9.89	9.73	190.90	189.30

Values are means of determinations on duplicate fermentations

The pH values of the fermenting soybeans (soaked or roasted dehulled) inoculated with *B. subtilis* SDA3 were similar throughout the fermentation (Table 2). The pH rose from 6.67 to 8.37 and from 6.71 to 8.35 in fermenting soaked dehulled soybeans and roasted dehulled soybeans, respectively. The increase in pH is a common feature in the fermentation of vegetable proteins (Steinkraus, 1996).

The changes in titratable acidity in the fermenting soybeans were similar in both fermentations. The titratable acidity increased significantly in the first 24 h and decreased significantly thereafter in both fermentations. The initial increase is indicative of carbohydrate hydrolysis leading to acid production during this period of fermentation. The slightly higher titratable acidity of the soaked dehulled soybeans at the onset of fermentation may be as a result of soaking, since soybean is reported to undergo acid fermentation during soaking (Mulyowidarso *et al*., 1989; Omafuvbe and Sanusi, 2005). The decrease in titratable acidity after the 24th h of fermentation coincided with the period when there was a sharp increase in pH towards alkalinity and ammonia concentration (Table 3).

The reducing sugar in the fermenting soybeans increased sharply in the first 24 hours and decreased thereafter in both fermentation procedures (Table 3). The period of rapid increase coincides with the period of increased viable cell count, α-amylase and β-fructofuranosidase activities in the fermenting seeds (Table 2, Fig. 1 and 2). The high activities of α-amylase and fructofuranosidase observed in the fermenting seeds are not unexpected since *B. subtilis* SDA3 is amylolytic and ferments sucrose (Table 1). The decrease in reducing sugar level of the fermenting seeds indicates that they are being used by *B. subtilis* for metabolism. The low concentration of reducing sugar in the soaked dehulled beans at the onset of fermentation is an indication that some sugars may have been leached out in the soak water, since reducing sugar in soybean soak water has been reported to increase (Omafuvbe and Sanusi, 2005).

The levels of ammonia, free amino acids (Table 3) and proteolytic activity (Fig. 3) were very similar in roasted and soaked dehulled soybeans fermentations. The free amino acid content of the fermenting seeds increased rapidly in the first 48 h and less rapidly thereafter in the fermentation of both roasted and soaked dehulled soybeans. The increase in free amino acids was due to the high

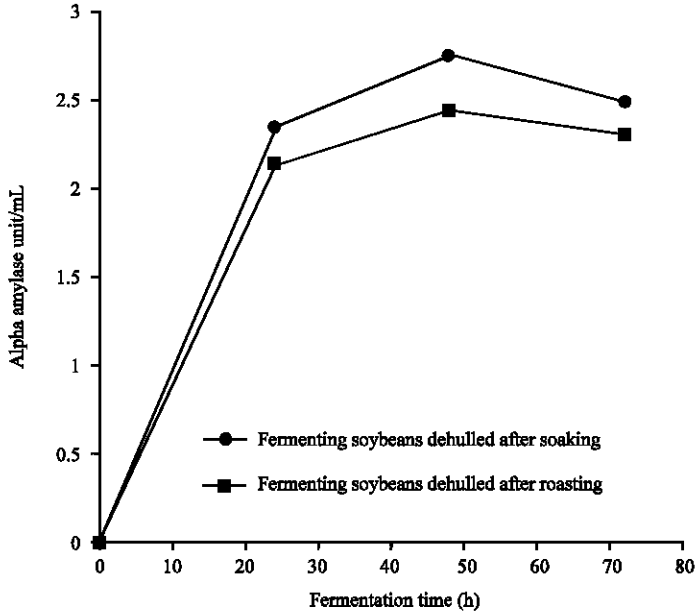

Fig. 1: Alpha amylase activity in soybean inoculated with *Bacillus subtilis* (SDA3) during fermentation for daddawa production

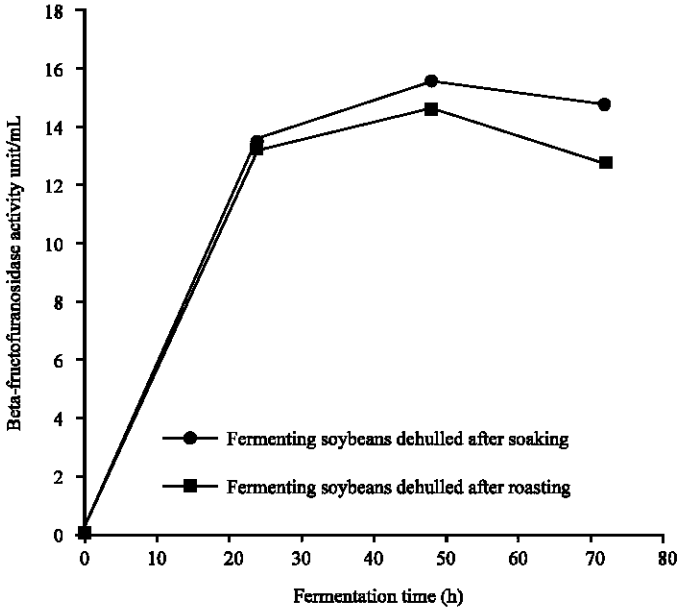

Fig. 2: Beta-fructofuranosidase activity in soybean inoculated with *Bacillus subtilis* (SDA3) during fermentation for daddawa production

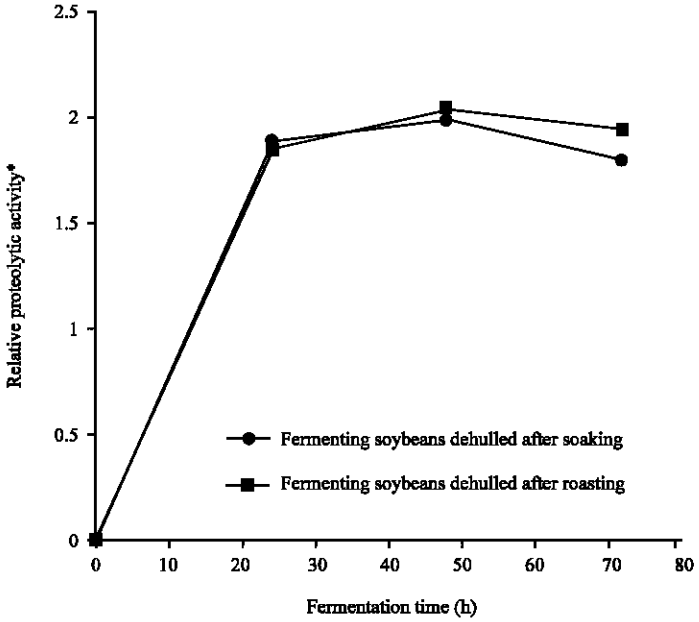

Fig. 3: Relative proteolytic activity in soybean inoculated with *Bacillus subtilis* (SDA3) during fermentation for daddawa production. * Measured as activity on azocasein

proteolytic activity of *B. subtilis* in the fermenting seeds (Fig. 3). The increase in ammonia content of the fermenting seeds indicates the utilization of amino acids as carbon and energy sources by the fermenting organism.

Table 4: Sensory evaluation of soy-daddawa produced by *Bacillus* fermentation of soybeans dehulled by soaking and roasting

	Organoleptic attributes					
Soy-daddawa samples	Color	Aroma	Stickiness	Texture	Taste	General acceptability
Soaked *	2.60±0.23^{a}	3.35±0.29^{c}	3.05±0.28^{c}	2.80±0.26^{a}	3.80±0.20^{c}	3.05±0.23^{a}
Roasted **	3.95±0.20^{b}	4.00±0.27^{c}	3.40±0.26^{c}	3.60±0.24^{b}	4.10±0.22^{c}	3.85±0.20^{b}

Values represent the mean scores ± standard error (n = 20), Means having the same superscripts within each column do not differ significantly ($p<0.05$), * Soy-daddawa produced from soybeans dehulled after soaking, ** Soy-daddawa produced from soybeans dehulled after roasting

Table 4 shows the sensory evaluation of soy-daddawa produced by *B.subtilis* fermentation of soybean dehulled after soaking or roasting. There was significant difference ($p<0.05$) in color, texture and general acceptability of the products. However, no significant difference was observed for aroma, stickiness and taste of the soy-daddawa products. In all the organoleptic attributes scored, there was preference for daddawa produced from roasted dehulled soybeans. The preference showed for the color of daddawa produced from roasted dehulled soybean may be related to the dark brownish color impacted on the product by roasting. Although, volatile aroma compounds were not evaluated in this study, roasting has been reported to impact good flavours (Manley *et al*., 1999). In this context, the preference for the roasted dehulled fermented soybean may be due to the roasting process before fermentation. Further studies on the volatile aroma compounds of the two products would be necessary to clarify the contribution of the roasting process.

The results of this study suggest that soybean dehulled after soaking and soybean dehulled after roasting undergo similar biochemical changes when fermented with *B. subtilis* SDA3 to produce daddawa. In addition, roasted dehulled soybean produced more organoleptically acceptable daddawa than soaked dehulled soybean. In conclusion, dehulling soybeans by soaking which takes longer time, has no advantage over dehulling by roasting. Therefore, roasting as a dehulling method can be used to optimize the production of soy-daddawa.

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