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Nutrient composition of soybean fermented with combined culture of *Bacillus subtilis* and *Bacillus licheniformis*

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ABSTRACT: Quantitative changes in the nutrient composition of soybeans during fermentation of soybean condiment by a combined culture of *Bacillus subtilis* and *Bacillus licheniformis* were investigated. The highest increase and the lowest decrease in the nutrient quantities were attained between 20 and 30h of fermentation. Significant ($P > 0.05$) increases were in the total soluble protein (356 $mg.g^{-1}$), free amino acids (354 $mg.g^{-1}$), ammonia (1.18 mmol/ml) and organic acid (3.5 molar acidity) relative to the values in unfermented soybean. Total sugars, reducing sugar, lipids and ash decreased by the end of the 72nd hour of fermentation. The resultant product of fermentation seems to be of higher quality than the unfermented soybean.

Key Words: Soybean fermentation; *Bacillus subtilis; Bacillus licheniformis*; Soydaddawa; Soyiru.

Introduction

'Soydaddawa' or 'soyiru' are appelations for fermented semi-solid products of dehysked soybean (*Glycine max* L. Merr.) in Nigeria. Like natto, it is added to soups and stews as condiments. Nigeria is the largest producer of soybeans in western and central Africa, and the bulk of the harvest is consumed as foos in the form of daddawa (Popoola and Akueshi, 1985) and fortified soyogi (Singh *et al*., 1987).

The seed primarily contains high protein and oil. The protein content of most cultivated varieties is between 40 and 41 percent and the oil is about 20% on a dry matter basis. The protein is well balanced in the essential amino acids. Proximate analysis by Wolf and Cowan (1975) showed that whole soybean consists of 40% protein, 21% fat, 34% carbohydrate and 4.9% ash while soybean cotyledon consists of 43% protein, 23% fat, 29% carbohydrate and 5% ash. Stachyose, raffinose, phosphatides, sterols, ash and other constituents are also present.

Soybean ferments into a condiment that is similar to 'iru' which is obtained from fermented African locust bean (Odunfa, 1985), and 'Ukpaka' which is also obtained from *Pentaclethra macrophylla* Benth (Deboye and Okonkwo, 1985). There are many fermented products of soybean in the Oriental countries. Fermentation process modifies the characteristic of the substrate nutritionally and chemically. In many cultures of the world all sorts of food and organic materials are allowed to ferment; either naturally or through controlled fermentation for the purpose of improving the end products. Changes are mediated through enzyme systems by which the final products are reduced or incompletely oxidised. In this way, fermentation may enhance the digestibility of the starting material by breaking down complex protein structures to peptides of varying lengths and free amino acids. Vitamins and amino acid levels many vary

or remain static, depending on the type of microorganisms involved in the fermentation and the material that was fermented.

The fermentation of many leguminous seeds into condiments in Africa is primarily for use as flavour enhancers and usually leads to improvement in organoleptic properties, while remaining wholesome. fermentation may also have detoxifying effect. In 'daddawa' preparation, oxalic and phytic acid levels are significantly reduced (Eka, 1980) during fermentation. Entrenchment of the fermenting organisms in the system, in addition to the production of certain metabolites creates competition for nutrients and antibiosis respectively; thus preventing other contaminants from developing (Fabian, 1951; Mensah *et al*., 1991). Suberu and Akinyanju 91996) reported that the combined cultures of *Bacillus subtilis* and *B. licheniformis* facilitated the production of soybean condiment with the best result compared with the single organism fermentation.

Unlike the one with fermented African locust bean, there has not been published data on the biochemical changes of fermented soybeans, especially with defined starter culture. The present study aims to report the changes in the nutrient composition of fermented soybean using a combined culture of *B. subtilis* and *B. licheniformis* as starter culture. Such studies are of potential advantage in food processing and fortification, especially in a developing country like Nigeria.

Materials and Methods

Preparation of 'soydaddawa'

Autoclaved soybean cotyledons were inoculated with a combination of the culture of *B. subtilis* and *B. licheniformis* and fermented in accordance with the procedure described by Suberu and Akinyanju (1996).

Sampling

Samples of autoclaved and fermented soybeans at 12, 24, 36, 48, 60 and 72h were obtained. Dried triplicate fermented samples were used in all assays except ammonia.

Total soluble protein

The extraction of protein from 1g sample was as described in the study by Kijowski and Niewiarowicz (1985). The soluble protein was estimated by the method of Lowry *et al.* (1951). Spectrophotometric readings were calculated against the standard curve with egg albumin to get the actual concnetration after multiplying by the dilution factor.

Total free amino acid

The ninhydrin colorimetric method as described by Odunfa 91985) was employed to estimate the total free amino acid in 1g of unfermented and fermented soybean.

Lipid

Extraction of groung 1g sample of the autoclaved and fermented soybean cotyledon was done in 15 ml chloroform-methanol (2:1 v/v) solvent. The mixture was allowed to stand for 24h. It was filtered through defatted filter paper. Non-lipid contents were removed in 2 ml of 0.73% NaCl solution, inside a separating funnel for 10 min. The lipid layer was washed three times wit the solution of chloroform:methanol:0.58% NaCl (3:48:47 v/v/v). the final separation was made in a conical flask of known weight. The solvent was evaporated off in a water bath at 60^{o}C with frequent shaking. The lipid content was the difference between the final weight and the initial weight of flask (Folch *et al.*, 1957).

Total sugar

Crude sugar from 1g per replicate of dried soybean cotyledon was extracted in 50 ml of 80% ethanol by heating at 65^{o}C for 3h. Basic lead (1%) in the ratio of 1:8 extract was added to purify sugar extract while excess lead was removed by the addition of 0.5 ml methanol to 8 ml of the mixture. The mixture was centrifuged at 5,000 rpm for 10 min (Faparusi, 1970). The optical density of the supernatant was read at 490 nm on a Spectronic 20 spectrophotometer. The actual concentration of sugar was calculated against the standard curve obtained with glucose (Dubois *et al*., 1956).

Reducing sugar

This was estimated by the dinitrosalicylic reagent method as described by Odunfa 91985). Five grams of the dried autoclaved and fermented soybean cotyledon in triplicates was used.

Ammonia

The method involving the use of the Microconway unit – dilute HCl – Nessler reagent reaction was used to assay for ammonia production. One gram of fermenting soybean cotyledon was placed in the inner chamber of the Conway unit, in triplicates. Two ml of 0.001M HCl was dispensed in the outer chamber and covered with the glass plate. In the control, one ml distilled water replaced the fermenting cotyledon. After one hour, one ml of the HCl was pipetted and diluted with 9 ml distilled water. One ml of Nessler's reagent was added to the solutions. The optical density of the solutions were measured at 450 nm against the control in an SP8-150 UV/Visible spectrophotometer. The concentration of ammonia in the samples were calculated against the standard curve obtained with serially diluted ammonia solution, multiplied by the dilution factor.

Total Organic Acid

The method of Philipson as described by Norris and Ribbon (1979) was used in the determination of organic acid content of fermented soybean cotyledons.

Ash

The method described by Ranjhan and Krishna (1980) was employed to assay for ash in the samples of soybean cotyledon.

Results

The autoclaved soybean cotyledon contained 296 mg.g^{-1} of total soluble protein. It increased to 358 mg.g^{-1} by the end of the 30th hour of the fermentation (Fig. 1). thereafter, the value dropped to 332 mg.g^{-1} by the 60th hour, and then remained constant until the fermentation was terminated. Free amino acid was 15.03 mg.g^{-1} in the autoclaved soybean cotyledon. Within the initial 30h of fermentation the free amino acid increased rapidly and substantially to 354 mg.g^{-1} (Fig. 2). the level dropped gently to 270 mg.g^{-1} at 72h fermentation. There was also a rapid increase in ammonia production; the level at 20h of fermentation was more that twice the level at 12h of fermentation (Fig. 3). The level of production dropped until after which the level was maintained. The lipid content of autoclaved soybean cotyledon was 116.0 mg.g^{-1}. This decreased to 72.0 mg.g^{-1} at the 20th hour of fermentation (Fig. 4), and then increased gently to 76 mg.g^{-1} at 72h of fermentation. the total organic acid content of autoclaved soybean was estimated at 0.25 molar acidity, increasing to 3.5 molar acidity at the 30th hour of fermentation (Fig. 5). There was a sharp drop afterwards to 1.03 molar acidity at the 48th hour and a gradual further decrease to about 0.8 at the 72nd hour. Total sugars in the soybeans was 43.4 mg.g^{-1}. It decreased to 33.8 mg.g^{-1} after 20h of fermentation and further to 30.5 mg.g^{-1} and 23.5 mg.g^{-1} at the 30th and 72nd hour respectively (Fig. 6). there was a slight increase in the reducing sugar from 29.0 µg.g^{-1} to 33.0 µg.g^{-1} during the initial 30h of fermentation and

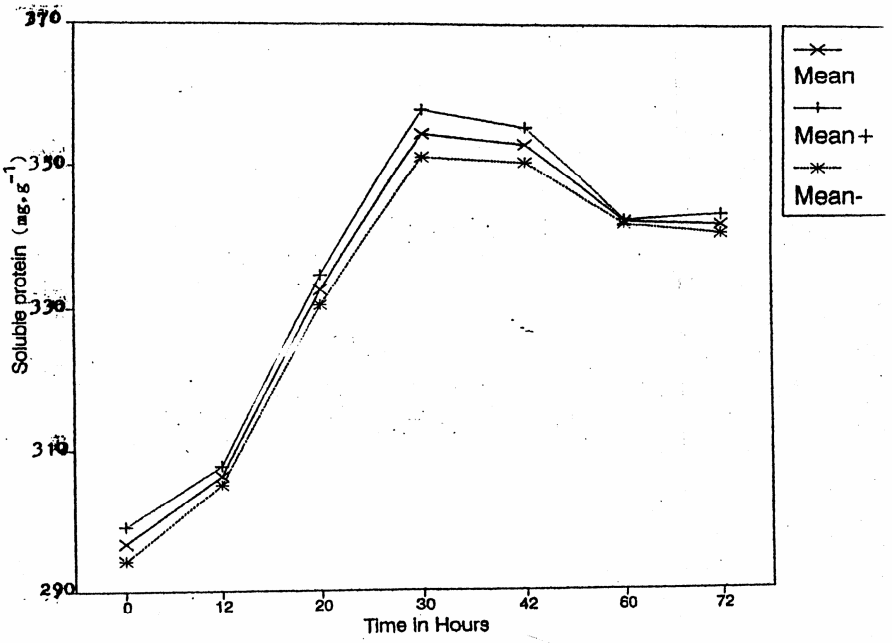

Fig. 1. Total soluble protein in fermenting soybean cotyledons inoculated with combined culture of B. subtilis and B. licheniformis.

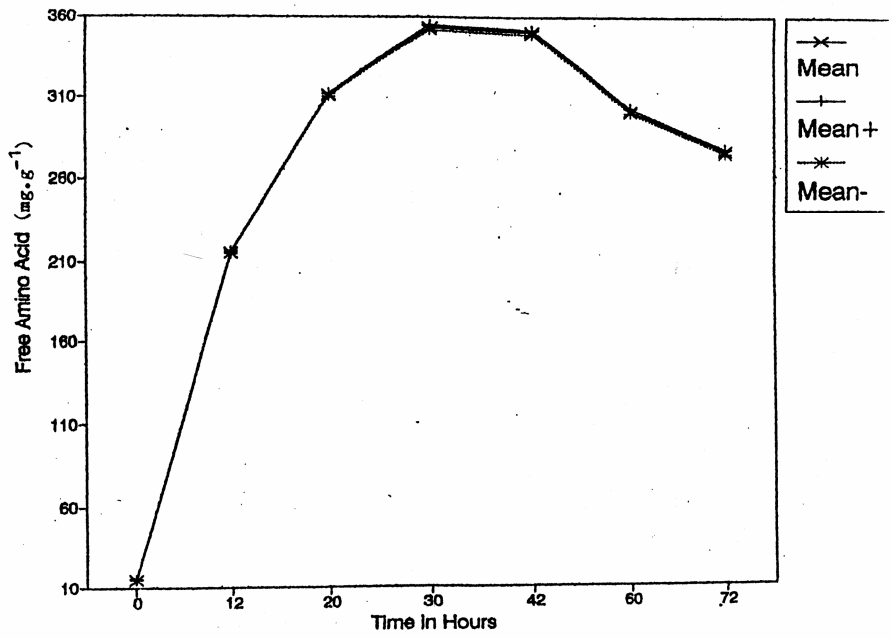

Fig. 2. Free amino acid of fermenting soybean cotyledons inoculated with the combined culture of B. subtilis and B. licheniformis.

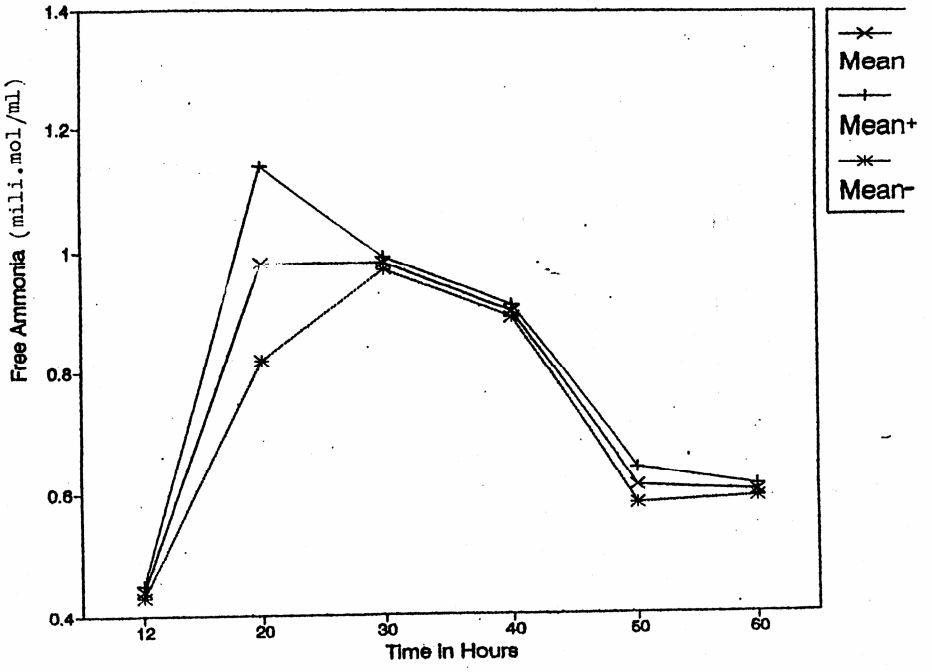

Fig. 3. Free ammonia produced in the fermenting soybean cotyledons inoculated with the combined culture of B. subtilis and B. licheniformis.

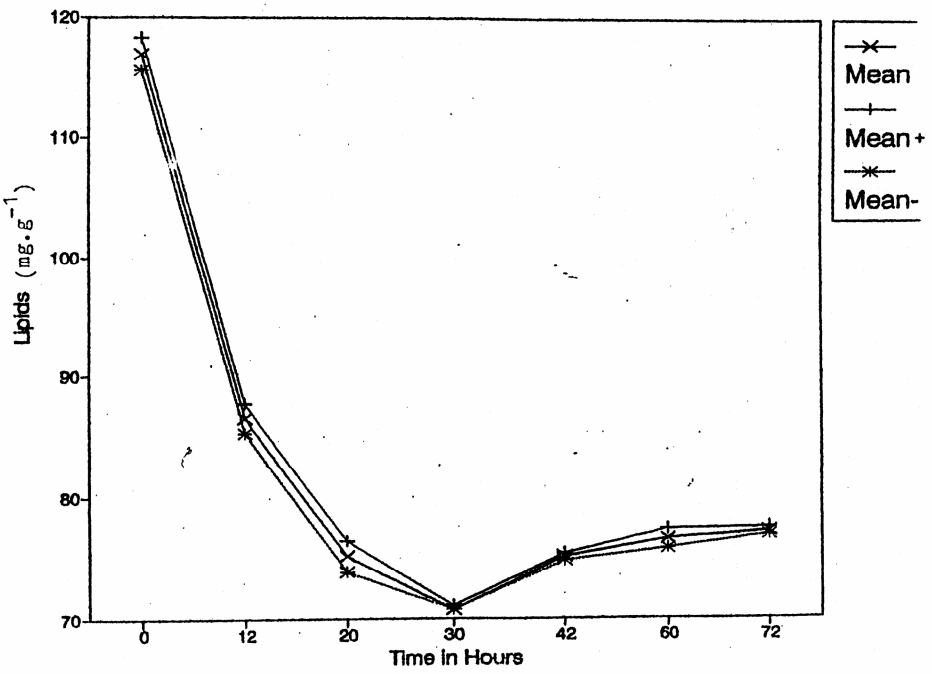

Fig. 4 Lipid in the fermenting soybean cotyledons inoculated with combined culture of B. subtilis and B. licheniformis.

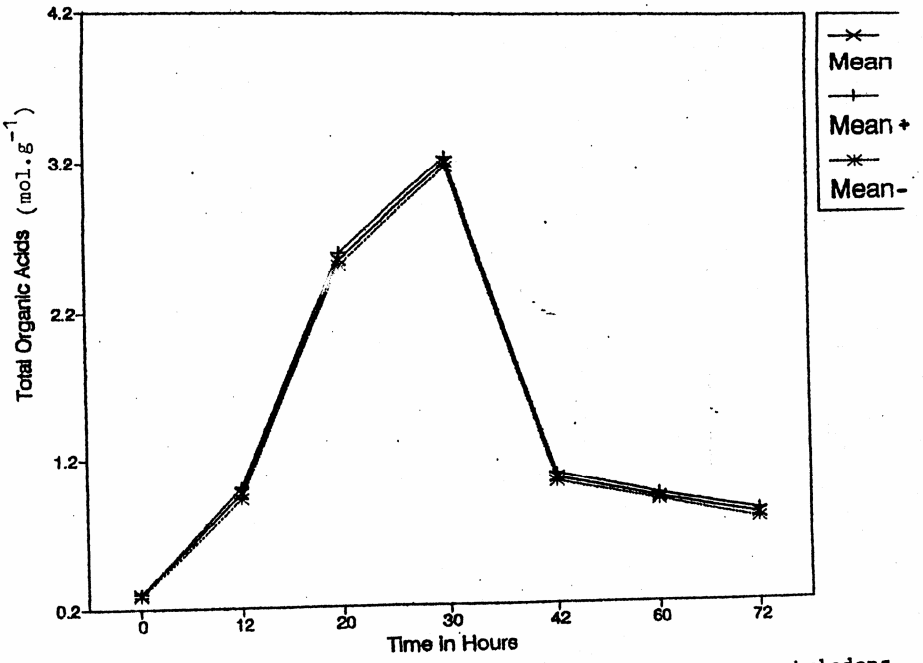

Fig. 5 . Total organic acids in the fermenting soybean cotyledons inoculated with the combined culture of B. subtilis and B. licheniformis.

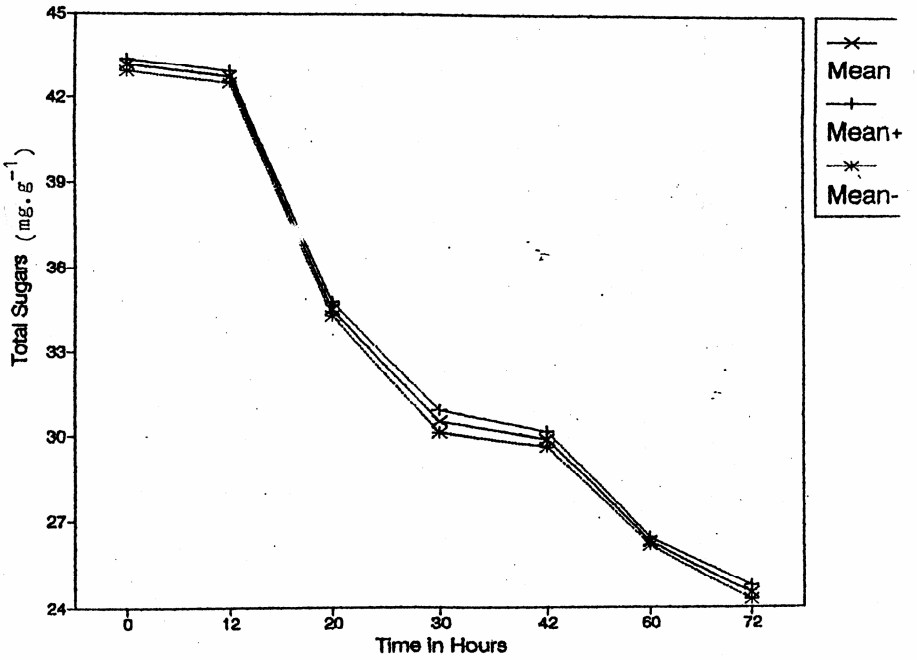

Fig. 6 . Total sugars in the fermenting soybean cotyledons inoculated with the combined culture of B. subtilis and B. licheniformis.

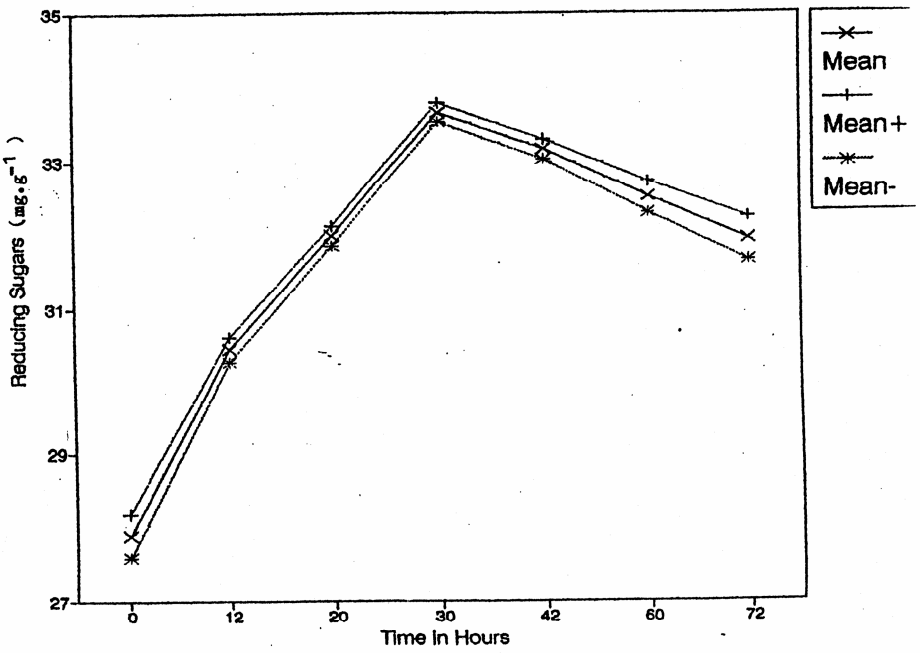

Fig. 7.. Reducing sugars in the fermenting soybean cotyledons inoculated with the combined culture of B. subtilis and B. licheniformis.

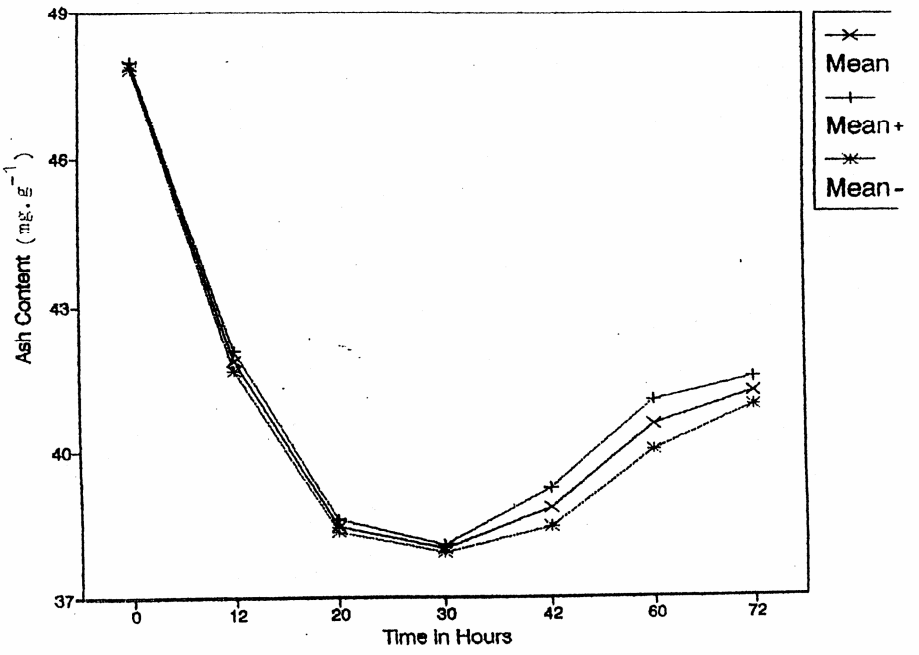

Fig. 8. Ash of fermented soybean cotyledons inoculated with the combined culture of B. subtilis and B. licheniformis.

subsequently the level was maintained till the 72nd hour (Fig. 7). The ash in the boiled soybean cotyledon was 48.0 mg.g^{-1}. It decreased with fermentation to 38.0 mg.g^{-1} after 30h and then a slight increase till 72h.

Discussion

The chemical composition of 'soydaddawa' in this study puts it among many other reported fermented soybean products in spite of the fact that the fermentation is absolutely mediated by the *Bacillus* spp. of bacteria. Enhancement of the products of proteolysis during the initial fermentation and then a reduction are reported in this study. there were increases in soluble protein and the free amino acids, similar to the reports of Young and Wood 91977) in their study of soysauce koji. the percentage increase of free amino acids of 'soydaddawa' are higher that has been reported in 'iru', a fermented condiment with the seeds of *Parkia biglobosa* (Odunfa, 1985). proteolysis is a common feature in the fermentation of many vegetable proteins, including products fermented by fungi and bacteria. In all cases the complex protein is hydrolysed into soluble protein and subsequently liberated in the form of free amino acids and ammonia gas. Decrease in amino acid towards the end of the fermentation should be a result of increased deamination of the amino acids produced, and perhaps its utilization for energy production. The quality of fermented products of soybeans , like soysauce, miso and tempeh are measured by the nitrogen yield, total soluble nitrogen and the ratio of amino nitrogen to total soluble nitrogen. Soluble amino nitrogen improves the taste of soybean fermented products (Yokotsuka, 1981).

The oligosaccharides and polysaccharides also hydrolysed to provide an increased level of sugar at the beginning of the fermentation. The sugar supplied the required source of carbon for the fermentation bacteria (Odunfa, 1983a,b) hence there was a decrease in the sugar level, especially at the most active period of fermentation.

The most active period of fermentation was more quickly attained with the combined cultures of *B. subtilis* and *B. licheniformis*, unlike the traditional chance fermentation. The peak activity in the production of a similar condiment by chance fermentation, with the seeds of *Parkia biglobosa,* was from 36h of fermentation (Odunfa, 1985). Presumably, all the changes in the nutrients are attained during the most active fermentation, and conventionally the fermentation is terminated immediately by the addition of salt. The enhancement of earlier fermentation in this study should be a result of the entrenchment of defined starter culture of the two *Bacillus* spp. It follows that soybean be terminated by the 30th hour of fermentation.

Although setailed amino acid analysis and animal studies are needed to enable unequivocal assertion in respect of the nutritional quality of the fermented product, the increase in total free amino acids and proteins is an encouraging preliminary result. the fermentation of locust beans enhanced increased number and quantities of amino acids (Odunfa, 1985). All fermented misos had much higher soluble nitrogen contents compared to the unfermented substrates (Carol-Shieh *et al*., 1982). It is hoped that further nutritional evaluation of the fermented product will be undertaken.

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