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Full Length Research Paper

Changes in enzyme activities during the fermentation of castor oil bean seeds using *B. subtilis* as monoculture starter

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Abstract

***B. subtilis* monoculture starter isolated from traditional fermenting castor oil bean condiment was used to inoculate three different samples of castor oil bean mash namely B_1 (0% NaCl/Lime), B_2 (2% NaCl), B_3 (3% Lime). The investigation into the enzyme activities during the fermentation of castor oil bean into *ogiri* revealed presence of proteinase, lipase and amylase enzymes using High Performance Liquid Chromatography. The sample containing 0% NaCl/lime had higher values for the three enzymes. The proteinase enzyme values ranged from 1.79 – 2.55 µg/ml for the three samples while the lipase enzyme activity ranged from 3.59 – 6.23 µg/ml. The amylase activity ranged from 2.35 – 4.20 µg/ml. It was also observed that the enzyme values increased as the fermentation time progressed from 0 – 96h in all the fermented castor oil bean samples.**

Keywords: Enzyme, castor bean, fermentation, *B. subtilis*

INTRODUCTION

Indigenous fermented foods developed through traditional or village art technologies which were preserved over the years in order to maintain their uniqueness and identity (Valyasevi and Rolle, 2002). Fermented foods are essential components of the diet in a number of developing countries and are consumed either as main dishes or as condiments (Steinkraus, 1996). They are prepared from both plant and animal materials, using processes in which microorganisms play active roles in the physical, nutritional and organoleptic modification of the starting material (Aidoo,1994). Fermentation enhances the nutrient content of foods through biosynthesis of vitamins, essential amino acids and proteins, by improving protein and fibre digestibility, by enhancing micronutrients bio-availability, and by degrading antinutritional factors. It also provides a source of calories when used in the conversion of substrates, unsuitable for consumption, to human foods.

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Fermentation processes enhance food safety by reducing toxic compounds such as aflatoxins and cyanogens, and producing antimicrobial factors such as lactic acid, bacteriocins, carbon dioxide, hydrogen peroxide and ethanol which facilitate inhibition or elimination of food-borne pathogens (Daeschel, 1989). Therapeutic properties of fermented foods have also been reported (Rolle, 1998).

Castor oil bean is a poisonous seed of the castor bean plant, *Ricinus communis* and belongs to the class of a plant called *Euphorbiaceae* and family *Angiosperm*. The castor oil plant probably indigenous to the south-eastern Mediterranean region and parts of East Africa is today widespread throughout the tropical regions of the world (Ibe and Orabuike, 2009). The castor oil bean is inedible because the seed contains a toxic protein, ricin and other toxic constituents, ricinine and ricinoleic acid but these can be removed by fermentation (Odunfa and Oyeyiola, 1985). The fat content of the seeds of *R.communis* is about 15% to 25% and consists of about 40-53% of fixed oil comprising glycosides of ricinoleic, isoricinoleic, stearic and dihydroxy stearic acids (Lin and Areinas,

2007). Also the seeds contain about 25% protein with 10 -20% carbohydrates, 2.2% ash and 5.1 – 6.5% moisture (Verscht et al., 2006).

Castor oil seed, *Ricinus communis* , is one of the well known oil seeds in Africa which forms important part of the diet. Other oil seeds like melon seed (*Citrullus vulgaris*) and sesame seed (*Sesamum indicum*) are fermented in similar manner like castor oil and ground into oily paste called 'ogiri'. These pastes have very strong putrid odour with some ammonia odour. 'Ogiri-Igbo' is produced from cooked-fermented castor oil bean cotyledons. It gives a pleasant flavour when added to soups and sauces. The product is consumed in eastern and mid-western parts of Nigeria by about five million people. As with other traditional arts there are no written records of origin of 'ogiri'. However, its history can be traced back several centuries to when castor oil seeds were traditionally planted around the areas of its consumption (Odunfa, 1985). In the urban areas the production and consumption of ogiri from castor oil seed is decreasing and being replaced by imported bouillon cubes like maggi cubes (Essien, 1983).

Ibe and Orabuike (2009) reported that the microorganisms involved in the fermentation of castor oil bean for 'ogiri' production were bacteria species of which *Bacillus subtilis* were mostly predominant. Other species identified were *B. licheniformis, B.megaterium* and *B.firmus*. All the *Bacillus* species were proteolytic and were capable of fermenting castor oil seed and producing the characteristic 'ogiri' aroma. This present research seeks to use High Performance Liquid Chromatography to study the changes in enzyme activities during the fermentation of castor oil bean seeds into 'ogiri' using *B.subtilis* as monoculture starter.

MATERIALS AND METHODS

Seeds

The castor oil bean seeds *(Ricinus communis)* used in this research were purchased from New Aba main market in Aba Abia State, Nigeria.

Organism

B. subtilis used as starter culture was previously isolated from naturally fermenting castor oil bean, *ogiri* and was maintained on nutrient agar slants in the refrigerator prior to use.

Preparation of *Bacillus* fermented castor oil bean seeds

The laboratory fermentation of castor oil bean was done using the method of Enujiugha (2009).

Determination of Enzymes in fermenting bastor bean using HPLC using waters 616/626 HPLC

The analysis was carried out using Waters 616/626 HPLC inter-phased with a computer system. The samples were weighed into a set of homogenized tubes and homogenized for 30 minutes. A quantity of 2.5g of the homogenized samples were transferred to a set of extraction tubes. 10.0 ml ultrapure water was added, followed by 10ml of acetone containing 5% (V/V) glycerol and 1.5% 2-mercaptoethanol. The set up was shaken on mechanical shaker (Edmund Buhler Model, USA). The sample solutions were quickly transferred to a set of polypropycene containers and caped properly. The samples were shaken again for 15 minutes, transferred to the centrifuge (Thermo Electron Corporation IEC Centra GP8 model, USA) and centrifuged for 20 minuts at 500rpm. Then samples were put into a set of auto-analyser tubes caped and stored for enzyme analysis using Waters 616/626 HPLC.

The accessories involved in the separation of the various analytes of the enzymes are:

i. Column (stationary phase) : Is Lichrosorb Si-60 7um.
ii. The mobile phase was isooctane / tertbutyl methyl ether (97.3ml).
iii. The detector was fluorescence detector (330nm).
iv. Interphased with a Digital Converter Software (DCS).
v. Flow rate used was 1.5ml/minutes.

The sequence of separation was as follows:

The sample solutions were arranged in a set of autoanalyser cups and mounted on the auto-sampler. All the necessary data/information were entered on the sample table of the softwares used (ie the laboratory numbers, the weight of sample, dilution factor, range of standards, concentrations, and all the information were saved accordingly.

The HPLC and the Autoanalyser sampler were put on. The probe of the auto-sampler picked the sample solution into the stationary phase (Is Lichrosorb Si-60 7um). The sample solution on suction to the stationary phase, mixed with the mobile phase (Iso octane / tertbutyl methyl ether (97:3)ml. The sample mixture with the mobile phase moved along the stationary phase. The analytes were eluted according to their molecular weight into a Fluorescence detector (FD) at the wavelength of 230nm. The intensity of the eluted analyte is fed on the Digital Converter Software 1473 series (DCS) Waters, starting from the analytes of a set of standards, and followed by the samples analytes.

Calculation:

The software stored the signal intensity from the standard concentration at the standards (0.0, 0.20, 0.4, 0.6, 0.8 ug/ml), and uses it to calculate the concentration of the unknown.

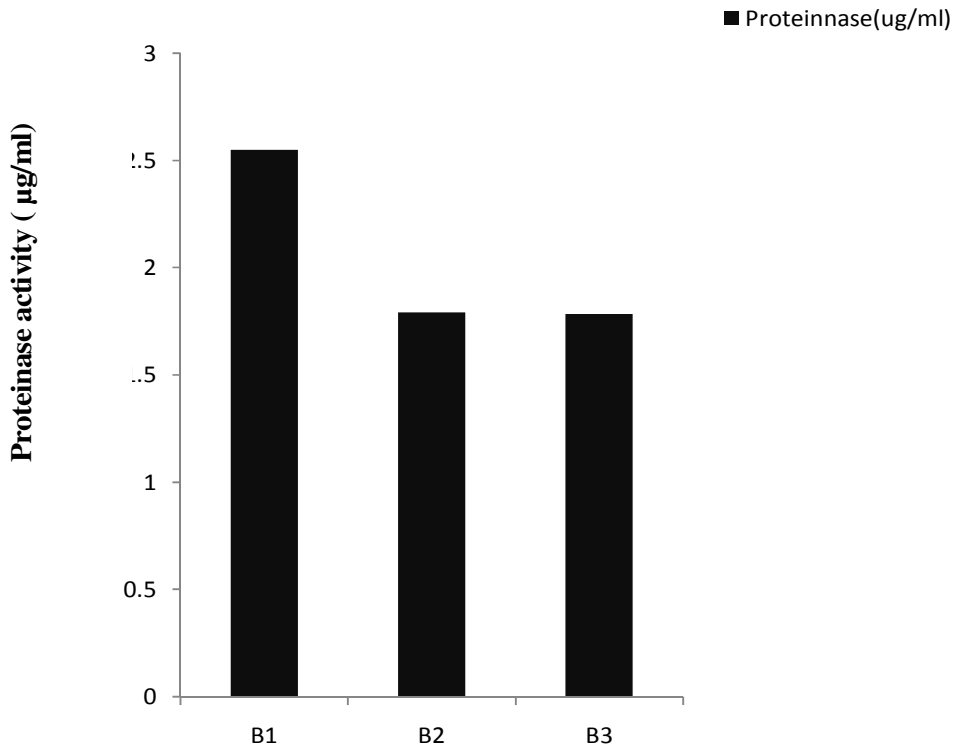

Figure 1. Mean of proteinase activity in fermenting castor bean samples

Where: ***B_1=O%NaCl/lime, B_2= 2% NaCl,B_3= 3%lime**

mg/ml enzyme (in Extract) = Dilution factor x peak height response

mg/ml enzyme (in sample) = ug/m/in extract x volume / Wt of sample

Statistical analysis

Statistical analysis was carried for each set of data obtained following the procedures of Steel and Torie (1984) for a Factorial Randomized Complete Block Design (Factorial RCBD) while GENSTAT discovery package (2006 edition) was used for the analysis of the data. Comparison of treatment means and significant differences between treatment means separated using Fisher's Least Significant Difference (LSD) as outlined by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Figure 1 shows the mean result for the proteinase enzyme activity of the *Bacillus* fermented castor oil bean samples. Sample B_1 (0%NaCl/Lime) had higher proteinase enzyme activity of 2.55µg/ml followed by samples B_2 (2% NaCl) and B_3 (3%Lime) with proteinase values 1.79 µg/ml and 1.78 µg/ml respectively. The difference could be attributed to the additives 2%NaCl and 3%Lime since the sample B_1 (0%NaCl/Lime) had the highest proteinase enzyme activity. The result in Figure 2 shows the mean results for the proteinase activity which increased as fermentation time progressed in the three samples. This increase in proteinase activity ranged from 1.73 – 2.50 µg/ml. This therefore is an indication that ogiri production by fermentation is undertaken by the activities of microbial enzymes secreted during the fermentation process. These enzymes catalysed a number of specific reactions during the fermentation which then produced changes in the food constituents, leading to enhancement of texture, safety, appearance, nutritional value and flavours in the food (Rolle, 1997). Detailed studies on the degradation of African locust bean proteins have been reported for pure cultures of Bacillus species isolated from different products of soumbala, a fermented African locust bean (Ouoba et al., 2003). Proteolysis has been reported as the main metabolic activity during the fermentation of African locust bean which also contributes to the development of texture and flavour of fermented products (Ouoba et al., 2003). Olajuyigbe and Ajele (2008) reported that protease enzyme was active over the entire pH range of 5 to 11 in the study of some properties of protease produced by *B.licheniformis*. Protease activity was also reported to have increased

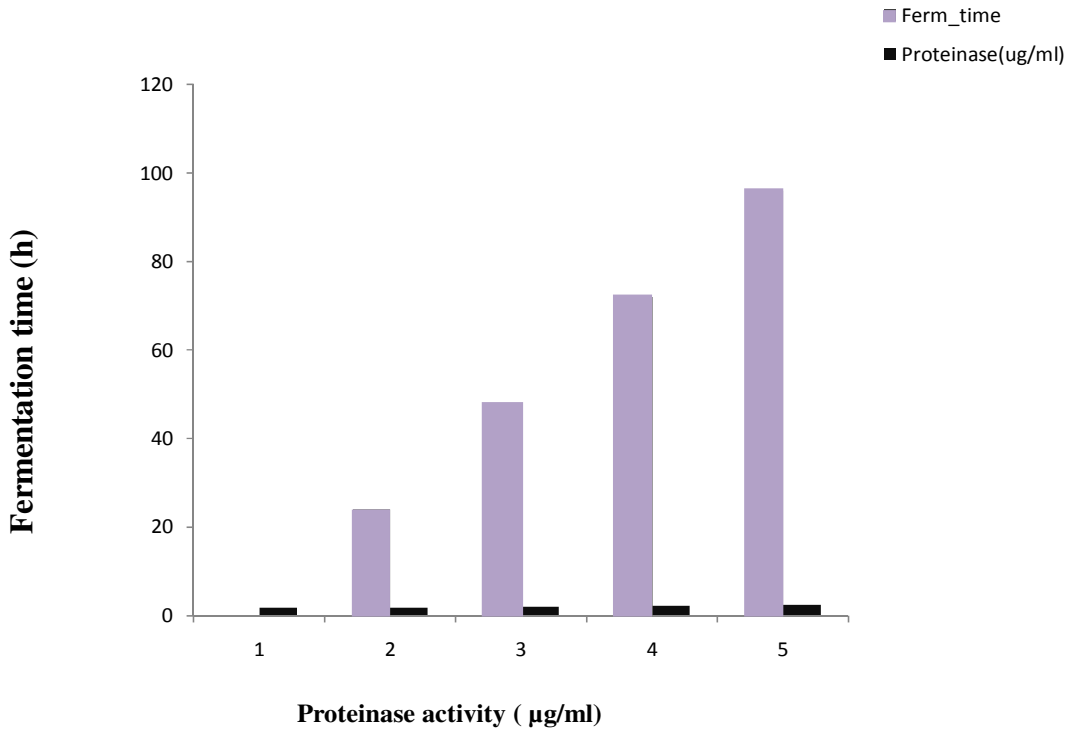

Figure 2. Effect of fermentation time on the proteinase activity in fermenting castor bean samples

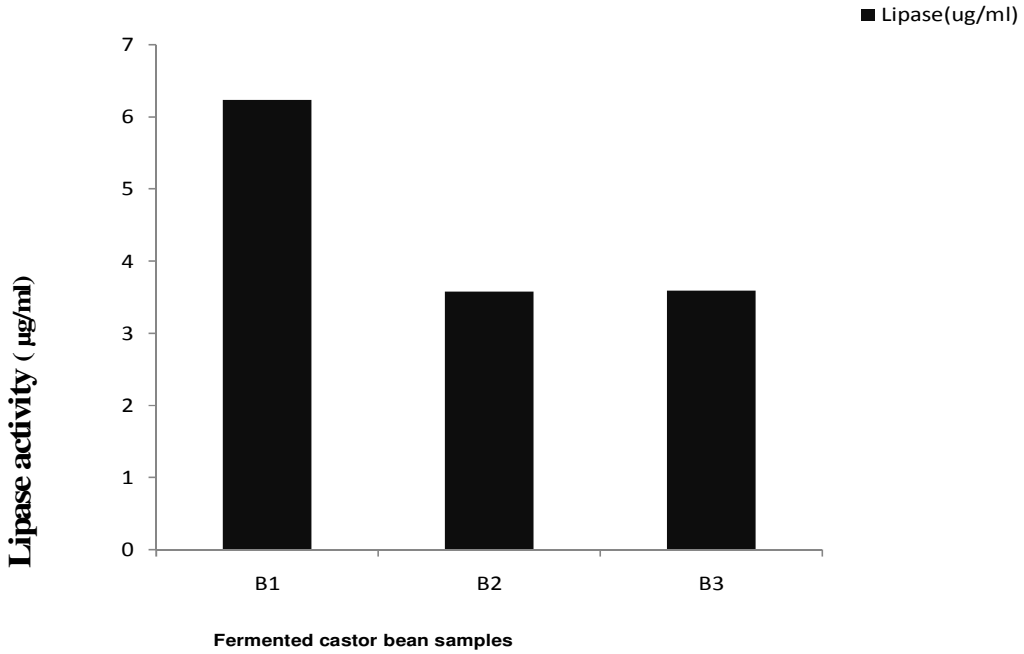

Figure 3. Lipase activity in fermenting castor bean samples

Where: ***B_1=O%NaCl/lime, B_2= 2% NaCl,B_3= 3%lime**

with temperature within the range of 30 to 60 ^{o}C in the study of the effect of temperature on protease activity (Olajuyigbe and Ajele, 2008).

Figure 3 shows the results for the mean of lipase activity of the three fermented castor oil bean samples. Samples B_2 and B_3 had the lowest value with 3.58 µg/ml

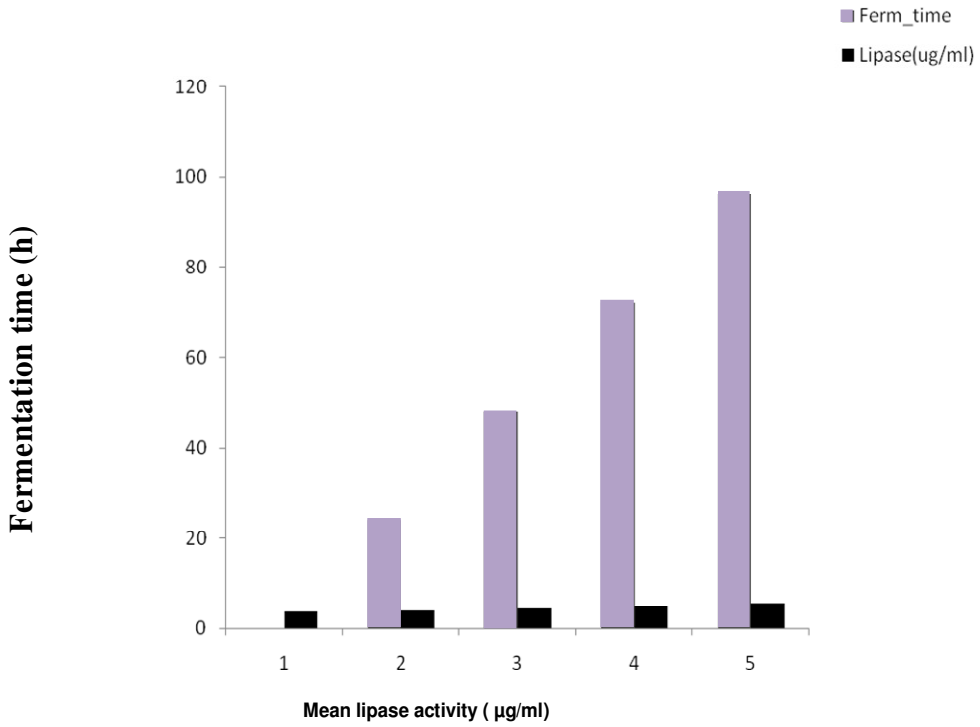

Figure 4. Effect of fermentation time on the lipase activity in fermenting castor bean samples

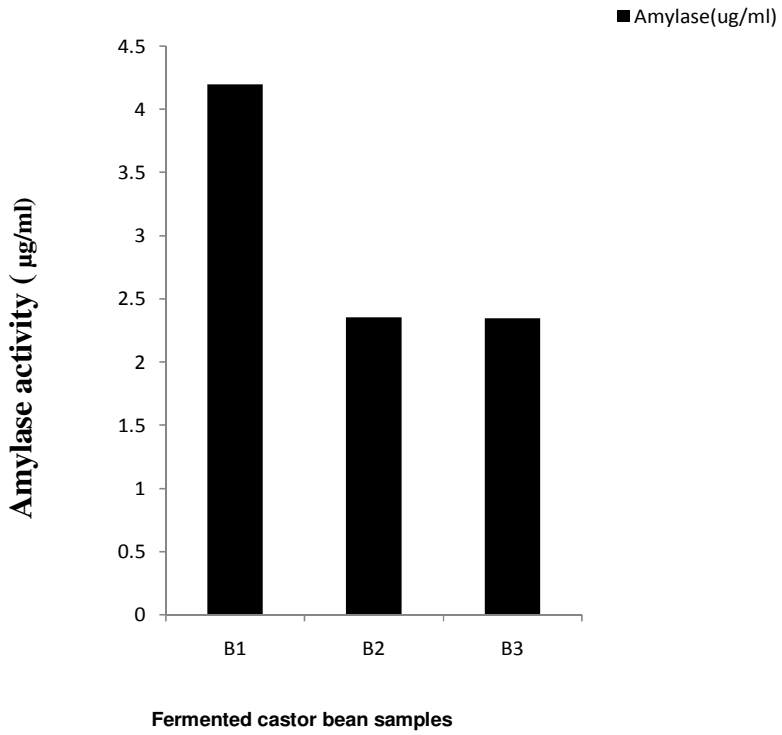

Figure 5. Amylase activity in fermenting castor bean samples

Where: *B_1=O%NaCl/lime, B_2= 2% NaCl,B_3= 3%lime

and 3.59 µg/ml respectively while sample B_1 with 0%NaCl/Lime had the highest lipase content of 6.23 µg/ml. The low values in B_2 and B_3 could be attributed to the 2% salt and 3% lime added differently to the fermenting castor oil bean mash before the onset of fermentation. However Figure 4 shows increased mean

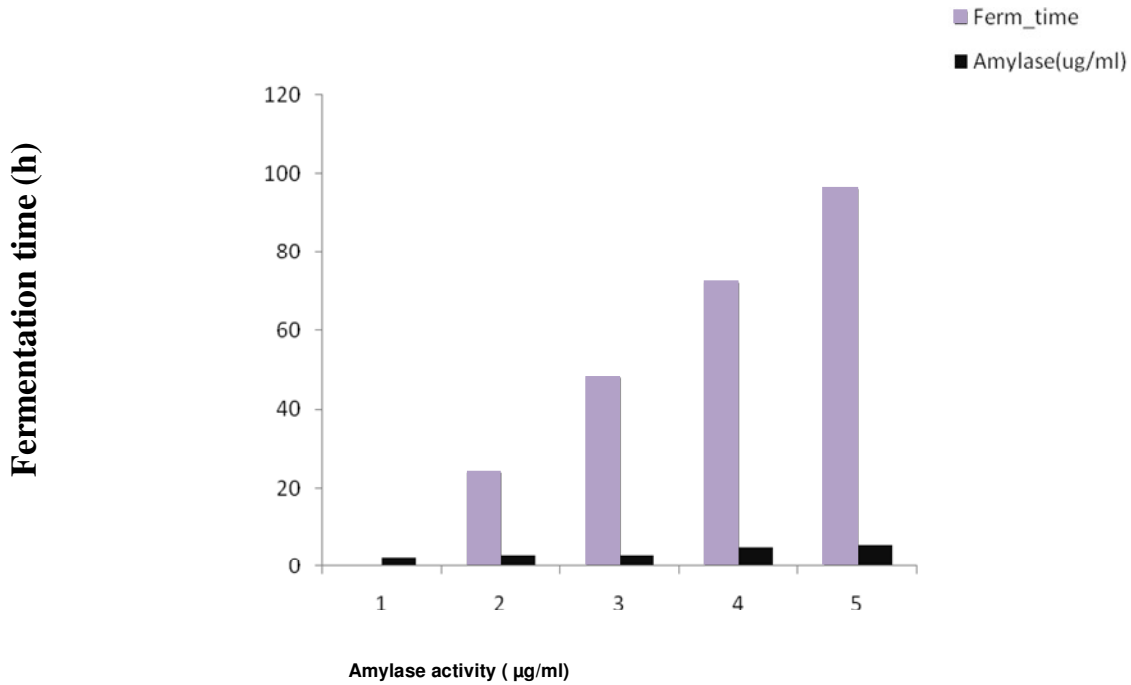

Figure 6. Effect of fermentation time on the amylase activity in fermenting castor bean samples

lipase activity as fermentation period progressed steadily from 3.64 µg/ml to 5.57 µg/ml. Kpikpi et al., (2009) also reported same increase in lipase activity during the fermentation of *Ceiba pentandra* seeds into 'kantong'. Chukeatirote et al., (2010) reported that lipase activity was detected with inconsistent result ranging from 0.78 – 1.31µ during the fermentation of *thua nao*. The low lipase activity was however unexpected by them considering that soya bean is an oil-rich seed. Similar observations have been described with other oil-rich seed fermentation (Omafuvbe et al., 2000; Odibo et al., 1990). Increasing NaCl concentration can decrease the activity of lipase in fermented African locust bean, castor bean seeds and African oil bean, indicating that NaCl is an inhibitor of lipase enzyme (Liman et al., 2010). Odunfa, (1985) reported that lipids are a major constituents of African locust bean and that lipolytic activity is reported to be fairly low and has been attributed to Staphylococcus species found in the fermenting African locust bean seeds. Ouoba *et al*. (2003) reported that lipolytic Bacillus species are expected to have a significant impact on the organoleptic quality of soumbala, fermented African locust bean. Significant differences have been reported in the lipase activities of fermented African locust bean. *Bacillus subtilis* have been shown to have high lipolytic activity (Ouoba et al., 2003). The lipase activity during the fermentation of African locust bean has been commonly attributed to *Staphylococcus or Leuconstoc* species (Odunfa, 1985; Ibrahim and Antai, 1986) and contradicting results have been published according to the changes of the quality of free fatty acid. Odunfa and Adesomoju (1985) reported a decrease in lipase during the fermentation of African locust bean and similar observations were also made by Ibrahim and Antai (1986) on a decrease in the lipase activity during fermentation of African locust bean into daddawa.

The mean results for amylase activity from Figure 5 shows that sample B_1 had the highest value of 4.20 µg/ml and the lowest value obtained was from sample B_3 with 2.34 µg/ml. Figure 6 shows the mean results of amylase for the period of fermentation in the three samples. A steady increase in the amylase activity of the samples was observed as fermentation time progressed from 2.43 – 3.56 µg/ml. *Bacillus* species have been reported as producers of certain enzymes such as amylase, galactanase, galactosidase, glucosidase and fructofuranosidase, which are involved in the degradation of carbohydrates (Aderibigbe and Odunfa, 1990; Kiers et al., 2000; Omafuvbe et al., 2000). Microbial amylases hydrolyze carbohydrates into sugars, which are then readily digestible by humans. Similarly, galactanases soften the texture of the seeds and liberate sugars for digestion (Achi, 2005). Most legumes contain large amounts of non-digestible carbohydrates which may include arabinogalactan, stachyose, sucrose and raffinose (Odunfa, 1983). But fementation is known to reduce total flatus factors from 16.5 to 2.0 mg/g in soybeans and from 0.16% to < 0.1% in groundnut (Fardiaz and Markakis, 1981). Sanni and Ogbonna (1991) reported an increase in reducing sugar during the first 24h of fermentation during the production of 'owoh' from cotton seed. This submission corroborates the

findings of Fadahunsi and Sanni (2010) in the production of tempeh from fermenting bambara nut (*Voandzeia subterranea*).

CONCLUSION

The proteinase and lipase activities were reduced by inclusion of 2% NaCl and 3% lime. Amylase activity which is indicative of carbohydrate utilization is reduced in the presence of 2% NaCl as well as 3% lime. NaCl has been shown to be an inhibitor of lipase enzyme.

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