A STUDY ON THE PHYSICOCHEMICAL PROPERTIES OF *BACILLUS* FERMENTED CASTOR OIL BEAN CONDIMENT

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ABSTRACT

A study was conducted to assess the physicochemical properties of three fermented castor oil bean samples. B.subtilis monoculture starter previously isolated from spontaneously fermenting castor oil bean was used to inoculate three different portions of castor oil bean mash : B_1 (0% NaCl/Lime), B_2 (2% NaCl), B_3 (3% Lime). Results of the physicochemical studies show that the pH, temperature, total viable count, ricin and ammonia contents increased steadily as fermentation period progressed from 0 – 96h. Sample B1 had higher pH and temperature values of 7.15 and 29.27°C than the other two samples. The ammonia content of sample B2 was significantly higher than B1 and B3 with 0.54 µg/ml. The ricin content of the three samples were all low (0.06 – 0.08 µg/ml) and are within the safe limit for human consumption.

Keywords: Castor oil bean, fermentation, condiment

INTRODUCTION

The knowledge and use of plants as spices and condiments is as old as the history of mankind (Garland, 1972). Plants used as spices and condiments are usually aromatic and pungent (Achinewu, *et al*, 1995). Spices and condiments are products of plants, which are mostly used for seasoning, flavouring and thus enhancing the taste of foods, beverages and drugs (Manandhar, 1995). Iwu (1993) had reported that these plants owe these properties to the presence of varying types of essential oils. Macmillan (1984) associated the antiseptic and preservative property of certain spices to these essential oils. In a more elaborate treatment Dziezak (1989) indicated that the rich presence of essential oils and oleoresins determine the aromatic, flavouring, colouring and pungent properties of spices and condiments. These spices and condiments apart from their culinary role as flavouring agents in foods have other functional uses as foods, raw materials for industries and pharmaceutical uses.

Condiments can be described as substances applied to food in the form of sauce, powder, spread or anything similar to enhance or improve the flavour (Oboh, 2006). Condiments are known to contribute to the calorie and protein intake and are generously added to soups as low-cost meat substitute by low-income families in parts of Nigeria (Eka, 1980; Odunfa, 1981).

There are reports on the production of fermented condiments from African locust bean 'iru' (Eka, 1980), melon seed, fermented Ogi (Odunfa, 1981, 1986; Barber and Achinewhu, 1992) and soybean Daddawa (Omafuvbe *et al.,* 2000). The proximate composition indicates these condiments could contribute to the daily intake of proteins, lipids and minerals when used liberally, as practiced in several homes, where expensive animal products are a luxury (Omafuvbe *et al.,* 2000, 2002). In view of the controversy surrounding the use of monosodium-based seasoning salt (Walker and Lupien, 2000), many homes in Nigeria are now using condiments produced from legumes as a flavouring agent in traditional soup preparation. Seeds of legumes may account for 80% of dietary protein and may be the only

ISSN-L: 2223-9553, ISSN: 2223-9944
Vol. 4 No. 4 July 2013

source of protein for some groups. Their cooked forms are eaten as meals and are commonly used in fermented form as condiments to enhance the flavour of foods (Oniofiok *et al.*, 1996).

The castor oil bean plant probably indigenous to the South-eastern Mediterranean region and parts of East Africa is today widespread throughout the tropical regions of the world. The castor oil bean is inedible because the seed contains a toxic protein, ricin and other toxic constituents, ricinic and ricinoleic acids but this can be reduced or be completely eliminated through fermentation (Odunfa and Oyeyiola, 1985). Castor oil bean seeds are fermented to give condiments. The fermented seeds can be ground to make an oily paste 'Ogiri' which has a characteristic ammoniacal odour. This characteristic flavour enhances the taste of traditional soups and sauces (Omafuvbe, 2006). It also contributes protein and essential fatty acid intake in West Africa (Oke and Umoh, 1998).

This present research seeks to study some of the physicochemical properties of *Bacillus* fermented castor oil bean condiment using *B.subtilis* as monoculture starter

MATERIALS AND METHODS

Castor Oil Bean

The castor oil bean seeds *(Ricinus communis)* used for this study were purchased from New Aba Main market in Aba Abia state, Nigeria

Sample Preparation and Isolation

The method of Ojimelukwe *et al.* (2011) was used in the spontaneous fermentation of castor oil bean into *ogiri*. About 300g of castor bean seeds were boiled for two hours, dehulled, drained and boiled again for about six hours. The seeds were ground in a clean mortar into paste, then wrapped with aluminum foil and allowed to ferment for four days at room temperature (29^{o}C).

Identification of Organisms

Identification of the isolates were carried out using the API strips according to manufacturer's instructions (API 50 CHB/E medium and API 20 E medium for *Bacillus*species while API 50 CHL medium was used for *Lactobacillus* species, - Montalieu, Vercieu France).

Preparation of Bacillus Fermented Castor Oil Bean Seeds

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

Sample Collection

Samples were collected at zero hour and every 24h of fermentation for a period of 96h

Determination of pH

The pH of the samples were determined by weighing 1g of the fermenting mash, and suspending it in 9ml of distilled water. The pH was measured after shaking with a pH meter (R1 – 02895 HANNA / Italy) on every 24 hours for four days.

Measurement of Temperature

This was determined by putting a sterile thermometer in the fermenting castor bean seed mash and taking the reading on the thermometer after two minutes.

Determination of the Viable Cell Counts

One (1) gramme of the sample mash was weighed into 9ml quarter strength Ringers solution. Further dilutions were made in quarter strength Ringers solution and 0.1ml of appropriate dilutions were spread on duplicate plates of sterile Plate Count Agar (Biolab). The plates were incubated at 37^{o}C for 24 hours. The colonies were counted after incubation.

Analysis of Ricin in Fermenting Castor Bean Seed Using Waters 616/626 HPLC

Procedure used for extraction of the samples

The analysis of ricin in fermenting castor bean sample was done using Waters 616/626 HPLC. Five (5) gram of castor bean sample was weighed out for blending with 10ml ultrapure water. Then 10mls of the homogenously blended sample was measured into sample extraction unit. 35.0ml of acetone was added and the extraction speed was set at 6000 rpm for 20 minutes.

The supernatant containing Ricin was separated from the precipitate. The sample solution was transferred to a set of centrifuge tubes and covered. The sample was homogenously shaken on a mechanical shaker for 15 minutes. The shaken set of samples was then transferred to a centrifuge set at 3000rpm for 10 minutes. The supernatant was then collected and stored for analysis using HPLC.

Preparation of working standards from the 1000ppm Ricin stock standard, the following working standards were prepared using the following formula:

$C_1V_1 = C_2V_2$

1000 x? = 100 250ml V. flask

$\therefore V_1 = 100 \times 250 / 1000$

Twenty-five (25) ml of 1000 ppm ricin in 250ml volumetric flask and made up to the mark with ultra pure water = 100 ppm ricin. The working standards of 0.0 ppm 2.0 ppm, 4.0 ppm, 6.0 ppm and 8.0ppm were prepared from the 100ppm stock ricin Standard, using the above formular :– $C_1V_1 = C_2V_2$

Running the Extract on HPLC (Waters 616/6266 Digital/Model)

The column size of 3.26mm diameter, and 35.0cm length was chosen as a stationary phase. Methanol was used as a mobile phase and the detector was a fluorescence detector. The instrument was programmed according to specifications. The tubing's were also primed with the reagents to avoid air bubbles. The instrument was inter-phased with both a computer unit and auto sampler. The samples were arranged in the auto-analyser cups and placed on the sampler. The sampler with the help of the probe picked the standard and analyzed them, then used the values of the standards to plot a standard curve equation with the ricin of the samples of unknown concentration determined and finally displayed on the computer as readout. The dilution factor column, volume and the weight of the samples were computed on the software which uses this information to calculate the final values of the analyte depending on the unit of choice.

Determination of Ammonia in Fermenting Castor Bean Seed Using Technicon Auto-Analyser (TECHNICON AAII)

The digests were run on Technicon auto-analyser (Technicon AAII Australia). This analytical Technique operates on the development of colour intensity of ammonia in the samples and quantizing it as being directly proportional to its concentration.

ISSN-L: 2223-9553, ISSN: 2223-9944
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a. Two (2) gram of each sample was weighed into a set of 50ml digestion tube.
b. 20.0 ml at digestion solution was added and shaken. (Alkaline phenol reagent + Ultra pure water + H2SO4) at 20oC. The samples were digested for 1 hour in the fume chamber.
c. It was made up 50ml with ultrapure water after cooling to room temperature.
d. It was transferred to a set of centrifuge tubes (Thermo Electron Corporation IEC Centra GP8 model, USA) and shaken for 15 minutes and centrifuged for 10 minutes at 5000 rpm.
e. The supernatants were sampled in a set of auto-analyzer cups arranged on a tray.

Determining the analyte (Ammonia) and calculation

The sample analyte was ran on the Technicon auto-analyzer by first running the standards of the known concentrations (0.0, 2.0, 4.0, 6.0, 8.0 ppm), which the software of the computer system, inter-phased with the instrument stores. The data was used to calculate the values of the analytes in the samples of unknown concentration.

Calculation: the software was used to carry out the calculation of the analyte values as follows: ppm (in extract) = Dilution factor x Readings or signal intensity

$$\text{ppm (in sample)} = \frac{\text{ppm in extract x volume of samples}}{\text{Wt. of samples}}$$

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 effects of the pH of the fermented castor bean samples. There was no significant difference ($P>0.05$) in the pH of the samples (B_1= 7.15, B_2=6.74, B_3=7.01) but there was significant difference ($P<0.05$) in terms of fermentation period (Table 1). The result shows that the pH values increased significantly as the fermentation time progressed from 6.12 to 7.92. The increase in pH would encourage the growth of *Bacillus sp*. which has been found to grow well at pH 7.0 to 8.0 (Odunfa and Oyeyiola, 1985). The increase in pH of the fermenting castor bean may be due to the abundant increase in ammonia production during the later stages of fermentation. Alkaline fermentation is defined as a fermentation process during which the pH of the substrate increases to alkaline values which may be as high as pH 9 (Aniche *et al.,* 1993; Sarkar and Tamang, 1995; Amadi *et al.,* 1999; Omafuvbe *et al.,* 2000). The increase in pH is due to degradation of proteins from the raw material into peptides, amino acids and ammonia (Kiers *et al*., 2000) or due to alkali treatment during production (Wang and Fung, 1996). Achinewhu, (1987) and Ogbadu and Okagbue (1988) in their studies involving other legumes, also observed a steady increase in the pH with fermentation period. The initial lower pH observed at the commencement of the fermentation can be caused by fermenting microbes which might have started fermentation by hydrolyzing available carbohydrates to acid before embarking on extensive proteolysis. Thus the acid produced initially lowers the pH of the fermenting mash and later leads to alkalinity by the hydrolyzing protein as illustrated by Whitaker (1978) or due to the protease and deaminase

enzymes produced by the *Bacillus* isolates (Heesseltine, 1979). The gradual development of ammoniacal odour is in agreement with the observed pH changes from acidic range to the reported alkaline pH range by Barimala (1994) in a similar experiment.

Figure 2 shows the mean effects of the temperature of the three fermented castor seed samples. There was no significant difference ($P>0.05$) in the mean of the temperatures of the three samples. The temperature values were 29.27°C, 28.51°C , and 28.78°C for B_1, B_2 and B_3 respectively. They all had high temperature values with the additive in B_2 and B_3 not having any effect on the temperature values. However there was significant difference ($P<0.05$) in the mean of the temperature values in terms of fermentation period (h). From Table 1, the mean temperatures were observed to have increased from 27.01°C to 30.50°C within the first 48h of fermentation and decreased gradually afterwards to 28.78°C to 28.67°C at the end of fermentation. This is in agreement with Ogueke *et al.,* (2010) during ugba fermentation. Thus fermented castor bean samples is exothermic and this initial increase in temperature has been attributed to the intense metabolic activities of the microorganisms (period of maximum microbial activity) and represents the most active and important period of the fermentation. This is because enzyme studies have revealed that alpha-amylase, proteolytic and lipolytic enzyme activities attained their maximum levels at 24 – 36h of fermentation (Njoku and Okemadu, 1989).

Figure 3 shows that there was significant difference ($P<0.05$) in the TVC of the three samples. 2% NaCl (B_2) and 3% lime (B_3) fermented castor bean samples had higher mean of TVC when compared with the sample containing 0%NaCl/Lime(B_1). The viable cell count was significantly higher in 2% salted *ogiri* and 3% lime added *ogiri*. This indicates that 2% salt concentration and 3% lime addition creates a favourable medium for the growth of *B. subtilis.* This has also been reported by Omafuvbe, (2006) in the use of 1% salted daddawa and Yabaya (2006) in fermented *Acacia nilotica* using *B.subtilis* monoculture starter. Table 1 shows significant difference also existed ($P<0.05$) in the mean of total viable count with regard to the fermentation time. There was also steady increase in the microbial load during the 96h fermentation of the castor bean seeds. The increase in the mean TVC can be attributed to the accumulation of compounds such as organic acids and other metabolites (David and Aderibigbe, 2010).

Figure 4 shows there is significant difference ($P<0.05$) in the mean of the ricin content of all the samples. Sample B_1 had the lowest value of 0.060 µg/ml followed closely by samples B_2 and B_3 with 0.072 µg/ml and 0.084 µg/ml respectively. All the samples had values within the safe limit for ricin consumption in foods. It was also observed in Table 1 that the ricin content reduced steadily as fermentation time progressed in all the samples thereby making the condiment safe for consumption as seasoning. Ricin being an anti nutrient is said to be among the compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods or animal feeds and they play a vital role in determining the use of plants for humans and animals (Soetan and Oyewole, 2009).

Figure 5 shows the mean result of ammonia production for the three fermented castor oil bean samples. Sample B_2 had the highest ammonia content with 0.54µg/ml followed closely by sample B_1 and B_3 with 0.20 µg/ml and 0.15 µg/ml respectively. Table 1 reveals there was a steady increase in the ammonia content of the samples as fermentation time progressed. This is in agreement with Omafuvbe (2006) who reported that ammonia concentrations in soy-daddawa increased significantly in the first 48h of fermentation after which the value remained stable. Ammonia concentration was shown to be significantly higher in 2% salted *ogiri* which may be a reflection of the enhanced proteolytic activity and release of ammonia following the utilization of amino acids by the increased population of *B. subtilis.* The result

ISSN-L: 2223-9553, ISSN: 2223-9944
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in Table 1 also shows a steady increase in the mean of the ammonia content with fermentation time from 0.18µg/ml at 0h to 0.36µg/ml at 96h fermentation for all the samples. In alkaline-fermented foods, the protein of the raw materials is broken down into amino acids and peptides. Ammonia is released during the fermentation, raising the pH of the final products and giving the food a strong ammonical smell. Visessanguan *et al*. (2005) observed similar trend during fermentation of *thua nao,* a traditional Thai fermented soy product. They observed marked changes in ammonia after 12, 18 and 36h of fermentation for the control and those inoculated with *B.subtilis* at 10^2 and 10^4 cfu/g respectively. Inoculation generally accelerated an increase in ammonia nitrogen. *B. subtilis* utilized protein in soybean and released amino acids and ammonia, leading to rise in pH (Ohta, 1986; Sakar *et al.,* 1993; Steinkraus, 1996).

CONCLUSION

The physiochemical studies reveal 2% NaCl and 3% lime was favourable for the growth of *B.subtilis* in the viable cell count determination. The 2% salted *ogiri* also reflected improved proteolytic activity which led to the release of ammonia following utilization of amino acids by the increased population of *B.subtilis.* It was observed that all the physiochemical properties studied increased as fermentation period progressed to 96h fermentation period.

REFERENCES

Achinewhu, S. C. (1987). Carbohydrate and Fatty Acid Composition of Fermented Melon Seed (*Citrullus vulgaris). J Food Sci, Tech., 24*, 16-19.

Achinewhu, S. C., Aniena, M. I. & Obomanu, F. G. (1995). Studies on spices of food value in the south eastern states of Nigeria 1: Antioxidants properties. *J. African Med. Plants, 18*, 135-139.

Amadi, E. N., Uneze, R., Barimalaa, I. S. & Achinewhu, S. C. (1999). Studies on the production of bambara groundnut (*Vigna subterranean*) tempe. Plant Foods. *Human Nutr., 53*, 199-208.

Aniche, G. N., Nwokedi, S. I. & Odeyemi, O. (1993). Effect of storage temperature, time and wrapping materials on the microbiology and biochemistry of *ogiri* – a fermented castor seed soup condiment. *World J. Microbiol. Biotechnol, 9*(6), 653-655.

Barber, L. I. & Achinewhu, S. C. (1992). Microbiology of *ogiri* production from melon seeds (*Citrullus vulgaricus*). *Nig. Food J., 10*, 129 – 135.

Barimala, I. (1994). *Vigna subterranean*. *J. Sci. Food & Agric., 66*, 443-446.

David, O. M. & Aderibigbe, E. Y. (2010). Microbiology and proximate composition of 'ogiri', a pastry produced from different melon seeds. *New York Science Journal 3*(4), 18-27.

Dziezak, J. D. (1989). Innovative food trends: species. *Food Technology, 43*(1), 102-116

Eka, O. U. (1980). Effect of fermentation on the nutritional status of African locust beans. *Food Chem., 5*, 303-588.

Enujiugha, V. N. (2009). Major fermentative organisms in some Nigerian soup condiments. *Pak. J. Nutr., 8*(3), 279-282.

Garland, S. (1972). *The herbs and spices book.* (pp. 20-27). London: Frances Lincoln Publishers.

Gomez, K. A. & Gomez, A. A. (1984). *Principles and procedures for agricultural research*. (2nd edition). New York: John Wiley and Sons.

Hesseltine, C. W. & Wang, H. L. (1979). Fermented foods. *Chem. Ind. Lond.,12*, 393-399.

Iwu, M. M. (1993). *Handbook of African medicinal plants*. (pp. 435). Boca Raton: CKC Press.

Kiers, J. L., Laeken, A. E. A. V., Rombouts, F. M. & Nout, M. J. R. (2000). In vitro digestibility of Bacillus fermented soy bean. *Int. J. Food Microbiol., 60*, 163-169.

Macmillan, H. F. (1984). *A handbook for tropical planting and gardening*. (pp. 326). London: Macnidlan Scientific Publishers.

Manandhar, N. P. (1995). Substitute spice in Nepal. *J. of Herbs, Spices and Medicinal Plants, 3*, 71-77.

Njoku, H. O. & Okemadu, C. P. (1989). Biochemical changes during the natural fermentation of the African oil bean *(Pentaclethra macrophylla*) for the production of ugba. *J. Sci. Food Agric., 49*, 457-465.

Oboh, G. (2006). Nutrient and antinutrient composition of condiments produced from some fermented underutilized legumes. *J. Food Biochem., 30*, 579-588.

Odunfa, S. A. (1986). *Daddawa,* In: Redd, N.R., Pierson, M.D. and Salunkhe, D.K. (eds). *Legume-based fermented foods.* Boca Raton: CRC Press Inc.

Odunfa, S. A. (1981). Microorganisms associated with fermentation of African locust beans (*Parkia filicoidea*) during iru fermentation. *J. Plant Foods, 3*, 245-250.

Odunfa, S. A. & Oyeyiola, G. F. (1985). Microbiological study of the fermentation of ugba, a Nigerian indigenous fermented food flavour. *J. Plant Foods, 6*, 155-163.

Ogbadu, L. J. & Okagbue, R. N. (1988). Fermentation of African locust bean (*Parkia biglobosa)* seeds: involvement of different species of Bacillus. *Food Microbiol., 5*, 195-199.

Ogueke, C. C., Nwosu, J. N., Owuamanam, C. I. & Iwouno, J. N. (2010). Ugba, the fermented African oil bean seeds: its production, chemical composition, preservation, safety and health benefits. *Pak. J. Biol. Sci., 13*(10), 489-496.

Ojimelukwe, P. C., Okechi, A. & Ojinnaka, M. C. (2011). Physicochemical characteristics of fermenting castor seeds containing lime and NaCl as additives. *Afr. J. Food Sci., 5*(14), 754-760.

Oke, O. C. & Umoh, J. B. (1998). Lesser known oil seeds: chemical composition. *Nutritional Institution, 17*, 293-297

Omafuvbe, B. O. (2006). Effect of salt on the fermentation of soybean (*Glycine max.)* into daddawa using *Bacillus subtilis* as starter culture. *Afr. J. Biotechnol., 5*(10), 1001-1005.

Omafuvbe, B. O., Abiose, S. H. & Shounkan, O. O. (2002). Fermentation of soybean (*Glycine max)* for soy-daddawa production by starter cultures of bacillus. *Food Microbiol., 19*, 561-566.

Omafuvbe, B. O., Shonukan, O. O. & Abiose, S. H. (2000). Microbiological and biochemical changes in the fermentation of soybean for soy-daddawa – Nigeria food condiments. *Food Microbiol., 17*, 469-474.

ISSN-L: 2223-9553, ISSN: 2223-9944
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Oniofiok, N. D. O., Nnanyelugo, & Ukwondi, B. E. (1996). Usage patterns and contributions of fermented foods to the nutrient intake of low income households in Emene Nigeria. *Plants Foods Hum. Nutr., 49*, 199-211.

Sarkar, P. K. & Tamang, J. P. (1995). Changes in the microbial profile and proximate composition during natural and controlled fermentations of soybeans to produce kinema. *Food Microbiol., 12*, 317-325.

Sarkar, P. K., Cook, P. E. & Owens, J. D. (1993). *Bacillus* fermentation of soybeans. *World J. Microbiol. & Biotechnol., 9*, 295-299.

Soetan, K. O. & Oyewole, O. E. (2009). The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds: A review. Afr. *J. Food Sci., 3*(9), 223-232.

Steel, R. G. D. & Torie, J. H. (1984). *Principles and Procedures of statistics.* (2nd Ed., pp 172-178). Singapore: McGraw Hill Book Co. Inc.

Steinkraus, K. H. (1996). *Handdbook of indigenous fermented foods.* (pp. 271-274). New York: Marcel Dekker.

Visessanguan, W., Benjakul, S., Potachareon, W., Panya, A. & Riebroy, S. (2005). Accelerated proteolysis of soy proteins during fermentation of thua-nao inoculated with *Bacillus subtilis*. *J. Food Biochem., 29*, 349-366.

Walker, R. & Lupien, J. R. (2000). The safety evaluation of monosodium glutamate. *J. Nutr.,* 130, 1049S-1052S

Wang, J. & Fung, D. Y. (1996). Alkaline fermented foods: A review with emphasis on pidan fermentation. *Critical Rev. Microbiol., 22*(2), 101-138

Whitaker, J. R. (1978). Biochemical changes occurring during the fermentation of high protein foods. *Food Technol., 32*, 175-190

Yabaya, A. (2006). Production of local dadawa seasoning and condiment from Acacia nilotica (Linn) seeds. *Science World Journal, 1*(1), 27-31

APPENDIX

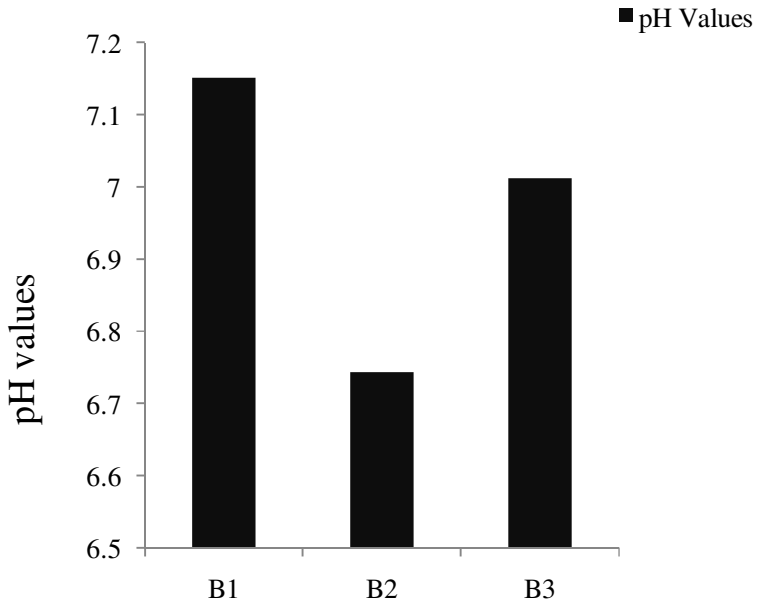

Figure 1. pH of Fermenting Castor Bean Samples

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

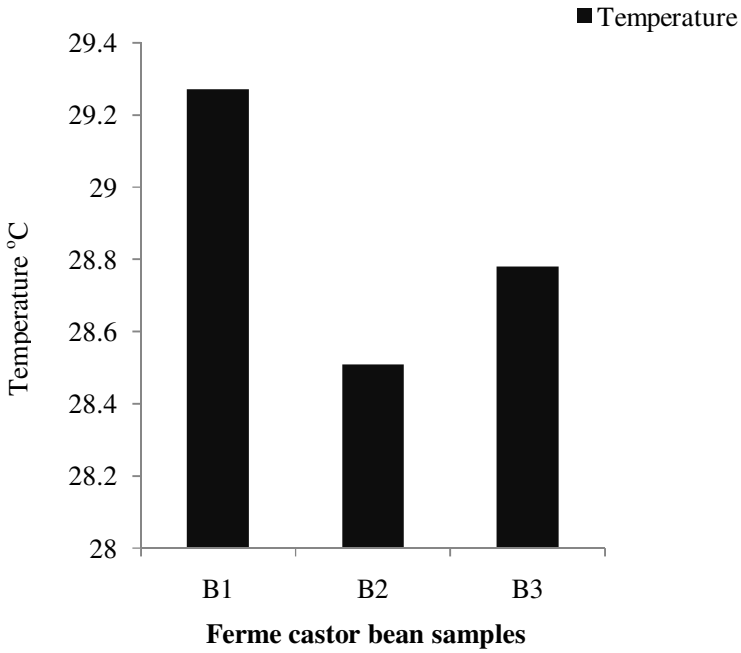

Figure 2. Temperature of Fermenting Castor Bean Samples

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

ISSN-L: 2223-9553, ISSN: 2223-9944
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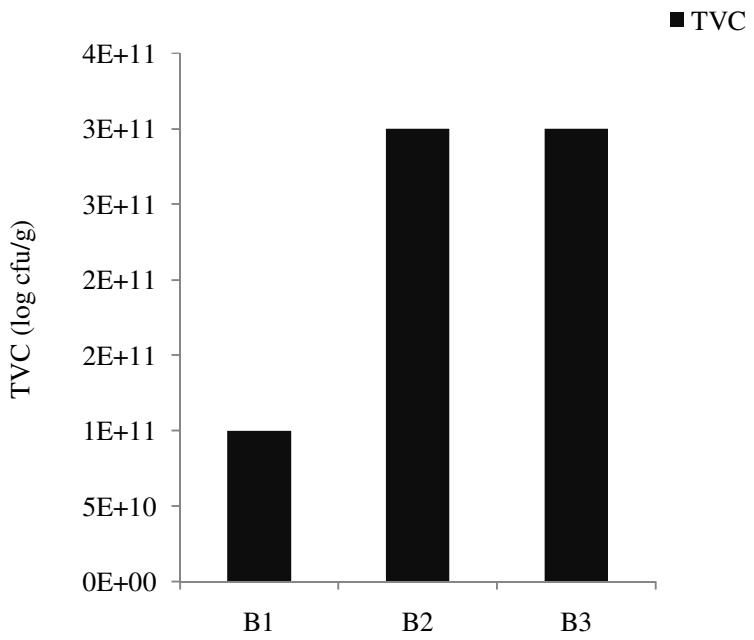

Figure 3. Total viable count of *B. subtilis* in fermenting castor bean samples given different processing treatments

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

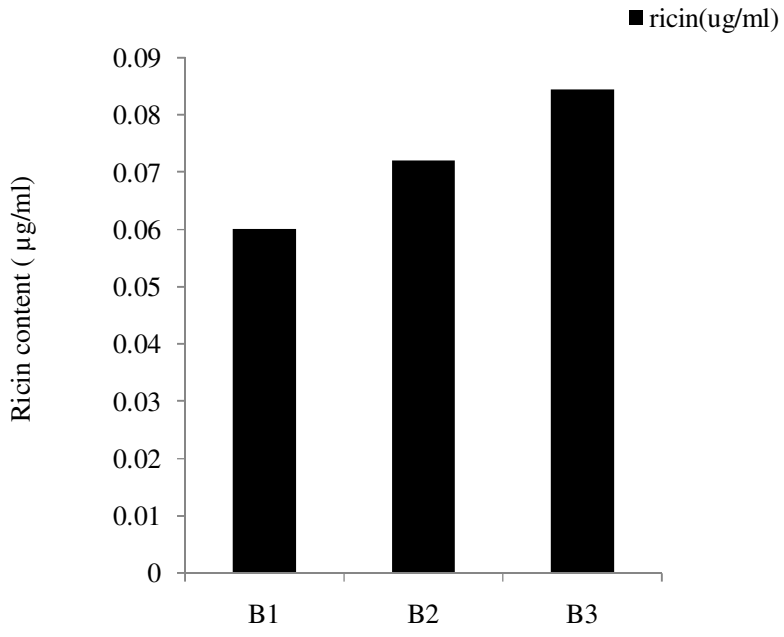

Figure 4. Ricin content in fermenting castor bean samples

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

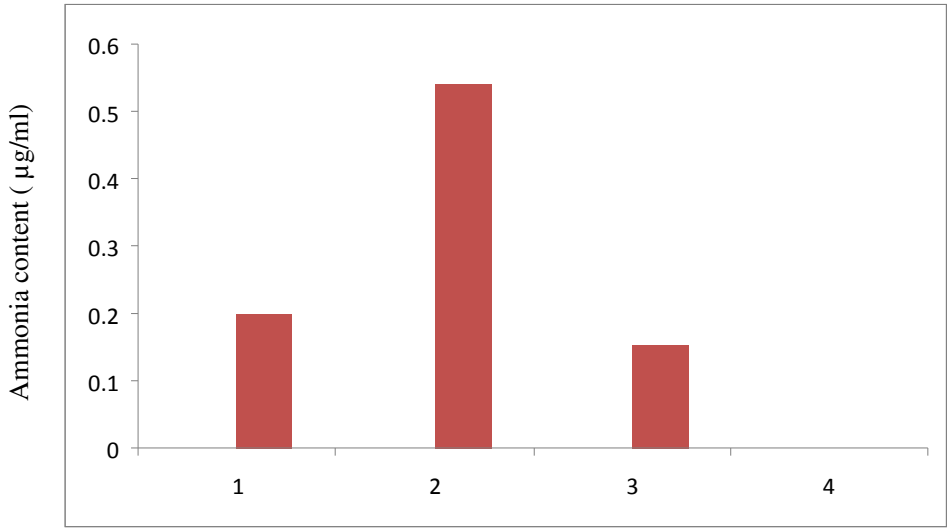

Figure 5. Ammonia content in fermenting castor bean samples

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

Table 1. Mean of changes in some physiochemical properties during fermentation of castor oil bean with *Bacillus subtilis*

Parameters Fermentation time (h)	*0*	*24*	*48*	*72*	*96*	*LSD*
PH(	6.12	6.63	6.89	7.29	7.92	0.47
Temperature(^{o}C)	27.01	29.30	30.50	28.78	28.67	1.19
TVC	9.E+08	4.E+09	3.E+11	7.E+11	1.E+11	7.883+10
Ricin(µg/ml)	0.08	0.08	0.07	0.07	0.06	0.01
Ammonia (µg/ml)	0.18	0.28	0.32	0.34	0.36	0.01