British Microbiology Research Journal
13(6): 1-10, 2016, Article no.BMRJ.24593
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Isolation and Screening of Microorganisms Associated with Locust Bean (IRU) for the Ability to Ferment Soya Bean to Produce Soy Iru

F. Titilayo Afolabi[1*], M. Abdulkadir[2] and A. Abiodun Onilude[1]

[1]Department of Microbiology, Faculty of Science, University of Ibadan, Nigeria.
[2]Department of Science Laboratory Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author FTA designed the study. Authors FTA and MA wrote the first draft of the manuscript with contribution from author AAO. Authors FTA and MA managed the literature searches and analyses of the data. Authors FTA, MA and AAO managed the experimental process. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/24593
Editor(s):
(1) Abha Sharma, Department of Microbiology, GB Pant Hospital, New Delhi, India.
Reviewers:
(1) A. O. Ajayi, Adekunle Ajasin University, Ondo State, Nigeria.
(2) Marcela Bianchessi da Cunha Santino, Universidade Federal de Sao Carlos, Brazil.
Complete Peer review History: http://sciencedomain.org/review-history/13862

Original Research Article

Received 26th January 2016
Accepted 11th March 2016
Published 25th March 2016

ABSTRACT

Aims: This study was conducted to isolate, identify and screen microorganisms associated with soy iru for the ability to be used as starter in the fermentation of soya bean (*Glycine max*) to produce soy iru.
Study Design: To control the fermentation of soya bean (*Glycine max*) to produce soya iru using functional starter culture in order to improve the quality of the product.
Place and Duration of Study: Soybeans (*Glycine max*) samples were collected from markets in Ibadan, in Oyo State Nigeria. Analyses of the samples were conducted in the Department of Microbiology, University of Ibadan and Central Multidisciplinary Research Laboratory in the University of Ibadan, Nigeria. The sampling and analyses were done between August 2014 to January 2015.
Methodology: Soybean (*Glycine max*) seeds were purchased from markets, in Ibadan, Oyo State,

**Corresponding author: E-mail: ft.afolabi@ui.edu.ng, folakeojo1@yahoo.com;*

Nigeria. Spontaneously fermented locust bean iru samples were also purchased from iru seller from markets, Ibadan, Oyo State and they were analysed using Nutrient agar, DeMann Rogosa and Sharpe medium (MRS), MacConkey Agar and Malt Extract Agar and isolates were also screened for enzymatic activity. The antibacterial activity of LAB metabolites against some indicator organisms was also determined;

Results: A total of thirty eight (38) strains of bacteria were isolated, seven (7) isolates from locust bean iru (LBI) and thirty one (31) isolates from soya bean iru (SBI). *Bacillus subtilis* SBI 13 and *Leuconostoc mesenteroides* SBI 15 produced the three enzymes and the highest protease activity was produced by *Bacillus subtilis* SBI 13. All the isolated LAB produced antimicrobial compounds; lactic acid, diacetyl and hydrogen peroxides with *Leuconostoc mesenteroides* SBI 15 producing the highest quantity of diacetyl (0.91 g/l) and hydrogen peroxide (1.10 g/l) while the highest quantity of lactic acid (1.61 g/l) was produced by *Lactobacillus plantarum*. *Bacillus subtilis* SBI 13 and *Leuconostoc mesenteroides* SBI 15 were chosen as starter cultures for further study.

Conclusion: *Bacillus subtilis* and *Leuconostoc mesenteroides* exhibit the best potentials of a good starter so they can be used as starter culture in the fermentation of soya beans to produce Soy Iru.

Keywords: Soybean; starter culture; fermentation.

1. INTRODUCTION

Soybean (*Glycine max* L.) is one of the nutritionally richest natural vegetable foods known to human kind, records of its food usage dates back to 2838 BC in China [1]. Although, having high protein content, minerals, vitamins and bio-actives, it has little direct use because of high satiety value caused by high oil content, poor digestibility, green beany taste, long cooking time and persistent bitterness. Soybeans as a food is used as soymilk, soy flour, soy oil, feed for livestock and poultry, soy concentrate, protein isolates, soy yoghurt, tofu and fermented foods such as Tempeh, soy sauce, Miso, Natto and Sufu [1].

In Nigeria, soyabean is fermented to make soy-dawadawa, a soup condiment and it is also used to make improvement in the diet of many Nigerians, particularly children and nursing mothers. Soyabean seed is rich in plant protein. Soy iru (soy dawadawa) is a food flavouring condiment prepared by fermenting whole beans. It is widely consumed by the people of Benue and Plateau States of Nigeria and its consumption is now extending to the Southern part of Nigeria. Locust beans (*Parkia biglobosa*) have been traditional raw material for the production of iru (dawadawa). However, emphasis has been shifted to the use of soyabean (*Glycine max*) as a substitute [2].

The microbiology of fermentation and some biochemical changes occurring during the production of this condiment have been reviewed. Fermentation is a proven method to improve flavour, texture and nutritional quality of the soybeans [3].

Bacillus spp. is the most dominant naturally fermenting agents in soybeans. These hydrolytic bacteria are associated with utilization and reduction of indigestible oligosaccharides and polysaccharides. The organism has also shown to reduce the activity of anti-nutrients that hinders availability of proteins and phytochemicals present in soybeans [4]. *B. subtilis* fermentation is accompanied by covering intact granules with white-coloured viscous substance, slimy appearance, softer texture, and unique rotten flavour. It also completely removes the beany odour of raw soybeans and increases sensory quality of the product [5]. Chemical studies have been carried out to know the nutritive value of soy-iru. According to [6], fermented soybean has the following chemical composition as expressed by 100 g dry matter, crude protein 49.51, fat 31.46, crude fibre 3.49, ash 3.97, carbohydrate 15.06 and organic matter 96.03. It also contains appreciable amount of minerals.

In recent years, attempts are being made in laboratories in several African countries to develop starter cultures along the lines used in modern food processing establishments to upgrade the production of various indigenous African fermented foods. These include mawe [7], kenkey, [8-10], pito, [11], kivunde [12], and dawadawa [13].

This research work was borne as a result of the fact that iru is being produced through uncontrolled fermentation process which does not give a quality final product and it could also be contaminated. This current research work therefore focused on the isolation of microorganisms associated with the fermentation

of soybeans and also to investigate and obtained the best starter cultures for the preparation of soy iru.

2. METHODOLOGY

2.1 Sample Collection

Soybean (*Glycine max*) seeds were purchased from Bodija market, Ibadan, Oyo State, Nigeria. Spontaneously fermented locust bean iru samples were also purchased from iru seller at Bodija market, Ibadan, Oyo State, Nigeria in a sterile plastic and were transported in ice packs to the laboratory.

2.2 Sample Processing

The seeds were picked carefully and boiled in water for 2 hours until very soft, it was then dehulled and boiled again for another 1 hour. 30 g of soybean was then wrapped with plantain leaves then placed in a covered plastic and allowed to ferment spontaneously for 5 days at 35°C.

2.3 Isolation and Identification of Microorganisms

One gram (1 g) of fresh spontaneously-prepared soy bean iru and purchased locust bean iru samples were weighed and serially diluted using test tubes containing 10 mL of sterile 0.1% peptone water. The mixture was homogenized. One milliliter of the dilution was pipetted into a sterile test tube containing 9 mL of 0.1% peptone water. The process was repeated for each of a set of nine tubes until a 10^{-10} dilution was achieved. One milliliter from the last three dilutions was plated in duplicate on nutrient agar for growth of *Bacillus* species, DeMann Rogosa and Sharpe medium (MRS) for the growth of Lactic acid bacteria and *Bacillus* species, MacConkey Agar for the growth of Enteric Bacteria, and Malt Extract Agar for the growth of fungi. Nutrient agar and MacConkey agar plates were incubated at 37°C for 24 hours. MRS plates at 37°C under anaerobic condition for 48 hours by using anaerobic jar and Malt Extract agar plates at 30°C for 5 days.

After incubation, the isolates were subcultured to obtain pure cultures [14,15]. Pure colonies were subjected to Gram staining, spore staining and some biochemical tests (oxidase, catalase, hydrogen sulphide test, nitrate reduction test, starch hydrolysis test, indole test, citrate utilization test, methyl red test, Voges Proskauer test, gelatin hydrolysis and urease test) and identification was based on Bergey's Manual of Determinative/Systematic Bacteriology [16,17].

2.4 Screening of Isolates for Enzyme Activity

The isolates were screened for their ability to produce protease, amylase and lipase enzymes respectively. The proteolytic activity was determined by the method described by [18]. The procedure of [19] and [20] were used to determine the amylolytic activity of the isolates. The lipolytic activity of the isolates was determined with slight modification of the method described by [21] and [22].

2.5 Quantitative Determination of Antimicrobial Compound Produced by Lactic Acid Bacteria

For these measurements the test organisms were grown in MRS broth for 72 h and centrifuged at 3000 g for 15 min. The supernatant was now used for the determination of the antimicrobials.

2.5.1 Quantitative estimation of lactic acid production

The production of lactic acid was determined by transferring 25 ml of supernatant fluid of test organisms into 100 ml flasks. This was titrated with 0.1 M NaOH and 1ml of phenolphthalein indicator (0.5 in 5% alcohols). The titratable acidity was calculated as lactic acid % w/v [22]. Each millimetre of 0.1 M NaOH is equivalent to 90.08mg of Lactic acid. The titratable acidity was then calculated as stated in [23] as;

$$\text{Titratable acidity} = \frac{\text{Ml NaOH} * \text{N NaOH} * \text{M.E} * 100}{\text{Volume of sample used}}$$

Where: Ml = Volume of NaOH used, N NaOH = molarity of NaOH solution, M.E = Equivalent factor.

2.5.2 Quantitative estimation of hydrogen peroxide production

20 mL of dilute H_2SO_4 acid was added to 25 mL of the supernatant fluid of the test organism. Titration was carried out with 0.1 M potassium permanganate (KMnO4). Each mL of 0.1 M.

Potassium permanganate is equivalent to 1.79 mg of Hydrogen peroxide solution. Decolourization of the sample was regarded as the end point. The volume of H_2O_2 produced was then calculated [24] as;

$$H_2O_2 \text{ produced} = \frac{\text{ml } KMnO_4 * N\ KMnO_4 * M.E * 100}{Ml\ H_2SO_4 * \text{volume of sample}}$$

Where; ml $KMnO_4$ = Volume of $KMnO_4$ used, N $KMnO_4$, ml H_2SO_4 = Volume of H_2SO_4 added, M.E = Equivalent factor.

2.5.3 Quantitative estimation of diacetyl production

Diacetyl production was determined by transferring 25 mL of the supernatant fluid of the test organisms into 100 mL flasks. Hydroxylamine solution (7.5 mL) of 1M was added to the flask and to a similar flask for residual titration. Both were titrated with 0.1M HCL to a greenish yellow end point using bromophenol blue as indicator [22] the equivalent factor of HCL to diacetyl is 21.52 mg. The concentration of diacetyl produced was calculated using the method of [23] as;

$$Ak = \frac{(b - s)\ (100E)}{W}$$

Where; Ak = percentage of diacetyl, b = No of 0.1mL HCL consumed in titration of sample, E= Equivalent factor, W = volume of sample.

2.6 Evaluation of Antagonistic Activity

Agar well and Disc diffusion methods were used to confirm the antagonistic activities of the LAB isolates. The methods of [25,26] were used to determine the antibacterial activities of the LAB isolates.

3. RESULTS AND DISCUSSION

Thirty eight bacterial isolates where obtained, nine (9) were gram -positive cocci, six were gram -negative rods, while 23 were gram -positive rods. *Bacillus* spp had the highest percentage of occurrence of 53%, *Staphylococcus* spp (16%), Enteric bacteria (16%), Lactic acid bacteria (LAB) (10%) and *Corynebacteria* (5%). The percentage of occurrence of these isolates is shown in Fig. 1. This is in agreement with the reports of [3,27] which showed that fermentation of vegetable proteins into condiments is usually initially mediated by diverse microbial flora, which at the long-run eventually becomes gram positive flora. This is also in accordance with the work of [28] in which they reported that organisms involves in the fermentation of soya bean seeds to produce iru (dawadawa) are mostly species of *Bacillus subtilis,* B. *licheniformis* and *B. pumilis*. [2] confirmed the presence of *Leuconostoc* sp in the fermenting soya beans. It was stated by [3] that coagulase –negative *Staphylococcus* species and *Bacillus* sp are often associated with the fermentation of food of plant origin.

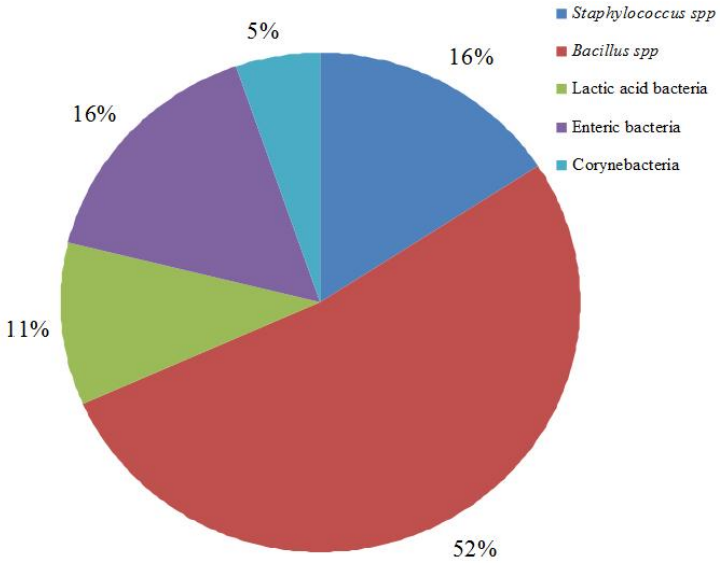

Fig. 1. Percentage occurrence of bacterial isolates from Iru sample

Fig. 2 shows the result of Enzyme production (amylase, protease and lipase) by bacteria isolates as depicted by clearance zones (millimetre) during screening on solid medium. *Bacillus subtilis* SBI 13 produced the highest proteolytic, amylolytic and lipolytic activities of 53 mm, 37 mm and 11 mm respectively. *Leuconostoc mesenteroides* SBI 15 also produced the three enzymes. In this study, 30 bacterial isolates were screened for their amylase, protease and lipase production. Results showed that, of 30 isolates tested, 2 isolates representing approximately 7% could produce the three enzymes. However, it was also evident that these bacteria had different enzyme activity; *Bacillus subtilis* SBI 13 produce the highest protease, amylase and lipase activity. *Leuconostoc mesenteroides* SBI 15 also produce the three enzyme activity. To group these enzyme-producing bacteria, the size of the diameter of the clear zone was used as an indicator as illustrated in Fig. 2. Only three isolates did not show proteolytic activity on skim milk agar. The result is in agreement with the study of [29] who reported *Bacillus subtilis* are widely known to produce protease in the fermentation of soya bean. The degradation of proteins has been previously described as the most important biochemical change occurring during the soya bean fermentation [3,30]. In addition, it has been suggested that such proteolysis is strongly relevant to dominating *Bacillus* species, especially for the iru case [3,31]. Previous investigations including this present study have also shown that the predominant microbes present in iru are capable of producing proteases, amylase and lipase [32-34]. [33] also showed that *Lactococcus raffinolactus, Pediococcus pentosaceus, Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus sp. and Pediococcus halophilus* were also involved in legume fermentation. Several workers have made mention of the presence of *Leuconostoc mesenteroides.*

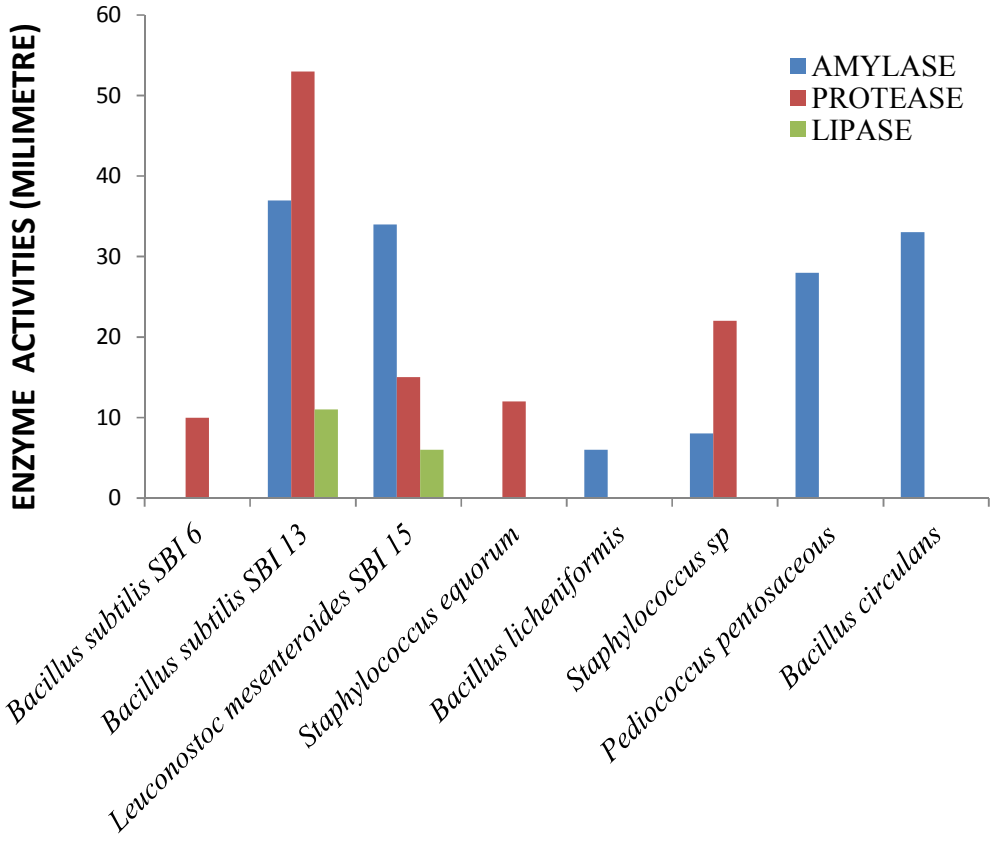

Fig. 2. Enzyme production as depicted by clearance zones (millimetre) during screening on solid medium

Tables 1, 2 and 3 shows the Quantity of diacetyl, lactic acid and Hydrogen peroxides produced by isolates of lactic acid bacteria. All the lactic acid bacteria (LAB) isolates produced diacetyl with the peak of production after 72 h of incubation. However, *Leuconostoc mesenteroides* SBI 15 which produced 0.91 g/l at 72 h, had the highest yield of diacetyl, while the least producer (0.83g/l) after 72 h of incubation was *Pediococcus pentosaceus* (Table 1). All the organisms also produced hydrogen peroxide to a varying degree. After 72 h incubation, *Leuconostoc mesenteroides* SBI 15 had the highest yield of 1.10 g/l (Table 2), whereas *Lactobacillus plantarum* had the highest, production of lactic acid of 1.61 g/l (Table 3). In this study, the isolated LAB produced antimicrobial compounds to a varying degree. *Lactobacillus plantarum* recorded the highest yield of lactic acid (1.61 g/l) after 72 h of growth in MRS broth while *Pediococcus pentosaceus* had the lowest value of 1.19 g/l. Although lactic acid production increased with time, it was observed that the production peak was reached at 72h of growth for all the test isolates (Table 3). The highest concentration of lactic acid produced by *L. plantarum* agreed with the findings of [22]. The increase in the production of lactic acid with time has been attributed to lower pH, which permit the growth of LAB to the detriment of the competing organism [35]. Diacetyl is important for the organoleptic quality of fermented products, such as cottage, cheese, butter and fermented cream. The antimicrobial properties of diacetyl are well-documented [25]. [22] reported the detection of hydrogen peroxide producing LAB, which are often searched for because of their antibacterial activity and also reported on the inhibition of *Pseudomonas fragi* and *Staphylococcus aureus* by hydrogen peroxide by certain LAB strains, which can contribute to their inhibitory activity against other microorganisms, including food–borne pathogens. Furthermore, the results observed in this study were in accordance with the work of [22] who reported that production of the primary metabolite, lactic acid and the resulting pH decrease is the main preserving factor in food fermentation.

Table 4 shows the antagonistic activity in millimeter, of LAB isolates obtained from Iru samples against different indicator microorganisms. Both disc and agar well methods were used for the analysis. Most of the LAB isolates tested were able to inhibit all the indicator organisms (*Escherichia coli*, *Salmonella* sp, *Staphylococcus* sp and *Listeria monocytogene*s) except *Bacillus subtilis* which was only inhibited by *Lactobacillus plantarum*. *Leuconostoc mesenteroides* SBI 15 had the highest inhibition zone of 30mm on *Listeria monocytogenes*. Lactic acid bacteria are known to inhibit the growth of unrelated organisms in a mixed culture (Table 4.). The antimicrobial effects of lactic acid and acetic acid have been extensively reviewed [36]. The effect is due to the un-dissociated form of the acids, which can penetrate the membrane and liberate hydrogen ions in the neutral cytoplasm, thus leading to inhibition of vital cell functions [37]. The inhibitory effect of hydrogen peroxide produced by LAB has also been reported [22]. The proliferation of spoilage organisms and food-borne pathogens can be prevented by low pH and high concentrations of organic acids.

Table 1. Quantity of diacetyl produced by isolates of lactic acid bacteria (g/l)

Isolates	Fermentation time (hours)/Diacetyl concentration (g/L)				
	18	24	36	48	72
Leuconostoc mesenteroides SBI 14	0.41	0.75	0.83	0.86	0.87
Leuconostoc mesenteroides SBI15	0.43	0.77	0.83	0.85	0.91
Lactobacillus plantarum	0.40	0.71	0.80	0.83	0.89
Pediococcus pentosaceus	0.44	0.76	0.84	0.87	0.83

Table 2. Quantity of hydrogen peroxide produced by isolates of lactic acid bacteria (g/l)

Isolates	Fermentation time (hours)/ Hydrogen peroxide (g/L)				
	18	24	36	48	72
Leuconostoc mesenteroides SBI 14	0.41	0.7 5	0.83	0.86	0.84
Leuconostoc mesenteroides SBI15	0.43	0.72	0.83	0.85	1.10
Lactobacillus plantarum	0.40	0.71	0.80	0.83	0.85
Pediococcus pentosaceus	0.44	0.76	0.84	0.87	0.88

Table 3. Quantity of lactic acid produced by isolates of lactic acid bacteria (g/l)

Isolates	Fermentation time (hours)/ lactic acid concentration (g/L)				
	18	**24**	**36**	**48**	**72**
Leuconostoc mesenteroides SBI 14	0.30	0.38	1.02	1.11	1.30
Leuconostoc mesenteroides SBI15	0.32	1.03	1.11	1.21	1.30
Lactobacillus plantarum	0.28	1.33	1.48	1.52	1.61
Pediococcus pentosaceus	0.33	1.08	1.13	1.20	1.19

Table 4. Antibacterial activity (mm) of LAB isolates obtained from iru samples against different indicator microorganisms

Isolates	Indicator organism/ method/ zone of inhibition (mm)									
	E. coli		***Staphylococcus* spp**		***Bacillus subtilis***		***Salmonella* spp**		***Listeria monocytogenes***	
	Disc	**Well**	**Disc**	**Well**	**Disc**	**Well**	**Disc**	**Well**	**Disc**	**Well**
Leuconostoc mesenteroides SBI 14	13	15	20	23	-	-	11	13	25	26
Leuconostoc mesenteroides SBI15	12	12	24	25	-	-	21	25	28	30
Lactobacillus plantarum	12	18	22	20	6	8	21	23	28	28
Pediococcus pentosaceus	11	12	20	21	-	-	10	12	20	21

Table 5. Sensory evaluation of soy iru produced using different inocula

Product	Taste	Aroma	Colour	Mucillage	Stickiness	General acceptability
Bacillus soy iru	4.22^{b}	3.67^{a}	3.33^{a}	4.22^{b}	4.44^{a}	3.89^{b}
LAB soy iru	4.50^{b}	3.50^{a}	3.44^{a}	3.33^{a}	3.67^{a}	3.25^{a}
bacillus + lab soy iru	4.78^{b}	4.44^{b}	3.33^{a}	4.33^{b}	4.44^{a}	4.78^{c}
Spontaneous fermentation	3.40^{a}	3.60^{a}	3.67^{a}	4.22^{b}	4.11^{a}	3.60^{ab}

Mean values followed with different lower case letters are statistically significantly different at p <0.05

The result of the sensory tests is shown in Table 5, there was no significant difference in the taste, colour and stickiness of the three starter based fermented soy iru. *Bacillus* + LAB soy iru scored the maximum point both in aroma and general acceptability. The soy iru treated samples were acceptable compared with the untreated soy iru. This study showed that soy-iru could be processed using *Bacillus subtilis* and *Leuconostoc mesenteroides* either singly or in combination at pH7 and 37°C for 72 hours of fermentation in calabash. The combined use of the two organisms proved to be the most appropriate starter culture for fermentation of soy-iru as it contained and recorded the highest protein content. It is also the most preferred by the sensory panelists when evaluated.

4. CONCLUSION

From this research, it has been confirmed that *Bacillus subtilis* and *Leuconostoc mesenteroides* can be used as starter culture in the fermentation of Soya beans to produce Soy Iru. Their use in combination produced improved aroma and increased percentage protein of Soy Iru which makes it an excellent diet supplement and can be used to combat malnutrition. The use of starter culture also improved the safety and consistency of the Locust bean (iru). Soybeans could be used as an alternative to *Parkia* spp. traditionally used for Iru production and thus eliminating the laborious dehulling and the time needed to ferment *Parkia* seed to Locust bean (iru).

Production of soy iru, if properly developed, has a strong potential in increasing food production and employment opportunity and also improving the nutritional status of the rural population.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Beuchat LR. Fermented soybeans foods. Food Technology. 1984;64-70.
2. Afolabi FT, Adewolu AM. Monitoring of the microbial profiles of soybeans (*Glycine max*) during fermentation for Soy Iru production. European International Journal of Applied Science and Technology. 2014; 1:140-148.
3. Odunfa SA. Biochemical changes in fermenting African Locus bean (*Parkia biglobosa*) during iru fermentation. Journal of Food Technology. 1985;20:295-303.
4. Esteves EA, Martino HSD, Olivieira FCE, Bressan J, Costa NMB. Chemical composition of soybeans cultivar lacking lipoxygenases. Food Chemistry. 2010;122: 238-242.
5. Hu Y, Ge C, Yuan W, Zhu R, Zhang W, Due L, Xue J. Characterization of fermented black soybeans *Natto* inoculated with *Bacillus Natto* during fermentation. Journal of Science Food and Agriculture. 2010;90:1194-1202.
6. Omafuvbe BO, Abiose SH, Shonukan OO. Fermentation of soybean (*Glycine max*) for soy-daddawa production by starter cultures of *Bacillus*. Food Microbiology. 2002;19: 561-566.
7. Hounhouigan DJ, Nout MJR, Nago CM, Houben JH, Rombouts FM. Starter cultures of lactobacilli and yeasts in the fermentation of mawe´ porridge. In: Hounhouigan, D.J. (Ed.), Fermentation of Maize (*Zea mays* L.) Meal for mawe´ Production in Be´nin. Doctorate thesis, Agricultural University of Wageningen, The Netherlands; 1994.
8. Nout MJR, Kok B, Vela E, Nche PF, Rombouts FM. Acceleration of the fermentation of kenkey, an indigenous fomented maize food of Ghana. Food Reserve International. 1996;28:599–604.
9. Halm M, Amoa-Awua WK, Jakobsen M. Kenkey an African fermented maize product. In: Hu YH, Meunier-Goddik L, Hansen AS, Josephsen J, Nip WK, Stanfied PS, Toldra F, (Eds.), Handbook of food and beverage fermentation technology. Marcel Dekker, Inc., New York. 2004;799–816.
10. Annan NT, Poll L, Sefa-Dedeh S, Plahar WA, Jakobsen M. Influence of starter culture combinations of *Lactobacillus fermentum, Saccharomyces cerevisiae* and *Candida krusei* on aroma in Ghanaian maize dough fermentation. European Journal of Food Reserve Technology. 2003;216:377–384.
11. Sefa-Dedeh S, Sanni AI, Tetteh G, Sakyi-Dawson E. Yeasts in the traditional brewing of pito in Ghana. World Journal of Microbiology and Biotechnology. 1999;15: 593–597.

12. Kimaryo VM, Massawe GA, Olasupo NA, Holzapfel WH. The use of a starter culture on the fermentation of cassava for the production of kivunde, a traditional Tanzanian food product. International Journal Food Microbiology. 2000;56: 179– 190.
13. Beaumont M. Flavouring composition prepared by fermentation with *Bacillus* spp. International Journal of Food Microbiology. 2002;75:189–196.
14. Barrow GI, Feltham RKA. Cowan and Steel's manual for the identification of medical bacteria. 3rd ed. Cambridge University Press. 1993;19-20.
15. Fawole MO, Oso BA. Laboratory Manual of Microbiology. 2007;34-35.
16. Baird-Parker, AC. Bergey's manual of determinative bacteriology. Ed. By Buchanan RE, Gibbons NE. The Williams and Wilkins Company, Baltimore. 1974; 478-489.
17. Paul D, George MG, Dorothy J, Noel RK, Wolfgang L, Rred AR, Karl-Heinz S, William BW. Bergey's manual of systematic bacteriology. Second edition. Volume three. The *firmicutes*. Ed. By William BW, Aidan CP. The Williams and Wilkins Company, USA. 2009;73-94.
18. Katekan D, Shannaphimon W, Phichaya T, Prapaporn B, Arunee A, Panupong S, Ekachai C. Comparative study of proteolytic activity of protease-producing bacteria isolated from *thua nao. Maejo* International Journal of Science Technology. 2009;3(2):269-276.
19. Harrigan WF, McCance ME. Laboratory methods in food and dairy microbiology. Rev. Edn., London, New York, Academic Press. 1966;33-200.
20. Akpomie OO, Akponah E, Okorawhe P. Amylase production potentials of bacterial isolates obtained from cassava root peels. Agricultural Science Research Journals. 2012;2:95-99.
21. Kumar M. Isolation of *Lactobacillus plantarum* from cow milk and screenin for the presence of sugar alcohol producing gene. Journal of Microbiology and Antimicrobials. 2012;4:16-22.
22. Ogunbanwo ST. Functional properties of lactic acid bacteria isolated from ogi and fufu, two Nigerian fermented foods. Advances in Food Sciences. 2005;27(1): 14-21.
23. AOAC. Official Methods of Analysis. 15th Edition. Association of Official Analytical Chemists, Washington DC; 1980.
24. AOAC. Official Methods of Analysis. 15th Edition. Association of Official Analytical Chemists, Washington DC; 1990.
25. Yusra FA, Novelina Periadnadi D. Antimicrobial activity of lactic acid bacteria isolated from Budu of West Sumatera to food biopreservatives. Pakistan Journal of Nutrition. 2013;12(7):628-635.
26. Girum T, Eden E, Mogese A. Assessment of the antimicrobial activity of lactic acid bacteria isolated from borde and shameta, traditional Ethiopian fermented beverage, on some food borne pathogen and affected of growth medium on the inhibitor activities. International Journal of Food Safety. 2010;5:13-20.
27. Achi OK. Traditional fermented protein condiments in Nigeria. African Journal of Biotechnology. 2005b;4:1612-1621.
28. Anosike EO, Egwautu CK. Biochemical changes during the fermentation of castor oil (*Ricinus communis*) seeds for use as seasoning agent. Quality Plant, Foods and Human Nutrition. 1981;30:181–185.
29. Amoa-Awua WK, Terlabie NN, Sakyi-Dawson E. Screening of 42 *Bacillus* isolates for ability to ferment soybeans into dawadawa. International Journal of Food Microbiology. 2006;106:343–347.
30. Visessanguan W, Benjakul S, Potachareon W, Panya A, Riebroy S. Accelerated proteolysis of soy proteins during fermentation of *thua-nao* inoculated with *Bacillus subtilis.* Journal of Food Chemistry. 2005;29:349-366.
31. Ouoba LII, Diawara B, Annan NT, Poli L, Jakobsen M. Volatile compounds of soumbala, a fermented African locust bean *(Parkia biglobosa)* food condiment. Journal Applied Microbiology. 2005;99:1413-1421.
32. Priest FG. Isolation and identification of aerobic endospore forming bacteria. In: *Bacillus*. Harwood CR, (Ed). Plenum Press, London. 1989;38-46.
33. Chantawannakul P, Oncharoen A, Klanbut K, Chukeatirote E, Lumyong S. Characterization of proteases of *Bacillus subtilis* strain 38 isolated from traditionally fermented soybean in Northern Thailand. Science Asia. 2002;28:241-245.

34. Aderibigbe EY, Odunfa SA. Growth and extracellular enzyme production by strains of *Bacillus* species isolated from fermenting African locust bean, Iru. Journal of Applied. Bacteriology. 1990;69:662-671.
35. Kandler O, Weiss N. In Sneath P, Mar NS, Sharpe ME, Hott J, (eds) Bergey's Manual of Systemic Bacteriology. 1986;2. Williams and Wilkins, Baltimore.
36. Ogunbanwo ST, Sanni AI, Onilude AA. Effect of bacteriocinogenic *Lactobacillus* spp on the shelf life of fufu, a traditional fermented cassava product. World Journal of Microbiology and Biotechnology. 2004; 20:57-63.
37. Corlett DA, Brown MH. In Silliker JH. Led microbiology. Academic Press, New York. Ecology of Foods. 1980;24.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13862