

Full Length Research Paper

Riboflavin enriched iru: A fermented vegetable protein

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African locust bean (*Parkia biglobosa*) cotyledon is fermented in most countries of West Africa to produce a soup condiment, known as 'iru' in Yoruba language, or 'dawadawa' in the predominant Hausa language. Iru is rich in minerals and serves as a source of protein supplement in the diet of poor families. Riboflavin (Vitamin B₂) is an essential component of basic cellular metabolism but its daily requirement is not met in Nigeria particularly among the rural dwellers. Therefore, the provision of a riboflavin enriched iru will help to eradicate problems encountered from riboflavin deficient diet. Iru was purchased from three different markets in Ibadan, Oyo State, Nigeria. From the iru, microorganisms were isolated, characterised, screened for riboflavin production and co-cultured for the production of riboflavin enriched iru. Sixty-three bacteria were isolated and identified as *Micrococcus varians* (9), *Staphylococcus* species (27), *Bacillus* species (24) and *Micrococcus luteus* (3). *Bacillus subtilis* IR50 produced highest riboflavin 25.77 mg/L, followed by *Staphylococcus* spp. strain IR26 23.37 mg/L, while *M. varians* IR49 had the least riboflavin production 6.35 mg/L. Mixed culture of *B. subtilis* IR50 and *Bacillus licheniformis* IR28 produced the highest riboflavin of 4.5 mg/L, *Staphylococcus aureus* IR06 and *B. subtilis* IR50 produced 2.3 mg/L, while *B. subtilis* IR50 produced 1.5 mg/L when used singly. The result shows that *B. subtilis* IR50 have the potential to increase the riboflavin content of iru and therefore will contribute to bioenrichment technology.

Key words: African locust bean, iru, riboflavin, *Bacillus subtilis*, bioenrichment.

INTRODUCTION

African locust bean (*Parkia biglobosa*) cotyledon is fermented in most West African belt countries to produce a soup condiment, known as 'iru', or 'dawadawa', depending on the ethnic group. Till date, the production process is a traditional art; and the fermentation is carried out by indigenous microflora derived from the immediate environment. Iru which is the Yoruba name is a product

of alkaline fermentation of African locust bean (*P. biglobosa*) which is rich in protein and usually fermented to a tasty food condiment used as a flavour intensifier for soups and stews and also adds proteins to a protein-poor diet. Apart from imparting flavour, it serves as a source of protein supplement in the diet of poor families (Odunfa, 1985).

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The pods are flat, large, irregular clusters from which the locust bean seeds are obtained (Omafuvbe et al., 2004). It contains about 40.4% protein, 31.5% fat, 3.1% fibre and 15% carbohydrate (Fetuga et al., 1974). The African locust bean is consumed mainly because of the flavouring attributes. Locust beans are not usually used for food in their natural state, because of the presence of non-digestible carbohydrates which may include arabinogalactan, stachyose, and raffinose (Odunfa, 1983) and the presence of anti-nutritional factors which are a diverse range of naturally occurring compounds in many tropical plants (Esenwah and Ikenebomeh, 2008). Fermentation makes the food to be more nutritious, digestible and safer with better flavour. The cooked African locust beans are unpalatable but when fermented into condiment, Iru, the physical, chemical and nutritional characteristics of the seeds change (Amoa-Awu et al., 2005).

The fermentation is brought about by strains of *Bacillus subtilis* (Odunfa, 1981). Many strains of the *B. subtilis* group have been isolated from iru samples obtained from different sources in southwestern. Quantity of iru consumed varies with the country and within the country. The average per capita per day consumption of iru in Togo and Ghana is 4 and 2 g, respectively, the Yorubas of Southwestern Nigeria consume 10 g per day per person (Dema, 1965), while the overall consumption estimated for parts of Nigeria range from 1 to 17 g per person per day (Nicol, 1959).

Riboflavin (vitamin B₂) is a water-soluble vitamin derived from plants and many micro-organisms. Because this biosynthetic capability is lacking in higher animals, they must therefore obtain this essential nutrient from their diet. Riboflavin is the precursor of the enzyme cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are vital in many of the body's enzymatic functions for the transfer of electrons in oxidation-reduction reactions (Burgess et al., 2006). Riboflavin deficiency is most commonly seen in developing countries (Blanck et al., 2002), among the elderly (McKinley et al., 2002), and in chronic alcoholics (Langohr et al., 1981). Riboflavin deficiency mainly manifests itself clinically in the mucocutaneous surfaces of the mouth, through the occurrence of cracks at the corners, and inflammation of the lips and tongue (Baker and Dickerson, 1996), but deficiency is also associated with vision deterioration and growth failure. In recent years the vitamin has been found to be effective in the treatment of migraine (Boehnke et al., 2004), malaria (Akompong et al., 2000) and Parkinson's disease (Coimbra and Junqueira, 2003). The recommended daily requirement of riboflavin is set at 1.3 mg (Food and Nutrition Board, 1999). The aim of this research work is to bioenrich iru using microorganisms that have the ability to produce riboflavin naturally and also ferment the locust beans.

MATERIALS AND METHODS

Sample collection

African locust bean seeds (*P. biglobosa*) and iru used for this study were purchased from retail markets in Ibadan, Oyo State, South-west Nigeria. They were transported to the Food and Industrial Microbiology Laboratory of the Department of Microbiology, University of Ibadan in clean polythene bags until further use.

Isolation of microorganisms from locust beans

The isolation of bacteria associated with the fermentation of locust beans to produce iru was done on Plate count agar, Mannitol salt agar, and tryptone soy agar using pour plate method according to Harrigan and McCance, (1966) and the viable populations were determined by plating out 1 ml of the 10⁻³, 10⁻⁵, 10⁻⁶ and 10⁻⁷ dilutions. The plates were incubated at 37° for 24 to 48 h. After incubation, pure isolates were obtained, and stored on Nutrient agar slants until further investigations. The cultural characteristics of each isolates were observed. Microscopic examination and several biochemical tests were also carried out for the purpose of identification.

Screening of isolates for riboflavin production

The standard medium used in screening the isolates for riboflavin production was composed of 25 g/L sucrose, KH₂PO₄, MgSO₄·7H₂O, ZnSO₄ (0.2% solution) and 10% sodium phosphate buffer 0.1 M, pH 7.0, according to the method of Suzuki et al. (2009). A volume of 15 ml of the medium was dispensed into McCartney bottles and autoclaved for 20 min at 121°C and 1 atm. An aliquot of 0.3 ml of each isolates were inoculated into the 15 ml culture medium, respectively. The incubation was carried out for 48 h at 30°C and 100 rpm on a rotary shaker, in aerobic condition. Riboflavin production was assayed in the culture broth after 48 h. The culture broth for each isolates was dispensed into 15 ml centrifuge tubes and was centrifuged at 1°250 × g for 15 min at 5°C. The experiment was carried out in the dark to avoid riboflavin oxidation. The supernatant were separated for riboflavin analysis.

Riboflavin assay

A 0.8 ml of the supernatant obtained from the culture broth was added to 0.2 ml of 1 M NaOH. A 0.4-ml volume of the resulting solution was neutralized with 1 ml of 0.1 M potassium phosphate buffer (pH 6.0). Using a Spectrum lab 752S UV VIS Spectrophotometer, the absorbance of the mixture for each isolates in the culture broth at 444 nm was measured (Ming et al., 2003). The riboflavin concentration was calculated using the extinction coefficient of 1.04 × 10⁻² M⁻¹cm⁻¹ (Sauer et al., 1996).

Laboratory production of iru and inoculation with screened isolates

Boiling

Locust beans seeds were boiled in a pressure pot in order to enhance good dehulling characteristics and high quality of final product. After 2 h of boiling, the locust bean seed was allowed to cool for 5 min after boiling before dehulling.

Dehulling

This was achieved by rubbing seeds in between palms to remove the seed coats.

Separation

The cotyledons were washed thoroughly in clean, potable water and the testae were removed using a sieve. The dehulled seeds were then cooked further for 30 min in the pressure pot to soften the cotyledons after which they are drained and cooled.

For the fermentation, 20 g of the locust bean seeds were placed aseptically into 6 sterile petridishes that were lined with aluminium foil which creates a warm environment for the fermentation. Using plating method, 1.7×10^7 cfu/ml of *Bacillus* species and *Staphylococcus* species that had the highest riboflavin concentrations, obtained from fermented iru were inoculated into the locust bean plates and incubated at 37°C for 4 days.

Estimation of riboflavin content in the inoculated locust beans

The riboflavin concentration of the locust bean samples that were inoculated with *Bacillus* spp. and *Staphylococcus* spp. was estimated at different time intervals of 12 to 96 h. The fermenting locust bean samples were washed in sterile distilled water and sodium borate buffer at pH 7.52; this was done to regulate the pH of the samples. The mixture was then filtered using Whatman filter paper no 1. The absorbance of the filtrate was determined and estimated using a JENWAY, 6405 UV/ Vis spectrophotometer at 444 nm. The riboflavin concentration of the fermented locust bean was calculated using the extinction coefficient $1.04 \times 10^{-2} \text{ M}^{-1} \text{ cm}^{-1}$ (Sauer et al., 1996).

Effects of different concentrations of NaCl on riboflavin concentration of iru

Twenty grams of the laboratory prepared iru was transferred into five sterile containers that contain different concentration of NaCl ranging from 0 to 10%. A volume of 1 ml from the 18 to 24 h old harvested cells of selected isolates which produced high concentration of riboflavin (inoculum size was determined to be 1.7×10^7 cfu/ml) was inoculated into the iru samples. The inoculated samples were covered properly and kept at room temperature for 4 days. At 24 h interval, 1 g of each sample at different concentration was transferred into 10 ml of already sterilized distilled water to serve as stock, and then it was serially diluted in subsequent 9 ml of sterile distilled water. Appropriate dilution factors were plated on sterile Petri dishes and incubated at 37°C for 18 to 48 h. After 24 to 48 h, the colonies that showed the characteristics of the organisms on the plates were counted and recorded. The riboflavin concentration of the locust bean sample was read by taking the absorbance of the samples using JENWAY, 6405 UV visible spectrophotometer at 444 nm and the estimated concentration was read from the standard curve.

Effect of different temperatures on the growth of isolates used as starters in enriching iru

Nutrient broth were inoculated with the appropriate starter and incubated at 30, 35, 40, 45 and 50°C for 24 h. After 24 h, the turbidity of each isolates was read as absorbance at 540 nm using JENWAY, 6405 UV visible spectrophotometer.

Effect of pH on the growth of isolates used in fermenting iru

Nutrient broth was dissolved in potassium phosphate buffer at different pH 3, 4, 5, 6, 7 and 8, after which it was dispensed in properly labelled test tubes for each isolate. Each test tube was sterilized at 121°C for 20 min and inoculated with appropriate starter. The turbidity of each isolate was read as absorbance at 540 nm using JENWAY, 6405 UV visible spectrophotometer after 24 h.

Statistical analysis

The data obtained in this research work were subjected to analysis of variance (ANOVA) and the Duncan's multiple range tests were used to separate the means while significant difference was obtained for sample treatments ($P \leq 0.05$).

RESULTS

Sixty-three bacteria strains were isolated from the fermenting iru. They were identified as *Staphylococcus aureus* (19), *Micrococcus varians* (9), *Staphylococcus* spp. (5), *Bacillus licheniformis* (4), *Bacillus alvei* (4), *Micrococcus luteus* (3), *Bufo marinus* (3), *Bacillus brevis* (2), *Bacillus megaterium* (2), *Bacillus pumilus* (2), *Bacillus sphaericus* (2), *Bacillus subtilis* (2), *Staphylococcus intermedius* (2), *Bacillus badius* (1), *Bacillus maequariensis* (1), *Bacillus polymxa* (1), and *Staphylococcus horminis* (1).

Screening of isolates for riboflavin production

The 63 isolates were screened for riboflavin production; it was observed that *Bacillus subtilis* IR50 produced the highest riboflavin of 25.8 mg/L, followed by *Staphylococcus* spp. IR26, while *M. varians* IR49 have the least of 6.4 mg/L.

Riboflavin concentration (mg/L) in locust bean fermented with selected starters that are capable of high riboflavin production

Figure 1 shows the mean riboflavin concentrations (mg/L) under laboratory fermentation of locust bean using *Bacillus* and *Staphylococcus* spp. for 12 to 96 h of fermentation. *B. subtilis* IR50 and *B. licheniformis* IR28 when used together in locust bean fermentation produced the highest riboflavin of 4.5 mg/L, combination of *S. aureus* IR06 and *B. subtilis* IR50 produced 2.3 mg/L riboflavin concentration, while *B. subtilis* IR50 alone produced the least riboflavin concentration of 1.5 mg/L. Figure 2 shows the growth response of selected isolate to temperature at 30, 35, 40, 45 and 50°C, respectively. At 35 and 50°C all the isolates had minimum growth. The optimum temperature for growth of all isolates is at 40°C.

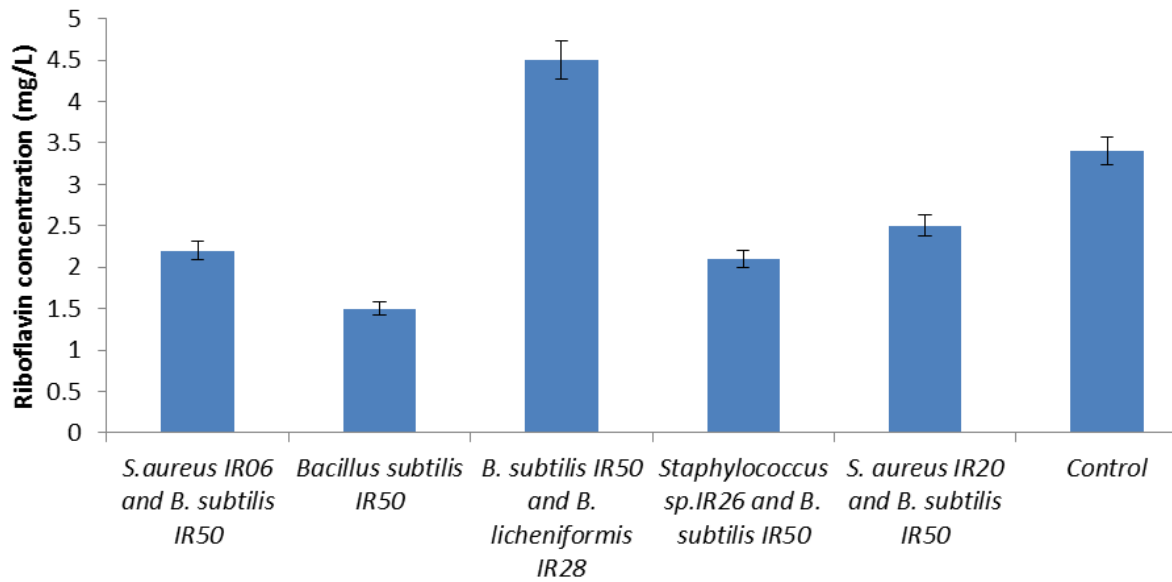


Figure 1. Mean riboflavin concentrations (mg/L) in locust bean fermented with *Bacillus* and *Staphylococcus* spp.

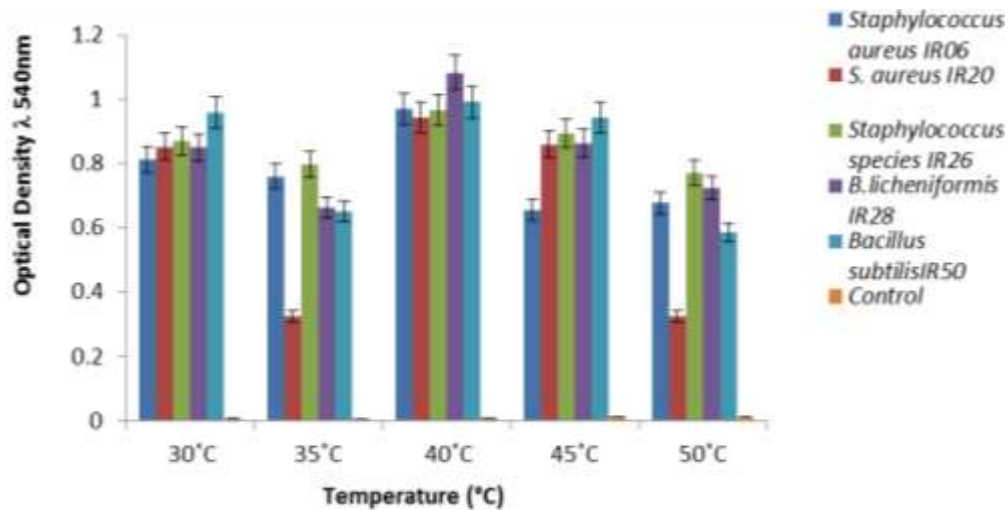


Figure 2. Growth response of selected isolates capable of high riboflavin production at different temperatures.

Also, the growth response of selected starters to different pH is shown in Figure 3. *S. aureus* IR06 and IR20 had optimum pH for growth at pH 6 while the optimum pH for *Staphylococcus* spp. IR26, *B. licheniformis* IR28 and *B. subtilis* IR50 is 7 and the least growth is obtained at pH 3 for all isolates. Isolates' growth response to different concentration of NaCl is as shown in Figure 4. All the isolates grew better at 0% NaCl and they had scant growth at 2.5% NaCl. *B. subtilis* IR50 in combination with *Staphylococcus* spp. IR26 had minimal growth at 7.5%

NaCl, while *B. licheniformis* IR28 in combination with *B. subtilis* IR50 were able to grow well at the different concentrations of NaCl compared to other isolates but had their least growth at 10% salt concentration. *B. subtilis* IR50 in combination with *S. aureus* IR06 survived at the different concentrations of NaCl but had their best growth at 0% NaCl and least growth at 5%. *B. subtilis* IR50 used singly was able to grow at different concentration of NaCl but had least growth at 7.5% NaCl and thrived best at 0% NaCl.

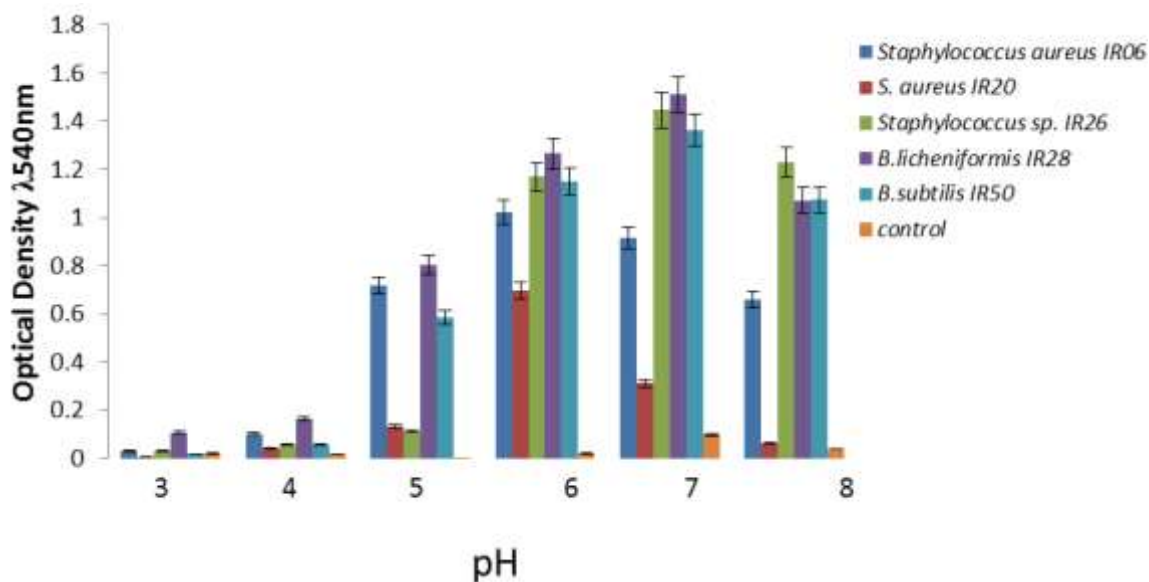


Figure 3. Growth response of selected starters capable of high riboflavin production at different pH.

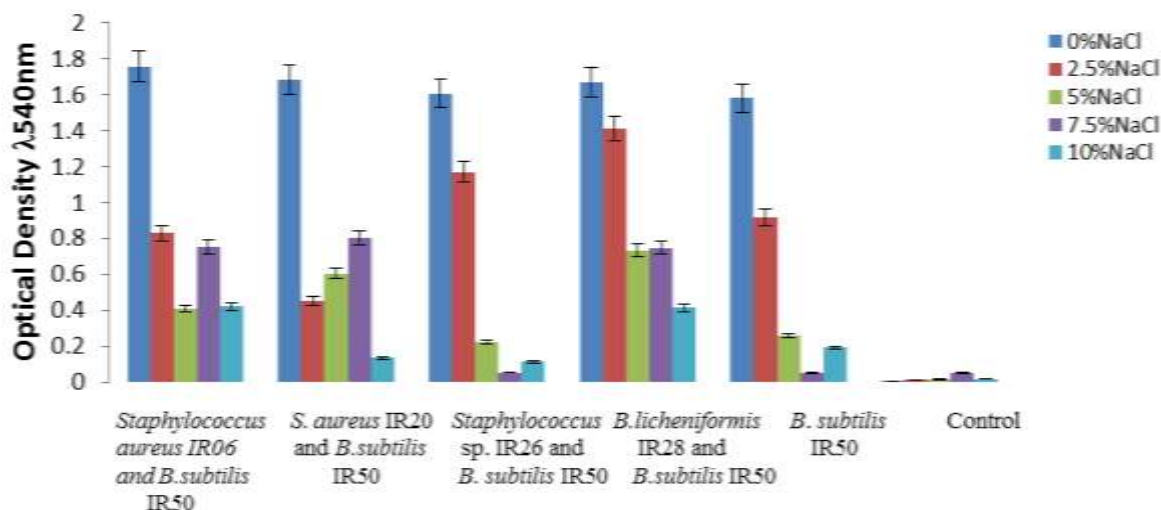


Figure 4. Growth response of selected starters capable of high riboflavin production to different concentrations of NaCl.

Effects of different concentrations of NaCl on riboflavin produced in locust bean fermented with *Bacillus* and *Staphylococcus* spp., respectively

The mean riboflavin concentrations of locust bean fermented with *Bacillus* and *Staphylococcus* spp. at different concentrations of NaCl is shown in Table 1. All the selected starters produced high concentration of riboflavin at 0% NaCl. There was a gradual decrease in riboflavin produced by the selected isolates at 2.5 to 10%

NaCl concentrations. *Staphylococcus* spp. IR26 combined with *B. subtilis* IR50 for the fermentation of locust beans at 0% NaCl produced highest riboflavin of 11.430 mg/L, while *B. subtilis* IR50 alone gave 3.890 mg/L .

DISCUSSION

The dynamics of fermentation in food or condiment such

Table 1. Mean riboflavin concentration of iru fermented with *Bacillus* and *Staphylococcus* species at different concentrations of NaCl.

Isolates code	Isolate	0% NaCl	2.5% NaCl	5% NaCl	7.5% NaCl	10% NaCl
IR06+IR50	<i>S. aureus</i> and <i>B. subtilis</i>	8.850	4.560	5.220	3.320	3.560
IR20+IR50	<i>S. aureus</i> and <i>B. subtilis</i>	8.580	8.800	4.820	4.280	3.520
IR26+IR50	<i>S. species</i> and <i>B. subtilis</i>	11.430	6.140	3.760	4.480	5.980
IR28+IR50	<i>B. licheniformis</i> and <i>B. subtilis</i>	10.310	5.070	4.720	4.550	4.40
IR50	<i>B. subtilis</i>	3.890	4.70	4.480	4.50	3.040
No isolate	Control	3.090	3.320	3.670	3.870	4.050

as iru is a complex microbiological process involving interactions among different microorganisms (Omafuvbe et al., 2003). The contribution of the accompanying flora of fermenting substrate is determined by the substrate composition and hygiene during production. The microorganisms isolated from iru samples at species level include different species of *Staphylococcus*, *Micrococcus*, and *Bacillus*. *Bacillus* is the most predominant because of its proteolytic ability and also their ability to break down oils. *B. subtilis* has been associated with iru fermentation (Enujiugha, 2009). *Staphylococcus* and *Micrococcus* were present in low numbers compared to *Bacillus*. These organisms do not appear to be important in the fermentation process. *Bacillus* spp. have been implicated in the fermentation of most vegetable oil protein seeds (Odunfa, 1981; Ogueke and Aririatu, 2004). *Staphylococcus* spp. and *Micrococcus* spp. have also been isolated from fermenting African oil bean seeds but from the health point of view, the presence and isolation of *S. aureus* indicated poor hygienic practices during production (Ibeabuchi et al., 2014).

B. subtilis was screened to produce the highest concentration of riboflavin among other isolates. Several studies have been established on the ability of this organism to produce riboflavin for feed and food fortification purposes (Stahmann et al., 2000; Burgess et al., 2006). Species of *B. subtilis* group have been reported to be generally regarded as safe (GRAS) by the U. S. Food and Drug Administration, and their role in the fermentation of locust bean to produce different condiments has been thoroughly investigated (Beaumont, 2002).

Riboflavin has been traditionally synthesised for food and feed fortification by chemical means but recently biotechnological processes which involve the use of various bacteria, yeast and fungi have been put in place (Stahmann et al., 2000). One of these biotechnological processes employs the use of *B. subtilis* and much work has been carried out in characterising the vitamins biosynthetic pathway (Perkins and Pero, 2002). *Bacillus subtilis* IR50 isolated in this study was screened to produce highest concentration of riboflavin.

Food fermentation processes mostly depend on co-culturing of microbes which act in conjunction to produce desired product characteristics. This study highlights the microbial diversity of riboflavin producing strains isolated from iru. Mixed culture of *B. licheniformis* IR28 and *B. subtilis* IR50 produced highest concentration of riboflavin; there could be a form of protocoperation interaction between the organisms which may mean that both interacting species gain fitness with each other thereby leading to an increase in riboflavin production (Sieuwert et al., 2008).

The use of high riboflavin producer as starters in locust beans fermentation contributes significantly to the functional value of iru produced and also will bring about enrichment of poor riboflavin diets; this will eventually lead to a decrease in riboflavin deficiencies among the populace of rural dwellers. Iru fermentation is solid substrate fermentation with an exothermic process, where the temperature of the fermenting seed increases gradually from ambient temperature to about 30°C to 45°C (Odunfa and Oyewole, 1986). The bacteria isolated from iru that are, *Staphylococcus* spp. and *Bacillus* spp. grew best at 35 to 40°C so this is evidence to their ability to ferment iru substrate.

One of the most common local/natural food preservatives is salt, which tends to improve the shelf life of processed African locust bean seeds by reducing the number of microbial load on the samples, which could have been agent(s) of deterioration or spoilage to the sample and reduce the shelf life (Ademola et al., 2013). There have been reports on methods of improving the shelf life of fermented locust beans (Omabuvfe, 2007; Kolapo et al., 2007), but little is known about its effect on the riboflavin content of the fermented product. In this study 0 to 10% concentration of NaCl was used. It has been reported in the findings of Odunfa et al., (1981) that a gradual decrease in growth rate of *Staphylococcus* spp. was observed with increasing concentration of sodium chloride. Ikenebomeh (1989) also states that variations in salt content influence the microbial development leading to lower microbial counts at salt addition above 3%. In this study, all the isolates used experienced a significant decrease in their microbial growth after exposure to 0%

NaCl. Furthermore, addition of NaCl to iru brought about a decrease in the riboflavin production due to inhibitory effect of NaCl on the selected isolates. This is in agreement with Ratnakar and Rai (2013) who observed reduction in riboflavin content of leaves of *Amaranthus polygamus* with increase in concentration of NaCl. This signifies that using salt as a means of preserving iru which is a common practice among the rural dwellers have a significant impact on the riboflavin content of the condiment and the practice should be discouraged.

Conclusion

B. subtilis IR50 and *B. licheniformis* IR28 isolated from fermented locust beans were able to increase the riboflavin content of iru *in vivo* significantly. However, the use of salt as a means of preservative has effect on both the growth of the isolates and the stability of the riboflavin content of the produced condiment (iru).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Ademola IT, Baiyewu RA, Adekunle EA, Awe AB, Adewumi OJ, Ayodele OO, Oluwatoke FJ (2013) Microbial load of processed *Parkia biglobosa* seeds: Towards enhanced shelf life. *Afr. J. Agricultural Research* 8 (1):102-105
- Akompong T, Ghorri N, Haldar K (2000). In vitro activity of riboflavin against the human malaria parasite *Plasmodium falciparum*. *Antimicrob Agents Chemother*, 44:88-96.
- Amoa-Awu WF, Owusu M, Feglo P (2005). Utilization of unfermented cassava flour for the production of indigenous African fermented Food, Agbelina. *World J. Microb. Biotechnol.* 21:1201-1207.
- Baker TK, Dickerson JWT (1996). Vitamins in Human Health and Disease. Wallingford, Oxon, CAB International, Bathua No. 1 *Journal of Stress Physiology Biochem.* 9(4):187-192.
- Beaumomt M (2002). Flavoring composition prepared by fermentation with *Bacillus* sp. *Int. J. Food Microbiol.* 75:189-196.
- Blanck HM, Bowman BA, Serdula MK, Khan LK, Kohn W, Woodruff BA (2002) Angular stomatitis and riboflavin status among adolescent Bhutanese refugees living in southeastern Nepal. *Am. J. Clin. Nutr.* 76:430-435.
- Boehnke C, Reuter U, Flach U, Schuh-Hofer S, Einhaupl KM, Arnold G (2004) High-dose riboflavin treatment is efficacious in migraine prophylaxis: an open study in a tertiary care centre. *Eur. J. Neurol.* 11:475-477.
- Burgess CM, Smid EJ, Rutten G, Sideren D (2006). A general method for selection of riboflavin overproducing food grade microorganisms. *Microbial Cell Factories.* 5:24
- Coimbra CG, Junqueira VB (2003) High doses of riboflavin and the elimination of dietary red meat promote the recovery of some motor functions in Parkinson's disease patients. *Braz. J. Med. Biol. Res.* 36:1409-1417.
- Dema JS (1965). Nutrition in Relation to Agricultural Production, Food and Agriculture Organisation, Rome. 129
- Enujiugha VN (2009) Major fermentative organisms in some Nigerian soup condiments. *Pak. J. Nutr.* 8(3):271-283.
- Esenwah CN, Ikenebomeh MJ (2008). Processing Effects on the Nutritional and Anti- Nutritional Contents of African Locust Bean (*Parkia Biglobosa* Benth) Seed. *Pak. J. Nutr.* 7(2):214-217.
- Fetuga BL, Babatunde GM and Oyenuga VA (1974). Protein quality of some unusual protein foodstuffs: Studies on the African locust beans (*Parkia filiciodea Welv*) Seed. *Brit. J. Nutr.* 32:27-36.
- Harrigan WF, McCance ME (1976). *Laboratory Methods in Food Dairy and Microbiology*, Academic Press, London, New York, San Francisco. P 342.
- Ibeabuchi JC, Olawuni IA, Iheagwara MC, Ojukwu M, Ofoedu CE (2014). Microbiological evaluation of Iru and Ogiri – Isi used as food condiments. *IOSR J. Environ. Sci. Toxicol. Food Technol.* 8:45-50.
- Ikenebomeh MJ (1989). The influence of salt and temperature on the natural fermentation of African locust bean. *Int. J. Food Microbiol.* 8:133-139.
- Kolapo AL, Popoola TOS, Sanni MO (2007). Evaluation of Biochemical Deterioration of Locust Bean Daddawa and Soybean Daddawa-Two Nigerian Condiments. *Am. J. Food Technol.* 2:440-445.
- Langohr HD, Petruch F, Schroth G (1981). Vitamin B 1, B 2 and B 6 deficiency in neurological disorders. *J. Neurol.* 225:95-108.
- McKinley MC, McNulty H, McPartlin J, Strain JJ, Scott JM (2002). Effect of riboflavin supplementation on plasma homocysteine in elderly people with low riboflavin status. *Eur. J. Clin. Nutr.* 56:850-856.
- Ming H, Pizarro AVL, Park EY (2003). Application of waste activated bleaching earth containing rapeseed oil on riboflavin production in the culture of *Ashbya gossypii*. *Biotechnology Progress.* 19:410-417.
- Nicol BM (1959). The protein requirements of Nigerian peasant farmer. *Br. J. Nutr.* 13:307
- Odufa SA (1981). Microorganisms association with fermentation of African Locust Bean during preparation. *J. Plant Foods.* 25: 245-250.
- Odufa SA (1983). Biochemical changes during the production of ogiri, a fermented melon (*Citrullus vulgaris* Schrad) product. *Plant Foods Human Nutr.* 32: 11-18.
- Odufa SA (1985). African Fermented Foods In: *Microbiology of fermented foods*. Ed: BJB Wood. Elsevier Applications. 2:155-191.
- Odufa SA, Oyewole OB (1986). Identification of *Bacillus* species from iru, a fermented African locust bean product. *J. Basic Microbiol.* 26:101-108.
- Ogueke CC, Ariariu LE (2004). Microbial and organoleptic changes associated with ugba stored at ambient temperature. *Niger. Food J.* 22:133-140.
- Omafuvbe BO, Abiose SH, Shonukan OO (2003). Fermentation of soybean (*Glycine max*) for soy-daddawa production by starter cultures of *Bacillus*. *Food Microbiol.* 19:561-566.
- Omafuvbe BO, Falade OS, Osuntogun BA, Adewusi SRA (2004). Chemical and biochemical change in African locust beans (*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds during fermentation to condiments. *Pak. J. Nutr.* 3:140-145.
- Perkins JB, Pero J (2002). Vitamin biosynthesis, p. 271-286. In A. Sonenshein, J. Hoch, and R. Losick (ed.), *Bacillus subtilis* and its closest relatives from genes to cells. ASM Press, Washington, D.C.
- Ratnakar Anjali, Rai Aruna (2013). Influence of NaCl Salinity on β -carotene, Thiamine, Riboflavin and Ascorbic Acid Contents in the Leaves of *Atriplex hortensis* L. var. Pusa. *Oct. J. Environ. Res.* 1(3):211-216.
- Sauer U, Hatzimanikatis V, Hohmann HP (1996). Physiology and metabolic fluxes of wild-type and riboflavin-producing *Bacillus subtilis*. *Appl. Environ. Microbiol.* 62(10):3687-3696.
- Sieuwerts S, de Bok FA, Hugenholtz J, van Hylckama Vlieg JE (2008). Unraveling microbial interactions in food fermentations: from classical to genomics approaches. *Appl. Environ. Microbiol.* 74:4997-5007.
- Stahmann KP, Revuelta JL, Seulberger H (2000). Three biotechnical processes using *Ashbya gossypii*, *Candida famata*, or *Bacillus subtilis* compete with chemical riboflavin production. *Appl. Microbiol. Biotechnol.* 53(5):509-516.
- Suzuki GT, Fleuri LF, Macedo GA (2009). Food Bioprocess Technology, In press: Influence of nitrogen and carbon sources on riboflavin production by wild strain of *Candida* sp.