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Comparative Chemical Composition of Iru Fermented spontaneously and with *Bacillus subtilis* A2 as Starter Culture

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ABSTRACT

The effect of using *Bacillus subtilis* A_2 as starter culture for iru production was examined by comparing the proximate and antinutritional composition with those fermented spontaneously and commercially available iru. The proximate composition result obtained for the spontaneously fermented iru using traditional method, iru fermented with Bacillus subtilis A_2 and commercially produced iru respectively are as follows; protein (26.43, 29.21 and 27.62)%, fibre: (9.73, 8.97 and 9.46)%, ash (2.68, 2.50 and 2.52)%, moisture (10.22, 11.98 and 10.33)%, fat (14.06, 13.98 and 13.82)% and carbohydrate by difference (36.88, 33.36 and 36.25)%.The antinutrient result; oxalate (0.192, 0.184 and 0.196)g/100g, phytic acid (7.69, 6.47 and 6.99)g/100g, phytate (0.183, 0.176 and 0.186)g/100g, total phenol (0.61, 0.86 and 0.56)g/100g and trypsin inhibitor (48.92, 41.73 and 45.33)g/100g. It can therefore be concluded that *Bacillus subtilis* A_2 has the potential to be used as starter culture for Iru fermentation since it hastens the process and it gives it an improved feature.

KEYWORDS: Fermentation, Iru, Locust bean, Antinutrient,Condiment

INTRODUCTION

African locust bean tree (*Parkia biglobosa*) originated from the family of *fabaceae* sub-family *mimosoidea* and genus *parkia*. The tree in West Africa is widely known to be an important multipurpose tree of west savannah land (Adekunle *et al*., 2014). The most essential part of the tree is found in its seeds and the processed is used as condiment for

soup. It is a source of natural nutritious condiment that features frequently in the traditional diet of the people. (Akande *et al*., 2010).

Besides the ability of the processed locust bean "iru" to add flavor to food, It also contribute immensely to the intake of protein, essential fatty acids, particularly vitamin B, riboflavin and vitamin A. (Oguntola, 2007). Locust bean originated from west Africa and it is also called by different local names in different localities; for instance, It is called "kinda" in Serria leone "kpalugu" among the inhabitants of Northern Ghana, "Nere" in Burkina Faso, "igi igba" in Yorubaland and "worku" in Ghana (Diawara *et al.*, 2000). Also in the dry area, locust bean trees function as potential sources of food, edible oil, fodder lumber, firewood and green manure. In Nigeria alone, it was estimated that about 200,000 tonnes of Africa locust beans seeds are gathered each year, with the large quantities being produced in the savannah region of south west, Nigeria. (Diawara *et al*., 2000), (Onnyi *et al*., 2004). The most esteemed product of the tree are seeds. For the farmers and women who are involved in its processing and marketing, it produces reliable and dependable income. National demand for various types of food condiments and seasonings in Nigeria is estimated to be 5,475 tonnes per annum (FIIRO, 2013). Among the popular brands in Nigeria market are "maggi", "royco", "ajinomoto", "iru" or "ogiri" (locust bean based), curry, thyme etc.

It is unfortunate that locust bean is fast losing its popularity to some other flavouring agents whose nutritive quality cannot measure up to its own (FIIRO, 2013). This could be because the product does not last, its odour and poor quality because of the processing practices. Despite the onset of science and technology, locust bean processing has been facing many challenges. Women still majorly do processing in a traditional and crude way; the production due to problems associated with the processing operations has not increased substantially. To determine the chemical composition of iru from African locust bean fermented with *Bacillus subtillis* A_2.

MATERIALS AND METHODS

Source of Materials: The African locust bean seeds *Parkia biglobosa* was purchased from King's market in Owo, Ondo state. It was collected in sterile container/polythene.

Pure culture of *Bacillus subtilis* typed A_2 was obtained from the Department of Microbiology, Ekiti State University, Ado Ekiti.

Preparation of Starter Culture: The starter culture was prepared by inoculating the Bacillus typed A_2 on nutrient agar (NA) plate from the stock and the plate was incubated at 37^0C for 24hrs.

Laboratory Preparation of Iru from African Locust Beans Seed using *Bacillus subtilis A2* : The method described by Omodara and Aderibigbe (2017) was adopted for the processing of the dried seeds of African Locust Beans. The fermented African Locust Beans seeds was subjected to chemical analysis.

Chemical Analysis of Fermented African Locust Beans Seeds (*Parkia biglobosa)*

Proximate Composition: The proximate composition of the fermented samples were determined using standard procedures of AOAC (2000). The parameters determined were protein, ash, crude fibre, fat and carbohydrate (by difference).

Determination of Anti-Nutritional Factors

Determination of Phytic Acid: The method of (Young and Greases 1940) was employed in the determination of phytic acid. 4g of finely grinded sample was soaked in 1litre of 2% HCl inside conical flask for 3hrs and indicator was added together with 50ml of distilled water. This was titrated against ferric chloride solution that contained 0.05mg of iron (Fe) per ml of $FeCl_3$. The iron equivalent was obtained and the phytate content in mg/100mg of dried samples were collected.

Determination of Trypsin Inhibitor: The trypsin inhibitor activity (T1A) in the sample was determined according to the method of (Smith *et al.,* 1980). The digest contained 1.0g of the sample 40*uf* of trypsin and 2mg of N-alpha-benzoyl-DL-Arginine-P-nitroanilide-hydrochloride (BAPA). The absorbance was read at 410*nm.*

Determination of Phytate :4g of grounded sample was weighed and soaked in 100cm^3 of 20% HCl for 3 hours and filtered. 25ml of the filetrate was transferred into 100ml conical flask and 5ml of 0.3ml NH_4SCN was added as indicator. 50ml of distilled water

was added to it for proper acidity. It was then titrated against $FeCl_2$ which contain 0.00195g/ml of Iron in $FeCl_2$ solution. Phytate content was calculated in mg/100g or g/100g.

Determination of Oxalate : 1g of the sample was weighed into 100ml conical flask. 50ml of 1.5N H_2SO_4 was added and stirred intermittently with a magnetic stirrer for 1 hour, it was filtered with whattman filter No1. and 25ml of the filterate was transferred into 100ml conical flask and titrated hot (80-90^{0}C) against 0.1$KMnO_4$ solution until a faint colour appeared which persisted for at least 30 seconds. Oxalate content was calculated in mg/g.

Total Phenol: The amount of total phenolics in extract was determined according to the Folin-Ciocalteu procedure. 2ml of each samples in triplicates were introduced into testubes; 1.0ml of Folin-Ciocalteu's reagent and 0.8ml of sodium carbonate (7. 5%) were added. The tubes were mixed and allowed to stand for 30 minutes. Absorption at 765nm was measured. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram dry material.

RESULT AND DISCUSSION

Table 1: Proximate composition of iru fermented with *Bacillus subtilis* $A_{2.}$

	Parameters					
Samples	Protein (%)	Fibre (%)	Ash (%)	Moisture (%)	Fat (%)	Carbohydrate by difference (%)
A	20.43	9.73	2.68	10.22	14.06	36.88
B	26.21	8.97	2.50	11.98	13.98	33.36
C	27.62	9.46	2.52	10.33	13.82	36.25

A= spontaneously fermented iru from traditional producer.

B= iru fermented with *Bacillus subtilis* $A_{2.}$

C= commercially produced iru.

Table 2: Anti-nutrient composition of iru fermented with *Bacillus subtilis* $A_{2.}$

	Anti-nutrient factors				
Samples	Oxalate	phytic acid	Phytate	total phenol	trypsin inhibitor
A	0.19	7.69	0.18	0.61	48.92
B	0.18	6.47	0.17	0.86	41.73
C	0.19	6.99	0.18	0.56	45.33

A= spontaneously fermented iru from traditional producer.

B= iru fermented with *Bacillus subtilis* $A_{2.}$

C= commercially produced iru.

Tables 1 and 2 showed the proximate composition and anti-nutrient composition of spontaneously fermented iru, iru fermented with *Bacillus subtilis* A_2 and commercially produced iru as samples A, B and C respectively. The moisture content of iru fermented using *Bacillus subtilis* A_2 was higher than the moisture content of the commercially produced iru and lower than the moisture content of spontaneously fermented iru. There was no significant difference in the ash content of the three fermented iru products. There was significant difference in the protein content of the three fermented iru products with that of iru fermented with *Bacillus subtilis* A_2 being the highest.

Decrease in the carbohydrate content of iru fermented with *Bacillus subtilis* A_2 when compared with the other two iru products may be as a result of the ability of the microorganisms to utilize it as their major carbon source during fermentation and this is in agreement with the works of (Amao *et al.,* 2013, Omafuvbe *et al.,* 2004 and Jonathan *et al.,* 2011) who individually reported decrease in the percentage carbohydrate contents of different proteinaceous beans after fermentation to produce different condiments; the reduction in carbohydrate content may as well be due to the hydrolytic effect of microbial amylase converting carbohydrate into sugars. There was a significant difference in the increased crude fibre after fermentation of the African locust beans. There was significant

difference in the protein content of the samples with the commercially produced iru having the highest crude protein (27.62) % followed by one fermented with *Bacillus subtilis* A_2 (26.21) % and then the spontaneously fermented iru from traditional producer (20.43) %. This may be due to the ability of *Bacillus subtilis* A_2 to carry out proteolysis more than the spontaneously produced iru products. This increase in the crude protein of the products is in agreement with the works of Pelig-Ba (2009), Dakwa *et al.,* (2005) and Omafuvbe *et al.,* (2004). The increase in the fat content of the fermented products may be attributed to the increased activities of enzymes, which hydrolyzed fats to glycerol and fatty acids. (Obizoba, 1991).

The level of anti-nutrient composition is shown in table 2. phytic acid, though considered an anti-nutritional factor, is a common storage form of phosphorus in seeds and in few tubers and fruits. The phytic acid content of the condiments ranged from 7.69mg/100g for that of spontaneously fermented iru to 6.47mg/100g and 6.99mg/100g for that of iru fermented with *Bacillus subtilis* A_2 and commercially produced iru respectively. Phytic acid, a hexaphosphate or inositol is an important storage form of phosphorus in plants. It is insoluble and cannot be absorbed in the human intestines. Phytic acid has 12 replaceable hydrogen atoms with which it can form insoluble salts with metals such as calcium, iron, zinc and magnesium. The formation of these insoluble salts renders the metals unavailable for absorption into the body (Akpabio *et al.,* 2012). Aregheore and Agunbiade (1991) reported that, cooking does indeed destroy anti-nutritional factors which are toxic to health and make dietary minerals available for absorption that phytate content is seen to reduce with fermentation and further reduction is expected with cooking. The minimum amount of phytic acid to cause negative effect on iron and zinc absorptions are 10-50mg/ml (Sanberg, 1991). While that of phytate is 0.183, 0.176 and 0.186 for the three products A, B and C respectively. Oxalate content in the three products A, B and C are 0.192, 0.184 and 0.196 respectively. These oxalate contents are present in low amount with no significant difference. Oxalates may be present in plants as soluble salts such as potassium, sodium or ammonium oxalate. Oxalic acid is a weak reducing agent that is readily oxidized to carbon dioxide and water by potassium permanganate in H_2SO_4 solution. It reduces calcium availability both in man and in non-ruminants, at higher dose of 1g to 2g/kg of body weight. Oxalic acid is toxic

to the kidney and heart. Symptoms of mild oxalate poisoning include abdominal pains and gastroenteritis. In severe cases, it can cause diarrhea, vomiting, convulsions, non coagulability of blood, coma and kidney disease (Akpabio *et al.,* 2012). Higher oxalate content contains more than 10mg per serving, while low content has less than 2mg per serving. Hence the oxalate content of the iru products is low. The phenol content of the product A, B and C also is very low 0.61g/100g, 0.86g/100g and 0.56g/100g respectively. Phenol protect plants from oxidative damage and perform the same functions for humans. (Okwu, 2005). The outstanding phytochemical features of phenol is their ability to specifically block enzymes that causes inflammations, they also modify the prostaglandin pathways, thereby protecting platelets from clumping (Okwu and Omodamiro, 2005). Phenolic compounds can enhance the body's immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood vessels (angiogenesis) that is necessary for tumour growth. They also attenuate adhesiveness and invasiveness of cancer cells thereby reducing their metastatic potential (Wahle *et al.,* 2010). The trypsin inhibitor content of the three products A, B and C are 48.92, 41.73 and 45.33 respectively. The trypsin inhibitor in a product reduces the protein efficiency and therefore results in the consumers' body not being

Conclusion

This work has shown that fermentation leads to appreciable reduction in the amount of anti-nutrient factors present in the seed with shorter fermentation period. Also the use of the strain of *Bacillus subtilis* A_2 to ferment the seed to produce iru yielded a high fat and protein content while that of ash and fibre is very low. The use of *Bacillus subtilis* A_2 also gave it improved features. The use of pressure cooker also reduced the cooking period of the seed.

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