Effect of Fermentation Method on Nutritional, Anti-Nutritional Composition and Microbial Properties of Mung Bean (*Vigna radiata*) '*Iru*"

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Abstract: The study investigated the effect of fermentation on the nutritional and anti-nutritional composition of fermented mung bean 'iru' as a protein condiment. The mung bean was processed to mung bean 'iru' using the local method of producing 'iru' from African locust beans. The mung bean was sorted, washed and boiled for 1 hr. The boiled seeds was dehulled to remove the seeds coat, washed and boiled again for 1 hr. The water was drained, spread and seed were spread on a sack bag to cooled and wrapped with enough banana leave (*Musa saplentum*) and packed in a cleaned plastic container, to ferment for 5 days in a warmed place at room temperature. Mung bean 'iru' were dried using an electric oven at 50^{O}C for 18 hrs used for chemical analysis. The fermented samples were cultured on the following medium; plate count agar, potato dextrose agar and nutrient agar to isolate microorganisms. The proximate composition was analysed using standard methods. The microbial load count of bacteria (8.85×10^6 CFU/ml), yeast (1.18×10^6 CFU/ml) and fungi (3×10^4 CFU/ml) respectively. The overall microorganisms isolated and identified from the sample were *Pediococus* sp, *Alcaligen* sp, *Bifidobacterium* sp, *Staphylococcus aureus*, *Lysinibacillus* sp, *Bacillus* sp, *Aspergillus flavus*, *Aspergillus niger*, *Penicillin* sp, *Candida* sp and *Geotrichum* sp.; ash content (2.080 ±0.06 %), crude fibre (2.857 ±0.02 %) were lower than moisture content (7.646 ±0.08 %), crude fat (6.683 ±0.13 %) while protein content (20.349 ±0.07 %) and carbohydrate content (60.385±0.12 %) were higher and energy (1527.566 ±0.15 kj/g) with the highest value. Anti-nutritional factors values were tannin (1.434 ±0.04 mg/g), phytic acid (1.915 ±0.09 mg/g) were lower than oxalate (2.701 ±0.07 mg/g), phytate (6.798 ±0.14 mg/g) and phenol (15.255 ±0.13 %) had highest value. Mung bean 'iru' is a good source of protein and energy.

Keywords: Mungbean, fermentation, microbial, proximate, antinutrient

INTRODUCTION

The mung bean (*Vigna radiata* (L.) R. Wilczek) is a legume cultivated for its edible seeds and sprouts across Asia. There are 3 subgroups of *Vigna radiata*: one is cultivated (*Vigna radiata* subsp. *radiata*), and two are wild (*Vigna radiata* subsp. *sublobata* and *Vigna radiata* subsp. *glabra*). The mung bean plant is an annual, erect or semi-erect, reaching a height of 0.15-1.25 m (FAO, 2012; Lambrides *et al*., 2006; Mogotsi, 2006). It is slightly hairy with a well-developed root system. Wild types tend to be prostrate while cultivated types are more erect (Lambrides *et al*., 2006). Whole cooked mung beans are generally prepared from dried beans by boiling until they are soft. Mung beans are light yellow when their skins are removed. Mung bean paste can be made by dehulling, cooking, and pulverizing the beans to a dry paste (Tomooka, *et al*., 2003).

Even though legumes are such a popular staple food, most of us are oblivious of the tremendous benefits this food class can offer to us. With the increase in awareness to make this class of food a part of our daily diet, several people are still ignorant of what they stand to gain by simply eating legumes. It is very heartbreaking that certain individuals, even in this day and age, still consume only starchy foods (for example, rice, maize, sugar etc) from morning till night, from week to week and from year to year and they still feel comfortable about it (Okpala, 2016). The choice of cowpea by Nigerian women is guided predominantly by the cooking time, swelling capacity, tastes and colour.

Fermentation can produce important nutrients or eliminate anti-nutrients. Food can be preserved by fermentation since fermentation uses up food energy and creates conditions unsuitable for spoilage microorganisms. For instance, in pickling, the acid produced by the dominant organism inhibits the growth of all other microorganisms (Egwim *et al*, 2013). The objectives of this research work are to prepare mung beans 'iru', carry out microbial analysis as well as proximate and antinutrients compositions.

MATERIALS AND METHODS

Sample collections; Mung bean was purchased from *Irepodun* market, Ekinrinadde, Ijumu Local Government Area of Kogi State, Nigeria and immediately processed.

Processing of Mung Beans to Produce 'Iru'

The local method used for the processing of mung beans 'iru' was adopted from the process highlighted by Egwin *et al*. (2013). Two hundred and fifty grams (250 g) of mung beans were sorted, washed and boiled for 1hr. The boiled seed was dehulled to remove the seeds coat, washed and boiled again for 1hr. The water was drained, spread on a sack bag to cooled and wrapped with banana leave (*Musa saplentum*) and packed in a cleaned plastic container, fermented for 5 days in a place at room temperature. The fermented products were isolated for microbial analysis. Mung bean 'iru' were dried using an electric oven at 50^{O}C for 18 hrs and the dried mung bean 'iru' were used for chemical analysis.

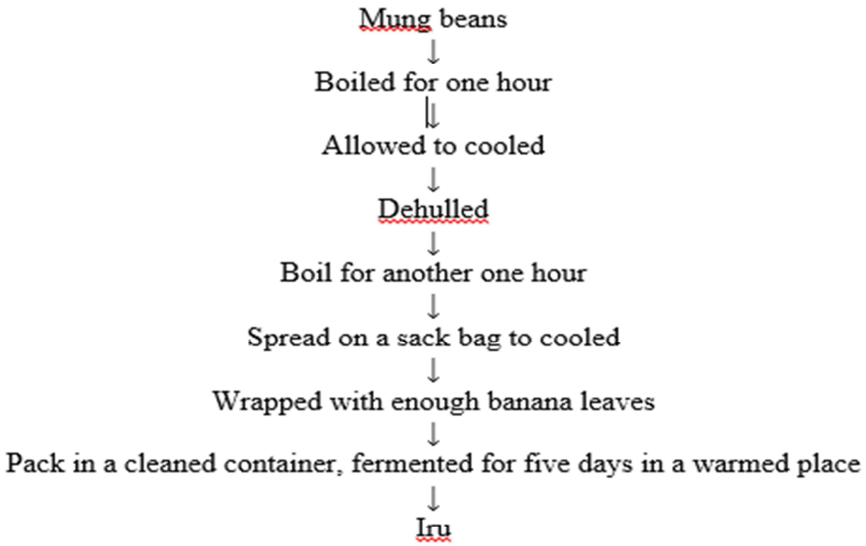

Figure 1: A flow chart for the production processes of Mung beans 'Iru'

Microbiological Analysis

Plate Count agar (PCA), Nutrient agar (NA) and Potato Dextrose agar (PDA) were used for total viable bacteria count, lactic acid bacteria, microbial culturing of bacteria and mould respectively. All media were prepared according to the manufacturer's specification and sterilized at 121^{o}C for 15 mins. The 1 ml from dilutions (10^{-4}) was pipetted from fermented mung bean, prepared by serial dilution, were a plate on sterile molten agar, swirled and allowed to set. NA and PCA plates were incubated aerobically at 35^{O}C for 24 hrs, while PDA was at 30^{o}C for 5 days. After incubation, colonies on each plate were counted and streaked out repeatedly until pure cultures of each were obtained and maintained on appropriate agar slants at 4^{o}C (Fawole and Osho, 2007). The isolates were characterized by cultural, morphological and biochemical tests (Lawal, 2021).

Characterization of Isolates

Bacteria isolates were characterized and identified based on their cultural, morphological, physiological and biochemical properties using Bergey's Manual of Systematic Bacteriology (Holt *et al*., 2000). The fungi isolated were identified according to the methods of Barnett *et al.* (2000).

Proximate Composition Determination of Mung Bean 'Iru'

Mung bean 'iru' were dried and analysed for moisture, crude protein, ash, fat and crude fibre contents according to the standard method of (AOAC, 2016) while the carbohydrate content of the samples was determined by difference as follows: % Carbohydrate = 100 – (% moisture + % protein+ % fat + % Crude fibre + % ash) (Lawal *et al*., 2017).

Antinutrient Analysis Determination of Mung Bean 'Iru'

Phytate and tannin were determined using AOAC (2016) methods. Oxalate content was by the titrimetric method (AOAC, 2016) while phenol was done as described by Anjum *et al.* (2012).

Statistical Analysis

The experiments were conducted in a completely randomized design with three replicates. All data were first subjected to analysis of variance (ANOVA), to determine significant differences at $p<0.05$. Statistical analyses were carried out using IBM SPSS 24.0 (SPSS Science, Chicago, IL, USA).

RESULTS AND DISCUSSION

Table 1 shown the microbial load count of mung bean 'iru' in plate count agar, potato dextrose agar and nutrient agar. The bacterial load was (8.85×10^6 ±0.01 CFU/ml) while yeast and fungi load counts were (1.18×10^6 ±0.01 and 3×10^4 ±0.00 CFU/ml) respectively. The results obtained for bacterial load count (8.85×10^6 ±0.01 CFU/ml) of fermented mung 'iru' was higher compared with the bacteria load count (4.16×10^5 to 2.30×10^5 CFU/ml) of fermented tiger nut while fungi and yeast microbial load count (1.18×10^6 ±0.01 and 3×10^4 ±0.00 SFU/ml) were lower compared with fermented tiger nut load count (4.43×10^5 and 9.33×10^4 CFU/ml) as reported by Agbaje *et al.* (2015).

Table 1. Microbial Load Count of Mung Bean 'Iru'

Fermentation method	Fermentation Time/h	Bacteria (CFU/ml)	Yeast (CFU/ml)	Fungi (SFU/ml)
Traditional fermentation	120 hrs	8.85×10^6 ±0.01	1.18×10^6 0.01	3.00×10^4 ±0.00

Values are expressed as mean ± standard deviation ($p < 0.05$)

The isolation and characterization of microorganisms were isolated from the fermented mung bean 'iru'; *Pediococus* sp, *Alcaligen* sp, *Bifidobacterium* sp, *Staphylococcus aureus*, *Lysinibacillus* sp, *Bacillus* sp, *Aspergillus flavus*, *Aspergillus niger*, *Penicilline* sp, *Candida* sp and *Geotrichum* sp. This is in agreement with the reports of Agbaje *et al.* (2015); Afolabi *et al.* (2016) which showed that the fermentation of tiger nut and locust bean (iru) for the ability to ferment soya bean to produce soy iru. This is in line with the submissions of Achi (2005) who reported that the fermentation of vegetable proteins into condiments is usually mediated by diverse microbial flora.

Organisms involve in the fermentation of soya bean seeds to produce iru (Dawadawa) have been reported to be mostly species of *Bacillus subtilis* (Afolabi *et al*., 2016) and that *Staphylococcus* sp and *Bacillus* sp are often associated with the fermentation of food plant origin. The presence of *Aspergillus niger*, *Aspergillus flavus* and *Penicilline* sp in this research was in line with the study conducted by Chukwu *et al.* (2013) which discovered the above fungi being associated with fresh and dry seeds.

Summarily from Table 2 shows the proximate compositions in terms of ash,

moisture, crude fibre and crude fat content were low while protein and carbohydrate content had higher values. The energy content calculated from the obtained parameters was also found to be higher. The ash content of fermented mung bean 'iru' had a high value (2.080 ±0.00 %) compared with the value obtained for co-fermented millet/cowpea (1.88 %) (Oyarekua, 2011).

Ash content is useful in assessing the quality of seeds (Otori and Mann, 2014). The moisture content (7.646 ±0.01 %) of dried mung bean 'iru' was lower than the (59.3 %) reported for soybean Omodara and Olowomofe (2013). The low moisture content of mung bean 'iru' may be due to the adequate drying of the sample after fermentation. The fat content of the sample (6.683 ±0.01 %) had a lower value compared to the value obtained for fermented African locust bean (29.08 %) (Omodara and Olowomofe, 2013). The decrease in fat content observed could be attributed to the activities of lipolytic enzymes during fermentation (Uvere *et al.*, 2010).

Crude fibre content value (2.857 ±0.00 %) is moderate compared to some other seeds such as *Alternanthera sessilis* (5.32 %), *Daniella oliveri* (8.21 %), fermented soybean (4.54%), fermented African locust bean (6.49 %) and *Olax subscorpoidea* (7.27 %) (Anhwange *et al.*, 2006: Omodara and Olowomofe, 2013: Otori and Mann, 2014). According to the Dietary Guideline for America (2005), the recommended limit for fibre intake is 1.4 % to 3.5 %. Based on this report the sample is a good source of dietary fibre.

Protein content (20.349 ±0.00 %) was lower compared to the values of fermented African locust bean and fermented soybean (37.41 and 45.53 %) (Omodara and Olowomofe, 2013). Carbohydrate content of (60.385±0.12 %) was higher compared with 52.42% reported for fermented mung bean flour by Onwurafor *et al.* (2014). The increase could be attributed to the use of carbohydrates as a source of energy by microorganisms. The energy value of the sample (1527.566 ±0.00 kj/g) is higher than that of *Olax subscorpoidea* and *Daniella oliveri* seeds (379.5 and 344.5 kj/g) (Otori and Mann, 2014).

Table 2. Proximate Composition of Mung Bean 'Iru'

Parameter	Values ± SD
Ash content (%)	2.080 ±0.00
Moisture content (%)	7.646 ±0.01
Crude fat content (%)	6.683 ±0.01
Crude fibre content (%)	2.857 ±0.00
Protein content (%)	20.349 ±0.00
Carbohydrate content (By diff) (%)	60.385 ±0.00
Energy value (kj/g)	1527.566 ±0.00

Values are expressed as mean ± standard deviation ($p < 0.05$)

The antinutritional factors can easily be reduced to tolerable limits by proper processing techniques such as handling, cooking and soaking (Anhwange *et al.*, 2006). Phytate content of (6.798 ±0.00 mg/g) was low compared to that of Otori and Mann (2014) who obtained 23.5 mg/g for *Daniella oliveri* seed.

The oxalate content of 2.701 ±0.00 mg/g was the lower than that of 21.3mg/g recorded for *Olax subscorpoidea* value by Otori and Mann (2014), this value is also lower than (6.95 mg/g) reported for dehulled seeds of African locust bean and 8.51 mg/g for seeds kernel of *Balanites aegyptiaca* (Lawal and Awe, 2020).

The value of tannin content (1.434 ±0.00 mg/g) was slightly higher than that of

fermented mung bean flour (1.13 mg/g) (Onwurafor *et al*., 2014). Ene-obong, (1995) reported that a decrease in tannin content could be achieved through soaking, dehulling, fermentation and germination. The decrease in tannin content in fermented mung beans could be attributed to enzymatic activity. The phenol content of 15.255 ±0.00 % for mung bean 'iru' was higher compared with 0.67% recorded for wheat cultivars (Anjum *et al*., 2012). The phytic acid content (1.915 ±0.00 mg/g) was high compared with the value (1.27 mg/g) reported for wheat cultivars (Anjum *et al*., 2012).

Table 3. Antinutrient Components of mung bean 'iru'

Parameter	Values ± SD
Phytate (mg/g)	6.798 ±0.00
Oxalate (mg/g)	2.701 ±0.00
Tannin (mg/g)	1.434 ±0.00
Phenol (%)	15.255 ±0.00
Phytic acid (mg/g)	1.915 ±0.00

Values are expressed as mean ± standard deviation ($p < 0.05$)

CONCLUSION

This study confirmed that *Bacillus* spp. is present during mung bean fermentation and produces mung bean oil. Mung beans can be used as a substitute for the *Parkia* species traditionally used in the production of 'iru' and, if properly developed, reduce competition between humans and animals for plant protein and It has a strong potential to improve the nutritional status of those improved by rural population.

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