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Effect of *Bacillus subtilis* Strains 3B and BC4333 Starter Cultures on the Quality of Fermented *Parkia biglobosa* Seeds, "iru"

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEY initiated the research, provided the research plan, protocol and starter cultures and vetted the final draft of the manuscript. Author OTR supervised the bench work and vetted the first draft of the manuscript. Author AIE performed the bench work, reported the raw data and prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A comparative study was conducted to determine the effect of field application of the starter cultures *Bacillus subtilis* strains 3B and BC4333 on the quality of fermented *Parkia biglobosa* seeds. The proximate composition, microbial load, and other physicochemical properties of the fermented products were determined. The result showed that the microbial load of the commercial 'iru' sample was higher than that of 'iru' fermented using starter culture. The pH, moisture content, protein, ash, fat and crude fibre of the 'iru' fermented using starter culture was significantly higher than the commercially produced 'iru' and the unfermented substrate. However, carbohydrate and total titratable acidity (TTA) in the commercial 'iru' sample was lower than those of the starter culture- fermented samples. This research proves that the starter culture helps to produce 'iru' of better quality. Hence, these strains (3B and BC4333) can be made available to local women for commercial production of 'iru'.

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1. INTRODUCTION

Fermentation of food has been in practice in many parts of the world since ancient times [1]. The main objectives of fermenting foods are food preservation and food safety [2]. Fermented foods are nourishing foods prepared from perishable, inedible or edible raw materials. Traditional methods are employed in the processing of these food products in developing countries, including Nigeria [3]. Fermented foods are highly appreciated for their sensory attributes and have been contributing to the nutritional diets of people in many regions of the world [4]. It has been a means of survival for man during winter seasons and drought period, in temperate and cooler regions of the world. In some regions, like Asia and African countries they are consumed either as main dishes or as condiments [5].

Parkia biglobosa (African locust bean) seeds are a popular soup condiment in Nigeria and other West African countries. It is called 'iru' among the Yoruba ethnic group. The processing of African locust bean include cooking, dehulling, washing, cooking for the second time and fermentation [6]. The fermentation is brings out the desired nutritional value and other organoleptic properties such as taste, flavour and texture. Previous studies have shown that fermentation improves the digestibility, nutritive value and flavour of the raw seeds [7]. Indigenous microorganisms are involved in the spontaneous fermentation; and the qualities of resulting fermented product do vary from one locality to another [8]. Strains of *Bacillus subtilis* group are the most predominant species and found to be responsible for the fermentation of *Parkia biglobosa* seeds [8].

All the twenty strains of *Bacillus* species isolated from commercial samples of 'iru' samples [9] which were characterized using DNA fingerprinting and RNA sequencing and reported to be closely related to *Bacillus subtilis*. The use of different strains of starter cultures resulted in varied qualities of 'iru'. A few strains were capable of producing 'iru' which were rated high, have been reported by Aderibigbe et al. [10]; thus it is necessary to develop these strains into starter cultures for use by the local commercial producers of 'iru'. The main objective of this study is to confirm the effect of the field application of some of these strains of *Bacillus* species as starter culture.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Pure cultures of *Bacillus subtilis* strains were obtained from the stock cultures [11] kept in the Laboratory of Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria. The commercial 'iru' samples and the African locust bean (*Parkia biglobosa*) seeds were purchased from Oja-Oba Market, Ado-Ekiti, Ekiti State.

2.2 Production of 'iru'

The method described by Ikenebomeh and Kok [12], on the production of 'iru' from *Parkia biglobosa* seeds was modified (Fig. 1). Five hundred grams (500 g) of dried African locust bean seeds were soaked in 4litres of water for 15 minutes and boiled using pressure pot for two hours (2 h). The cooked seeds were dehulled and washed thoroughly to remove the testa. The cotyledons were boiled for the second time under pressure for 45 mins then spread into calabash trays in which the *Bacillus subtilis* strains were inoculated into it and covered with sacks and fermented at 35°C for 36 h.

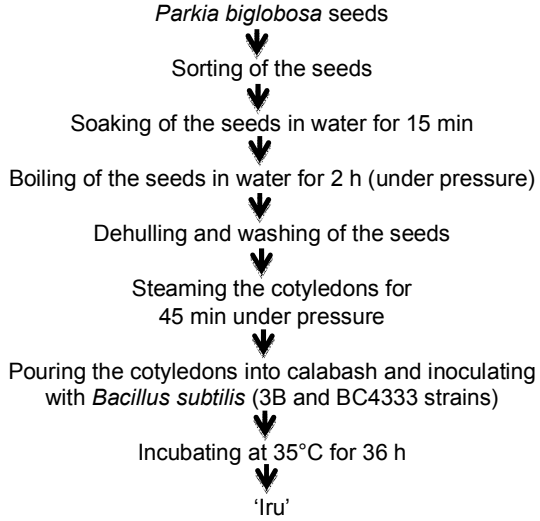

Fig. 1. Flow-chart for laboratory production of 'iru" from African locust bean (*Parkia biglobosa*) seeds

2.3 Determination of Microbial Load of the Samples

The microbial load of the samples was determined by the method of Fawole and Oso

[13]. One gram (1 g) of each sample was diluted serially in 9 ml sterile water to 10^{-6} dilution factor. An aliquot (0.1 ml) from the 10^{-6} dilution tube was spread on sterile nutrient agar plates. The plates were incubated at 35°C for 24 h. The colonies were counted and the microbial load was calculated and expressed as colony forming per gram (CFU/g). The representative colonies were subcultured onto nutrient agar plates by streaking to obtain pure cultures. The pure isolates were grown at 35°C and stored at 4°C on agar slants in McCartney bottles.

2.4 Physicochemical Analysis

The following parameters were determined: pH, titratable acidity (TTA) and moisture content.

2.5 Determination of pH

The pH of then sample was determined accordingly to the method of AOAC [14]. Five grams (5 g) of each sample was weighed into a sterile mortar and mashed with clean pestle and 50ml of distilled water was added. It was mixed thoroughly to form slurry. A standard buffer solution (pH 6.0) was prepared and this was used to standardize the pH meter (Checker, produced by Hanna instruments, Model no-16607). The electrode of the digital pH meter was dipped in the slurry. The pH readings were recorded in triplicates.

2.6 Determination of Total Titratable Acidity

The amount of titratable acidity (lactic acid) in the fermenting mass was determined as described by Pearson [15]. Twenty (20 ml) filtrate obtained from the slurry during pH determination was titrated against 0.1 M NaOH, using phenolphthalein as indicator. The titre value was then used to calculate the titratable acidity as percentage lactic acid using the formula:

$$M_1V_1 = M_2V_2.$$

Determination of moisture content: Five grams (5 g) of the samples were weighed and transferred to the oven set at 105°C and left for 3 h, then were cooled in the desiccator and reweighed.

Total mass of the moisture = (Mass of the sample before drying + Dish) – (Mass of the sample after drying + Dish)

$$\text{Moisture content \%} = \frac{\text{Total mass of moisture x 100}}{\text{Mass of the sample}}$$

Proximate analysis: The proximate composition of the fermented and unfermented samples was determined by the methods of AOAC [14]. The following parameters were determined: protein, crude fibre, ash and carbohydrate. All determinations were in triplicates.

3. RESULTS AND DISCUSSION

The bacterial count of the commercially produced 'iru' was significantly higher than the bacterial count of the starter culture-fermented products. The pH of the starter-culture fermented samples was higher than the pH of naturally fermented products. The total titratable acidity (TTA) of the starter culture-fermented products were comparable to unfermented cotyledons. However, the unfermented substrate had a titratable acidity value of 0.29 N. The moisture content of 'iru' produced using *Bacillus subtilis* strains BC4333, and 3B were higher than the moisture content of the naturally produced 'iru', while the unfermented sample had the lowest moisture content.

The protein and contents of 'iru' produced using *Bacillus subtilis* strains 3B (28.21% and 14.24%) and BC4333 (33.00% and 16 13%), respectively.

Studies on the effect of field application of starter cultures on the quality of fermented *Parkia biglobosa* revealed that microbial load was higher in the commercial 'iru' sample. The higher microbial load could be due to the presence of other microorganisms (contaminants) which were not responsible for the fermentation process; which agrees with the findings of Oladunmoye [16]. Starter culture fermented samples were effectively fermented within 36 h compared to indigenous fermentation process which required 72 h. There was significant reduction in the period of fermentation when starter culture was introduced. The increase in pH of the fermented *Parkia biglobosa* might be due to breakdown of protein and the release of ammonia through the process of deamination [5,17]. There was corresponding decrease in total titratable acidity (TTA) where there was increase in pH. The increase in the moisture content of the fermented products may be due to hydrolysis of macro-molecules in the beans by the extracellular enzymes produced by the fermenting organisms during fermentation. Similar observations were reported by Achinewu and Isichei [18].

Table 1. Microbial load and physiochemical properties of fermented and unfermented *Parkia biglobosa* seeds

Sample	Microbial load (log CFU/g)	pH	TTA(N)	Moisture content (%)
USF	6.84^{c}±0.06	6.80^{c}±0.10	$2.9^{a} \times 10^{-1}$	53.00^{c}±2.00
F3B	7.03^{b}±0.05	8.40^{ab}±0.00	$2.2^{b} \times 10^{-1}$	59.67^{b}±1.53
FBC4333	7.10^{ab}±0.50	8.53^{a}±0.15	$2.0^{c} \times 10^{-1}$	63.33^{a}±1.16
CPI	7.18^{a}±0.03	8.23^{b}±0.06	$2.1^{b} \times 10^{-1}$	55.33^{c}±1.53

Key: USF: unfermented sample, F3B: 'iru' fermented with Bacillus subtilis strain 3B, FBC4333: 'iru' fermented with Bacillus subtilis strain BC4333, CPI: commercially produced 'iru'

Table 2. Proximate composition of fermented and unfermented *Parkia biglobosa* seeds

Sample	Protein (%)	Crude fibre (%)	Ash (%)	Fat (%)	Carbohydrate (%)
USF	26.50^{c}±0.03	9.79^{a}±0.06	2.75^{a}±0.02	14.00^{c}±0.05	43.51^{a}±0.06
F3B	28.21^{b}±0.10	9.09^{d}±0.01	2.52^{c}±0.01	14.24^{b}±0.08	42.93^{a}±0.04
FBC4333	33.00^{a}±0.03	9.35^{c}±0.05	2.41^{d}±0.01	16.13^{a}±0.08	33.75^{c}±0.04
CPI	27.10^{bc}±0.14	9.50^{b}±0.08	2.66^{b}±0.03	14.04^{c}±0.06	41.29^{b}±0.07

Key: USF: unfermented sample, F3B: 'iru' fermented with Bacillus subtilis strain 3B, FBC4333: 'iru' fermented with Bacillus subtilis strain BC4333, CPI: commercially produced 'iru'

The higher percentage of protein in the starter culture fermented products of *Parkia biglobosa* might also be due to deamination of proteins and its complexes to release free amino acids. Similar observation was reported by Osman [19]. The increase in fat content of the fermented products may be attributed to the increased activities of enzymes, which hydrolyzed fats to glycerol and fatty acids. Microorganisms will readily utilize simple sugars for growth, before amino acids, fatty acids and glycerol; thus the presence of fatty acids in the fermented products [20].

4. CONCLUSION

This research has confirmed that fermentation improves the nutritional quality of *Parkia biglobosa,* The use of starter cultures during fermentation of *Parkia biglobosa* produced 'iru' of comparable nutritional quality within a shorter fermentation period. Hence, the *Bacillus* strains may be recommended for use as starter cultures in the commercial production of 'iru'.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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