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Archives of Applied Science Research, 2014, 6 (1):115-120
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Physicochemical properties of powdered condiments of *P. biglobosa* fermented with and without mixed *Bacillus* species A and B as inocula and subjected under different drying conditions

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ABSTRACT

Two kilograms of P.biglobosa seeds were purchased from Sabongari market Zaria, Kaduna state of Nigeria. These seeds were transported to the laboratory, Department of Microbiology, Ahmadu Bello University Zaria. P.biglobosa seeds were precleaned and processed for fermentation. Processed unfermented seeds of P.biglobosa (300g) were transferred in three earth pots lined with aluminum foil. Mixed Bacillus species (5%) (B.subtilis and B.pumilus) both standard and test strains were prepared as inocula and was calibrated using McFarland standard 7. Pot A was inoculated with standard strains of B.subtilis and B.pumilus (Bacillus spp A) while pot B was inoculated with test strains of B.subtilis and B.pumilus (Bacillus spp B), pot C was allowed to ferment without Bacillus species. Fermentation in all the earth pots was allowed to progress at room temperature (28±2 ^{0}C). It was observed that fermentation in earth pots with Bacillus species A and B fermented faster (48hrs) as compared to natural fermentation (72hrs). Freshly fermented seeds were subjected to different drying conditions (solar drying, hot air oven drying, vacuum drying, direct sunlight drying protected with a net and direct sunlight drying without net). Fermented dried seeds of P.biglobosa were converted into powdered form using a sterile blender. Physicochemical analyses were carried out on powdered form under different conditions of drying. It was observed that P.biglobosa powdered condiment subjected to hot air oven drying gave lowest values of moisture content, titratable acidity, peroxide values as oppose to higher values in other drying conditions. Therefore, P.biglobosa powdered condiment dried using hot air oven can be preserved for use over a longer period of time.

Keywords: *P.biglobosa*, fermentation, inocula, *Bacillus* spp earth pots, aluminum foil, solar dryer, vacuum dryer, hot air oven.

INTRODUCTION

Locust bean seeds (*Parkia biglobosa*), when fermented are very good source of dietary proteins. Traditionally fermented condiments (“*daddawa”* and *“okpehe*”) are based on vegetable proteins, and are consumed by different ethnic groups in Nigeria [1]. They are commonly used in fermented forms as condiments to enhance flavours and taste of foods [2] . Bacteria of the genus *Bacillus* are commonly involved in the fermentation of legume seeds. [3] Dakwa *et al.*, (2005) and [4] Gberikon *et al.*, (2009) reported the involvement of the genus *Bacillus* in the fermentation of locust bean seeds and other legumes like soya bean, African mesquite and castor oil seeds.[5] Omafuvebe *et al.*, (2003) also reported that the organisms involved in fermentation of locust bean seeds have been identified as *Bacillus* species with species of *Bacillus subtilis* being the predominant microorganisms. There is need

to apply modern biotechnological techniques such as starter cultures in improving traditional food processing technologies [6]

For preservation purposes, drying of freshly fermented *P.biglobosa* seeds is necessary to extend shelf life during storage [7] (FAO, 2013). Traditionally, direct sun drying has been the basic drying method used. This is practiced in the rural setting and is characterized by direct solar radiation and natural air circulation on the products [7] (FAO, 2013). Apart from sun drying, improved approaches can also be developed such as the use of solar dryer, hot air oven and vacuum drying. These drying methods can reduce microbial contamination and to a larger extent, markedly extend shelf life stability of condiments.

MATERIALS AND METHODS

Two kilograms of *P.biglobosa* seeds were purchased from Sabon gari market Zaria, Kaduna state of Nigeria. These seeds were packaged into cleaned polythene bags and transported to the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria.

Revalidation and characterization of *Bacillus* species

Preliminary characterization of isolates: Test strains of *Bacillus* species ; *B. subtilis* (TS001) *and B. pumilus* (TS002) obtained from the Department of Microbiology Ahmadu Bello University Zaria were compared by re-culturing in nutrient agar broth. The strains were incubated at 37^0C for 24hours. Compared cells were sub-cultured on aerobic plates of nutrient and plate count agars and were incubated at 37^0C for 24hours. This was carried out along side with standard strains of *B.subtilis* (SX1BS) and *B.pumilus* (SX1BP) obtained from Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, which was used as control.

Representative colonies of microorganisms which developed on the aerobic plates of both nutrient and plate count agar were subjected to initial staining and microscopic examinations.

The isolates were subjected to the following biochemical tests using standard methods as described by [8]Gordon *et al*, (1973).

Preparation of inoculum (*Bacillus* species)

The inoculum used for each fermentation contained 2.7×10^7 cells/ml; the cell population was calibrated using McFarland standards (No 7) which was prepared by adding 0.7ml of 1% anhydrous barium chloride ($BaCl_2$) to 9.3ml of 1% sulphuric acid (H_2SO_4) [9]

The inoculum used formed 5.0% of fermenting materials and consisted (15ml of 24hr old cultures of organism into 300g of unfermented seeds) *Bacillus* spp A (standard strains mixture of *B.subtilis* and *B.pumilus* combined) and *Bacillus* spp B (Test strains mixture of *B.subtilis* and *B.pumilus* combined).

Cleaning and preparation of locust bean (*P.biglobosa*) seeds for fermentation

The seeds obtained from the market were pre-cleaned by sorting out stones and debris. This was followed by washing and boiling in water for12 hours, renewing the water intermittently until the seeds became soft [10] (Ogbadu, 1988). The soft seeds were de-hulled by pounding lightly in a wooden mortar using a wooden pestle. The seed coats were removed, and the cotyledons boiled again for two hours. The re-boiled cotyledons were allowed to cool to 35^0C in an earthen pot lined with sterile aluminum foil.

Controlled fermentation of *P.biglobosa* seeds using mixed *Bacillus* species A and B as inocula

The fermentation process was set up using *Bacillus* spp A and B separately. The organisms were inoculated into 300g of unfermented seeds of *P.biglobosa* and were wrapped with sterile aluminum foil and placed in an earthen pot with cover. Fermentation was allowed to progress at room temperature (28 ± 2^0C) in the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria.

Microbiological monitoring of fermentation.

Microbiological analysis was carried out at intervals of 12hrs to monitor growth of inoculum from the start to the end of the fermentation process.

During fermentation, a sample of ten grams was taken aseptically at intervals of 12 hours and was transferred into 90ml sterile peptone water. The suspension was shaken vigorously to dislodge microorganisms, thus forming the stock concentration. A tenfold serial dilution was prepared to obtain dilutions up to ten folds. Aliquots of 0.1ml of 10^{-5} and 10^{-6} dilutions were plated in duplicates on nutrient agar plates (Oxiod), plate count agar (Oxiod); for isolation and determination of count of bacteria. Potato dextrose agar containing chloramphenicol (0.5mg/ml) to suppress growth of bacteria was used for isolation of fungi. The plating was done using a hockey glass stick spreader. The nutrient and plate count agar plates were incubated at 37^0C for 24 hours. Potato dextrose agar plates were incubated at room temperature (27 ± 2^0C) for one week.

Drying of freshly fermented seeds of *P.biglobosa* using different methods

- **Hot air oven - drying**

Fifty grams of freshly fermented seeds of *P.biglobosa* containing both inocula, and a control that fermented naturally, were weighed into Petri dishes cleaned with ethanol. The Petri dishes containing the fermented samples were placed in a hot air oven at a temperature of 45^0C for a period of one week. The samples were re-weighed again and again until a constant weight was obtained.

- **Direct sun - drying**

Fifty grams of freshly fermented seeds of *P.biglobosa* containing both inocula, and a control that fermented naturally, were weighed into Petri dishes cleaned with ethanol. The dishes containing the samples were exposed to direct sunlight on a pouch in the Department of Microbiology, Ahmadu Bello University, Zaria for a period of two weeks to ensure total drying. Atmospheric temperature of the environment was also taken by placing a thermometer in a beaker containing distilled water. The products were reweighed until a constant weight was obtained.

- **Drying using a vacuum pump**

Fifty grams of freshly fermented seeds of *P.biglobosa* containing both inocula, and a control that fermented naturally, were weighed into Petri dishes cleaned with ethanol. The samples contained in the Petri dishes were placed in desiccators with a vacuum pump machine connected to it, and were dried for a period of one week. The samples were reweighed until a constant weight was obtained.

- **Drying using a solar dryer**

Fifty grams of freshly fermented seeds of *P.biglobosa* containing both inocula, and a control that fermented naturally, were weighed into Petri dishes cleaned with ethanol. The samples were placed in a solar dryer box, and a thermometer was also placed in the box to monitor temperature changes. Another thermometer was placed in a 100ml beaker containing distilled water outside the box to monitor atmospheric temperature. The fermented fresh samples dried within a period of five days, and they were repeatedly weighed until a constant weight was obtained.

- **`Sun drying of seeds protected with net**

Fifty grams of freshly fermented seeds of *P.biglobosa* containing both inocula, and a control that fermented naturally, were weighed into Petri dishes cleaned with ethanol. The fermented samples were exposed to direct sunlight but were protected with a soft net with little meshes. Atmospheric temperature was also taken by placing a thermometer in a 100ml beaker containing distilled water around the drying environment. Drying was done in a period of two weeks and repeated weighing was carried out, until a constant weight was obtained

Blending and packaging of dried fermented seeds of *P.biglobosa* using different drying conditions into powdered form

Dried fermented seeds of *P.biglobosa* were blended into powdered form using a sterile blender. Ten grams of powdered condiment was packaged into small plastic containers with seals sterilized with 70% ethanol. The packaged condiments were stored at refrigeration temperature (9 ± 2^0C). Fermented samples purchased from Sabongari were also analysed with laboratory samples.

Determination of physicochemical properties of *P.biglobosa* seeds after drying

pH

A Pye Unicam pH meter, model 291 equipped with a glass electrode was first calibrated using standard buffers of pH 4.0 and 9.2. Readings were also taken at intervals of 12 hours. This was done by mixing one gram of powdered condiment of fermented *P.biglobosa* into 10ml of sterile distilled water. The pH of the suspension was then determined.

Moisture content, peroxide value and titratable acidity were analyzed adopting the methods of [11] AOAC, (2007).

RESULTS

Table 1: Physicochemical properties of powdered condiments of *P.biglobosa* fermented with and without mixed *Bacillus* species A and B as inocula subjected under different drying conditions

Drying conditions	Powdered condiment of *P.biglobosa*	Means of fermentation	pH	Moisture content (%)	Peroxide value(meqkg)	Titratable acidity(mg lactic acid g)
Solar	*P.biglobosa*	*Bacillus* spp A	4.21±0.01	0.19±0.01	4.25±0.01	1.11±0.01
	P.biglobosa	*Bacillus* spp B	5.20±0.02	0.22±0.00	4.28±0.00	1.12±0.02
	P.biglobosa	NF	4.24±0.00	0.21±0.01	4.30±0.03	1.14±0.00
Oven	*P.biglobosa*	*Bacillus* spp A	4.21±0.01	0.10±0.00	3.10±0.01	1.01±0.00
	P.biglobosa	*Bacillus* spp B	5.25±0.00	0.10±0.00	2.10±0.04	1.02±0.00
	P.biglobosa	NF	5.20±0.00	0.11±0.00	3.10±0.00	1.10±0.01
Vacuum	*P.biglobosa*	*Bacillus* spp A	5.19±0.00	0.22±0.02	4.31±0.00	1.11±0.00
	P.biglobosa	*Bacillus* spp B	4.20±0.01	0.27±0.00	4.30±0.01	1.15±0.00
	P.biglobosa	NF	5.20±0.02	0.25±0.01	4.20±0.05	1.12±0.00
Sun(N)	*P.biglobosa*	*Bacillus* spp A	5.17±0.05	0.25±0.00	4.30±0.02	1.10±0.00
	P.biglobosa	*Bacillus* spp B	5.25±0.05	0.22±0.01	4.29±0.01	1.14±0.11
	P.biglobosa	NF	4.25±0.01	0.25±0.02	4.26±1.01	1.12±0.00
Sun(O)	*P.biglobosa*	*Bacillus* spp A	5.21±0.05	0.21±0.05	4.33±0.01	1.11±0.00
	P.biglobosa	*Bacillus* spp B	6.21±0.00	0.29±0.02	4.30±0.00	1.10±0.01
	P.biglobosa	NF	5.25±0.00	0.27±0.00	4.28±1.02	1.11±0.00
Market samples	*P.biglobosa*		5.25±0.00	1.28±0.02	6.20±0.04	1.22±0.02

Values are means of triplicate determinations

Bacillus spp (A)- P.biglobosa seeds fermented with standard strains of mixed B.subtilis and B.pumilus; Bacillus spp (B)- P.biglobosa seeds fermented with test strains of mixed B.subtilis and B.pumilus; NF- naturally fermented; Sundrying (O)- sundrying without net; Sun drying (N)- sundrying with net.

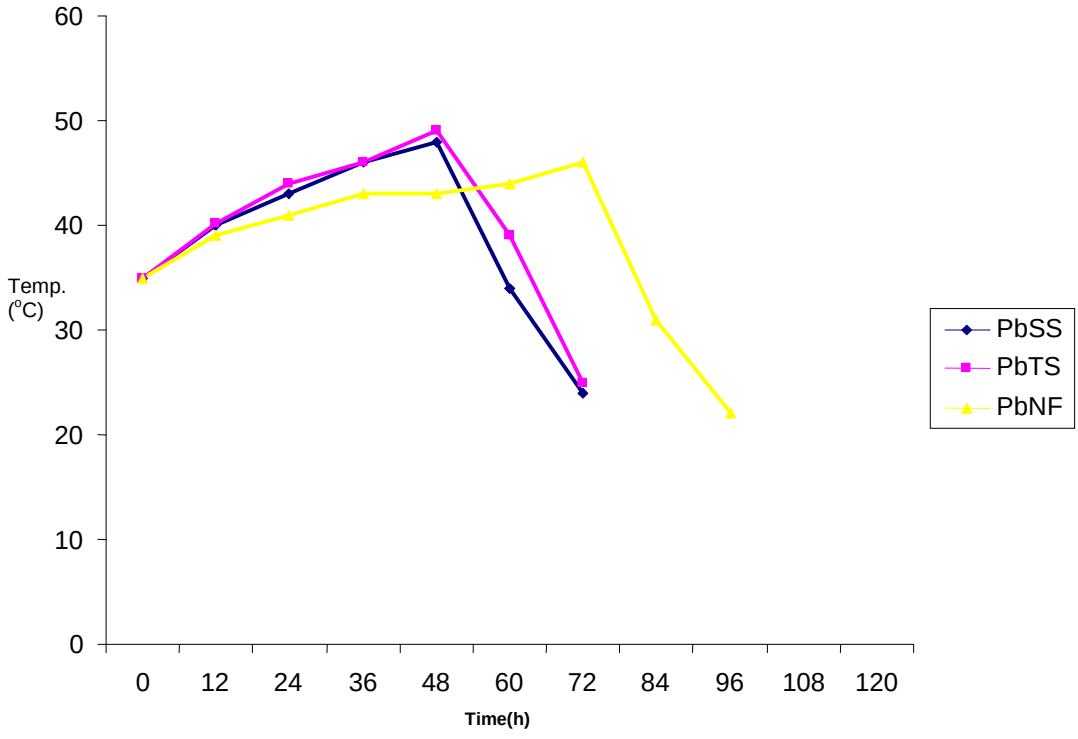

Figure 1: Temperature changes during natural and inoculated fermentations of *P.biglobosa* seeds

PbSS- P.biglobosa seeds fermented with mixed culture of standard strains of B.subtilis and B.pumilus (A); PbTS- P.biglobosa seeds fermented with mixture culture of test strain of B.subtilis and B.pumilus (B); PbNF- P.biglobosa seeds undergoing natural fermentation

DISCUSSION

Species of *Bacillus*, namely *Bacillus subtilis, Bacillus circulans, Bacillus licheniformis and Bacillus pumilus* are implicated in fermentation of locust bean seeds [12] (Ouoba *et al*., 2003). [13] Odunfa, (1981) reported that a single species of *Bacillus* species, *Bacillus subtilis* can initiate and end fermentation of locust bean seeds. Experience has shown that mixed species of *Bacillus* enhances fermentation activities more than single species.

Utilization of inocula (*Bacillus species* A and B)
There are immense benefits in using inocula (combined *Bacillus* species) during fermentation of *P.biglobosa*. It helps speed up fermentation activities as shown in figure 1. It was observed in this study that fermenting mashes inoculated with *Bacillus* spp A and B fermented faster, 48hrs. Fermentation mash that was allowed to ferment without *Bacillus* spp fermented within 72hrs (Figure1). This is because starter cultures optimize production processes and they speed up fermentation by their abilities to break down protein to amino acids faster than seeds that fermented naturally.

Effect of drying methods on physiochemical properties of powdered condiments of *P.biglobosa* fermented with *Bacillus* spp A and B and under natural conditions.
Different drying methods were employed (solar drying, sun drying with net protection, direct sun drying without net, vacuum drying and oven drying) to dry fermented seed condiments of *P.biglobosa*. Powdered condiments from seeds dried in hot air oven at a temperature of 45^0C for seven days had lowest moisture, peroxide and titratable acidity values as compared to fermented seeds dried under solar drying, vacuum drying, sun drying with and without net (Table 1). Fermented *P.biglobosa* seeds dried using hot air oven gave products with optimum physicochemical properties. This is because the hot air released by the oven dried the environment and did not leave room for residual moisture which can be absorbed back into the product.

Peroxide and titratable acidity values which are indices for deterioration were not significantly high in oven dried condiments as opposed to others. High peroxidation value is a good indicator for fat deterioration [14] (Kolapo *et al*., 2007), and can be used as an indicator for condiments spoilage especially at values 20-40 meq kg. Therefore powdered condiments from fermented seeds of *P.biglobosa* dried using hot air oven can be preserved longer than powdered condiments from other drying conditions.

CONCLUSION

It has been concluded from the analyses of this research that at 5% inoculum *P.biglobosa* seeds fermented faster (48h) as oppose to (72h) fermentation without inoculum. Hot air oven out of all the different drying methods used (solar dryer, vacuum dryer, sun drying without net and sun drying with net) used in drying freshly fermented *P.biglobosa* seeds fermented with 5% *Bacillus* species inocula A and B gave powdered condiments with lower physicochemical values of moisture, titratable acidity and peroxide values as compared to higher values obtained from other drying methods. Therefore hot air oven produced powdered condiment of *P.biglobosa* with optimum physicochemical properties that can have longer shelf life stability during storage.

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