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Effect of drying conditions on physicochemical parameters of powdered *Prosopis africana* condiment fermented with or without consortia

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Seeds of African mesquite (*Prosopis africana*) were purchased from Otukpo market in Benue state of Nigeria. *P. africana* seeds were precleaned and processed for fermentation. Processed unfermented seeds of *P. africana* (300 g) were transferred in three earth pots lined with aluminum foil. Mixed *Bacillus* species (5%) (*Bacillus subtilis* and *Bacillus pumilus*) of both standard and test strains were prepared as consortia and were calibrated using McFarland standard 7. Pot A was inoculated with standard strains of *B. subtilis* and *B. pumilus* (consortium A), while pot B was inoculated with test strains of *B. subtilis* and *B. pumilus* (consortium B), pot C was allowed to ferment without consortium. Fermentation in all the earth pots was allowed to progress at room temperature (25°C). It was observed that fermentation in earth pots with consortia fermented was faster (84 h) as compared to natural fermentation (96 h). Freshly fermented seeds were subjected to different drying conditions (solar drying, oven drying, vacuum drying, direct sunlight drying protected with a net and direct sunlight drying without net). Fermented dried seeds of *P. africana* 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. africana* powdered condiment subjected to hot air oven drying differed significantly from other drying methods and gave lowest values of moisture and peroxide contents as opposed to higher values in other drying conditions. Therefore, condiment of *P. africana* dried using oven will have extended shelf life stability during storage than *P. africana* condiment dried using other methods of drying.

Key words: *Prosopis africana*, fermentation, consortia, *Bacillus* earth pots, aluminum foil, solar dryer, vacuum dryer, hot air oven.

Introduction

In Nigeria, legume seeds such as African mesquite (*Prosopis africana)* are very good source of dietary proteins. Fermented condiment of *P. africana* is used as seasoning, in Africa and other parts of the world

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(Oniofiok et al., 1996). Fermentation of *P. africana* is carried out by the genus *Bacillus,* and the predominating species is *Bacillus subtilis* (Oguntoyinbo, 2010). Ouoba et al. (2003) and Dakwa et al. (2005) opined that, the genus *Bacillus* in the fermentation of locust bean seeds and other legumes like soybean, African mesquite and castor oil seeds has been established. Organism consortia if developed and used as starter cultures, will reduce fermentation time and inconsistencies that arises during fermentation of *P. africana*, thereby guaranteeing product quality that will be appreciated as a result of improved fermentation processes (Holzapfel, 2002). Fermentation improves digestibility, nutritive value and flavour of the raw seeds (Ogunshe et al., 2006).

Removal of moisture content from freshly fermented legume seeds of *P. africana* must be carried out to extend shelf life stability of products (FAO, 2013). Direct sun drying method is usually practiced in villages because of lack of availability of modern drying facilities. The use of solar dryer, hot air oven and vacuum drying have been developed to dry and produce products devoid of contaminants as opposed to direct sun drying methods. Therefore, the use of modern drying techniques for freshly fermented seeds of *P. africana* is recommended for obtaining dried and hygienic products.

MATERIALS AND METHODS

Seeds of African mesquite (2 kg) were purchased from Otukpo market in Benue 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* isolates (consortia)

Preliminary characterization of isolates

Test strains of *Bacillus* species: *B. subtilis* (TS001) *and Bacillus 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°C for 24 h. Compared cells were sub-cultured on aerobic plates of nutrient and plate count agars and were incubated at 37°C for 24 h. 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 (catalase, coagulase, motility, indole, hydrogen sulphide production, growths at different sodium concentrations and sugar utilization) using standard methods as described by Gordon et al. (1973).

Preparation of *Bacillus* inoculum

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 0.7 ml of 1% anhydrous barium chloride ($BaCl_2$) to 9.3 ml of 1% sulphuric acid (H_2SO_4) (Todder, 2009). The inoculum used formed 5.0% of fermenting materials and consisted of (15 ml of 24 h old cultures of organism into 300 g of unfermented seeds) consortium A (standard strain mixture of *B. subtilis* and *B. pumilus* combined) and consortium B (test strain mixture of *B. subtilis* and *B. pumilus* combined).

Preparation of *P. africana* seeds for fermentation

P. africana seeds obtained from the market were pre-cleaned by sorting out stones and debris. This was followed by washing and boiling in water for 24 h, renewing the water intermittently until the seeds became soft. The soft seeds were dehulled by removing seed coats with finger tips (Ogbadu, 1988). The cotyledons were reboiled for four hours and were allowed to cool to 35°C in an earthen pot lined with sterile aluminum foil.

Controlled fermentation of *P. africana* seeds with and without consortia

The fermentation process was set up using both consortium A and B, separately. The organisms were inoculated into 300 g of the unfermented seeds of *P. africana* and were wrapped with sterile aluminum foil and placed in an earthen pot with cover. Thermometers were inserted in each of the earth pot to monitor fermentation temperature. Initial temperature of earth pots with unfermented seeds was 35°C. Another fermentation process of *P. africana* was set up to ferment without consortia. Both fermentation processes of *P. africana* were allowed to progress at room temperature (24°C) in the laboratory of Department of Microbiology, Ahmadu Bello University, Zaria.

Microbiological monitoring of fermentation

Microbiological analysis was carried out at intervals of 12 h to monitor growth of starter cultures from the start to the end of the fermentation process. During the 120 h of fermentation, samples of ten grams of *P. africana* with consortia were taken aseptically at intervals of 12 h and were transferred into 90 ml sterile peptone water. The suspension was shaken vigorously for one minute to dislodge microorganisms, thus forming the stock concentration. Serial dilution was prepared to obtain dilutions up to ten folds. Aliquots of 0.1 ml 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 (0.5 mg/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°C for 24 h. Potato dextrose agar plates were incubated at room temperature (24°C) for one week.

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

Fifty grams of freshly fermented seeds of *P. africana* containing consortia, and a control without consortia, were weighed into Petri dishes cleaned with ethanol. Petri dishes containing fermented samples of *P. africana* were subjected to drying conditions using the following methods; oven drying, vacuum drying, direct sun drying, drying using a solar dryer and sun drying protected with a net.

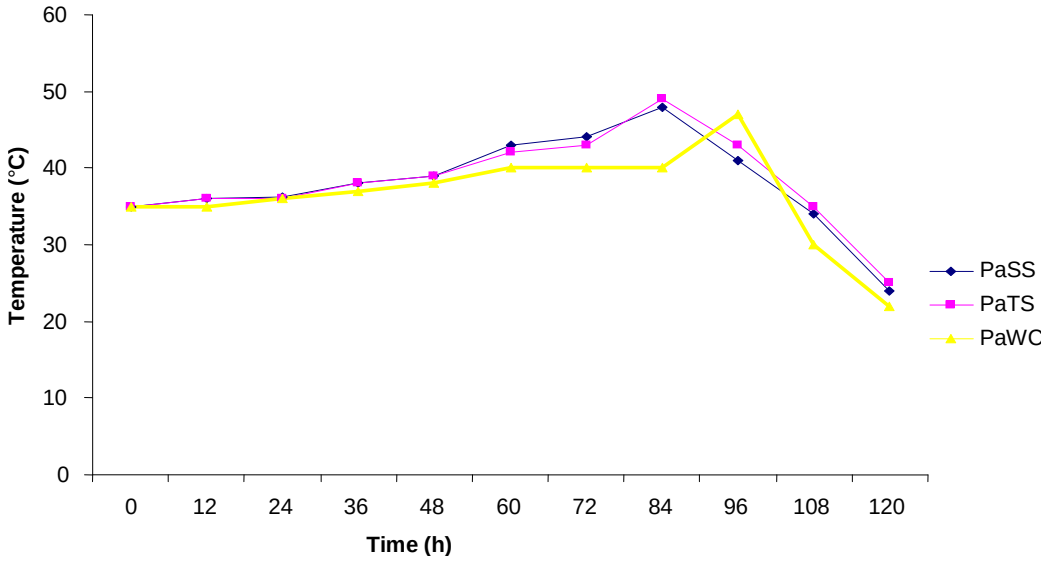

Figure 1. Temperature changes during fermentation of *P. africana* seeds with and without consortia. PaSS- *P. africana* seeds fermented with mixed culture of standard strain of *B. subtilis* and *B. pumilus* (consortium A); PaTS- *P. africana* seeds fermented with mixed culture of test strain of *B. subtilis* and *B. pumilus* (consortium B); PaWC- *P. africana* seeds fermented without consortia.

Powdering, blending and packaging of dried fermented seeds of *P. africana*

Dried fermented seeds of *P. africana*, were blended into powder using a sterile blender. Ten gram of each type of powder was packaged into small plastic containers with seals sterilized with 70% ethanol. The packaged condiments were stored at refrigeration temperature (9±2°C).

Determination of physicochemical parameters of *P. africana* powdered condiment

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 h. This was done by mixing one gram of *P. africana* powder in 10 ml 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 AOAC (2007).

RESULTS AND DISCUSSION

Microbial consortia can be found everywhere in nature and are implicated in processes of great importance to human, one of which is assistance in food processing (Brenner et al., 2008). Microbial consortia can perform complicated task and endure more changeable environment than monoculture (Brenner et al., 2008). A monoculture of *Bacillus,* namely *B. subtilis* can initiate and end fermentation of legume seeds as reported by Odunfa (1981). Experience has shown that *Bacillus* consortia enhance fermentation activities more than monoculture. Holzapfel (2002) reported that *Bacillus* species were found to be associated with fermentation of plant seeds, such as *P. africana* and other legume seeds. The development and introduction of combined *Bacillus* species as consortia is to speed up fermentation activities.

Figure 1 shows that as fermentation time progressed, there was a rise in temperature from 35°C to the peak (49°C) with seeds inoculated with consortia and 47°C with seeds that were allowed to ferment without consortia. The increase in temperature is due to increase in metabolic activities during which heat was evolved (Odunfa, 1981). Temperature dropped drastically to 22°C for seeds with consortia and 20°C for seeds without consortia. The drop in the temperature is as a result of reduced metabolic activities (Odunfa, 1981).

It was also shown in this study that fermenting mashes inoculated with consortia A and B fermented faster (84 h). Mashes that fermented without consortia, completed fermentation process within 96 h (Figure 1). This is because, *Bacillus* consortia developed as starter cultures optimized production processes and they speed up fermentation by their abilities to break down protein to amino acids faster than seeds fermented without consortia. Another reason for fermentation been faster with consortia may be due to the fact that, each species brought unique set of enzymes or metabolic pathways. They may not be able to break down protein in the seeds alone, but together, all the necessary enzymes are present for breaking down proteins to amino acids

Table 1. Effect of drying conditions on physicochemical parameters of *P. africana* fermented with or without consortia.

Drying condition	Powdered condiment of *P. africana*	Means of fermentation	pH	Moisture content (%)	Peroxide value (meqkg)	Titratable acidity (mg/actic acid g)
	P. africana	Consortium A	5.23±0.00	0.19±0.02	4.29±0.01	1.10±0.00
Solar	*P. africana*	Consortium B	5.23±0.00	0.20±0.00	4.27±0.04	1.12±0.00
	P. africana	Without consortia	5.24±0.00	0.20±0.02	4.30±0.02	1.11±0.01
	P. africana	Consortium A	6.24±0.00	0.10±0.02	3.10±0.01	1.02±0.05
Oven	*P. africana*	Consortium B	6.23±0.00	0.10±0.01	3.11±0.04	1.05±0.00
	P. africana	Without consortia	5.29±0.00	0.14±0.00	3.15±0.00	1.10±0.01
	P. africana	Consortium A	5.20±0.00	0.20±0.02	4.30±0.00	1.10±0.00
Vacuum	*P. africana*	Consortium B	5.20±0.00	0.21±0.02	4.31±0.01	1.11±0.00
	P. africana	Without consortia	5.21±0.00	0.25±0.02	4.29±0.00	1.10±0.01
	P. africana	Consortium A	5.23±0.05	0.27±0.05	4.33±0.02	1.11±0.03
Sun (N)	*P. africana*	Consortium B	5.20±0.05	0.20±0.05	4.30±0.02	1.12±0.13
	P. africana	Without consortia	5.25±0.00	0.24±0.02	4.27±1.01	1.12±0.00
	P. africana	Consortium A	5.23±0.05	0.20±0.05	4.32±0.02	1.12±0.03
Sun (D)	*P. africana*	Consortium B	5.20±0.05	0.24±0.01	4.30±0.02	1.12±0.03
	P. africana	Without consortia	5.25±0.01	0.24±0.01	4.26±1.04	1.10±0.01

Values are means of triplicate determinations. Consortium A- seeds fermented with standard strains of *B. subtilis* and *B. pumilus*; consortium B- seeds fermented with test strains of *B. subtilis* and *B. pumilus;* Sun (D)- direct sun drying; sun (N)- sun drying covered with net.

Table 2. Mean separation using Duncan multiple range table for different drying methods used in drying freshly fermented seeds of *P. africana*

Duncan grouping	Mean	N	Factor
A	5.5767	27	Oven drying
B	5.2726	27	Vaccum drying
BC	5.2681	27	Solar drying
BC	5.2578	27	Sun drying (N)
C	5.2541	27	Sun drying (D)

(N), Sun drying covered with net; (D), Direct sun drying.

faster, resulting to shorter fermentation time.

Different drying conditions (solar drying, sun drying with net protection, direct sun drying without net, vacuum drying and oven drying) were used to dry freshly fermented condiment of *P. africana*. Interactions of drying conditions on condiment fermented with consortium A (standard strains of *B. subtilis and B. pumilus*) and consortium B (test strains of *B. subtilis and B. pumilus*) showed that physicochemical analyses on condiments with and without consortia showed no significant difference with one another. Highest pH value of 6.24±0.00 was recorded in oven dried condiment. Lowest pH value of 5.20±0.00 was recorded in vacuum drying. Yeast and moulds grow best at lower pH. A higher pH of 6.24±0.00 will affect growth of yeast and moulds. Lowest moisture value of 0.10±0.10 was recorded in oven dried condiment; growth of microorganisms is always affected when moisture content is low. Peroxide and titratable acid values were low in oven dried condiment (Table 1). Peroxide and titratable acid levels when high (20-40 meq kg) in condiment posed spoilage threat to condiment (Kolapo et al., 2007). The fact that oven drying condition differed among other drying conditions (Table 2), makes physicochemical parameters obtained from oven dried condiment also optimal. Using Duncan multiple range table, oven drying differed greatly from other drying methods with the highest mean of 5.5767 and Duncan grouping of A. The lowest mean value of 5.2541 was recorded in direct sun drying with Duncan group of C (Table 2).

Conclusion

From the analyses of this research work, it has been concluded that *P. africana* seeds inoculated with 5% *Bacillus* consortia fermented faster (84 h) as opposed to fermentation without consortia (96 h). Oven drying, out of the different drying conditions (solar dryer, vacuum dryer, sun drying without net and sun drying with net) used in drying freshly fermented *P. africana* seeds gave powdered condiment with lower values of moisture, titratable

acid and peroxide values as compared to higher values obtained from other drying conditions. Powdered condiment of *P. africana* obtained from oven drying methods produced condiment with physicochemical properties that will contribute in preserving the condiment for a very long time.

Conflict of Interest

The authors have not declared any conflict of interest.

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