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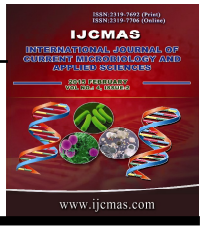

Original Research Article

Nutritional composition of Fermented Powdered *Prosopis africana* Soup Condiment with and without Inocula

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Inocula, *P.africana*, *B.subtilis*, *B.pumilus*, Biological ennoblement, Cotyledons, Fermentation, and Aluminum foil

A B S T R A C T

Analyses to ascertain the nutritional information of fermented powdered African mesquite (*Prosopis africana)* was carried out. Two kilograms (2kg) of *P.africana* seeds were purchased from Otukpo market, Benue state of Nigeria. These seeds were collected in small polythene bags and were transported to the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria. Seeds of *P. africana* were processed by cleaning and boiling for 24h to remove seed coats. Three hundred grams of *P. africana* cotyledons were subjected to fermentation with inocula (5% mixed *B. pumilus* and *B. subtilis*) and another 300g without inocula in an earth pot lined with sterile aluminum foil at room temperature (27 ± 2^0C) for 72 and 96h. The freshly fermented *P. africana* seeds were subjected to drying using hot air oven at a regulated temperature of 45^0C for seven days. Dried samples were weighed repeatedly to ensure absolute dryness. Dried fermented *P. africana* seeds with and without inocula were converted to powdered form using a sterile blender. Powdered condiments were subjected to proximate analysis to determine nutrient content of the powdered condiment with and without inocula. Results of the analyses showed that *P. africana* powdered condiment with inocula fermented faster (72h) and gave highest nutritional values than condiment without inocula *viz.*, crude protein (24.70–30.79%), crude lipid (10.01–11.03%), carbohydrates (26.01–28.17%), fibre (5.01–8.50%) and ash (5.01–7.07%). The enhanced nutritional content of powdered condiment with inocula is as a result of inoculated organisms. The contributions of these organisms in fermentation as "biological ennoblements" showed increased nutrients in powdered condiment of *P. africana* with inocula over the fermented powder of *P. africana* condiment without inocula. Powdered condiment of *P.africana* with inocula can be used as food supplements and flavour enhancers in soups and other dishes.

Introduction

Prosopis africana is a flowering plant species of the genus *Prosopis* found in Africa. Its common names include African mesquite and iron tree (Barminas *et al.*, 1998). Seeds of *P. africana* are used in Nigeria to prepare traditional fermented soup condiment or as flavour enhancers known as "*okpehe"* (Omafuvbe *et al.*,

1999). Fermented condiments remain key constituents of diets throughout many parts of Asia and Africa. Traditional diets in West Africa often lack varieties and consist of large quantities of staple foods (cassava, yam and maize) which provide the calories, but are poor in other nutrients. Soups are the main source of protein and minerals and one of the ways to improve the diet is to improve the nutrients in the soup through the use of condiments such as fermented legume seeds (Achi, 2005).

Fermentation markedly improves shelf life, digestibility, nutritive value and flavours of the raw seeds. When fermented, the seeds become tasty and protein rich seasoning. They are added to dishes directly or used as thickening agents in soups and stew (Ogbadu, 1988). Organism actively involved in the fermentation of *P. africana* is *Bacillus* species, predominantly are *B. subtilis, B. pumilus, B. licheniformis* and *B. megaterium* (Oguntoyinbo *et al.,* 2010). Undoubtedly, traditionally fermented condiments have not been incriminated in food poisoning. There is need to apply modern biotechnological techniques such as the use of starter cultures (inocula) in improving traditional food processing technologies (Achi, 2005). It has been suggested that even though most fermentations in developing countries do not use inocula or extrinsic cultures, these processes could be improved by using inocula (Holzapfel, 2002). Inocula have been found to speed up fermentation time of legume seeds as well as guarantee product quality.

Materials and Methods

Sample collection

Two kilograms of African mesquite *(Prosopis africana*) seeds were purchased from Otukpo market in Benue State of Nigeria. Seeds were transported to the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria in polythene bags.

Characterization of *Bacillus* isolates

Bacillus pumilus and *Bacillus subtilis* isolates were obtained from Department of Microbiology, Ahmadu Bello University, Zaria. The following biochemical test: catalase, coagulase, motility, indole, hydrogen sulphide, starch hydrolysis, growth at different sodium chloride level concentration and sugar utilization were carried out on the isolates as described by Gordon *et al.* (1973).

Preparation of unprocessed *P. africana* 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 for 24 hours, renewing the water intermittently until the seeds became soft. The soft seeds were dehulled by removing seed coats with finger tips. The cotyledons were reboiled for four hours and were allowed to cool to 35^0C in an earthen pot lined with sterile foil (Ogbadu, 1988).

Preparation of *Bacillus* inocula

The inocula (mixed strains of *B. subtilis* and *B. pumilus*) used for 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) (Todder, 2009).

The inocula used formed 5.0% of fermenting materials and consisted (15ml of 24hr old cultures of organisms into 300g of unfermented *P. africana* seeds).

Controlled fermentations of *P. africana* with and without inocula

Fermentation process of *P. africana* was set up using inocula. The organisms were inoculated into 300g of the unfermented seeds and were wrapped with sterile aluminum foil and placed in an earthen pot with cover. Thermometer cleaned with ethanol was inserted in the fermenting mash to monitor fermentation temperature. Another fermentation process was set up without inocula. Fermentation of *P. africana* with and without inocula was allowed to progress at room temperature (27 ± 2^0C) for 72 and 96 hrs respectively in the Department of Microbiology, Ahmadu Bello University, Zaria.

Microbiological monitoring of fermentation

Microbiological analysis was carried out at intervals of 12hrs to monitor growth of inocula from the start to the end of the fermentation process. During 72 and 96 hours of fermentation, ten grams of the samples were taken aseptically at intervals of 12 hours and added 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. 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.

Drying of freshly fermented *P. africana* seeds with and without inocula using hot air oven

Fifty grams of freshly fermented seeds of *P. africana* with inocula, and without inocula, were weighed into Petri dishes cleaned with ethanol. The Petri dishes containing the fermented samples were placed in a hot air oven at a regulated temperature of 45^0C for a period of one week. The samples were re-weighed repeatedly, until a constant weight was obtained.

Powdering blending of dried fermented seeds of *P. africana* with and without inocula

The dried fermented seeds of *P. africana* were blended into powdered form using a sterile blender. Ten grams of the powdered condiment was packaged into small plastic containers with seals sterilized with 70% ethanol. The packaged condiment was stored at refrigeration temperature (9 ± 2^0C) for further use. Dried condiments of *P. africana* purchased from Sabongari market was also converted to powdered form packaged and stored under the same condition.

Proximate analysis of powdered condiments of *P. africana* with and without inocula

Proximate analyses for crude protein, carbohydrates, crude lipids, fiber, ash and moisture content were carried out on powdered condiment of *P. africana* according to AOAC (2007) methods.

Results and Discussion

Bacteria of the genus *Bacillus* was isolated from fresh samples of fermented *P. africana*. Species that were commonly isolated were *B. subtilis, B. pumilus, B. licheniformis* and *B. circulans* with *B. subtilis* predominating over other species of *Bacillus* (Omafuvebe *et al.,* 2002). In this study only *B. subtilis* combined with *B. pumilus* were used as inocula to initiate

fermentation of *P. africana* seeds. Results from earlier studies showed that these two organisms fermented and produced products with very high nutritional and sensory qualities**.** *Bacillus* species have been found to be associated with fermentation of plant seeds for production of condiments. Oguntoyinbo *et al.* (2007) isolated a number of *Bacillus* species from fermenting African oil bean tree (*Pentaclethra macrophylla)* for '*ugba'* production. Achi (1999) isolated *Bacillus* species in fermentation of *P.africana* seeds for production of '*okpehe'*. Achi (2005) isolated *Bacillus* species in the fermentation of "*okpehe* and *daddawa*". Oguntoyinbo *et al.* (2010) also isolated *Bacillus subtilis* in fermenting mash of *P. africana* for *"okpehe"* production. Results of proximate analyses carried out in this study with fermented powdered condiment of *P. africana* with and without inocula showed that crude protein (24.70–30.79), crude lipids (10.01–11.03), carbohydrates (26.01–28.17), fibre (5.01–8.50) and ash (5.01–7.07) increased with powdered of *P. africana* with inocula (Table 2). It can be concluded that fermentation of legume seeds results in enhanced nutritional quality of the seeds as compared to seeds fermented without inocula and market samples.

The enhanced nutritional qualities are as a result of the activities of the inoculated microorganisms. Platt (1980) referred to this contribution by organisms in fermentations as "biological ennoblement," showing increased nutrients in fermented foods over the unfermented counterparts. Similarly, (Tamang, 2009) stated that increased nutritional content during fermentation is as a result of probiotic functions. Nutrient enhancement in fermented foods was also reported in an Indian cereal protein (Ogbadu, 1988). Odunfa (1984) and Omafuvbe *et al.* (1999) also reported that protein, fats, vitamins especially riboflavins increased significantly during fermentation of legume seeds.

Table.1 Temperature changes during fermentation of *P. africana* seeds with and without inocula

Time (hr)	Temperature change during fermentation of *P. africana* seeds with inocula (^{0}C)	Temperature change during fermentation of *P. africana* seeds without inocula(^{0}C)
0	35	35
12	38	36
24	39	37
36	39	38
48	41	38
60	44	41
72	50*	43
84	21	45
96	15	48*
120	13	17

Key: * - peak of fermentation

Table.2 Proximate composition of powdered condiment of *P. africana* with and without inocula, and market samples

Nutrients	Powdered condiment of *P. africana* without inocula	Powdered condiment Market samples of *P. africana* with inocula	
	NPA	LPA	MPA
Moisture content	0.04 ±0.01	0.02±0.00	0.03±0.00
Ash	5.01 ± 0.00	7.07±0.00	0.05±0.01
Crude lipid	10.01±0.00	11.03±0.04	10.00±0.02
Crude protein	24.70±0.02	30.79±0.00	24.50±0.04
Crude fiber	5.01 ± 0.00	8.50 ± 0.02	4.00±00
Soluble carbohydrate	26.01±0.02	28.17±0.00	24.02±0.03

Values are means of triplicate determinations

Key: NPA- fermented powdered *P. africana* condiments without inocula, LPA- fermented powdered *P. africana* condiment with inocula; MPA- market samples of *P. africana.*

The development and introduction of combined *Bacillus* species as inocula is to speed up fermentation activities by their abilities to break down protein to amino acids faster than seeds that fermented without inocula (Table 1), thereby enhancing nutritional content of the fermented seeds (Holzapfel, 2002; Gberikon *et al.,* 2009).

It was concluded from this research work that, *P. africana* seeds inoculated with mixed *Bacillus* species (*B. subtilis* and *B. pumilus*) constituting 5% inocula was inoculated into 300g of processed unfermented *P. africana* seeds to initiate fermentation. It was observed that seeds with inocula fermented faster than seeds without inocula. Oven dried fermented seeds that were converted to powdered form was subjected to proximate analysis. Results from proximate analysis showed that powdered condiment with inocula had highest nutritional contents than powdered condiment without inocula. Therefore, powdered condiment from fermented *P. africana* seeds with inocula, can be use as flavor enhancer in soups and other dishes.

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