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Benefits of Utilizing Starter Cultures in the Fermentation of *Glycine max* for Production of Condiment in the Food Industry

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ABSTRACT

Two kilograms of soya bean seeds (*Glycine max*) were purchased from Kasuwan mata, Sabon gari market, Zaria. These seeds were packaged in polythene bags and were transported to the laboratory, Department of Microbiology, Ahmadu Bello University, Zaria. Seeds were processed by precleaning to remove debris, roasting and dehulling to remove seed coats. Cotyledons were boiled in water for 5th, until it became soft and was allowed to cool to 35°C in an earth pot lined with aluminum foil. Bacterial isolates (5% mixed *B. subtilis* and *B. pumilus*) prepared as starter cultures was inoculated into 300 g of processed unfermented soft cotyledons of *G. max* seeds in an earth pot lined with aluminum foil. Thermometer cleaned with ethanol was inserted for temperature readings. Another fermentation process of *G. max* seeds (300 g) was also set up without starter cultures. Fermentation was allowed to proceed at room temperature (25°C) in the laboratory. As fermentation progresses, it was observed that *G. max* seeds with starter cultures fermented faster (48 h) than *G. max* seeds without starter cultures (72 h) as indicated by fermentation temperatures 50°C highest temperature at 48 h for seeds with starter cultures and 49°C highest temperature for seeds without starter cultures at 72 h. Results from proximate analyses showed that, fermented *G. max* seeds with starter cultures have highest nutritional values as compared to fermented seeds without starter cultures. The benefits of utilizing starter cultures in food industry cannot be over emphasized as they hasten fermentation processes as well as guarantee product quality by nutrients enhancement.

Key words: Fermentation, legume seeds, inocula, *Glycine max*, *B. subtilis*, *B. pumilus*, cotyledons, testa, earthpot, aluminum foil, condiments

INTRODUCTION

Legume seeds such as soya bean seeds (*Glycine max*) contains high levels of protein (38-45%) as well as its high (approximately 20%) oil content (FAO., 2013). Their fermented forms are commonly used in the Orient as seasoning and in Africa as condiments to enhance flavour and taste of foods (Riaz, 2007). Fermented condiments remain key constituents of diets throughout many parts of Asia and Africa.

The fermentation process for condiment production is still being carried out by the traditional village art (Achi, 2005). There is need to apply modern biotechnological techniques such as the use of starter cultures in improving traditional food processing technologies. 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 starter cultures (Holzapfel, 2002). Starter cultures have

been found to reduce fermentation time as well as guarantee product quality of fermented products. In the traditional method of manufacture, fermentation of legume seeds is achieved by indigenous microbial flora or the addition of fermented materials from previous production. Thus it may be assumed that undefined starter cultures have traditionally been employed in the production of these products (Omafuvbe *et al*., 2002; Dakwa *et al*., 2005).

A defined starter culture is essential for controlled fermentations. Most authors now agree that there is a predominant development of *Bacillus* species during the various legume fermentation processes (Dakwa *et al*., 2005; Gberikon *et al*., 2009; Ouoba *et al*., 2003). For soya bean fermentation, single or combined cultures of *Bacillus subtilis*, *B. licheniformis* have been successfully used (Sarkar *et al*., 1993). The use of a mixture of microorganisms with complementary physiological properties as starter cultures seems to be the best approach for obtaining a product with the nutritional and sensory properties desired (Omafuvbe *et al*., 2002).

MATERIALS AND METHODS

Two kilograms of soya bean seeds (*G. max*) were purchased from Kasuwan Mata market, Sabon gari, Zaria. These seeds were packaged into polythene bags and were transported to the laboratory, Ahmadu Bello University, Zaria for cleaning, processing and fermentation.

Preparation of fermentable part of *G. max* seeds for fermentation: Soya bean seeds obtained from the market were pre-cleaned by sorting out stones and debris. They were roasted on a hot pan for 5 min. This was followed by dehulling to remove seed coats followed by boiling in water for 5 h; the water was renewed intermittently until the seeds became soft (Ogbadu, 1988). The cotyledons were allowed to cool to 35°C in an earthen pot lined with sterile aluminum foil.

Preparation of *Bacillus* species (mixed *B. subtilis* and *B. pumilus*) as starter cultures: The starter culture used for fermentation contained 2.7×10^7 cells mL^{-1} 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) (Todar, 2009).

Starter cultures used formed 5.0% of fermenting materials which consisted of 15 mL of 24 h old cultures of organism into 300 g of unfermented seeds of *G. max*.

Controlled fermentations of *G. max* seeds with and without starter cultures in the laboratory: The fermentation process was set up and organisms were inoculated into 300 g of processed unfermented seeds of *G. max*, another fermentation process was set up using 300 g of unfermented *G. max* seeds without starters. All the seeds were wrapped with sterile aluminum foil and placed in different earth pots with covers. Thermometers cleaned with ethanol were inserted in the fermenting mashes to monitor fermentation temperature. Controlled fermentation was allowed to progress at room temperature (25°C) in the laboratory.

Microbiological monitoring of fermentation with starter cultures and natural fermentation: Microbiological analysis was carried out at intervals of 12 h to monitor growth of starters from the start to the end of the fermentation process.

During the 48 h fermentation, 10 g of the sample was taken aseptically at intervals of 12 h and was transferred into 90 mL 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 0.1 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. 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.

RESULTS AND DISCUSSION

Bacillus subtilis can initiate and end fermentation of locust bean and soya bean seeds as reported by Odunfa (1984). Experience has shown that mixed species of *Bacillus* enhances fermentation activities more than single species.

Starter cultures are used to initiate soya fermentations in Asia and some African countries. Fermentations that do not require conscious introduction of starters would depend on chance inoculation by the microbial flora of the fermenting environments. *B. subtilis* and *B. pumilus* used in this study was developed as starters to enhance fermentation activities by reducing fermentation time and increasing nutritional values thereby enhancing product quality. The development and introduction of combined *Bacillus* species as starters is to speed up fermentation activities (Holzapfel, 2002). It was observed in this study that fermenting mash of *G. max* seeds with starters fermented within 48 h at a temperature of 50°C while *G. max* seeds without starters fermented within 72 h at a temperature of 49°C (Table 1). This is because starters optimize production processes and they speed up fermentation by their abilities to break down protein to amino acids faster than seeds that fermented without starters. The increase in temperature is due to increase in metabolic activities during which heat was evolved (Odunfa 1984). Temperature dropped drastically as soon as fermentation processes was completed as a result of reduced metabolic activities as shown in Table 1. Nutritional qualities of *G. max* condiment fermented using starters have highest values of crude protein (40.00), crude lipid (18.03), crude fiber (8.50) soluble carbohydrate (7.12) and ash (3.50). Nutritional qualities of *G. max* condiments fermented without starters had lowest values of crude protein (38.70), crude lipid (15.01), crude fiber (7.01), soluble carbohydrate (6.01) and ash (3.01). Market samples were also compared and there was no significant difference with natural fermentation (Table 2). Seeds fermented with starters gave significantly higher nutritional values than seeds that fermented naturally. Platt (1964) 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. Odunfa

Table 1: Temperature changes during fermentation of *G. max* seeds with starter cultures and natural fermentation

Time (h)	Starters (°C)	Natural fermentation (°C)
0	35	35
12	38	36
24	41	38
36	45	40
48	50*	44
60	30	47
72	20	49*
84	18	30
96	15	19

*Peak of fermentation

Table 2: Proximate composition of fermented condiment of *G. max* seed with starters, natural fermentation and market samples

	Fermented condiment of *G. max* with natural fermentation	Fermented condiment of *G. max* Market samples with starters	
Nutrients	NGM	GMS	MGM
Moisture content	52.04±0.01	52.02±0.00	53.03±0.00
Ash	3.01±0.00	3.50±0.00	1.05±0.01
Crude lipid	15.01±0.00	18.03±0.04	15.00±0.02
Crude protein	38.70±0.02	40.00±0.00	37.50±0.04
Crude fiber	7.01±0.00	8.50±0.02	6.00±000
Soluble carbohydrate	6.01±0.02	7.12±0.00	4.02±0.03

Values are means of triplicate determinations, NGM: Fermented *G. max* condiments with natural fermentation, GMS: Fermented *G. max* condiment with starters, MGM: Market samples of *G. max*

(1984) and Omafuvbe *et al.* (2002) also reported that protein, fats, vitamins especially riboflavins increased significantly during fermentation of legume seeds. This study thus, is in agreement with these findings. There was increase in protein, lipids, fiber, ash and soluble carbohydrates particularly with seeds that were inoculated with starters.

From experience, single starters can be used to initiate fermentation. The use of a mixture of microorganisms with complementary physiological properties as starter cultures seems to be the best approach for obtaining products with good sensory properties (Omafuvbe *et al.*, 2002).

CONCLUSION

Starter cultures from different sources have retained similar physiological properties over sometime. These starter cultures at 5% inoculum, when used on *G. max* seeds carry out fermentation faster, achieving 48 h fermentation as oppose to 72 h in natural fermentation. Nutritional values of seeds inoculated with starters were higher as compare to seeds without starters. Therefore, use of starters in fermentation industry for production of condiments has immeasurable benefits which can be maximally utilized.

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