

Dehydration of Freshly Starter Assisted and Naturally Fermented Seeds of *G.max* Using Different Dehydrating Methods and its Physicochemical Effects

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Abstract

Four kilograms of soya bean seeds (*Glycine max*) were purchased from Kawo market, Kaduna, Kaduna state of Nigeria. These seeds were transported to the laboratory, Department of Microbiology, Ahmadu Bello University Zaria. *G.max* seeds were pre-cleaned and processed for fermentation. Processed unfermented seeds of *G.max* (300g) were transferred into three earth pots lined with aluminum foil. 5% mixed *Bacillus* species (*B.subtilis* and *B.pumilus*) both standard and test strains were prepared as starter cultures and were calibrated using McFarland standard 7. Pot A was inoculated with standard strains of *B.subtilis* and *B.pumilus* (starter A) while pot B was inoculated with test strains of *B.subtilis* and *B.pumilus* (starter B), pot C was allowed to ferment naturally. Fermentation in all the earth pots was allowed to progress at room temperature ($28\pm 2^{\circ}\text{C}$). It was observed that fermentation in earth pots with starters A and B fermented faster (48hrs) as compared to natural fermentation (pot C), (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 *G.max* 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 *G.max* powdered condiment subjected to vacuum drying gave lowest values of moisture content, titratable acidity, peroxide values as oppose to higher values in other drying conditions. *G.max* powdered condiment dried using vacuum dryer can be preserved for use over a longer period of time.

Keywords: *G.max*, fermentation, starter assisted, *B.pumilus*, *B.subtilis*, earth pots, aluminum foil, solar dryer, vacuum dryer, hot air oven

Introduction

Remarkably, soya bean seeds (*Glycine max*) contains very high levels of protein (38-45%) as well as its high (approximately 20%) oil content (FAO, 2013). The beans can be processed in a variety of ways. Common forms of soya include soy meal, soy flour, soy milk and soy oil (Riaz, 2006). Their fermented forms are commonly used in the Orient as seasoning and in Africa as condiments to enhance flavours and taste of foods (Riaz, 2006). Fermented condiments remain key constituents of diets throughout many parts of Asia and Africa. Bacteria of the genus *Bacillus* are commonly involved in the fermentation of legume seeds. Dakwa *et al.*, (2005) and Gberikon *et al.*, (2009) reported the involvement of the genus *Bacillus* in the fermentation of locust bean seeds and other legumes like soya bean seeds, African mesquite and castor oil seeds. Modern biotechnological techniques such as the use of starter cultures are important in improving traditional food processing technologies and guaranteed product quality (Holzapfel, 2002).

Dehydration of freshly fermented legume seeds is often employed to reduce high moisture content, contamination and markedly extend shelf life during storage (FAO, 2013). The basic traditional drying method is called sun drying. This is practiced in the rural setting and is characterized by direct solar radiation and natural air circulation on the products (FAO, 2013). Apart from sun drying, improved approaches have been developed such as the use of solar dryer, hot air oven and vacuum drying. These dehydration methods can effectively dry fermented seeds, reduce microbial contamination to a minimal level and extend shelf life stability of product.

Materials and Methods

Soya bean seeds (4kg) were purchased from Kawo market, Kaduna, 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* Isolates (starter cultures)

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°C for 24hours. Compared cells were sub-

cultured on aerobic plates of nutrient and plate count agars and were incubated at 37⁰C 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 Gordon *et al*, (1973).

Preparation of Starter Cultures (*B.subtilis* and *B.pumilus*)

Starter cultures 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 (BaCl₂) to 9.3ml of 1% sulphuric acid (H₂SO₄) (Todder, 2009).

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

Preparation of Soya Bean 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 five minutes. This was followed by dehulling to remove seed coats followed by boiling in water for five hours; the water was renewed intermittently until the seeds became soft. The cotyledons were allowed to cool to 35⁰C in an earthen pot lined with sterile aluminum foil .

Controlled Fermentations of Starter Assisted *G.max* Seeds

The fermentation process was set up using both starter cultures A and B separately. 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. Fermentation was allowed to progress at room temperature (28 ± 2⁰C) 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 the starter cultures from the start to the end of the fermentation process.

During fermentation, samples of ten grams were taken aseptically at intervals of 12 hours and 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 37⁰C for 24 hours. Potato dextrose agar plates were incubated at room temperature (27±2⁰C) for one week.

Dehydration of Starter Assisted and Naturally Fermented Seeds of *G.max* Using Different Methods

- **Hot Air Oven - Drying**

Fifty grams of freshly fermented seeds of *G.max* containing both starters, 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 45⁰C for a period of one week. The samples were re-weighed repeatedly until a constant weight was obtained.

- **Direct Sun - Drying**

Fifty grams of freshly fermented seeds of *G.max* containing both starters, 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 *G.max* containing both starters, 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 *G.max* containing both starters, 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 *G.max* containing both starters, 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

Powdering Blending and Packaging of Dried Fermented Seeds of *G.max*.

Dried fermented seeds of *G.max* were blended into powdered form, using a sterile blender. Ten gram of powder was packaged into small plastic containers with seals sterilized with 70% ethanol. The packaged condiments were stored at refrigeration temperature ($9\pm 2^{\circ}\text{C}$). Fermented samples of *G.max* purchased from Sabongari market were also analysed with laboratory samples.

Determination of Physicochemical Properties of *G.max* Seeds after Dehydration

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 taken from powdered form into 10ml of sterile distilled water. The pH of the suspension was then determined.

Moisture content, peroxide value and titratable acidity were analysed adopting the methods of AOAC, (2007).

Results and Discussion

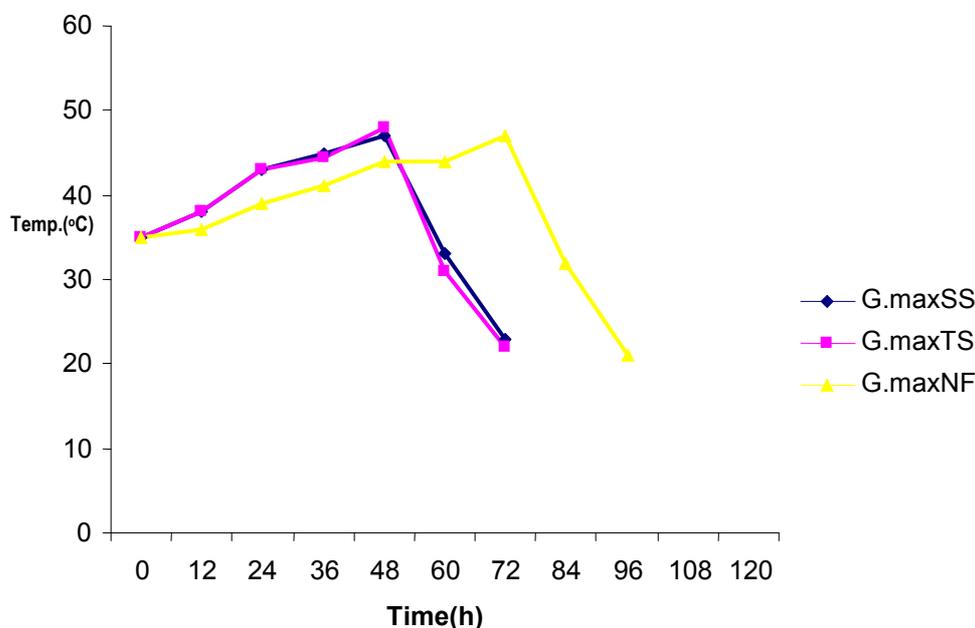


Figure 1: Temperature Changes During Natural and Starter Assisted Fermentation of *G.max* Seeds

Key

GmSS- *G.max* seeds fermented with mixed culture of standard strains of *B.subtilis* and *B.pumilus* (starter A);

GmTS- *G.max* seeds fermented with mixture of test strains of *B.subtilis* and *B.pumilus* (starter B); GmNF- *G.max* seeds fermented under natural conditions.

Table 1: Effect of Dehydration Methods on the Physicochemical Properties of Starter Assisted Fermented *G.max* Powder and Natural Fermentation

Drying conditions	Powdered condiment of <i>G.max</i>	Means of fermentation	pH	Moisture content (%)	Peroxide value(meqkg)	Titrateable acidity(mglactic acid)
Solar	<i>G.max</i>	Starter A	6.20±0.00	0.19±0.01	4.26±0.01	1.12±0.00
	<i>G.max</i>	Starter B	6.21±0.01	0.23±0.00	4.22±0.00	1.13±0.02
	<i>G.max</i>	NF	5.24±0.00	0.21±0.02	4.30±0.01	1.13±0.01
Oven	<i>G.max</i>	Starter A	5.24±0.02	0.21±0.00	4.16±0.00	1.14±0.06
	<i>G.max</i>	Starter B	6.23±0.01	0.19±0.05	4.11±0.02	1.13±0.00
	<i>G.max</i>	NF	5.29±0.00	0.20±0.01	4.18±0.01	1.12±0.01
Vacuum	<i>G.max</i>	Starter A	6.20±0.00	0.10±0.02	3.30±0.00	1.10±0.00
	<i>G.max</i>	Starter B	4.20±0.00	0.11±0.02	4.11±0.01	1.10±0.00
	<i>G.max</i>	NF	5.21±0.00	0.15±0.02	3.10±0.00	1.10±0.01
Sun(N)	<i>G.max</i>	Starter A	5.23±0.02	0.20±0.05	4.23±0.01	1.14±0.03
	<i>G.max</i>	Starter B	4.20±0.05	0.10±0.01	4.20±0.00	1.12±0.00
	<i>G.max</i>	NF	5.25±0.00	0.24±0.02	4.27±1.00	1.12±0.00
Sun(O)	<i>G.max</i>	Starter A	5.22±0.02	0.20±0.00	4.12±0.02	1.13±0.01
	<i>G.max</i>	Starter B	6.20±0.01	0.22±0.02	4.20±0.01	1.14±0.03
	<i>G.max</i>	NF	5.25±0.01	0.21±0.01	4.22±1.04	1.10±0.01
Market samples	<i>G.max</i>		4.23±0.01	1.26±0.01	5.21±0.00	1.22±0.05

Values are means of triplicate determinations

Key

Starter A- seeds fermented with standard strains of *B.subtilis* and *B.pumilus*; starter B-seeds fermented with test strains of *B.subtilis* and *B.pumilus*; NF- naturally fermented; Sundrying (O)- sundrying without net; Sun drying (N)- sundrying with net.

Discussion

Bacillus, species namely, *Bacillus subtilis*, *Bacillus circulans*, *Bacillus licheniformis* and *Bacillus pumilus* are implicated in fermentation of *G.max* seeds and other legumes (Ouoba *et al.*, 2003). 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. In this study, standard and test strains of *B.subtilis* and *B.pumilus* were used as starters to enhance fermentation activities

Advantages of Utilizing Starter Cultures

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

Effect of Dehydrating Methods on Physicochemical Properties of Powdered Condiments of *G.max* Assisted with Starters and Under Natural Conditions.

Different dehydrating methods were employed (solar drying, sun drying with net protection, direct sun drying without net, hot air oven drying and vacuum drying) to dehydrate fermented seed condiments of *G.max*. Powdered condiments from seeds dried in a vacuum dryer for seven days had lowest moisture, peroxide and titrateable acidity values as compared to fermented seeds dried under solar drying, vacuum drying, sun drying with and without net (Table 1). Fermented *G.max* seeds dried using vacuum dryer gave products with optimum physicochemical properties. This is because *G.max* seeds been an oil seeds were dried in a heat free environment (vacuum dryer) which did not leave room for residual moisture and oil coming out of seeds as a result of heat that can be absorbed back into the product.

Peroxide and titratable acidity values which are indices for deterioration were not significantly high in dried vacuumed condiments as opposed to others. High peroxidation value is a good indicator for fat deterioration (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 *G.max* dried using vacuum dryer can be preserved longer than powdered condiments from other drying conditions.

Conclusion

It has been concluded from the analyses of this research that, when fermentation is assisted with starter cultures, fermentation is usually faster than when seeds are allowed to ferment naturally. Vacuum drying out of all the different dehydrating methods used (solar dryer, hot air oven, sun drying without net and sun drying with net) used in dehydrating freshly fermented *G.max* seeds with and without starter cultures gave powdered condiments with lower values of moisture, titratable acidity and peroxide values as compared to higher values obtained from other dehydrating methods. Therefore vacuum dryer produced powdered condiment of *G.max* with optimum physicochemical properties that can have longer shelf life stability during storage.

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