

Research Article

An Evaluation of Shelf Life of Fermented Soybean (Soy "Daddawa") Under Different Storage Conditions

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ABSTRACT

Fresh fermented soybean cake (soy "Daddawa") is highly perishable, limiting its shelf life, consumer acceptability and commercialization. This study evaluated the effects of different storage environments on the shelf life and quality of soy "Daddawa". Two laboratory-produced samples (one inoculated with *Bacillus subtilis* and one uninoculated control) and a commercially purchased sample were investigated. Following fermentation, proximate composition was determined, and samples were stored under oven (low-moisture) and humid conditions for 12 days. Temperature, relative humidity, pH, aerobic mesophilic bacterial count, sensory quality, and bacterial contaminants were monitored throughout storage. The oven maintained a constant temperature of $27 \pm 0.00^\circ\text{C}$ throughout the 12-day storage period, whereas the ambient storage temperature gradually increased to $28.33 \pm 0.33^\circ\text{C}$ by day six of storage. No significant differences ($p > 0.05$) were observed in proximate composition among the fermented samples; however, the *B. subtilis*-inoculated sample had the highest protein content (45.28%). Relative humidity decreased from 21% to 10% under oven storage and from 48.33% to 40.67% under humid conditions, while pH increased progressively, reaching 8.17 in oven-stored samples. Aerobic mesophilic bacterial counts increased during storage without significant differences ($p > 0.05$) among treatments. Freshly fermented laboratory-produced samples achieved 100% consumer acceptance, with the inoculated sample receiving the highest sensory score (8.6 ± 0.6). Sensory acceptability declined markedly after 6 days and was completely lost by day 12. *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. were detected in stored samples. These findings show that Moisture-free storage prevents "Daddawa" spoilage.

Keywords: Daddawa; Fermentation; Shelf life; Storage conditions; Moisture content; Sensory evaluation

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INTRODUCTION

Fermentation is one of the oldest and most widely applied biotechnological processes for improving the nutritional quality, safety, shelf life, and sensory characteristics of foods. Through the metabolic activities of beneficial microorganisms, complex food constituents are transformed into simpler, more digestible compounds, resulting in enhanced nutrient bioavailability, reduced anti-nutritional factors, improved organoleptic properties, and the formation of bioactive metabolites with potential health benefits (Marco *et al.*, 2021; Owusu-Kwarteng *et al.*,

2022). Beyond food preservation, fermentation contributes significantly to human nutrition by promoting gut health, improving immune function, and increasing the bioavailability of essential amino acids, vitamins, minerals, and other functional compounds (Yang *et al.*, 2022).

Among fermented foods, alkaline-fermented soybean condiments occupy an important position in many Asian and African diets because of their high protein content, distinctive flavour, and nutritional value. In West Africa, "Daddawa" is a traditional fermented condiment widely consumed as a flavouring agent in

soups and stews. Traditionally, “Daddawa” is produced from African locust bean (*Parkia biglobosa*); however, soybean (*Glycine max*) has increasingly become an alternative raw material owing to its year-round availability, affordability, high protein content, and favourable amino acid composition. Soybean also represents a sustainable alternative capable of supporting food security while reducing dependence on locust beans, whose availability continues to decline due to deforestation and environmental degradation.

Soybean contains approximately 35–40% protein and 18–20% oil, making it one of the most nutritionally valuable legumes. However, its direct utilization is limited by the presence of anti-nutritional compounds such as trypsin inhibitors, *phytates*, *lectins*, and *oligosaccharides*, as well as its characteristic beany flavour, which reduces consumer acceptance. Alkaline fermentation effectively overcomes these limitations by degrading anti-nutritional factors, hydrolysing proteins into peptides and free amino acids, and generating desirable volatile compounds that improve flavour, aroma, digestibility, and overall nutritional quality (Qiao *et al.*, 2022).

The fermentation of soybean into “Daddawa” is mediated predominantly by species of *Bacillus*, particularly *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus*. These microorganisms produce extracellular proteases, amylases, lipases, and other hydrolytic enzymes that catalyse the degradation of proteins, carbohydrates, and lipids into simpler metabolites. Proteolysis during fermentation releases peptides, amino acids, ammonia, and other nitrogenous compounds, leading to the characteristic increase in pH that defines alkaline fermentation and contributes to the development of the distinctive aroma, flavour, texture, and nutritional properties of “Daddawa” (Tersoo *et al.*, 2021; Owusu-Kwarteng *et al.*, 2022). In addition, fermentation promotes the formation of bioactive peptides with antioxidant, antimicrobial, antihypertensive, and immunomodulatory activities, thereby increasing the functional value of the condiment.

Despite its nutritional importance, traditional “Daddawa” production relies largely on spontaneous fermentation, where microorganisms originate from raw materials, utensils, processing surfaces, and the surrounding environment. Such uncontrolled fermentation often results in inconsistent product quality, prolonged fermentation time, variable sensory characteristics, and an increased risk of contamination by spoilage organisms and foodborne

pathogens. These challenges limit product standardization, consumer confidence, shelf stability, and opportunities for large-scale commercial production.

To address these limitations, recent research has focused on the application of defined starter cultures, particularly *Bacillus subtilis*, to achieve controlled fermentation. The use of selected starter cultures has been reported to shorten fermentation time, improve process reproducibility, enhance nutritional quality, produce more desirable sensory characteristics, and reduce contamination by undesirable microorganisms (Kolapo *et al.*, 2023). Controlled fermentation also facilitates product standardization and represents an important step toward industrial-scale production of soybean “Daddawa” with consistent quality and improved food safety.

Although several studies have investigated the microbiology and nutritional composition of soybean “Daddawa”, limited information is available regarding the influence of controlled fermentation using *Bacillus subtilis* on the shelf life and storage stability of the product under different storage conditions. Understanding the microbiological, physicochemical, and sensory changes that occur during storage is essential for developing appropriate preservation strategies and improving the commercial potential of soybean “Daddawa”.

Therefore, this study aimed to evaluate the effect of *Bacillus subtilis* inoculation on the nutritional composition, microbiological quality, sensory characteristics, and shelf life of soybean “Daddawa” stored under different environmental conditions. The findings are expected to provide useful information for optimizing soybean “Daddawa” production and developing safer, nutritionally improved, and commercially viable fermented soybean condiments.

MATERIALS AND METHODS

Source and Identification of Soybean Seeds (*Glycine max*)

Glycine max seeds (1000g) were purchased from Jogana Market, a town in Gezawa local Government Area, Kano State, Nigeria. The seeds were identified at the Department of Plant Biology Bayero University Kano as *Glycine max* with BUKHAN number 0088.

Isolation of *Bacillus subtilis* from Previously Fermented Soybean “Daddawa”

The *Bacillus subtilis*, used as starter culture, was isolated from previously fermented soybean “Daddawa” (Omabuvfe *et al.*, 2000). Mannitol Yolk Polymyxin (MYP) agar was used in isolation of *Bacillus subtilis*.

Preparation of Food Homogenate

Twenty five grams (25g) of previously fermented soybean “Daddawa” (*Glycine max*) cake was homogenized by blending in 225ml of peptone water. Six test tube containing 9ml of peptone water each were labeled as (10^1 to 10^6). One milliliter (1ml) of the homogenate was introduced into the test tube labeled as 10^{-1} and then serially diluted to five other test tubes labeled (10^2 to 10^6). One milliliter (1ml) each from test tubes labeled (10^4 to 10^6) was pipetted and added to appropriately labeled sterile petri dishes in triplicates; warm MYP agar was added and swirled so that the aliquot were evenly distributed, allowed to gel and the plates were incubated at 37°C for 24 hours (Pour- plate technique). Morphologically, different colonies were selected from the agar plates and repeatedly sub cultured on MYP agar plates. The plates were incubated at 37°C for 24 hours. The isolates were inoculated unto nutrient agar slant and stored in the refrigerator for further analysis.

Biochemical Characterization of Isolate

Biochemical characterization of bacterial isolates was carried out for the purpose of identifying the isolates. *Bacillus* species (mainly *Bacillus subtilis*) was identified in accordance with the techniques of Cheeseborough (2012).

Gram Staining

A heat fixed smear was prepared on a clean glass slide from an 18hr old bacterial culture. The smear was stained with crystal violet solution for 1 minute. The slide was rinsed with tap water; Gram’s iodine solution was added. The iodine solution was allowed to stay for 60 seconds. The slide was rinsed with water and 95 % ethanol was added, this was allowed to stay for 60 second to decolorize the crystal violet stain. The slide was rinsed with water and counter stained with Safranin solution, which was left for 30 seconds. The slide was rinsed with tap water and allowed to dry. The slide was observed under the microscope with immersion oil (Cheeseborough, 2012).

Catalase Production test

A loopful of bacterial culture was emulsified with hydrogen peroxide on a clean slide. The slide was observed for effervescence caused by the liberation of free gas bubbles and the result was recorded (Cheeseborough, 2012).

Oxidase Production Test

Few drops of 1% aqueous solution of tetramethyl-p-phenylenediamine hydrochloride were added to a loopful of bacterial culture on a clean slide. The slide was observed for the development of purple color

within 5 seconds and the result was recorded (Cheeseborough, 2012).

Starch Hydrolysis Test

Solidified starch nutrient agar plates were inoculated with bacterial culture by streaking once across the surface of the agar. The plates were incubated at 35°C for 24hrs. After incubation, the plates were flooded with Gram’s iodine solution and starch hydrolysis was observed as a clear zone on the plates (Cheeseborough, 2012).

Citrate Utilization Test

Simmon citrate medium was dispensed into clean test-tubes and sterilized in an autoclave at 121°C for 15 mins. The sterile medium was inoculated with bacterial isolate using a sterile wire loop. The inoculated medium in tubes was incubated at 35°C for 24hrs. After incubation, the tubes were observed for turbidity indicating growth and utilization of citrate (Cheeseborough, 2012).

Methyl Red Test

Methyl red voges proskauer (MR-VP) broth was prepared, dispensed into tubes and sterilized in an autoclave at 121°C for 15mins. The sterile medium was inoculated with bacterial culture and incubated at 35°C for 4 days. After incubation, five drops of methyl red indicator were added to 5ml of broth culture in tubes. The tubes were observed for the development of red color which was an indication of positive result (Cheeseborough, 2012).

Voges- Proskauer Test

Sterile glucose phosphate broth was inoculated with bacterial culture and incubated at 35°C for 24hrs. After incubation, a pinch of creatine was added to the culture followed by 5ml of 40% sodium hydroxide. The tubes were shaken very well and allowed to stand for 30mins. The tubes were observed for the development of red color within 30mins which is an indication of the production of acetyl methyl carbinol from glucose (Cheeseborough, 2012).

Sporulation Test

A drop of water was added to a clean free grease slide using a sterile wire loop. The isolated colony of the test organism was picked and emulsified gently in the drop of water on the glass slide. The smear was then allowed to air dry and then heat fixed by the brief passage of the slide over a burning spirit lamp three times. The heat fixed smear was then allowed to cool and then flooded with 5% malachite green solution and heated until steam rises then left for 5mins. The stain was rinsed well with water. After tipping off the excess water, the smear was counter stain with 0.5% Safranin for 30min. The stain was then washed with water blotted and air-dried. The air dried smear was

then examined microscopically with the oil immersion objective and the spores appeared green while the vegetative bacteria appeared red (Cheeseborough, 2012).

Egg Yolk Reaction Test

Egg yolk agar plates was prepared by mixing 50ml of eggs emulsion (oxidized) with sterilized nutrient agar containing 1% sodium chloride at 45 degrees Celsius and the mixture was immediately poured into sterile petri dishes. An opaque zone around the colonies, after 4 days of incubation at 30°C was considered to be a positive test.

Molecular characterization of the Bacterial Isolate

Genomic DNA Extraction

DNA extraction was carried out on the samples using the Zymo Research Fungal bacterial DNA preparation kit. The bacterial isolate was inoculated into 5ml Nutrient Broth and incubated in a round-bottom culture tubes with shaking at 30°C for 18 or 22 hours. The bacterial cell was centrifuged at 4000rpm at 4°C for 10 minutes. Cell pellets were thoroughly suspended in 250µl suspension Solution and transferred to a sterile 1.5ml micro centrifuge tube. DNA was isolated from one half of the sample using the zymo research preparatory DNA purification system protocol. The sample was treated with 100µl of lysozyme (10mg/ml) for 15 minutes at 30°C prior to adding the cell lysis solution from the DNA purification system and proceeding with the protocol described in technical bulletin.

Polymerase Chain Reaction

Polymerase chain reaction was carried out to amplify the 16S rRNA gene of the bacteria using the primer pair 27F-5'-AGAGTTTGATCCTGGCT CAG-3', and 1492R 5'-GGTACCTTACGACTT-3'. The PCR reaction was carried out using the Solis Biotec 5X*HOT FIREPol Blend Master mix. The PCR was performed in 25µl of a reaction mixture and the reaction concentration to 1x concentration containing 1x Blend Master mix buffer (Solis Biotec), 1.5mM MgCl₂, 200µM of each deoxynucleoside triphosphate (dNTP) (Solis Biotec), 25pMol of each primer (Jena Bioscience, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biotec), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in a Peltier thermal cycler (PTC100)(MJ research series) for an initial denaturation of 95°C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95 °C, 1 minute at 61°C and 1 minute 30 seconds at 72 °C. This was followed by a final extension step of 10 minutes at 72 °C. The amplification product was separated on a

1.5% agarose via and electrophoresis, DNA bands were visualized by ethidium bromide staining and 100bp DNA ladder (Solis Biotec) was used as DNA molecular weight marker.

Sequencing

All PCR products (10µl of primer, 15µl of purified PCR, 12µl of plasmids and 15µl of gDNA) were sent to Inqaba biotech lab (South Africa) via courier for quantification of the template. The Brilliant Dye terminator™ is blended with the ABI 3500XL genetic analyzer for sequencing reactions.

Preparation of 0.5 McFarland Turbidity Standard

To make 1% sulfuric acid, 90ml of distilled water was added to a 100ml volumetric flask, 1ml of concentrated H₂SO₄ was pipetted and added to the flask, it was then mixed thoroughly. To make 1% barium chloride, 1.175g of BaCl₂ was weighed and placed in a 100ml volumetric flask, 50 ml of distilled water was added and mixed until dissolved. A 0.5 McFarland standard was prepared by mixing 0.05ml of 1.175% barium chloride (BaCl₂.2H₂O) with 9.95ml of 1% sulfuric acid (H₂SO₄). The standard can be compared visually with a suspension of isolate in a sterile saline or nutrient broth. If the isolate is too turbid, it can be diluted with more diluents. If the suspension is not turbid enough, the isolate can be added.

Standardization of Inoculum of Test Bacteria

The inoculums of the bacterial isolate tested was standardized by the use of overnight nutrient broth culture prepared by inoculating three loopfuls of well isolated colonies of test bacteria in 10ml of nutrient broth which was incubated at 35°C for 24hrs. A loopful of overnight broth culture was diluted in sterile physiological saline (0.8% w/v), such that its turbidity matched with that of 0.5 McFarland Standard (1.5 × 10⁻⁸ cfu/ml).

The Production of *Glycine max* (Soybean Seeds) cake

The method of Omabuvfe *et al.* (2006) was used for the fermentation. *Glycine max* seeds (1Kg) were roasted with sands and dehulled by rubbing with palms wearing disinfected hand gloves. The traditional method of boiling for 12hrs (Omabuvfe *et al.*, 2000) was replaced with autoclaving at 121 °C for 15min to obtain sterile beans and boiled in a pressure cooker for 2hrs (Omabuvfe *et al.*, 2004). The cotyledons were later drained through a sieve and cooled to room temperature. The cooked *Glycine max* seeds were poured into two different BAGCO sack disinfected with 95% ethanol for sterilization and then separately covered with sterile aluminium foil. *Bacillus subtilis* of density (1.5 × 10⁻⁸ cfu/ml) was inoculated to one of the treatments. The inoculation

was carried out in single culture and was done inside the sack, and the other treatment without the inoculation of the organism served as the control. Both were covered and allowed to ferment in a sterilized safety chamber for 2 days (48hr). The completion of the fermentation process was indicated by the formation of mucilage and overtones ammonia produced as a result of the breakdown of amino acids during fermentation (Onyenekwe *et al.*, 2012). At the end of 48hr (fermentation), the *Glycine max* seeds were pounded in a mortar and pestle sterilized with ethanol and shaped into desirable shapes using sterile hand gloves in the safety chamber. The "Daddawa" inoculated with *Bacillus subtilis* (ID), uninoculated (UID) and freshly produced soybean "Daddawa" (FPD) were divided into two groups. One set of the treatments were stored in the oven at 27 °C and the other set of treatments were stored at ambient temperature. All treatments in the oven and ambient temperature were analyzed at 2 days interval for 12 days and 6 days respectively.

Determination of Temperature and Relative Humidity of the Storage Environment

Determination of Temperature of Oven and when kept at ambient temperature

The temperature was determined by inserting a sterile thermometer (wiped with alcohol) into the storage facility (oven). The mercury in glass thermometer was used (AOAC, 2000). All analyses were carried out in triplicate.

Determination of Relative Humidity of Oven and when kept at ambient temperature

Wet and dry hygrometer was placed into the storage facility (oven). The difference in temperature between the dry bulb and wet bulb was used to calculate the relative humidity of the air. To work out the level of humidity, dry bulb temperature and wet bulb temperature was recorded. From the chart given, the wet bulb depression was read across the dry bulb temperature to where both meets. This gave the relative humidity as a percentage (State of New South Wales, Department of Education and training, 2009).

Determination of Proximate Composition

The method of AOAC (2023) was used for the determination of protein, fat, moisture, ash, crude fiber and carbohydrate after fermentation of inoculated and uninoculated together with the freshly purchased "Daddawa". All analysis were carried out in triplicates.

Determination of pH of the Soybean "Daddawa"

A pH meter (model Denwer 20) was used. It was used to measure the acidity and alkalinity of "Daddawa"

samples. The electrode was rinsed with distilled water and immersed into the samples. Five grams from each sample (inoculated, uninoculated and freshly purchased soy "Daddawa") were grounded thoroughly in a mortar. They were each then suspended in 100ml of distilled water. The pH of the suspension was measured using pH meter at 0,2,4,6,8,10 and 12 days of storage. All analysis was carried in triplicate.

Determination of Aerobic Mesophilic Bacterial Counts of the Soybean "Daddawa"

Samples of fermented *Glycine max* seeds were prepared according to the method described by Food and Agriculture Organization (FAO) (2003). Five gram (5g) each of all treatments both stored in the oven and humidity chamber were homogenized by blending in 225ml of peptone water. Five different test tubes for each treatment were labeled from 10^{-1} to 10^{-5} each containing 9ml of peptone water. One milliliter from the prepared homogenate of all treatments was introduced into the different sets of test tube and serially diluted. One millilitre aliquot of each sample was pipetted and added to appropriately labeled sterile duplicate Petri dishes for each sample. Molten Nutrient Agar (NA) was added and swirled so that the inoculum was evenly distributed, allowed to gel and the plates were incubated aerobically at 37°C for 24hrs (Pour plate technique) after which the bacteria colonies were counted, multiplied by the inverse of the dilution factor and expressed as colony forming unit per gram (CFUg⁻¹). The same procedure was carried out at 2 days interval for 12 and 6 days. All analysis was carried out in triplicates.

Organoleptic/Sensory Evaluation of the Soybean "Daddawa"

Inoculated, uninoculated and freshly purchased soybean "Daddawa" were made into *Yajin* "Daddawa". Judges were offered clean water to rinse their mouth in between servings of different "Daddawa". The organoleptic/sensory evaluation of (*Yajin* "Daddawa") was carried out using 9-point hedonic scale (David, 2005). The samples were evaluated for colour, aroma, texture, taste and general acceptability by a panel of 10 regular consumers of soybean seeds *Yajin* "Daddawa". They were requested to express their feelings about the serving by scoring the organoleptic attributes using the hedonic scale (David, 2005).

Detection of Bacterial Contaminants of the Soybean "Daddawa"

Some bacterial contaminants were detected at 6 and 12 days of storage. Suspected organisms were *E. coli*,

Staphylococcus aureus and *Salmonella*. Certain biochemical tests were done to confirm the organisms.

RESULTS AND DISCUSSION

Morphological, Biochemical and Molecular Characterization of Bacterial Isolate

The morphological and biochemical characteristics of the bacterial isolate are presented in Table 1. The isolate exhibited typical characteristics of *Bacillus subtilis*, including Gram-positive reaction, catalase positivity, indole negativity, cream-colored sparse colonies, short rod-shaped cells, and centrally located endospores. Figure 1 shows partial sequencing of the isolate having a sequence ID gi/507147976/NR, length 1555 and plate 1 shows the Agarose gel depicting the amplicons of the isolate showing the ladder -ve control and the isolates with base pair of 1500 for *Bacillus subtilis*. These characteristics are consistent with the established phenotypic profile of *B. subtilis* commonly associated with the alkaline fermentation of soybean "Daddawa". The successful isolation of *B. subtilis* from previously fermented soybean "Daddawa" confirms its dominance during fermentation and supports its suitability as a starter culture. Similar findings have been reported by Odunfa (2002); Popoola *et al.* (2007), who identified *B. subtilis* as the predominant fermentative organism responsible for protein hydrolysis, flavour development, and product stabilization during "Daddawa" fermentation.

The produced "Daddawa" was dark brown coloured paste with a characteristic pungent smell and sticky when touched.

Proximate Composition

The proximate composition of inoculated, uninoculated, and freshly purchased soybean "Daddawa" is presented in Table 2. The inoculated "Daddawa" contained significantly ($p < 0.05$) higher ash, moisture, protein, and crude fibre contents than the uninoculated and freshly purchased samples. In contrast, the freshly purchased "Daddawa" exhibited the highest carbohydrate content (26.77%). The lower carbohydrate content observed in the fermented laboratory samples may be attributed to microbial utilization of carbohydrates as energy sources during fermentation, resulting in their

conversion into simpler metabolites. Similar reductions in carbohydrate content following fermentation have been reported in fermented sorghum and locust bean products by Chavan and Kadam, (2000) and El-Tinay *et al.* (2001).

The inoculated "Daddawa" recorded the highest protein content (45.28%), indicating enhanced proteolytic activity of *B. subtilis*. During alkaline fermentation, extracellular proteases hydrolyse complex soybean proteins into peptides and amino acids, thereby increasing protein availability and improving nutritional quality. This finding agrees with previous studies that reported improved protein quality following controlled fermentation with *Bacillus* species.

Fat content ranged from 5.97% to 8.16%, with freshly purchased "Daddawa" recording the lowest value. The reduction in lipid content may be associated with thermal processing during boiling and soaking as well as enzymatic degradation during fermentation. Similar observations were reported by Fadahunsi *et al.* (2013); Kazanas and Fields (2001). Overall, the significant differences ($p < 0.05$) among treatments demonstrate that controlled fermentation with *B. subtilis* positively influenced the nutritional composition of soybean "Daddawa".

Storage Temperature and Relative Humidity

The storage temperature and relative humidity under oven and ambient conditions are presented in Table 3. The oven maintained a constant temperature of $27 \pm 0.00^\circ\text{C}$ throughout the 12-day storage period, whereas the ambient storage temperature gradually increased to $28.33 \pm 0.33^\circ\text{C}$ by day six. Conversely, relative humidity declined under both storage conditions, decreasing from $21 \pm 0.00\%$ to $10 \pm 0.00\%$ in the oven and from $48.33 \pm 0.33\%$ to $40.67 \pm 0.67\%$ under ambient conditions.

The inverse relationship between temperature and relative humidity is expected because warmer air possesses a greater moisture-holding capacity, thereby lowering relative humidity. Such environmental changes influence microbial growth, moisture migration, and product stability during storage. The present findings are consistent with Ajadi and Sanusi (2013), who reported similar relationships between storage temperature and relative humidity in food drying systems.

Table 1: Morphological and biochemical characterization of isolate

	Microscopic observation/ Biochemical tests
Colony morphology	Cream, sparse colonies
Cell morphology	Short rods, central spores
Grams reaction	+
Indole test	-
Catalase test	+
Oxidase test	+
Starch hydrolysis	+
Citrate utilization	+
Methyl red	+
Egg yolk reaction test	+
Sporulation test	+
Voges proskeur test	+
Identity of the isolate	<i>Bacillus subtilis</i>

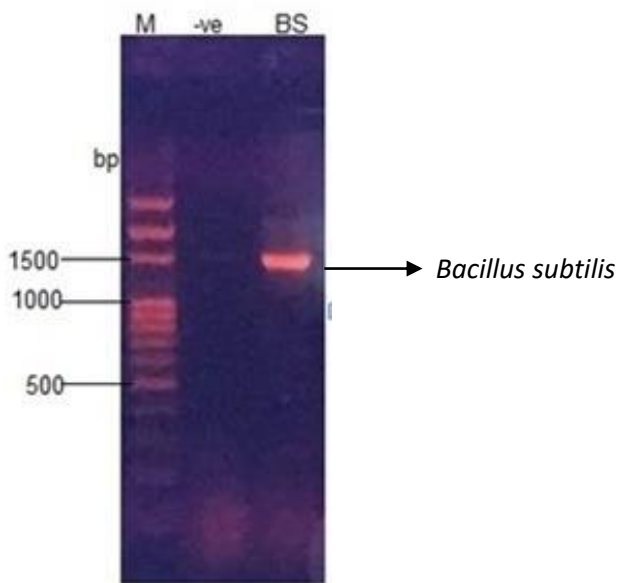


Plate 1: Agarose Gel showing Amplicon of *B. subtilis*

Key: M = Molecular ladder

-ve = Negative

BS = *Bacillus subtilis*

Bacillus subtilis strain 168 16S ribosomal RNA gene, complete sequence
 Sequence ID: [gi|507147976|NR_102783.1](#) Length: 1555 Number of Matches: 1

Range 1: 82 to 877 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1353 bits(1500)	0.0	778/797(98%)	1/797(0%)	Plus/Minus
Query 17	CGTTTGTCTGCAGCACTAAAGGGCGGAAACCCCTCTAACACTTAGCAYTCATCGTTTACGGC			76
Sbjct 877	CGTTAGCTGCAGCACTAAAGGGCGGAAACCCCTAACACTTAGCACTCATCGTTTACGGC			818
Query 77	GTGGACTACCAAGGTAICTAATCCTGTTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGT			136
Sbjct 817	GTGGACTACCAAGGTAICTAATCCTGTTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGT			758
Query 137	TACAGACCAGAGAGTTCGCTTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTACCGG			196
Sbjct 757	TACAGACCAGAGAGTTCGCTTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTACCGG			698
Query 197	CTACACGTGGAATTCCTACTCTCCTCTTCTGCACTCAAGTTCCTCCAGTTTCCAATGACCCT			256
Sbjct 697	CTACACGTGGAATTCCTACTCTCCTCTTCTGCACTCAAGTTCCTCCAGTTTCCAATGACCCT			638
Query 257	CCCCGGTTGAGCCGGGGGCTTTTCACATCAGACTTAAGAAACCGCCTGCGCGCGCTTTACG			316
Sbjct 637	CCCCGGTTGAGCCGGGGGCTTTTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACG			578
Query 317	CCCAATAATTCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTT			376
Sbjct 577	CCCAATAATTCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTT			518
Query 377	AGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTRCCGCCCTATTGAAACGGTACTTGTTC			436
Sbjct 517	AGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGGCCCTATTGAAACGGCACTTGTTC			458
Query 437	TTCCCTAACAAACAGAGTTTACGATCCGAAAACCTTCATCACTACGCGGCGTTCCTCCG			496
Sbjct 457	TTCCCTAACAAACAGAGCTTACGATCCGAAAACCTTCATCACTACGCGGCGTTCCTCCG			398
Query 497	TCAGACTTTCGTCATTGCGGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCG			556
Sbjct 397	TCAGACTTTCGTCATTGCGGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCG			338
Query 557	TGTCTCAGTCCCAGTGTGGCCGATCACCCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGT			616
Sbjct 337	TGTCTCAGTCCCAGTGTGGCCGATCACCCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGT			278
Query 617	GAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCTRAA			676
Sbjct 277	GAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCCG-A			219
Query 677	AGCCACCTTTTATGATTGAACCATGCGGTTCAATCAAGCATCCGGTATTAGCCCCGGTTT			736
Sbjct 218	AGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTT			159
Query 737	CCCCGAGTTATCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCT			796
Sbjct 158	CCCCGAGTTATCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCT			99
Query 797	GACCTAAGGGAGCAAGC	813		
Sbjct 98	AACATCAGGGAGCAAGC	82		

Figure 1: 16S rRNA partial sequence *Bacillus subtilis*

Table 2: Proximate composition of inoculated, uninoculated and freshly purchased soybean “Daddawa” after production

(%)	UID	ID	FPD	SA
Ash	10.85±0.00	10.59±0.00	8.38±0.12	P < 0.05
Moisture	11.3±1.26	12.03±0.33	11.03±0.00	P < 0.05
Fat	8.16±0.00	8.03±0.33	5.97±0.00	P < 0.05
Protein	44.14±5.02	45.28±0.00	40.43±0.00	P < 0.05
CHO	17.30±0.00	14.46±0.00	26.77±0.00	P < 0.05
Fibre	8.23±0	9.64±0.00	7.24±0.00	P < 0.05

Key: values are means ± SE of triplicates, ID=inoculated “Daddawa” UID=uninoculated “Daddawa”, FPD=freshly purchased “Daddawa”, SA=statistical analysis, P < 0.05=significantly different

Changes in pH During Storage

The pH values of soybean “Daddawa” during storage are presented in Table 4. Under oven storage, pH increased progressively from approximately 6.1 to

values above 8.1 in all treatments, with the uninoculated sample reaching the highest value (8.17 ± 0.23). Similarly, pH increased under ambient

storage, although the final values remained lower than those observed under oven storage.

The gradual increase in pH reflects the alkaline nature of “Daddawa” fermentation. During storage, *Bacillus* species continue to degrade proteins into peptides, amino acids, and ammonia, resulting in increased alkalinity. This alkaline environment contributes to the characteristic flavour and aroma of fermented soybean products. The observed trend agrees with the findings of Omafuvbe *et al.* (2000), who reported progressive increases in pH during alkaline fermentation of legumes.

Aerobic Mesophilic Bacterial Count

The aerobic mesophilic bacterial counts of the stored products are presented in Table 5. Microbial counts increased progressively during storage under both oven and ambient conditions, indicating continued microbial proliferation throughout the storage period. The increase was more pronounced under ambient conditions, suggesting that higher temperature and humidity favoured bacterial growth. Although *B. subtilis* contributes positively to fermentation, prolonged storage encourages the growth of spoilage microorganisms, leading to deterioration in product quality.

Sensory Evaluation

The sensory evaluation results are presented in Table 6. Freshly prepared inoculated and uninoculated “Daddawa” received high overall acceptability scores, with inoculated “Daddawa” receiving the greatest preference among the panelists. The improved acceptability may be attributed to enhanced proteolytic activity of *B. subtilis*, which generates peptides and amino acids responsible for desirable flavour, aroma, and texture. Similar observations have been reported by Omafuvbe *et al.* (2006).

After storage, sensory quality declined markedly. Samples stored for six and twelve days developed objectionable odours, undesirable texture, and visible spoilage, resulting in complete rejection by the sensory panel. These findings indicate that prolonged storage adversely affects the sensory attributes and consumer acceptability of soybean “Daddawa”.

Occurrence of Foodborne Pathogens During Storage

The occurrence of selected foodborne pathogens during storage is presented in Table 7. After six days of storage, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* species were not detected in oven-stored samples, whereas *E. coli* and *S. aureus* were detected in some ambient-stored samples. Notably, the inoculated “Daddawa” remained free from these pathogens during this period, suggesting that fermentation with *B. subtilis* may have exerted inhibitory effects against contaminating microorganisms through competitive exclusion and production of antimicrobial metabolites.

By day twelve, *S. aureus* was detected in the inoculated and freshly purchased samples but remained absent in the uninoculated sample. *Escherichia coli* was detected only in the freshly purchased “Daddawa”, which may indicate contamination arising from poor hygienic handling during production or marketing. Similar observations were reported by Bukar *et al.* (2010), who identified *E. coli* on the hands of food handlers in small-scale food processing facilities. *Salmonella* species remained undetected in most treatments throughout the storage period, suggesting satisfactory microbiological safety with respect to this pathogen. Overall, the microbiological findings demonstrate that controlled fermentation with *Bacillus subtilis*, combined with proper storage conditions and

hygienic handling practices, enhances the microbiological safety and shelf stability of soybean

“Daddawa” while minimizing contamination by foodborne pathogens.

Table 3: Changes in Temperature and Relative humidity of the Oven and when kept at ambient temperature

Parameters	Days	Oven	Ambient temperature	SA
Temperature	0	27±0.00	27±0.00	P > 0.05
	2	27±0.00	27.33±0.33	
	4	27±0.00	28±0.00	
	6	27±0.00	28.33±0.33	
	8	27±0.00		
	10	27±0.00		
	12	27±0.00		
Relative humidity	0	21±0.00	48.33±0.33	P < 0.05
	2	20±0.00	45.67±0.33	
	4	18±0.57	43.33±0.67	
	6	15.33±0.33	40.67±0.67	
	8	14±0.00		
	10	12.33±0.66		
	12	10±0.00		

Key=values are mean ± SE of triplicates, UID=uninoculated “Daddawa”, ID=inoculated “Daddawa”, FPD=freshly purchased “Daddawa”, SA= statistical analysis, P > 0.05=no significant difference, P < 0.05 =significantly different

Table 6: Sensory scores of uninoculated (UID), inoculated (ID), and freshly purchased (FPD) soybean “Daddawa” after fermentation and at 6 and 12 days of spoilage during storage

SEAF	UID	ID	FPD				SA
	8.2±0.13 (100)	8.6±0.16 (100)	6.0±0.37 (60)				P < 0.05
SEAS	UIDO	IDO	FPDO	UIAT	IAT	FPAT	
6	3.2±0.51(0)	3.3±0.33(0)	4.6±0.16(0)	1.4±0.16(0)	1.5±0.16(0)	1.6±0.16(0)	P < 0.05
12	2.4±0.33(0)	2.2±0.39(0)	2.9±0.38(0)				P < 0.05

Key: UID = uninoculated “Daddawa”, ID = inoculated “Daddawa”, FPD = freshly produced “Daddawa”, SEAS=sensory evaluation at spoilage, UIDO = uninoculated “Daddawa” in oven, IDO = inoculated “Daddawa” in oven, FPDO = freshly produced “Daddawa” in oven, UIAT = uninoculated “Daddawa” at Ambient temperature, IAT = inoculated “Daddawa” at Ambient temperature, FPAT = freshly produced “Daddawa” at Ambient temperature

Table 4: Changes in pH of Inoculated, Uninoculated and freshly Purchased Soybean “Daddawa” under Different Storage Condition

Parameter	Days	Oven			AT			SA
		UID	ID	FPD	UID	ID	FPD	
pH	0	6.12±0.03	6.12±0.00	6.11±0.00	6.19±0.04	6.12±0.00	6.23±0.01	P > 0.05
	2	6.12±0.03	6.23±0.01	6.43±0.01	6.54±0.16	6.12±0.00	6.32±0.01	
	4	6.42±0.00	6.42±0.00	6.46±0.00	6.84±0.12	6.19±0.04	6.48±0.12	
	6	7.26±0.00	6.55±0.00	6.64±0.03	7.96±0.24	7.15±0.03	7.29±0.33	
	8	7.96±0.24	7.12±0.01	7.13±0.00				
	10	8.14±0.00	7.33±0.01	7.33±0.01				
	12	8.17±0.23	8.14±0.00	8.12±0.00				
							P < 0.05	

Key: values are mean ± SE of triplicates, UID = uninoculated “Daddawa”, ID = inoculated “Daddawa”, FPD = freshly purchased “Daddawa”, AT = ambient temperature, SA = statistical analysis, NSD = no significant difference, SD=significantly different

Table 5: Changes in Aerobic mesophilic Bacterial count of inoculated, uninoculated and freshly purchased soybean “Daddawa” under different storage conditions.

Storage time(days)	Oven			Ambient temperature			SA
	UID	ID	FPD	UID	ID	FPD	
0	6.45±0.53	6.12±30	5.96±1.19	6.62±0.57	6.51±0.56	6.62±0.45	P > 0.05
2	6.57±0.55	6.40±0.57	6.53±0.58	6.69±0.55	6.54±0.57	6.67±0.54	
4	6.64±0.57	6.55±0.57	6.55±0.56	6.71±0.57	6.65±0.57	6.70±0.53	
6	6.67±0.56	6.64±0.57	6.56±0.56	6.65±0.59	6.73±0.57	6.70±0.53	
8	6.71±0.56	6.65±0.58	6.61±0.57				
10	6.73±0.57	6.66±0.57	6.71±0.56				
12	6.75±0.58	6.68±0.57	6.72±0.56				
							P > 0.05

Key=values are mean±SE of triplicates, UID=uninoculated “Daddawa” ID=inoculated “Daddawa”, FPD= freshly purchased “Daddawa”, SA=statistical analysis NSD=No significant difference (P > 0.05).

Table 7: Detection of some bacterial Contaminants at 6 and 12 days of storage

Stored Products	Days	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Salmonella</i> sp	No. of isolates
Oven	UID	6	-	-	0
		12	-	+	1
	ID	6	-	-	0
		12	+	-	1
	FPD	6	-	-	0
		12	+	+	3
HC	UID	6	-	+	2
	ID	6	+	-	1
	FPD	6	+	-	1

Key= UID = uninoculated *daddawa*, ID = inoculated "*Daddawa*" FPD = freshly purchased "*Daddawa*", HC = humidity chamber, += Positive, - = Negative.

CONCLUSION

The isolation of *Bacillus subtilis* from previously fermented soybean "*daddawa*" was carried out. The proximate composition of all products when compared shows that they are significantly different with inoculated "*daddawa*" having the highest composition of ash, protein, moisture and fibre. Carbohydrate and fat are higher in freshly purchased "*daddawa*" and uninoculated "*daddawa*" respectively. The products were characterized by increase in pH throughout the storage period. The temperature of the oven recorded was 27±00 and that of humidity chamber reached 28.33±0.33. The aerobic mesophilic bacterial count observed was found to increase throughout the storage period. Some bacterial contaminants were detected at 6 and 12 days of storage which include *E. coli*, *Staph aureus* and *Salmonella*. Sensory properties of the soybean *daddawa* after 48hrs of fermentation with *Bacillus subtilis* revealed 100% likeness by all the judges. Soybean "*Daddawa*" should be stored under dry conditions to prevent moisture absorption and extend its shelf life. The use of a standardized *Bacillus subtilis* starter culture is recommended for industrial Production to ensure consistent product quality, safety, and fermentation efficiency. Adequate drying of soybean "*Daddawa*" before storage is recommended to reduce moisture content and enhance its storage stability.

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