Vol 7 No 1 March 2026 Pages 59-64 e-ISSN 2708-5694

Journal of Agriculture, Food and Environment (JAFE)

Journal Homepage: https://journal.safebd.org/index.php/jafe

https://doi.org/10.47440/JAFE.2026.7107

Research Article

OPEN ACCESS

Fermenting soymilk with starter culture improves the physiochemical, proximate, and sensory properties of Nigerian soy curd (*Beske*)

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Article History

Received: 28 October 2025

Accepted: 05 January 2026

Published: 31 March 2026

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Keywords

Plant-based food, Food fermentation, Soybean, Lactic acid bacteria, Starter culture

How to cite: Olorunleke Y, Oladipupo AO, Ogunremi OO, Olawoye B, Ogunremi OR 2026: Fermenting soymilk with starter culture improves the physiochemical, proximate, and sensory properties of Nigerian soy curd (*Beske*). J. Agric. Food Environ. 7(1): 59-64.

A B S T R A C T

This study investigated the effect of fermentation of soymilk with starter-culture on the physicochemical, proximate and sensory properties of derived indigenous soy curd. The pH of starter fermented (FSM) and uninoculated (UFSM) soymilks respectively decreased at different rates from 6.56 to 5.57 and 6.07 after 8 hours of fermentation. The total titratable acidity of FSM sample is significantly higher (2.24 g/L) than that of UFSM (0.09 g/L). Greater proportions of carbohydrate (9.85%), protein (20.42%), fat (6.31%) and fiber (0.25%) were present in soy curd derived from starter culture fermented soymilk (FSC). The sensory evaluation panelists preferred FSC to soy curd derived from uninoculated soymilk (UFSC), with an overall acceptability score of 7.9. Specifically, the panelist preferred the appearance, flavor and texture of FSC. The fermentation of soymilk with starter culture improved proximate properties and acceptability of derived soy curd. The starter culture improved the physicochemical properties of the soymilk used to produce the soy curd. Therefore, the development and use of starter culture is required for the production of high-quality and safe soy curd.

INTRODUCTION

Soybean is among the most valuable and versatile legumes worldwide. The high demand for soybeans stems from its consumption in fermented forms like soy sauce, miso, and tempeh. The fermentation process involves microorganisms such as moulds, yeasts, and bacteria, breaking down complex substrates, which can enhance flavor, nutritional value and health benefits of the products, while serving as a preservation method (Elhalis *et al.*, 2024). However, traditionally fermented soybean products, that are mostly produced by spontaneous fermentation may pose certain risks, including the presence of food borne pathogens, elevated levels of mycotoxins and biogenic amines.

Soy curd is one of most common traditional foods made from soybean (Li *et al.*, 2021). Traditional soy curd is locally known as *Awara*, *Beske* and *Wara soya* in Nigeria. It has a smooth and firm texture, with a rich complement of flavour, nutritive and bioactive compounds, including organic acids, alcohols, esters, hydrolyzed proteins, essential amino acids, omega-6 polyunsaturated fatty acids, saccharides, B-group vitamins, minerals and isoflavones (He *et al.*, 2020; Cai *et al.*, 2021; Liu *et al.*, 2022). *Beske* is commonly consumed as snack or cheap and healthy meat substitutes. Some of the health benefits associated with its consumption are prevention of anemia, obesity, cardiovascular disease and cancer (Raji *et al.*, 2023).

Typically, *Beske* is prepared by the coagulation of soymilk protein with the addition of coagulants. This is followed by

pressing the precipitated protein into a firm and smooth mass that is cooked before consumption (Raji *et al*., 2023). The process is without a fermentation step after filtration. Besides improving the safety and shelf-life of *Beske*, fermentation has the potentials to complement the removal of antinutritional, allergenic and flatulence factors, such as trypsin inhibitor, lipoxygenase, phytic acid, lectin, glycinins and oligosaccharides (Chourasia *et al*., 2022; Elhalis *et al*., 2024; Li *et al*., 2020). Fermentation also offers the benefits of improved sensory attributes and health benefits of *Beske.*

Compared to the traditionally employed spontaneous fermentation process, controlled fermentation using starter cultures is required for predictable and reproducible improvement in quality, safety and stability of *Beske* (Elhalis *et al*., 2024; Sirilun *et al*., 2017). Therefore, the aim of this study is to investigate the effect of fermentation of soymilk with starter culture on the physicochemical, microbiological and proximate properties of derived *Beske*.

MATERIALS AND METHODS

Collection of materials

Soybean (Glycine max) and commercial starter culture (Yogourmet, Lellemand, La Ferté-sous-Jouarre, Paris, France*) was purchased from a local market in Ibadan, Oyo State, Nigeria.*

Production of *Beske* (soy curd)

Soy curds were produced following the modified method of Poysa and Woodrow (2002). *Soybean was sorted and washed to remove dust, dirt and other extraneous materials. Exactly 400 g of* soybean was steeped in water (1:3 v/w) for 12 hours, de-hulled and mill with the same volume of water. *The slurry was* sieved with a sterile muslin and the soymilk milk was boiled for 10 minutes. The soymilk was allowed to cool to 40°C and 500 mL each was measured separately into two different conical flasks. The soymilk in one of the conical flasks was aseptically inoculated with 1.5g of starter culture, mixed thoroughly, and incubated at 30°C for 8 hours for fermentation to occur. The soymilk in the other conical flask was not inoculated. *Samples were taken from both conical flasks at 0,* 2, 4, 6, and 8 hours of fermentation *and subjected to physicochemical and microbiological analyses. After fermentation,* 25 mL of lime fruit juice was gradually added to both soymilks, while stirring until curds were formed. The mixtures were left for 20-30 minutes to allow for complete curd formation. The soy-whey was decanted and the soy-curd was mechanically pressed. The resulting soy curd was cut into 2.0 mm thick circular pieces, *packed into* polyethylene-laminated aluminum foil *bags and stored for further analysis.*

Physicochemical analyses of soymilk

Determination of pH

pH of soymilk samples was determined using a calibrated pH meter (Corning Pinnacle M530 pH meter, Illinois, United States).

Determination of total titratable acidity (TTA)

Total titratable acidity (TTA) was determined using the method of AOAC (2012), *by measuring* 10 ml of soymilk samples and titrating it with 0.1N NaOH to pH 8.3. The volume of NaOH used was recorded for calculating the TTA, using the formular below:

$$TTA\ g/L\ lactic\ acid = \frac{(\text{Volume of NaOH used} \times \text{Molarity of NaOH}) \times 90}{\text{Volume of sample}}$$

Microbiological analyses of soymilk

The enumerations of lactic acid bacteria (LAB) in soymilk samples were carried out by pour plate method using the De-Man Rogosa and Sharpe (MRS) agar and incubated anaerobically at 37 ºC for 48 h. Exactly 1 mL of each soymilk sample was subjected to serial dilution in sterile distilled water and 1 mL of appropriate dilution was aseptically dispensed in Petri dishes. The colonies were counted after incubation, and recorded as colony forming units per milliliter (CFU/mL) (Wang *et al*., 2023).

Proximate analyses of *Beske* (Soy curd)

The proximate composition, expressed in dry weight (g/100 g dry weight (D.W.) of each *Beske* sample was determined as described by AOAC (2012). The parameters determined include moisture, protein, fat, crude fiber, ash and carbohydrate contents. The moisture content was determined by calculating the percentage weight difference of *Beske* after oven drying it at 105°C until constant weight was obtained. The crude protein content was calculated using Kjeldahl method, by multiplying the total nitrogen with a factor 6.25. Fat content was determined after Soxhlet extraction and use of standard methods. Standard methods were used to determine the crude fiber and ash contents, while carbohydrate content was calculated by the difference of the sum of percentage moisture, protein, fat, crude fiber and ash contents from 100%.

Sensory evaluation of *Beske* (Soy curd)

Exactly 10-membered sensory panelists were randomly recruited among students of Abiola Ajimobi Technical University, Ibadan, Nigeria. The eligibility criteria for participation were as prescribed by Olawoye and Gbadamosi (2020). These include history of consumption of *Beske*, absence of history of allergy or intolerance to the *Beske* and willingness and availability to participate by completing appropriate consent forms. The panelists rated the *Beske* samples for color, appearance, flavor, texture, and overall acceptability based on a nine-point Hedonic scale (9 and 1 representing "like extremely" and "dislike extremely" respectively). Panelists were provided with water to rinse their mouth before evaluating a new sample (Iwe *et al*., 2017).

Statistical analysis

The data obtained from the observations were analyzed statistically and presented as mean with standard deviation of replicate values. Data obtained from sensory studies were subjected to ANOVA and the significant differences were compared using Tukey's HSD test. Values of $P < 0.05$ were considered statistically significant. The statistically package used is SPSS version 23 software.

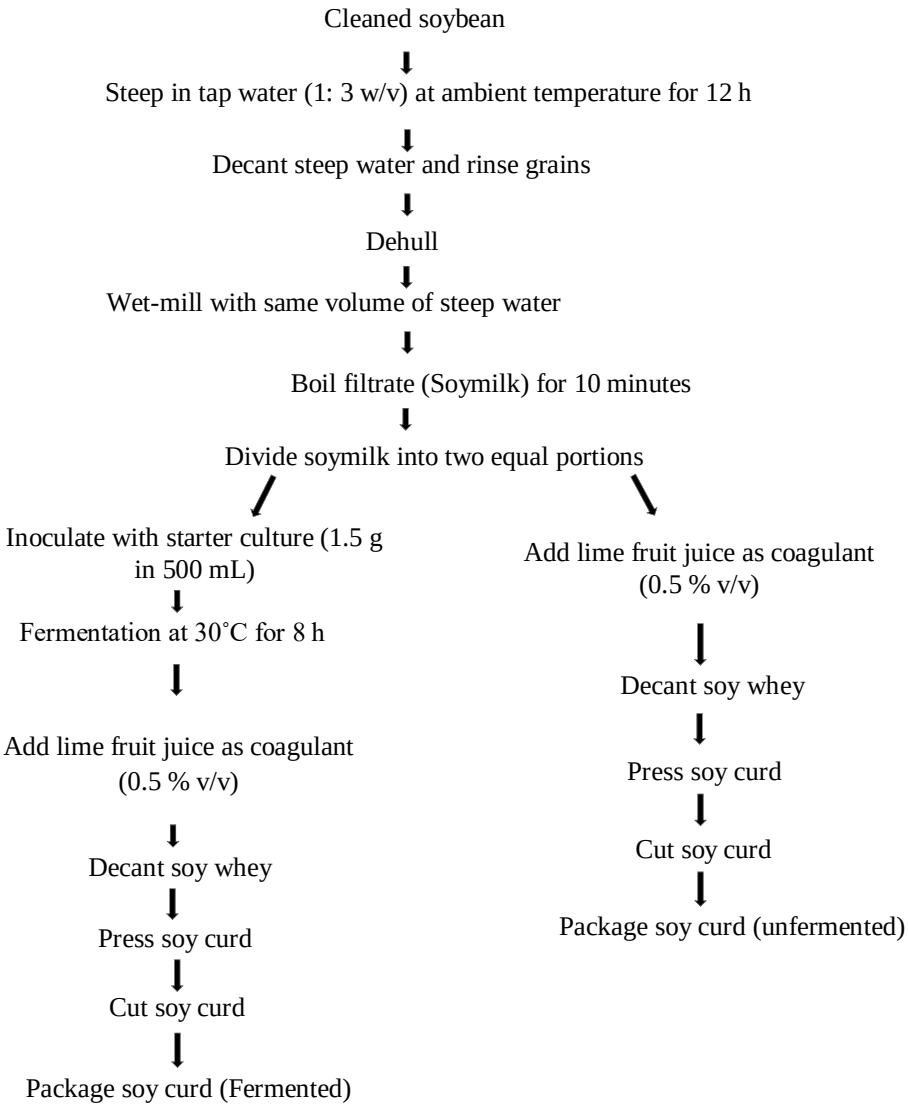

Figure 1: Flow chart for the production of *Beske* (soy curd) from fermented and unfermented soymilk

RESULTS AND DISCUSSION

Physicochemical properties of soymilk

The metabolism of sugars in food substrate by LAB derived enzymes have the potentials to produce organic acids, particularly lactic acid. This results to the acidification and reduction in the pH of the product. The pH of both inoculated and uninoculated soymilks reduced significantly from pH 6.56 to pH 5.57 and pH 6.07 respectively after 8 hr (Fig. 2). Production of organic acid was also demonstrated by significant increase in the total titratable acidity of soymilk fermented with starter culture over 8 hr (Fig. 3). Similar decrease in pH and increase in TTA have been previously reported during lactic fermentation of soy substrates to produce soy-based products. Probiotic strains of LAB decreased the pH of soymilk from about pH 6.8 to less than pH 5.5 over different period of fermentation, ranging from 9.5 hours to 22 hours (Li *et al*., 2012). The use of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* alone or in combination to ferment soymilk for 12 hours reduced the pH of the fermented soymilks to less than pH 4.5 (Zong *et al*., 2022). Obadina *et al*. (2013) reported the reduction in the pH of spontaneously fermented soymilk from pH 6.90 to pH 4.09 after 72 hours. The TTA increased significantly in all the instances of decreased pH (Li *et al*., 2012; Obadina *et al*., 2013; Zong *et al*., 2022). Increased acidity contribute to improved microbiological safety, extended shelf-life and enhanced sour flavour of fermented food products (Allwood *et al*., 2021). This substantial increase in TTA during fermentation indicates microbial conversion of sugars into organic acids, underscoring the crucial role of fermentation in modifying acidity, which subsequently affects the curd's texture, flavor, and shelf life (Singh *et al*., 2021).

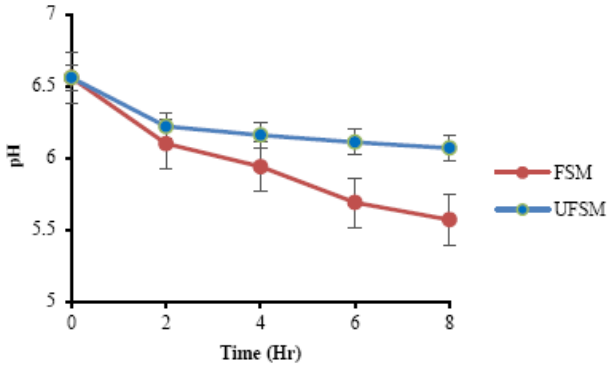

Figure 2: pH of soymilk used for soy curd production

FSM- Fermented soymilk, UFSM- Unfermented soymilk

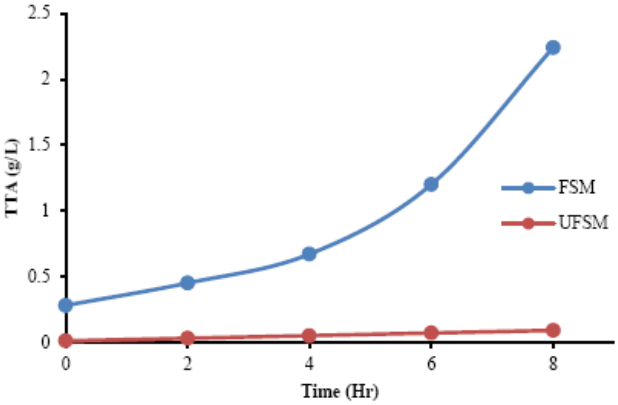

Figure 3: Total titratable acidity (TTA) of soymilk used for soy curd production. FSM- Fermented soymilk, UFSM- Unfermented soymilk

Microbiological properties of soymilk

The LAB counts of both fermented soymilk and unfermented soymilk increased over the fermentation period (results not shown). It was particularly higher in the sample inoculated with starter culture, implying a more robust microbial activity, hence breaks down soy proteins to release bioactive peptides known for their various health benefits and desirable flavour (Saad *et al.*, 2025).

Proximate properties of Beske (Soy curd)

Enzymes can break down soy curd through biochemical processes, yielding various fatty acids, amino acids, alcohols, organic acids, aldehydes, and esters. These compounds contribute desirable aromas and textures, and also cause significant physicochemical changes in the fermented curd (He *et al.*, 2022; Liu *et al.*, 2022; Xu *et al.*, 2020). Proximate composition of fermented (FSC) and unfermented soy curd (UFSC) as presented in Table 1 shows notable differences across several components, reflecting the impact of fermentation on nutritional content. The unfermented soy curd (UFSC) has a higher moisture content (63.81%) and ash content (3.74%) compared to the fermented one. On the other hand, the fermented curd (FSC) shows higher carbohydrate (9.85%), protein (20.42%) and fat content (6.31%). These differences suggest that fermentation enhances the carbohydrate, protein and fat levels likely due to increased microbial activity that breaks down complex food compounds and releases more bioavailable forms (Wang *et al.*, 2023b). The microbial populations generate proteolytic enzymes that hydrolyze these proteins into peptides and free amino acids, thereby enhancing digestibility and contributing antioxidant activity (Sanjukta and Rai, 2016). The slight increase in ash content in fermented soy curd may have resulted from the greater mineral retention after fermentation. These results highlight the nutritional enhancement that fermentation can offer, making fermented soy curd a potentially better option for protein enrichment in diets.

Table 1: Proximate composition of *Beske* (soy curd) produced from fermented and unfermented soymilk

Sample	Moisture (%)	Carbohydrate (%)	Protein(%)	Fat (%)	Fiber (%)	Ash (%)
FSC	59.82 ±0.04	9.85 ±0.02	20.42 ±0.06	6.31±0.03	0.25±0.02	3.41±0.04
UFSC	63.81±0.04	7.95 ± 0.02	18.63±0.05	5.63±0.02	0.21±0.02	3.74±0.02

FSC- Fermented soy curd, UFSC: Unfermented soy curd

Table 2: Sensory evaluation of *Beske* (soy curd) produced from fermented and unfermented soymilk

Sample	Colour	Appearance	Flavor	Texture	Overall acceptability
FSC	7.8±0.70	7.6±1.00	7.8±1.13	7.6±1.07	7.9±0.74
UFSC	8.0±0.80	7.4±1.10	7.2±1.23	7.0±1.22	6.7±0.73

FSC- Fermented soy curd, UFSC: Unfermented soy curd

Sensory properties of *Beske* (Soy curd)

The sensory evaluation shows a preference for fermented soy curd, especially in flavor, texture and overall acceptability (Table 2). This support previous findings that fermentation improves sensory attributes by enhancing flavor complexity and texture (Liu *et al.*, 2022; Tamang *et al.*, 2020) with more favorable product overall (Yao *et al.*, 2021). The flavor profiles of fermented soy curds are shaped by a combination of physicochemical conditions and the microorganisms present during fermentation. The findings of this study offer valuable insights for understanding FSC flavor and can guide the industry in selecting optimal production strains to enhance flavor quality (Liu *et al.*, 2022).

CONCLUSION

The current research revealed the influence of fermentation of soymilk with starter culture on improving the proximate properties and acceptability of soy curd. The starter culture improved the physicochemical properties of the soymilk used to produce soy curd. Besides, food processing risks mainly stem from contaminants, which are associated with spontaneous fermentation. The development and use of starter culture is required for the production of high-quality and safe soy curd.

Acknowledgement: Authors acknowledge all participants that volunteered to participate in the sensory evaluation.

Ethics Statement: Not applicable. However, consents were obtained from all participants in the sensory evaluation study.

Conflict of Interest: All authors declare that they do not have any conflicts of interest that could have appeared to influence the work reported in this paper.

Data Availability: All data are included in this paper.

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