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ORIGINAL RESEARCH ARTICLE

Fermenting Soymilk with Starter Culture Improves the Physicochemical, Proximate, and Sensory Properties of Nigerian Soy Curd (Beske)

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ABSTRACT

This study investigated the effect of fermentation of soymilk with starter-culture on the physicochemical, microbiological, proximate and sensory properties of derived indigenous soy curd. The pH of starter culture-fermented (FSM) and uninoculated (USM) soymilks respectively decreased at different rates from 6.56 to 5.57 and 6.56 to 6.07 after 8 hours of fermentation. The total titratable acidity (lactic acid equivalent) of FSM sample is significantly higher (2.24 g/L) than that of USM (0.09 g/L). Greater proportions of carbohydrate (9.85±0.02%), protein (20.42±0.06%), fat (6.31±0.03%) and fiber (0.25±0.02%) were present in soy curd derived from starter culture-fermented soymilk (FSC) than in soy curd derived from uninoculated soymilk (USC). The sensory evaluation panelists preferred FSC to USC, in appearance, flavour, texture and overall acceptability. The color score was slightly higher for USC. 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 may contribute to enhanced quality attributes of soy curd.

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1. 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 traditional production of *Beske* is typically without a fermentation step after filtration. However, the incorporation of fermentation offers the potential benefits of improving the safety and shelf-life of *Beske,* and complementing 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). This was demonstrated for some African indigenous foods such as *Kunu zaki* and *Kunu aya* produced from millet and tiger nut respectively (Ogunremi et al., 2022; Ogunremi et al., 2024). Therefore, the aim of this study was to investigate the effect of fermentation of soymilk with starter culture on the physicochemical, microbiological, proximate and sensory properties of derived *Beske.*

2. MATERIALS AND METHODS

2.1 Collecton of materials

Soybean (*Glycine max*) and commercial freeze-dried starter culture (Yogourmet, Lallemand, *La Ferté-sous-Jouarre, Paris, France*) were purchased from a local market in Ibadan, Oyo State, Nigeria. The composition of strains in the starter culture includes Lactobacillus bulgaricus, Lb. acidophilus and Streptococcus thermophilus.

2.2 Production of *Beske* (soy curd)

Soy curds were produced following the modified method of Poysa & 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 w/v) for 12 hours and the steep water was decanted and discarded. The steeped soybean was de-hulled and milled with water equivalent to the volume of the steeping water. The slurry was sieved with a sterile muslin and the soymilk was boiled for 10 minutes. The soymilk was allowed to cool to 40°C and 500 mL was measured separately into each of two different conical flasks. The soymilk (500 mL) 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, 2.5 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 cm thick circular pieces, packed into *polyethylene-laminated aluminum foil* bags and stored at 5*℃* for further analysis within 24 hours. This was carried out in triplicates and two independent trials.

2.3 Physicochemical analyses of soymilk

2.3.1 Determination of pH

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

2.3.2 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 formula below:

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

Where “90” represent the standard correction factor, being the molecular weight of lactic acid.

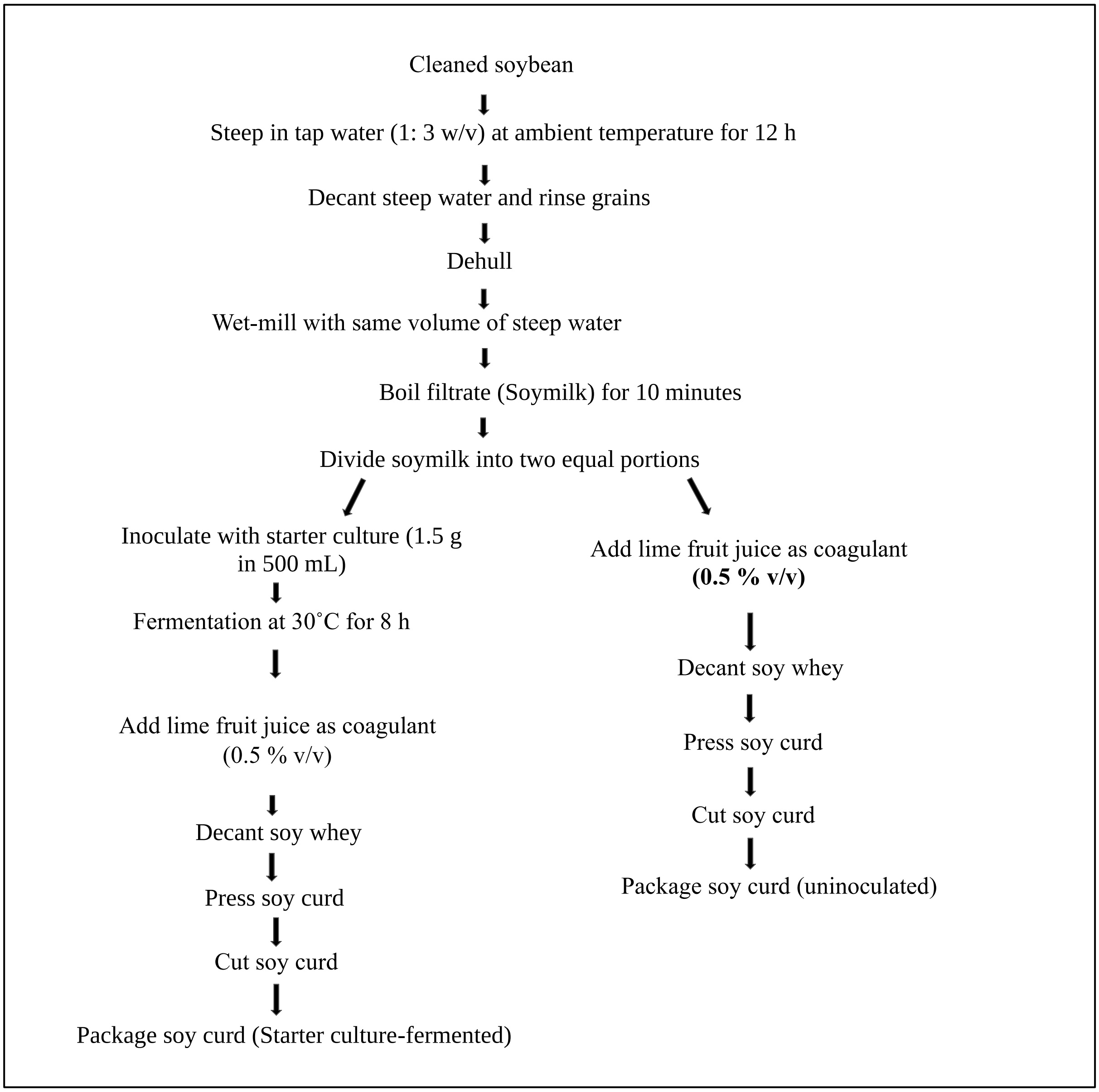

Fig. 1: Flow chart for the production of *Beske* (soy curd) from starter culture-fermented and uninoculated soymilk

2.4 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).

2.5 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%.

2.6 Sensory evaluation of *Beske* (Soy curd)

A 10-member naive sensory panel was 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*, no history of allergy or intolerance to Beske, or intolerance to the *Beske* and willingness and availability to participate by completing appropriate consent forms. The panelists blindly 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).

2.7 Statistical analysis

The data obtained from the observations were analyzed statistically and presented as mean with standard deviation of replicate values. Data obtained from proximate and 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 statistical package used was SPSS version 23 software.

3. RESULTS AND DISCUSSION

3.1 *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 starter culture-fermented and uninoculated soymilks reduced significantly from pH 6.56 to pH 5.57 and pH 6.56 to 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).

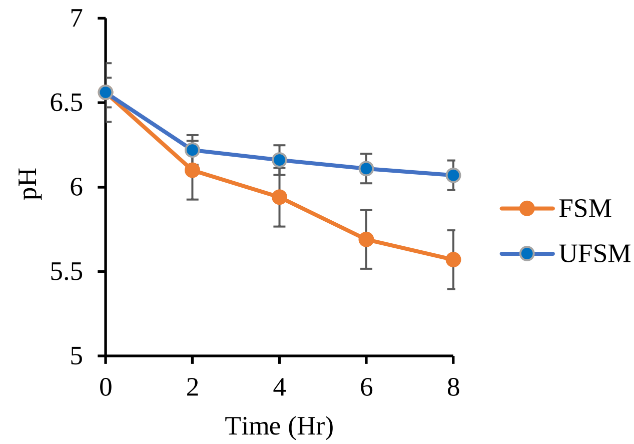

Fig. 2: pH of soymilk used for soy curd production. FSM- Starter culture-fermented soymilk, USM- Uninoculated soymilk

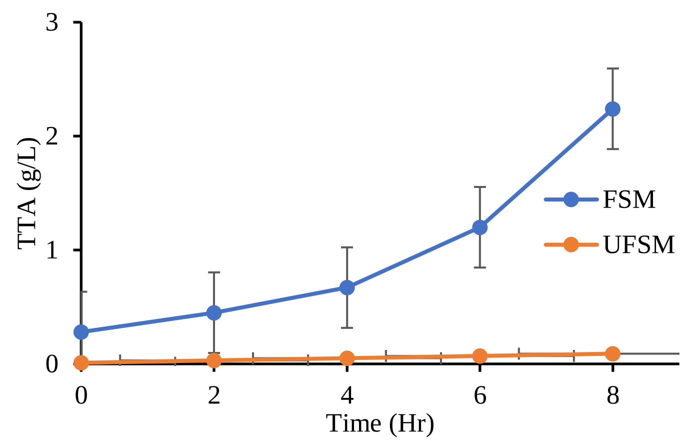

Fig. 3: Total titratable acidity (TTA) of soymilk used for soy curd production. FSM- Fermented soymilk, USM- Uninoculated soymilk

3.2 *Microbiological properties of soymilk*

The LAB counts of both starter culture-fermented soymilk and uninoculated 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 and metabolism of compounds in soymilk.

3.3 *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 starter culture-fermented (FSC) and uninoculated soy curd (USC) as presented in Table 1 shows notable differences across several components, reflecting the impact of fermentation on nutritional content. The uninoculated soy curd (USC) has significantly higher moisture content (63.81%) and ash content (3.74%) compared to the fermented sample. On the other hand, the starter culture-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., 2023). The microbial populations generate proteolytic enzymes that hydrolyze these proteins into peptides and free amino acids, thereby enhancing digestibility and contributing antioxidant activity (Sanjukta & Rai, 2016). 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 starter culture-fermented and uninoculated soymilk

Sample	Moisture (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)
FSC	59.82 ±0.04^{b}	9.85 ±0.02^{a}	20.42 ±0.06^{a}	6.31±0.03^{a}	0.25±0.02^{a}	3.41±0.04^{b}
USC	63.81±0.04^{a}	7.95 ± 0.02^{b}	18.63±0.05^{b}	5.63±0.0^{b}	0.21±0.02^{a}	3.74±0.02^{a}

FSC- Starter culture-fermented soy curd, USC: Uninoculated soy curd. Values are mean±SD of triplicate values. Values not sharing common superscript within a column differ significantly at p<0.05 by Tukey's HSD test.

Table 2. Sensory evaluation of *Beske* (soy curd) produced from starter culture-fermented and uninoculated soymilk

Sample	Colour	Appearance	Flavor	Texture	Overall acceptability
FSC	7.8±0.70^{a}	7.6±1.00^{a}	7.8±1.13^{a}	7.6±1.07^{a}	7.9±0.74^{a}
USC	8.0±0.80^{a}	7.4±1.10^{a}	7.2±1.23^{a}	7.0±1.22^{a}	6.7±0.73^{b}

FSC- Starter culture-fermented soy curd, USC: Uninoculated soy curd. Values represent rating of *Beske* samples based on a 9-point hedonic scale, where "9" and "1" represent "like extremely" and "dislike extremely" respectively. Values are mean±SD of replicate (n=10) values. Values not sharing common superscript within a column differ significantly at p<0.05 by Tukey's HSD test.

3.4 Sensory properties of *Beske* (Soy curd)

The sensory evaluation shows a significant preference for fermented soy curd, especially in 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's sensory attributes and can guide the industry in selecting optimal production strains to enhance 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 use of a defined starter culture improved selected physicochemical, proximate and sensory attributes of Beske in this study and may contribute to more consistent quality. Further work, including comprehensive microbial safety assessment, is needed before firm recommendations can be made. Further limitation that to highlight that this study is a proof of concept include the use of a single type of starter culture, limited replicates and a 10-membererd naive sensory panel. Therefore, this provides a basis for more robust studies on the application of starter culture in indigenous food fermentation.

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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.

ETHICS STATEMENT

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

DATA AVAILABILITY

The data used to support the findings of this study are available upon reasonable request from the corresponding author.

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