



Controlled fermentation with starter culture enhanced the physicochemical and microbiological properties of soy-yoghurt



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Abstract

This study investigates the effects of controlled fermentation using a specific starter culture on some properties of soy-yoghurt, focusing on the physicochemical and microbiological properties after production and storage. The results showed that the starter culture improved both the physicochemical properties and shelf life of soy-yoghurt. Specifically, pH dropped in controlled fermented soy-yoghurt (CFSY) from 6.18 to 5.40 after 8 hours of fermentation, whereas spontaneously fermented soy-yoghurt (SFSY) had a slight pH decrease from 6.51 to 6.04. Fermentation influenced significant increase in Total Titratable Acidity (TTA) for CFSY from 0.38 to 2.08 g/L, while the TTA of SFSY only slightly increased from 0.04 to 0.10 g/L. Proximate analysis revealed that SFSY has higher moisture content (87.09%), crude fat (3.16%), crude fibre (0.05%) and carbohydrate (5.46%) compared to CFSY. Sensory evaluations showed that while SFSY scored higher in colour, while CFSY scored higher in flavour, texture and appearance. During storage, the pH of CFSY declined with respect to storage days, while the TTA increased. In conclusion, controlled fermentation improved the physicochemical, nutritional and sensory properties in soy-yoghurt by enhancing the storage stability of the product.

Keywords:

Soymilk; Fermentation; Starter culture; Sensory evaluation, Yoghurt

1. Introduction

Soybeans (*Glycine max*), a leguminous crop, is widely recognised for its exceptional nutritional profile. It is rich in high-quality plant protein, essential amino acids, dietary fibre, and bioactive compounds such as isoflavones, which have been linked to cardiovascular health, cancer prevention, and hormonal balance (Falcao et al., 2019). Due to its versatility and nutrient density, soybean has become a cornerstone of plant-based diets globally.

One of the most prominent derivatives of soybean is soymilk, an aqueous extract obtained by soaking, grinding, and straining soybeans. Soymilk serves as a lactose-free alternative to dairy milk and is suitable for individuals with lactose intolerance, dairy allergies, or those following vegan diets (Liu et al., 2025). In its unfermented form, soymilk contains valuable nutrients, however, it harbours anti-nutritional and flatulence factors such as phytic acid, trypsin inhibitors, and oligosaccharides, which can impair nutrient absorption and cause digestive discomfort (Liu et al., 2025). Other important limitations to soymilk, include proneness to rapid deterioration and beany flavour (Falade et al., 2015; Pahane et al., 2017).

To enhance the nutritional and functional properties of soymilk, fermentation has emerged as an affordable, energy efficient and sustainable value addition process. Using beneficial microorganisms, particularly lactic acid bacteria, fermentation removes flatulence factors, mask or remove unwanted flavour and enriches soymilk with bioactive metabolites such as aglycone isoflavones, γ -aminobutyric acid (GABA), and organic acids (Sun et al., 2022). These modifications have the potentials to enhance the digestibility, flavour, texture, safety and shelf stability of soy-yoghurt. Besides, it confers antioxidant, anti-inflammatory, and antidiabetic benefits on soy-yoghurt. However, most traditional fermentation processes are uncontrolled, being carried out spontaneously, with the

risk of generating products with inconsistent and unappealing quality, short shelf-life and safety concerns (Adekoya et al., 2017; Sanni et al., 1999). Change from spontaneous to controlled fermentation with indigenously developed or appropriate commercially available starter cultures is required to mitigate these challenges. This study, therefore, aims to investigate the effect of controlled fermentation on the physicochemical, nutritional, microbial and sensory properties of derived soy-yoghurt.

2. Materials and Methods

2.1 Sample collection

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

2.2 Production of controlled fermented soy-yoghurt (CFSY) and spontaneously fermented soy-yoghurt (SFSY)

Firstly, soymilk was produced following a modified method described by Ani et al. (2018). Briefly, Soybean (1 kg) was cleaned and soaked for 24 hours in 4 L of warm distilled water in a ratio of 1:4. After soaking, the soybean was drained, de-hulled and subsequently blanched at 100 °C for 5 min. Following blanching, the soybean was milled with one litre of water using a portable Bajaj blender. The slurry obtained after milling was filtered using sterile muslin cloth, and the extract (soymilk) was obtained.

Soy-yoghurt was produced following a modification of the method described by Ani et al. (2018) as presented in Figure 1. Briefly, soymilk was first pasteurized at 90 °C for 6 mins, allowed to cool (40 °C) and 500 mL each was

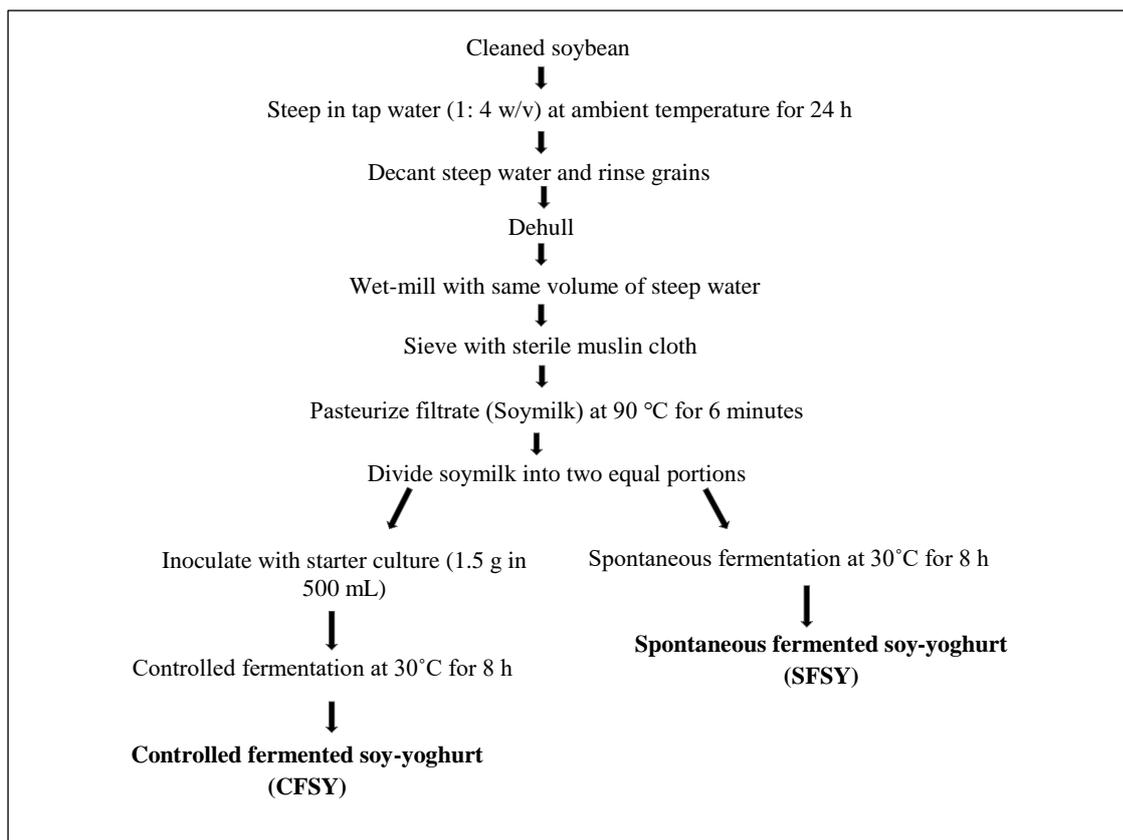


Figure 1: Flow chart for the production of soy-yoghurt

measured separately into two different conical flasks. For controlled fermentation, the soymilk in one of the conical flasks were aseptically inoculated with 1.5g of starter culture, mixed thoroughly, and incubated at 30°C for 8 hours for fermentation to occur. For spontaneous fermentation, the soymilk in the other conical flask was not inoculated but allowed to incubated at 30°C for 8 hours. Samples were taken from both conical flasks at 0 and 8 hours of fermentation and subjected to further analyses.

2.3 Physiochemical analyses of soy-yoghurt samples

2.3.1 pH

The pH of the samples was determined following AOAC (2005). The pH meter (Corning Pinnacle M530 pH meter, Illinois, United States) was first calibrated using a 4.0 and 7.0 pH buffer solution and rinsed with water. The pH meter was then put in 20 mL of the milk samples, and reading was recorded following stabilisation.

2.3.2 Total titratable acidity (TTA)

The method described by AOAC (2005) was used to determine the TTA of the milk samples. Five (5) mL of the milk sample was dispensed into a 250 mL conical flask containing two drops of phenolphthalein as an indicator. The mixture was titrated against 0.1 N NaOH until a pink colour persisted. The acidity of the milk samples was reported as percentage lactic acid as shown in the equation below:

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

2.4 Enumeration of microorganisms

One (1) mL of each sample was aseptically transferred into a test tube already containing nine (9) mL of sterile peptone water and shaken vigorously. The sample solutions were further serially diluted until a 10^6 dilution was obtained. From appropriate serial dilutions, a 0.1 mL aliquot was taken and spread-plated on the surface of solidified nutrient agar (NA), MRS agar and MacConkey agar (MCA), for the cultivation of total bacteria, lactic acid bacteria and coliforms, respectively. For total mould count, 0.1 mL of appropriate serial dilutions were spread-plated onto potato dextrose agar (PDA) that was supplemented with antibiotics to prevent bacterial growth. The NA and MacConkey agar plates were incubated for 24 hrs at 37°C, MRS agar plates were incubated anaerobically for 48 hrs at 37°C, while the PDA plates were incubated at 25 °C for 96 hrs. The colonies were counted after incubation, and recorded as colony forming units per milliliter (CFU/mL) (Zong et al., 2022).

2.5 Proximate composition of soy-yoghurt samples

The proximate compositions of the samples were determined in triplicate using the standard procedures of the Association of Official Analytical Chemists (AOAC, 2005). Moisture content, crude protein (Kjeldahl method), crude fat (solvent extraction), crude fibre and ash were determined using standard methods (AOAC, 2005). The carbohydrate content was determined by difference, that is, the addition of moisture, crude fat, crude protein, ash and crude fibre, which was subtracted from 100%. Carbohydrate. Total carbohydrate content was determined by the difference.

Carbohydrates = (100 – (%Crude Proteins + %Lipids + %Ash + %crude fibre + %moisture)).

2.6 Sensory evaluation of soy-yoghurt samples

The method described by Olawoye et al. (2023) was used in evaluating the sensory properties of the soy-yoghurt samples. Twenty semi-trained panellists who are students of Abiola Ajimobi Technical University and familiar with soy-yoghurt were recruited to evaluate the soy-yoghurt samples. The panellists assessed the soy-yoghurt based on a 9-point Hedonic scale for its colour, flavour, texture, appearance and overall acceptability.

2.7 Determination of storage stability of soy-yoghurt samples

The soy-yoghurt samples were stored at 4 °C for 7 days while the pH and TTA of the samples were monitored.

2.8 Statistical analysis

The data obtained from the study were analysed 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 using XLstat ver. 21 (Addinsoft Inc. USA).

3. Results and Discussion

3.1 Physicochemical properties of the soy-yoghurt samples

Table 1 shows the results of the physicochemical properties of the CFSY and SFSY samples, with initial pH 6.18 and 6.51, respectively at 0 hours. The reduction in pH of both soy-yoghurt samples in the course of fermentation is due to the metabolic activities of the fermenting microorganism. A similar trend of pH reduction was also reported by Ani et al. (2018) for fermented soymilk. More significant reduction in the pH was recorded for CFSY compared to SFS. This could be due to the variation in the fermenting microorganism.

The fermentation process to produce CFSY was dominated by strains of lactic acid bacteria (LAB) that constitute the starter culture. LAB metabolise sugars present in food substrates to produce lactic acid and CO₂, thereby reducing the pH of the fermented product. These reports align with the findings of Zong et al. (2022), who also reported a decrease in the pH of soymilk and milk mixture as the fermentation time prolongs. Fukuda et al. (2017) also reported a pH drop from 6.50 to 5.90 over a similar fermentation period.

Table 1 shows the result of the total titratable acidity of the CFSY and SFSY samples. Unlike pHs, the TTAs increased, with a significantly higher increase recorded for CFSY from 0.38 to 2.08 g/L after 8 hrs of fermentation. This indicates the significant production of lactic acid by the starter culture. This finding is consistent with findings of Basharat et al. (2020), who observed an increase in the TTA of fermented soymilk from 0.35 to 2.15 after 8 hours of fermentation. The increased TTA is expected to contribute to the characteristic sour taste and shelf stability of soy-yoghurt, which is desirable attributes for consumers. A slight increase in TTA for SFSY from 0.04 to 0.10 g/L was recorded. An almost similar TTA increase from 0.05 to 0.12 was also reported by Li et al. (2025),

after spontaneous fermentation of soymilk for 10 hrs. The minimal change has implications for safety concerns, rapid deterioration and unappealing flavour in the soy-yoghurt. This underscores the importance of optimal fermentation, using starter culture to significantly boost acid content and enhance both flavour and preservation properties of fermented product (Ogunremi et al., 2017).

3.2 Microbial counts in soy-yoghurt samples

Table 1 shows the result of the enumeration of microorganisms during fermentation of soymilk to produce CFSY and SFSY. The total bacterial counts (TBC) of CFSY and SFSY at the onset of fermentation was not within detectable limit. However, after 8 hours of spontaneous and controlled fermentation, the TBC was 1.62 and 1.63 log CFU/mL, respectively. These relatively low values are because the soymilk was subjected to a pasteurization-like treatment before fermentation.

The TBCs in this study are consistent with the values reported by Li et al. (2014), where 1.6–1.8 log CFU/mL TBCs were counted from fermented soymilk. The LAB counts in CFSY was significantly higher than the counts in SFSY. Coliform was not detected in both samples.

3.3 Proximate composition of the soy-yoghurt samples

The results of the proximate composition of SFSY and CFSY are shown in Table 2. The result revealed that SFSY has higher contents of moisture (87.09%), crude fat (3.16%), crude fibre (0.05%) and carbohydrate (5.46%) compared to CFSY. The crude protein and ash content were, however, found to be higher in CFSY. The significantly higher protein content is suggested to be due to the accumulation of LAB biomass and breakdown of complex proteins into simpler peptides, which are more digestible. In agreement with this study, Obadina et al. (2013) reported that fermentation increased protein availability in soy products.

The ash contents indicate the minerals available in the soy-yoghurts. The activities of enzymes produced by microorganisms and the evolving conditions during fermentation brings about the liberation and availability of minerals, especially from antinutritional compounds (Ogunremi et al., 2020).

The reduction in the carbohydrate content of the fermented soymilk could be attributed to the utilisation of carbohydrate as a source of energy for the fermenting microorganisms (Ghumman et al., 2016). Similar results have been reported by Akinola et al. (2015), where the moisture content of fermented soy milk decreased slightly, as the fermentation process typically reduces water retention. However, Niamah et al. (2017) reported a larger reduction in moisture, suggesting that the strain of microorganisms can influence moisture levels due to water metabolism.

A slight increase in fat content is observed in this study, which aligns with the findings of Olasupo et al. (2010), who also reported increased fat content after fermentation. They attributed this to microbial synthesis or the release of bound fat during fermentation. However, Akabanda et al. (2010) in their study reported a slight decrease in fat content after fermentation, likely due to differences in fermentation time or microbial strains.

Table 1: Physicochemical chemical properties of soy-yoghurt

Parameter	0 hr	8 hr	
	0 hr	SFSY	CFSY
pH	6.35±0.17	6.04±0.07	5.40±0.09
TTA (g/L)	0.21±0.07	2.08±0.22	3.80±0.15
TBC (log CFU/mL)	ND	1.62± 0.27	1.63± 0.16
LABC (log CFU/mL)	-	2.15 ± 0.04	7.25± 0.21
Coliform (log CFU/mL)	ND	ND	ND

TTA: Total titratable acidity, TBC: Total bacterial count, LABC: Lactic acid bacterial count, ND: Not detected; SFSY: Spontaneously fermented soy-yoghurt, CFSY: Controlled fermented soy-yoghurt

Table 2: Proximate composition of spontaneously and controlled fermented soy-yoghurts

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)	Carbohydrate (%)
SFSY	87.09±0.049	3.73±0.085	2.92±0.036	0.77±0.022	0.054±0.0029	5.46±0.0467
CFSY	86.75±0.036	4.23±0.064	3.16±0.022	0.68±0.022	0.020±0.0022	5.18±0.0262

SFSY: Spontaneously fermented soy-yoghurt, CFSY: Controlled fermented soy-yoghurt, Values are given as means ±SD

3.4 Sensory evaluation of soy-yoghurt samples

The results of the sensory evaluation of the soy-yoghurt samples are shown in Figure 2. The results revealed that fermentation enhanced the colour, flavour, texture and overall acceptability of the soy-yoghurt. This enhancement of flavour and texture could be due to the respective production of flavour compounds, particularly organic acids and texturizing compounds, particularly exopolysaccharides, by the fermenting microorganism during the fermentation process (Ogunremi et al., 2022). The relatively higher scores attributed to CFSY by the panellists are an indication that fermentation did not significantly diminish visual appeal. This is consistent with Kim et al. (2012), where the colour of fermented soymilk was similarly rated positive (7.8 on a 9-point scale), as the fermentation process typically does not drastically change colour unless pigment-producing strains are used. The preference for CFSY in terms of flavour in this study aligns with findings by Zong et al. (2022) due to the sourness introduced by lactic acid bacteria. This sour taste tends to be preferred by consumers who are more accustomed to the sourness and tanginess of foods as suggested by Giri and Mangaraj (2012). The overall acceptability scores of the soy-yoghurt are an indication of the acceptance of soy-yoghurt by the panellists.

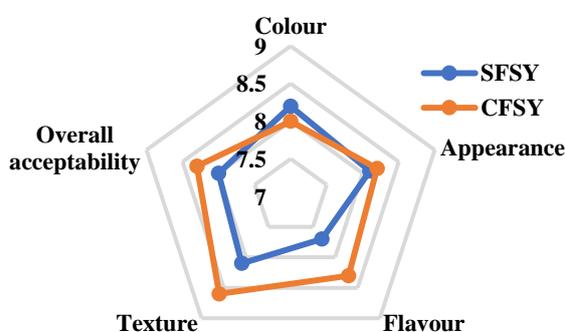


Figure 2: Sensory evaluation of soy-yoghurt.
SFSY: Spontaneously fermented soy-yoghurt, CFSY: Controlled fermented soy-yoghurt

3.5 Changes in the pH of stored soy-yoghurt samples

The result of the effect of storage on the pH of the soymilk samples is shown in Figure 3. The result revealed that the pH of CFSY decreases from 5.40 on Day 1 to 4.50 on Day 7, indicating continued microbial metabolism and production of organic acids during storage. This trend is similar to findings from Mäkinen et al. (2017), where the pH of fermented soymilk decreased to 4.40 over 7 days of storage. They attributed the continuous decrease in the pH value with respect to storage days to the metabolic activity of lactic acid bacteria (LAB), even post-fermentation, which contributes to further product acidification and improves shelf life by inhibiting spoilage microorganisms. There was also a slight decrease in the pH value of SFSY over the period of storage. This could be attributed to the metabolic activities of natural microflora of the product.

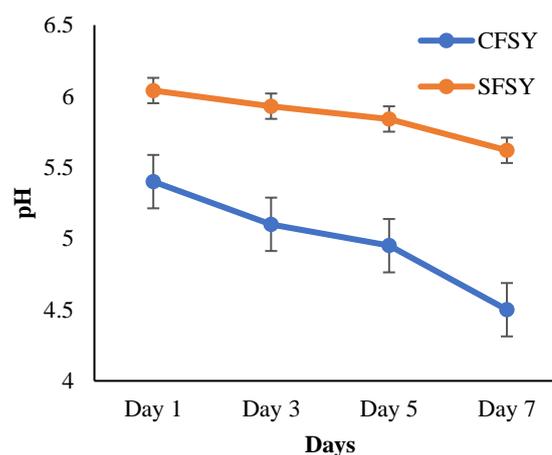


Figure 3: Changes in the pH of soy-yoghurt samples over 7 days of storage at 4°C.

SFSY: Spontaneously fermented soy-yoghurt, CFSY: Controlled fermented soy-yoghurt

Conclusion

The fermentation of soy milk using a selected starter culture significantly enhances its physicochemical, microbiological, sensory and storage stability properties.

The fermentation process lowered the pH and increased the acidity, thereby improving flavour and texture, while enhancing the digestibility and bioavailability of proteins and nutritionally important minerals. The reduction of anti-nutritional factors, such as phytic acid, further contributes to its nutritional value. These findings suggest

that controlled fermentation of soymilk, using starter culture is a viable strategy to obtain predictably and consistently nutritious, safe, shelf-stable and appealing soy-yoghurt. This findings of this study will contribute to the further development of the dairy beverage alternative industry.

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