

Sensory and nutritional evaluation of soy yoghurt fermented with indigenous lactic acid bacteria

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ABSTRACT

This study evaluated the sensory properties and nutritional composition of soy yoghurt produced using a combination of indigenous lactic acid bacterial strains, focusing on improving protein content, viscosity, and overall consumer acceptability. Lactic acid bacteria were isolated from 24-hour fermented cow milk using the pour plate method and incubated at 37°C for 48 hours. The isolates were characterized based on cultural, morphological, physiological, and biochemical identification. Eleven (11) treatments were prepared from combinations of starter cultures. The soy yoghurt was fermented at 30°C for 12 hours and then stored at 4°C for proximate analysis and sensory evaluation. The proximate composition of soymilk (moisture, fat, carbohydrate, ash content, and protein) was 91.82%, 8.07%, 14.95%, 0.80%, and 5.28%, respectively. The evaluation of the soy yoghurt's nutritional quality showed that the fat (8.49%), moisture (91.75%), ash (3.75%), and protein content (7.95%) were higher than those of a popular dairy yoghurt brand (8.38%, 84.39%, 1.35%, and 2.45%, respectively). Sensory evaluation revealed that the consistency of SM+Laf2+Lal was preferred to CY. The soy yoghurts produced were creamy in color, with different flavors depending on the type and ratio of the bacterial combinations used. This study demonstrates that soy yoghurt produced using starter cultures from nunu competes favorably with popular dairy yoghurt brands. Furthermore, soy yoghurt, with its high protein content and probiotic properties, could be introduced into public health programs, particularly in regions where dairy consumption is limited or unaffordable.

Keywords: Plant-based yoghurt, soymilk, protein content, carbohydrate content, ash content, sensory evaluation.

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INTRODUCTION

Owing to their potential health benefits, consumers' demand for functional foods has increased dramatically in recent years. Improving nutrition and health in underprivileged communities through locally sustainable probiotic distribution remains a significant challenge. Despite their potential in developing nations, the use of staples as probiotic delivery systems has received little attention. To be beneficial, probiotics must contain a sufficient number of live bacteria (10^6 – 10^7 cfu/g), allowing them to thrive in the intestine and withstand the acidic environment of the upper gastrointestinal tract (Arjamand, 2011).

Recent studies on the effects of soy meals and soybeans, which contain various bioactive components,

have gained attention for their potential health benefits. Soybeans provide complete protein (Henkel 2009). A complete protein contains all essential amino acids, which the body cannot produce on its own. Among cereals and legumes, *Glycine max* (soybean) has the highest protein content (40%), followed by other legumes. While cereals contain 8–15% protein, some legumes contain 20–30%. The protein composition of soybeans (40%) is higher and more affordable than that of groundnuts (23%), beef (19%), chicken (20%), and fish (18%).

In Nigeria, the cost of animal protein and milk has risen, making it unaffordable for most yoghurt producers. The growing demand for plant-based food products has

sparked interest in developing dairy alternatives that accommodate various dietary preferences and restrictions.

“Nunu” is a spontaneously fermented milk product from Nigeria and other West African countries, such as Ghana and Burkina Faso that resembles yoghurt. Occasionally, goat’s milk is used instead of cow’s milk. Fresh milk is fermented for 24 hours at room temperature in a covered calabash. Several bacterial species naturally contaminating the milk contribute to nunu’s fermentation. Notable microorganisms include *Lactobacillus acidophilus*, *Streptococcus lactis*, and *Streptococcus cremoris* (Wouters et al., 2002).

Soymilk is a creamy, aqueous extract from soybeans that resembles cow milk in appearance and consistency, though the amino acid profile differs (Dauda and Adegoke, 2014). Soymilk contains about 40% protein, 27% complex carbohydrates, 20% oil, 8% moisture, and 5% minerals. It is affordable, nutrient-dense, and suitable for individuals with lactose intolerance. Soymilk is also used for feeding infants and supplementing the diets of preschool children, young adults, and the elderly (Adebayo-Tayo et al., 2008).

Soy yoghurt is a nutritious fermented food with more calcium and protein than soymilk, thanks to the addition of calcium salts and bacterial cultures during production. It is prepared under anaerobic conditions using soymilk, lactic acid bacteria, and sometimes sweeteners such as fructose, glucose, honey, or raw sugar (Le-Ngoc and Cao, 2000). Soymilk’s distinctive beany flavor, caused by lipooxygenase enzymes, can be unappealing to some consumers. However, studies show that this flavor can be reduced through heating and fermentation (Salminen et al., 1998).

Further research is needed to explore various LAB strains, alone or in combination, to develop a standardized soy yoghurt fermentation process. The random selection of efficient LAB strains from nunu can help ferment soy milk into a nutritious and palatable yoghurt. Soybeans offer an affordable protein source for nursing mothers and infants due to their accessibility and low cost. This study aims to improve plant-based yoghurt formulations, potentially increasing their adoption among health-conscious and low-income populations seeking dairy alternatives.

MATERIALS AND METHODS

Area and duration of study

The study was conducted for a period of 3 months (May-July 2024) in the Microbiology laboratory Rivers State University Nkpolu Oroworukwo Port Harcourt. Sample collection was done at Mammy Market (Bori Camp) situated between latitude 4°51’51” N and longitude 7°0’24” E. After that the bean was kept at 4°C in a clean polyethylene bag until it was needed.

Collection of fresh cow milk

The fresh cow milk was purchased from Fulani women at mammy market military barracks Bori camp in Port Harcourt Rivers State. The fresh samples were collected in sterile Mac Cartney bottles. Within 24 hours the samples were promptly transported in ice packs to the laboratory for analysis.

Isolation of lactic acid bacteria

At room temperature (29±2°C) fresh samples were covered and placed on the laboratory bench through the night. 1 ml of sample was serially diluted up to ten-fold in 0.85% normal saline. Using pour plate method aliquots of 1 ml from 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵ test tubes were poured into De Mann Rogosa and Sharpe (MRS) agar in triplicates. The culture plates were incubated anaerobically for 48 hrs at room temperature (28°C). Discrete colonies that developed were counted and expressed in cfu/ml.

Isolation of pure cultures of isolates

Different distinct colonies from the incubated plates were picked and subcultured on De Mann Rogosa and Sharpe (MRS) agar plates using a sterile wire loop. These were incubated at 37°C for 24 hours for colony development/growth.

Characterization and identification of isolates

The cultural characteristics such as shape, size, pigmentation, opacity, surface, and elevation were observed and recorded. This was followed by microscopic examination of cell types, arrangement, Gram’s reaction, and motility. Following this, the biochemical characteristics of the isolates as regards sugar and other chemical utilization were assessed. The results of the tests were entered into the search dialogue of the online bio-database software “Advanced Bacterial Identification Software (ABIS)” at https://www.tgw1916.net/bacteria_logare.html, revealing the presumed identity of all isolates.

Preparation of soy milk

Soybeans were sorted and cleaned to eliminate stones and damaged seeds. Next, 500 grams of dry soybeans were washed and soaked in 1 liter of distilled water for 12 hours. After soaking, the soybeans were decoated, thoroughly rinsed with distilled water, and boiled in distilled water at 96–100°C for 15 minutes to remove some

lipoxygenase enzymes present in the beans. The drained beans were hand-washed to remove their testa; this was followed by grinding in a sterile food blender into a paste. This paste was diluted with distilled water at a 1:6 ratio. The resulting slurry was filtered through a fine layer of cheesecloth. The soy milk obtained was transferred to a flask and stored at 5°C (Kohli et al. 2016).

Soy yoghurt production

To the produced soy milk, 3% (w/v) sugar and 0.5% (w/v) gelatin were added and thoroughly mixed. The mixture was pasteurized at 60°C for 30 minutes and then cooled to 42°C. A 50 ml portion of this soy milk was transferred into various sterilized beakers labeled 'A' through 'K' as seen in Table 1. To each beaker, soymilk was inoculated with 1 ml (0.5 McFarland standard) of a 24-hour-old

culture of different isolates in various ratios and combinations. The mixture was stirred for two minutes and incubated at 30°C for 12 hours. The resulting soy yoghurt was stored at 4°C until sensory analysis was performed.

Proximate analyses

The yoghurt samples were analyzed for moisture content using the drying method, protein content using the semi-Kjeldahl method, fat content via Soxhlet extraction, ash content by ashing in a muffle furnace at 550°C, and carbohydrate content by difference. These procedures were conducted in the Food Science and Technology laboratory at Rivers State University following the methods outlined by the Association of Official Analytical Chemists (AOAC) 2010.

Table 1. Formulation of soy yoghurt.

Treatment	Components	Combination ratio
A	SM+Laf1	Single
B	SM+Laf2	Single
C	SM+Lym	Single
D	SM+Lal	Single
E	SM+Laf1+Laf2	1:1
F	SM+Laf1+Lym	1:1
G	SM+Laf1+Lal	1:1
H	SM+Laf2+Lym	1:1
I	SM+Laf2+Lal	1:1
J	SM+Lym+Lal	1:1
K	SM+Laf1+Laf2+Lym+Lal	1:1:1:1

SM (Soymilk), Laf1 (*Lactobacillus fermentum* MT186598, *Lactobacillus fermentum* MN907811 (Laf2), Lym (*Lysinibacillus macroides*), Lal (*Lactococcus lactis*).

Sensory evaluation

The different soy yoghurt samples and the commercial yoghurt (control) were stored at 6±2°C until evaluation. A panel of 20 judges familiar with yoghurt consumption but not trained in sensory evaluation assessed the samples. Each sample was labeled and evaluated for taste, color, flavor, mouth feel, thickness, sourness, and overall acceptability using a 9-point Hedonic scale.

Statistical analysis

All analysis was carried out using the Statistical Package for the Social Sciences (SPSS v27). Mean values were compared using Duncan's Multiple Range Test, and differences were evaluated using Analysis of Variance (ANOVA) at a 5% significance level. Data were presented in tables as mean ± standard deviation.

RESULTS

Proximate composition of soymilk

The proximate analysis revealed that the soymilk had a mean moisture content of 91.82 ± 0.03%, total fat of 8.07 ± 0.01%, carbohydrate content of 14.95 ± 0.07%, ash content of 0.80 ± 0.00%, and protein content of 5.28 ± 0.0% (Figure 1).

Identity of isolated lactic acid bacteria

Four lactic acid bacterial isolates were obtained from the 24-hour fermented nunu. These isolates exhibited a range of colony morphologies, from small to medium-sized colonies (Table 2). As shown in Table 3, the isolates demonstrated diverse sugar utilization patterns, fermenting glucose, sucrose, fructose, and lactose.

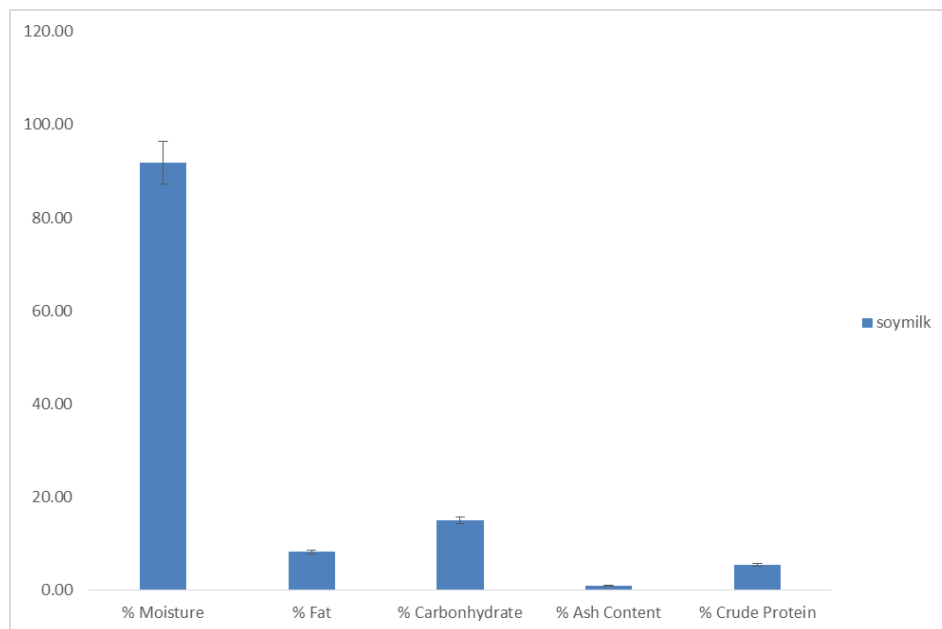


Figure 1. Proximate composition of soymilk.

Table 2. Colonial morphology of randomly selected lactic acid bacteria.

Isolate code	Elevation	Color	Surface	Opacity	Shape	Size (mm)
A	Concave	Creamy	Smooth	Clear	Circular	Small
B	Concave	Creamy	Smooth	Clear	Circular	Medium
C	Concave	Cream	Smooth	Clear	Circular	Small
D	Flat	Cream	Smooth	Clear	Circular	Small

Table 3. Biochemical characteristics of selected lab isolates.

Isolate code	Gram reaction	Shape	Catalase	Oxidase	Mortality	MR	VP	Indole	Sucrose	Fructose	Glucose	Lactose	Gelactose	Starch	Probable organisms
A	+	Rod	-	-	-	+	-	-	+	+	+	+	+	-	<i>Lactobacillus</i> sp
B	+	Rod	-	-	-	+	+	-	+	+	+	+	+	-	<i>Lactobacillus</i> sp
C	+	Rod	+	+	+	-	-	+	+	+	+	-	+	+	<i>Lysinibacillus</i> sp
D	+	Cocci	-	-	-	+	-	-	+	+	+	+	+	-	<i>Lactococcus</i> sp

Key: + positive, - negative.

Proximate composition of yoghurt

As shown in Table 4, Sample 'I' had the highest crude protein content at 7.95%, followed by Sample 'H' with 5.90%, while Sample 'CY' recorded the lowest value at 2.35%. Sample 'A' had the highest moisture content at 91.75%, whereas the control (CY) had the lowest at 84.39%. The highest fat content of 8.48% was observed in Samples 'G' and 'H,' while the lowest fat value of 7.89% was recorded for Sample 'B.' Carbohydrate content ranged from 14.5% (Sample 'A') to 15% (Sample

'CY'). The highest ash content was observed in Sample 'B' (3.75%), followed by Sample 'CY' (1.35%), while Sample 'I' recorded the lowest ash value (0.40%).

Sensory properties of the produced yoghurt

Sensory evaluation results (Table 5) showed that Sample 'I' had the highest mean score of 6.25 for taste. For color, Samples 'I' and 'K' both achieved a high mean score of 7.65, while Sample 'H' had the lowest score at 6.30.

Table 4. Proximate composition of the produced yoghurt.

Treatments	Treatment code	(%) Protein	(%) Moisture	(%) Fat	(%) Carbohydrate	(%) Ash content
CY	CY	2.45±0.07 ⁱ	84.39±0.01 ^e	8.38±0.00b	15.00±0.00 ^a	1.35±0.07 ^b
A	SM+Laf1	2.35±0.07 ⁱ	91.75±0.04 ^a	7.98±0.01 ^g	14.50±0.01 ^g	1.20±0.00 ^c
B	SM+Laf2	5.40±0.00 ^c	91.15±0.01 ^{ab}	7.89±0.00 ^h	14.69±0.01 ^f	3.75±0.07 ^a
C	SM+Lym	4.95±0.07 ^e	90.26±0.00 ^b	8.03±0.03 ^f	14.70±0.00 ^{ef}	0.73±0.04 ^{ef}
D	SM+Lal	5.25±0.00 ^{cd}	91.06±0.00 ^{ab}	8.12±0.01 ^d	14.72±0.01 ^e	0.69±0.01 ^{ef}
E	SM+Laf1+Laf2	3.83±0.04 ^g	87.8±1.13 ^{cd}	8.15±0.01 ^c	14.81±0.01 ^c	0.81±0.01 ^e
F	SM+Laf1+Lym	3.53±0.11 ^h	87.08±0.11 ^d	8.02±0.01 ^f	14.78±0.01 ^d	0.77±0.02 ^{ef}
G	SM+Laf1+Lal	3.69±0.01 ^g	84.53±0.01 ^e	8.49±0.01a	14.79±0.01 ^{cd}	0.64±0.06 ^{ef}
H	SM+Laf2+Lym	5.90±0.00 ^b	84.8±1.13 ^e	8.48±0.00a	14.81±0.01 ^c	1.05±0.07 ^d
I	SM+Laf2+Lal	7.95±0.07 ^a	90.34±0.00 ^b	8.48±0.00a	14.81±0.01 ^c	0.40±0.01 ^h
J	SM+Lym+Lal	4.00±0.14 ^f	88.54±0.00 ^c	8.49±0.00a	14.80±0.00 ^c	0.55±0.07 ^{fg}
K	SM+Laf1+Laf+Lym+Lal	5.15±0.07 ^d	90.86±0.00 ^{ab}	8.08±0.00 ^e	14.88±0.01 ^b	0.50±0.14 ^{gh}
P- value		<0.001	<0.001	<0.001	<0.001	<0.001

Values are means ±SD, n=3, SM (Soymilk), Laf1 (*Lactobacillus fermentum* MT186598), Laf2 (*Lactobacillus fermentum* MN907811), Lym (*Lysinibacillus macroides*), Lal (*Lactococcus lactis*), CY (commercial yoghurt).

* The means reported with the same superscript in each column indicated no significant difference (p≤0.05).

Table 5. Sensory properties of yoghurt samples.

Treatment	Treatment code	Taste	Color	Flavor	Mouth feel	Thickness	Sourness
CY	CY	8.30±0.86 ^a	8.40±0.60 ^a	8.45±0.76 ^a	8.40±0.82 ^a	4.80±2.50 ^{cd}	7.30±1.78 ^a
A	SM+Laf1	5.60±1.27 ^{bc}	7.05±0.69 ^b	5.75±1.16 ^{cd}	5.55±1.50 ^{bc}	4.70±1.59 ^d	6.45±1.70 ^{ab}
B	SM+Laf2	6.10±1.33 ^{bc}	6.70±0.80 ^{bc}	5.45±0.69 ^{cd}	5.15±1.76 ^{bc}	6.20±1.54 ^{ab}	6.25±1.02 ^b
C	SM+Lym	5.60±1.19 ^{bc}	6.55±0.83 ^{bc}	5.65±1.31 ^{cd}	5.05±1.61 ^{bc}	6.30±1.17 ^{ab}	5.90±1.07 ^b
D	SM+Lal	5.65±1.90 ^{bc}	7.05±1.23 ^b	6.15±1.18 ^{bc}	5.55±1.93 ^{bc}	6.30±1.42 ^{ab}	6.30±1.53 ^{ab}
E	SM+Laf1+Laf2	6.05±1.36 ^{bc}	6.80±1.01 ^{bc}	6.35±1.18 ^b	5.70±1.78 ^b	5.80±1.51 ^{ab}	6.40±0.99 ^{ab}
F	SM+Laf1+Lym	5.85±1.09 ^{bc}	6.85±0.67 ^{bc}	6.15±1.35 ^{bc}	5.45±1.57 ^{bc}	5.70±1.26 ^{bc}	6.30±1.34 ^{ab}
G	SM+Laf1+Lal	5.80±1.51 ^{bc}	6.65±0.93 ^{bc}	5.85±1.63 ^{cd}	5.95±1.61 ^b	5.50±1.40 ^{bc}	6.35±1.57 ^{ab}
H	SM+Laf2+Lym	5.70±1.13 ^{bc}	6.30±0.80 ^c	5.90±1.33 ^{bc}	5.75±1.74 ^b	6.20±1.28 ^{ab}	6.25±1.33 ^b
I	SM+Laf2+Lal	6.25±1.62 ^b	7.65±1.09 ^{ab}	5.70±1.53 ^{cd}	5.80±1.70 ^b	6.65±1.35 ^a	6.05±1.43 ^b
J	SM+Lym+Lal	5.25±1.37 ^c	6.90±0.72 ^{bc}	5.50±1.54 ^{cd}	5.40±1.50 ^{bc}	6.10±1.12 ^{ab}	5.65±1.14 ^b
K	SM+Laf1+Laf2+Lym+Lal	5.15±1.31 ^c	7.65±0.88 ^{bc}	4.90±1.33 ^d	4.40±1.76 ^c	5.70±1.34 ^{bc}	4.65±1.93 ^c
P- value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are means ±SD, n=3, SM (Soymilk), Laf1 (*Lactobacillus fermentum* MT186598), Laf2 (*Lactobacillus fermentum* MN907811), Lym (*Lysinibacillus macroides*), Lal (*Lactococcus lactis*), CY (commercial yoghurt).

* The means reported with the same superscript in each column indicated no significant difference (p≤0.05).

Sample 'E' recorded the highest mean flavor score (6.35), while Sample 'K' had the lowest flavor score (4.90). The mean mouthfeel scores ranged from 4.40 (Sample 'K') to 5.95 (Sample 'G'). For gel thickness, Sample 'I' had the highest score (6.65), while Sample 'A' had the lowest (4.70). Sourness was most preferred in Sample 'A' (6.45), with Sample 'K' receiving the lowest score (4.65).

DISCUSSION

The composition of foods significantly influences their physical, nutritional, sensory, and shelf-life characteristics (Zubeir, 2009). The soymilk produced in this study was creamy-white, comprising water, sugar, and salt (Opara et al., 2013). Acidity impacts the product's flavor, shelf life, and microbial stability. Soy yoghurt benefits from the high protein content of soybeans. During fermentation,

lactose, a key carbohydrate in milk, is converted into lactic acid, leading to the reduced carbohydrate content observed in yoghurt (Ihemeje et al., 2015). Ash content reflects the mineral composition, which is essential for the development of teeth, bones, and other bodily functions.

Fermentation produces metabolites that enhance flavor, nutrition, and organoleptic qualities while inhibiting undesirable microorganisms, thereby extending shelf life and improving consumer acceptance. Organic acids, naturally occurring or produced during fermentation, play a vital role in determining the product's stability, nutritional quality, and sensory appeal (Soyer et al., 2003; Karadeniz, 2004). They play significant roles in influencing organoleptic properties, stability, nutrition, acceptability and overall quality (Santalad et al., 2007). Fat content of the yoghurt produced ranged from 7.89-8.49% which was above the standard for low fat yogurts (<3.5%) (Saint-Eve et al., 2008). Although higher fat

content improves sensory properties such as creaminess, it may negatively affect shelf stability (Ndife, 2014; Weerathilake et al., 2014).

The protein content in yoghurt increases due to the proteolytic activity of lactic acid bacteria, which breaks down proteins into smaller peptides and amino acids. The protein content of Samples 'B' through 'K' was within the limits of the CODEX Standard for Fermented Milks (CODEX STAN 243-2003, FAO/WHO 2001). However, Samples 'A' and 'CY' did not meet the recommended minimum of 2.7%.

The highest protein content (7.95%) was recorded in Treatment I (SM+Laf2+Lal), surpassing that of the commercial yoghurt control (CY), which had 2.45%. This demonstrates that the combination of *Lactobacillus fermentum* and *Lactococcus lactis* significantly enhances the protein content of soy yoghurt, making it a nutritionally superior alternative to dairy yoghurt.

Moisture content affects yoghurt texture and mouth feel. Treatment A (SM+Laf1) had the highest moisture content (91.75%), resulting in a more watery product. Conversely, treatments with lower moisture levels (e.g., CY, H, G) produced thicker yoghurts, which consumers generally prefer.

The fat content varied significantly across treatments, with the highest (8.49%) observed in Treatments G, H, I, and J, close to that of the control (CY) at 8.38%. Higher fat content enhances creaminess and mouthfeel, increasing consumer acceptability.

Carbohydrate levels remained relatively consistent across treatments, ranging from 14.5% (Treatment A) to 15% (CY), indicating that fermentation did not significantly alter carbohydrate content. This stability ensures a consistent energy profile in the final product.

Ash content, representing mineral levels, varied significantly across treatments. Treatment B (SM+Laf2) recorded the highest ash content (3.75%), likely due to enhanced mineral uptake during fermentation. Products with higher mineral content offer better nutritional value, making them attractive to health-conscious consumers.

The enhanced protein and fat content in Treatments I (SM+Laf2+Lal) and G (SM+Laf1+Lal) suggests that these formulations provide better mouth feel and nutritional value. These findings highlight the potential of soy yoghurt as a viable alternative to commercial dairy yogurt, particularly for lactose-intolerant individuals and those seeking plant-based options.

CONCLUSION

This study demonstrates the potential of using LAB strains isolated from nunu to produce soy yoghurt with enhanced nutritional quality. The results indicate that specific combinations of LAB strains can significantly improve protein, fat, and ash content in soy yoghurt, contributing to a product with better nutritional value and

sensory appeal. The elevated protein content in the soy yoghurt can be attributed to the inherently high protein content of soybeans. This finding could have important implications for producing affordable and nutritious plant-based yoghurt alternatives, particularly in low-income communities where access to dairy products is limited.

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