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# Improving the Nutritional and Functional Qualities of Fermented Soymilk by *Lactobacillus rhamnosus*

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## ABSTRACT

Fermentation is widely used to enhance the nutritional and functional qualities of soy products. This study evaluated the improvements in soymilk fermented by *Lactobacillus rhamnosus* Yoba. Soymilk was fermented at 40°C for 16 hours, and samples were analyzed for proximate and anti-nutrient composition. The amino acid and phenolic acid profiles were evaluated using Gas Chromatography techniques, while the gamma-aminobutyric acid (GABA) and the isoflavone content were evaluated using High Performance Liquid Chromatography. Results showed that fermentation significantly increased fiber (0.89% to 1.48%), protein (3.15% to 5.25%), and carbohydrate (0.46% to 10.64%) contents, while moisture (88.73% to 75.86%) and ash (and 3.24% to 3.00%) contents decreased ($p < 0.05$). The concentration of six out of the ten anti-nutrients examined namely: Oxalate, Cardiac Glycoside, Phytate, Hemagglutinin, anthocyanin and Cyanogenic glycoside significantly decreased from 0.51mg/g to 0.39mg/g, 6.36% to 5.94%, 0.54% to 0.12%, 0.08mg/kg to 0.05mg/kg, 3.43% to 1.94% and 4.05mg to 3.24mg respectively. Nutritionally, fermentation enhanced six out of the nine essential amino acids namely: histidine, isoleucine, lysine, methionine, threonine, and tryptophan. Fermentation improved GABA from 1.34g/100g to 3.79g/100g. Most free phenolic acids increased, while the glucoside forms of isoflavones were significantly reduced. These findings suggest that fermentation with *Lactobacillus rhamnosus* substantially improves the nutritional and functional qualities of soymilk, supporting the incorporation of well-documented probiotics into food matrices for health benefits.

**Keywords**: soymilk, *Lactobacillus rhamnosus*, fermentation, polyphenols, amino acid, anti-nutrient

## INTRODUCTION

Fermented foods are generally defined as those made through controlled microbial growth and enzymatic conversions of major and minor food components (Maria *et al*., 2017). There are two main techniques for fermenting foods; natural fermentation, often called "spontaneous fermentation," which occurs due to microorganisms naturally present in raw food such as sauerkraut, ukpaka, and some fermented soy products and the other technique which involves adding starter cultures to the food, known as "culture-dependent ferments," such as natto, kefir, and kombucha (Shah *et al*., 2023). For Africans, traditional food fermentation is important for enhancing the flavors of existing staples like cereals and root crops, providing an affordable means of food preservation, and improving the nutritional quality and digestibility of raw products. Fermented foods are often considered health-promoting and are believed to help control various diseases, particularly intestinal disorders, in many African regions (Franz *et al*., 2014).

Fermentation is commonly used to enhance the physicochemical and sensory qualities of various soy products. Non-dairy probiotic products have gained global significance due to increasing vegetarianism trends and the high prevalence of lactose intolerance worldwide (Saadiya *et al*., 2018). Soymilk, a traditional and affordable oriental beverage, offers a rich source of protein and energy (Kumari *et al*., 2018). Soy products have garnered considerable interest for their beneficial health effects, including enhanced bioavailability of isoflavones, improved protein digestion, increased soluble calcium content, promotion of intestinal health, boosted antioxidant activity, and support for the immune system. Additionally, soy milk-based yogurts, such as sogurt, have become increasingly popular as alternatives to traditional dairy-based yogurts due to their lower levels of cholesterol and saturated fat, as well as their lactose-free nature (Kumari *et al*., 2018). Soy milk, offers additional advantages such as a rich nutrient composition and natural prebiotic properties that support the growth and survival of probiotic cultures (Hatice *et al.*, 2025).

Fermentation enhances the nutritional quality of foods by boosting the levels of vitamins, essential amino acids, and fatty acids, while also aiding in the detoxification and elimination of anti-nutritional compounds. In addition to proteins and isoflavones, soybeans contain a variety of other functional and nutritional substances that are enhanced through microbial transformations during fermentation. Microorganisms that produce specialized hydrolytic enzymes like proteases, amylases, and β-glucosidases are particularly instrumental in augmenting the functional characteristics of fermented soybean products (Kumar *et al.*, 2016). It is essential to incorporate well-documented probiotics like *Lactobacillus rhamnosus,* known for their health benefits, into our food matrix. Given that most fermented foods in Nigeria are produced spontaneously, developing probiotic products specifically for household food preparation could profoundly impact the health and well-being of both adults and children. Since milk is either not readily available or is expensive in many Nigerian communities, soymilk, a very cheap and popular household drink produced and marketed by local women as a source of

livelihood, could serve as an alternative. Therefore, the aim of this work is to use the inexpensive Yoba culture (*Lactobacillus rhamnosus*) in the fermentation of soymilk derived from local soybeans and to determine its potential as an enhancer of the nutritional and functional qualities of soymilk.

## Materials and Methods

### Production of soymilk

Soymilk was prepared as described by Ajogwu *et al*. (2025). Local soybean (300g) procured from Eke-Awka market Anambra state was submerged in 2L of distilled water for 12 h and then dehulled. The dehulled soybeans were grounded with distilled water using a blender (ratio 1:4 w/v) soybean/water and filtered with cheesecloth to obtain soybean milk. The soybean milk was boiled for 30mins at 100$^{o}$C and cooled to 40$^{o}$C to obtain the soymilk slurry. The soymilk slurry was placed in sterilization jars and sterilized in an autoclave at 121$^{o}$C for 15mins.

### Proximate composition of the fermented and unfermented soymilk

The moisture content, ash content, crude fibre, crude protein of the fermented and unfermented soymilk was determined using the AOAC (1990) method. The crude fat content of the fermented and unfermented soymilk was determined using the Soxhlet Fat Extraction Method as described by ISO (1998). Carbohydrate content was estimated through substitution. The content of the moisture, protein, fat, ash and crude fibre were summed up and subtracted from 100% as.

Carbohydrate (%) = 100 – (Moisture + Protein + Fat + Ash + Crude Fibre).

### Anti-nutrients determination of the fermented and unfermented soymilk

The oxalate was determined in three major steps: digestion, oxalate precipitation and permanganate titration as described by Harborne (1993). The Saponin, Cardiac Glycosides, Tannins, Phytate, total phenolic content (TPC), Hemagglutinin content, cyanogenic glycosides, anthocyanin content and steroid content were determined as described by Obadoni and Ochuko, (2001), Wang and Filled method, follins dennis titrating method modified by Pearson (1976), the Young and Greaves method as adopted by Lucas and Markakes (1975), the Folin-Ciocalteu assay as adopted by Akomeng and Adusei (2021), Doris and Grace (2022), the AOAC (1990) acid titration method, the gravimetric method of Harborne (1993) and the modified Liebermann-Burchard test as adopted by Attarde *et al*. (2010) respectively.

### Amino acid profile determination of the fermented and unfermented soymilk

The extraction and instrumental analysis were performed using a modified version of AOAC Method 982.30 (2006) and the method described by Danka *et al.,* (2012). The dried and pulverized sample was made to be free of water by ensuring constant weight for a period of time in the laboratory. A 0.5g of the sample was weighed into a 250 ml conical flask. The sample was defatted by extracting the fat content of the sample with 30 ml of petroleum spirit three times, with Soxhlet extractor that was equipped with a thimble. The sample was hydrolyzed three times for complete hydrolysis to be achieved for the totality of amino acids recovery. The pulverized and defatted sample was soaked with 30 ml of the 1M potassium hydroxide solution and was incubated for 48 h at 110°C in a hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralized to get pH in the range of 2.5-5.0. The solution was purified by cation-exchange solid-phase extraction. To increase volatility and reduce polarity during Gas Chromatography analysis, the amino acids in purified solution were derivatized

with ethyl chloroformate by the established mechanism.

**Determination of gamma-aminobutyric acid (GABA) in the fermented and unfermented soymilk**

The Gamma-Aminobutyric Acid (GABA) extraction and analysis were performed by a modified method of Zhang *et al.* (2023). Protein of the liquid sample was removed by sampling 1 ml aliquot of the samples, which was diluted five times with sterile $H_2O$, and 250 µl of zinc acetate and ferrous cyanide were added and mixed thoroughly. After standing for 1 h, samples were centrifuged, 5000 g at 25°C to completely precipitate proteins and peptides. A GABA analysis was performed after samples were stored at 4°C. The obtained supernatant containing GABA was derivatised by taking 200 µl of supernatant and mixing with 200 µl of acetonitrile, 200 µl of $NaHCO_3$ (pH 9.8), 200 µl of $H_2O$, and 100 µl 40g/l of Dansyl chloride was added at 60°C in the dark for 1 h. After derivation, 100 µl of 20 µl/ml acetic acid was added to stop the reaction. Subsequently, the sample was centrifuged at 12,000 g at 25°C for 5 min. Moreover, the supernatant was passed through a 0.22 µm filter with a membrane and was stored in a brown vial. The thoroughly washed micro syringe was employed to pipette 5.0 µl of the derivatized sample into the HPLC column.

**Isoflavone assay of the fermented and unfermented soymilk**

The isoflavones extraction and analysis was performed by modified method of Molamma *et al.* (2005). An amount of test sample that contains approximately 1 g protein was accurately weighed into a 250ml Erlenmeyer flask with ground-glass stopper. To this, 40ml of the extraction solvent (80% methanol in de-ionised water) was added. The flask with its contents was shaken in a 65°C water bath for 2 hours, cooled to room temperature, and 3ml of 2M NaOH were added. They were stirred well at room temperature for 10 min and 1ml of glacial acetic acid was added. The contents were swirled and poured into a 50ml volumetric flask, diluted to mark with the extraction solution and mixed well. The contents were centrifuged for 10 min at 5000 rpm. An aliquot of the supernatant was filtered through a 0.45 mm polytetrafluoroethylene filter unit and analyzed by High Performance Liquid Chromatography (HPLC). After the extraction of isoflavones from the samples, they were immediately analyzed by HPLC. A linear HPLC gradient consisting of (A) 0.1% glacial acetic acid in filtered MilliQ water and (B) 0.1% glacial acetic acid in acetonitrile was used. The injection volume was 20 ml, and the components were eluted using the following solvent gradient: from 0 to 5 min 10% B and 90% A; from 5 to 50 min 10-35% B and 90-65% A; then held at 35% B and 65% A for another 10 min and re-equilibrated back to 10% B and 90% A. The flow rate was 0.8 ml/min and the UV detector wavelength was set at 260 nm.

**Phenolic compounds extraction from the fermented and unfermented soymilk**

Two stage extraction procedures followed for the effective removal of the polyphenols/phenolic compounds. The first stage involved the extraction of 50.0 mg of the sample with 5 ml of 1M NaOH for 16 hours on a shaker at ambient temperatures as described by Kelley *et al*. (1994) and Provan *et al*. (1994). After extraction, the sample was centrifuged (5000 × g), rinsed with water, centrifuged again, and the supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2 h to release the conjugated phenolic compounds (Whitehead *et al*.,1983). The heated extract was cooled, titrated with 4 M HCl to pH < 2.0, diluted to 10 ml, with deionised water, and centrifuged to remove the precipitate. The supernatant was saved for subsequent purification and the residue was extracted further in stage 2. The residue from stage 1 above was extracted with 5 ml of 4 M NaOH, heated

to 160°C in Teflon as described by Provan *et al*. (1994). After cooling, the mixture was filtered, supernatant was collected and the residue washed with water (deionised). The supernatants were combined and adjusted to pH <2.0 with 4 M HCl. The filtrates were combined for further purification. An aliquot (5-15 ml) of the various supernatants was passed through a conditioned Varian (Varian Assoc., Harbor City, CA) Bond Elut PPL (3-ml size with 200-mg packing) solid-phase extraction tube at 5 ml/min attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under a vacuum (-60 kPa) until the resin was thoroughly dried after which the PAs were eluted with 1 ml of ethyl acetate into gas chromatography auto sampler vials. The PPL tubes were conditioned by first passing 2 ml of ethyl acetate followed by 2 ml water (pH <2.0). The phenolic acid standards used were purchased from Aldrich (Aldrich Chemical Co., Milwaukee, WI).

**Statistical analysis**

This was carried out using the analysis tool pack of Microsoft excel. Tables and bar charts were used to summarize each parameter descriptively using averages and their levels of deviation. ANOVA and t-tests were used to check for significant differences. A significant difference was assumed for a p-value < 0.05.

## Results

**Proximate composition of the soymilk**

Figure 1 shows the proximate composition of the fermented and unfermented soymilk. After fermentation there was a drastic decrease in the mean moisture and ash content of the fermented soymilk from 88.73% to 75.86% and 3.24% to 3.00% respectively. However, there was an increase in the fibre content, protein, fat and carbohydrate content of the soymilk after fermentation from: 0.89% to 1.48%, 3.15% to 5.25%, 3.53% to 3.77% and 0.46% to 10.64% respectively. Except for the fat content, the difference in the mean values of all other parameters were statistically significant at p< 0.05.

**Anti-nutrient Determination in the Soymilk**

Table 1 shows the variations in the concentrations of anti-nutrients present in the soymilk and the fermented probiotic soymilk. It was observed that there was a decrease in the concentration of six out of the ten examined anti-nutrients after fermentation namely: Oxalate, Cardiac Glycoside, phytate, Hemagglutinin, anthocyanin and Cyanogenic glycoside, from 0.51mg/g to 0.39mg/g, 6.36% to 5.94%, 0.54% to 0.12%, 0.08mg/kg to 0.05mg/kg, 3.43% to 1.94% and 4.05mg to 3.24mg respectively. The difference in all the values is statistically significant at p< 0.05.

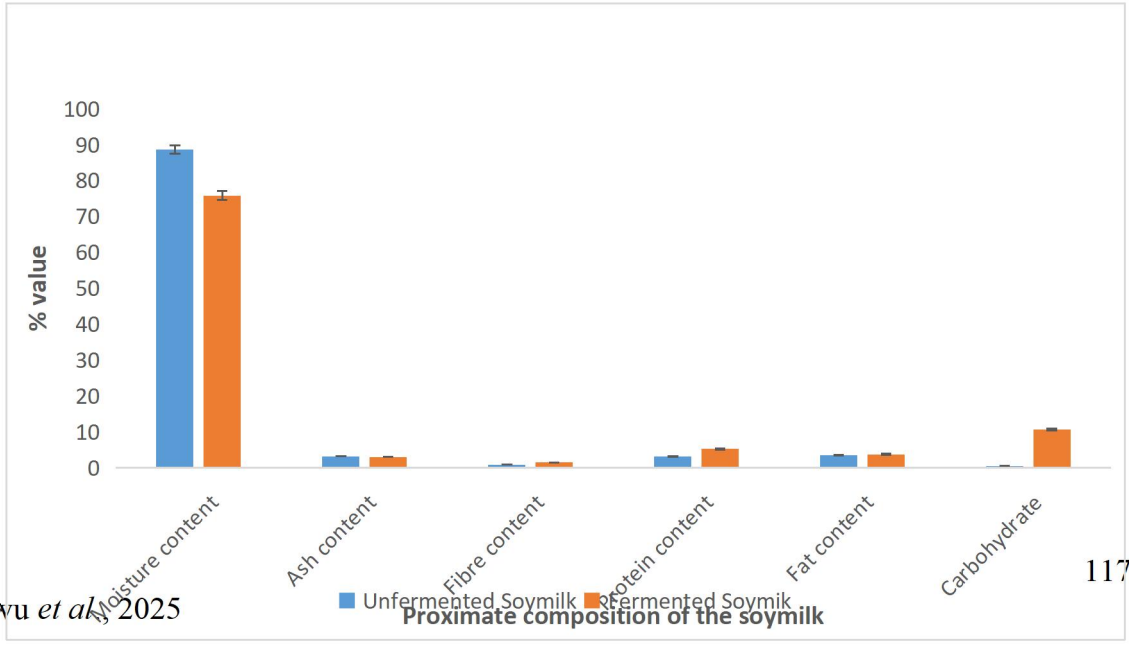


**Figure 1.** Mean proximate composition of the unfermented soymilk (A) and the fermented soymilk(R). All measurements were performed in triplicate.

**Table 1**: Mean anti nutrients in unfermented Soymilk (A) And Fermented Probiotic Soymilk(R)

| Parameters | A | R |
|---|---|---|
| Oxalate (mg/g) | 0.51 | 0.39 |
| Saponin (%) | 1.74 | 2.82 |
| Cardiac Glycoside (%) | 6.36 | 5.94 |
| Tannin (%) | 11.92 | 12.79 |
| Phytate (%) | 0.54 | 0.12 |
| Phenol (mg/kg) | 2.83 | 2.94 |
| Hemagglutinin (mg/kg) | 0.08 | 0.05 |
| Cyanogenic glycoside (mg) | 4.05 | 3.24 |
| % anthocyanin | 3.43 | 1.94 |
| Steroid (mg/kg) | 2.94 | 4.30 |

**All measurements were performed in triplicates.**

**Amino Acid Profile of the Soymilk**

The amino acid profile of the fermented and unfermented soymilk is shown in Table 2. There was an increase in all the amino acids in the fermented soymilk (R) when compared with the unfermented soymilk (A). Fermentation significantly improved the total amino acid of soymilk from 88.22g/100gprot in the unfermented to 95.00g/100gprot in the fermented soymilk. Lysine was the most concentrated essential amino acid (7.27g/100gprot in the unfermented and 7.43g/100gprot in the fermented), followed by Leucine (6.64g/100gprot in the unfermented and 7.43g/100gprot in the fermented), then Phenylalanine which was 4.82g/100gprot in unfermented soymilk and 5.35g/100gprot in the fermented soymilk. The increase in the values of six out of the nine essential amino acids (g/100gProt) after fermentation namely; threonine, isoleucine, lysine, methionine, histidine, and tryptophan from 3.51 to 3.53, 3.21 to 3.55, 7.27 to 7.65, 1.07 to 1.14, 3.18 to 3.39 and 1.22 to 1.28 were statistically significant at $p< 0.05$ while that of valine, phenylalanine and leucine are not statistically significant at $p< 0.05$.

**Gamma-aminobutyric acid (GABA) composition of the soymilk**

Table 3 shows the Gamma-aminobutyric acid (GABA) composition of the fermented and unfermented soymilk. There was a significant increase of GABA in the fermented soymilk (R) when compared with the unfermented soymilk.

The GABA increased from 1.34 g/100g to 3.79 g/100g after fermentation.

**Isoflavone contents of soymilk**

Table 4 shows the isoflavone content of the fermented and unfermented soymilk. The glucoside forms of soy isoflavones (daidzein, Glycitin and genistin) which are easily absorbable in humans were significantly reduced after fermentation. Daidzin, Glycitin and Genistin were reduced from 6.78 g/100g to 6.55 g/100g, 9.14 g/100g to 8.46 g/100g, and 8.21 g/100g to 7.95 g/100g respectively. The effect on the easily absorbable aglycones (genistein, glycitein and daidzein) varied. There was a significant increase in Glycitein from 2.45 g/100g to 2.50 g/100g after fermentation.

**Phenolic acid profile of soymilk**

Table 5 shows the results for the analysis of the phenolic content of the fermented and unfermented soymilk. From the result, there was a significant improvement in thirteen out of the fifteen phenolics present, only Cinnamic, O-cumaric acid and chlorogenic acid showed a decrease after fermentation.

**Table 2:** Amino acid profile of unfermented soymilk (A) and fermented probiotic soymilk(R)

| **Parameters** | **A (**g/100gProt) | **R (**g/100gProt) |
|---|---|---|
| Glycine | 3.52 | 3.77 |
| Alanine | 3.35 | 3.63 |
| Serine | 5.73 | 6.39 |
| Proline | 3.06 | 4.23 |
| Valine | 3.42 | 3.73 |
| Threonine | 3.51 | 3.53 |
| Isoleucine | 3.21 | 3.55 |
| Leucine | 6.64 | 7.43 |
| Aspartate | 10.74 | 11.48 |
| Lysine | 7.27 | 7.65 |
| Methionine | 1.07 | 1.14 |
| Glutamate | 17.10 | 17.56 |
| Phenylalanine | 4.82 | 5.35 |
| Histidine | 3.18 | 3.39 |
| Arginine | 6.00 | 6.24 |
| Tyrosine | 2.98 | 3.09 |
| Tryptophane | 1.22 | 1.28 |
| Cysteine | 1.40 | 1.54 |
| Total | 88.22 | 95.00 |

**Table 3**: Gamma-aminobutyric acid (GABA) composition of unfermented soymilk (A) and fermented probiotic soymilk(R)

| **Parameters** | **A (**g/100g) | **R (**g/100g) |
|---|---|---|
| GABA (e-1) | 1.34 | 3.79 |

**Table 4:** Isoflavone contents of unfermented soymilk (A) and fermented probiotic soymilk (R)

| Parameters | A (g/100g) | R **(**g/100g) |
|---|---|---|

| | | |
|---|---|---|
| Daidzin | 6.78 | 6.55 |
| Glycitin | 9.14 | 8.46 |
| Genistin | 8.21 | 7.95 |
| Daidzein | 3.89 | 3.75 |
| Glycitein | 2.45 | 2.50 |
| Genistein | 2.26 | 1.83 |

**Table 5:** Phenolic acid profile of unfermented soymilk (A) and fermented probiotic soymilk(R)

| Parameters | A (mg/100g) | R (mg/100g) |
|---|---|---|
| Phenylacetic acid | 2.0879e-4 | 2.48635e-4 |
| Salicylic acid | 5.06673e-4 | 5.37379e-4 |
| Cinnamic acid | 1.30375e-3 | 1.30167e-3 |
| Protocatechuic acid | 6.15772e-2 | 6.89064e-2 |
| O-coumaric acid | 9.65492e-7 | 8.21131e-7 |
| Vannilic acid | 1.37968e-1 | 1.46982e-1 |
| P-Hydoxy benzoic acid | 1.19393e-1 | 1.35927e-1 |
| P-coumaric acid | 2.37308e-2 | 2.69448e-2 |
| Syringic acid | 1.85060e-1 | 1.96384e-1 |
| Gallic acid | 1.99434e-1 | 2.37309e-1 |
| Caffeic acid | 1.42110e-1 | 1.65608e-1 |
| Sinapic acid | 3.73956e-5 | 3.78862e-5 |
| Ferulic acid | 8.08473e-2 | 9.16862e-2 |
| Ellagic acid | 3.62587e-5 | 3.37450e-5 |
| Chlorogenic acid | 2.14109e-1 | 2.07563e-1 |
| Totals | 1.16632 | 1.27947 |

**Discussion**

The results of the study to determine the improvement of the nutritional and functional qualities of soymilk fermented by *Lactobacillus rhamnosus* Yoba are presented on different tables and figures. Figure 1 showed the proximate composition of the fermented soymilk as compared to the unfermented soymilk, the moisture content and ash content decreased after fermentation. This low ash content could be a result of the solubilisation of minerals in the cases of steeping and boiling. Conversely, the fibre content, protein content, fat content and carbohydrate content increased after fermentation. Fermentation can increase the protein levels of soy milk due to the presence of protein from these bacterial cells. This was similar to the report of Darimiyya and Annis (2021), where the unfermented soymilk had the lowest protein content compared to other treatments. Also, Obadina *et al.* (2013), observed an increase in protein content of

soymilk as it was being fermented. The improvement reported in protein content of fermented soymilk as compared to soymilk might be due to some anabolic processes leading to polymer build-up or due to microbial cell proliferation. The increase in the carbohydrate content contrasts the findings of Fathy *et al*. (2023) where soymilk fermented with *Lactobacillus plantarum* KU985432 and *Saccharomyces boulardii* CNCMI-745 had less carbohydrate content than the unfermented soymilk. Carbohydrates are needed for the survival or even the growth of lactic acid bacteria, which leads to lactic acid production. Armistice *et al*. (2022) while evaluating the probiotic potential of novel fermented cowpea-peanut milk with *Lactobacillus rhamnosus* Yoba reported that the carbohydrate content of the control sample (4.25%) was lower as compared to the fermented cowpea-peanut milk samples (5.18–6.05%). The increase in the carbohydrate content of the soymilk after fermentation could be a result of breakdown of starch by the enzymes produced during fermentation.

Table 1 shows the effect of fermentation on anti-nutrients in soymilk. Although, soy milk is a very good source of nutrient with high biological value, the presence of anti-nutritional factors affects its nutrition quality and limits bioavailability of the nutrients. In this study, fermentation significantly reduced the phytate in soymilk. It is very essential to reduce phytate in soy milk or soy food because phytate inhibits calcium absorption, it reduces iron, zinc, magnesium and calcium absorption, and results in poor iron absorption in soy foods (Humyra *et al*., 2018). Iron may be better absorbed from fermented soy foods like tempeh and miso than soymilk (Mazumder and Hogsprabhas, 2016). The oxalate, cardiac glucosides, cyanogenic glucosides and anthocyanin also decreased after fermentation. Functional soy milk is defined as soy milk which its beneficial bioactive components are increased or its undesired compounds are decreased (Neda *et al*., 2022). Amino acids are the main components of protein, and are divided into two groups, namely essential and non-essential amino acids. Essential amino acids cannot be produced in the body so they often have to be added in the form of food, while non-essential amino acids can be produced in the body (Danang, *et al.,* 2021). The effect of fermentation on the amino acid profile of soymilk was reported in table 2, where fermentation significantly improved the total amino acid of soymilk from 88.21507g/100gprot in the unfermented to 94.99695g/100gprot in the fermented soymilk indicating proteolysis by microorganisms. All nine essential amino acids were present in both fermented and unfermented soymilk. Lysine was the most concentrated essential amino acid (7.27178g/100gprot in the unfermented and 7.43408g/100gprot in the fermented) followed by Leucine (6.64467g/100gprot in the unfermented and 7.43408g/100gprot in the fermented), then Phenylalanine which was 4.81542g/100gprot in unfermented soymilk and 5.35138g/100gprot in the fermented soymilk. According to a study by Kumar *et al*. (2016), leucine (108.5 mg/g protein) was the most abundant essential amino acid observed in soy "Okara" protein followed by Phenylalanine (97.5 mg/g protein) and the concentration of lysine content per gram of "Okara" protein was slightly lower (54.0 mg/g protein). From this current study, although glutamate and aspartate are non-essential amino acid, they produced the highest yield out of all the amino acids in this study 17.09519g/100gprot in the unfermented soymilk and 17.56118g/100gprot in the fermented soymilk was recorded for glutamate followed by Aspartate with 10.74241g/100gprot in the unfermented soymilk and 11.47757g/100gprot in the fermented soymilk. These high concentrations of amino acids shows that the nutritional quality of protein in legumes, and primarily in soybean is very

high, since the composition of soybean protein is close to that of animal protein. (Modgil, *et al.*, 2021).

Gama-aminobutyric acid (GABA) is a four-carbon non-protein amino acid which is synthesized by irreversible decarboxylation of L-glutamic acid (Glu), in a reaction catalyzed by glutamate decarboxylase (GAD), in the presence of pyridoxal-5-phosphate (PLP) as a co-factor (Phuong *et al.*, 2021). GABA is well known as an inhibitory neurotransmitter in mammals. This amino acid has been widely studied because of its physiological functions and positive effects on human health such as; treating neurological disorders, enhancing immunity under stress conditions, controlling nerve cell hyperactivity associated with anxiety, stress and fear thereby producing a calming effect, regulating blood pressure, inhibiting leukemia cell proliferation and growth of some cancerous cells (Diana and Rafecas, 2014). Table 3 showed the effect of fermentation on GABA concentration in soymilk. There was a significant increase from 1.34084e-1g/100g (unfermented) to 3.79259e-1g/100g (fermented). The liberation of GABA by decarboxylation reaction is involved in Krebs cycle utilizing glutamate as a substrate, Glutamate is converted into GABA, and the GAD enzyme catalyzes the reaction (Pham *et al.*, 2016). The significant increase in GABA in the current study could be due to the ability of the probiotics to produce glutamic acid decarboxylase that degraded the high glutamate in the soymilk which was the highest concentrated amino acid in this study. Phuong *et al*. (2021), observed that both the free Glu and the GABA content were significantly increased in soymilk hydrolysate, a product obtained after enzymatic hydrolysis of soymilk. After 72 h of fermentation, the GABA content in soymilk hydrolysate reached 859 mg/L while the remaining Glu was 2467 mg/L.

Isoflavones are bioactive peptides that can improve functional properties of fermented food, having different therapeutic values and act as an antioxidant (Sanjukta *et al*., 2015), antihypertensive, anti-tumor, antidiabetic and are also being recognized to preclude atherosclerosis. This allows them to act as an alternate to synthetic drugs. The administration of fermentation in soy milk is effective to enhance the aglycones isoflavones in soy milk that include genistein and daidzein. Aglycone forms of soy isoflavone (daidzein and genistein) are the most active forms and gets easily absorbed in higher quantity in humans compared to that of the glucoside forms of soy isoflavones (daidzin and genistin) (Sasithorn *et al*., 2017). Table 4 shows the isoflavone profile of fermented and unfermented soymilk. Fermentation significantly reduced the glucosidic forms; Diadzin, glycitin and genistin. On the other hand, except for genistein, fermentation could not improve the concentration of the other isoflavone aglycones. Although there was no significant increase in the aglycones, concentration of 3.74788mg/100g, 1.82689mg/100g and 2.49890mg/100g of daidzein, genistein and glycitein respectively were observed. This was higher than the quantity Ekkapon *et al*. (2017) in their study on the concentration of isoflavone aglycone in four different brands of soymilk in Thailand. In their study, the highest quantity for daidzein, genistein, and glycitein was 2.0mg/250ml, 2.2mg/250ml and 0.4mg/250ml respectively. The result of this current study is in contrast with the study of Fathy *et al.* 2023, where fermentation with *Lactobacillus plantarum* KU985432 and *Saccharomyces. boulardii* CNCMI-745 affected the change in isoflavones. Isoflavone aglycones, daidzein, and genistein, increased after 48 h fermentation (Fathy *et al.,* 2023).

Free phenolic acids such as p-hydroxybenzoic acid, salicylic acid, p-coumaric acid, and ferulic acid have been

reported in soybeans (Kumari and Chang, 2016). Cinnamic acid and its derivatives, particularly those with the phenolic hydroxyl group, are well-known antioxidants with various health benefits attributed to their high free radical scavenging abilities. Protocatechuic acid, and protocatechuic aldehyde have been shown to have pharmacological effects both *in vitro* and *in vivo*, which include antioxidant activity (Zhang *et al.,* 2021). The result of the current study shows that O-coumaric acid and ferulic acid were the most predominant phenolic acid in the soymilk samples followed by protocatechoic acid, salicyclic acid, sinapic acid and ellagic acid. Then, p-cumaric acid, phenylacetic acid, gallic acid and chlorogenic acid. However, cinnamic acid, vanillic acid, hydroxybenzoic acid, syringic acid, and caffeic acid were present in relatively smaller amounts. This observation is in contrast with the studies of Xiaoming *et al*. (2020), where gallic acid was the most predominant phenolic acid in the soymilk samples followed by p-hydroxybenzoic acid, protocatechuic acid, 2, 3, 4-trihydroxybenzoic acid, vanillin, syringic acid and p-hydroxybenzoic acid. In this current study, fifteen phenolic acids were present and only O-cumaric acid, chlorogenic acid and ellagic acid did not improve after fermentation, all other free phenolic acids present increased after fermentation. Fermentation enhances bioactivity in polyphenols by converting high-molecular-weight polyphenols such as punicalagin into smaller, glycoside-free, more biologically active molecules like ellagic acid (Yang *et al*., 2023). This study highlights the possibility of improving the functional and nutritional qualities of soymilk by fermentation using *Lactobacillus rhamnosus.*

## Conclusion

Since milk is either not readily available or is expensive in many Nigerian communities. Soymilk, a very cheap and popular household drink produced and marketed by local women as a source of livelihood, could serve as an alternative. Given that most fermented foods in Nigeria are produced spontaneously, developing probiotic products with improved nutritional and functional qualities specifically for household food preparation could profoundly impact the health and well-being of both adults and children. It is essential to incorporate well-documented probiotics like *Lactobacillus rhamnosus,* known for their health benefits, into our food matrix such as soymilk.

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