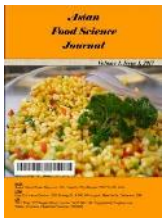

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Effect of Thermal Processing on the Nutritional Status of Soy Milks and Yoghurts Produced by Enhanced Spontaneous Fermentation

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This research was carried out to determine the effects of thermal process of boiling of soy bean seeds on the nutritional status of soy milk and soy yoghurt produced by fermentation. The soybean seeds were de-hulled, boiled and thermally processed to soymilk. Food sweetener (Glucose -D), cassava stabilizer and starter cultures were added to the samples to enhance the spontaneous fermentation. *Staphylococcus aureus, Bacillus* spp*, Escherichia coli, Streptococcus Lactobacillus* spp, *Aspergillus* spp and *Saccharomyces* spp were isolated from the fermenting samples. The proximate composition showed that the soy yoghurt had more protein content than soymilk while the soymilk had more crude fiber, moisture content, fat content, ash content and carbohydrate. The soy yoghurt was more acidic pH 3.7 than the soy milk pH 6.4. The acidic nature of the soy yoghurt together with other metabolites of Lactic acid bacteria prevented the continued growth of food borne pathogens and spoilers thus ensuring the safety of soy yoghurt. Soy yoghurt can be used as a close substitute for cow milk which is relatively expensive to the rural dwellers.

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1. INTRODUCTION

Soy bean is a high protein legume grown as food and is common in the orient and other parts of the world. Aside from protein, it contains good amounts of carbohydrates and fats. It also contains antioxidants and phyto-nutrients that have been linked with various health benefits. *Glycine soja* is the wild ancestor of *Glycine max*, and grows wild in china, Japan, Korea, Taiwan and Russia. The subgenus Glycine consists of at least 16 wild perennial specie, for example *Glycine canascens* [1].

Recently, in Nigeria, a stimulated interest appears to have developed in the use of soybean as human food. One of such steps is commercial production of soy-ogi, a protein fortified Nigeria-made beverage which was derived, perfected and produced at the Federal Institute of Industrial Research, Oshodi Nigeria [1].

The main products from soybeans are meal and oil in the orient, alongside a variety of fermented soy foods and non-fermented soy foods. Fermented soy is a form of soy that has gone through a lengthy fermentation process that makes it digestion-friendly. Examples are soy yoghurt, *tempeh, natto* and *miso*. Unfermented, soy comes in the form of *tofu*, soymilk, fresh raw or cooked soybeans, soy chips, soy flour and the countless number of processed foods that contain soy derivatives or soybean oil. Soy milk, the water extract of soybean offers a promising performance as a carrier of probiotics [2]. Furthermore, it is rich in nutritive elements like proteins, unsaturated fatty acids, lecithins, isoflavones, mineral substances, free amino acids and polypeptides [3], while containing only a small amount of saturated fatty acid and it lacks cholesterol or lactose [4]

Soy contains high levels of toxins or anti-nutrients that can cause gastric distress, growth depression, pancreatic hypertrophy, hyperplasia and adenoma in experimental animals. These anti-nutrients include potent enzyme inhibitors that block the action of trypsin, an enzyme needed for protein digestion [5]. Excessive amount of these anti-nutrients can lead to stomach upset and a deficiency of nutrients, especially amino acids. When soy is fermented, these compounds are deactivated. Fermented soy has a wide range of health benefits like anti cancer effects, reduction of menopausal syndrome frequency, osteroprosis protection, coronary heart disease prevention due to anti-atherogenic properties and high amounts of vitamin K_2 [5].

Despite the benefits derived from soybean, it can be an easy medium or route for transmitting food borne bacteria and enteric bacteria pathogens as well as fungal pathogens; identified with food poisoning, gastric enteritis, dysentery and enteric fever. These pathogens can be balanced by probiotics which are constituents of fermented milks and yoghurt. Dairy milk used in the production of yoghurt contains lactose and since soymilk made from soybean is milk alternative for vegans and people who are lactose intolerant, therefore, it was necessary to use soymilk in yoghurt production, knowing that soybean comes from plants, making it naturally free of cholesterol and low in saturated fat.

The microbial load and proximate composition of soybean present prior to thermal application and fermentation definitely differs from the microbial load and proximate composition after thermal processing and fermentation. This study aimed at the assessing the effect of thermal process of boiling on the nutritional status of soy milks and yoghurts produced by enhanced spontaneous fermentation.

2. MATERIALS AND METHODS

2.1 Sample Collection

The soybean seeds and the freeze-dried yoghurt starter culture used in the soy yoghurt production were purchased from umuahia. This study was conducted in the laboratory of the Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

2.2 Production of Soymilk

The method of Onwumere J. U [6] was modified in the production of plain soymilk. Hot extraction method was used in the soymilk production. 250 g of soy bean seeds were sorted, cleaned and transferred into 1 litre boiling water and boiled for 10 minutes. It was then manually dehulled and wet-milled with 1 liter of borehole water using Kenwood BL 440 electric blender. Little quantity was blended at a time to achieve thorough

blending which enhances milk extraction. The slurry was sieved using a doubly folded sterile muslin cloth. The mashy residue called *okara* was discarded. The soy milk was stored in 1 litre clean glass beaker in the refrigerator at 4°C.

2.3 Production of Soy Yoghurt

The method of Ohakwe N [7] was adopted with slight modifications in the production of soy yoghurt. 1g of sweetener (Glucose D) which equals 10% of the soymilk was gradually dissolved into the soymilk by first dissolving some portion of the sweetener with some quantity of the soymilk at a time in a beaker before carefully transferring it into the entire lot (soymilk) in the container. Then, the same step was taken in mixing 1 g of the stabilizers which equals 1% of the soymilk. The soymilk samples were separately and thoroughly homogenized by blending at higher speed using the electric blender. The homogenization was to help break down the globules which will enhance thorough fermentation process. Part of the homogenized soymilk samples was pasteurized at 81°C for 20 minutes before cooling to 42°C to serve as control sample. 5 g of the starter culture was dissolved in 1000 ml of homogenized soymilk using the same method used in dissolving the sweetener. The sample was carefully and thoroughly shaken to mix it well. It was covered very well and incubated at 37°C for 12 hours to allow for fermentation. The soy milk was later stored at 4°C in the refrigerator.

2.4 Microbiological Analysis of Samples

2.4.1 Isolation of organisms

Spread plate method [5] was used in plating the samples of the soymilk and soy yoghurt. 1ml of each of the samples was serially diluted in sterile peptone water and 0.1 ml was aseptically inoculated onto suitable media in triplicates by Spread plate method [8] for the isolation of bacterial and fungal cultures. The bacterial plates were incubated at 37°C for 24-48 hours while the fungal plates were incubated at 22°C for 5 days. The various bacterial colonies isolated were sub-cultured to obtained pure cultures. The total viable counts and coliform counts were determined [1]. The isolates were later identified based on their colonial morphology, Gram staining and biochemical characteristics [9].

2.5 Proximate Analysis of Fermented Soy Beans

The proximate analyses of the soybean seeds (fat, crude protein, carbohydrate, crude fiber and moisture content) of the fermenting samples were carried out according to [10].

2.5.1 Moisture content determination

This was carried out by the determination of percentage moisture loss according to [10].

2.5.2 Ash content determination

A clean silica dish was weighed to a constant weight. 2.0 g of each sample was measured into the dish. The sample was ignited using a heating mantle in the fume cupboard until charred and no more smoke given off (pre-ashing). Using a pair of tong the sample was transferred into a muffle furnace at a temperature of 550°C until fully ashed. The percentage ash was then calculated as follows:

$$\% \text{ Ash} = = \frac{\text{Weight } of \text{ } ash}{\text{Weight of original sample}} \times 100$$

$$= \frac{W3-W1}{W2-W1} \times 100$$

Where:

W_1 = weight of empty dish
W_2 = weight of dish + sample before drying
W_3 = weight of dish + ash

2.5.3 Fat content determination

The method of solvent extraction in a Soxhlet reflux apparatus was used. 250 ml boiling flasks were thoroughly washed and dried using an oven. They were transferred into the desiccators to cool and each was weighed accordingly after which the Soxhlet reflux apparatus was set up. 5.0 g of the sample was accurately weighed and put into a labeled thimble with a cotton wool. 200 ml of petroleum ether was filled in the boiling flasks and heated using a heating mantle set at a temperature of 100°C, the Soxhlet apparatus was allowed to reflux for about 6 hours. The thimble was carefully removed and the petroleum ether was recollected using a rotary evaporator and drained into a bottle for re-use. The flask was removed and dried at 105°C for 1 hour in an oven. The flask was then transferred from the oven into desiccators using a pair of tong and

allowed to cool. After cooling, the flask was then weighed (containing the oil).

Calculation:

$$\% \text{ Crude Fat Content} = \frac{W2 - W1}{W3} \times 100$$

Where:

W_1 = weight of the empty extraction flask
W_2 = weight of the flask and the oil extracted
W_3 = weight of the sample.

2.5.4 Carbohydrate content determination

The Nitrogen free extract method was used. The carbohydrate was calculated as weight by different between 100 and the summation of other proximate components as nitrogen free extract (NFE).

Calculation:

% N F E = 100 - % (a + b + c + d + e)

Where: a = protein content; b = fat content; c = fibre content; d = ash content; e = moisture content.

2.5.5 Crude fibre determination

This was determined using the ashing method of [10]. 2.0 g of mashed sample was placed in a 600 ml beaker and 200 ml of 1.25% H_2SO_4 solution was added into the beaker. The sample was heated for 30 minutes on a heating mantle. The sample was cooled before filtering. The residue was placed on a weighed crucible and allowed to dry in an oven at 150°C until a constant weight was obtained. The dried sample was burnt to ashes in a muffle furnace, then cooled in a desiccator and re-weighed.

% Crude fiber = weight loss on ashing/Weight of original sample x100

$$\frac{\text{Weight } loss\ on\ ashing}{\text{Weight of original sample}}\ x\ 100$$

The percentage crude fibre was calculated as follows:

$$\% \text{ crude fibre} = \frac{W2 - W3}{W1} \times 100$$

Where; W_1 = weight of sample; W_2 = Weight of sample + crucible; W_3 = Weight of crucible + ash.

2.5.6 Protein content determination

The protein content was determined by Kjeldahl method according to [10]. 0.2 g of the sample was weighed into a filter paper and transferred into a neat dried Kjeldahl flask. Exactly 25 ml of Conc. Sulphuric acid was added to the flask and 2 tablets of selenium catalyst. The flask was heated gently in a fume cupboard using a heating mantle in an inclined position and allowed to digest (digest is complete when the liquid is clear and free from black or brown colour). The flask was allowed to cool and was diluted with 200 ml of distilled water. A distillation apparatus consisting of 500 ml flask with stopper carrying a dropping funnel and a splash head adaptor and a vertical condenser in which a straight delivery tube is attached was used. Approximately 50 ml of boric acid solution was measured into 500 ml Erlenmeyer titration flask and a few drops of screened methyl red indicator were added and the Erlenmeyer flask placed on the receiving end of the delivery tube dipping just below the level of the boric level. Some anti-bumping agent granules and 75 ml of NaOH solution were added to the distillation flask. Exactly 50 ml of distilled water was added and was gently shaken to ensure mixing of contents. The flask was connected to distillation bulb and boiled vigorously until about 100 ml of the distillate was obtained. The distillate was titrated with 0.1 M Hcl till first trace of pink colour.

Calculation:

$$\% \text{ N} = \frac{Tv \times 1.4 \times 0.1}{W}$$

Where:

W = weight of sample in grams
Tv = Titre value
% crude protein = N x conversion factor

2.6 Determination of Total Titratable Acidity

The titratable acidity was determined by the alkaline titrimetric method expressed in percent (%). The acid produced was determined by the titration of 10 ml of fermenting *ugba* sample dissolved in deionized water with 0.1 N NaOH using phenolphthalein as an indicator until the end point (pink colour) is achieved. The percentage total titratable acidity (%TTA) was calculated as:

100/volume of sample x Normality NaOH used (0.0002) x Titre value.

The acidity was calculated using the formula below:

$$\% \text{ TTA} = = \frac{100}{W} \times titre \times N$$

Where: N = normality of titrant, W = weight of sample used

2.7 Determination of pH

A Hanna size pH meter was used. The meter was switched on and immersed into the sample up to the maximum immersion level. The sample was stirred gently until the display stabilized and the value was recorded.

2.8 Statistical Analyses

Data obtained were analyzed by finding the standard deviation.

3. RESULTS

The microbiological changes associated with the effects of thermal processing and fermentation was determined. The bacterial isolates are shown in Table 1 and they include *Staphylococcus aureus, Bacillus* spp, *Escherichia coli, Streptococcus* spp and *Lactobacillus* spp. Table 2 shows the bacterial succession during the fermentation time. Only *Lactobacillus* spp and *Streptococcus* spp were isolated at the end of the fermentation. The fungal isolates (*Aspergillus* spp and *Saccharomyces cerevisiae*) are shown in Table 3 while Table 4 shows the fungal succession during the fermentation. The Proximate compositions of the soymilk and soy yoghurt are shown in Table 5. Raw soy bean had higher values in most of the parameters compared to the soymilk and soy yogurt. Table 6 shoes the total titratable acidity of the products indicating a drop in pH value of soy yoghurt.

4. DISCUSSION

This research was carried out to determine the effect of thermal process of boiling on the nutritional status of soy milk and soy yoghurt through enhanced spontaneous fermentation. The absence of coliform in the soy yoghurt signifies that the samples were free from faecal contaminants, therefore the microbial status of the soy-yoghurt sample conforms to the accepted standard as reported by Oyeniyi et al. [11]. The yeast and mould counts were comparable with the results of [12]. The official microbiology standard of foods requires the complete absence of coliform and organisms such as *Staphylococcus aureus, Bacillus* species and other spore forming revivable microorganisms [1].

The proximate composition shows that the soy yoghurt is more proteinuous than the soymilk. Although the raw soybean seed had higher values, according to [1], it shouldn't be consumed raw due to the presence of anti-nutrients which can be reduced by heating and fermentation. The increase in total titratable acidity and decrease in pH could be attributed to the number of the lactic acid bacteria and the amount of lactic acid produced by LAB and the low pH values and other antimicrobial substances they produce such as hydrogen peroxide, bacteriocins, diacetyl, CO_2 which helped in eliminating food borne pathogens like *S. aureus, E. coli, Streptococcus* spp found at the early stage of the fermentation from the soy yoghurt. This ensures food safety thus making the food wholesome for consumption. The values recorded in this work are in agreement with those previously reported for fermented milk by Abou-Dobara Mi et al. [13].

The decrease in the fat content of soymilk could be as a result of increased activities of the lipolytic enzymes during fermentation which led to the hydrolysis of fat components (triacylglycerol) into fatty acid and glycerol. Similar report was made by Obadina AO et al. [14]. The decrease in fat content of the soy yoghurt indicates that the food will not contribute to health issues associated with high fat contents.

Similar decrease in the crude fiber of the soy yoghurt could be due to metabolism of the fiber by fermenting microbes. This finding agrees with the report of [1].

The decrease in the crude protein value of the soy milk shows that thermal processing of boiling of the soy bean leads to great loss in the protein content of soybean. However, fermentation increased the protein content to an extent possibly due to the increase biomass of single microbial cell proteins and synthesis of total free amino acids during the log phase of growth of the starter cultures [15]. According to [16], unfermented soybean derivatives such as soy milk contain high levels of toxins or anti-nutrients such as phytate that can cause gastric distress

Table 1. Morphological and Biochemical characteristics of Bacterial isolates

			Biochemical tests						Sugar fermentation									
Colonial Morphology	**G Gram reaction**	**Spor stain**	**Motility**	**Catalase**	**coagulase**	**Oxidase**	**Methyl red**	**Indole**	**Starch hydrolysis**	**Glucose**	**Lactose**	**Sucrose**	**Maltose**	**Frutose**	**Galactose**	**Manitol**	**Xylose**	**Probable isolates**
Shinny surface on NA	+ Cocci	-	-	+	+	-	+	-	+	A/-	A/G	A/-	A/G	A/-	A/G	A/-	A/-	*Staphylococcus aureus*
Large round colony on NA	+ Cocci	-	-	-	-	-	+	-	+	A/-	A/G	A/G	A/-	-	-/G	-	-	*Streptococcus* spp
White creamy rough colony on MRSA	+ rod in chain	-	-	-	-	-	+	-	+	A/-	A/G	A/-	A/G	A/G	A/-	A/-	A/G	*Lactobacillus* spp
White dry surface on NA	+ Straight long rods	+	-	+	-	-	-	-	+	A/G	-	A/-	-	-	A/G	A/G	A/-	*Bacillus* spp
Smooth pink colonies on MacConkey	+ Short rods	-	-	+	-	=	+	-	-	A/-	A/G	A/G	A/-	A/-	A/G	-	-	*Escherichia coli*

Key: G – Gas production A – Acid production. + = positive, - = negative

and can lead to mineral deficiency especially in vegetarians. It also contains hemagglutinin that promotes unwanted blood clotting. Unfermented soybean derivatives also contain goitrogens that can impair the thyroid function. It also contains phyto-estrogens that can mimic and even block estrogen. Thus, soy yoghurt is a better derivative of soybean seeds for human consumption.

Table 2. Bacterial succession on different agar

Isolates	Time (hr)							
	RSB	SM	FSM					SY
		0	2	4	6	8	10	12
Nutrient Agar (Bacteria)								
Staphylococcus aureus	+	-	-	-	-	-	-	-
Bacillus spp	+	-	-	-	-	-	-	-
Escherichia coli	+	-	-	-	-	-	-	-
MacConkey Agar								
Escherichia coli	+	-	-	-	-	-	-	-
De Man Rogosa Sharpe Agar								
Lactobacillus spp	+	-	+	+	+	+	+	+
Streptococcus spp	-	-	+	+	+	+	+	+

Key: RSB: Raw Soybean, SM: Soymilk, FSM: Fermenting soymilk, SY: Soy yogurt

Table 3. Morphological characteristics of fungal isolates

Surface	Elevation	Spore colour	Type of mycelium	Mode of reproduction	Septation	Probable isolate
Powdery	Raised	Black	Conidiophore	Sexual	Septate	*Aspergillus* spp
Cottony	Raised	Black	Sporangiospore	Sexual	Septate	*Sacchomyces cerevisiae*

Table 4. Fungal succession

	Time (hr)							
	RSB	SM	FSM					SY
Isolates		0	2	4	6	8	10	12
(Yeast and Mold)								
Aspergillus spp.	+	-	-	-	-	-	-	-
Saccharomyces spp.	+	-	-	-	-	-	-	-

Key: RSB: Raw Soybean, SM: Soymilk, FSM: Fermenting soymilk, SY: Soy yoghurt

Table 5. Proximate composition of soy bean seed, soy milk and soy yoghurt (%)

Samples	Moisture	Crude Protein	Crude fibre	Fat	Ash	Carbohydrate
RSB	8.00±0.00	41.00±1.41	4.00±0.00	19.00 ±1.41	7.00 ±0.00	21.00 ±0.00
SM	82.16±0.01	5.00±0.01	0.07 ±0.01	4.00 ±0.02	0.77 ±0.01	8.00 ±0.07
SY	80.35±0.01	7.87 ±0.01	0.02 ±0.01	3.35 ±0.01	0.55 ±0.01	7.86 ±0.01

Key: RSB: Raw Soybean, SM: Soymilk, SY: Soy yoghurt

Table 6. Total titratable acidity and pH values of soymilk and soy yoghurt

	Soy milk (0 hr)	Soy yoghurt (12 hr)
pH	6.4	4.0
TTA (%)	0.11	1.81

Key: TTA= Total titratable acidity

The mean moisture content of the soybean was lower than the soymilk. This possibly was due to boiling of the soy beans leading to water absorption and ultimate increase in size through swelling. The absorbed water was then carried over to the soy milk. However, fermentation led to loss in water content possibly as a result of microbial hydrolytic utilization of water in their various metabolic activities. Fermentation occurs at raised temperature due to increased metabolic activities. This could also lead to loss of water through evaporation. Similar result was recorded by Thingom P and Chhetry GKN [15] and Rachel OB and Oluwamodupe EG [1].

Boiling could have led to reduction in carbohydrate content of the soymilk due to thermal hydrolysis of starch to simple sugars. The further reduction in the carbohydrate value of the soymilk was due to enzymatic hydrolysis of carbohydrate to simple sugars which were used for energy generation and formation of bio-molecules via transformation by the microbes involved in the fermentation process [17]. Obadina AO et al. [14] also reported a decrease in carbohydrate content of soy-yoghurt fermented with starter cultures. Soy yoghurt could serve better as an adequate weaning food for babies and a protein supplement as it provides adequate amount of protein, less fat and a good amount of carbohydrate.

The reduction in ash content of both soy milk and soy yoghurt shows that boiling might have contributed to great loss in the ash content of soy bean seeds. Microbial utilization of the nutrients for metabolic activities could also have contributed to the significant reduction in ash content.

5. CONCLUSION

This study revealed that soy yoghurt is free from food borne pathogens and food spoilers. It contains more protein and less fat compared to soymilk. Soy yoghurt is cheap and could be easily accessed and processed in developing countries. Therefore, the consumption of soy yoghurt is encouraged due to its safe and nutritional relevance compared with soy milk and the inedible soy beans. The addition of starter culture assisted in the spontaneous fermentation.

6. RECOMMENDATION

In view of the continued increase in cost of dairy milk beyond the reach of poor families in rural localities, increased production and consumption of soy yoghurt as a suitable substitute for such people as well as those that are lactose intolerant is highly recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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