

Production of Yoghurt Analogue from Soy Milk and Different Fermented Cereal Based Water Filtrates (Omi ogi)

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Abstract
Yoghurt analogue was produced by fermenting soy milk with different selected fermented cereal based filtrates. The water filtrate was obtained from fermented 100% *Zea mays* (yellow variety) 100% Quality Protein Maize (yellow variety), 100% Sorghum (red variety) and 100% Millet (light cream variety). The pH, titratable acidity (TTA), microbial load, microbial type, proximate composition and mineral content of the yoghurt samples were determined. The samples were also subjected to sensory evaluation. The pH ranged from 4.68 in soy milk fermented with millet to 5.20 in soymilk fermented with quality protein maize. Total viable count was highest in soymilk fermented with sorghum filtrate (9.08 log cfu/ml) while Lactic acid bacteria count was highest in soymilk fermented with millet filtrate (7.50 log cfu/ml). *Lactobacillus plantarum, L. fermentatum* and *Saccharomyces cerevisiae* were found to occur in all the four yoghurt samples. There was no significant difference ($p > 0.05$) in the moisture content of all the yoghurt samples. Soymilk fermented with sorghum filterate contained the highest crude protein (3.57%) while the highest fat content was recorded in soymilk fermented with yellow maize filtrate (2.97%). Highest calcium and magnesium content was found in soymilk fermented with millet filterate. Commercial yoghurt was the most preferred in all the sensory attributes tested followed by soy milk fermented with millet filterate. All the yoghurt samples were accepted organoleptically. Soymilk and fermented cereal filterate could serve as good alternative to the expensive soymilk and commercial starter for production of good quality yoghurt analogue. Filtrate from sorghum appear to be the best starter for soy yoghurt production in terms of nutrients content while filterate from millet was most accepted by sensory panelists.
Keywords: Yoghurt, soymilk, maize, millet, sorghum, filtrate.

1. Introduction

The word Yoghurt is derived from the Turkish word "Jugurt" meaning fermented foods with an acidic taste (Younus *et al*., 2002). Yoghurt is made by adding culture of acid forming bacteria to milk which is then homogenized, pasteurized and fermented and the microorganism used to initiate the fermentation are referred to as starter cultures (Opara *et al*. 2013). Yoghurt popularity is increasing due to its characteristic taste and aroma. It has high nutritional value and is a rich source of carbohydrates, protein, fat, vitamins, calcium, and phosphorus (Sanchez-Segarra *et al*., 2000). Yoghurt is easily digested because milk protein, fat and lactose components undergo partial hydrolysis during fermentation (Sanchez-Segarra *et al*., 2000). Yoghurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to the people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, it also aids in immune function and weight control (Mckinley, M. C., 2005). Yoghurt is conventionally produced from cow milk and commercial starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophillus.* Both cowmilk and commercial starters are expensive and not within the reach of the poor populace. Soy milk and maize steep water have been reported as good alternatives to these expensive raw materials for yoghurt production (Farinde *et al*. 2008; 2009; 2010).

Soy yoghurt is produced from soy milk. Soy milk is an aqueous extract of soy beans (Glycine max). Fermentation has been found to reduce the beany or soy flavor in soy milk (Jimoh and Kolapo, 2007).

Cereals are one of the staple food crops widely grown and consumed worldwide. Some of the cereals grown in Africa include maize, rice, wheat, sorghum and millet. Cereal consumption accounted for about 77 % of calorie intake in Nigeria (Mitchen and Boustani, 1993).

Maize (*Zea mays*) ranks third after rice and wheat as the most important cereal crop mainly used as staple food and animal feed in most developing countries (Akande and Lamidi, 2006; Olakojo *et al*., 2007; Mboya *et al*., 2011). It is an important staple for over 1.2 billon people in Sub Sahara Africa and Latin America. Maize is one of the most widely utilized cereals in Nigeria and other West African countries. This is because of its high yielding potentials, storability and versatility in processing. It is a good source of digestible and high calorific starch, and dietary fibre. The grains are also rich in phosphorous, magnesium, manganese, zinc, copper, iron and selenium.

The discovery of the quality protein maize (QPM) varieties that contained about twice the levels of lysine and tryptophan and 10% higher grain yield than the most modern varieties of tropical maize (Vasal, 1993) brought a great hope in the effort of maize improvement as human and animal nutrition (Akande and Lamidi, 2006; Olakojo *et al*., 2007). A high level of these two amino acids not only enhances the manufacture of complete proteins in the body, but also offers 90% of the nutritional value of skim milk, thereby alleviating malnutrition (Olakojo *et al*., 2007; Upadhyay *et al*., 2009).

Sorghum (*Sorghum bicolor*) has achieved the highest growth rate of any major food crops in Western Africa and it is believed to have the greatest potential among food crops for attaining technological breakthroughs that will improve food production in any region (Manyong *et al.*, 1996). Sorghum constitutes about 75% of the cereals consumed in all parts of Nigeria today (Ekpenyong *et al.*, 1977). The grain is often processed into a fermented product known as ogi which can also be called as "ogi-baba" is consumed in many parts of West Africa

Millet (*Pennisetum americanum*) is one of the cereals produced extensively in Nigeria. Nigeria produces 21% of the World's total millet (FAO, 2002). Millet contains about 67% carbohydrate, and 12% protein. The seed is high in ash, iron, phosphorus and is an important source of the B group of vitamins (FAO, 1995). The essential amino acid profile of millet indicate that it rich in lysine, threonine, methionine and cysteine (FAO, 1995). Millet is traditionally processed into household porridge-type breakfast gruel (akamu) consumed in the western part of Nigeria and and dough (fura) consumed in the northern part of Nigeria.

The above cereals serve as good raw materials for processing cereal gruel (*ogi*) or porridge (akamu) in Nigeria. Maize steep water or filtrate obtained during fermentation of maize into ogi has been used as starter as alternative to the expensive commercial starter culture to ferment soy milk into yoghurt (Farinde *et al*. 2008; 2009; 2010). Maize steep water/filtrate has been found to contain lactic acid bacteria (Adegoke, 2004)

Nutritional composition of different cereal based beverages was reported by Ibironke *et al*. (2013). The objective of this study therefore is to use different cereal based water filtrates to ferment soy milk into yoghurt with a view to determine which one will be best as starter for quality yoghurt production so that the technology can be affordable and easily adopted by household community for improved nutrition and income generation.

2.0 Materials and methods

2.1 Processing of raw materials and ingredients into yoghurt

Soy milk was processed according to the method used by Fasoyiro, (2014). Cereal filtrate was obtained using the traditional method of processing cereals into gruel (*ogi*).

2.1.1 Soymilk processing

Five cups (500g) of soybean grains were soaked in 2.5 litre of water for 8 h, the soaked beans were boiled for 15 minutes after which the beans were ground with 3 litre of water for 5 min at high speed using a blender (Magic blender pengguman, Nikai, Japan) to obtain a slurry. The slurry was strained through a clean muslin cloth to extract the milk and the residue discarded. The milk was boiled for 20 minutes.

2.1.2 Cereal filtrate processing

One kilogram each of cereals (Ordinary maize (yellow variety), Quality protein maize (yellow variety), Sorghum (red variety) and millet (light cream variety)) were soaked in five litres of water for 72 hours to ferment. The fermented cereals were wet milled into paste and the paste was sieved in water using muslin cloth. The slurry was allowed to sediment for 48 hour during which further fermentation occurred. The filtrate was decanted after sedimentation. The filtrate for each cereal sample was stored in a clean plastic bottle.

2.1.3 Soy yoghurt processing

Soy yoghurt processing was carried out according to the method used by Farinde *et al.* (2010). Sugar was added to boiled soymilk to taste. The milk was cooled to 45^{o}C, the cooled milk was inoculated with cereal filtrate/steep water in ratio 10: 1. The inoculated soy milk was mixed thoroughly, vanilla flavor (1/2 teaspoon) was added and mixed again. The inoculated milk was then poured into clean plastic containers with well screwed caps. The containers and the contents were incubated at 45 ^{o}C for 8 to 10 hours. The fermentation was stopped by putting the fermented milk immediately in refrigerator for 30 minutes. The soy yoghurt produced was mixed and dispensed into clean packaging bottles for analysis.

Note: the water used for all the processing was boiled and cooled in a covered container.

2.2 Sample analysis

2.2.1 Chemical analysis

The crude protein, fat, ash, moisture and total solid were determined using the method (AOAC, 2000). Carbohydrate content was determined by subtracting the sum of protein per cent, fat per cent and ash per cent from total solids per cent. The solid non fat (SNF) was determined by subtracting fat from the total solids. Mineral content of the samples were determined using the method of AOAC (2000).

2.2.2 pH and titratable acidity

pH or hydrogen ion concentration of each sample were measured with a standard pH meter (ATC, Model HI-8915). Titratable acidity was determined using the method of Ikenebomeh (1989).

2.2.3 Microbial analysis

Microbial load of the yoghurt samples was determined using the method of Uzeh *et al*. (2006). Sample (1 ml) of appropriate dilution was plated out using appropriate agar. Nutrient agar was used to plate for total viable count, Man, Rogsa and Sharpe (MRS) (DeMan et al, 1960) medium was used to plate for lactic acid bacteria, Macconkey agar was used to plate for *coliform*, Manitol salt was used to plate for *Staphilococcus*, while acidified potato dextrose agar was used to plate for yeast .The plates were incubated at 37°C for 48 h for the growth of bacteria and 25°C for 72 h for that of yeasts. Isolation and identification of bacteria in the yoghurt samples were carried out using the method of Buchanan and Gibbons (1974), while yeasts were isolated and identified following the method of Deak and Beuchart (1987).

2.2.4 Sensory evaluation

Freshly prepared soy yoghurt samples (produced by fermenting soy milk with each of the selected fermented cereal filterate/steep water) as well as the commercial yoghurt (Ruvic) were presented to 20 untrained panel of judges who are regular yoghurt consumers. The panelists were given water for mouth rinsing after each tasting and they were asked to score the yoghurt samples for colour, appearance, flavour, mouthfeel, after taste and overall acceptability using 9 point hedonic scale, where 9 = like extremely and 1= dislike extremely (Larmond, 1977).

2.2.5 Statistical analysis

The results obtained were analyzed using descriptive and inferential statistics (ANOVA) using SPSS version 17. Means were separated using Duncan Multiple Range Test.

3.0 Results and Discussion

3.1 pH and *titratable acidity*

The ph of the soy yoghurt samples ranged from 4.68 in soy milk fermented with fermented millet filtrate to 5.20 in soy milk fermented with fermented quality protein maize filtrate (Table 1). A corresponding trend was observed for titratable acidity (TTA). TTA ranged from 0.10 in soy milk fermented with fermented millet filtrate to 0.21 in soy milk fermented with fermented quality protein maize filtrate. The values for the acidity in the yoghurt samples are as a result of the activities of the microorganisms in the fermented cereal filtrates (Nwoku and Oyeka, 1998)

3.2 Microbial analysis

The results of the microbial count of the yoghurt samples are shown in Table 2. Total viable count of microorganisms in the yoghurt samples ranged from 5.1 log cfu/ml in soy milk fermented with quality protein maize filtrate to 9.0 log cfu/ml in soy milk fermented with sorghum filtrate. The low count of microorganisms in the yoghurt fermented with filtrate of quality protein maize is similar to the report of Ijabadeniyi (2007). There was no significant difference (p > 05) in the total viable count of soymilk fermented with filtrate from ordinary maize and that of millet. Lactic acid bacteria (LAB) count was highest in soymilk fermented with filtrate from millet (7.5 log cfu/ml) while soy milk fermented with quality protein maize filtrate recorded the least LAB count (7.5 log cfu/ml). There was no coliform and mould count in all the yoghurt samples. However Staphylococcus was found in soymilk fermented with ordinary maize and soy milk fermented with sorghum filtrates (0.5 log cfu/ml and 0.60 log cfu/ml respectively). The *Staphylococcus* count in each of these samples was negligible, less than the standard set by FAO/WHO Expert Consultation on microbiological specification for minimum *Staphylococcus* count in foods (Frazier and Westhoff, 2005) and as such the product is safe for consumption.

Table 1: pH and Titratable acidity of the yoghurt samples

Sample	PH	TTA (g/100g)
SAP	4.96	0.12 ± 0.07^b
SAT	5.20	0.21 ± 0.10^a
SAS	4.88	0.18 ± 0.03^a
SAM	4.68	0.10 ± 0.05^b

Means followed by different superscript within the same column are significantly different ($p < 0.05$).
SAP - Soymilk fermented with maize filtrate SAT - Soymilk fermented with QPM filtrate
SAS - Soymilk fermented with sorghum filtrate SAM - Soymilk fermented with millet filtrate

Table 2: Microbial count of the yoghurt samples (log cfu/ml)

Sample	Total viable count	Coliform count	Lactic acid bacteria(LAB) count	Staphylococcus count	Mould count	Yeast count
SAP	6.2 ± 0.18^{b}	Nil	4.2 ± 0.05^{b}	0.5 ± 0.06^{a}	Nil	2.8 ± 0.16^{b}
SAT	5.1 ± 0.07^{c}	Nil	3.6 ± 0.17^{b}	Nil	Nil	3.3 ± 0.05^{a}
SAS	9.0 ± 0.11^{a}	Nil	7.1 ± 0.08^{a}	0.60 ± 0.1^{b}	Nil	2.1 ± 0.10^{c}
SAM	6.5 ± 0.20^{b}	Nil	7.5 ± 0.10^{a}	Nil	Nil	2.7 ± 0.05^{b}

Means followed by different superscript within the same column are significantly different ($p < 0.05$).
SAP - Soymilk fermented with maize filtrate SAT - Soymilk fermented with QPM filtrate
SAS - Soymilk fermented with sorghum filtrate SAM - Soymilk fermented with millet filtrate

Yeast count ranged from 2.1 log cfu/ml in soy milk fermented with sorghum filtrate to 3.3 log cfu/ml in soy milk fermented with quality protein maize filtrates. There was no significant difference (p > 05) in the yeast count of soymilk fermented with sorghum and soymilk fermented with millet. According to the Codex standards for fermented milk (Codex, 2003), yoghurt should contain a minimum of 7.0 log cfu/ml as the total microorganisms contained in the yoghurt sample. All the yoghurt samples met this requirement.

The result of the occurrence of isolates in the yoghurt samples are shown in Table 3. *Lactobacillus plantarum, L. fermentatum* and *Saccharomyces cerevisiae* were found to occur in all the four yoghurt samples. This is an indication that these three microbial species were actually present in all the fermented cereal filtrates. *Lactobacillus plantarum* and *L. fermentatum* might probably be very active in the fermentation process because Lactic acid bacteria count were far higher than yeast counts in the fermented yoghurt samples (Table 2), they might thus be majorly responsible for the fermentation of the soymilk into yoghurt. Nwosu and Oyeka reported that the activities of the microorganisms during fermentation of cereal into ogi provided fermentable sugar for the second part of ogi fermentation, and that the microorganisms responsible for these activities are the lactic acid bacteria and yeasts. These organisms produce organic acids, lower the pH and contribute to flavor development of the final product. Since the cereal filtrates used for the fermentation of the soy milk into yoghurt was found to contain Lactic acid bacteria and yeasts, it agrees with the report of Nwosu and Oyeka. These organisms must have been responsible for the acidity and the sour taste reminiscent of yoghurt. Lactobacillus isolates have been reported as important microflora of African fermented foods (Odunfa *et al*., 1996; Olasupo *et al*., 1995; Olasupo *et al*., 1997)

3.3 Chemical composition

The chemical composition of the soy yoghurt samples are shown in Table 4. Protein content ranged from 3.20 % in the soymilk fermented with ordinary maize to 3.57% in the soymilk fermented with sorghum filtrate. There was no significant difference (p> 0.05) in the protein content of soymilk fermented with sorghum and that of millet filtrate. There was also no significant difference (p> 0.05) in the protein content of soymilk fermented with ordinary maize

Table 3: Occurrence of Isolates in the yoghurt samples

Sample	***Lactobacillus plantarum***	***L. fermentatum***	***Leuconostoc meseteroides***	***Streptococcus thermophilus***	***Staphylococcus aureus***	***Saccharomyces cerevisae***
SAP	+	+	Nil	Nil	+	+
SAT	+	+	Nil	Nil	Nil	+
SAS	+	+	Nil	+	+	+
SAM	+	+	+	Nil	Nil	+

SAP - Soymilk fermented with maize filtrate SAT - Soymilk fermented with QPM filtrate
SAS - Soymilk fermented with sorghum filtrate SAM - Soymilk fermented with millet filtrate

Table 4: Chemical composition of the yoghurt samples

Sample	Moisture %	Crude protein %	Crude fat %	Total ash %	Carbohydrate %	SNF %	Total solids (g/100g)
SAP	75.61 ± 0.09[a]	3.28 ± 0.12[b]	2.97 ± 0.09[a]	0.77 ± 0.10[b]	6.66 ± 0.03[a]	10.71 ± 0.11[b]	13.68 ± 0.11[a]
SAT	75.70 ± 0.05[a]	3.36 ± 0.11[b]	2.89 ± 0.05[b]	0.86 ± 0.08[a]	6.49 ± 0.10[a]	10.71 ± 0.0[b]	13.60 ± 0.12[a]
SAS	72.72 ± 0.08[a]	3.57 ± 0.11[a]	2.92 ± 0.21[b]	0.84 ± 0.08[a]	7.77 ± 0.10[c]	12.18 ± 0.22[a]	15.10 ± 0.32[a]
SAM	73.09[a] ± 0.05	3.49 ± 0.19[a]	2.89 ± 0.03[b]	0.92 ± 0.10[a]	7.60 ± 0.10[b]	12.01 ± 0.03[a]	14.90 ± 0.03[a]

Means followed by different superscript within the same column are significantly different ($p < 0.05$).
SAP - Soymilk fermented with maize filtrate SAT - Soymilk fermented with QPM filtrate
SAS - Soymilk fermented with sorghum filtrate SAM - Soymilk fermented with millet filtrate

and that of quality protein maize filtrate. The protein content of soy yoghurt samples in this report is similar to the report of Farinde *et al*. (2008) and Opara *et al*. (2013). The Codex standard for milk and fermented milk products specifies a minimum of 2.7 % protein (Codex 2003). All the yoghurt samples contained higher protein content than the specified minimum value for protein by Codex. The highest fat and carbohydrate contents were recorded in soymilk fermented with ordinary maize (2.97% and 9.61% respectively) (Table 4). Similar report was given by Ibironke *et al*. (2013) in which fat and carbohydrate content was higher in ordinary maize filtrate than that of sorghum filtrate. Maize has also been reported to contain higher fat and carbohydrate than sorghum and millet (Apena et *al.*, 2015). Total solids and solids non fat were highest in soymilk fermented with sorghum filtrate (15.10% and 12.18% respectively). There was no significant difference ($p > 0.05$) in the total solids of all the yoghurt samples (Table 4).

The result of the mineral content of the yoghurt samples are shown in Table 5. There was no significant difference ($p > 0.05$) in the calcium contents of all the yoghurt samples. Soymilk fermented with sorghum filtrate was significantly high ($p < 0.05$) in phosphorus and zinc compared with all the other yoghurt samples. Soy milk fermented with millet filtrate contained the highest calcium and magnesium

The result of the sensory evaluation of the yoghurt samples is shown in Table 6. The commercial yoghurt was significantly different ($P < 0.05$) in colour compared to all the other yoghurt samples. There was no significant difference ($p > 0.05$) in the appearance of the commercial yoghurt and all the developed soymilk yoghurt samples (Table 6). There was no significant difference ($p > 0.05$) in the mouthfeel of the commercial yoghurt and yoghurt developed from soymilk and millet filtrate. There was also no significant difference ($P < 0.05$) in the taste of yoghurt developed from soymilk and millet filtrate and yoghurt developed from soymilk and sorghum filtrate. The commercial yoghurt was most preferred in terms of overall acceptability. However all the yoghurt samples were accepted.

Table 5: Mineral composition of the yoghurt samples (mg/kg)

Sample	Ca	Mg	P	Fe	Zn
SAP	877.50 ± 0.12[a]	1325 ± 0.11[b]	600.20 ± 0.15[ab]	18.50 ± 0.12[a]	27.70 ± 0.11[b]
SAT	779.50 ± 0.11[a]	1429 ± 0.19[b]	632 ± 0.10[ab]	18.50 ± 0.10[a]	25.40 ± 0.10[b]
SAS	945.75 ± 0.18[a]	1835 ± 0.20[a]	725.70 ± 0.09[a]	17.30 ± 0.15[a]	40.20 ± 0.16[a]
SAM	965.25 ± 0.09[a]	1875 ± 0.21[a]	688.5 ± 0.14[b]	17.30 ± 0.20[a]	27.80 ± 0.16[b]

Means followed by different superscript within the same column are significantly different ($p < 0.05$).
SAP - Soymilk fermented with maize filtrate SAT - Soymilk fermented with QPM filtrate
SAS - Soymilk fermented with sorghum filtrate SAM - Soymilk fermented with millet filtrate

Table 6: Sensory Evaluation of soy-yoghurt samples

Sample	Colour	Appearance	Flavour	Mouth feel	After taste	Overall acceptability
SAT	6.5 ± 0.34^{a}	6.5 ± 0.39^{a}	5.6 ± 0.71^{a}	4.7 ± 0.65^{b}	4.7 ± 0.84^{c}	5.4 ± 0.40^{a}
SAM	6.9 ± 0.45^{a}	7.2 ± 0.41^{a}	6.2 ± 0.57^{ab}	6.9 ± 0.70^{a}	5.5 ± 0.80^{b}	7.2 ± 0.32^{b}
SAP	6.6 ± 0.74^{a}	7.4 ± 0.37^{a}	4.7 ± 0.71^{a}	5.0 ± 0.44^{b}	4.1 ± 0.84^{c}	5.6 ± 0.37^{a}
SAB	8.3 ± 0.21^{b}	7.2 ± 0.29^{a}	7.9 ± 0.40^{b}	7.2 ± 0.81^{a}	8.4 ± 0.22^{a}	8.8 ± 0.13^{c}
SAS	6.7 ± 0.47^{a}	6.4 ± 0.52^{a}	5.4 ± 0.56^{a}	5.5 ± 0.60^{ab}	6.1 ± 0.56^{b}	6.3 ± 0.49^{ab}

Values are mean scores ± standard error where n = 20
Means followed by different superscript within the same column are significantly different ($p < 0.05$).
SAP - Soymilk fermented with maize filtrate SAT - Soymilk fermented with QPM filtrate
SAS - Soymilk fermented with sorghum filtrate SAM - Soymilk fermented with millet filtrate
SAB = Commercial yoghurt (Ruvic yoghurt)

Conclusion

This study showed that yoghurt analogue of good quality and which met the minimum standard for protein content in milk and fermented milk products, specified by Codex could be produced from soymilk and fermented cereal filtrates (cheap and available raw materials). Filtrate from sorghum appear to be the best starter for soy yoghurt production in terms of nutrients content while filterate from millet was most accepted by sensory panelists out of the three cereal filterate samples. Lactic acid bacteria (*Lactobacillus plantarum and L. fermentatum*) which are probiotic microorganisms were found to be present in all the soy yoghurt samples. Combination of fermented cereal filtrate from sorghum and millet to ferment soy milk should further be studied to develop a starter of high nutrients and high consumer acceptability.

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