Research Journal of Food Science and Quality Control (RJFSQC) E-ISSN 2756-5483
P-ISSN 2695-2459 Vol 11. No. 1 2025 www.iiardjournals.org Online Version

Effect of Fermentation Time on the Physicochemical and Proximate Composition of Germinated and Ungerminated Soybean and Tigernut Yoghurt, Soursop Yoghurt and Cow Milk Yoghurt

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DOI: 10.56201/rjfsqc.vol.11.no1.2025.pg31.49

Abstract
The aim of this work was to determine the effect of fermentation time on the physicochemical and proximate composition of germinated and ungerminated soy and tigernut (GSY, UGSY, GTNY, and UGTNY), soursop and cow milk yoghurt. Germinated and ungerminated soy and tigernut yoghurts were prepared from milk reconstituted from flour, soursop yoghurt (SSY) was prepared from the pulp juice. Cow milk yoghurt (CMY) (control) was prepared from cow milk. Fermentation was at 42 ℃ for 6-24 h with two strains of commercial starter cultures: Lactobacillus bulgaricus and Streptococcus thermophillius. The samples were analyzed using standard methods. Result showed an initial pH that ranged from 5.60 to 6.54 but significantly (p<0.05) decreased to 4.11 - 4.82 and 3.53 - 3.96 after 6 and 12 h. Similarly, total soluble solids (TSS) at initial range of 12.90 to 16.90 decreased to a range of 7.20 to 11.90 °Brix while Total titatible acidity (TTA) increased from 0.52 to 0.86 %. Effect of fermentation time on viscosity and syneresis of yoghurt significantly (p<0.05) increased from 0.6 to 0.73 Pa.S and 3.72 to 8.90 %, respectively, after 6 and 12 h of fermentation. Effect of fermentation time on proximate composition at initial, 6 and 12 h fermentation showed moisture and fat content that ranged from 84.74 to 90.08 % and 2.34 to 3.58 %, correspondingly. Protein content increased significantly (p<0.05) from 3.32 to 6.81 % whereas ash and crude fibre varied from 0.61 to 0.99% and 0.02 to 0.08 % after 6 and 12 h of fermentation. Carbohydrate increased as fermentation time increased, the highest carbohydrate content (8.48 %) was found in GTNY while the lowest values were established in CMY. The highest energy value (81.09 Kcal/100g) was found in GSY while the lowest value (48.90 Kcal/100g) was established in SSY. Fermentation time increased the physicochemical and proximate composition of yoghurts was affected.

Keywords: *Fermentation Time, Physicochemical, Proximate, Yoghurt, Germinated and Ungerminated*

1. Introduction

Yoghurt is a dairy product produced by fermentation of milk by two species of bacteria (*Lactobacillus* sp. and *Streptococcus* sp.) (Aktar, 2022). Yoghurt is one of the most popular fermented dairy products which have a wide acceptance worldwide whereas it's nutritional and health benefits are well known for centuries. Yoghurt is made with a multiplicity of ingredients with milk being the main ingredient. The type of milk used depends on the variety or type of the yoghurt to be prepared. Other ingredients include sweeteners, stabilizers, flavours, and bacterial cultures (Corrieu *et al.,* 2016). The nutritional composition of yoghurt is generally like that of milk. Yoghurt is said to be a dense food and is rich in protein, carbohydrates, amino acids, minerals (Calcium and Phosphorus) and vitamins (thiamin-B1, riboflavin-B2, niacin-B3, folate-B9, cobalamin-B12, and vitamin C), but is lacking in iron. Fat content of yoghurt depends on the fat content of the mixture and the type of milk used (Weerathilake *et al.,* 2014). The interest of consumers for vegetable milks is growing in many countries as they are free of cholesterol**.** Animal milks are more expensive in Nigeria and not easily affordable for yoghurt production, this necessitates the need to seek for ways of producing yoghurts from plant protein such as soybeans, Soursop, and tigernut which are rich sources of proteins, minerals, vitamins, fats with some medicinal properties.

In yoghurt production, yoghurt quality is affected by fermentation time, for instance, when the yogurt starter is introduced to the pasteurized milk, the bacteria begin to feast on the lactose in the milk and produce lactic acid, and the culture proliferates and spreads throughout the milk (Oladimeji, Oyinlola and Odigure, 2016). The longer the fermentation time progressed, the longer the culture has time to multiply, thereby increasing the amount of bacteria and acids in the yoghurt while decreasing the lactose content of the milk, (Shafiee, 2010). Fermentation time is crucial for yoghurt because it directly impacts the final taste, texture, and acidity of the yoghurt; prolonged fermentation time, leads to an increase in TTA and a decrease in pH due to the metabolic activity of the lactic acid bacteria used, lactose converts into lactic acid which coagulates milk proteins along with the production of certain volatile compounds that gives its characteristic flavour and aroma (Murugkar, 2014).

Soybeans (*Glycine max*) have been an important crop for thousands of years. Soymilk is an aqueous extract of soya beans commonly characterized by beany flavor, which can be improved by lactic acid fermentation, as in yoghurt-like products for acceptability (Aydar, Tutuncu, & Ozcelik, 2020). Soymilk can serve as a very good alternative to the expensive cow milk as it contains all the essential amino acid, the milk is an ideal hypoallergenic foodstuff for infants who are allergic to human milk and cow's milk (Mañes & Molto, 2022).

Tiger-nuts (Cyperus esculentus L.) are one of the underutilized tubers in Nigeria, with high levels of soluble fibers, vitamins and minerals. It is rich in protein with considerable amounts of essential amino acids (Airaodio, and Ogbuagu, 2020). It is rich in mono saturated fatty acids (MUFA) making it an excellent anti-diabetic agent. Tigernut is reported to have therapeutic properties as it contains phytonutrients such as quercetin which may boost libido in men (Barber, Kabisch, Pfeiffer and Weickert,2020). Tigernut can be consumed fresh or in dried form as snacks or in form of beverage referred to as tiger nut milk (Adejuyitan, Otunola, Akande, Bolarinwa, and Oladokun, 2009). The development of yoghurt based on tigernut provides a healthy advantage, when compared with some conventional yogurts.

Soursop is considered a healthy fruit because of its bioactive compounds such as polyphenols and acetogenins that offer attractive health benefits such as anti-inflammatorry, anti-diabetic and anti-tumoral activities (Coria-Téllez et al., 2018). Extract from soursop (Annona muricata) fruit consists of 67.5% fruit flesh, 20% fruit skin, 8.5% fruit seeds, and 4% fruit core. After water, the nutrient content in soursop is 16.3 g carbohydrates, 1.0 g protein, 0.3 fat and the most dominant vitamin in soursop fruit is vitamin C, which is about 20 mg per 100 grams of fruit flesh (Tiara Wacana: Yogjakarta; 2010). The content of vitamin C in soursop is an excellent antioxidant to increase endurance and slow the aging process. The need for vitamin C per person per day (ie 60 mg) can be fulfilled by consuming 300 grams of soursop meat. Sour taste in soursop comes from nonvolatile organic acids, especially malic acid, and isositric acid.
Cow milk is an excellent source of nutrients except iron and ascorbic acid. It has been reported to be an important food for infants, teens and adolescents (Aydar, Tutuncu, and Ozcelik, 2020). The high cost of milk has necessitated the development of alternative sources of milk from plants.
The importance of germination of seeds cannot be overstressed; Germination alters the nutritional profile and morphological structure of grain seeds, eventually improving the digestibility and bioaccessibility of nutrients. (Chu *et al*., 2020, Li et al., 2022). Germination has also been shown to improve the bioactivities of food components generating compounds such as polyphenols and flavonoids, responsible for antioxidant activities in grain seeds (Xu et al., 2019, Chu et al., 2020).
The objective of this work was to investigate the 'Effect of fermentation time on the physicochemical and proximate composition of germinated and ungerminated soybean and tigernut yoghurt, soursop yoghurt and cow milk yoghurt''

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2. Materials and methods

2.2.1. Materials

2.2.2. Source of raw materials

The plant raw materials (Soybean, tiger nut and soursop) and powdered cow milk (peak brand) were purchased from Mile 3 Market, Port Harcourt, Rivers State, Nigeria. Starter culture used *Lactobacillus bulgaricus* and *Streptococcus thermophiles* manufactured by NP Selection LTD, London, UK.

2.2.3. Chemicals and reagents

Microbial media and chemicals of analytical grade were obtained from the Department of Food Science and Technology, Rivers State University, Port Harcourt.

2.2.4. Production germinated soy flour

100 g of soybean were sorted to remove stones and broken seeds, washed and soaked in distilled water for 12 h. After which the soaked seeds were washed and allowed to drain. The seeds were spread on jute bag with water sprinkled at intervals at room temperature to allow sprouting for 27 h. Then, the soybean seed was oven-dried at 60℃ for 30 min. The dried beans were dehulled, winnowed and milled using locally fabricated attrition mill. The flour obtained was sieved using 0.45 mm mesh size and packed in an air tight container until required for use as in Fig 1

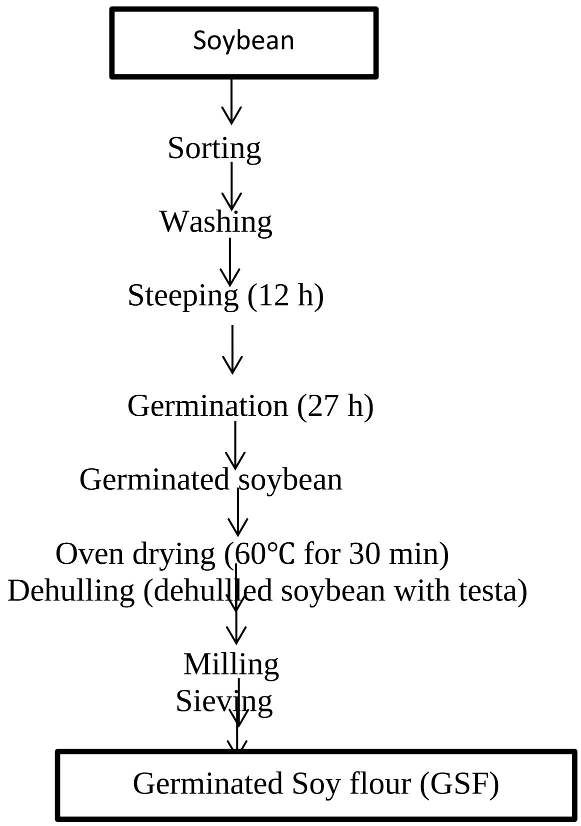

Fig 1: Production of germinated soybean flour
Source: Barber *et al*. (2020)

2.2.5. Production of ungerminated soy flour

Soybean were sorted to remove stones and broken seeds, washed and soaked in distilled water for 12 h to soften. After 12 h, the seeds were washed and allowed to drain. The seeds were decanted and precooked, dehulled, washed, draining and oven-dried at 60℃ for 30 min and milled. Resulting flour was packed in air tight container after sieving through 0.45 mm mesh.

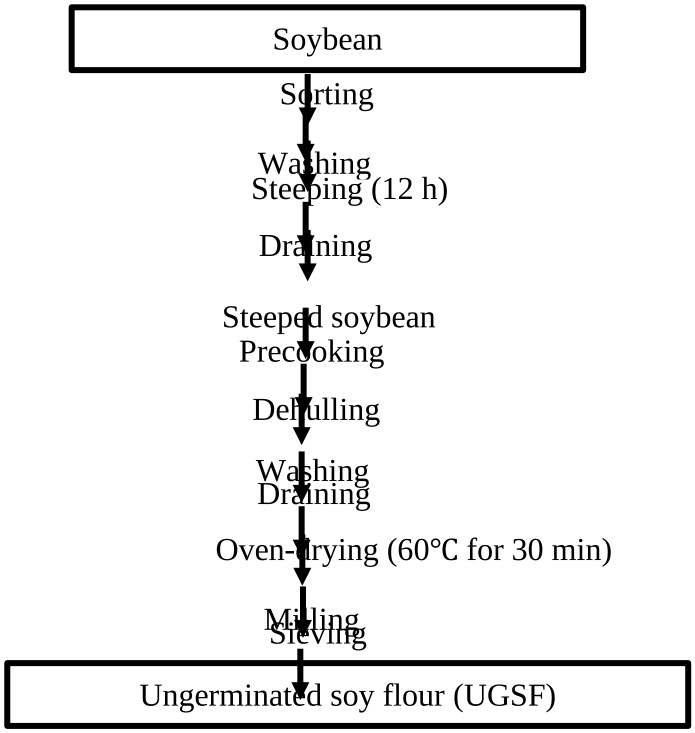

Fig. 2: Production of ungerminated soy flour
Source: Barber *et al.* (2020) with some modification.

2.2.6. Production of germinated/ ungerminated soy milk from soy flour

Germinated/ungerminated soy milk was produced from soy flour using the method described by Barber *et al.* (2020). 100 g of the flours was reconstituted in 400 mL of distilled water, and was allowed to stand for 30 min before straining using a muslin cloth as in Fig 3 below.

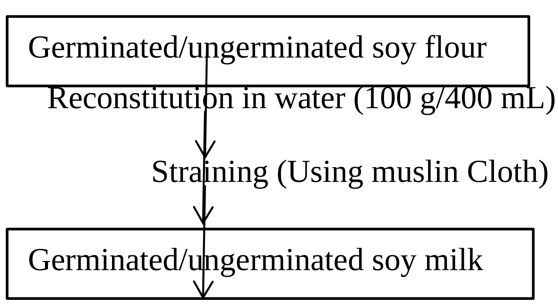

Fig 3: Production of germinated soy milk from germinated soy flour
Source: Barber *et al*. (2020)

2.2.7. Production of germinated tiger nut flour

The method described by Obinna-Echem *et al.* (2020) with modification as in Fig 4 was employed to produce germinated soy flour. 100 g of dried tigernut tubers was weighed, and sorted to remove unwanted matter by visual observation before washing. Washed tuber was soaked in distilled water for 12 h at room temperature. After which the tubers were spread on a jute bag and water was sprinkled at intervals to initiate sprouting at room temperature for 96 h. Sprouted tubers were oven dried, allowed to cool, milled and sieved (0.45 mm) mesh size. The flour obtained was packaged in a container, and stored in refrigerator till required for production.

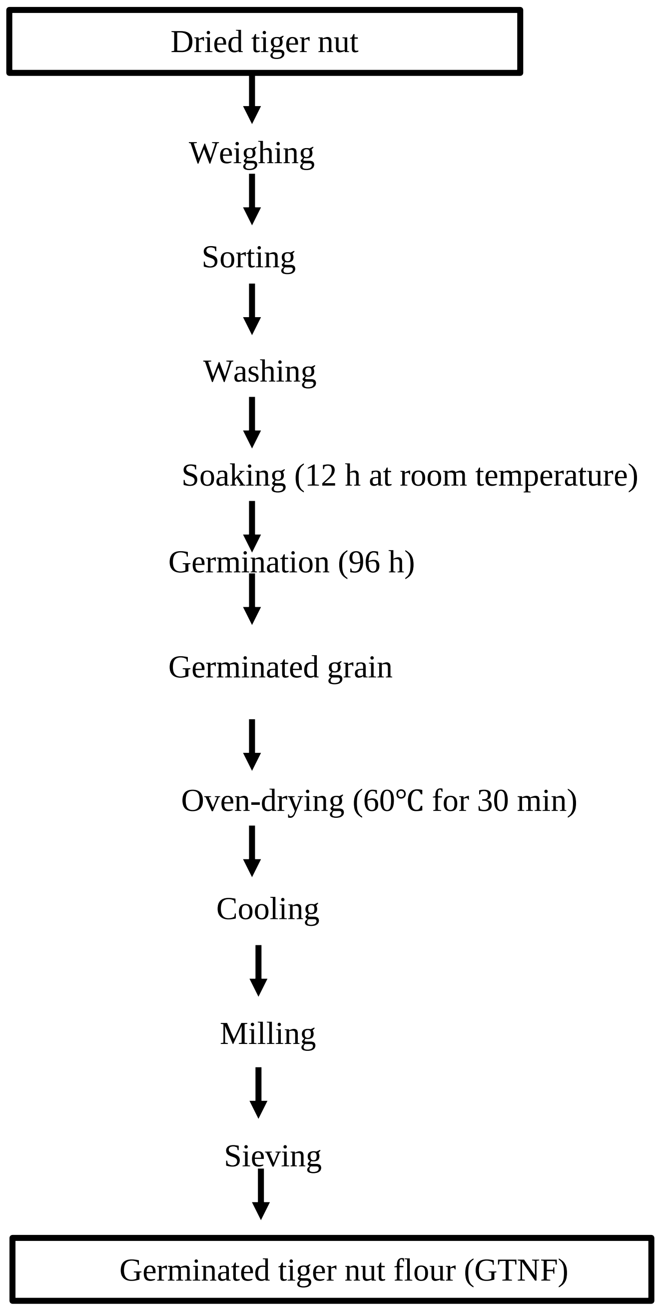

Figure 5: Production of germinated tiger nut flour
Source: Obinna-Echem *et al*. (2020)

2.2.8. Production of ungerminated tiger nut flour

The method described by Obinna-Echem *et al.* (2020), was used in the production of tiger nut flour. Fresh tiger nuts was sorted, washed with distilled water, oven dried on a sterile foiled tray at 60℃ for 30 min and was milled into powder using attrition mill. The powder was sieved using a 0.45 mm mesh size and packaged in an air tight container until required as in Fig 6

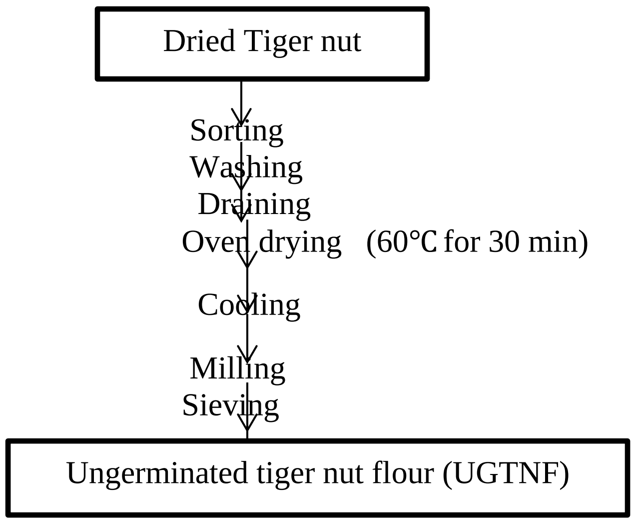

Figure 6: Production of ungerminated tiger nut flour
Source: Obinna-Echem *et al*. (2020)

2.2.9. Production of milk from germinated/ ungerminated tigernut flour

Germinated and ungerminated milk from tigernut flour was produced using the method described by Obinna-Echem *et al.* (2020). 100 g of the flours was reconstituted in 400 mL of distilled water, and was allowed to stand for 30 min before straining using a muslin cloth as in Fig 5.

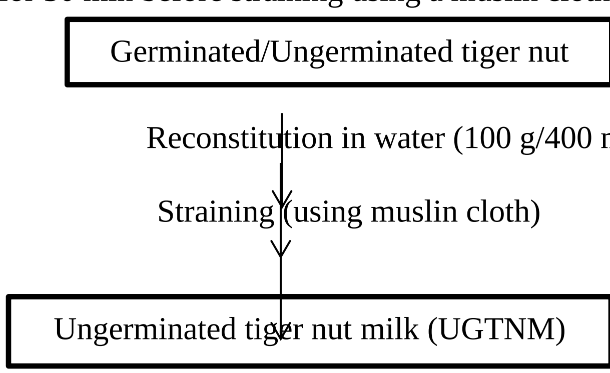

Fig.7: Production of Germinated and ungerminated milk from tigernut flour
Sourcee: Obinna-Echem *et al.* (2020)

2.2.10. Production of ungerminated and ungerminated soy and tigernut milk from flour

Germinated and ungerminated tigernut milk was produced using the method described by Barber *et al.* (2020). 100 g of the flours was reconstituted in 400 mL of distilled water, and was allowed to stand for 30 min before straining using a muslin cloth as in Fig 6 below.

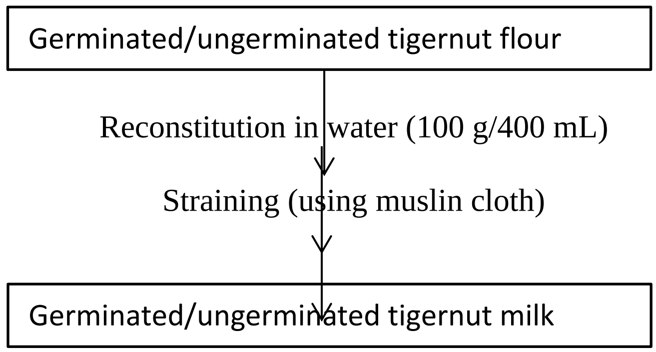

Fig 8: Production of Germinated/ungerminated tigernut milk flour
Source: Barber *et al*. (2020).

2.2.10. Production of soursop puree

The method described by Belewu *et al*. (2005), was used to produce the soursop puree. Mature and fresh soursop fruit was sorted and washed severally with clean water and then hand peeled. The seeds were removed and the fruit were blended to obtain slurry which was filtered using a muslin cloth to obtain the puree as in Fig 6 below.

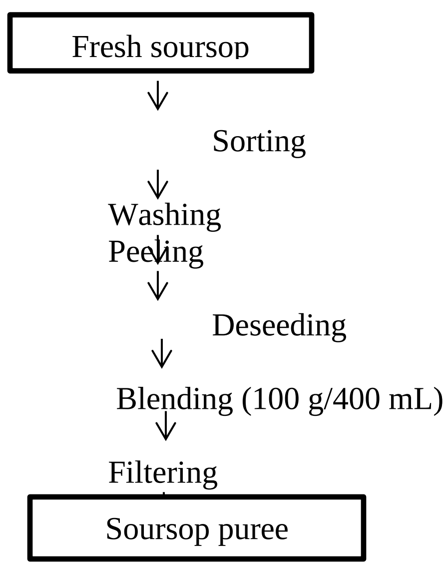

Fig .9: Production of soursop puree
Source: Belewu *et al.* (2005).

2.2.11. Preparation of cow milk for cow milk yoghurt

The method adopted by Bristone, (2006), with modification, was used for the preparation of cow milk for cow milk yoghurt. 100g of peak milk powder was reconstituted in 400 mL of distilled water and was pasteurized at 72℃ for 15 min, then allowed to cool at 42℃.

2.2.12. Production of date syrup

Hundred (100 g) grams of date fruit was deseeded, and was taken into a wide shallow vessel and were washed under running tap water. The dates were soaked in 300 mL hot water (70℃) for 30-45 min, and thereafter blended using a blender and extracted by straining using a cheese cloth to squeeze out the date syrup. The date residue was discarded, and the syrup was collected in saucepan and boiled in a saucepan until reaches 53 °Brix by measurement of the refractometer. The flame was kept low to medium and stirred occasionally to avoid burning at the bottom. The syrup was placed in a cool condition for storage (4 ℃) as shown in Fig 10 below.

Date fruit
↓
Sorting
↓
Deseeding
↓
Washing
↓
Size reduction
↓
Soaking (100g fruit, 300 mL boiling water for 30-45 min)
↓
Blending
↓
Filtration (using cheese cloth)
↓
Boiling (until thick and reaches a consistency, 53 °Brix)
↓
Cooling (4℃)
↓
Date syrup

Fig 10: Production of date syrup
Source: Barber *et al*. (2020)

2.2.13. Yogurt Preparation

2.2.13.1. Production of yoghurt from germinated /ungerminated soy and tigernut milk, soursop puree and cow milk

The method described by Barber *et al*., (2020) with some modification was followed**.** Required quantity of milk from germinated /ungerminated soy and tigernut milk, soursop puree and cow milk was used with 10 mL of date syrup and 0.1 g of xanthan gum added. The mixture was homogenized, pasteurized at 72℃ for 15min, and allowed cooling at 42℃. After which, the mixture was inoculated with commercial starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) in a ratio 1:1 while maintaining the temperature of 40℃ for approximately 6, 12 and 24 h until desired degree of acidity was achieved. Finally the mixture was cooled to room temperature of about 27°C. The yoghurt sample was kept in sterile plastic bottles in the refrigerator until further analysis.

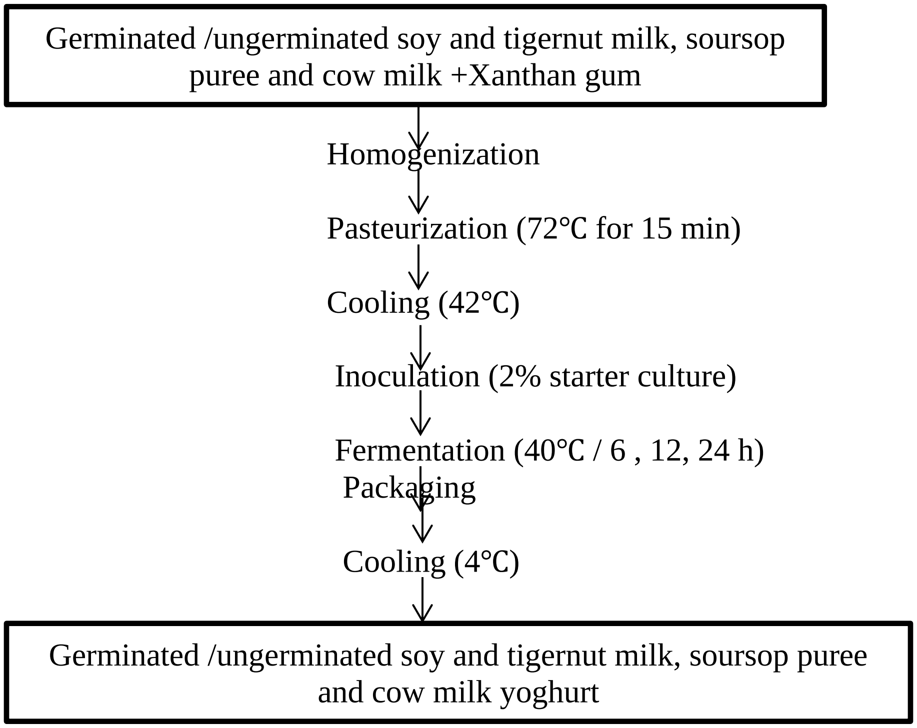

Fig 11: Production of yoghurt
Source: Belewu *et al.* (2005)

3.0 Physicochemical Analyses

3.1. Determination of pH

A calibrated potentiometer prior to determination was used. Calibration was done using pH buffer of 4.0, 7.0 calibration kit Thermo Fisher Scientific Orion (Waltham, MA, USA). Ten (10 g) grams of homogenized yoghurt in 100 mL of distilled water as described by Peralta-Ruiz *et.al* (2020) was followed.

3.2 Determination of Titratable acidity (TTA)

Titratable acidity was determined by the method described by Obinna-Echem and Torporo (2018) with some changes. 1 mL of each yoghurt was diluted to 10 mL with distill water and titrated against 0.1N sodium hydroxide solution using phenolphthalein as indicator. The result was expressed as % lactic acid as given in the equation below.

$$\text{TTA (\%)} = \frac{\text{Vol.NaOH used(mL) x 0.1N NaOH x milliequivalent factor x 100}}{mass\ of\ sample\ (g)} \qquad \text{Eq. (1)}$$

3.3. Determination of Total soluble solid content (TSS)

Total soluble solid (°Brix) was determined using Abbe hand refractometer as specified by AOAC, (2012). Prism of the refractometer was cleaned and a drop of the sample was placed on the surface and result read from the refractometer scale.

3.4. Determination of Viscosity

The viscosity of the yoghurt was determined with the aid of a rotary digital viscometer (NDJ-85, China) at 20^{O}C. The rate of flow of 150 mL of each yoghurt in a beaker introduced directly unto the rotating spindle was displayed on the LCD screen in Pa.s was recorded as the viscosity (Obinna-Echem and Torporo (2018) with some modifications.

3.5. Determination of Syneresis

The degree of syneresis is expressed as the proportion of spontaneous release of the watery part of yoghurts due to gel contraction (free whey). It was measured by the method described by Wijesinghe *et al*. (2018). Ten (10 mL) of each yoghurt was placed separately on a centrifuge tube and was placed on a centrifuge (L-600 China centrifuge) at 5000 rpm for 10 min. After which supernatant was weighed was calculated as the syneresis using the equation given below.

$$\text{Free whey (\%)} = \frac{\text{weight of initial sample} - \text{weight of sample after filtration}}{\text{weight of initial sample}} \times 100 \qquad \text{Eq. (2)}$$

3.6. Proximate analysis of fermented yoghurt: The proximate composition (moisture, ash, fat, protein, fibre and energy value) was done using standard analytical methods for food analysis described by (AOAC, 2019).

3.7. Statistical analyses: All data were expressed as means of two independent trials with standard deviation. Minitab (19 versions) statistical software was used to assess differences between treatments and the data subjected to analysis of variance (ANOVA). Means was compared and Turkey pairwise comparisons was used to separate means where differences existed and significance was accepted ($p<0.05$).

4.0 Results

4.1. The effect of fermentation time on the physicochemical and proximate composition of germinated and ungerminated soybean and tigernut yoghurt, soursop yoghurt and cow milk yoghurt is shown in Tables 1 and 2.

From table 1 the highest pH value (6.54) was found in the control sample (CMY) at initial fermentation while the lowest pH (3.79) was observed in SSY after 12 h fermentation time. Total titatible acidity (TTA) was lowest (0.52%) in GTNY at initial fermentation and highest (0.86%) after 12 h fermentation. TSS was highest (16.90%) in CMY and significantly ($p<0.05$) lowest (7.20%) in GSY. viscosity and Syneresis were respectively, highest 0.73 PaS (UGSY) and 8.90 % (GSY) and lowest 0.67 PaS (GTNY) and 3.99 % (SSY).

Parameter	**Sample code**	**Fermentation time**		
		initial	**6 h**	**12 h**
pH	GSY	6.09±0.01^{b}	4.50±0.02^{b}	4.46±0.01^{b}
	UGSY	6.14±0.03^{b}	4.36±0.01^{c}	4.28±0.01^{c}
	GTNY	5.60±0.02^{c}	4.23±0.05^{d}	4.12±0.01^{c}
	UGTNY	6.01±0.03^{b}	4.11±0.01^{e}	4.32±0.01^{c}
	SSY	6.06±0.11^{b}	4.30±0.01^{cd}	3.79±0.02^{e}
	CMY	6.54±0.01^{b}	4.82±0.00^{a}	4.11±0.01^{d}
TTA (%)	GSY	0.42±0.00^{b}	0.76±0.00^{a}	0.80 ±0.00^{a}
	UGSY	0.48±0.00^{b}	0.70±0.00^{c}	0.72±0.00^{c}
	GTNY	0.48±0.00^{b}	0.63±0.00^{d}	0.65±0.00^{c}
	UGTNY	0.32±0.00^{c}	0.54±0.00^{c}	0.55±0.00^{c}
	SSY	0.53±0.00^{a}	0.85±0.00^{b}	0.86±0.00^{d}
	CMY	0.62±0.00^{a}	0.66±0.00^{a}	0.67±0.00^{b}
TSS (%)	GSY	12.90±0.14^{d}	8.90±0.14^{c}	7.20 ^{d}±0.28
	UGSY	12.90±0.14^{d}	12.10±0.14^{a}	10.95±0.07^{b}
	GTNY	13.90±0.14^{c}	11.90±0.14^{a}	11.10±0.14^{b}
	UGTNY	13.95±0.07^{c}	10.95±0.07^{b}	11.10±0.14^{b}
	SSY	14.90±0.14^{b}	12.20±0.14^{a}	11.90±0.14^{a}
	CMY	16.90±0.14^{a}	8.05±0.07^{d}	7.90±0.14^{c}
Viscosity (PaS)	GSY	0.69±0.00^{d}	0.72±0.00^{b}	0.71±0.00^{d}
	UGSY	0.68±0.00^{c}	0.71±0.00^{d}	0.73±0.00^{a}
	GTNY	0.67±0.00^{b}	0.72±0.00^{c}	0.73±0.00^{a}
	UGTNY	0.69±0.00^{b}	0.72±0.00^{c}	0.73±0.00^{a}
	SSY	0.62±0.00^{b}	0.71±0.00^{d}	0.71±0.00^{c}
	CMY	0.64±0.00^{a}	0.72±0.00^{b}	0.72±0.00^{b}
Syneresis (%)	GSY	6.05±0.07^{b}	8.10±0.14^{b}	8.90±0.14^{a}
	UGSY	4.95±0.07^{c}	6.90±0.14^{c}	8.05±0.00^{b}
	GTNY	3.72± 0.01^{f}	6.90±0.14^{f}	7.40±0.14^{c}
	UGTNY	7.22±0.01^{a}	5.15±0.00^{a}	5.25±0.00^{d}
	SSY	4.05±0.00^{e}	3.99±0.02^{e}	4.06±0.14^{e}
	CMY	4.64±0.00^{d}	5.52±0.00^{b}	6.63±0.02^{c}

Table 1 Effect of fermentation time on physicochemical properties of yoghurts
Values are means ±standard deviation of duplicate determination. Means that do not share the same superscript number along the column for each parameter are significantly different ($p<0.05$).
Key: GSY = Germinated soybean yoghurt, UGSY = Ungerminated soybean yoghurt, GTNY = Germinated tiger nut yoghurt, UGTNY = Ungerminated tiger nut yoghurt, SSY =Soursop yoghurt, CMY = Cow milk yoghurt

Table 2 shows results for the Effect of fermentation time on proximate composition of yoghurts. Moisture content was highest at initial fermentation time and lowest after 6 and 12 h of fermentation for all the yoghurt samples. Protein, crude fibre, ash, carbohydrate and energy values were highest after 6 and 12 h fermentation while fat content decreased after 6 and 12 h.

Parameter	Fermentation time	Yoghurt samples					
		GSY	UGSY	GTNY	UGTNY	SSY	CMY
Moisture (%)	0	84.74^{f}±0.01	85.15^{e}±0.01	86.65^{d}±0.01	86.71^{c}±0.01	90.08^{a}±0.01	88.53^{b}±0.01
	6	83.05^{f}±0.01	84.24^{e}±0.01	85.92^{d}±0.01	86.12^{c}±0.08	88.02^{a}±0.01	87.12±0.01
	12	82.56^{f}±0.01	83.45^{e}±0.01	84.82^{d}±0.01	85.66^{c}±0.01	87.79^{a}±0.01	86.77^{b}±0.01
Protein (%)	0	5.22^{b}±0.01	5.13^{c}±0.01	3.47^{e}±0.01	3.32^{f}±0.01	4.04^{d}±0.01	6.51^{a}±0.01
	6	5.37^{b}±0.01	5.33^{c}±0.01	3.51^{e}±0.01	3.37^{f}±0.01	4.08^{d}±0.01	6.73^{a}±0.01
	12	5.46^{b}±0.01	5.48^{b}±0.01	3.57^{d}±0.01	3.45^{e}±0.02	4.14^{c}±0.01	6.81^{a}±0.01
Fat (%)	0	3.33^{b}±0.01	3.31^{b}±0.01	2.44^{d}±0.01	2.76^{c}±0.01	2.34^{e}±0.01	3.58^{a}±0.01
	6	3.21^{c}±0.01	3.30^{b}±0.01	2.42^{e}±0.01	2.57^{d}±0.02	2.31^{f}±0.01	3.46^{a}±0.02
	12	3.13^{c}±0.01	3.26^{b}±0.01	2.35^{d}±0.01	2.36^{d}±0.01	2.19^{e}±0.01	3.44^{a}±0.02
Crude Fibre (%)	0	0.07^{a}±0.01	0.05^{a}±0.01	0.08^{a}±0.01	0.08^{a}±0.01	0.02^{b}±0.01	0.02^{b}±0.01
	6	0.08^{ab}±0.01	0.06^{b}±0.01	0.09^{a}±0.01	0.10^{a}±0.01	0.03^{c}±0.01	0.03^{c}±0.01
	12	0.10^{ab}±0.01	0.08^{b}±0.01	0.10^{ab}±0.01	0.11^{a}±0.01	0.03^{c}±0.01	0.03^{c}±0.01
Carbohydrate (%)	0	5.72^{c}±0.03	5.47^{d}±0.03	6.66^{a}±0.06	6.49^{b}±0.03	2.92^{e}±0.03	0.77^{f}±0.03
	6	7.34^{a}±0.06	6.15^{c}±0.01	7.35^{a}±0.04	7.18^{b}±0.03	4.92^{d}±0.02	2.02^{e}±0.04
	12	7.77^{b}±0.01	6.85^{c}±0.06	8.48^{a}±0.01	7.72^{b}±0.04	5.23^{d}±0.07	2.29^{e}±0.03
Ash (%)	0	0.94^{a}±0.01	0.91^{a}±0.01	0.71^{b}±0.01	0.65^{c}±0.01	0.61^{d}±0.01	0.61^{d}±0.01
	6	0.96^{a}±0.01	0.94^{a}±0.01	0.73^{b}±0.01	0.67^{c}±0.01	0.63^{d}±0.01	0.66^{cd}±0.01
	12	0.99^{a}±0.01	0.97^{a}±0.01	0.74^{b}±0.01	0.71^{c}±0.01	0.65^{d}±0.01	0.68^{cd}±0.01
Energy (Kcal/100g)	0	73.69^{a}±0.12	72.13^{b}±0.02	62.48^{d}±0.04	64.04^{c}±0.01	48.90^{f}±0.04	61.32^{e}±0.01
	6	79.69^{a}±0.11	75.56^{b}±0.04	65.16^{d}±0.08	65.29^{d}±0.36	56.73^{e}±0.08	66.08^{c}±0.05
	12	81.09^{a}±0.13	78.66^{b}±0.41	69.35^{c}±0.24	65.92^{e}±0.01	57.17^{f}±0.07	67.30^{d}±0.11

Table 2 Effect of fermentation time on proximate composition of yoghurts

Values are means ±standard deviation of duplicate determination. Means that do not share the same superscript number along a row for each process are significantly different ($p<0.05$). Key: GSY = Germinated soybean yoghurt, UGSY = Ungerminated soybean yoghurt, GTNY = Germinated tiger nut yoghurt, UGTNY = Ungerminated tiger nut yoghurt, SSY =Soursop yoghurt, CMY = Cow milk yoghurt

5.0. Discussions

The effect of fermentation time on pH of the yoghurts (Table 1) showed initial fermentation pH range of 5.60 to 6.54 that significant (P<0.05) decreased to a range of 4.11 - 4.82 and 3.53 - 3.96 after 6 and 12 h fermentation. All the yoghurts analyzed had low pH. Low pH may conceivably be the result of amount of lactic acid produced from the fermentation of milk lactose by bacteria (Oladipo et al., 2014). The pH value from the study matched the common pH of yoghurt. The pH of all six yoghurt samples complied in accordance with Food and drug administration (FDA) specifications which state that yoghurt should have a maximum pH of 4.5 (Weerathilake et al., 2014). One physico-chemical parameter that makes a significant difference when altering fermentation time is pH value. Decrease in pH value is due to fermentation mechanism of lactic acid bacteria (LAB) to produce lactic acid (Matter, Mahmoud, Zidan, 2016). During fermentation, lactic acid decomposes into H+ ions and CH CHOHCOO- ions, thus the longer the fermentation time, the higher H+ ions content causing a reduction in pH as reported by Corrieu and Beal,. (2016). Fermenting plant milk for 12 h in this study reached a pH of 3.53. Lower pH affects the casein (milk protein), causing it to coagulate and precipitate, thereby forming the solid or thick curd that made up the yoghurt (Falade et al., 2015).

TTA values of the yoghurt samples ranged between 0.52 and 0.80%. The TTA (%lactic acid) values obtained from this study is similar to the findings of Isanya and Zhang, (2007) who obtained TTA (% lactic acid) that ranged from 0.5 to 0.75. It was observed in this study that TTA values of yoghurt increased as pH value decreased. This development could be ascribed to the fermentation process, during fermentation, microorganism utilize sugar (lactose and glucose) for metabolic activity and produce acids as by-products. According to Obi et al. (2010), the International Dairy Federation has recommended that the minimum value of acidity in yogurt is 0.70% and obtained from this study achieve the minimum value.

Total soluble solids (TSS) of the yoghurt samples ranged from 7.20 to 14.90% as presented in Table 2. According to Bristone, Badau, Igwebuike, & Igwegbe, 2015), total soluble solid is the mass fraction of substances remaining after completion of the heating process It is an important parameter in yoghurt production because it has consequence on the viscosity, increase in the total solids led to an increase in viscosity, which is good for the rheological property of a yoghurt sample (Falade et al., 2015). The yoghurt samples produced had higher total solids than the control sample.

The viscosity of the yoghurt samples are shown in Table 2 and varied from 0.69 and 0.73 Pas. The yoghurt from UGSY had the highest value (0.73Pas) after 12 h of fermentation while GSY sample had the least value (0.69 Pas) at the initial fermentation. There was no significant (P>0.05) variation after 6 and 12 h of fermentation among the yoghurt samples. The result obtained was closely related to viscosity value reported by Obinna-Echem, (2024) for Utilization of Tigernut Milk in Yoghurt Production: Physicochemical Properties and Growth of Lactobacillus bulgaricus and Streptococcus thermophiles in Tigernut Yoghurt. Viscosity is a vital rheological property of a yoghurt sample (Falade et al., 2015). Viscosity is affected by the strength and number of bonds between casein micelles in yoghurt, as well as their structure and spatial distribution (Izadi et al., 2014). This is because the coagulation of milk proteins was induced by thermophilic bacteria (Streptococcus thermophilus and Lactobacillus bulgaricus), which propagated at high temperatures. Viscosity is an important parameter that correlates with the consistency, texture and

flow of yoghurt. Viscosity of yoghurt has been found to be dependent on the lactic acid production as the concentration of lactic acid increased, the proteins present in milk formed gel to give the end result as the viscous yogurt.
Table 2 shows the results for syneresis where the highest point of syneresis (8.90 %) was obtained in the GSY after 12 h fermentation. Syneresis is a defect due to the appearance of two phases, whey, and water. Syneresis results from low milk quality, high incubation temperature, low acidity, enzymes that clot the protein and low viscosity. The syneresis values may increase as the pH decreases because the yogurt continues to produce lactic acid during storage (Rebollar, 2017).
The proximate composition such as moisture, protein, carbohydrates and fat of all yogurt bite samples is summarised in Table 2. The moisture content of the yoghurts varied from 82.56-86.77% after 12 h fermentation time. Moisture content was affected by fermentation time, moisture decreased as fermentation time increased. The moisture content of the produced yoghurts was much higher than control (CMY) yoghurt. Results obtained were lower than values by Sagita et al. (2020), for moisture content of commercial yoghurt was 75-80%. This was due to a lot of additional ingredients which could make the water content of commercial yoghurt have lower value.
Milk fat is a chemical compound that is included in the ester group which is composed of various fat and glycerol. Ninety percent of the milk fat components are fatty acids which are divided into unsaturated fatty acids and saturated fatty acids. Fat content decreased after 12 h of fermentation time. The decrease in the fat content was probably due to the growth of the mixed culture used as energy requirement for the fermentation process (Zakaria, Novita and Delima, 2010). The more active bacteria in the fermented milk will accelerate the breakdown of fats by bacteria as an energy supply for the growth of these bacteria.
The protein content of the fermented yoghurts with the different fermented times are presented in table 2. Protein content increased from a range of 3.32- 6.51% after initial fermentation time increased to a range of 3.45-6.81% after 12 h fermentation time. The 12 h of fermentation time showed the high protein content. The protein in the media increased because the protein is a constituent of microbes. This is in line with the research conducted by Kartikasari and Nisa, (2014). Increase in protein with increase in fermentation time could also be due to the breakdown of protein by proteolytic organisms, producing a larger amount of free amino groups (Li et al., 2019)
The carbohydrate present in raw milk is normally lowered by 20-30% during the fermentation. During the fermentation process, the carbohydrate present in milk in the form of glucose, as well as other sugar such as lactose, galactose, fructose, sucrose, and maltose is broken down into simpler form of glucose and galactose due to the metabolic activity of lactic acid bacteria creating yoghurt, this is good for lactose intolerant persons (Ramandeep *et al.*, 2017).

6.0. Conclusion

Fermentation time showed significant ($P<0.05$) effect on the physicochemical properties of yogurt produced from different plant and cow milk, pH decreased from a range of 5.60-6.14 to 3.79 – 4.82 with an increase in TTA from a range of 0.52-0.83% to 0.72-0.86% after 12 h fermentation time making the yoghurt more acidic and sour. TSS with a range of 12.90-16.90% decreased to a range of 7.20-11.90% whereas viscosity showed no significant ($p>0.05$) difference after 12 h fermentation time. On the other hand, protein, crude fibre, ash content and energy value of the yoghurts increased significantly after 12 h fermentation time. It was observed that 12 h fermentation time had effect on the physicochemical and proximate composition of the yoghurts studied. 12 h fermentation time is therefore recommended.

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