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CHEMICAL COMPOSITION, SENSORY AND MICROBIAL ANALYSIS OF YOGHURT-LIKE PRODUCT FROM BREADFRUIT, COCONUT AND SOYBEAN EXTRACT.

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ABSTRACT

Yoghurt, a dairy product with established nutritional and health benefits. However, food security coupled with high cost of animal milk and its products has prompted the need for the use of plant milk, hence the need for alternative products. This research is aimed at investigating the chemical, microbial and sensory attributes of yoghurt-like products made from breadfruit (B), coconut (C), and soybean (S) extract in the ratio: B20:C20:S60, B20:C30:S50, B30:C20:S50 and B30:C30:S40 fermented with Lactobacillus acidophilus using standard methods. The percentage proximate composition of the yoghurt-like product revealed the following ranges for moisture (70.06±0.01 to 78.02±0.11), crude protein (11.08±1.32 to 20.87±0.02), crude fat (2.01±0.31 to 3.01±0.31), crude fiber (4.37±0.03 to 6.01±0.16) and carbohydrate (3.10±0.02 to 5.51±0.03). There was no significant difference (p>0.05) in the protein values of B20: C20: S60 when compared with the commercial (100%) cow milk yoghurt. All the yoghurt-like products contained appreciable ranges of essential minerals, namely: Ca (30.02±0.05 to 40.31±0.03), Mg (1.22±0.22 to 11.2± 36), Na (2.80±0.12 to 12.38±0.03) and P (5.15±0.62 to 20.02± 0.52). The microbial results under refrigeration temperature at 4°C showed that the microbial load increases with the blend with high percentage of breadfruit. The sensory attributes of the yoghurt-like products composed of B20:C30:S50 was rated closest to the control (100% cow milk yoghurt). The results obtained from this research has shown that its feasible to produce non-dairy yoghurt derived from blend of breadfruit, coconut, and soybean extract of comparable nutritional and health benefits with the whole milk yoghurt.

Keyword: Breadfruit, coconut, soybean extract, yoghurt-like product

INTRODUCTION

Yoghurt is a product derived from milk fermentation involving *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Sanful, 2009). It is often produced from a buffalo milk or partially skimmed cow milk (El- Batawy, 2014). The consistent geometric increase in population of developing countries and inadequate supply of basic nutrients has inadvertently increased the occurrence of malnutrition. Over the years, man has depended on milk as one of its sources of essential nutrients for their growth and developments (Abdel-Moneim *et al*., 2006). Yoghurt have evolved empirically some century ago by allowing naturally contaminated milk to sour at warm temperature (Ihekoronye and Ngoddy, 1985).

The fermentation process involved the use of microorganisms often referred to as the "Starter Culture".

Presently, food security coupled with high cost of animal milk and lactose intolerant individuals has prompted the need for the use of plant milk as substitute for animal milk and its products. Investigation on the use of extract from plant source to produce yogurt-like product as an alternative to animal milk having close quality attributes (taste, flavor, texture, shelf-life stability) health benefit and variety in diet (Bamishaiye and Bamishaiye, 2011). Several researches have been carried out in a bid to produce an alternative to diary yogurt or yoghurt-like products from plant, that will meet the consumer expectation with regards to nutritional value and sensory attributes. Plant with high protein content have been used to improve other deficient diets for nutritional value and economic significant.

Soybean extract can be referred to as aqueous extract of soya beans (*Glycine max*) and has close similarities with cow milk in appearance (Agure-Dam, 1997). It is often characterized with beany, grassy or soy flavor, which studies has proven can be improved by lactic acid fermentation, as in yoghurt-like products (Jimoh and Kolapo, 2007).

Breadfruit (*Artocarpus altilis*) belong to the Moraceae family and has more than fifty species. According to Amusa *et al*. (2002) breadfruit can be cultivated by stem-cuttings and the fruiting period is an average four to six years. Breadfruit is known to be high in carbohydrate and protein (2.2–5.9%) on a dry weight basis. It is equally a good source of amino acids, especially lysine and histidine which are essential nutrients for infant growth and development. Breadfruit is a very good source of vitamins and minerals like Mg, Cu, Fe, Ma, K and Ca (Oladunjoye *et al*., 2010). Breadfruit is also known to contain bioactive compounds such as phytate, oxalate, and tannin (Oladunjoye *et al*., 2010), ascorbic acid and carotenoids (Azevedo-Meleiro, 2004).

Coconut (*Cocos nucifera* Linn) is often referred to as the fruit of life because of its numerous nutritional attributes and health benefits (Kahn *et al*., 2003). Coconut kernel or coconut meat, is the endosperm of the fruit covered by a brown-colored layer called testa. The fluid found in the cavity has been a popular drink in different parts of the world, believed to control hypertension (Alleyne *et al*., 2005). Currently, Coconut milk is being used by industries to produce pastry products, confectionaries, biscuits and ice cream worldwide in order to enhance the flavor and taste of the products (Persley, 1992).

Dairy milk and products are very expensive and only within the reach of selected few in developing countries, resulting in low consumption necessitating the demand for the processing of milk from plant parts (Belewu and Belewu, 2007). This study is therefore carried out to evaluate the physio-chemical, microbiological and sensory attributes of yoghurt-like products from a blend of breadfruit, coconut, and soybean milk.

MATERIALS AND METHODS

Source of raw materials

Soybean seeds and Coconut were purchased from Choba Market, while breadfruit was purchased from Oil-mill market, both in Rivers State.

Preparation of raw materials

Five hundred grams (500g) of fresh coconut of about seven to eight months old was used in this research-work. The coconuts were cracked open and the juice stored in a refrigerator at 4^{o}C. The coconut flesh was

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thereafter removed from the shell then grated and homogenized together with the coconut juice using a mechanical blender. The mixture was filtered using a fine sieve twice and the volume made up to 1.5 L (the filtrate final seeds to water ratio of 1:3 (w/v) before storing in a refrigerator at 4^{o}C. The filtrate (coconut milk) was then pasteurized 90°C for 30 min and allowed to cool gradually to a temperature of 43°C. It was kept at this temperature for 12 h before it was finally cooled to room temperature of about 29±2°C (Sanful, 2009).

Soybean (500g) were washed and fermented in 2.5 L of water for 16 h. They were deshelled manually, rinsed and wet milled into paste using a high-speed blender. The slurry was sieved with added water through muslin cloth. The milk was then pasteurized at 85^{o}C for 20 min with continuous stirring to avoid coagulation (Ugwona *et al*., 2018).

Five hundred grams (500g) of breadfruit seeds were washed with water, after which foreign materials and damaged seeds were removed. This was followed by boiling at 100^{o}C for 1 h, air drying and deshelling before soaking in water for 6 h (the water was changed intermittently, this is to avoid foul smell and greasy substances). The seeds were repeatedly washed with water after soaking before wet-milling using a blender (SB-736, Sonic, Japan) with intermittent addition of water. The slurry obtained was then filtered using the clean double layer linen cloth, the filtrate final seeds to water ratio of 1:3 (w/v). The filtrate was again boiled for 20 min with continuous stirring and re-filtered to obtain plain breadfruit milk (Udeozor, 2012).

Table 1: Yogurt Formulation

Yogurt Ratio	Description
B20: C20: S60	Breadfruit extract 20%, Coconut extract 20% and Soybean extract 60%
B20: C30: S50	Breadfruit extract 20%, Coconut extract 30% and Soybean extract 50%
B30: C20: S50	Breadfruit extract 30%, Coconut extract 20% and Soybean extract 50%
B30: C30: S40	Breadfruit extract 30%, Coconut extract 30% and Soybean extract 40%
Cow milk 100	Cow milk 100%

Four yoghurt formulations were prepared by varying the proportion of breadfruit, coconut and soybean. The proportion was carried out in line to give a good nutritive protein intake. Formulation five (5) has 100% cow milk.

Production of composite yoghurt drink

Breadfruit extract, Coconut Extract and Soybean milk

↓

Homogenize

↓

Pasteurize (95^{o}C at 15 min)

↓

Cooling

↓

Inoculated at 42^{o}C with 2% *Lactobacillus acidophilus*

↓

Fermentation (24 h)

↓

Cooling and refrigerator

Fig 1: The production of the composite yogurt drink.

Production of composite breadfruit, coconut and soymilk yoghurt: Production of composite yoghurt was based on the recipe as shown in Table 1 where Breadfruit milk, Coconut milk and soymilk was blended and processed according to Figure 1.

Microbial Analysis

The total viable bacteria and mould counts of yogurt formulations were determined using aseptic technique as described by Perkins and Campbell (1990), Harrigan and McCance (1976), Diliello (1982), Balogun and Olatidoye (2010) and Badau *et al.* (2001). Ten millilitre of sample was prepared and transferred into a bottle containing 90 ml of distilled water to form the stock solution as described by Badau *et al.* (2001). This was followed by a 10-fold serial dilution and the plating of 0.1 ml of appropriate dilutions on Nutrient and Potato dextrose agars Badau *et al.* (2001). Plates were incubated at 29±2^{o}C for 18 to 24 h for bacteria and 48 to 72 h for mould. Discrete colonies were counted and reported as colony forming unit per millilitre (Balogun and Olatidoye, 2010).

Proximate analysis of yoghurt: Moisture, fat, crude protein, carbohydrate ash and crude fiber contents were determined by the methods described by AOAC (2006).

Sensory evaluation of composite: A 20-member panel consisting of students who were familiar with the conventional cow milk yoghurt.

Statistical analysis Results obtained from proximate and sensory analyses were subjected to analysis of variance (ANOVA). The sensory scores were then subjected to ANOVA using one factor randomized design according to Mahony (1986).

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RESULTS AND DISCUSSION

The results of the proximate composition of the composite yoghurt-like drink is shown in Table 2 using 100% cow milk yoghurt as control. There was a significant difference in the percentage moisture content with the highest observed with the ratio B30:C30:S40 (78.02±0.11), followed by B30:C20:S50 (76.20±0.09), B20:C30:S50 (74.20±0.03), B20:C20:S60 (70.06±0.01) with the control having the lowest moisture content of 70.00±0.03%. This finding is comparable to the range of 75.25 to 86.06% reported by Belewu *et al.* (2005) but lower than 86.93 to 90.67% reported for soy-walnut milk drinks (Bristone *et al*., 2015). There was no significant difference (p>0.05) in the protein values of B20:C20:S60 when compared with the (100% cow milk yoghurt), there was also no significant difference (p>0.05) in the fibre and carbohydrate contents of B30 C30 S40% and B30 C20 S50% when compared with the 100% cow milk yoghurt. The percentage ash for all the yoghurt-like blend was extremely low, this is in line with the work of Bristone *et al.* (2015). The result from the percentage carbohydrate is in correlation with the work Bolarinwa *et al*. (2018). The percentage ash content ranging from 0.28 to 0.50% is comparable to 0.36 to 0.60% for Soymilk and breadfruit milk blend reported by Okwunodulu *et al*. (2021). The percentage fat content was equally observed to have increased with increase soybean milk; this shows that the soymilk is oily legume which was equally reported by Okwunodulu *et al*. (2021).

Table 2: Proximate composition of the various yoghurt- like composite (%)

Parameters	B20:C20:S60	B20:C30:S50	B30:C20:S50	B30:C30:S40	100% Cow milk yogurt
Moisture (%)	70.06 ± 0.01[d]	74.20±0.03[c]	76.20±0.09[b]	78.02±0.11[a]	70.00±0.03[d]
Protein (%)	20.87 ± 0.02[a]	17.02 ±0.08[b]	15.09 ±0.02[c]	11.08± 1.32[d]	21.00± 0.02[a]
Fat (%)	3.21± 0.08[c]	3.01±0.31[b]	2.81±0.31[d]	2.00± 0.44[e]	3.00 ± 0.17[a]
Fibre (%)	4.01±0.16[a]	4.37±0.03[b]	3.37±0.03[d]	4.15±0.10[c]	4.20± 0.14[c]
Ash (%)	0.38±0.01[b]	0.28±0.08[c]	0.28±0.04[c]	0.21±0.23[d]	0.50±0.12[a]
Carbohydrate (%)	2.10±0.0[c]	2.23±0.19[c]	4.23±0.19[b]	4.51±0.03[a]	4.22±0.22[b]

Values are means ± standard deviation of triplicate determinations. Mean values in the same column with different superscript are significantly different (p<0.05).

The table equally showed that the protein content decreased significantly (p>0.05) from 20.87% (B20:C20:S60) to 11.08% (B30:C30:S40) as the level of soybean extract substitution in the yoghurt decreases, while the control (100% cow milk) recorded 21.00% which agrees with the work of Belewu *et al*. (2005), who stated that the higher inclusion of soymilk in soymilk coconut yoghurt the higher the protein content.

All the yoghurt-like products contain appreciable quantity of minerals, there was an increase in calcium (Ca) content with a decrease in percentage coconut, this agreed with the work of Belewu *et al*. (2005). The iron (Fe) content present was observed to be in a range, 0.5 to 0.12 mg which is very low and does not meet the requirement for recommended daily allowance (RDA) of 8 – 18 mg (Szefer and Grembecka, (2007), Mohammed and Mathew (2020), this agrees with the work of Mohammed and Mathew (2020). The magnesium (Mg) content of the yoghurt-like blend was observed to be between 14.2 to 24.92 mg/100g with significant differences at P<0.05. This is equally less than the required recommended daily allowance (RDA) of 200-400 mg (Szefer and Grembecka, 2007). The sodium (Na) concentration from the various yoghurt-like blend were between 6.0 and 22.28 mg/100g with significant differences at P<0.05. This finding is not in agreement with the work of Usman and Bolade (2020) which range from 21.1 to 58.6 mg/100g. The phosphorus (P) content of the yoghurt-like blend was observed to be between 21.01 to 44 mg/100g with significant differences at P<0.05. Sample B30:C30:S40 was observed to have the highest phosphorus concentration when compared with other samples, though the concentration is less than the recommended daily allowance (RDA) of 800-1300 mg (Szefer and Grembecka, 2007), this did not agree with the work of Usman and Bolade (2020), whose phosphorus range from 48.5 to 80.6 mg/100g. The mineral values are all less than the recommended daily allowance (RDA) but can serve as a serve as a complementary source for the mineral element.

Table 3: Mineral composition of the various yoghurt composite (mg/100g)

Parameters	B20:C20:S60	B20:C30:S50	B30:C20:S50	B30:C30:S40	100% Cow milk yogurt
Calcium	24.12 ± 0.51^{c}	20.02 ± 0.05^{c}	24.31 ± 0.3^{a}	20.31 ± 0.4^{a}	21.05 ± 0.13^{c}
Iron	0.52 ± 0.8^{a}	0.10 ± 0.24^{b}	0.08 ± 0.52^{c}	0.03 ± 0.52^{c}	0.12 ± 0.51^{b}
Magnesium	14.2 ± 36^{d}	21.22 ± 0.22^{b}	20.22 ± 0.24^{c}	24.92 ± 0.24^{a}	22.76 ± 0.22^{b}
Zinc	1.03 ± 0.32^{a}	1.14 ± 0.10^{a}	1.12 ± 0.01^{a}	1.02 ± 0.01^{a}	0.87 ± 0.23^{b}
Potassium	42 ± 0.51^{a}	29.52 ± 0.01^{b}	22.01 ± 0.18^{d}	26.01 ± 0.18^{c}	28.53 ± 0.17^{b}
Sodium	12.38 ± 0.03^{c}	15.80 ± 0.12^{b}	14.0 ± 0.21^{b}	6.0 ± 0.21^{d}	22.08 ± 0.23^{a}
Phosphorus	5.15 ± 0.62^{d}	15.14 ± 0.08^{c}	18.02 ± 0.52^{b}	20.02 ± 0.52^{a}	3.69 ± 0.15^{d}

Values are means ± standard deviation of triplicate determinations. Mean values in the same column with different superscript are significantly different (p<0.05).

Sensory evaluation

The results of the sensory evaluation of the yogurt composite samples are shown in Table 4. The results showed no significant (P<0.05) difference in flavor, texture, taste, aroma and mouthfeel among the composite blends. Though there was significant difference between B20:C20:S60 and the control (Cow 100%) in the aroma, sweetness and mouthfeel. This can be as a result of the high beany aroma of the soymilk, which is in correlation with the report of Mahony (1986). The sensory attributes of the yoghurt-like products of B20:C30:S50 was rated closest to the control (Cow 100%) and was significantly acceptable.

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Table 4: Sensory evaluation of the various yoghurt composite

Yoghurt ratio	Aroma	Mouthfeel	Sweetness	Sourness	Lingering After Taste	Overall Acceptability
B20:C20:S60	4.10+1. 05^d	4.12+1.11^d	4.30+1.06^d	5.80+1.01^c	5.14+1.08^c	4.88+1.01^d
B20:C30:S50	5.91+1.01^c	6.00+1.01^b	6.03+0.20^b	6.21+0.9^b	5.80+1.30^c	6.08+0.9^b
B30:C20:S50	5.08+0.65^c	5.12+1.07^c	5.00+1. 11^c	5.60+1.12^c	5.07+1.09^c	5.00+1.12^e
B30:C30:S40	5.12+0.65^c	4.99+1.07^d	5.00+1. 11^c	5.40+1.12^d	5.08+1.09^c	5.08+1.12^c
Cow 100%	7.40+1.14^a	7.10+0.95^a	7.02+1.0^a	7.18+0.9^a	7.10+1.40^a	7.19+0.9^a

Values are means + standard deviation of triplicate determinations. Mean values in the same column with different superscript are significantly different ($p<0.05$).

Microbial analysis

Total viable bacterial and mould count

The results of the microbial analysis are presented in Table 5 for refrigerated temperature. Total viable Count (TVC) showed there were no visible growth of TVC during the day zero (0) for all the yoghurt blend and commercial cow milk yoghurt, this could be attributed to good manufacturing practices during the processing. The resurfacing back of the bacterial could be that they have repaired themselves from the damage during the pasteurization (Badau *at al*., 2001) this does not agree with the work of Ifediba and Ozor (2018), which showed growth. However, the second week of refrigeration temperature showed growth rate with B20:C20:S60 having the lowest microbial growth, while B30:C20:S50 recorded the highest microbial growth, same scenario was observed the fourth blend, as at the fourth week, B20:C:20:S60 equally recorded the lowest microbial growth, while B30:C20:S50 recorded the highest microbial growth.

The mould growth of the samples and the commercial cow milk yoghurt during the first week showed similar trend with the total viable count, as there was no growth. The results from week two to the fourth week of the storage period also showed that the total fungal counts increase with the increasing level of the breadfruit. This is apparently due to higher acidity and total solid. The mould count for yoghurt blend of B30:C20:S50 and B30:C30:S40 is $4.5x10^2$ cfu/ml, same with that of commercial milk yoghurt on day 14 of storage and were all still within the limit of acceptance ($2.0x10^5$ cfu/ml) for dairy products by Codex Alimentarius Commission (FAO/WHO, 2002).

Table 5: Microbiological quality of yoghurt samples during 28 days storage

Yoghurt Ratio	DAY 1		DAY 7		DAY 14		DAY 21		DAY 28	
	TVC	**Mould**	**TVC**	**Mould**	**TVC**	**Mould**	**TVC**	**Mould**	**TVC**	**Mould**
B20:C20:S60	**0**	**0**	**<10**	**<16**	**$2.00x10^2$**	**$3.58x10^2$**	**$3.35x10^3$**	**$3.0x10^3$**	**$4.0x10^3$**	**$3.8x10^3$**
B20:C30:S50	**0**	**0**	**<8**	**<19**	**$3.02x10^2$**	**$3.0x10^2$**	**$4.35x10^3$**	**$2.0x10^3$**	**$4.04x10^3$**	**$3.90x10^3$**
B30:C20:S50	**0**	**0**	**$3.20x10^2$**	**$2.22x10^2$**	**$4.52x10^2$**	**$4.50x10^2$**	**$6.75x10^3$**	**$5.10x10^3$**	**$6.07x10^3$**	**$5.50x10^3$**
B30:C30:S40	**0**	**0**	**$3.00x10^2$**	**$3.10x10^2$**	**$4.00x10^2$**	**$4.50x10^2$**	**$5.75x10^3$**	**$4.10x10^3$**	**$6.07x10^3$**	**$4.50x10^3$**
Cow 100%	**0**	**0**	**0**	**<12**	**$4.20x10^2$**	**$4.5x10^2$**	**$5.35x10^3$**	**$4.0x10^3$**	**$5.04x10^3$**	**$4.60x10^3$**

CONCLUSION

The results obtained from this study have demonstrated that yoghurt-like sample produced from breadfruit, coconut and soybean extract competes favorably with dairy yoghurt. Nutritionally, yoghurt-like composite blend of ration B20:C20:S60 was the closest as there was no significant difference in the percentage moisture, protein and dietary fiber, though other composite blend equally had appreciable quantity of the nutritive value. The organoleptic property shows that yoghurt-like products of B20:C30:S50 was rated closer to the control (Diary yoghurt). However, the objectionable bean flavor from the products must be reduced to minimum so it does not affect the consumer acceptability. Despite the findings from this research, further studies are necessary to enhance the quality for overall acceptability of the yoghurt-like. The results obtain from this research has shown that its feasible to produce non-dairy yoghurt derived from blend of breadfruit, coconut, and soybean extract which will serve same close nutritional and economic significance and also the definitional problem protein malnutrition will be solved.

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