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Effect of Cocoa Powder, Banana Pulp and Bambara Nut Inclusion on Nutritional and Sensory Acceptability of Yoghurt

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Abstract: This study was to investigate the effect of utilizing milk powder with addition of Cocoa powder, Banana pulp and Bambara nut extract for the production of functional yoghurt. The yoghurt samples were produced by 10% inclusion of each of the food item into reconstituted skimmed milk powder, inoculated with starter culture (*streptococcus.thermophilus* and L*actobacillus. Bulgaricus) and* allowed to ferment for 9hr. The nutritional properties (Proximate, Brix, pH, Flavonoid, Vitamin C, antioxidant activity) and sensory characteristics (colour, taste, aroma and over all acceptability) were determined. Significant differences ($p<0.05$) existed among the nutritional and sensory properties of the yogurts. The results showed that the inclusion of used food items improves the Protein, Ash and Energy content of the products. The sample with Cocoa powder had the highest antioxidant activity (2.75mg/100g) which is significantly higher at ($p<0.05$) than Banana pulp (2.64mg/100), Bambara nut extract (2.44mg/10g) and control (2.25mg/100g) which was the least. The sample with Banana pulp inclusion has highest score (5.70) for taste and overall-acceptability (5.90) using 7-point score ranking and was the most preferred. The microbial load of all the samples were within the limits specified of acceptable counts, making the products safe for consumption as at the time the products were evaluated.
Keywords: Yoghurt; Milk powder; Food items; Sensory properties; Consumer acceptance.

1. Introduction

Yoghurt is a type of dairy product produced from milk or milk products by lactic acid fermentation through the action of starter culture (Streptococcus salivarius subsp. thermophilus and Lactobacillus dellbruekii sub-spp. *bulgaricus* [1, 2]. It is one of the most famous fermented dairy products widely consumed in many countries including Nigeria. Yoghurt is a healthy food for both adult and children. For children, it is a balance source of protein, fats, carbohydrates, and mineral while for the older people who are usually characterized by more sensitive colons, Yoghurt is also a valuable food [3]. It is a nutritiously balanced food containing almost all the nutrients present in milk but in a more assimilable form. Human consumption of yoghurt has been associated with tremendous health benefits due to their effect on improvement of gastrointestinal functions and disease risk reduction [4].

The live bacteria in yoghurt could act as probiotic, contributing to microbial balance in the host's gastrointestinal tract which is capable of providing significant health benefits when yogurt product is consume in adequate quantity [5]. The regular consumption of yoghurt with live cultures which is also known as (probiotic) has been suggested to be effective in reducing serum cholesterol level, improves lactose digestion in case of lactose intolerance, prevention of gut infections, inflammation, diarrhea and colon cancer [6].

The use of different fruits to enrich yoghurt has been found to improve its nutritional and sensory properties. Fruits such as strawberry, apple, watermelon, mango, and grape are rich sources of vitamins, mineral, fibers and anti-oxidants, which could be incorporated in the making of yogurt to increase their nutrients and nutritional benefits [7]. It also has the potential to boost their market distribution due to increasing demand for functional food products rich in essential nutrients and bioactive compounds with strong biological activities which are richly available in fruits and vegetables [8]. However, in this case Cocoa powder, Banana pulp, and Bambara nut extracts were being partially incorporated with aim to improve nutritional values and have more appeal to consumers.

Cocoa powder is a refreshing food item characterized by rich source of flavanols which improves dermal blood flow and contributes to the maintenance of skin health [9, 10]. Banana is an important fruit consume daily for its

immense nutritional and medicinal values. It provides high quantity of potassium, which is useful for muscles and controlling of blood pressure [11, 12]. Owing to its high iron content, banana increases the production of haemoglobin in the blood and therefore very good for patients suffering from shortage of blood [13]. The health benefits of banana are attributed to array of bioactive compounds mainly phenols and flavanoids which help bananas to have a greater antioxidant capacity than some herbs and vegetables [14]. Bambara nut is regarded as underutilized legume with high concentration of protein; it plays an important role in diets of young children as it helps in overcoming problem of protein deficiency. Its milk was acceptable to consumers and recommended as weaning food. The aim of this study was to develop yogurts from reconstituted milk powder with inclusion of 10% Cocoa powder; banana pulp and Bambara nut extract and evaluate their effects on nutritional properties and sensory acceptability.

2. Materials and methods

2.1 Raw materials procurement

The Cocoa powder, banana, and Bambara nut, commercial full-cream milk (Dano) and Yogourmet freeze-dried starter culture (containing Streptococcus thermophillus and Lactobacillus delbrueckii subsp. bulgaricus were purchased from Bodija market, Ibadan, Nigeria. The yoghurts were prepared using standard production procedure as described by [15].

1) Preparation of re-constituted milk powder

The 20% reconstituted of milk powder was prepared by dissolving 200g of the milk powder in 1litre of water (200g/l). The reconstituted milk was used to prepare yoghurt samples. The yoghurt without inclusion of food item and ready- made yoghurt product bought from supermarket serves as first and second control respectively.

2) Preparation of cocoa powder added to the re-constituted milk

Cocoa powder was weighed and dissolved in warm water and then filtered using cheese-cloth to remove un-dissolved particles. Then 10% Cocoa powder equivalent was added to re-constituted milk to prepare Cocoa yogurt

3) Preparation of Bambara nut extract added to the re-constituted milk

Bambara nuts were weighed and soaked overnight, dehulled and wet-milled. The slurry was filtered using muslin cheese-cloth. The 10% of Bambara nut extract equivalent was added to reconstituted milk.

4) Preparation of banana pulp added to the re-constituted milk

Few fingers of banana fruit were washed, peeled and cut into pieces. It was blended into slurry and about 10% equivalent was taken and added to reconstituted milk.

5) Market yoghurt

This is ready–made plain yoghurt bought from supermarket and was used as second control.

2.2 Yogurt formulations

Sample A (reconstituted milk + 10% sugar syrup only) serves as first control

Sample B (reconstituted milk + 10% Cocoa powder +10% sugar syrup)

Sample C (reconstituted milk + 10% Banana + 10% sugar syrup)

Sample D (reconstituted milk + 10% Bambara + 10% sugar syrup)

Sample E (yogurt purchased from supermarket) serves as second control

After the mixing the ingredients used in the production of the yoghurt, the samples (A to D) were heated to 80^0C for pasteurization and then allowed to coo to temperature between 42-45°C before inoculated with Starter culture (*S. thermophilus* and *L. bulgaricus)* and incubated for 9 hours. At the end of the incubation period, the products were allowed to cool in a bath of ice water to stop the fermentation process; they were packaged in sterilized plastic bottles for analysis.

2.3 Analysis

1) Determination of proximate properties of the yoghurt samples

Moisture, fat and ash content were determined using AOAC methods [16]. The protein content was determined by Kjeldahl method (N×6.25). Total carbohydrate was calculated by difference as Carbohydrate = 100 – (Moisture + Ash + Fat +Protein).

The energy value in kilocalorie (Kcal) was calculated using a converting factor thus:

Energy (kcal) = [protein *4] + [CHO *4] + [fat * 9].

2) Measurement of ᴘH and TSS (%brix) values

The physical properties of yoghurt like pH, and TSS (%brix) were determined. The pH analysis was carried out using a pH meter (Hanna HI 8314, HannaInc., Italy) and refractometer was used for total soluble solid (%brix).

3) *Determination of Vitamin C*

Vitamin C was determined through titration method using 2, 6-dichlorophenol-indophenol [17].

4) Determination of total phenols

Phenolic compounds were determined using the method described by [18]. About 1 mL of aqueous extract of yoghurt, obtained as described above, was added to 1 mL of Folin-Ciocalteau reagent diluted 1:2 with water. After 3 min, 2 mL of 10% Na_2CO_3 was added and the samples were incubated for 15 min at room temperature. At the end of this step, the absorbance was measured at 750 nm. A calibration curve was performed with gallic acid and the results were expressed as micrograms of gallic acid equivalents per 100 mL of sample (GAE).

5) Sensory determination

Sensory quality of yoghurt products was evaluated by a jury of 20 panelists randomly selected from staff of National Horticultural Research Institute (NIHORT) Ibadan, scoring was using seven-point hedonic scale where 7 represent like very much and 1 dislike very much. The following parameters: color, taste aroma and overall acceptability were determined. The Subjects who tasted the samples were asked to keep the yoghurt in the mouth for 12 seconds before scoring. Water was used for rinsing mouth between sample evaluations.

6) Microbial load evaluation

A microbial test on yoghurt products was conducted to ascertain the suitability or otherwise of the products for human consumption by storing. For coliform test, the Sample of yoghurt was is cultured on medium VRBA and counted after incubation for 48 hours at 37°C while mold and yeast tests was done after preparation of dilution (0.1 and .001) of yogurt samples and were cultured on PDA medium and counting was done after 72hrs incubation at 22°C.

7) Statistical analysis

All the data obtained from three replications were analyzed using the general procedure of the SPSS statistical package program (SPSS, Inc., Chicago, IL). Duncan's multiple range test was used to measure the significant difference between means ($P<0.05$).

3. Results and discussion

The results of proximate composition are presented in Table 1. Moisture content ranged from (73.07-80.47%), protein (2.30%-4.97%0, fat (2.07-2.27%), ash (0.53 to 1.00%), CHO (14.77-19.07%) and energy (87.18kcal/100g -112.33Kcal/100g). The sample which was partially fortified with Bambara nut extract has the highest protein value (4.97%) which was significantly higher at ($p<0.05$) than control (4.23%) and yoghurt product bought from supermarket 2.30% which had the least and served as second control. The sample with banana had the highest ash concentration (1.00%) which was also significantly higher than control (0.60%) and product bought from supermarket (0.53%) respectively. The sample with addition of Cocoa powder has the highest energy value (111.36kcal/100g) which was significantly higher than control (102.33kcal/100g) and the product bought (87.18kcal/100g). The protein which improved significantly from the addition of Bambara nut and banana is an essential food nutrient that consists of various amino acids essential for growth and development especially in children. It plays an important role in building and maintaining body tissue; they are essentially needed during infancy and childhood periods for optimal growth and development [19]. Protein deficiency could precipitate multiples of clinical syndromes such as low serum cells, impaired anti-oxidative reactions, growth stunting in young children and poor cognitive development [20]. However, consumption of yoghurt fortified with Bambara nut extract (milk) can improve protein nutritional requirements. Bambara nut is rich in protein which could be utilized in the formulation of protein rich diet or supplementation of diets low in protein [21]. Ash content is an important component for in food, it represents a measure of aggregate mineral present within a food [22]. The partial fortification with fruits and rich food items and regular consumption yoghurt can boost the dietary minerals available for body use. For daily energy requirement of a person which is averagely put at about (1,800kcal) according to[19].Partial fortification of yoghurt with Cocoa powder, banana and Bambara nut milk could serve as energy drink that can contribute to daily energy needs.

Table 1. Proximate properties of partially supplemented yoghurt (%composition)

Samples	Moisture	Protein	Fat	Ash	CHO	Energy(kcal/100g)
Control (A)	73.07 ± 0.90^{e}	4.23 ±0.19^{cd}	2.27 ± 0.23^{a}	0.60 ±0.03^{d}	19.07±00^{a}	102.33 ± 0.99^{b}
10%Cocoa(B)	76.20 ± 0.40^{c}	4.27 ±0.12^{c}	2.04 ± 0.06^{d}	0.67 ±0.29^{c}	19.28±0.15^{b}	111.86 ± 0.57^{a}
10%Banana(C)	77.80 ± 5.29^{b}	4.59±0.08^{b}	2.12 ± 0.10c	1.00 ±0.00^{a}	15.30 ± 0.10^{c}	97.93 ± 1.00^{c}
10%Bambara(D)	74.67 ±2.00^{d}	4.97 ±0.50^{a}	2.26 ± 0.06^{a}	0.83 ±0.29^{b}	17.96 ± 0.01^{d}	111.58 ± 1.00^{a}
Market- product (E)	80.47 ± 1.70^{a}	2.30 ±0.20^{e}	2.19 ± 0.12^{b}	0.53 ±0.29^{e}	14.77 ± 0.01^{e}	87.18 ± 0.99^{d}

*Values are means ± SD triplicate determinations. Values with different superscript within the same column are significantly different ($P < 0.05$)

The ^{o}Brix (TSS %) and $_{P}$H results are presented in (Fig 1). The Brix was between (13%-22%) and $_{P}$H (4.30-4.70). The yoghurt sample with addition of banana had ^{o}Brix level (18.00%) and this was higher than sample Bambara (16.00%) and cocoa powder (14.00%) respectively. The product bought from super market has the lowest

Brix level (13%). The Brix value are important being one of the subjective criteria that influences consumer preferences [23] . The ^{o}Brix value is a measure of sugar in a given sample and represents the degree of sweetness. The sample partially fortified with banana with ^{0}Brix level (16%) was most preferred by consumers. pH represents a critical quality control step in production of dairy products, especially yoghurt; it offers an indication to estimate acid development of a dairy product. Yoghurt producers preferred their product to set (formed) at pH (4.0 and 4.6) because the amount of lactic acid present at this pH range which is good for the product. It gives yogurt its characteristic tartness, aids in thickening and preserves the product against undesirable strains of bacteria [24]. The pH of all the yoghurt samples analyzed fell within the range of 4.60 and 4.70.

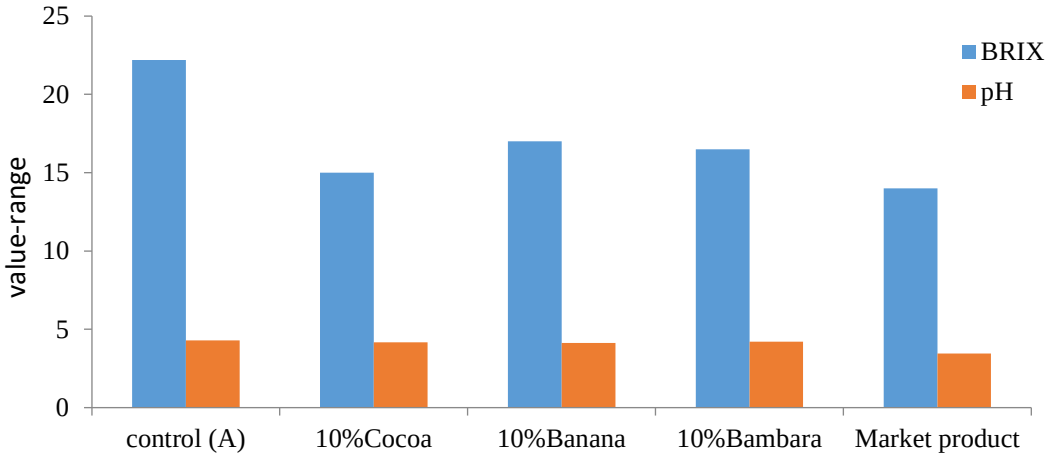

Fig 1. The ^{O}Brix(TSS %) and $_{P}$H measurement of the yoghurt

The results of total phenol, flavonoids, Vitamin C and antioxidant activity are presented in Table 2. The value of phenol ranged from (1.01-2.15mg/100g), flavonoids (3.34mg/100ml-3.88mg/100g), Vitamin C (2.2-8.1mg/100g) and anti-oxidant activity (2.25-2.75mg/100g).

The sample with Cocoa powder has highest vitamin C (8.1mg/100g). This was significant higher than control (6.6%) and yoghurt product bought from supermarket which had the least (2.2mg/100g). Similarly, sample with inclusion cocoa powder has highest flavonoids (3.88mg/100g) and antioxidant activity (2.75mg/100g). Its antioxidant activity (2.75mg/100g) was significantly higher than sample with banana (2.64mg/100ml) and Bambara nut (2.44mg/100ml) respectively. Vitamin C which is also known as ascorbic acid, is an essential dietary nutrient with vital roles in some biological functions, it is fundamental in the biosynthesis of collagen in bones, cartilage, muscle, and blood vessels, it aids in absorption of iron. Vitamin C is a potent free radical scavenger in the plasma, protecting cells against oxidative damage. It has a strong antioxidant property attributed to its ability to reduce potentially damaging free radicals [25]. The daily recommended allowance for vitamin C is 90mg/day for adult male and 75mg/day for women [26]. Sufficient consumption of yogurt partially fortified with fruits and other nutritious food items can contribute significantly to daily requirement of vitamin C. Anti-oxidants are natural compounds that play important role in reducing the damaging effect of free-radicals against body cells. They are present in foods as vitamins, minerals, carotenoids, and polyphenols. Frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular diseases and cancers in which yogurt fortified with fruits and other nutrient rich food items could be a medium of delivery and sufficient bioavailability [27,28].

Table 2. Nutritional and antioxidant activity of yoghurt

Samples	Total Phenol (mg/100g)	Flavonoid (mg/100g)	Vitamin C (mg/100g)	Anti-Oxidant activity (mg/100g)
Control(A)	2.06 ± 0.09^{ab}	3.35 ± 0.69^{c}	6.6 ± 0.00^{c}	2.25 ± 0.27^{e}
10%Cocoa(B)	1.01 ± 0.24^{e}	3.88 ± 0.17^{a}	8.1 ± 0.02^{a}	2.75 ± 0.13^{a}
10%Banana(C)	2.15 ± 0.24^{a}	3.64 ± 0.61^{b}	7.2 ± 0.12^{ab}	2.64 ± 0.36^{b}
10%Bambara(D)	1.54 ± 0.41^{c}	3.82 ± 0.14^{a}	4.5 ± 0.04^{d}	2.44 ± 0.14^{cd}
Market- product (E)	1.37 ± 0.30^{d}	3.34 ± 0.32^{c}	2.2 ± 0.04^{e}	2.49 ± 0.13^{c}

*Values are means ± SD triplicate determinations. Values with different superscript within the same column are significantly different ($P < 0.05$).

The sensory property which includes color, taste, aroma and overall acceptability are summarized in Table 3. The seven-point (7) hedonic scale was used for score ranking. The average values for color ranged between (4.37-

6.20), taste (3.5-5.70) aroma (4.40-5.20) and over acceptability (4.40-5.90). Significant differences ($p < 0.05$) were observed between the control and the different yoghurt samples for all the sensory parameters evaluated. Sample with partial inclusion of banana has the highest average score for taste (5.70), aroma (5.70 and over acceptability (5.90) which was higher than samples with cocoa powder with average score for taste (3.50), aroma (4.40), over acceptability (4.940) and Bambara with taste (5.10), aroma (4.00 and over acceptability (4.95) respectively. Sensory properties offers quality control criteria [29].it helps to provide guide and information as to any critical variable of production to be adjusted. The sample with inclusion of banana has the highest overall acceptability. The addition of banana imparted positively and significantly on its taste and aroma. Thus making it most preferred in term of overall acceptability. The addition of fruit and other nutritious food items to fortified yogurt may not only improve nutritional constituents, it could impact positively on sensory properties which can influence the consumer's preference and acceptability. Although, the sample with cocoa have significant flavonoid, vitamin C and antioxidant properties, however, the impact of cocoa on taste affect its consumer preference which is relatively low compared with sample with inclusion of banana and this I believe can be improved upon.

Table 3. Sensory characteristics of yoghurt

Samples	Colour	Taste	Aroma	Over-all acceptability
Control(A)	6.20 ± 0.69^{a}	5.40 ± 0.81^{b}	5.50 ± 0.88^{b}	5.40 ± 0.91^{b}
10%Cocoa(B)	4.39 ± 1.29^{e}	$3.50\pm1.31e$	4.40 ± 1.40^{d}	4.40 ± 1.56^{e}
10%Banana(C)	5.80 ± 0.95^{b}	5.70 ± 0.93^{a}	5.70 ± 1.03^{a}	5.90 ± 0.97^{a}
10%Bambara(D)	5.20 ± 1.20^{c}	5.10 ± 1.03^{c}	4.80 ± 1.20^{d}	4.95 ± 1.20^{c}
Market- product(E)	5.10 ± 1.40^{d}	4.50 ± 1.53^{d}	5.00 ± 1.70^{c}	4.90 ± 1.33^{c}

*Values are means ± SD triplicate determinations. Values with different superscript within the same column are significantly different ($P < 0.05$).

The results of microbial load are presented in Table 4. The microbial test was performed to evaluate the suitability or otherwise of the products for human consumption. All counts are recorded as number of microbial colonies formed per 1ml (cfu/g) of sample. The total microbial count (TMC) was between ($0.2x10^3$cfu/ml-$1.4x10^3$cfu/ml), total fungi count (TCF) ($0.2x10^3$cfu/ml-$0.2x10^3$cfu/ml) and there was no coliform detected. All counts recorded are within the limits of specified acceptable counts; this could be attributed to the pasteurization and hygienic conditions under which the production was done. Hence, the products are safe for consumption.

Table 4. Microbial load of the yoghurt

Samples	TMC (cfu/ml)	TFC(cfu/ml)	TCC(cfu/ml)
Control(A)	$1.4\ x10^3$	$0.9X10^3$	N.D
10%Cocoa(B)	$0.6x10^3$	$0.2X10^3$	N.D
10%Banana(C)	$1.3x10^3$	$0.2X10^3$	N.D
10%Bambara(D)	$0.9x10^3$	$0.3X10^3$	N.D
Market-product(E)	$0.2x10^3$	N.D	N.D

Note: Each count is recorded as the total number of microbial colonies formed per 1ml of yoghurt sample (cfu/ml)
Key: TMC= Total microbial count, TFC=Total Fungal count**,** TCC=Total coliform count, ND=No microbial growth detected

4. Conclusion

Yoghurt is a healthy food for both adult and children. For children, it is a balance source of protein, fats, carbohydrates, and mineral while for the elderly who are usually characterized by more sensitive colons, Yoghurt is also a valuable food. Nowadays, consumer interest in disease prevention and health promotion is shifting towards the potential health benefits of functional foods. Hence, the need for regular consumption of yoghurt.

Based on the findings of this study, it can be concluded that, the partial inclusion of cocoa powder, Banana pulp and Bambara nut extract could improve the nutritional and sensory value of yoghurt. The sample partially fortified with banana has the best acceptance and mostly preferred because of the impact of banana inclusion on aroma and taste.

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