Chemical Composition, Sensory Attributes and Microbial Properties of Yoghurt Enhanced with Fresh Tropical Fruits

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Abstract

Yoghurt is a milk product of high nutritional and culinary value to humans. Its quality and acceptability by consumers can improve with addition of tropical fruits. In this study, fruit pulp prepared from banana, pineapple, mango and orange were added separately to yoghurt at 0.1g/mL each. Dry matter content of yoghurt varied from 15.3-17.6%; fat, 2.74-3.95%; protein, 3.00-3.56%; crude fibre, 0.00-0.30%; and soluble carbohydrates, 6.55-10.2%, respectively. Fat and protein of yoghurt decreased with addition of fruit pulp while crude fibre and carbohydrates content increased. Acceptability scores indicates that banana-yoghurt was the most preferred, followed by yoghurt only, mango-yoghurt, orange-yoghurt and pineapple-yoghurt. Total bacterial count varied from 1.8×10^4 - 6.5×10^5 cfu/g; coliform count, 6.20 - 96.3 cfu/g; and total fungal count, 5.50 - 98.4 cfu/g. Microbial load of yoghurt increased with addition of fresh fruit pulp and days in storage On the average, total bacterial count increased from 3.52×10^4 - 2.43×10^5 cfu/g; coliform count, 15.0- 64.3 cfu/g; and total fungal count, 30.7- 69.1 cfu/g as storage progressed from 7 - 28 days. While addition of fruit pulp has potential to enhance the quality and acceptability of yoghurt, there is need to pre-heat the fruit pulps to reduce microbial contamination and ensure safety of the consuming public.

Keywords: Acceptability, Fruit pulp, Microbial load, Yoghurt quality.

Introduction

Yoghurt is a dairy food derived from fermentation of milk by two symbiotic bacteria, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Schmidt *et al*., 2001). Yoghurt is considered to be highly nutritious with ample supply of carbohydrates, protein, fat, vitamins, calcium and phosphorus (Tamime and Robinson, 1999). It is also considered to have therapeutic properties and increases immunity to disease in the human body (Desobry-Banon *et al.,* 1999; Meydani and Ha, 2000). These perceived nutritional properties and health benefits have led to increased public interest in the consumption of yoghurt. Although yoghurt consumption is known to have existed for a long period, its worldwide acceptance as a regular part of the diet is only recent. In traditional yoghurt consuming populations, addition of fruits had little influence on acceptance of the product, whereas, in non-traditional yoghurt-consuming communities, addition of fruits greatly enhanced the acceptance and consumption of yoghurt (Tarakçi and Küçüköner, 2003).

Fruits and vegetables are important components of a healthy diet which could help prevent major diseases such as cardiovascular diseases and certain cancers if consumed in sufficient amounts (WHO, 2003). Fruits contain a large number of naturally occurring vitamins, minerals and phyto-chemicals that confers health benefits on the consumer. Fruits also provide dietary fibre which helps to reduce cholesterol and fats in the body, aid bowel movement and offer relief from constipation (Morton, 1987; Sarkiyayi *et al.,* 2013). Anti-oxidants in fruits help to boost immunity and protect the human body from oxidant stress and diseases (Benzie and Chow, 2014). Edible fruits have high culinary value and sensory properties that makes them highly desirable in yoghurt preparations (Barnes *et al*., 1991; Yousef *et al*., 2013; Ali, 2016).

Apart from additional nutritional and therapeutic value of fruits, their incorporation into yoghurt preparations has been reported to greatly enhance consumer acceptance (Yousef *et al*., 2013).

The purpose of this study therefore, was to evaluate the chemical composition, sensory attributes and microbial properties of yoghurt enhanced with different freshly prepared tropical fruit pulp.

Materials and Methods

Location and treatments

This study was conducted at the Dairy Unit of the Teaching and Research Farm, University of Ibadan with the following experimental treatments: yoghurt only (control); yoghurt with banana fruit pulp; yoghurt with pineapple fruit pulp; yoghurt with orange fruit pulp. The experiment was replicated three times using the completely randomized design.

Preparation of fruit pulp

Fresh banana, pineapple, mango and sweet orange fruits were obtained from the open market and washed. All the fruits were peeled using a sharp knife and cut into small pieces of 2-3cm. Seeds inside the orange fruits were picked using the tip of the knife. The cut fruits were blended (Qlink®, China) to obtain a uniform pulp.

Milk collection and yoghurt preparation

Raw milk was collected from ten Zebu cows at early to mid-lactation on the farm, clarified using a muslin cloth and preserved in the refrigerator at 4°C until required for processing. During processing, milk was pre-heated to 60°C and homogenized using a high speed mixer (Qlink®, China). The milk was pasteurized by heating through a water bath to a temperature of 75 °C for 20 minutes with continuous stirring and later cooled to a temperature of 45°C. The milk was inoculated with a starter culture (Yo-gourmet, Lyo-San Inc., Canada) containing *Streptococcus thermophilus, Lactobacillus bulgaricus* and *Lactobacillus acidophilus* at the rate of 25 g/L and placed inside an incubator at 43°C until a soft curd was formed. Sugar (sucrose) was stirred into the curd at the rate of 60 g/L. The stirred yoghurt was decanted into 500ml plastic cups with or without 50g of fresh fruit pulp (banana, pineapple, mango and orange). Each cup was covered with a lid and stored in the refrigerator at 4°C for 7, 14, 21 and 28 days.

pH of yoghurt samples

The pH of yoghurt samples at 7 days of refrigerated storage was determined using an electronic pH meter (PHS-3C, TBT, Jiangsu, China). The pH meter was calibrated with buffer standards of pH 4 and 10 prior to use.

Chemical Analysis

Dry matter in yoghurt at 7 days of storage was determined by drying in an oven at 105°C to constant weight. Crude protein was determined using Kjeldahl method (N x 6.38), crude fibre with a Soxhlet apparatus, ash with a muffled furnace and fat by a modified Rose Gottlieb method, following the general procedures of AOAC (1995). The carbohydrate fraction of the samples was determined as the difference between the dry matter and other solids in the yoghurt (protein, fibre, fat and ash).

Sensory Evaluation

All the samples were evaluated for sensory characteristics and overall acceptability by a 10-man panel selected from a pool of students trained for yoghurt evaluation in the Department of Animal Science, University of Ibadan, Nigeria. Yoghurt samples were identified by three-digit random numbers and presented to the panel in a random manner. A nine-point hedonic scale ranging from 9 (highest score) to 1 (lowest score) was used (Iwe, 2002). Sensory characteristics evaluated include: colour, aroma, taste and texture. Overall acceptability of yoghurt was determined as the average score for sensory characteristics.

Preparation of Media

Nutrient Agar (NA)

Powdered nutrient agar (28 g) was accurately weighed into clean, dry 1L flask and 1000 ml of distilled water was added and placed inside water bath (Classic Equipment, Mumbai, India) set at about 90°C to allow the agar to dissolve. The dissolved agar was then distributed into MacCartney bottles and placed inside an autoclave (Systec GmBh, Germany) set at 121°C for 15 min.

MacConkey Agar (MCCA)

MacConkey agar (55 g, Sigma-Aldrich) was accurately weighed and 1000 ml of distilled water added and boiled to dissolve the agar. The dissolved agar was then distributed into MacCartney bottles and autoclaved as previously described.

Potato Dextrose Agar (PDA)

Potato Dextrose Agar (39 g, BD Worldwide) was accurately weighed and 1000 ml of distilled water added and brought to boil to dissolve the agar. The dissolved agar was then distributed into MacCartney bottles and autoclaved as previously described.

Microbiological analysis

The pour plate technique (Adegoke, 2000) was used for the microbiological examination of the various yoghurt samples. Distilled water (9 ml) was pipetted into clean test tubes and plugged with cotton wool and wrapped with aluminum foil. This was then sterilized in the autoclave at 121°C for 15 min. Each of the yoghurt samples (1ml) was measured into a clean test tube containing 9ml of sterile distilled water and serially diluted until a dilution factor of 10^{-5} was achieved and 1ml of the last dilution factor plate out into sterile plates. The media was poured individually; that is, NA, MCCA and PDA into separate plates and each was duplicated. The plate for total bacteria count (NA) and coliform counts (MCCA) were allowed to cool and set; inverted and incubated at 37°C for 48 h. However, the plates for fungal counts (PDA) were inverted and incubated at 28- 30°C for 72h.

Statistical analysis

All data were subjected to Analysis of Variance using procedures of SAS (1995). Means were separated using Duncan's Multiple Range test at 5 % level of probability.

Results

Chemical composition

Chemical composition of yoghurts changed slightly with addition of fruits (Table 1). Dry matter content varied from 15.3 to 17.6%; fat from 2.74 to 3.95%; protein, 3.00 to 3.56%; crude fibre, 0.00 to 0.30% and soluble carbohydrates from 6.55 to 10.19%. The pH also varied from 3.95 to 4.54. Addition of banana and mango fruit pulp increased the dry matter and carbohydrate content of yoghurts while pineapple and orange pulp did not significantly (P>0.05) affect dry matter content but slightly altered carbohydrate fractions. Fat and protein content in yoghurts decreased with addition of fruit pulps while fibre content increased. Ash content and pH values in yoghurts did not follow a defined trend with addition of fruit pulps.

Sensory attributes

The colour of ordinary yoghurt was most acceptable, followed closely by banana-yoghurt while orange and mango-enhanced yoghurts were least acceptable (Table 2).

Aroma of banana-enhanced yoghurt was judged to be most acceptable while pineapple-enhanced yoghurt was least acceptable. Taste scores also showed banana-yoghurt to be more acceptable than other yoghurts, followed by mango, and least by pineapple. Texture scores also favoured banana-yoghurt above other yoghurts. Overall acceptability of the yoghurts, taken as the average of all sensory parameters, showed that banana-yoghurt was more acceptable than ordinary yoghurt and other fruit yoghurts while mango-yoghurt had equal acceptability with ordinary yoghurt.

Table 1: Chemical composition of yoghurt and fruit-enhanced yoghurt at 7 days of refrigerated (4°C) storage

Parameters (%)	Yoghurt only	Yoghurt enhanced with				SEM
		Banana	Pineapple	Mango	Orange	
Dry matter	15.3^{b}	17.6^{a}	15.4^{b}	17.2^{a}	15.3^{b}	0.23
Fat	3.95^{a}	2.74^{b}	2.92^{b}	3.05^{b}	2.90^{b}	0.06
Protein	3.56^{a}	3.09^{b}	3.00^{b}	3.05^{b}	3.04^{b}	0.08
Ash	1.41^{ab}	1.35^{b}	1.42^{ab}	1.53^{a}	1.20^{b}	0.04
Crude fibre	0.00^{b}	0.22^{a}	0.29^{a}	0.15^{ab}	0.30^{a}	0.02
Carbohydrates	6.55^{c}	10.19^{a}	7.77^{b}	9.43^{a}	7.86^{b}	0.51
pH	4.54^{a}	3.95^{b}	4.42^{a}	4.10^{b}	4.41^{a}	0.09

Means with different superscripts within the same row are significantly different (p<0.05).

Table 2: Sensory attributes of yoghurt and fruit-enhanced yoghurt at 7 days of refrigerated (4°C) storage on a 0-9 hedonic scale

Parameters	Yoghurt only	Yoghurt enhanced with				SEM
		Banana	Pineapple	Mango	Orange	
Colour	7.65^{a}	7.55^{a}	6.40^{b}	5.80^{c}	5.15^{c}	0.68
Aroma	6.05^{c}	7.80^{a}	5.35^{d}	7.15^{b}	6.25^{c}	0.67
Taste	6.61^{c}	7.75^{a}	3.00^{d}	7.25^{b}	6.65^{c}	0.68
Texture	7.35^{b}	7.85^{a}	4.60^{d}	7.30^{b}	6.60^{c}	0.66
Overall acceptability	6.92^{b}	7.74^{a}	4.84^{d}	6.88^{b}	6.16^{c}	0.62

Means with different superscripts within the same row are significantly different (p<0.05).

Microbial Counts

Total bacterial count (TBC) (including lactic acid bacteria) for yoghurts in this study varied from 1.8×10^4 to 6.5×10^5 cfu/g (Table 3).

Bacterial count increased with days in storage and addition of fruits increased bacterial load in the yoghurt.

The coliform count in yoghurt stored for 7 to 28 days inside a refrigerator is presented in Table 4. As with TBC, coliform count in this study increased with progression in storage days and addition of fruit pulp. The highest coliform count was observed in yoghurt fortified with banana fruit and least in yoghurt without fruit pulp.

Total fungal count (TFC) in yoghurts enhanced with fruits and stored in a refrigerator for 7- 28 days is presented in Table 5. The TFC in yoghurt samples increased with length of days in refrigerated storage. At 7 days of storage, TFC ranged from 5.50 to 55.4 cfu/g while at 28 days, TFC ranged from 25.3 to 98.4 cfu/g. Irrespective of days in storage, TFC was highest in mango, followed by orange, banana, pineapple-yoghurts and least in yoghurt-only.

Table 3: Total bacterial count (cfu/g) in yoghurts from 7 to 28 days of refrigerated (4°C) storage

Storage period (days)	**Yoghurt only**	Yoghurt enhanced with				**SEM** ($\times 10^3$)
		Banana	**Pineapple**	**Mango**	**Orange**	
7	$1.8 \times 10^{4d,z}$	$6.0 \times 10^{4a,z}$	$4.3 \times 10^{4b,z}$	$3.3 \times 10^{4c,z}$	$2.2 \times 10^{4d,z}$	1.92
14	$3.5 \times 10^{4d,y}$	$8.1 \times 10^{4a,y}$	$6.1 \times 10^{4b,y}$	$4.9 \times 10^{4c,y}$	$4.2 \times 10^{4c,y}$	2.43
21	$6.6 \times 10^{4c,x}$	$2.3 \times 10^{5a,x}$	$9.0 \times 10^{4b,x}$	$7.8 \times 10^{4c,x}$	$7.1 \times 10^{4c,x}$	2.86
28	$9.8 \times 10^{4d,w}$	$6.5 \times 10^{5a,w}$	$2.1 \times 10^{5b,w}$	$1.6 \times 10^{5c,w}$	$1.0 \times 10^{5d,w}$	3.17
SEM ($\times 10^3$)	0.18	0.26	0.28	0.32	0.40	

Means with different superscripts within the same row are significantly different ($p<0.05$).

Table 4: Coliform count (cfu/g) in yoghurts from 7 to 28 days of refrigerated (4°C) storage

Storage period (days)	**Yoghurt only**	Yoghurt enhanced with				**SEM**
		Banana	**Pineapple**	**Mango**	**Orange**	
7	$6.20^{d,z}$	$28.7^{a,z}$	$18.3^{b,z}$	$14.3^{c,z}$	$7.60^{d,z}$	0.45
14	$8.40^{d,y}$	$51.0^{a,y}$	$40.0^{b,y}$	$31.8^{c,y}$	$9.30^{d,y}$	0.62
21	$15.5^{e,x}$	$75.6^{a,x}$	$64.8^{b,x}$	$58.2^{c,x}$	$23.3^{d,x}$	0.74
28	$30.8^{e,w}$	$96.3^{a,w}$	$82.4^{b,w}$	$73.1^{c,w}$	$38.8^{d,w}$	0.90
SEM	0.40	0.80	0.75	0.53	0.50	

Means with different superscripts within the same row are significantly different ($p<0.05$).

Table 5: Total fungal count (cfu/g) in yoghurts from 7 to 28 days of refrigerated (4°C) storage

Storage period (days)	**Yoghurt only**	Yoghurt enhanced with				**SEM**
		Banana	**Pineapple**	**Mango**	**Orange**	
7	$5.50^{d,z}$	$26.8^{a,z}$	$24.3^{a,z}$	$55.4^{b,z}$	$41.7^{c,z}$	0.65
14	$7.50^{e,y}$	$40.9^{a,y}$	$35.1^{b,y}$	$62.7^{c,y}$	$58.1^{d,y}$	0.78
21	$14.1^{e,x}$	$54.0^{a,x}$	$46.8^{b,x}$	$75.9^{c,x}$	$68.5^{d,x}$	0.87
28	$25.3^{e,w}$	$70.7^{a,w}$	$65.3^{b,w}$	$98.4^{c,w}$	$85.8^{d,w}$	0.98
SEM	0.58	0.80	0.78	0.91	0.88	

Means with different superscripts within the same row are significantly different ($p<0.05$).

Discussion

Addition of pineapple and sweet orange fruit pulp did not alter the dry matter content in yoghurt while addition of banana and mango fruit pulp significantly increased the dry matter content. This was due to the similarity in dry matter content of yoghurt, pineapple and orange fruit; and disparity in yoghurt, banana and mango fruit. The dry matter content of pineapple and sweet orange fruits which was reported to range between 13 and 14% (Morton, 1987; Hemalatha and Anbuselvi, 2013) is similar to 15% reported in this study for stirred yoghurt. However, higher dry matter content of 20 to 28% were reported for banana and mango fruits (Adeyemi and Oladiji, 2009; Sarkiyayi *et al*., 2013), leading to higher dry matter content of yoghurt mixed with these fruits. The fat and protein content in fruit-enhanced yoghurts were lower than yoghurt only. This was probably a reflection of lower fat and protein content in these fruits. Crude fibre content of yoghurts increased with addition of fruit pulps. This was expected since milk from which yoghurt is derived lacks any form of fibre while fruits are known to be good sources of dietary fibre. Dietary fibre promotes gut health and bowel movement hence, fruits in yoghurt is expected to confer beneficial effects on consumers of such products. The pH value of yoghurts in this study varied from 3.95 to 4.54 which were within acceptable range for good yoghurt (Tamime and Robinson, 1999). The pH of banana and mango-enhanced yoghurts were however slightly lower than other yoghurts, indicating higher acidity. This is thought to be a result of higher carbohydrate levels in these fruit yoghurts which provided suitable substrates for fermentative activity of lactic acid bacteria.

Colour assessment of yoghurts showed that ordinary yoghurt was rated above fruit enhanced yoghurts in the following order; yoghurt-only > banana-yoghurt > orange-yoghurt> mango-yoghurt. This may be attributed to the white colour of yoghurt and banana-yoghurt which was shown to be more acceptable to yoghurt consumers (Olorunnisomo, 2008). Addition of fresh pineapple fruit pulp to yoghurt resulted in a slightly bitter taste after 7 days of storage. The reason for this is not fully understood but an unpublished observation showed that boiling the pineapple fruit pulp before adding to yoghurt removes the bitter taste. This suggests the presence of active plant enzymes in the fresh fruit which were deactivated when the pulp was heated (Helmalatha and Anbuselvi, 2013). Texture score was highest for banana-yoghurt, followed by yoghurt-only and mango-yoghurt. The higher texture score attributed to banana-yoghurt may be due to the natural smooth-feel of ripened banana compared to the coarse-feel of other blended fruits in the yoghurt. Overall acceptability of yoghurts was in the following order: banana-yoghurt > yoghurt-only > mango-yoghurt > orange-yoghurt > pineapple-yoghurt. The smooth feel, aroma and sweet taste of banana contributed to the high sensory rating of banana-yoghurt by panelists. Pineapple-yoghurt was the least acceptable of all yoghurt mixture due to its low aroma, taste and texture scores among the panelists.

The TBC for yoghurts in this study (1.8×10^4 - 6.5×10^5 cfu/g) is lower than the standard 1.0×10^6 cfu/g set as maximum for yoghurts under Indian food regulation (Pal *et al*., 2015). It is however, higher than $1.63 – 1.77 \times 10^4$ cfu/g reported by Igbabul *et al*. (2014) and comparable to $3.1 – 5.1 \times 10^5$ cfu/g reported by Dirisu *et al.* (2015) for some yoghurts sold in Nigeria. These figures suggest that total bacterial count in yoghurt during this study was within acceptable limits. Although TBC is an index of hygienic conditions during food preparation (Pal *et al*., 2015), high TBC in yoghurts does not necessarily indicate food contamination, since the organisms employed for yoghurt production (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) are also bacteria. Alli *et al.* (2010) have shown that a high proportion of the bacterial population found in commercially prepared yoghurt in Ibadan, Nigeria is lactic acid bacteria with beneficial properties. The increased bacterial count in yoghurt with added fruits and length of storage however suggests extra bacterial load from fresh fruits and sustained bacterial growth during refrigerated storage. Since fruits used in this study were neither heated nor irradiated before use, the extra bacterial load may be inherent in the fruits, handling utensils or personnel during pulp preparation. In order to reduce bacterial contamination during production of fruit yoghurts, extra attention needs to be paid to hygienic conditions in the preparation of fresh fruit pulps.

The presence of coliform bacteria in yoghurt is an indication of poor hygiene during production (Dirisu *et al.*, 2015).

According to FSSAI (2015), coliform count in yoghurt should not exceed 100 cfu/g. The coliform count in yoghurt made in this study varied from 6.20 to 96.3 cfu/g. While these are within acceptable limits, less than ideal hygienic conditions during yoghurt preparation are implied. Since all yoghurts contain some levels of coliform bacteria, it may be induced that contamination resulted from yoghurt handling, utensils, personnel, and the fruit pulp. Yabaya and Idris (2012) reported a coliform count of 1.0×10^2 to 4.0×10^3 cfu/g for yoghurts sold in Kaduna metropolis while Younus *et al.* (2002) reported a coliform count of $0 – 3.39 \times 10^3$ cfu/g for some yoghurt sold in Islamabad, Pakistan. These were much higher than those reported in this work. Dirisu *et al.* (2015) however, reported a lower coliform count of 0 - 1.0 cfu/g in yoghurts sold in some schools in Rivers State, Nigeria. Fungal count in this study (5.50 – 98.4 cfu/g) was within the limits of 50 -100 cfu/g set by FSSAI (2015) for yoghurts and much lower than fungal count of 10^2 - 5.0×10^3 cfu/g and $3.2 – 4.9 \times 10^3$ cfu/g reported by Varga (2007) and Dirisu et al. (2015) respectively. The presence of molds and yeasts is not desirable in yoghurts and signifies aerobic spoilage. Fungal count in this study increased with addition of fruit pulp to yoghurt. The least count was observed in ordinary yoghurt and highest count in yoghurt fortified with mango fruit pulp. The relatively high fungal count in fruit-enhanced yoghurts may have resulted from contamination during the preparation of fresh fruit pulps. Fungal count in yoghurts increased with days in storage, indicating that molds continued to grow in the yoghurt even under refrigerated storage.

Conclusions

Addition of fruits increased dry matter content of banana and mango-yoghurts. Fat and protein content of yoghurts decreased with addition of fruit pulps while fibre and carbohydrate content increased. Banana-yoghurt was the most acceptable by panelists while mango-yoghurt had equal acceptability with ordinary yoghurt. Acceptability of orange and pineapple-yoghurt were lower than ordinary yoghurt. Addition of fresh fruit pulp increased microbial load of yoghurt. Microbial load of yoghurt also increased with days in storage. While the addition of banana fruit pulp improved acceptability of yoghurt, the tendency of fresh fruit pulps to increase microbial contamination in yoghurt pose a serious concern for public health. It is recommended that fruit pulp used as additives in yoghurt preparation should be subjected to appropriate heat treatment to deactivate unwanted plant enzymes, reduce microbial contamination from fruits and exclude pathogenic organisms from yoghurt sold to the public.

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