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Effect of Hibiscus Sabdariffa (Calyce) Extract on Biochemical and Organoleptic Properties of Yogurt

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Abstract: Previous studies have shown that substances incorporated into milk before and after fermentation elicit profound influence on the physicochemical properties of yogurt and consumers' sensory perception of their acceptability as healthy and refreshing drinks. This study examined the effect of *Hibiscus sabdariffa* calyx extract on pH, titratable acidity (TA), syneresis, ash and moisture contents in two brands of low fat milk (LFM) yogurts when incorporated before and after fermentation. Results were compared to those of plain and strawberry yogurts with the sensory profiles of the yogurts after manufacture and on days 3, 5 and 7 of storage under refrigeration temperature (8±1°C) determined and compared on a 9 point-hedonic scale using 10 independent yogurt accustomed panelists. Incorporation of *Hibiscus sabdariffa* calyx extract into the reconstituted LFM (500g) resulted in significant ($P < 0.05$) decreases in coagulation time 5.5±0.4 vs. 6.2±0.8 h), pH 4.4-4.16 vs. 4.54-4.31± (0.003-0.007), syneresis 0.8-1.9 vs. 1.3-2.6 ± (0.01-0.04) and β galactosodase activity (1.02-1.86 vs. 0.57-0.98±0.02-0.05 U/g) after manufacture and during storage. Microbial analysis revealed the presence of non-starter isolates: *L. plantarum, L. brevis* and higher total LAB count (2.5-3.1 x 10^9 vs. 1.2-1.5 x 10^7 cfu/ml; $P < 0.05$) on day 7 in the sabdariffa yogurts. Sensory evaluation recorded significantly ($P < 0.05$) lower scores on aroma (2.6-4.1 vs. 5.3-7.7), taste (2.7-4.7 vs. 6.1-7.7), and overall acceptability (4.1-5.9 vs. 6.4-8.4) for the sabdariffa yogurts after manufacture and during storage. On the whole, the strawberry and sabdariffa (pre-added) yogurts were the most and the least preferred with retention of freshness on day 7 of storage at 8±1°C. Results indicate the need to improve on the sensory attributes of the more probiotic sabdariffa yogurts in order to optimize consumer's perception on their acceptability.

Key words: *Hibiscus sabdariffa*, yogurts, starter culture, non-starter culture, sensory analysis

Introduction

Fermented milk drinks, yogurts are diary products produced from milk fermented with viable and retainable lactic acid bacteria (Adolfsson *et al*., 2004). Human consumption of yogurts has been associated with tremendous health benefits due to improvement of gastrointestinal functions and disease risk reduction (Heyman, 2000). These health benefits include longevity (Metchnikoff, 1908), improved lactose digestion and elimination of lactose intolerance symptoms among maldigesters (Vesa *et al*., 1996), lowered cholesterol level and reduction in the risk of hypertension (Taylor and Williams, 1998; Takano, 1998), lowered cancer tendency (van't Veer *et al*., 1989), diarrhea prevention and control as well as maintenance of gastrointestinal microflora (Boudraa *et al*., 1990). The probiotic elements in yogurts are lactic acid bacteria (LAB), which include *Lactobacillus* species, Bifidobacteria and *Streptococcus thermophillus* have severally been demonstrated as the cause of their health benefits and the reasons for their description as functional foods (Adolfsson *et al*., 2004). A search of the literature has shown that strains of *Lactobacillus acidophilus* either singly or in combination with other LABs have persistently being used as models for studying the biology and mechanisms of probiotic functions in diary products including yogurts (Gaon *et al*., 1995; Savaiano *et al*., 1984; Marteau *et al*., 1992).

The consumption of yogurts in developed countries of the world has increased tremendously in the last 10 years owing to the ability of diary industries to meet the challenges of producing different brands of yogurts with variable consumer appeals in constant competition with carbonated drinks (Trap *et al*., 1993; Karagul-Yuceer *et al*., 1999; Deis, 2000). In developing countries including Nigeria, many yogurts and related products are still produced on a small scale at household/cottage level and production is primarily meant for weaning children, generating household income and refreshment (ILRI, 1998; Bayemi *et al*., 2005). Consequently, few yogurt varieties exist and probiotic composition of most products is undefined coupled with their poor hygienic preparations and short lifespan (Younus *et al*., 2002). This is so because fermentation of source milk materials for many of the yogurt products is accomplished naturally (i.e. by chance fermentation) at unregulated temperatures and often in the absence of additives for texture, flavor and curd stabilization. Meanwhile, the inclusion of these non-diary ingredients have been found to improve yogurt quality, create new brands of yogurts and modulate perception of consumers in developed countries and Asia (Deeth and Tamine, 1981; Haddadin, 2005). This approach

simultaneously evokes an economic constraint of increased cost of production with a subsequent decrease in affordability by consumers (Dorota *et al*., 2002). Sourcing of non-diary alternatives from nutritional plants may provide a means of breaking this economic barrier. Earlier studies on dahi, a yogurt product made from milk co-fermented with the juice of *Musa sapientum* have revealed this possibility coupled with inherent nutritional, health and probiotic benefits (Ayebo and Shahani, 1980; Kamruzzaman *et al*., 2002).

Hibiscus sabdariffa is a medicinal ephemeral plant belonging to the *Malvaceae* family. The plant also called Roselle or Karkade, has a long traditional history of usage as a therapy against hypertension, diabetes, skin disorder and bacterial diseases (Ali *et al*., 2005). The flower extract of the plant is rich in flavonoids and natural colorants, which include anthocyanins and catechins (Falade *et al*., 2005). The plant is also rich in ascorbic acids and acid polysaccharides (Falade *et al*., 2005; Muller and Franz, 1992). This nutraceutical properties of *H. sabdariffa* have been exploited in the production of zobo drink and furundu, consumed as a meat substitute by humans (Kolawole and Maduenyi, 2004). However, there is lack of data concerning the incorporation of *H. sabdariffa* flower extract in yogurt production and its effects on the physicochemical and sensory qualities of yogurt products. It is hypothesized that 'zobo' would supplement yogurt with nutrients, optimize its probiotic functions and modifies its qualities as well as alter consumers perception of yogurt as a diary product in a cost-effective way.

In the present study, four brands of yogurts-plain, strawberry, pre-added and pot-added sabdariffa yogurts were prepared from reconstituted low fat powdered milk fermented with *Lactobacillus acidophilus* and *Streptococcus thermopillus*. The physicochemical and sensory attributes of the yogurt products were also determined and compared.

Materials and Methods

Hibiscus sabdariffa: Dry calyces of *Hibiscus sabdariffa* were brought from Alaba market in Ojo Local Government of Lagos State and transported to the laboratory in a closed sterile plastic container. The calyces were identified and authenticated at the Pharmacognosy Department of the College of Medicine University of Lagos, Nigeria. They were then further dried in an oven at 50°C for 24 h. This was followed sieving to remove impurities using a 3 - 5 mm mesh. The dried calyces were then ground to powder.

Preparation of the Aqueous Extract of *Hibiscus sabdariffa* calyce: Ground powdered form of *Hibiscus sabdariffa* calyce (50g) were extracted under reflux with sterile water (350 ml) at 100°C for 4 h. The resulting extract was filtered using cheesecloth and the sub-filtrate was sterilized using a 45µm Millipore filter to remove microbial contaminants. The resulting filtered extract was reconstituted with sterile water to 50mg/L final concentration and kept in air -tight container at 4°C prior to use as a flavouring agent.

Low-fat Powdered Milk (LFPM) and Processing: The low-fat powdered milk (Fat, 1%; protein 34.5%; Lactose, 39%, minerals 6.5%, moisture, 3.5%, soya lecithin 0.2% and vitamins (A, 1800 IU, B2 1.4mg, B12, 3mg, D3 400IU) with an energy value of 490kcal/100g was purchased from a local market in Lagos. The powdered milk was reconstituted as a homogenous milk solution at 10.5% with sterile water. This was followed by pasteurization at 85°C for 20 min. After pasteurization, the milk was cooled to 42°C in ice bath.

Starter culture: A mixture *Lactobacillus acidophilus* YA015 and *Streptococcus thermophillus* strain as lyophilized sample (0.03g% final concentration) was used as the starter culture. The bacteria were recovered from yogurt products sold in Lagos metropolis and maintained as viable cells in milk suspension at -20°C. *L. acidophilus* YA015 has previously been characterized as hydrogen peroxide and bacteriocin producing homolactic thermophillic organism, exhibiting mild tolerance to bile salt, taurocholate (Iwalokun *et al*., 2005).

Prior to use, the organism was tested for viable by growth on De Mann Rogosa Sharpe (MRS) medium under anaerobic condition using a candle jar at 37°C for 24 h (De Mann *et al*., 1960). Colonies were further biochemically validated based on catalase test, oxidase reaction, mannitol and lactose fermentation (Cowan and Steel, 1974). The viability of *S. thermophillus* strain was tested by growth of lyophilized sample in a modified J8 medium containing (in g/L) lactose 5g, beef extract 2g, peptone 7g, yeast extract 2g, Na_2PO_4 5.5g, KH_cPO_4 5.0g, ascorbic acid 0.5g and $MgCl_2$ 0.2g (Appendix 1) according to Thomas and Crow (1984). Both bacterial cells were washed twice with normal saline (0.90% NaCl), pelleted by centrifugation at 1,500g for 10 min and then reconstituted to a cell density of 10^8cfu/ml in the low fat milk solution (50ml each). The resulting cultured milk samples were mixed in ratio vol/vol 1:1 to become a starter culture. The starter culture was subsequently equilibrated to 42°C and used immediately for milk fermentation.

Yogurt preparation: To the pasteurized milk solution was added the starter culture suspension at 2%. The resulting mixture was stirred, transferred into an airtight container and incubated at 42°C in a hot air oven under static condition to enable lactose fermentation and setting of the yogurt.

After coagulation, the yogurt samples were stirred and

the following were added, sugar at 30g/l, 0.8ml/l of strawberry flavour for strawberry yogurt and 20ml/l *Hibiscus sabdariffa* calyx extract for the sabdariffa yogurts. To test the effect of the extract on the physicochemical properties of plain yogurt, the extract was added prior to fermentation. The preparation of the various brands of yogurt is presented in Fig. 1.

Physicochemical analysis

pH determination: The pH of low fat milk suspension before and after fermentation was determined in the four brands of yogurts prepared in this study using a pH meter model ARHI CE (Myron L Company, USA). Each sample was diluted in ratio 1: 5 (vol/vol) with distilled water. The pH meter was calibrated with sample solutions of pH 4 and 10 prior to use. It was also rinsed with distilled before pH measurement of another yogurt brand. Changes in pH of fermented milk products on days 0, 3, 5 and 7 at the storage refrigeration temperature (4°C) were subsequently monitored and recorded.

Titratable acidity: Titratable acidity (TA) was measured according to the International diary Federation (IDF, 1991) standard. Here, 9g of each of the yogurt products were mixed with 0.5ml of 1% phenolphthalein indicator. The resulting mixture was then titrated with 0.1M sodium hydroxide until the yogurt product turned pink, which persisted for 30 sec. TA was measured after production and on days 3, 5 and 7 of storage under refrigeration temperature (8±1°C). It is expressed as grams of lactic acid per one hundred grams of the product, using the equation:

Titratable acidity = V x 0.9/m

Where V = volume (in mL) of 0.1 M sodium hydroxide solution require to titrate a sample of yogurt to a pH of 8.3, m = mass (in g) of the tested yogurt sample, and 0.9 is the conversion factor for lactic acid.

Moisture content determination: The moisture content of the yogurt products was determined by the Association of Official Analytical Chemists (AOAC) method (1995). Each yogurt product (15g) was placed in an oven at 100°C for 48 h. The moisture content is expressed as the percentage (%) of the weight of yogurt during drying.

Ash content determination: The organic content in each of the dry yogurt samples was burnt off by incineration at 550°C for 1 h. the ash content is expressed as the inorganic residue left as a percentage of the total weight of yogurt incinerated (AOAC, 1995).

Syneresis determination: Syneresis was determined by dispensing 125g of yogurt into a cheesecloth line funnel placed on top of a graduated cylinder. The amount of whey, 'milk serum' in mL was measured after manufacture and on days 3, 5 and 7 of storage at refrigeration temperature (8±1°C). The amount of whey drained off (expressed as milliliters per 125g of sample) was calculated as the syneresis index (Marshal, 1993).

β- Galactosidase assay: The activity of β-galactosidase in each of the yogurt products after manufacture and during storage at 8±1°C was carried out according to Hughes and Hoover (1995). To 1.0 ml of yogurt sample were added 4.0ml of 0.05M sodium phosphate buffer (pH 7.0) and chloroform (0.2ml). The mixture was then centrifuged at 29, 000 x g for 15 min. After the removal of supernatant, the cell debris was mixed 1% Triton X -100 (1.0ml) in sodium phosphate buffer (2.0ml) and incubated at 37°C for 15 min. this was followed by the addition of 15mM 0-nitrophenyl-β-galactoside substrate (1.0ml) and further incubation at 37°C for 10 min. the reaction was stopped by the addition of 1.0ml of 0.0M Na_2CO_3 followed by centrifugation at 29,000 x g for 15 min to remove cell debris. The absorbance of the recovered supernatant was then measured at 405nm using a Colorimeter E120 (Boreinger, Germany) to determined the concentration of 0-nitrophenol liberated by extrapolation from a standard o-nitrophenol curve (50 - 3000µmol). One unit of β-galactosidase activity is defined as the amount of enzyme required to liberate 1 µmole of o-nitrophenol per min at 37°C.

Microbial analyses: Samples were diluted in ratio 1:10 (vol/vol) and used for total bacterial Lactobacillus and mould counts on plate count, MRS and potato dextrose agar media. The lactobacilli were speciated biochemically and macroscopically according to Cowan and Steel (1974)

Sensory evaluation: The qualities of the coded (01- 04) four yogurt brands produced - taste, flavor, appearance, sourness, thickness, smell, aroma and creaminess were organoleptically determined according to Meilgaard *et al.* (1999). This involves the use of ten (10) independent yogurt accustomed panelists to examine the listed attributes of the yogurts products that were blinded by coding. Evaluation was based on a 9 point-hedonic scale with very poor (score = 0) and excellent (score = 9) as extremes.

Statistical analyses: Data on physicochemical parameters and sensory profiles were computed as mean ± standard error of mean (SEM) and analyzed by one - way analysis of variance (ANOVA). Coagulation times of the yogurts were expressed as mean ± SD and evaluated by student's t-test. The relationships between sensory attributes were determined by regression analysis using Microsoft Excel Statistical analysis Program. Outcomes were interpreted at 95% confidence limits and $P < 0.05$ was considered significant.

Results

The coagulation (set) time of the plain and pre-added sabdariffa yogurt products: The set times of plain yogurt product prior to incorporation of strawberry flavor and *H. sabdariffa* calyce extract (10.0mL) to generate two additional varieties and zobo yogurt made by inclusion of the extract prior to fermentation of LFM are summarized in Table 1. The pre-added zobo yogurt was observed to set significantly ($P < 0.05$) at a shorter time (5.5 ± 0.4 h) compared to the plain yogurt (6.2 ± 0.02 h) (Table 1).

Table 1: Comparative analysis of set times of plain and pre-added zobo yogurts

Yogurt brand	Set time (h)	t	P
Plain yogurt	5.5±0.4		
Pre-added zobo yogurt	6.2±0.02	2.37	< 0.05

Data are presented as mean ± SD of three measurements and analyzed by student's t-test. $P < 0.05$ indicates significance.

Comparison between ash and moisture contents of sabdariffa and non-sabdariffa yogurt products: The ash and moisture contents of the resulting four yogurt brands after manufacture and on days 3, 5 and 7 of storage under refrigeration temperature (8±1^{0}C) are presented in Table 2. Ash contents of 0.84 - 0.86, 0.86 - 0.89, 0.94 - 1.20 and 0.89 - 1.10g/100g were recovered from plain, strawberry and sabdariffa yogurts (pre-added and post-added) after the 1h incineration at 550^{0}C. The zobo yogurts elicited a significantly higher ($P < 0.05$) ash content throughout the study period. While disparity observed in ash content between the zobo yogurt products was significant ($P < 0.05$) after manufacture (i.e. on day 0) (Table 1). Moisture content ranges of 88.7 - 88.8g/100g on day 0 and 88.6 - 89.1g/100g during storage were observed in the four yogurt brands produced with no significant ($P > 0.05$) difference in their moisture content (Table 2).

Variations in pH and titratable acidity of the four yogurt products: The variations in the pH values of the four brands of yogurts with storage time at 8+10C are presented in Fig. 1. pH value variations of 4.53 - 4.32, 4.53 - 4.30, 4.41 - 4.11 and 4.50 -4.10 were observed after manufacture and during storage for plain, strawberry, pre-added and post-added yogurts respectively. Pre-added and plain yogurts elicited the highest and lowest decline in pH with time respectively. Furthermore, disparity in pH values between sabdariffa and non-sabdariffa yogurts was significant ($P < 0.05$) throughout the study period. While the significantly ($P < 0.05$) reduced pH of pre-added zobo yogurt became non-significant (4.10-4.15 vs. 4.11 - 4.16; $P > 0.05$) from day 5 of storage when compared with the post-added yogurt. No significant difference ($P > 0.05$) was observed in the pH values of plain and strawberry yogurts.

The ability of the four yogurt brands to whey off after manufacture and during storage at 8±1^{0}C was analyzed in Table 2. Prior incorporation of *H. sabdariffa* calyx extract resulted in a significant ($P < 0.05$) Syneresis reduction after yogurt production with a mean syneresis index of 0.7 ± 0.03 mL/125g sample compared to 1.7 ± 0.05 mL/125g sample in non-sabdariffa yogurts and post-added sabdariffa yogurt (Table 2). However, the generally observed increased syneresis with days of storage was higher in non-zobo yogurts (1.8 - 2.9 mL/125g) than their sabdariffa counterparts (0.8 - 1.6 mL/125g). The order of susceptibility to syneresis was plain > strawberry > post-added > pre-added zobo yogurts with significant ($P < 0.05$) disparity observed between plain and strawberry throughout the storage days and between pre-added and post-added zobo yogurts on day 3 of storage.

Titratable acidity of the four yogurt brands produced a range of 0.85 - 1.07% lactic acid after manufacture and during the 7 days storage period for the non-zobo yogurts. While the zobo yogurts exhibited a significantly ($P < 0.05$) higher TA values (1.2 - 1.39% lactic acid) on days 5 and 7 post storage (Table 3). Further analysis of TA values between pre-added and post-added zobo yogurts revealed significance ($P < 0.05$) only on days 0 (0.9 ± 0.05 vs. 1.03 ± 0.02) and 3 (0.98 ± 0.05 vs. 1.2 ± 0.02% lactic acid) respectively (Table 3).

β-galactosidase activity of the yogurt products after manufacture and during the 7-day storage period at 8±1^{0}C: β galactosidase activities of 1.02 - 1.27 ± 0.03 - 0.05 and 0.71 - 0.73 ± 0.02 U/ml ($P < 0.05$) were exhibited by sabdariffa and non - sabdariffa yogurts respectively. Both yogurts brands also exhibited decrease in enzyme activity with time but greater decrease (0.58 vs. 1.01 - 1.02 U/ml; $P < 0.05$) was observed in the non-sabdariffa varieties (Table 3).

Microbial analysis of the yogurt products: Microbial assessment of the yogurts products revealed besides the starter culture isolates, the presence yeast in all the four brands after production and occurrence of *L. plantarum* and *L. brevis* isolates in the zobo yogurts (Table 3). A general decline (6.6-228.1 folds) in lactic acid bacteria count but increase in yeast count was observed in the four yogurt products with lactic acid bacteria count of < 10^7cfu/mL attained from day 5 of storage of the non -sabdariffa yogurts. Yeast counts of 2.5 - 2.6 x 10^3 and 0.94 - 1.1 x 10^3 cfu/mL were recorded in the non-zobo and zobo yogurts respectively on day 7 -post storage (Table 4).

Sensory Evaluation of the yogurt products: The sensory qualities of the sabdariffa and non-sabdariffa yogurts after manufacture and during storage are summarized in Table 5. The sabdariffa yogurts exhited

Table 2: Ash and moisture contents of the four yogurt brands after manufacture and storage for 7 days at refrigeration temperature (8±1^{0}C)

Yogurt brand	Day 0	Day 3	Day 5	Day 7	SEM
@Ash con tent (g/100g)					
Plain	0.84^a	0.86^a	0.85^a	0.86^a	0.003 - 0.005
Strawberry	0.86^a	0.88^a	0.89^a	0.89^a	0.001 - 0.007
Pre-added zobo	0.94^b	0.98^b	1.10^b	1.20^b	0.002 - 0.007
Post-added zobo	0.89^b	0.96^b	0.99^b	1.10^b	0.003 - 0.005
Moisture con tent (g/100g)					
Plain	88.7^a	88.7^a	89.0^a	88.7^a	0.06 - 0.09
Strawberry	88.7^a	88.9^a	88.6^a	88.6^a	0.08 - 0.1
Pre-added zobo	88.8^a	89.0^a	89.0^a	89.1^a	0.08 - 0.12
Post-added zobo	88.8^a	89.1^a	89.1^a	89.1^a	0.04 - 0.13

Data are presented as mean ± SEM of three determination per yogurt brand and analyzed using ANOVA statistics. Superscripts of the same letter in each column indicate no significance (P > 0.05) and significance (P < 0.05) if otherwise. @Ash content was recorded on dry weight basis.

Table 3: β-galactosidase activity of the yogurt products after manufacture and during the 7-day storage period at 8±1^{0}C

Yogurt sample	β- Galactosidase actiicty (U/g) Day 0	Day 3	Day 5	Day 7
1	0.73±0.02	0.98±0.07	0.65±0.01	0.58±0.02
2	0.71±0.02	0.98±0.02	0.66±0.03	0.58±0.03
3	1.27±0.03^{ab}	1.86±0.05^a	1.41±0.07^a	1.02±0.08^a
4	1.03±0.05^a	1.65±0.08^a	1.32±0.02^a	1.01±0.05^a

1 = Plain yogurt, 2 = strawberry yogurt, 3 = Pre-added zobo yogurt, 4 = Post-added zobo yogurt. Data are presented as mean ± SEM of three determinations and analyzed by one way ANOVA. ^{a}P < 0.05 (Sabdariffa vs. non-sabdariffa yogurts), ^{b}P < 0.05 (Pre-added vs. post-added sabdariffa yogurts). P < 0.05 = significant.

significant (P < 0.05) reduction in scores for aroma (2.6 - 3.7 vs. 5.3 - 7.7), taste (2.7 - 4.7 vs. 6.1 - 7.7) and overall acceptability (4.1 - 5.9 vs. 6.4 - 8.4) when compared to their non-sabdariffa counterparts. Disparity between smells was only significant for the strawberry yogurts. While the sabdariffa yogurt elicited lesser reduction in textural score (8.1 to 7.2) compared to the non-sabdarifa varieties (7.8 to 5.2). The thickness scores of the sabdariffa yogurts were higher than those of non-sabdariffa yougurts during storage with sabdariffa yogurt (pre-added) significantly (P < 0.05) thicker (8.4 vs. 7.1 - 7.7) than other varieties after manufacture.

Irrespective of the type of yogurt, multiple regression analyses revealed an inverse significant (P < 0.05) relationship between syneresis, texture, thickness and overall acceptability of a yogurt product. While titratable acidity also associated significantly (r = 0.04; P < 0.05) with taste (Table 6).

Discussion

Yogurts, probiotic protein-based hydrocolloidal gel systems, exhibit variations based on their characteristic physicochemical (e.g. pH, titratable acidity, Syneresis) and rheological properties (e.g. viscosity, texture, firmness), which interact to modulate their sensory qualities and acceptability by consumers (Trachoo and Mistry, 1998; Salvador and Fiszman, 2004).

The present study reports the effects of *Hibiscus sabdariffa* calyx extract on biochemical and sensory profiles of yogurt when incorporated before and after fermentation of fermentation of pasteurized low fat milk (LFM).

The coagulation time of the pre-added sabdariffa yogurt was observed to be shorter by ~ 0.7 h (42 min) than that of the plain yogurt, suggesting that *Hibiscus sabdariffa* calyx extract interacts with either fermentation components or fermentation products to accelerate curd formation. Different coagulation times have been reported for yogurt products depending on the composition of the starter culture, milk type and quantity. Haddadin (2005) recently reported a coagulation time of 4 h for labneh using *Streptococcus thermophillus* and *Lactobacillus delbrueckii subsp. Bulgaricus* as starter culture at 40^{0}C holding temperature. The same starter culture composition was used by Muhammed et al (2005) who reported a coagulation time range of 45 - 120 min at varying culture concentrations and holding temperatures using cow, powdered and skim milk as source materials. In this study, the substitution of a lactose hydrolyzing, bile tolerant and bacteriocin producing strain of *L. acidophilus* for *L. bulgaricus* in the starter culture mix may further explains disparity between the coagulation time for our plain yogurts and those of the previously reported plain yogurts (Sodini *et al.*, 2004). Generally, coagulation or curdling occurs as result of the interaction of denatured whey proteins with disrupted casein micelles at pH ≤ 4.6 mediated primarily by lactic acid (Lee and Lucey, 2004). The abundance of organic acid in *H. sabdariffa* (Lee and Lucey, 2004), the observed occurrence of lactic acid bacteria such as *L. plantarum and L. brevis* and recovery of higher density of the starter isolates in the resulting sabdariffa yogurt may acts in synergy to accelerate pH reduction and time at which coagulation occurs. Unlike, *L. acidophilus* in the starter culture, *L. plantarum* has been observed to be a faster lactic acid producer (Adolfsson *et al.*, 2004) and reported to form smooth homogenous curds in white cheeses made from sheep milk (Haddadin, 2005). The

Table 4: Microbial isolates, lactic acid bacteria and yeast counts in the four yogurt brands after manufacture and storage for 7 days at refrigeration temperature (8±1^{0}C)

	Microbial isolates			
Yogurt brand	Day 0		Day 7	
1	*L. acidophilus, S. thermophilus,* Yeast		*L. acidophilus, S. thermophilus,* Yeast	
2	*L. acidophilus, S. thermophilus,* Yeast		*L. acidophilus, S. thermophilus,* Yeast coliforms.	
3	*L. acidophilus, S. thermophilus,*		*L. acidophilus, S. thermophilus,*	
	L. plantarum, L brevis, Yeast		*L. plantarum, L. brevis*, Yeast	
4.	*L. acidophilus, S. thermophilus,*		*L. acidophilus, S. thermophilus,*	
	L. plantarum, L brevis, Yeast		*L. plantarum, L. brevis*, Yeast	
	Lactic acid bacteria count (cfu/ml)			
Yogurt brand	Day 0	Day 3	Day 5	Day 7
1	7.3 x 10^7	1.1 x 10^7	2.8 x 10^6	3.2 x 10^5
Change in count		-(6.6)	-(26.1)	-(228.1)
2	7.1 x 10^7	1.3 x 10^7	3.7 x 10^6	4.2. x 10^5
Change in count		-(5.5)	-(19.2)	-(169.0)
3	5.6 x 10^9	3.1 x $10^{8\backslash}$	7.2 x 10^7	2.5 x 10^7
Change in count		-(18.1)	-(77.8)	-(224.0)
4	2.7 x 10^8	1.3 x 10^8	5.2 x 10^7	1.4 x 10^7
Change in count		-(2.1)	-(5.2)	-(19.3)
	Yeast count (cfu/ml)			
Yogurt brand	Day 0	Day 3	Day 5	Day 7
1	< 50	2.8 x 10^2	1.7 x 10^3	2.5 x 10^3
2	< 50	2.4 x 10^2	1.8 x 10^3	2.6. x 10^3
3.	1.8x 10^2	4.8 x 10^2	6.6 x 10^2	9.4 x 10^2
4.	1.0 x 10^2	2.7 x 10^2	8.5 x 10^2	1.1 x 10^3

1 = Plain yogurt, 2 = strawberry yogurt, 3 = Pre-added zobo yogurt, 4 = Post-added zobo yogurt. Data on microbial count are presented as average of three determinations per yogurt brand. Figures in brackets represent decrease (-) in lactic acid bacterial count relative to count on day 0 (After manufacture).

observed higher lactic acid bacterial count in the sabdariffa yogurts after manufacture may also be attributed to the bifidogenic action of the reported acidic polysaccharides such as arabinogalactan in *Hibiscus sabdariffa* (Muller and Franz, 1992). This possibility emerged from the studies by He *et al.* (1992) and Kelly (1999) in which arabinogalactans recovered from *Ganoderma lucidum*, herbs of the *Echinacea* species and gum Arabic were found to be immunogenic and bifidogenic.

The observed pH range of 4.41 - 4.53 observed in our four yogurt brands after manufacture are still within the ranges reported for yogurts in previous studies (Haddadin, 2005; Muhammad *et al.*, 2005; Salvador and Fizman, 2004). However, our sabdariffa yogurts from day 5 became more acidic than these related yogurt products. This may be due to the involvement of *L. plantarum* and *L. brevis* to lactic acid formation and the contribution of other acidic components to pH reduction. Although the observed patterns of reduction in pH and increase in titratable acidity are different in the four yogurt brand, the reported acidic components and the lactic acid microflora due to *H. sabdariffa* (Mueller and Franz, 1992) may still be used to explain the observed higher titratable acidity in the zobo yogurts compared to their non-sabdariffa counterparts. The observed TA values of 0.9 - 1.2% lactic acid on days 0 and 3 were still within the acceptable range set by the International diary guidelines (IDF, 1991). While the > 1.2 - 1.39% values recorded from day 5 are lower than 1.9% reported for labneh (Haddadin, 2005) but comparable to 1.31 - 1.51% reported for skim milk yogurt by Muhammed *et al.* (2005). Titratable acidity as high as 6.8% lactic acid has also been reported for skimmed yogurt after 91 days of storage at 10^{0}C (Salvador and Fizman, 2004). Taken together, it appears that starter culture composition, and fermentation temperature and duration could influence the overall level of lactic acid and resulting titratable acidity in the yogurt product. In this study, *L acidophilus* and *S. thermophillus* at a starting density of 10^8 cfu/mL was used to ensure that at least 10^7 cfu/mL is retained in the yogurt product after manufacture in compliance with international standards (Chandan and Shabani, 1993). This is of probiotic importance since at this dose both *L. acidophilus, L. brevis* and *S. thermophillus* are capable of neutralizing toxins of pathogens and promoting their faecal shedding (Saaveda *et al.*, 1994), and stimulating both innate and humoral immunity in humans and animals (Tejada-Simon *et al.*, 1999; Schiffrin *et al.*, 1995). In this study, LAB density $\geq$ 10^7cfu/ml was achieved up to day 3 in all the four yogurt brands and throughout the study period for the

Table 5: Sensory qualities of the sabdariffa and non-sabdariffa yogurts after manufacture and during storage

Sensory quality	Day 0				Day 3			
	1	2	3	4	1	2	3	4
Aroma	6.4^{a} (0.3)	7.7^{b}(0.3)	3.7^{c} (0.2)	4.1^{c} (0.2)	6.1^{a} (0.2)	7.4^{b} (0.4)	3.3^{c} (0.2)	3.9^{d} (0.1)
Taste	7.3^{a} (0.1)	7.7^{a} (0.2)	4.7^{b} (0.3)	4.6^{b} (0.1)	7.2^{a} (0.3)	7.6^{a} (0.4)	4.4^{b} (0.2)	4.2^{b} (0.6)
App	6.9^{a} (0.2)	8.1^{b} (0.2)	6.6^{a} (0.3)	6.5^{a} (0.3)	6.9^{a} (0.3)	8.0^{b} (0.3)	6.1^{a} (0.2)	6.1^{a} (0.3)
Texture	7.5^{a} (0.3)	7.8^{a} (0.2)	8.1^{a} (0.3)	7.9^{a} (0.2)	6.9^{a} (0.4)	7.1^{a} (0.3)	7.9^{a} (0.3)	7.8^{a} (0.1)
Smell	6.9^{a} (0.1)	7.9^{b} (0.2)	6.7^{a} (0.1)	6.7^{a} (0.3)	6.5^{a} (0.2)	7.8^{b} (0.2	6.5^{a} (0.2)	6.4^{a} (0.2
Thickness	7.1^{a} (0.2	7.2^{a} (0.1)	8.4^{b} (0.2)	7.7^{a} (0.1)	7.1^{a} (0.2)	7.3^{a} (0.2)	8.6^{b} (0.2)	8.1^{b} (0.2)
OA	7.1^{a} (0.2)	8.4^{b} (0.2)	5.9^{c} (0.2)	5.9^{c} (0.2)	6.8^{a} (0.1)	7.8^{b} (0.2)	5.2^{c} (0.2)	5.3^{c} (0.2)
Sensory quality	Day 5				Day 7			
	1	2	3	4	1	2	3	4
Aroma	5.8^{a} (0.1)	6.7^{b} (0.3)	2.8^{c} (0.6)	2.9^{c} (0.3)	5.3^{a} (0.2)	5.9^{a} (0.2)	2.6^{b} (0.3)	2.7^{b}(0.4)
Taste	7.0^{a} (0.4)	7.2^{a} (0.7)	3.9^{b} (0.1)	3.9^{b} (0.3)	6.6^{a} (0.5)	6.1^{a} (0.7)	3.2^{b} (0.4)	2.7^{b} (0.2)
App	6.4^{a} (0.4)	7.5^{b} (0.3)	5.3^{c} (0.2)	5.2^{c} (0.2)	6.1^{a} (0.4)	6.9^{a} (0.4)	5.1^{b} (0.2)	5.2^{b} (0.2)
Texture	5.6^{a} (0.2)	5.7^{a} (0.3)	7.5^{b} (0.3	7.5^{b} (0.2)	5.2^{a} (0.3)	5.2^{a} (0.3)	7.4^{b} (0.3)	7.3^{b} (0.1)
Smell	6.4^{a} (0.2)	7.8^{b} (0.2)	6.3^{a} (0.2)	6.3^{a} (0.2)	6.3^{a} (0.1)	7.6^{b} (0.2)	6.3^{a} (0.1)	6.2^{a} (0.2)
Thickness	6.8^{a} (0.2)	6.8^{a} (0.2)	8.5^{b} (0.3)	7.9^{b} (0.2)	6.1^{a} (0.2)	6.2^{a} (0.2)	7.9^{b} (0.2)	7.7^{b} (0.2)
OA	6.5^{a} (0.2)	7.2^{b} (0.1)	4.3^{c} (0.2)	4.2^{c} (0.1)	6.4^{a} (0.1)	6.9^{b} (0.2)	4.1^{c} (0.2)	4.1^{c} (0.1)

1 = Plain yogurt, 2 = strawberry yogurt, 3 = Pre-added zobo yogurt, 4 = Post-added zobo yogurt. Data on microbial count are presented as average of three determinations per yogurt brand. App = Appearance, OA = Overall acceptability, Figures in parentheses represent SEM of the mean values of data. Different superscript letters per row indicate significant ($P < 0.05$) difference between data (ANOVA).

sabdariffa yogurts. Our observation may not be unconnected with the toxic effect of lactic acid and colonization and spoilage tendency of yeast in the yogurt products (Marteau and Rambaud, 1993; El-Samargy *et al.*, 1988; Tamine and Robinson, 1999; Yamani and Abu-Jaber, 1994). The retention of > 10^{7}cfu/mL lactic acid bacteria count in the sabdariffa yogurts may be due to the microflora contribution by *H. sabdariffa*. Kneifel *et al.* (1993) also reported a lactobacilli count of 5.5 x 10^{7} - 6.5 x 10^{8} cfu/ml after manufacture and 4.0 x 10^{5} - 2.6 x 10^{8} cfu/ml after a 2 -week storage at 6^{0}C of yogurts and yogurt - related milk products using 44 commercially available starter cultures. Apart from acidification, contamination of yogurt products by pathogens and food spoilage organisms may also reduce the number of viable starter culture. Molska *et al.* (2003) analyzed 153 diary samples and found *L. delbrueckii subsp. bulgaricus* counts < 10^{7}cfu/ml in 40% of yogurt products and 48% of kefir samples failing FAO/WHO requirements concerning the number of yeasts. However, the higher yeast counts observed in the sabdariffa yogurts cannot be said to indicate spoilage possibility since novel yeast cells of fermentative importance namely *Kodamae nitidulidarum, K. acthophila* and *Candida restingae* have recently been recovered from Roselle (Rosa *et al.*, 1999). Therefore, the use of yeast count and associated taste as a determinant of the shelf life of our sabdariffa yogurts is questionable and requires further studies. Meanwhile, the day 7 retention of freshness of our non-sabdariffa yogurts at 8±1^{0}C could also mean that the recorded yeast counts in these yogurts have not reached a spoilage threshold. Increase in the number of psychotropic yeast cells with storage times has also been reported in other related yogurt products, which include labneh, kefir and koumassi (Zurivarachchi and Fleet, 1981).

Syneresis, the exudation of whey proteins: α-lactabulbumin and β-lactoglobulin from yogurts is a well-established rheological factor responsible for decrease nutritional quality and organoleptic failure of yogurt products (Salvador and Fiszman, 2004; Trachoo and Mistry, 1998). In this study, a higher degree of Syneresis characterized by high syneresis index values was observed for non-zobo yogurts compared to sabdarffa-yogurts after manufacture and during the 7 -0 day study period. These values were are comparable to those reported by Salvador and Fiszman (2004), Lee and Lucey (2004) and van Vliet *et al.* (1991) for plain yogurts. Low fat yogurts are known for poor textural characteristics owing to their low total solid contents, which make them susceptible to syneresis unless they are heavily stabilized (Trachoo and Mistry, 1998). To prevent whey separation in low fat yogurts substances such as sweet buttermilk powder, gelatin, starch, whey protein concentrate and gum Arabic have been incorporated into nonfat or low fat milk to serve as thickeners and stabilizers prior to fermentation (Morris *et al.*, 1995; Trachoo and Mistry, 1998). Pre-fermentation methods including pasterurization, ultrafiltration, pressure application and homogenization have also been used to improve body texture and syneresis in yogurt products after manufacture and during storage (Savello and Dargan, 1995). In this study, our reconstituted low fat powder milk was also subjected to pasteurization prior fermentation in order to achieve denaturation of whey proteins, disruption of casein

Table 6: Relationships between sensory parameters, syneresis and acidity of the yogurt products

Sensory parameters	Syneresis index (mL/125g sample)	Acidity	
		pH	Titratable acidity (g lactic acid/100g sample)
Aroma	0.28	0.17	-0.14
Appearance	-0.15	0.12	-0.47
Texture	-0.03*	0.18	-0.09
Taste	-0.002*	0.03*	0.04*
Smell	-0.07	0.08	0.08
Thickness	-0.02*	-0.05*	0.07
Overall acceptability	-0.001*	0.06	0.06

Data represent correlation coefficient (r) values. Values obtained for each of the sensory parameters after manufacture and during storage were pooled and used as correlates of syneresis and acidity of yogurt irrespective of type. *P < 0.05

micelle and reduction in surface hydrophobicity as previously reported (Lee and Lucey, 2004). This would subsequently enable interactions between α-lactalbumin and casein and formation of whey protein complexes to form a strong and stable gel network of curds in yogurts with good texture (Mottar *et al.*, 1989; Lucey and Singh, 1997). The overwhelming effect of low solid on pasteurization, which resulted in higher syneresis in plain and strawberry yogurts, was averted by *Hibiscus sabdariffa* extract incorporation in the two sabdariffa yogurts produced culminating in their observed lower syneresis index values. Therefore, our observation provides a strong indication for the presence of stabilizing agents in *H. sabdariffa*. The stabilization factors may be contributed by the dietary fibres endowment in *Hibiscus sp* (Punna *et al.*, 2004) and non-starter lactic acid bacteria isolated from the plant extract and its associated sabdariffa yogurts. A prior study by Fernandez-Garcia *et al.* (1998) revealed the body texture improvement effect of oat fibre fortification in plain yogurts. While Frengova *et al.* (2002) reported substantial contribution of exopolysaccharides secreted by lactic acid bacteria during fermentation to the subsequent texture improvement of diary products including yogurts. Apart from *Streptococcus thermophillus*, strains of *L. acidophilus and isolates of L. plantarum* and *L. brevis*, which were found as non-starter cultures in this study have been reported to secrete exopolysacharides and found to produce good texture from curds with reduced susceptibility to syneresis during storage (Haddadin, 2005).

However, from post-acidification viewpoint, the observed lesser degree of syneresis in the zobo-yogurts during storage is at variance with the observations of Schmidt and Bouma (1992) and Richmond *et al.* (1985). Both workers found a positive correlation between acid production and degree of syneresis in cottage cheese and yogurt. There is thus a high possibility that the fibre and non-starter LAB of *H. sabdariffa* work in synergy to inhibit the syneresis reduction function that could have resulted from the higher acidity of the sabdariffa yogurts.

The acceptance of yogurts as a healthy food also involves their ability to serve as sources of minerals, which are constituted by their ash content (Closa *et al.*, 2003). The higher ash content in the sabdariffa yogurts observed in this study may also be due to the mineral contribution of *H. sabdariffa* (Punna *et al.*, 2004) and this may offer an additional nutritional advantage in terms of supply of minerals such as zinc, calcium, phosphorus and copper to consumers (Closa *et al.*, 2003).

Furthermore, the lower organoleptic scores recorded for flavor, taste, smell and overall acceptability of the zobo yogurts may also be explained by the previously reported off flavor and taste defect tendencies of excessive acidification, fibres and secreted exopolysaccharides in diary products (Fernandez-Garcia *et al.*, 1998; Frengova *et al.*, 2002).

Nevertheless, the observed organoleptic attributes of zobo yogurts, which make them less desired compared to non-zobo yogurts does not imply non-fitness for human consumption since the cut-off scores for failure vary for each of the organoleptic attributes tested. Using a 5-point hedonic scale, Randell *et al.* (1995) and Piga *et al.* (2000) used scores of 2 and 3 as markers for quality failure of yogurts. With respect to flavor, a cut-off score of 2.5 for one product and a score of 3.5 for another product were used to depict failure (Gacula, 1975).

Taking together our findings and previous observations, the need to improve on the flavor, smell and taste of the sabdariffa yogurts are critical for their overall acceptability. Elsewhere, incorporation of sweeteners such as sucrose, stabilizers and diary ingredients such as such as ultrafiltered sweet buttermilk powder and lactose hydrolyzing milk has been effected to improve the flavor and overall acceptability of yogurts (Keating and White, 1990; Trachoo and Mistry, 1998; Fernandez-Garcia *et al.*, 1998).

The international regulatory bodies for diary products have recommended a minimum of 10^6 cfu/g of viable lactic acid bacteria for effective probiotic functions in unpasteurized yogurts throughout their shelf life (Adolfsson *et al.*, 2004). Several studies have reported locally made yogurt products having either above or below 10^6 cfu/ml viable LAB counts with the former limiting their fitness for human consumption and probiotic benefits (Tamine and Robinson, 1999; Sodini *et al.*, 2004; Adolfsson *et al.*, 2004) Therefore, the

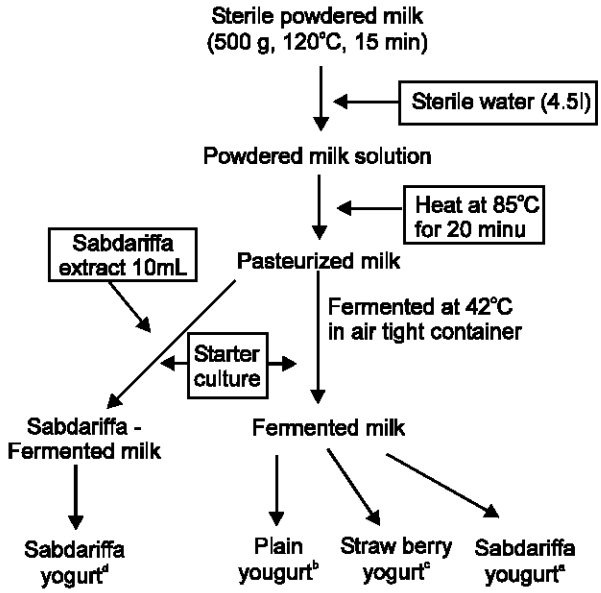

Fig. 1: Schematics of yogurt preparation

observed higher LAB count of 2 - 5 x 10^9/ml after manufacture and retention of 10^7cfu/ml viable cells on day 7 of storage at 8±1°C in the zobo-yogurts would undoubtedly offer a probiotic advantage to these yogurt brands. Nutritional advantage in terms of enhanced lactose digestion would further be conferred by the observed higher β -galctosidase activity in the zobo yogurts with values comparable to those of yogurts made with *Bifidobacterium sp* (Lamoreux *et al*., 2002). The sabdariffa yogurts are thus highly desirable for consumption by lactose maldigesters and intolerant individuals.

In conclusion, the results of this study found higher β-galactosidase activity, and viable lactic acid bacteria of probotic importance in the sabdariffa yogurts compared to the non-sabdariffa counterparts. However, the present sensory qualities of sabdariffa yogurts need improvement to further enhance their acceptability.

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