INNOVARE JOURNAL OF FOOD SCIENCE

Vol 2, Issue 1 , 2014

Original Article

PRODUCTION AND EVALUATION OF FLAVORED YOGHURT FROM GRADED LEVELS OD SOURSOP (*ANNONA MURICATA*) PULP

MBAEYI-NWAOHA*, EKERE K.S.

Department of Food Science and Technology, University of Nigeria, Nsukka. Email: miphie2003@yahoo.co.uk or ifeoma.mbaeyi-nwaoha@unn.edu.ng

Received: 28 May 2014, Revised and Accepted: 10 August 2014

ABSTRACT

Yoghurt was produced and flavoured with graded levels of soursop pulp. Soursop pulp was used to substitute 0, 10, 20, 30, 40 and 50% of yoghurt. The chemical, sensory and microbiological properties of the yoghurts were determined. The results showed that the pH of the yoghurt ranged from 4.30 to 4.60. The protein content varied from 2.68%-5.83% and the ash content ranged from 1.21% - 1.38%. The fat and moisture contents decreased and the values varied from 2.21% - 4.12% and 74.57%- 79.26% respectively. However, the carbohydrate and micro-nutrient (Ca and Vitamin C) content increased with increased level of soursop in the yoghurt. The total viable count and lactic acid bacteria count values were also inversely proportional to the concentration of soursop. The values for Total viable count (TVC) ranged from 2.0×10^5-4.5×10^5 while the values for Lactic acid bacteria (LAB) varied from1.3×10^5-3.9×10^5. There was no significant difference ($p < 0.05$) in the overall acceptability of all the products. The most acceptable flavoured yoghurt contained 60% yoghurt and 40% soursop pulp and had a general acceptability of 7.15. Soursop could be used to produce acceptable beverage.

Keywords: Flavoured yoghurt, Micro-organisms, Micro-nutrient, Soursop pulp, Sensory evaluation

INTRODUCTION

Yoghurt is a diary product manufactured by bacterial fermentation of milk. The bacteria used to make yoghurt are known as yoghurt cultures. These cultures include *Lactobacillus delbrueckii* subspecie *bulgaricus* and *Streptococcus salivarius* subspecie *thermophilus*. In addition, other Lactobacilli and Bifidobacteria are also sometimes added during or after culturing the yoghurt. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yoghurt its texture and characteristic tang (Anon, 2013a). Yoghurt is a Turkish word for milk that has been curdled with lactic starter (Fias Co. Farm, 2006). It can also be referred to as pasteurized full cream or low fat milk coagulated to custard-like consistency with a mixed lactic acid culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Potter and Hotchkiss, 2007). It is a probiotic product since it contains live active micro-organisms which upon ingestion in sufficient number exert health benefits beyond the inherent basic nutrition (Guarner and Shaafsma, 1998). This benefit has increased the consumption of yoghurt.

However milk, the major ingredient in yoghurt production, is manufactured by a number of animals, although in terms of commercial quantity, milk from cow is the most popular. Cow's milk protein which comprises mainly of casein is most commonly used to make yoghurt but milk from goat, water buffalo, ewe, mares, camels and yaks can also be used. Goat milk has been reported to be a good raw material for yoghurt processing as it compared well with cow milk in terms of nutrient composition (Ohiokpehal, 2003; Obatolu *et al.,* 2007).

In mechanized production of yoghurt, skimmed milk is mixed with whole or full cream milk and heated at 82 - 93^{0}C for 30 – 60 minutes to destroy pathogenic/spoilage micro-organisms and to destabilize Kappa-casein. It is inoculated with a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* Initially, *S. thermophilus* grows rapidly to produce diacetyl, lactic, acetic and formic acids (Fellows, 2009). *L. bulgaricus* possesses weak protease activity which releases peptides from the milk proteins. These stimulate the growth of *S. thermophilus* (Fellows, 2009). The increased acidity then slows down the growth of *S. thermophilus* and promotes *L. bulgaricus,* which is stimulated by formate produced in the initial stage. *L. bulgaricus* produces most of the lactic acid and acetaldehyde which together with diacetyl, gives the characteristic flavour and aroma in yoghurt (Fellows, 2009). Yoghurt is generically known as cultured milk as they all derive from the action of bacteria on all or part of the lactose to produce lactic acid, carbon (IV) oxide, acetic acid, diacetyl, acetaldehyde and several other compounds that give the product the characteristic fresh taste and smell (Anon, 2013b).

Types of yoghurt vary from set and stirred to frozen, drinking, concentrated, sweetened, dried and flavoured yoghurt (Anon, 2013c). Flavoured yoghurt is made by adding flavoured food stuff such as fruits or other flavoured substances to a coagulated milk product obtained by lactic acid fermentation of milk under *L. bulgaricus* and *S. thermophilus*. Flavored Yoghurt with various flavors and aroma has become very popular. The flavors are usually added at or just prior to filling into pots. Common additives are fruit or berries, usually as a puree or as whole fruit in syrup. These additives often have as much as 50% sugar in them. However with the trend towards healthy eating gaining momentum, many manufacturers offer a low sugar and low fat version of their products. Low or no sugar yoghurts are often sweetened with saccharin or more commonly aspartame. The use of "fruit sugars" in the form of concentrated apple juice is sometimes found as a way of avoiding added sugar on the ingredients declaration; this tends to be a marketing ploy and has no real added benefit (Anon, 2013c).

Yoghurt is most often flavoured with fruit preserves or other ingredients to mellow down or offset its natural sourness, the flavours are usually added at or just prior to filling into pots. Presently, only flavoured yoghurts from exotic fruits such as vanilla, strawberry, peach, raspberry, and banana are commercially available. However, there are some underutilized tropical fruits that can be used in place of these exotic ones, for instance, soursop can also be used as a flavouring agent in yoghurt.

Soursop (*Annona muricata*) is a member of the family of custard apple trees called Annonaceae and specie of the genus Annona known mostly for its edible fruits. *Annona muricata* produces fruits that are usually called soursop due to its slightly acidic taste when ripe. Soursop trees grew natively in the Caribbean and Central America but are now widely cultivated and in some areas, escaping and living on their own in tropical climates throughout the world (Anon, 2013d). The soursop fruit has a rich, creamy and fruity flavour with an underlying citrus taste. The fruit is covered by a green skin and contains a white sop and black cone-shaped seeds. The sop is soft when ripe and can be eaten fresh or made into soursop juice. The fruit pulp is a rich source of carbohydrate particularly fructose, and contains significant amounts of vitamin C, B_1, and B_2 (Rice *et al.*, 1991). Also, soursop is a juicy, acidic and highly aromatic fruit. It also has many therapeutic and nutritive properties. However, the ripe fruits are highly perishable or susceptible to spoilage as they become easily bruised, soften rapidly and become spongy and difficult to consume fresh.

To combat this spoilage and as such improve the use of the nutritive and therapeutic properties of the fruit, soursop could be used as a flavouring agent in yoghurt production. When incorporated into yoghurt, the fruit would add to the nutritional quality of the product by providing essential vitamins and minerals. It would also contribute to the protein and calorific value of the product. Addition of soursop would improve the taste of the yoghurt and the micronutrient requirements of the consumers thereby playing a considerable role in consumption and sales. It would add variety to the already existing flavoured product thereby establishing a new niche.

However, yoghurt is produced from milk which contains a reasonable quantity of fat globules referred to as milk fat. Yoghurt therefore is prone to oxidation and production of off-flavour. However, fruits are rich sources of antioxidants that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species (Shi *et al.,* 2001). The most abundant antioxidants in fruits are polyphenols and vitamins A, B, C, and E, while carotenoids are present to a lesser extent in some fruits. These polyphenols with antioxidant activities mostly belong to flavonoids (Flueriet and Macheix, 2003). Meanwhile, soursop (*Annona muricata*) is one of the tropical fruits that demonstrate antioxidant properties. This plant contains annonaceous acetogenins which display antitumor, pesticidal, antimalarial, antihelminthic, antiviral and antimicrobial effects, thus suggesting many potential useful applications.

Furthermore, most Nigerian fruits are seasonal. They are abundant during their season (wet season) and as such most of them are wasted or lost either to spoilage or pests. To avoid these post harvest losses, these fruits (for instance soursop) could be incorporated into yoghurt as a flavouring agent. Soursop, which is one of the fruits neglected by the populace, is highly nutritious and therapeutic. As such, its use as a flavourant in yoghurt which is the most widely consumed fermented dairy product would increase the number of consumers that derive from the enormous benefits of soursop. Therefore, main thrust of this study was to produce and evaluate yoghurt flavoured with graded levels of soursop pulp.

MATERIALS AND METHODS

Source of Raw Materials: Skimmed milk, starter culture Yoghurmet), sugar and stabilizer were purchased from Ogige main market, Nsukka Local Government Area of Enugu State, Nigeria. The soursop fruit was obtained from Faculty of Agriculture farm, University of Nigeria, Nsukka.

Processing of Soursop Fruit Juice: The soursop fruits were sorted to remove the bad ones after which they were washed and peeled. Fruit juice was extracted using a juice extractor and pasteurized for 85^{0}C for 3 minutes. It was then cooled. The flow chart for soursop juice production is shown in Figure 1.

Sour sop Flavoured Yoghurt Production

Soursop juice flavoured yoghurt was produced using the modified method described by Ihekoronye (1999). The raw materials (whole milk, Carboxy methyl cellulose (CMC) and sugar) were appropriately weighed and mixed with water. The mixed product was then homogenized to obtain a creamy and uniform product. Pasteurization was then carried out at 85^{0}C for 30 minutes as shown in Figure 3 to reduce the number of spoilage microorganisms in the raw materials to provide a better environment for the growth of the starter culture. The product was then cooled to a temperature of 43-46^{0}C which is the ideal growth temperature of the starter culture. The fruit pulp was added and the starter inoculated. Fermentation was then carried out for 16 hours after which the yoghurt was set. Figure 2 shows the unit operations involved in the processing of formulated soursop flavoured yoghurt samples.

CHEMICAL ANALYSIS OF SAMPLES

Determination of Moisture Content

The moisture content of the samples was determined using the hot oven method of AOAC (2010). Two millilitres (2mls) of each sample was put into a washed and dried crucible dish and placed in a Phoenix oven at a temperature of 70-80^{0}C for 2 hours and at 100-105^{0}C until the weight is constant. The samples were cooled in a desiccator and weighed. The weight loss was obtained as the moisture content and was calculated as:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

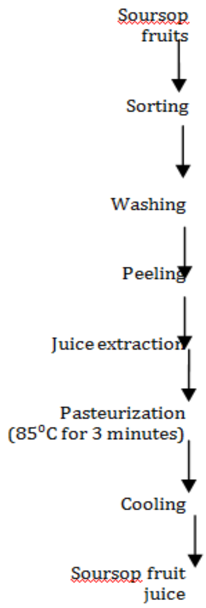

Fig. 1: Production of soursop fruit juice.

Source: Abazu (2011)

Where; W_1 = initial weight of empty crucible; W_2 = weight of crucible + sample before drying; W_3 = final weight of crucible + sample after drying

Determination of Fat Content

The Solvent extraction method as described by AOAC (2010) was used. The extraction flasks were washed with petroleum ether, dried and cooled and weighed. Two millilitres (2mls) of the sample were weighed into the extraction thimble. It was then placed back in the Soxhlet apparatus. The washed flask was filled to about three quarter of its volume with petroleum ether (that has the boiling temperature range of 40-60^{0}C). The apparatus was then set-up and extraction carried out for a period of 4-6 hours after which complete extraction was made. The petroleum ether was recovered leaving only oil in the flask at the end of the extraction. The oil in the extraction flask was dried in the oven, cooled and finally weighed. The fat content was expressed as a percentage of raw materials. The difference in weight of empty flasks and the flask with oil content which was calculated as:

$$\% \text{ Fat content} = \underline{C - B} \times 100 \quad A$$

Where; A = Weight of sample; B = Weight of empty flask; C = Weight of flask + Oil.

Determination of Crude Protein

The crude protein of the samples was determined by the semi-micro Kjeldahl technique described by AOAC (2010). A millilitre (1.0ml) of the sample was put into a Kjeldahl flask. Three grams (3g) anhydrous sodium sulphate and one (1g) of hydrated copper sulphate (catalyst) were added into the flask. Then 20ml of concentrated tetraoxosulphate (IV) acid (H_2S0_4) was added to digest the sample. The digestion continued under heat until a solution was observed. The clear solution was then cooled and made up to 100ml with distilled water and a digest of about 5ml was collected for distillation. Also, 5ml of sodium hydroxide (NaOH) was put into the distillation flask and distillation was allowed to take place for some minutes. The ammonia distilled off was absorbed by boric acid indicator and this was titrated with 0.01M hydrochloric acid (HCl). The titre value of the end point at which the colour changed from green to pink was taken. The crude protein was calculated as:

$$\% \text{ Crude protein} = \frac{0.0001401 \times T \times 100 \times 6.25}{W \times 5}$$

Where: T= titre value; W= weight of sample dried

Determination of Ash Content

The ash content of the sample was determined by the method recommended by AOAC (2010). A silica dish was heated to about 60^{0}C, cooled in a desiccator and weighed. Five milligrams (5ml) of the sample was put into the silica dish and transferred to the furnace. The temperature of the furnace was then allowed to reach about 525^{0}C after placing the dish in it. The temperature was maintained until whitish-grey colour was obtained indicating that all

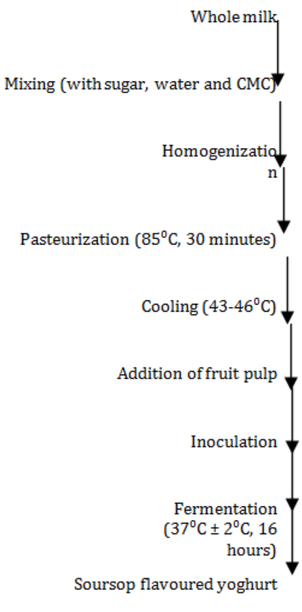

Fig. 2: Production of soursop flavoured yoghurt.

Source: Ihekoronye (1999)

the organic matter content of the sample has been destroyed. The dish was then brought out from the furnace and cooled in the desiccator and re-weighed. The percentage ash content was the calculated as:

Where: A = weight of empty dish; B = weight of empty dish + sample before ashing;

C = weight dish + ash

Determination of Carbohydrate

Carbohydrate was determined as the nitrogen free extraction calculated by difference as described by Oyenuga (1968). The formula below was used:

% Carbohydrate = 100 % - (protein + fat + fibre + ash + moisture)

Determination of Total Titrable Acidity (TTA) of the Formulated Flavoured Yoghurt

The total titrable acidity was determined by the method described by AOAC (2010). Then, 10ml of the sample was measured into a conical flask and about 3 drops of phenolphthalein indicator was added to the sample and titrated with 0.1N Sodium hydroxide (NaOH) until colour change was observed. The end point was taken and the TTA expressed as % lactic acid was given as % TTA as lactic acid =

$$\frac{m(NaOH) \times N(NaOH) \times 0.09 \times 100}{\text{Volume of sample}}$$

Determination of Total Solids of Formulated Flavoured Yoghurt

The total solid content of the formulated flavored yoghurt samples was determined by drying 5ml of the sample to constant weight in a hot air oven (Gallenkamp) at 130^{0}C. The total solid content was obtained as percentage (%) total solids (AOAC, 2010).

$$\% \text{ Total solids} = \frac{\text{Weight of dried sample}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of Milk Solid Non Fat (MSNF)

The milk solid nonfat content of the formulated flavored yoghurt samples was determined by AOAC (2010) procedure. About three milliliters of concentrated formalin was added into the already titrated total titrable acidity solution and mixed well in order to change the color back to white. It was then titrated with 0.1M NaOH. Then the titre value was recorded and MSNF was calculated as

%MSNF=5.67(X-Y)

Where: X= titre value and Y=titre value of blank

Determination of pH

The pH was determined using a pH meter. The electrode was dipped into the yoghurt solution and then the pH was determined.

DETERMINATION OF MICRONUTRIENTS

Determination of Vitamin B_1 Content

Five milligrams (5ml) of the sample was homogenized in 50ml ethanoic Sodium hydroxide. Its 10ml filterate was added to 10ml potassium dichromate and absorbance was recorded at 360nm after colour had developed (Barkat *et al.,* 1973).

Determination of Vitamin B_2 Content

Five milligrams (5ml) of the sample was extracted with 100ml ethanol for 1hour. Then 10ml of this filtered extract was added 10ml of 5% potassium permanganate and 10ml of 30% hydrogen peroxide (H_2O_2) and allowed to stand on hot water bath for 30 minuites. To this, 2ml of 40% Sodium sulphate was added. The volume was made up to 50ml and absorbance was recorded at 510nm. This was used to calculate the vitamin B_2 content (Bhandange, 2010).

Determination of Vitamin C Content

The ascorbic acid was determined using the method of (Osborne and Voogt, 1978). Two milligrams (2ml) of the sample was weighed and 100ml of distilled water was added to it. It was then filtered to get a clear solution. Also, 10ml of the clear solution was pipette into small flask in which 2.5ml acetone was added. It was then titrated with indophenols solution (2, 6-dicholorophenolindophenol) to a faint pink colour which persists for 15 seconds. The Vitamin C was calculated as:

Vitamin C (mg/ 100ml of sample) = 20 x V x C

Where: V= indophenols solution in titration (ml); C= mg Vitamin C/ml indophenols

Calcium Content Determination

It was determined by titration method according to Kirk and Sawyer (1991). Two milligrams (2ml) of the ashed sample was diluted with 3ml of distilled water and 1ml of 50% ammonium oxalate. One drop of methyl red indicator was made alkaline with ammonia drops and drops of glacial acetic acid until colour changes to pink. It was stood for 4 hours and centrifuged for 5 minutes, followed by decantation of the supernatant. About 1ml of hydrogen sulphate was added to the residue which was diluted with 4ml of distilled water. The solution was boiled and titrated with 0.02N potassium permanganate.

Determination of Phosphorus

The already prepared ash solution was boiled with 10ml HCl and the solution was washed into a flask with water. It was neutralised by drop wise addition of 0.88 ammonia (the volume of the solution at this stage was 50ml). Dilute nitric acid, and 25ml of vanadate-molybdate solution was added and the volume was made up, thereafter optical density was measured after 10 minutes that it was allowed to stand (Kirk and Sawyer, 1991).

MICROBIAL ANALYSIS

Preparation of Ringer Solution

One Ringer tablet was dissolved in distilled water (500ml). The clear solution formed was sterilized by autoclaving for 15 minutes at 121^{0}C and 15lb pressure. The Ringer solution was allowed to cool completely to a temperature of about 28±2^{0}C.

Determination of Total Viable Count

The total viable count test was carried out using Prescott *et al.* (2005). Using of sample and sterilized quarter strength ringer solution as diluents, 1 ml of the sample and 9 ml ringer solution was made serial dilutions (10^{-4}). The diluted sample was pipetted into a marked Petri dish, swirled to mix and incubated at the temperature of about 37°C for 24 hours. After incubation, the number of colonies was counted and represented as colony forming unit per milliliter.

Determination of Lactic Acid Bacteria (LAB) using deMan Rogosa Sharpe (MRS) Agar

The microbial count of lactic acid bacteria (LAB) in the formulated yoghurt was determined using deMan Rogosa Sharpe (MRS) Agar (CM361) as described by Oxoid Manual (Oxoid, 1982). Samples were serially diluted in duplicates using the surface pour plate method. The plates were incubated under anaerobic conditions at 37^{0}C for 48 hours (Harrigan and McCance, 1976).

Sensory Evaluation of the Formulated Flavored Yoghurt

Sensory properties of the samples were evaluated by 20 semi-trained panelists who are conversant with yoghurt and consisting of students of University of Nigeria, Nsukka for various sensory attributes (colour, taste, flavour, texture and overall acceptability). The extent of differences between the yoghurt samples for each sensory quality was measured on a nine- point Hedonic scale where "9" represents extremely like and "1" represents extremely dislike (Ihekoronye and Ngoddy, 1985).

Data Analysis of the Formulated Flavored Yoghurt

Data analyses were carried out using SPSS (Statistical Package for Social Science) version 17. The mean and standard deviation were calculated. The mean and standard error of mean was calculated using Analysis of Variance separated by Duncan's new multiple range test. Significance was accepted at (P< 0.05) according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Proximate Composition of Yoghurt Flavoured with Graded Levels of Soursop Pulp

Table 1 shows the proximate composition (%) of graded levels of soursop flavored yoghurt samples.

Table 1: Proximate composition (%) of soursop flavoured yoghurt samples

Sample	Moisture	Carbohydrate	Crude Protein	Fats	Ash
PY+SP (100:0)	$79.26^a \pm 0.17$	$9.41^e \pm 0.25$	$5.83^a \pm 0.14$	$4.12^a \pm 0.23$	$1.38^a \pm 0.17$
PY+SP (90:10)	$78.41^{ab} \pm 0.41$	$12.83^d \pm 0.18$	$4.60^b \pm 0.18$	$2.84^b \pm 0.33$	$1.32^a \pm 0.18$
PY+SP (80:20)	$78.26^b \pm 0.33$	$13.74^c \pm 0.23$	$4.08^c \pm 0.16$	$2.63^b \pm 0.17$	$1.29^a \pm 0.33$
PY+SP (70:30)	$77.82^b \pm 0.62$	$15.57^b \pm 0.23$	$3.12^d \pm 0.27$	$2.23^b \pm 0.30$	$1.26^a \pm 0.18$
PY+SP (60:40)	$77.80^b \pm 0.18$	$15.71^b \pm 0.31$	$3.03^d \pm 0.11$	$2.22^b \pm 0.34$	$1.24^a \pm 0.11$
PY+SP (50:50)	$74.57^c \pm 0.27$	$19.33^a \pm 0.35$	$2.68^d \pm 0.30$	$2.21^b \pm 0.18$	$1.21^a \pm 0.25$

Values are mean ± standard deviation of duplicate readings. Means on the same column with different superscripts are significantly different (p<0.05).

PY= Plain yoghurt. SP= Soursop pulp

The moisture content of the flavoured yoghurt samples ranged from 74.57% - 79.26%. (Table 1) There was no significant difference (p<0.05) in the moisture contents of samples with PY+SP ratios of 80:20, 70:30 and 60:40 respectively. However, sample PY+SP (50:50) had the lowest moisture content while the plain yoghurt PY+SP (100:0) had the highest moisture content. This showed that moisture levels decreased with increase in the concentration of the soursop pulp added. The moisture content differed significantly (p<0.05) from the range (85.00-89.01%) reported by Mbaeyi and Awaziem (2007) and 87.76% reported by Anon(2012b) probably due to the difference in formulation such as quantity of stabilizer used (30g per litre).

Table 1 showed that the crude protein content ranged from 2.68%-5.83% with plain yoghurt having the highest crude protein content. The protein content of the flavoured yoghurt decreased with increase in the level of soursop pulp added. This decrease could be attributed to the lower protein content of soursop pulp compared to milk. Morton (1987) reported that soursop has a protein content of 1% while Enweani *et al.* (2004) reported a protein content of 2.91. Animal milk on the other hand, has a high protein quality as it provides all of the amino acids the body needs to function correctly. The protein content of the flavoured yoghurts concurred with the value (4.9) reported by Dlamini *et al.* (2009) for vanilla flavoured and strawberry fruit yoghurt. Also, the protein content of the plain yoghurt agreed with the range (3.4-5.6%) reported by Janhoj *et al.* (2006).

The maximum fat content (4.12%) was seen in plain yoghurt [sample PY+SP (100:0)] and fat content was found to gradually decrease with addition of the fruit pulp (Table 1). Generally, soursop contains low level of fat therefore the addition of pulp might have decreased the fat percent of flavoured yoghurt. The differences in fat percentage between plain yoghurt and yoghurt containing fruit pulp were significant (p<0.05) while the fat content found in flavoured yoghurts were significantly different. The fat content of yogurts varies depending on the product, ranging from approximately 10% fat for full fat Greek style yogurts, 3% fat for whole milk yogurts, 1.7% fat for low fat yogurts and non-fat varieties containing less than 0.3% fat (Dairy council, 2013). Therefore, the formulated soursop flavoured yoghurt which had a fat content ranging from 2.21 to 2.84% could be referred to as whole milk yoghurt. The fat content of the samples observed in this study agreed with the range of 2.6%-3.24% for brands of commercial yoghurt reported by (Orakwue, 2007).

Table 1 showed that the ash content ranged from 1.21% in sample PY+SP (50:50) to 1.38% in sample PY+SP (100:0). This was in agreement with the ash content in beetroot flavored yoghurt (0.94%-1.49%) as reported by Mbaeyi-Nwaoha and Nwachukwu (2012). The ash content of soursop flavoured yoghurt was somewhat lower than that of plain yoghurt but differences in ash content between samples was not significant ($p<0.05$). The ash content of the samples was observed to decrease with increasing level of soursop pulp added. This could be as attributed to the decrease in the volume of milk using soursop pulp as a substitute. According to McClements(2003), ash could be the residue remaining after water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals present within a food. Milk is highly rich in minerals some of which are not found in soursop. This therefore could have instigated that the decrease in ash content with the addition of soursop pulp. However, this decrease in ash content was minimal.

Carbohydrate content ranged from 9.41% in sample PY+SP (100:0) to 19.33% in sample PY+SP (50:50) as shown in Table 1. The carbohydrate content increased with increase in the concentration of the pulp in the formulated flavored product. This corresponds with the report by Hossain *et al.* (2012a,b) and could probably be due to the fact that soursop had high carbohydrate content 14.63g in 100g (Morton, 1987). Rice *et al*. (1991) also reported the fruit pulp as a rich source of carbohydrate particularly fructose. There was no significant difference ($p<0.05$) between the carbohydrate content of sample with PY+SP ratio of 90:10 (12.83%) and the value (12.4%) reported by Anon (2012d) for strawberry flavoured yoghurt.

Physicochemical Properties of Flavoured with Graded Levels of Soursop Pulp

Table 2 shows some selected physicochemical of yoghurt flavoured with graded levels of soursop pulp.

Table 2: Physicochemical properties of soursop flavoured yoghurt samples.

Sample	pH	Titrable Acidity	Total Solids (%)	SNF (%)
PY+SP (100:0)	$4.31^{a}±0.31$	$0.83^{a}±0.17$	$20.74^{c}±0.31$	$16.63^{d}±0.20$
PY+SP (90:10)	$4.35^{a}±0.16$	$0.80^{a}±0.24$	$21.59^{b}±0.58$	$18.76^{c}±0.31$
PY+SP (80:20)	$4.40^{a}±0.17$	$0.78^{a}±0.14$	$21.74^{b}±0.17$	$19.11^{c}±0.11$
PY+SP (70:30)	$4.45^{a}±0.20$	$0.77^{a}±0.17$	$22.19^{b}±0.31$	$19.96^{b}±0.31$
PY+SP (60:40)	$4.51^{a}±0.25$	$0.75^{a}±0.21$	$22.19^{b}±0.24$	$19.97^{b}±0.31$
PY+SP (50:50)	$4.60^{a}±0.11$	$0.69^{a}±0.30$	$23.22^{a}±0.30$	$23.22^{a}±0.28$

Values are mean ± standard deviation of duplicate readings. Means on the same column with different superscripts are significantly different (P<0.05). PY= Plain yoghurt, SP= Soursop pulp; SNF=Solids nonfat;

Table 2 showed that the pH of the flavoured yoghurt samples ranged from 4.31 in sample PY+SP (100:0) to 4.60 in sample PY+SP (50:50). This pH range is suitable for yoghurt marketed in the tropics because of the expected effect of poor storage conditions such as high temperature and epileptic power outage. The pH range was similar to the range of 3.19-4.2 reported by Dlamini *et al.* (2009). The flavoured yoghurt samples had slightly higher pH values than plain yoghurt but this difference was not significant ($p<0.05$). The pH was observed to be directly proportional to the concentration of soursop pulp and inversely proportional to the titrable acidity. Addition of soursop pulp slightly lowered the acidity of the yoghurts and this was however in contrast with the report given by Dlamini *et al.* (2009) whose results indicated that the use of indigenous fruits as flavours resulted in a slight increase in the acidity of yoghurts. The decrease in acidity could probably be attributed to fact that the acidity of fruits decrease as they ripen. Also, the citric acid contained in the fruit might have been oxidized due to exposure to atmosphere and heat. Increases in acidity might also be perceived as a negative attribute in yoghurt processing if the acidity results in very low pH values, (below pH 3.5) as reported by Salvador *et al.* (2004). Results presented in Table 2 have shown that the pH values obtained in this study were in accordance with FDA specifications (of 4.6 or lower) for the pH of yoghurt (FDA, 2009).

Total solids increased with increase in concentration of the soursop pulp in yoghurt. Sample PY+SP (100:0) had the lowest total solids content (20.74%) while sample PY+SP (50:50) had the highest total solids content (23.22%). Dublin-Green and Ibe (2005) reported values for fruit yoghurts ranging from 15.0-22.8%. Similar results (20.74-23.22%) were obtained from this study. Expectedly the SNF content increased with increase in soursop content of the yoghurt. According to Food Standards, yoghurt should not contain less than 8.25% SNF (USDA, 2001) and (FDA 2009). The result of this study therefore shows that the SNF content of the formulated product was within the stipulated standard.

MICRO-NUTRIENT COMPOSITION OF SOURSOP FLAVOURED WITH GRADED LEVELS OF SOURSOP PULP.

The selected vitamins and minerals composition of the flavored yoghurt samples are shown in Table 3.

Table 3: Selected vitamins and mineral composition of soursop flavored yoghurt.

Sample	Vitamin B_1 (mg/100g)	Vitamin B_2 (mg/100g)	Vitamin C (mg/100g)	Phosphorus (mg/100g)	Calcium (mg/100g)
(PY+SP) 100:0	$0.57^{a} ± 0.18$	$0.84^{a}± 0.13$	$6.72^{c}±1.05$	$5.67^{a}±0.18$	$8.02^{a}±0.48$
(PY+SP) 90:10	$0.56^{a} ± 0.07$	$0.83^{a}±0.17$	$6.86^{c}±0.64$	$3.33^{b}±0.24$	$7.99^{a}±0.01$
(PY+SP) 80:20	$0.47^{a} ± 0.07$	$0.82^{a}±0.18$	$7.43^{bc}±0.59$	$3.32^{b}±0.15$	$7.98^{a}±0.04$
(PY+SP) 70:30	$0.45^{a} ± 0.03$	$0.81^{a}±0.20$	$8.82^{ab}±0.66$	$3.00^{b}±0.31$	$6.80^{b}±0.44$
(PY+SP) 60:40	$0.43^{a} ± 0.25$	$0.75^{a}±0.17$	$9.30^{a}±0.42$	$2.73^{bc}±0.31$	$6.40^{b}±0.03$
(PY+SP) 50:50	$0.42^{a} ± 0.17$	$0.75^{a}±0.10$	$9.31^{a}±0.11$	$2.29^{c}±0.33$	$5.20^{c}±0.31$

Values are mean ± standard deviation of duplicate readings. Means on the same column with different superscripts are significantly different (P<0.05).

PY= Plain yoghurt; SP= Soursop pulp;

Table 3 showed that thiamine content ranged from 0.42-0.57 mg/100g and plain yoghurt sample had the highest thiamine content while sample PY+SP (50:50) had the lowest. However, there was no significant ($p<0.05$) difference in the thiamine content of the samples. The thiamine content reduced with the addition of soursop. This could be attributed to losses during heat treatment. Thiamine is unstable to heat as such, losses could have occurred during sterilization of the soursop pulp and pasteurization of the milk.

The riboflavin (Vitamin B_2) content of the formulated products was found to be inversely proportional to the quantity of soursop added. The decrease in riboflavin content (0.84 mg/100g in plain yoghurt to 0.75 mg/100g in sample flavored with 50% soursop) could probably be as a result of the substitution of milk with soursop which was lower than milk in riboflavin content. Morton (1987) stated that soursop has a riboflavin content of 0.05mg/100g while according to Anon (2012c), plain yoghurt has a riboflavin content of 0.12mg/100g. However, there was no significant difference in the thiamine and riboflavin content of all the samples respectively.

The ascorbic acid content (Table 3) had a range between 6.72mg/100g in plain yoghurt to 9.31mg/100g in the sample flavored with 50% soursop. The ascorbic acid content increased with the addition of soursop. This could probably be due to the high ascorbic acid content of soursop. Soursop is a kind of fruit rich in antioxidants with vitamin C which helps destroy free molecular radicals (Anon, 2011). According to Morton (1987), soursop has an ascorbic acid content of 29.6mg/100g. Soursop is rich in vitamin C and help bladder weakness in children, especially if the heart of the fruit is eaten (Anon, 2012d). This implies that by incorporating soursop into yoghurt which is more widely consumed, the benefit would be made easily accessible.

Phosphorus (P) content is ranged from 2.29mg/100g in the sample containing with 50% soursop to 5.67 mg/100g in sample plain yoghurt (Table 3). Phosphorus content decreased with increase in concentration of the soursop content of the yoghurt. Plain yoghurt had the highest phosphorus content (5.67mg/100g). There was no significant difference ($p< 0.05$) in phosphorus content of the flavoured samples.

Table 3 showed that the calcium (Ca) content of the samples ranged between 5.20mg/100g to 8.02mg/100mg. Plain yoghurt had the highest calcium content. There was no significant difference ($p< 0.05$) in the calcium content of the plain yoghurt sample and the samples containing 10% and 20% soursop. The lower level of calcium in the samples containing soursop pulp could be attributed to the substitution of milk with soursop which has lower calcium content. According to Morton (1987), soursop has a calcium content of 10.3mg/100g. Plain Yoghurt however contains 120.8mg of calcium per 100g (Anon, 2012c).

Microbial Count (cfu/ml) of Yoghurt Flavoured with Graded Levels of Soursop Pulp

Table 4 shows the microbial population of the formulated products.

Table 4: Lactic acid bacteria (LAB) and total viable count (TVC).

SAMPLE(ml)	TVC (cfu/ml)	LAB (cfu/ml)
PY+SP (100:0)	4.3×10^5	3.9×10^5
PY+SP (90:10)	3.5×10^5	2.8×10^5
PY+SP (80:20)	3.0×10^5	2.1×10^5
PY+SP (70:30)	4.5×10^5	3.2×10^4
PY+SP (60:40)	2.1×10^5	1.5×10^5
PY+SP (50:50)	2.0×10^5	1.3×10^5

PY= Plain yoghurt; SP= Soursop pulp; LAB= Lactic acid bacteria TVC= Total viable count

As shown in Table 4 the samples showed a total viable count of between 2.0×10^5 in sample PY+SP (50:50) to 4.5×10^5 cfu/ml in plain yoghurt [sample PY+SP (100:0)]. The total viable count was observed to decrease with increase in soursop pulp with the exception of the sample PY+SP (70:30) which showed an increase in TVC. This increase could be as a result of proliferation since the samples are rich in nutrients.

The samples showed a lactic acid bacteria count of between 1.3×10^5-3.9×10^5cfu/ml. With the exception of the sample containing 30% soursop, the LAB count was also observed to decrease with increase in soursop concentration. This could be as a result of the decrease in milk content which contains the lactose which acts as a substrate for the growth and multiplication of the lactic acid bacteria (LAB). This decrease could also be responsible for the increase in pH observed.

Scientific studies have shown that soursop contains a class of compounds known as bioactive acetogenins of Annonaceae, which has antimicrobial properties (Varien-Moos, 2013). These anti-microbial properties may have affected the microorganisms thereby leading to reduction in their level with increased addition of soursop.

Sensory Scores of Yoghurt Flavoured with Graded Levels of Soursop Pulp

Table 5 shows the sensory scores of the yoghurt flavoured with graded levels of soursop.

Table 5: Sensory properties of yoghurt flavoured with graded levels of soursop pulp.

Sample	Colour	Flavour	Taste	Aftertaste	Consistency	Mouthfeel	Overall Acceptability
PY+SP (100:0)	$8.35^a \pm 0.93$	$7.45^a \pm 1.61$	$6.95^a \pm 1.47$	$7.05^a \pm 1.32$	$7.85^a \pm 1.53$	$7.55^a \pm 1.50$	$8.05^a \pm 1.05$
PY+SP (90:10)	$7.80^{ab} \pm 1.00$	$5.95^{bc} \pm 1.90$	$5.60^{ab} \pm 2.26$	$5.30^{bc} \pm 2.15$	$7.05^{ab} \pm 1.61$	$7.10^a \pm 1.62$	$6.25^b \pm 1.97$
PY+SP (80:20)	$7.10^{bc} \pm 1.41$	$5.75^{bc} \pm 1.97$	$5.50^b \pm 1.96$	$4.95^c \pm 2.26$	$6.75^{ab} \pm 1.86$	$6.45^a \pm 1.93$	$6.05^b \pm 2.01$
PY+SP (70:30)	$6.75^c \pm 1.62$	$5.35^c \pm 2.18$	$6.20^{ab} \pm 2.07$	$6.10^{abc} \pm 2.05$	$6.55^b \pm 1.67$	$6.70^a \pm 1.98$	$6.75^b \pm 1.65$
PY+SP (60:40)	$7.50^{abc} \pm 1.19$	$6.35^{abc} \pm 2.06$	$6.40^{ab} \pm 2.01$	$6.35^{ab} \pm 1.95$	$6.50^b \pm 1.61$	$7.10^a \pm 1.45$	$7.15^{ab} \pm 1.53$
PY+SP (50;50)	$7.10^{bc} \pm 1.74$	$6.75^{ab} \pm 1.74$	$6.55^{ab} \pm 2.09$	$6.40^{ab} \pm 2.09$	$6.60^b \pm 1.90$	$6.95^a \pm 1.85$	$6.95^{ab} \pm 2.04$

Values are mean ± standard deviation. Means on the same column with different superscripts are significantly different P<0.05.

PY= Plain yoghurt; SP= Soursop pulp

The mean scores for quality attributes of the flavoured yoghurt samples are shown in Table 5. The mean scores of colour ranged from 6.75 in the sample containing with 30% soursop to 8.35 in sample plain yoghurt. Plain yoghurt had the highest score for colour but there was no significant difference ($p<0.05$) in the colour of the plain yoghurt sample and the samples containing 10% and 40% soursop respectively. The range values observed for colour was similar to 7.19 and 7.76 reported for soursop drink without sugar syrup and soursop drink with sugar syrup respectively (Onyechi *et al.,* 2012). The value for mouthfeel ranged from 6.95-7.55 and this was not significantly different ($p<0.05$) from the values (5.05-7.80) reported for beetroot flavoured yoghurt (Mbaeyi-Nwaoha and Nwachukwu, 2012).

The values for consistency ranged from 6.50 in sample PY+SP (60:40) to 7.85 in sample plain yoghurt. Plain yoghurt had the highest score (7.85) for consistency. The preference for the consistency of the products reduced with increasing level of soursop addition. This is in agreement with the report that apart from the processing method, the highly influencing attributes for texture/consistency are fat content and addition of milk powder or milk proteins (Stolz *et al.,* 2011).

The results show that there was no significant difference ($p<0.05$) in mouthfeel and general acceptability. Although plain yoghurt had the highest mean score for taste (6.95) from the panelists, the flavored yoghurt samples competed favourably with it presenting sample PY+SP (50:50) and PY+SP (60:40) as good substitutes for commercial production.

The mean scores for overall acceptability ranged from 6.05in sample PY+SP (80:20) to 8.05 in plain yoghurt. High mean values (>6.9) were obtained for the plain yoghurt for all sensory attributes (color, flavor, taste, aftertaste, consistency and mouth feel) therefore making it the most preferred sample with an overall acceptability of 8.0. Sample PY+SP (60:40) had the highest mean for general acceptability (7.15) compared to the other flavored yoghurt samples and was second in overall preference. Low means were observed in the sample containing 20% soursop for aftertaste (4.95) and general acceptability (6.05). Generally, the mean values for overall acceptability of all soursop flavored yoghurts were more than 'slightly like' (6.0). The results of the sensory evaluation implied that yoghurt flavored with up to 40% soursop pulp could be produced without having a negative impact on the consumer acceptability of the product.

These quality findings may be useful for yoghurt industries to produce new variety of yoghurts. Plain yoghurt was more highly rated than soursop flavored yoghurt in all the sensory attributes. This could be attributed to the fact that the panelists were more accustomed to plain yoghurt rather than the soursop flavored yoghurts that had never been used in yoghurt production before.

CONCLUSION

In summary, the addition of soursop pulp to yoghurt had a positive impact on the proximate, micronutrient and sensory properties of the formulated product. From the results obtained in this study, it can be concluded that the yoghurt combined with soursop at a ratio of 60:40 was the most preferred among the flavored yoghurt samples formulated. It had a general acceptability of 7.15. The crude protein content of the most preferred sample was 3.03% while its ash content was 1.24. It also had a fat content of 2.22 and a total solids content of 22.19. Increased levels of soursop led to increase in the ascorbic acid and a decrease in the titrable acidity.

Based on the findings, it is recommended that soursop pulp should replace the use of essence in the production of flavored yoghurt to improve the nutrient value of the product. Consumers should be enlightened on the nutritional and health benefits of soursop. A similar study targeting panelists that are more conversant with soursop fruit could be done to confirm the findings from this study. Further studies should be done on the shelf stability of soursop flavored yoghurt.

REFERENCES

1. Abazu, J. C. (2011). Production and Evaluation of probiotic Soymilk Flavoured with Soursop (*Annona muricata*) Juice. A B.Sc. Thesis. Department Food Science and Technology, University of Nigeria, Nsukka.
2. Anon. (2011) YoBi Sterilized drinking yoghurt: Soursop. www.bidrico.com. Accessed August 3, 2013.
3. Anon. (2012a). Yoghurt Varieties www.aboutyoghurt.com. Accessed 20th March, 2013.
4. Anon. (2012b). 10 reasons yoghurt is a top health food. www.drsearswellnessinstitute.org. Retrieved 20th March, 2013.
5. Anon. (2012c). Dairy Food and Receipes; Nutritional Information. www.dairyaustralia.com. Retrieved 20th March, 2013.
6. Anon. (2012d). *Annona muricata* L. (Soursop): Health Benefits and Uses www.healthypix.com Accessed 4th April, 2013.
7. Anon, (2013b) Health and Fitness. www.lifestyle.iloveindia .com. Retrieved 20th March, 2013.
8. Anon. (2013c). Yoghurt. www.Askdrsears.com. Retrieved 4th January, 2013
9. Anon. (2013d). Soursop en.wekipedia.org/wiki/soursop. Accessed 4th January, 2013
10. AOAC Official methods of Analysis of Association of Official Analytical Chemists International 18th ed., Published by AOAC International, Gaithersburg, Maryland, USA, 2010.
11. Ashurst, P.R. Fruit Juices. In: Food Flavouring. Ashurst, P. R. (Ed). Blackie Academic and Sons Ltd, London. pp 87-144, 1994.
34. Ohiokpehai, O. Processed food products and nutrient composition of goat milk. Pak J Nutr 2003; 2(2): 68 – 71.
12. Barkat, N.Z., Shehab, S.K., Darwish, N. and Zahermy, E. I. Determination of Ascorbic Acid from Plants. J Analyt Biochem 1973; 53:225-245.
13. Bhandange, D.G. (2010). Phytochemical and Antimicrobial Studies of some Ethnomedicinal Plants from Akola and Washin Districts. A thesis submitted to SGB, Amravati University, and Amravati.
14. Dairy council, (2013), Nutrients in yoghurt. www.milk.co.uk. Retrieved on 5th April, 2013.
15. Dlamini, A.M., Mamba, R. and Silaula, S.M. Attributes and Consumer Acceptance of Yoghurt Flavoured with Non–Cultivated Indigenous Swazi fruits. Afri J Food Agric Nutr Dev 2009; 9 (1): 636-651.
16. Dublin-Green, M. and Ibe, S.N. Quality evaluation of yoghurts produced commercially in Lagos, Nigeria. Afric J Appl Zool Environ Biol 2005; 7:78-82.
17. Enweani, I. B., Obroku, J., Enahoro, T. and Omoifo, C. The biochemical analysis of soursop (*Annona muricata* L.) and sweetsop (*A. squamosa* L.) and their potential use as oral rehydration therapy. Food Agric Environ 2004; 2(1): 39-43. Also cited online: www.world-food.net.
18. FDA Milk and cream products and Yoghurt Products. Food and Drug Administration Federal Register 74: 24-48, 2009.
19. Fellows, P.J. Food Processing Technology: Principles and Practice 3rd Ed., CRC Press, Florida, U.S.A. pp 173-175, 2009.
20. Fias Co. Farm. Home Dairy and Cheese Makings: Yoghurt recipe. Http://fiascofarm.com/dairy/yoghurt.htm). Probiotics. Intl J Food Microbiol 2006; 39:237-238.
21. Fleuriet, A. and Macheix, J.J. Phenolic acids in Fruits and Vegetables. In: C.A. Rice-Evans and L. Packer (Eds.). Flavonoids in Health and Disease. Marcel Dekker Inc, 2003.
22. Harrigan, W.E. and McCance, M.E. Basic Methods. In: Laboratory Methods in Food and Dairy Microbiology. Academic Press, London, pp 3-16, 1976.
23. Hossain, M.N., Fakruddin M. and Islam M.N. Development of Fruit Dahi (Yoghurt) Fortified with Strawberry, Orange and Grapes Juice. Amer J Food Technol 2012a; 7:562-570.
24. Hossain, M.N, Fakruddin, M and Islam, M. [illegible])12b). Quality Comparison and Acceptability of Yoghurt [illegible] Different Fruit Juices. J Food Proc Technol 2012b; 3:3-9.
25. Ihekoronye, A.I. Manual of Small-Scale Food Processing. Macmillan Publishers Ltd, London, pp 108-212, 1999.
26. Ihekoronye, A.I. and Ngoddy P.O. Integrated Food Science and Technology for the Tropics. Macmillan Publishers Ltd, London, pp 368-369, 1985.
27. Janhoj, E., Charlotte B. and Micheal B. (2006). Sensory and Rheological Characterization of Low Fat Stirred Yoghurt. J Tex Stud 37:276-299.
28. Kirk, R.S. and Sawyer, R. Pearsons' Composition and Analysis of Foods, 9th ed., Longman Group Ltd, London, pp 126-130, 1991.
29. Mbaeyi, I.E. and Awaziem, N.E. (2007). Effects of Fermentation on the Physio-Chemical Sensory Qualities and Storage Stability of Locally Produced Yoghurt in Enugu Metropolis. Proceeding of the 31st Annual Conference/AGM of the Nigerian Institute of Food Science and Technology (NIFST), National Universities Commission, Abuja, October 22nd -25th, 2007.
30. Mbaeyi-Nwaoha, I.E. and Nwachukwu G.O. (2012). Production and Evaluaton of Yoghurt Flavoured with Beetroot (*Beta vulgaris* L.). J Food Sci Engr 2012; 2:583-592.
31. McClements, D. J. (2003) Analysis of food products (Food Science 581). www.people.umass.edu/581Ashandminerals
32. Morton, J. (1987). Soursop. In: Fruits of Warm Climates. Available at: http://www.hort.purdue.edu/newcrop/morton/index.html. Accessed 4th April, 2013.
33. Obatolu, V. A., Adebowale, E. A., Omidokun, F. A. and Farinde, E. O. Comparative evaluation of yoghurt samples from goats and cowmilk and commercial retail outlet. Nig J Anim Prod 2007; 34(1): 163 – 171.
35. Orakwue, R.C. (2007). Quality characteristics of some brands of probiotic yoghurt sold in Nsukka. A B. Sc. Thesis. Department Food Science and Technology, University of Nigeria, Nsukka.

36. Osborne, D.R. and Voogt, P. The analysis of Nutrients in Foods. Academic Press, New York, 6th ed. p 251, 1978.
37. Onyechi,U. A., Ibeanu, V. N., Eme, P. E., Madubike K. Nutrient, Phytochemical Composition and Sensory Evaluation of Soursop (*Annona muricata*) Pulp and Drink in South Eastern Nigeria. Intl J Basic Appl Sci IJBAS-IJENS 2012; 12 (06): 1240-8787.
38. Oyenuga, V.A. Nigeria's Foods and Feeding Stuff, their Chemistry and Nutritive Value. University Press, Ibadan, Nigeria, pp 9-16, 1968.
39. Oxoid Oxoid Manual: Culture Media, Ingredients and Other Laboratory Services, 5th ed. Oxoid Limited, Basingstoke. pp 73-91, 1982.
40. Potter, N.N and Hotchkiss, J.H. Food Science, CBS Publishers and Distributors Pvt. Ltd., New Delhi, India, pp 76-84, 2007.
41. Prescott, L.M., Harley, J.P. and Klein, O.A. Microbial nutrition, types of media, in: Microbiology, 6th Ed., McGraw Hill Publishers, New York, pp. 95-105, 2005.
42. Rice, R.P., Rice, L.W. and Tindal, H.D. Fruit and Vegetable Production in Warm Climates. Macmillan Education Ltd, p 363, 1991.
43. Salvador, A. and Fiszman, S.M. Textural and sensory characteristics of whole and skimmed flavoured set-type yoghurt during long storage. J Dairy Sci 2004; 87: 4033 – 4041.
44. Shi, H. L., Noguchi, N. and Niki, E. Introducing natural antioxidants. In: J. Pokorny *et al*. Antioxidants in food: Practical applications. Woodhead Publishing Ltd, New Delhi, India, pp 134-152, 2001.
45. Steel, R.G.D. and Torrie, J.H. (1980), Principles and Procedures of Statistics, 2nd ed, McGraw-Hill publishers, New York. pp 176-183.
46. Stolz, H., Espig F., Kretzschmar, U. (2011). Fact Sheets on all the tasted products in the appropriate language. Research institute of Organic Agriculture (FiBL), Frick, Switzerland.www.fibl.org.
47. USDA Specifications for Yoghurt, Nonfat Yoghurt and Low fat Yoghurt. Document 21CFR, Part 131: 200-203, 2001.
48. Varien-Moos, (2013). Leaves and Soursop Pulp. www.cancer.vg. Retrieved 18th February, 2013.