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EFFECT OF STORAGE TEMPERATURE ON SHELF-LIFE OF YOGURT-LIKE PRODUCT FROM SOYBEAN (*Glycine max*)

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ABSTRACT

Soy yogurt was produced from soy milk using commercially freeze-dried samples of Streptococcus thermophilus and Lactobacillus bulgaricus at a ratio of 1:1 as starter culture. Evaluation of nutritional quality of the soy yogurt showed that the fat content (3.27%), moisture (89.42%) and ash content (0.96%) were higher than those of a popular brand of dairy yogurt (1.78%, 82.66% and 0.93%) respectively. However, the protein content (3.20%) and carbohydrate content (3.15%) were lower than values of (3.51%) and (11.12%) respectively for dairy yogurt. Soy yogurt had significantly lower acceptability ($P \leq 0.05$) than the dairy yogurt. Shelf life study was carried out at room temperature (27±3^oC) and refrigeration temperature (7±2^oC). A product shelf life of four days at 27±3^oC and ten days at 7±2^oC was obtained without additives. There was decrease in pH from 3.8 to 3.3 and increase in titratable acidity from 1.21% to 1.93% during storage. Total aerobic bacterial and yeast counts reached 6.3× 10^3 cfu/ml and 4.5× 10^5 cfu/ml at 27±3^oC respectively compared to 3.4 × 10^2 cfu/ml and 1.1× 10^3 cfu/ml at 7±2^oC. The counts for the starter cultures at 10^7 cfu/ml dropped two log cycles at 27±3^oC and a log cycle at 7±2^oC. The spoilage yeasts in soy yogurt were mainly Saccharomyces sp. and Candida sp. Soy yogurt spoilage was evident from the curdling of the yogurt, increased moisture and yeasty odour.

Key words: Soy yogurt, soy milk, shelf-life, spoilage, temperature.

INTRODUCTION

Soybean is a species of legumes native to East Asia but is now grown in northern parts of Nigeria namely Benue, Bauchi, Plateau, Borno and Kaduna States (Onuorah *et al*., 2007). It is an oilseed rather than a pulse and is a source of complete protein since it contains sufficient amount of essential amino acids that are needed by the body but the body cannot produce them (Henkel, 2000). The oil is used for vegetable oil production and the brands marketed in Nigeria include Grand, Sunola and Lahda. There are many varieties of soybean. The shape and size of seeds vary from small round pea to large elongated beans and the colour also varies from cream, yellow, brown, green to black (Onuorah *et al*., 2007). Soybean is consumed in a ground and fried form 'soya cake' in the northern parts of Nigeria.

Soy milk contains about the same proportion of protein as cow milk around 3.5%, 2% fat, 2.9% carbohydrate and 0.5% ash. Staple cereal foods such as fermented maize called 'ogi' are fortified with soy milk powder and marketed as 'soy-ogi' (Akinrele and Muller, 1971). In addition, soymilk and its fermented products like soy yogurt constitute an alternative protein source for lactose intolerant people (Cavallini and Rossi, 2009; McGee, 2004). The beany flavour of soy milk can

be eliminated by heating in a solution of sodium bicarbonate at 80^{o}C before washing and dehulling (Nwanekesi, 1984).

Yogurt is considered as food containing probiotics such as lactic acid bacteria and is good for people with stomach upset. It is usually produced using milk. The type of milk used determines the type of yogurt obtained: whole milk for full-fat yogurt, low fat-milk for low fat yogurt and skim milk for non fat yogurt (Trachoo, 2002). Large dietary intake of yogurt was found to lower cholesteremia because yogurt contains a factor that inhibits the synthesis of cholesterol from acetate (Jay, 2005). In addition to the high quality protein in soy yogurt, it contains a considerable amount of isoflavone compounds which have been known as a natural substance to replenish the female hormone oestrogen in order to relieve the menopausal symptoms (Hughes, 2003 and Setchell, 1998). Health benefits of soy yogurt include its dietetic properties of having lower contents of saturated fats, cholesterol and no lactose. It can also reduce the risk of cardiovascular diseases (Sarker, 2006).

Shelf-life studies on dairy yogurt have been carried out extensively (Suriyaracchi and Fleet, 1981, Green and Ibe, 1987 and Moreira *et al*., 2001). Yeasts were the main spoilage flora. Yogurts marketed in Lagos, Nigeria had shelf life of six days at 10^{o}C and 2 days at 30^{o}C and the spoilage yeast genera found included *Kluveromyces, Candida* and *Saccharomyces* (Green and Ibe, 1987). Amakoroma *et al*., (2012) studied the shelf-life of non dairy yogurt-like product from another legume, African yam bean and found a shelf life of four days at 29±3^{o}C and twenty one days at 4^{o}C. Information on shelf life of soy yogurt is sparse. Therefore, the objective of this study was to determine the shelf life of soy yogurt stored at room and refrigeration temperatures and evaluate the nutritional, sensory and microbiological qualities of fresh and spoilt soy yogurt.

MATERIALS AND METHODS

Source of Raw Materials

The starter cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from commercially freeze-dried yogurt cultures prepared in the ratio 1:1 bought at Aba, Abia State, Nigeria. The freeze dried starter culture was reconstituted by suspending 5g in 45ml of soy milk prepared as shown below. Purity of the starter cultures was ascertained by serial diluting of the inoculums in 1% (w/v) peptone water and spread-plating of 0.1ml of dilution on de Mann Rogosa and Sharpe (M.R.S) agar, then gram staining, biochemical test such as oxidase test, catalase test, sugar utilization and fermentation tests were carried out.

Cream coloured soy beans used were purchased from Choba market, Port Harcourt and were transported to the laboratory where they were processed.

The dairy yogurt used was purchased from Everyday Supermarket, Port Harcourt.

Preparation of Soymilk and Soy Yogurt

Soy milk was prepared as was described by Shurtleff and Aoyagi (2000) with modification (Fig.1). Two hundred and fifty grams of beans were weighed, sorted, cleaned and washed, then soaked in hot water overnight. The ratio of beans to water was 1:4. The bean seeds were dehulled and wet milled with Phillips blender for 3-5 minutes. Additional 1.5 litres of boiled tap water was added to the resulting thick slurry and the diluted slurry was filtered using a double-layered cheese cloth into a sterile 2000ml flask. Then 5% (w/v) sucrose was added before the one litre of soy milk was heated at 95^{o}C for 10 minutes, in a water bath in order to improve its nutritional value

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and flavour by heat inactivation of the beany flavour and breaking down of complex sugars which cannot be digested by humans. The heat pasteurization reduced the level of contaminants and toxic compounds such as trypsin inhibitor (a substance that inhibits proteolytic activity of certain enzymes), flavonoids, alkaloids, non protein amino acids and decreased the oxidation-reduction potential of the medium making it suitable for the growth of starter cultures. An aliquot of 5ml of the soy milk was removed to determine the following indices: total bacterial count, total coliform count, pH and titratable acidity. The soy milk was cooled to inoculation temperature of 42-43^{o}C in a water bath.

The reconstituted starter cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (50mls) were added to one litre of soy milk. The soy milk was held at that temperature (42-43^{o}C) for 6 hours for fermentation to occur. Thirty millilitre of the soy yogurt was dispensed into new sterile 50ml plastic bottles and held at 27±3^{o}C and 7±2^{o}C.

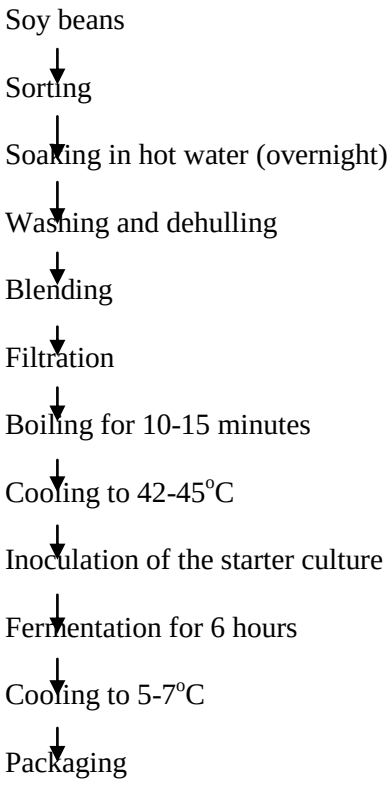

Fig.1: **Flow Chart of Production of Soy yogurt**

Proximate Analysis of Soy Yogurt

Proximate analysis of samples of dairy yogurt, soy yogurt after preparation, soy yogurt after storage at 27±3^{o}C and 7±2^{o}C was carried out. The carbohydrate content of soy yogurt was determined using Cleg Anthrone method, an air-oven method as was described by Osborne and Voogt (1978) was used for moisture content while Kjeldahl method(AOAC, 1980) was used for protein determination and fat and ash contents were determined according to AOAC (1980).

Determination of Titratable Acidity and pH.

Titratable acidity of the soy yogurt during fermentation at hourly interval and during storage was determined according to AOAC (1980) guidelines while the pH was measured using a digital pH meter (Hana Instrument).

Sensory Evaluation of Soy Yogurt

A nine-point hedonic scale was used to evaluate the coded samples of fresh dairy yogurt as control and soy yogurt organoleptically for general acceptability, aroma, taste, texture and overall appearance with the highest point (nine) for 'like extremely' and the lowest (one) for 'dislike extremely'. The test was conducted by ten- member panelists and evaluated statistically. The panellists were graduate students of University of Port Harcourt.

Shelf Life Studies on Soy Yogurt

Shelf-life study of the soy yogurt was conducted to ascertain its keeping time. The samples of soy yogurt in bottles were stored at room temperature ($27\pm3^{o}C$) for five days and refrigeration temperature ($7\pm2^{o}C$) for ten days. The dairy yogurt was stored at $27\pm3^{o}C$ for 21 days. The dairy yogurt and soy yogurt samples stored at room and refrigeration temperatures were sampled every 24 hour throughout the storage period. Physico-chemical studies carried out include pH, titratable acidity and viscosity by visual examination along with microbiological evaluation every 24 hours. Samples were evaluated for spoilage characteristics which included gas production, curdling and production of off odour and off colour.

Enumeration of Bacteria and Fungi

Total bacterial and fungal counts were enumerated using spread plate method technique. Ten millilitres of the sample were diluted with 90ml sterile 0.1% (w/v) peptone water giving 1:10 dilution and further tenfold serial dilution was done up to 4^{th} dilutions. From appropriate dilutions, 0.1ml was spread-plated on duplicate plates of nutrient agar, de Mann, Rogosa and Sharpe (MRS) agar and potato dextrose agar (PDA). Plates were incubated at $37^{o}C$ for 48 hours except for PDA plates held at room temperature. The MRS plates were incubated in an anaerobic jar. Colonies with the same cultural characteristics were counted together and average count of the duplicate plates was calculated as colony forming units (cfu) per millilitre. Counts on nutrient agar represented total bacterial count, on de Mann Rogosa and Sharpe (MRS) agar for the starter cultures and PDA for yeasts counts.

Total coliform count was enumerated using five tube most probable number (MPN) technique. From the 1:10 dilution of sample, 10ml, 1ml and 0.1ml aliquots were inoculated into 10mls of MacConkey broth containing inverted Durham tubes, incubated at $37^{o}C$ for 24-48 hours. Double strength MacConkey broth was used for the 10ml inoculum. Presence of gas in the Durham tubes and colour change from purple to yellow indicated positive presumptive result. The presumptive test was negative.

Identification of Isolates

Subcultures were made to obtain pure cultures. Gram's staining, spore staining, capsule staining, motility test, and biochemical tests such as catalase test, oxidase test, starch hydrolysis test, were conducted for the identification and characterization of the isolates. *Bacillus* sp. was identified by stabbing gelatin medium. (Cheesebrough, 2006 and Buchanan and Gibbons, 1974). Yeasts were identified using microscopy, growth morphology (cultural characteristics) and sugar tests (Green and Ibe, 1987).

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Statistical Analysis

Means and standard deviations were calculated to analyze sample attributes, proximate analysis and sensory attributes. Analysis of variance (ANOVA) was used to determine significant difference between means at $P \leq 0.05$ (Steel and Torries, 1980).

RESULTS

Proximate Composition of Soy Yogurt

Table 1 shows values for the nutritional composition of laboratory made soy yogurt and a popular brand of dairy yogurt marketed in Port Harcourt, Nigeria. Fat content (3.27%), moisture (89.42%) and ash content (0.96%) of soy yogurt were higher than those for the dairy yogurt 1.78%, 82.66% and 0.93% respectively while the protein and carbohydrate contents of soy yogurt were lower than those for the dairy yogurt. There was slight decrease of all the components of soy yogurt except moisture which increased slightly during storage. There was significant difference ($P \leq 0.05$) in fat, moisture and carbohydrate contents of dairy and soy yogurt.

pH and Titratable Acidity

Fermentation was complete with titratable acidity of 1.21% (Fig. 2) and pH of 3.8(Fig. 3) There was a gradual increase in titratable acidity and a decrease in pH of the soy yogurt with storage time. The increase was more rapid at room temperature compared to refrigeration temperature (Fig. 4). Acidification rate of lactic acid bacteria varies with type of milk (Vargas *et al*., 2008).

For the sample stored at room temperature, the pH decreased gradually through the five days of storage, from 3.8 to 3.3 (A0 to A5). The pH decreased slightly after the first day (3.8 to 3.7) for the sample stored under refrigeration temperature then remained constant for the next 7 days (R8) and decreased again.

Then it remained constant for the 9^{th} day and decreased a point on the tenth day (R10).

For the dairy yogurt, the pH remained constant (3.5) through 20 days of storage and decreased a point (3.4) which was the final pH on the last day, 21^{st} day of storage (Fig. 5).

Sensory Quality of Soy Yogurt

The result of the sensory evaluation of soy and dairy yogurt showed that dairy yogurt had higher acceptability in taste, texture, visual appearance, aroma and general acceptability than soy yogurt. There was significant difference ($P \leq 0.05$) between the two products after subjecting the result of the evaluation to analysis of variance (Table 2).

Microbiological Succession

The results obtained indicate that fresh soy milk contained total bacterial load of 2.5×10^1 cfu/ml mainly *Bacillus* spp, no coliform and no staphylococci. Viable bacterial count of fresh yogurt was maximal after six hours fermentation yielding a level of 6.5×10^7 cfu/ml which comprised the starter cultures. Starter cultures decreased two log cycles after five day storage at room temperature (Fig. 6). Reduction in acidity led to growth of yeasts after one day storage (Fig. 6) with *Saccharomyces* spp attaining a level of 3.0×10^5 cfu/ml and *Candida* spp 2.5×10^3 cfu/ml after five days.

Under refrigeration temperature only the yeast *Saccharomyces* spp appeared after 3 day storage and increased to 1.1×10^3 cfu/ml by ten days (Fig. 7) while the starter cultures dropped one log cycle. There was a marked change in physical and organoleptic characteristics of the soy yogurt which was more evident in sample stored at room temperature.

Table 1: Percent proximate composition of dairy yogurt, soy yogurt and spoilt soy yogurt samples

Components (%)	Ko	SYo	A5	R10
Protein	3.51[a]	3.20[a]	3.05[a]	3.07[a]
Fat	1.78[a]	3.27[b]	3.12[b]	3.16[b]
Ash	0.93[a]	0.96[a]	0.89[a]	0.91[a]
Moisture	82.66[a]	89.42[b]	89.92[b]	89.83[b]
Carbohydrate	11.12[a]	3.15[b]	3.02[b]	3.03[b]

Key: Ko = Fresh dairy yogurt, SYo = Fresh soy yogurt, A5= Soy yogurt after five day storage at 27±3^{o}C and R10 = Soy Yogurt after 10 days storage at 7±2^{o}C. Mean values having different superscript letters in a row for each sample are significantly different (P≤0.05)

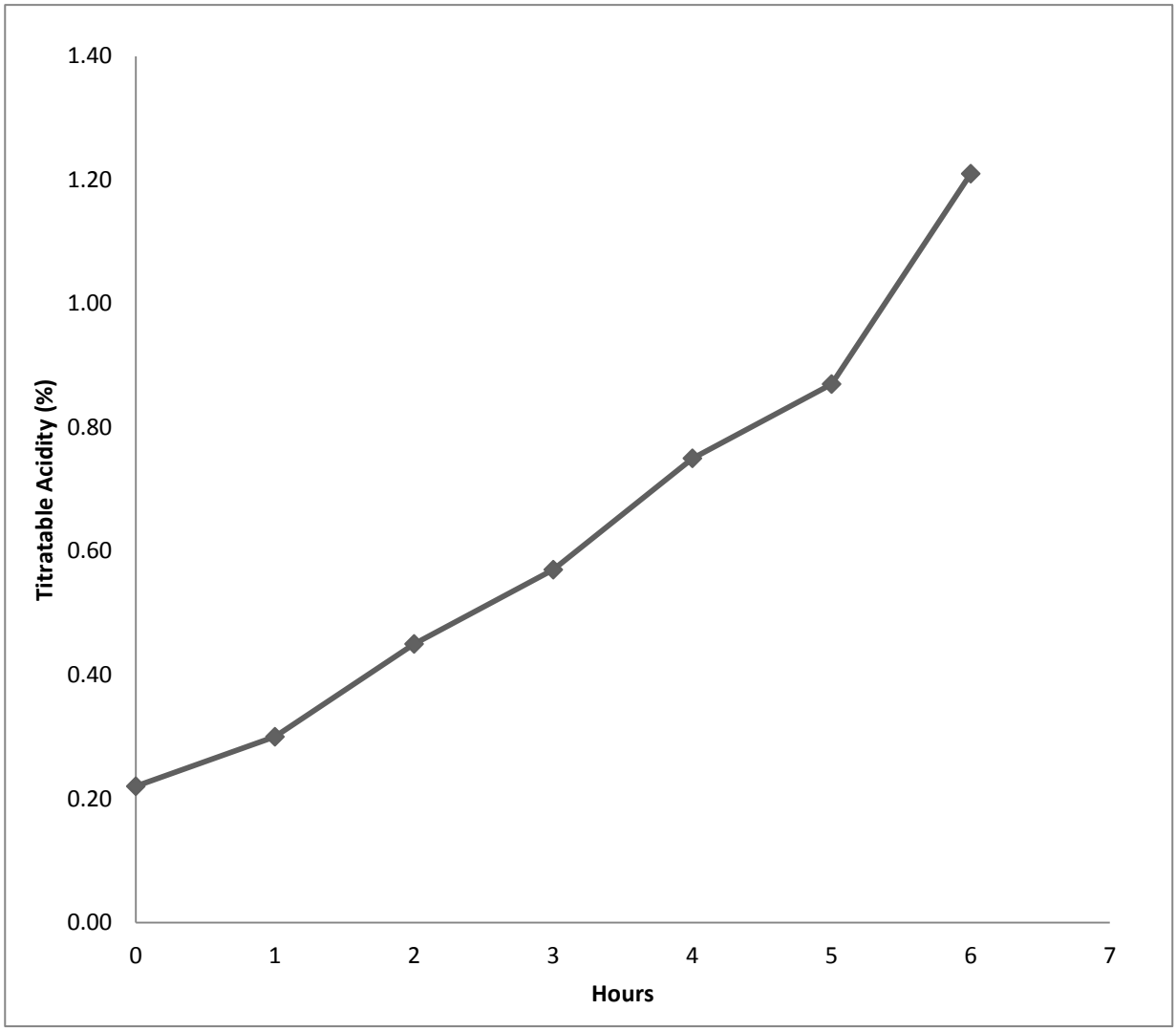

Fig.2: **Changes in Titratable Acidity during Fermentation**

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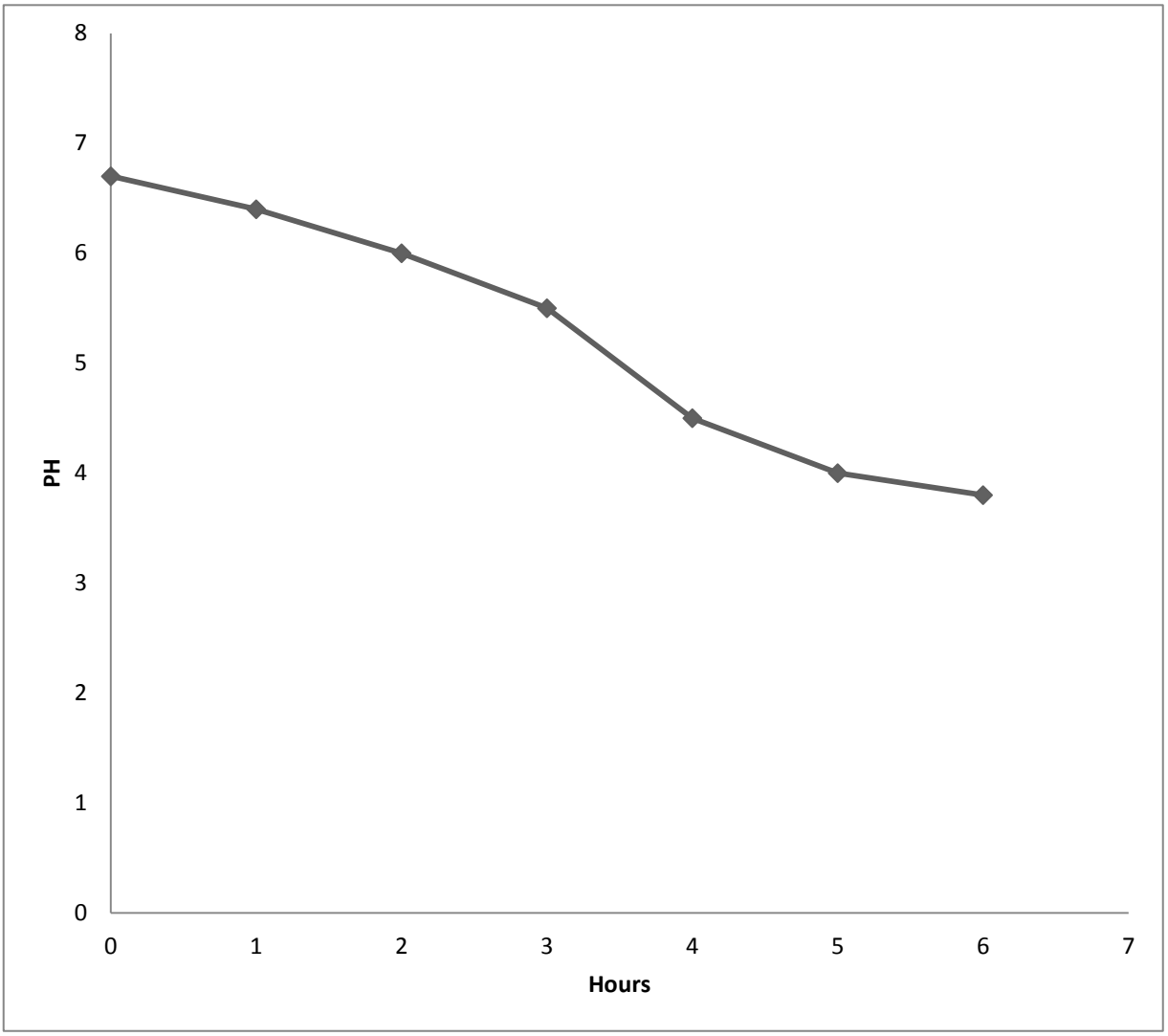

Fig.3: Changes in pH during Fermentation of Soy Yogurt

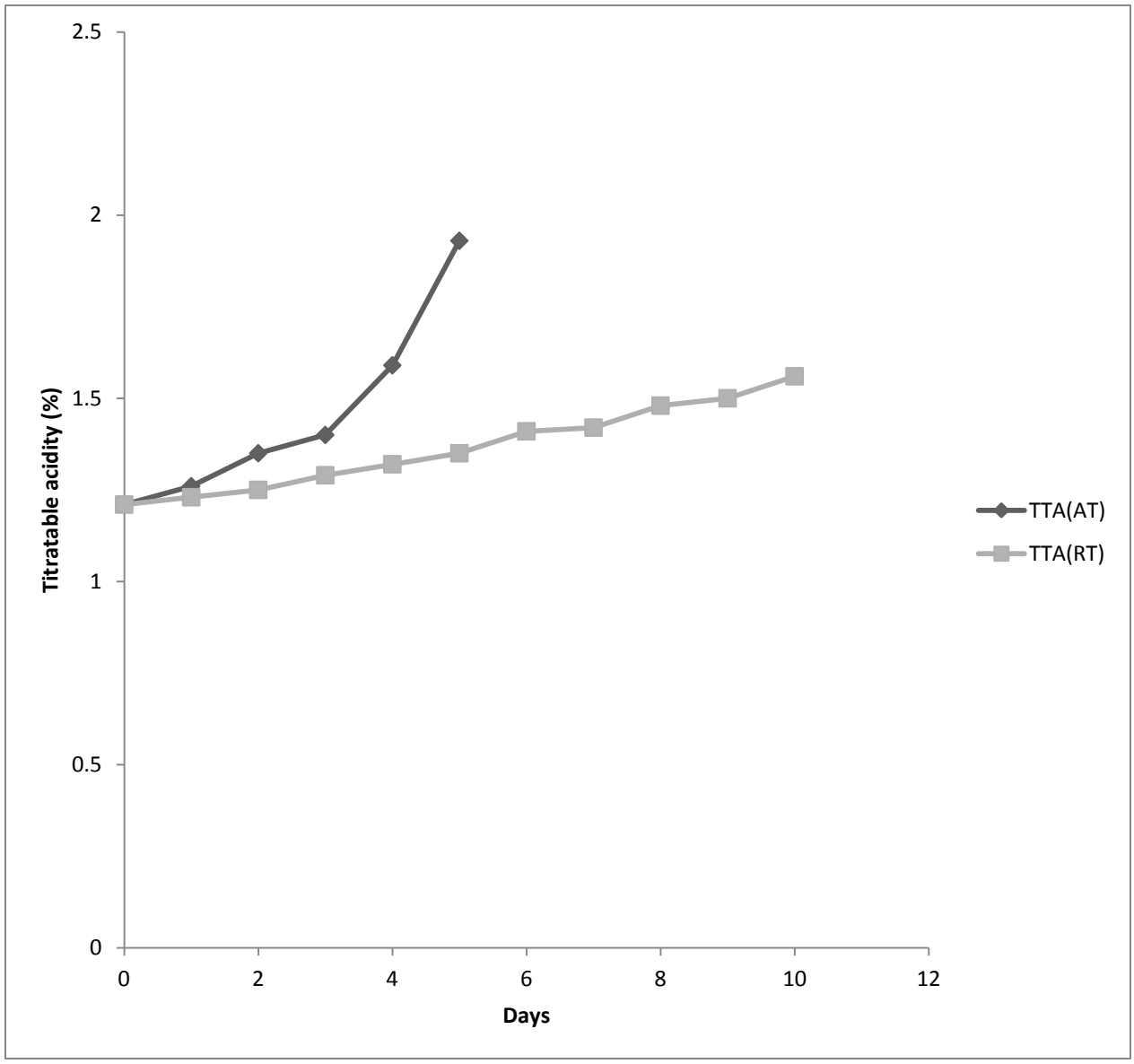

Fig. 4: Changes in titratable acidity during storage of soy yogurt at room TTA(AT) and refrigeration TTA(RT) temperatures (27±3^{o}C and 7±2^{o}C respectively). Values are means of three replicates.

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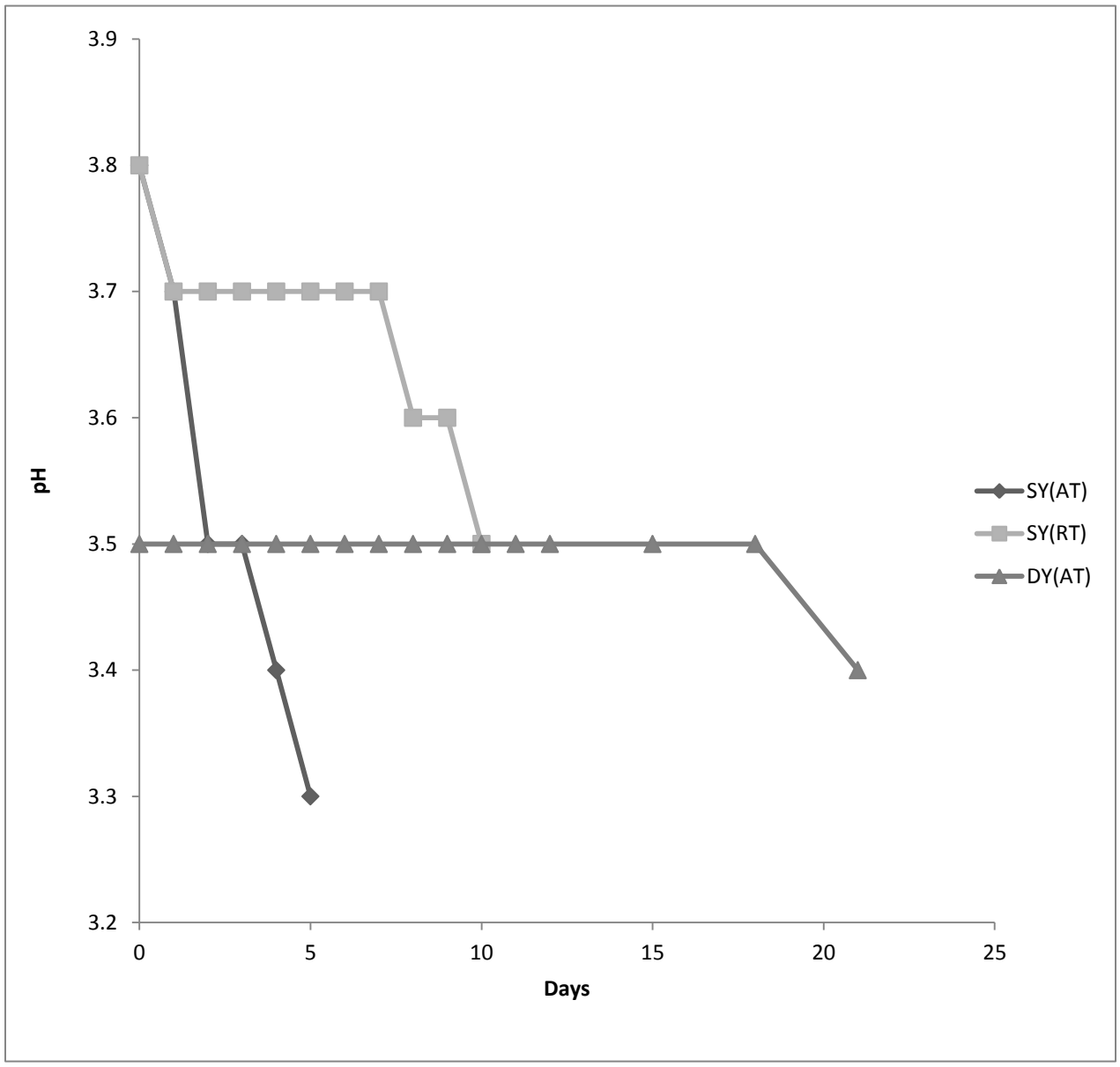

Fig. 5: Changes in pH during storage of dairy (DYAT) and soy yogurt samples at room (SYAT) and refrigeration (SYRT) temperatures (27±3^{o}C and 7±2^{o}C respectively). Values are means of three replicates.

Table 2: Sensory properties of Dairy Yogurt and Soy Yogurt.

Samples	Taste	Texture	Visual Appearance	Overall Appearance	Aroma
Dairy Yogurt	$8.0^{a} \pm 1.05$	$7.7^{a} \pm 1.06$	$8.2^{a} \pm 0.79$	$8.3^{a} \pm 0.82$	$7.7^{a} \pm 0.67$
Soy Yogurt	$5.3^{b} \pm 1.77$	$5.9^{b} \pm 1.60$	$6.4^{b} \pm 1.84$	$6.0^{b} \pm 1.49$	$6.0^{b} \pm 1.41$

Values are means ± standard deviation. Values having different superscript letters in a column for both samples are significantly different ($P<0.05$).

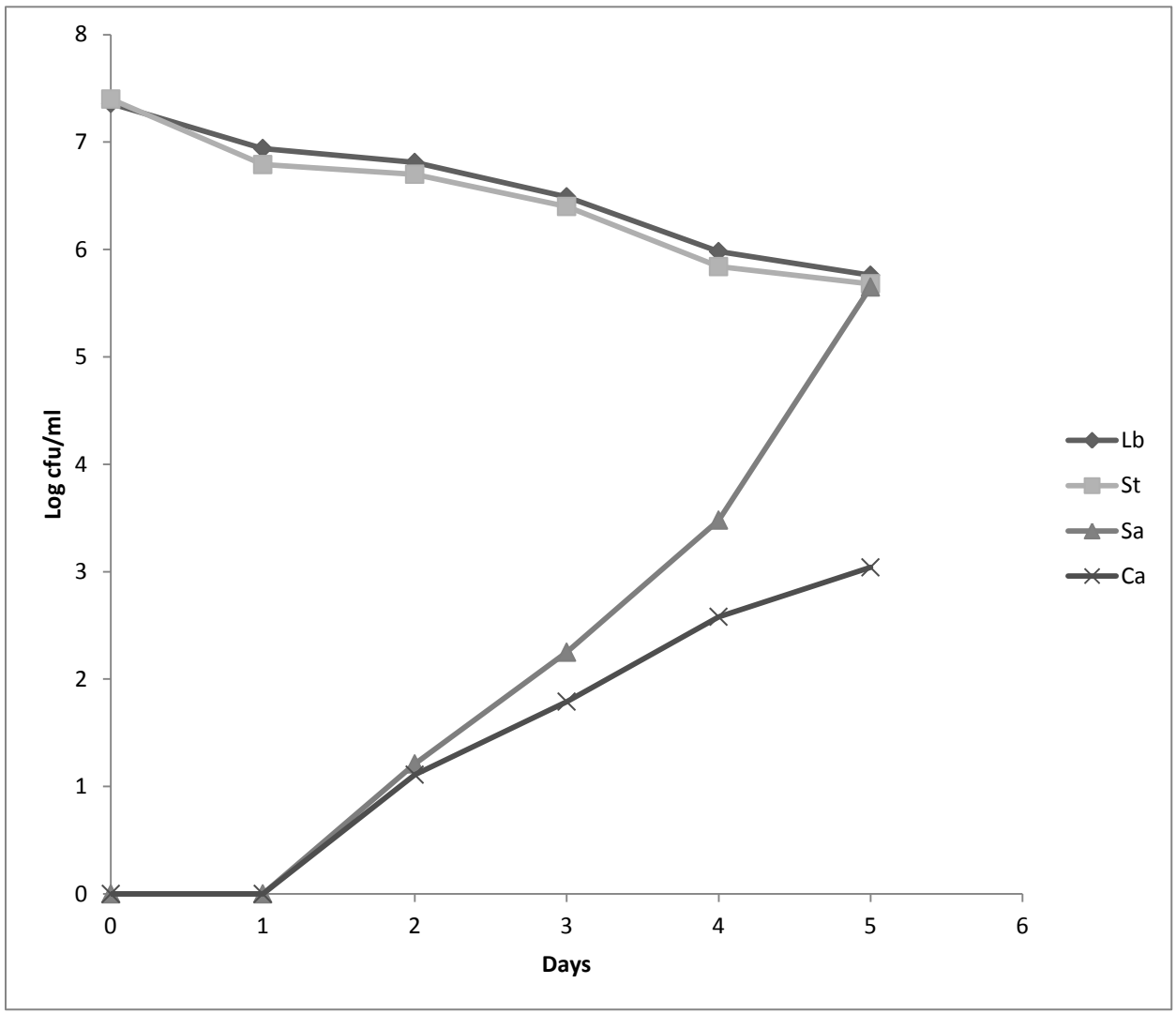

Fig. 6: **Succession of *Lactobacillus bulgaricus*(Lb), *Streptococcus thermophilus*(St), *Saccharomyces*(Sa) and *Candida*(Ca) species in soy yogurt stored at 27±3°C**.

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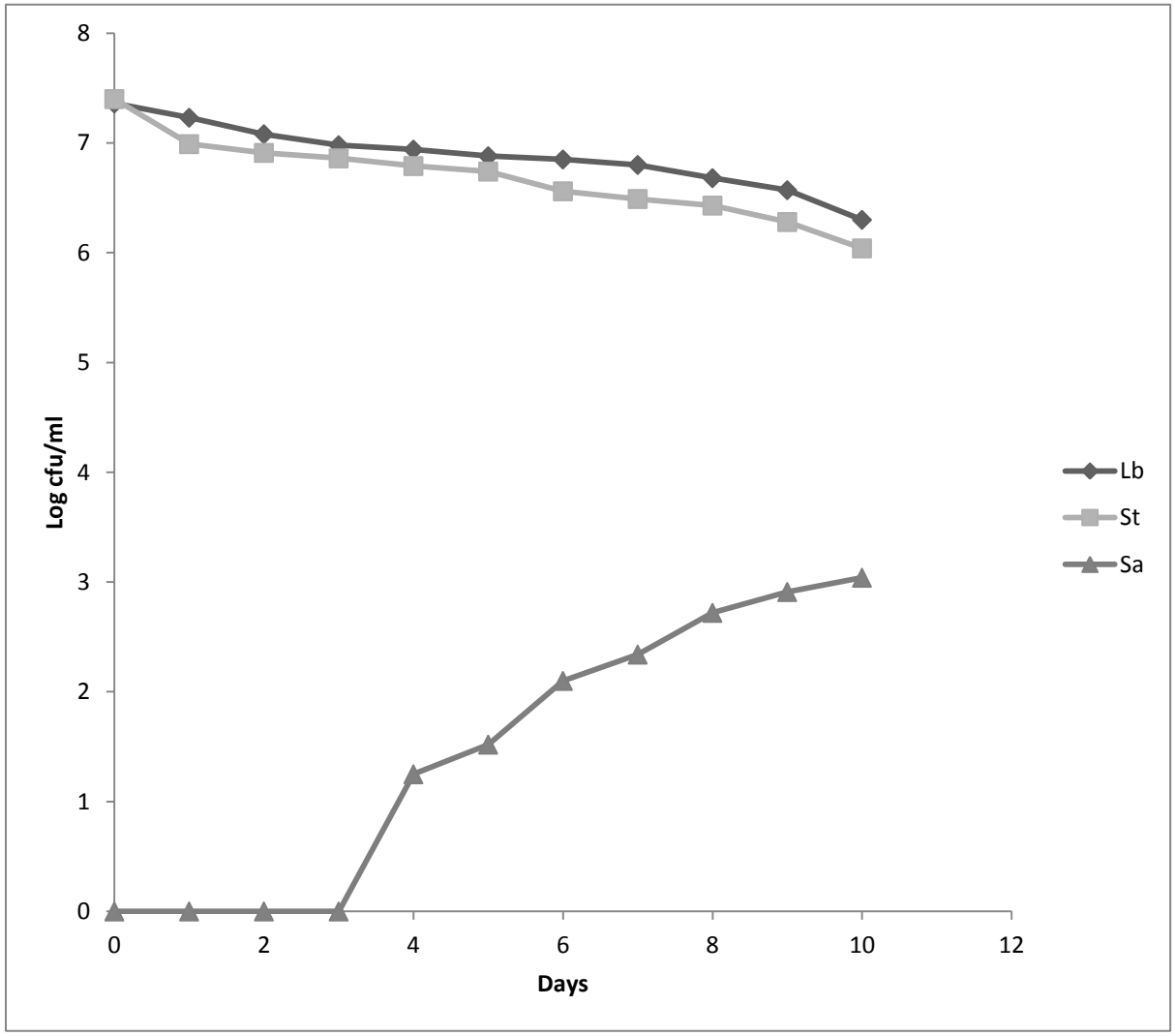

Fig. 7: **Succession of *Lactobacillus bulgaricus*(Lb), *Streptococcus thermophilus*(St) and *Saccharomyces*(Sa) *species* in soy yogurt stored at 7±2°C**

DISCUSSION

The increase in moisture was as a result of decrease in carbohydrate, protein and fat contents of soy yogurt during storage. This could be attributed to the fact that the spoilage organisms metabolized these components to produce water and also from the curdling of the yogurt (separation of water from solids). The higher moisture and lower carbohydrate content of soy yogurt in comparison to dairy yogurt (Table 1) could be as a result of additives (starch) present in dairy yogurt which increased the viscosity in dairy yogurt resulting in less available water and higher carbohydrate content. The difference in fat content of both yogurts could be as a result of the type of milk used for the dairy yogurt which was not stated. Amanze and Amanze (2011) reported low protein (2.02%), fat (1.30%) and ash (0.51%) contents and higher carbohydrate (4.79%) and moisture (91.63%) contents in soy yogurt

compared to values obtained in this work. Green and Ibe (1987) reported lower protein and fat values (1.2% and 1.0%) and higher carbohydrate and ash contents (12.7%, and 1.1%). The moisture value (82.66%) obtained in this work is comparable to the value (83%) reported by Green and Ibe (1987).

The decrease in pH of soy yogurt which was more rapid at ambient temperature could be attributed to higher metabolic activities producing more acid. The spoilage organisms thrived more than the starter cultures in acidic environment. The decrease in pH observed in this work was in agreement with the report of Osman and Razig (2010) although the values obtained differs. They also reported an increase in titratable acidity with storage time. Green and Ibe (2005) reported a mean pH and titratable acidity of 3.82 and 3.0% respectively. This was higher than the values obtained in this work.

The difference observed in the sensory properties (taste and aroma) of soy and dairy yogurt (Table 2) could be attributed to the slight beany flavour of the soy yogurt which was absent in dairy yogurt. The texture of the soy yogurt could have been affected by the increased moisture and absence of additives which would be enhanced by adding stabilizers such as gelatin and edible starch. Acceptance would also be enhanced by adding flavour such as vanilla and fruit flavours.

As the count of spoilage organisms (*Saccharomyces* and *Candida* sp.) increased, there was a slight decline in the counts of starter cultures (Fig. 4). This could be attributed to the fact that the environment (pH) and temperature was favourable for the growth of the spoilage yeasts which the starter cultures cannot compete with. Green and Ibe (1987) reported an initial increase in the counts of the starter cultures before a decline, although, growth of *Streptococcus thermophilus* stopped at pH 4.2 to 4.4 whereas that of *Lactobacillus bulgaricus* stopped at pH 3.5 to 3.8.

Previous studies have shown that yeasts were the primary contaminants of yogurts due to the low pH and high sugar content (Suriyaracchi and Fleet, 1981; Moreira *et al*., 2001). The genera of *Saccharomyces* and *Candida* were isolated during the storage of soy yogurt. Spoilage by these genera have been well documented (Green and Ibe, 1987; Suriyaracchi and Fleet, 1981 and Moreira *et al*., 2001). The growth of the spoilage yeast in soy yogurt stored at room temperature was more rapid than in refrigeration temperature. This could be attributed to the fact that cold environment slowed their metabolic and multiplication ability. Also only the genus *Saccharomyces* sp was isolated from the sample stored at refrigeration temperature in comparison to genus *Saccharomyces* and *Candida* sp (Fig. 6 and 7). This also could be attributed to the ability of *Saccharomyces* sp to thrive well at lower temperature.

Absence of coliform and staphylococci in the fresh yogurt signifies a safe and high quality product. This could be due to the pasteurization process during processing. The transient presence of *Staphylococcus* sp on the third and sixth day of storage at room and refrigeration temperatures respectively suggests that it could have been transferred from the skin or nose to the yogurt at any point during sampling process, since there was no replication.

The fact that the dairy yogurt stayed more than ten days at room temperature suggests presence of additives which was not indicated on the label. It became unacceptable after 20 days at room temperature while soy yogurt became unacceptable after four days and ten days at room and refrigeration temperature respectively.

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Suriyaracchi and Fleet (1981) reported that yogurt stored at 20^{o}C became unacceptable after 7 days. These observations illustrate the importance of storage of yogurts at low temperature in order to increase shelf life.

The onset of spoilage of soy yogurt in this study was indicated by off odour, separation of water and increase in microbial load suggest a product shelf life of four days at ambient and 10 days at refrigeration temperature. More work should be carried out on how to extend the shelf life of the product stored at room temperature using chemical preservatives as most families either do not have refrigerators or do not have constant power supply in Nigeria.

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