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RESEARCH ARTICLE

PROXIMATE AND SHELF LIFE EVALUATION OF SOYMILK YOGHURT WITH ADDED SACCHAROMYCES BOULARDII

Okafor S.O. and Anyalogbu E.A.
Department of Biotechnology, Federal University of Technology, Owerri, P.M.B. 1526 Owerri, Imo State, Nigeria.

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Abstract

The effect of adding Saccharomyces boullardii in soya yoghurt was studied. The control was made with soya milk and traditional starter culture (Lactobacillus bulgaricus and Streptococcus thermophilus) while the other three treatments were made by adding 1%, 2%, 3% of S. boulardii with traditional yoghurt starter. Proximate composition of all yoghurt treatments were determined after fermentation time. Shelf-life evaluation of yoghurt treatment were observed during the storage time. During the proximate composition evaluation, treatment with 3% S. boulardii had highest moisture and protein content at 83.43±0.03 and 92±0.3 but least ash and carbohydrate content at 1.2±0.18 and 4.27±0.3. During shelf-life evaluation, titratable acidity and syneresis values of yoghurt with S. boulardii were slightly increased while pH and water holding capacity decreased compared with control yoghurt. After 21 days, S. boulardii counts were 5.89, 6.07 and 6.03 log.cfu/ml for yoghurt with 2% and 3% S. boulardii respectively whereas L. bulgaricus and S. thermophilius of yoghurt with 3% S. boulardii were 7.45 and 8.38 log.cfu/ml respectively. The addition of S. boulardii improved the survivability of the bacteria starter culture.

Introduction:-

The primary role of diet is to provide sufficient nutrient to meet the nutritional requirement of an individual.The increasing scientific evidence that support the hypothesis of some foods and food components having beneficial physiological and psychological effects over and above the provision of basic nutrients gave rise to the concept of functional foods. Functional foods provide biological and therapeutic properties beyond their basic nutritional value (Hasler, 2002) which incorporates readily into diet food and proposed to reduce diseases risk (Buckler *et al*., 2001). Yoghurt is considered as a functional food because of its probiotic components. Yoghurt is a semi-solid fermented milk product made by fermentation process of fresh milk using lactic acid starter culture containing *Lactobacillus delbrueckis*ubsp. *bulgaricus* and *Streptococcus thermophilus* at a ratio of 1:1 to give acidity value of 0.7-1.1% lactic acid with pH approximately 3.8-4.6. According to the Codex Alimentarius Commission (2003) both bacteria strain must remain above in the final product with at least 10 million bacteria per gram. Yoghurt is one of the most popular fermented milk products and the consumption is increasing worldwide (Shiby and Mishra, 2013).

Soya milk is plant-based milk produced from soy beans. It is a stable mixture of protein, oil and water. It hasseveral health benefits which include low content of cholesterol and sugar but contains high proteins and isoflavone (Chang,

Corresponding Author:- Okafor S.O.
Address:- Department of Biotechnology, Federal University of Technology, Owerri, P.M.B. 1526 Owerri, Imo State, Nigeria.

1988). Soymilk improves health, bones, risk of heart disease and menopausal symptoms (Amanze, 2011). Soymilk is mostly used as a substitute for dairy milk by individuals who are lactose intolerant.

In the present study, *Saccharomyces boulardii* is investigated. *Saccharomyces boulardii* is a tropical strain of yeast first isolated from lychee and mangosteen forest by Henri Boulard (Hui, 2004). It is related to *Saccharomyces cerevisiae* but differing in several taxonomic metabolic and genetic properties (Champagne *et al*., 2011). It has been discovered that *S. boulardii* maintains and restores the natural floral in the large and small intestine, so it is classified as a probiotic. It has been shown to be non-pathogenic, non-systemic (it remains in the gastrointestinal tract rather than spreading to other parts of the body) (McFarland, 2010). It grows mostly at a high temperature of 37^0C. It tolerates differential pH levels which makes its survivability higher than that of bacteria. *S. boulardii* is recognized to have probiotic effectiveness when used alone or in combination with other probiotics to support digestion (Bisson*et al*., 2010)

Materials and Methods:-

Collection of Samples

Soya Beans was purchased from Eke-Awka market in Awka, Anambra State. Nigeria.Yoghurt Starter containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1) (Yogourmet, Lyo-San Inc., Canada) was purchased from Shoprite Supermarket Lekki, Lagos State. Nigeria. Flora Norm containing *Saccharomyces boulardii* (Prisma Pharmaceutical Ltd.) was obtained from NAFDAC Agulu, Anambra State. Nigeria.

Activation of Organisms

Lactobacillus bulgaricusand Streptococcus the rmophilus

The 5g sachet of yoghurt starter (containing*L. bulgaricus* and *S. thermophilus*) was swabbed with 70% Ethanol and cut using sterile scissors. A 2.5g of the yoghurt starter was collected and used to inoculate the MRS broth (Merck KGaA)and M17 broth (Merck KGaA, Darmstadt, Germany) respectively. Both Broth weremaintained at 37^0C and 40^0C for 16hrs in an aseptic environment to obtain cells at the stationary phase. The cells were harvested by centrifugation (Selecta Medifridger centrifuge, Spain) and the pellet was washed once in sterile distilled water and re-suspended in 100ml distilled water. The bacterial cells were standardized using McFarland Standard ampule (bioMérieux, France).

Saccharomyces boulardii

The 1g sachet of flora norm (containing *S. boulardii*) was swabbed with 70% Ethanol and cut using sterile scissors. The floranorm was used to inoculate the PDA broth. The culture was maintained at 25^0C for 24hrs in an aseptic environment to obtain cells at the stationary phase. The cells were then harvested by centrifugation (Selecta Medifridger centrifuge, Spain) and the pellet was washed once in sterile distilled water and re-suspended in 100 mL distilled water. The bacterial cells were then standardized using McFarland Standard ampule (bioMérieux, France).

Preparation of Soya Bean Powder

A 500g of soya bean was soaked overnight in 3ltrs of water. The soybean was blanched in a cooking pot for 15 min. The bean was dehulled and oven dried at 80^0C for 21h. The dried bean was roasted for 10mins under medium heat and milled into powder using an industrial processor. The Soya bean powder was stored in a dry airtight jar and kept in the refrigerator.

Experimental Design

Four (4) portions of soya milk (SM) were measured out and labeled: $SM_{\mathbf{10/0}}$, $SM_{9/1}$, $SM_{\mathbf{8/2}}$ and $SM_{\mathbf{7/3}}$ respectively. Each of the labeled portions was treated as milk sample throughout the experiment. The subscript represent the different doses of the conventional yoghurt starter culture (1:1 ratio of *L. bulgaricus* and *S. thermophilus*) and *S. boulardii* adjunct culture used correspondingly as shown in the Table below.

Table 2.1:- Percentage (%) inclusions of culture and adjunct culture in the milk samples.

MILK SAMPLES	Percentage (%) inclusion	
	L. bugaricus &S. thermophilus (1:1)	*S.boulardii* adjunct culture
SM 10/0	10:0	0:0
SM9/1	9:0	0:1
SM8/2	8:0	0:2

SM7/3	7:0	0:3

Preparation of Yoghurt From The Milk Sample

Yoghurt was prepared from soya bean powder as follows; four (4) sterilized 250ml capacity flask (representing the 4 milk samples) were appropriately labeled and the contents added as shown in Table 2.1. A 20g of soya bean powder was homogenized in 100ml of sterile water, heated for 10 minutes and sieved to remove lumps and debris for each sample. The milk was pasteurized at 110^0C for 10 minutes and rapidly cooled to 40^0C by suspending the flask in an ice bath for each treatment. The milk samples were inoculated with the yoghurt starter culture (1:1 of *L.bulgaricus* and *S. thermophilus*) and adjunct *S. boulardii*. After inoculation, the flasks were placed in an incubator of 40^0C for 8hrs after which the desired custard consistency was reached (Faladeet.al., 2015). After 8hrs, the flask were brought out of the incubator without shaking or stirring and placed in the refrigerator for 21 days at 4^0C

Determination of Proximate Parameters

Moisture Content

The moisture content was determined as described by AOAC (2005).A clean crucible was oven dried and weighed as (W1), then about 10ml of the yoghurt was dispensed into it and both the crucible and the yoghurt sample were weighed and recorded as (W2). The crucible and its content was then dried at 105oC in an oven for 24 hours after which it was removed and weighed again as (W3) which gave a constant and final weight. This was done in duplicates and the average or mean was taken. The loss in weight represents the moisture content and the percentage was calculated and expressed in percentage.

Ash Content

Total ash was determined according to AOAC (2005).A. 10ml of the yoghurt sample was added into the clean dried crucible. The crucible and its contents was then transferred into the muffle furnace set at 600oC for about 6 hours, the colour change to ash showed that it was fully ashed. The crucible and its contents were removed from the furnace and placed inside desiccators to cool. The ash content of each of the samples was calculated in percentage.

Protein Content Determination

The macro kjeldah method as described by AOAC (2005) was used to determine the crude protein content. 2g of the samples was introduced into the digestion flask. 10g of copper sulphate and sodium sulphate in the ratio of 5:1 and 25ml of concentrated sulphuric acid was added to the digestion flask. The flask was placed into digestion block in fume cupboard and heated until frothing ceased given a clear and light blue colouration. The mixture was allowed to cool and was diluted with distilled water until it reached 25ml of volumetric flask.10ml of the mixture was poured into the distillation apparatus and 10ml of 40% sodium hydroxide was added. The released ammonia by boric acid was allow to continue until 10ml of boric acid is treated with 0.02m of hydrochloric acid until the green colour change to purple. The nitrogen in the sample was then determined. The percentage nitrogen of the sample was calculated and multiplied by 6.25 to get the crude protein.

Fat Content Determination

The soxhlet solvent extraction method as described by AOAC (2005) was used to determine the fat content. In this method 2g of the sample was weighed into a flat bottom flask of known weight with the extractor mounted on it. The thimble was held half way into the extractor and the weighed sample was carefully transferred into the thimble and the thimble was plugged with cotton wool. The extraction was carried out at the temperature of 40 – 60^0C for 8hours. The solvent was removed by evaporation and then, the remaining part of the flask was dried in the oven at 80^0C for 30minutes and was finally cooled in a desiccator. The flask was reweighed and the percentage fat was calculated.

Determination Of Carbohydrate Content

The content of carbohydrate was determined by difference as described by Ihekoronye and Ngoddy (1985).

CHO = 100 - %(ash + protein + fat +moisture)

Determination of Shelf-Life

Determination of Ph

The pH was determined by the method described by AOAC (2005). This was determined using a pH meter.

Titratable Acidity
The titratable acidity was determined by method described by AOAC (2005). The amounts of acid in the yoghurt drinks were determined by titrimetric method. The titratable acidity of yoghurt sample was determined by titration with 0.1N sodium hydroxide solution.

A 10g of sample was weighed into a clean conical flask and diluted with 10ml of distilled water. Three drops of phenolphthalein indicator were added to the diluted sample and titrated against 0.1N sodium hydroxide solution. A faint but permanent pink colour change marked the end point.

Determination Of Water Holding Capacity (Whc)
Water holding capacity (WHC) Water holding capacity was measured as described by Parnell-Clunies, *et al*., (1986). Yogurt incubated in the sterile centrifuge tubes were centrifuged at 10 °C at 13500 × g for 30 min (Marathon 21000R, Fischer Scientific). The supernatant fluid was drained for 20 min by inverting tubes at 24 °C ±1. Water holding capacity was expressed as percent pellet weight over original yogurt weight.

Determination of Syneresis
Susceptibility of yoghurt to syneresis was determined by centrifuging 10g of sample at 2500rmp for 5mins and weighing the supernatant (Guzman-Gonzalez, *et al*., 2000). Then measuring the weighing the supernatant recovered. Syneresis was expressed in percentage.

Total Viability Count of L. bulgaricus, S.thermophilus and S.boulardii
The viable cells count of *L.* bulgaricus,*S.thermophilus*and *S.boulardii*was measured during storage time. The sample was mixed thoroughly by shaking vigorously so that uniform consistency is obtained. A 1g of each sample was serially diluted.

A 1ml of an appropriate dilution was innoculated on MRS, M17 and PDA agar and plateswere Inverted and incubated at37°C (MRS and M17) and 25°C (PDA) for 24 hrs. After incubation, the plates were examined for typical colonies of the bacteria.

Results:-

Proximate Evaluation of Yoghurt Samples
The proximate composition of the yoghurt samples from soya milk are presented in Table 1. The inclusion of the adjunct culture exhibited the same pattern of effect on the milk samples either increasing or decreasing the constituent concentration with increased concentration.

Table 3.1:- Proximate Composition of Dairy + Non-Dairy Yoghurt from milk samples.

PROXIMATE CONSTITUENT	COMPOSITION (%) OF SAMPLES			
	SM10/0	SM9/1	SM8/2	SM7/3
Moisture	82.55±0.15[a]	81.07±0.03[b]	80.32±0.02[c]	83.42±0.03[d]
Ash	1.4±0.1[a]	1.6±0.1[b]	1.4±0.2[a]	1.2±0.1[c]
Protein	7.9±0.4[a]	8.8±0.4[b]	9.1±0.4[c]	9.2±0.3[d]
Fat	1.4±0.2[a]	1.2±0.3[b]	1.4±0.3[a]	1.2±0.4[b]
Carbohydrate	6.75±0.2[a]	7.38±0.3[b]	7.82±0.3[c]	4.27±0.3[d]

Values are means ± SD of triplicate determinations
Values in the same row with the same superscript are not significantly different at 5% level ($p>0.05$).

Shelf Life Evaluation Of Yoghurt Samples
The change in shelf life parameter of yoghurt produced from Soy milk with time are presented in Figures below.

These figures represent the following parameters; pH, Titratable Acidity, Water Holding Capacity, Syneresis and Microbial viability respectively. The shelf life evaluation determines the longevity of the yoghurt.

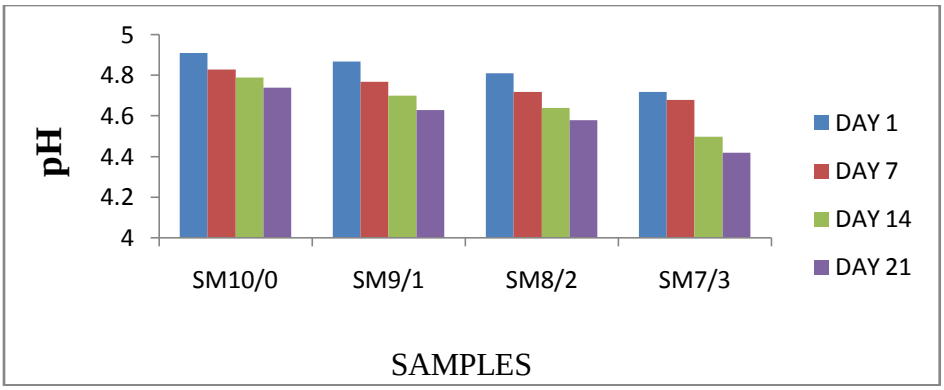

Fig 4.1:- Change in pH of yoghurt from soy milk sample during the storage.

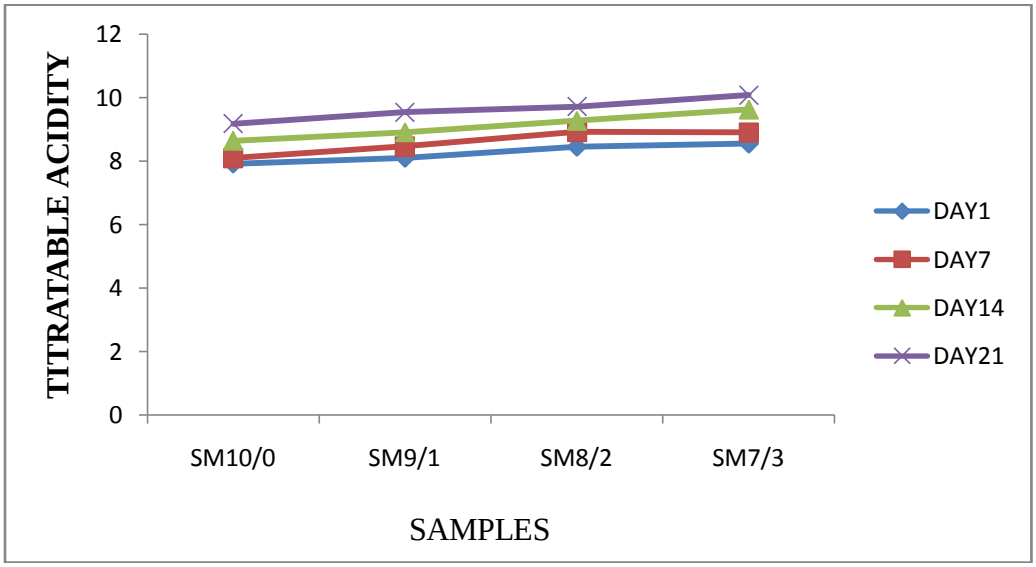

Fig 4.2:- Change in titratable acidity content of yoghurt from soy milk sample during the storage.

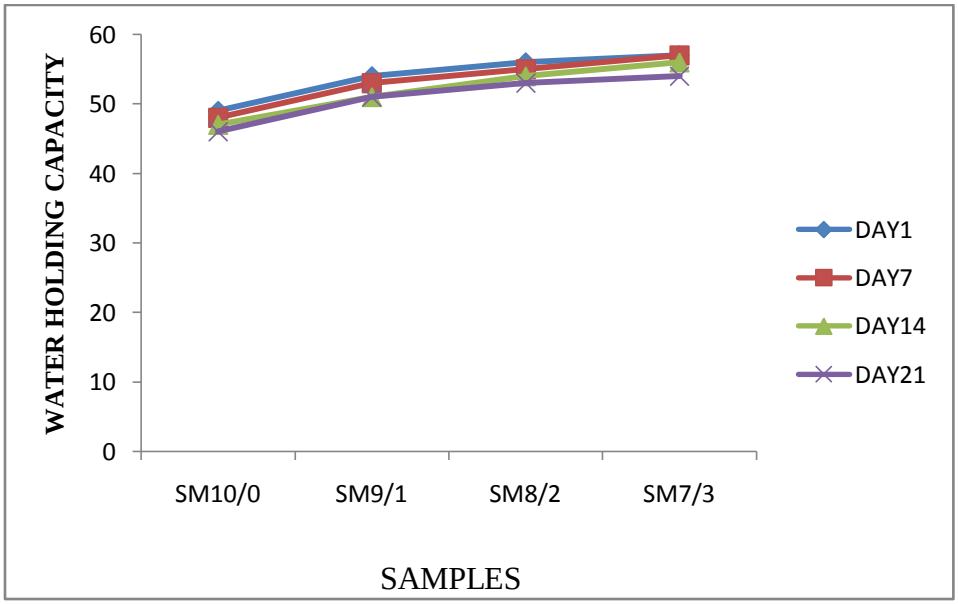

Fig 4.3:- Change in Water Holding Capacity contentyoghurt from soy milk samples during the storage.

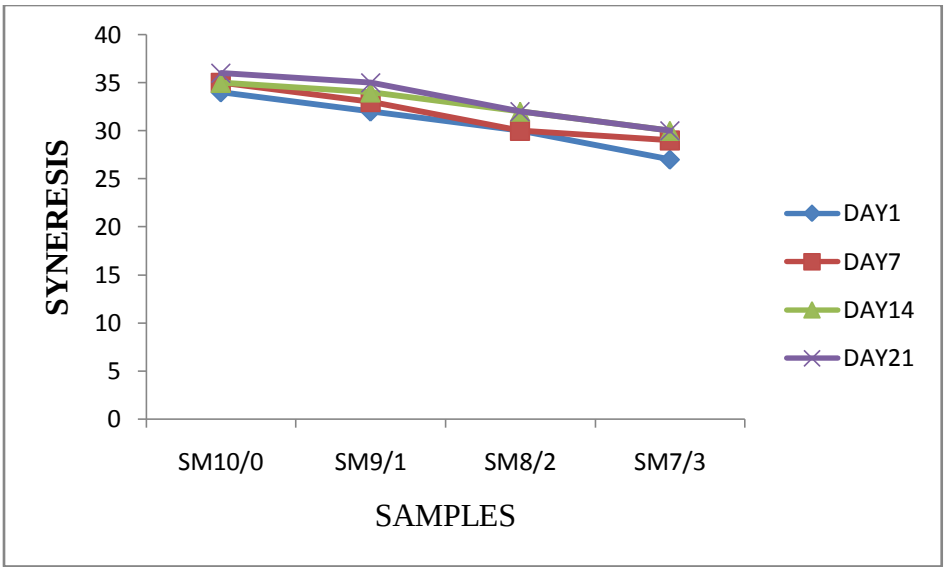

Fig 4.4:- Change in Syneresis of yoghurt from soymilk samples during the storage.

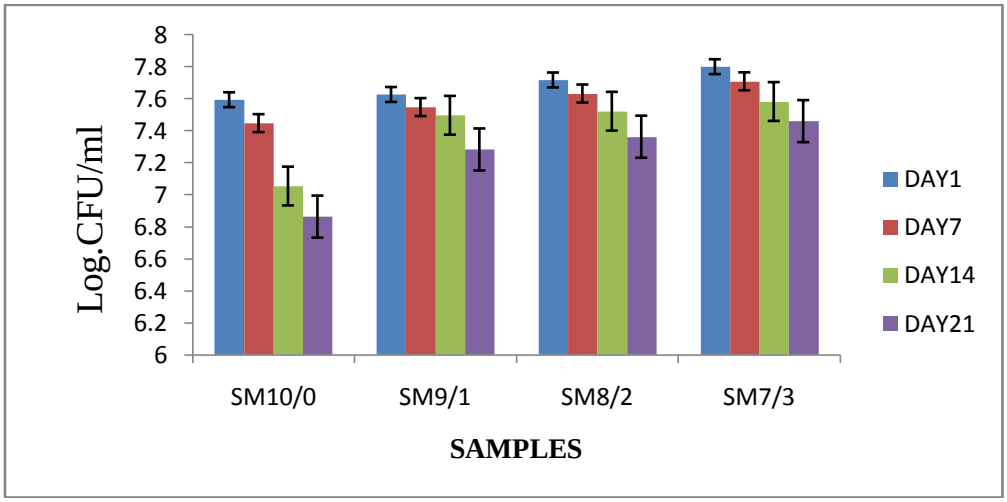

Fig 4.5:- Change in Viable count of *L. bulgaricus* (log.cfu/ml) of yoghurt from soy milk samples during storage.

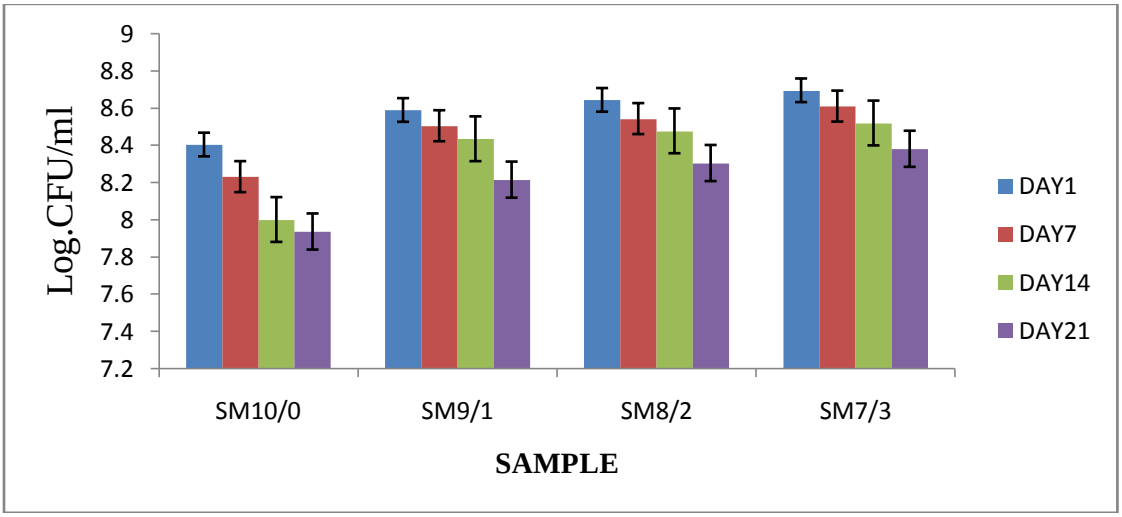

Fig 4.6:- Change in viable count of *S. thermophilus* (log.cfu/ml) of yoghurt from soy milk samples during storage.

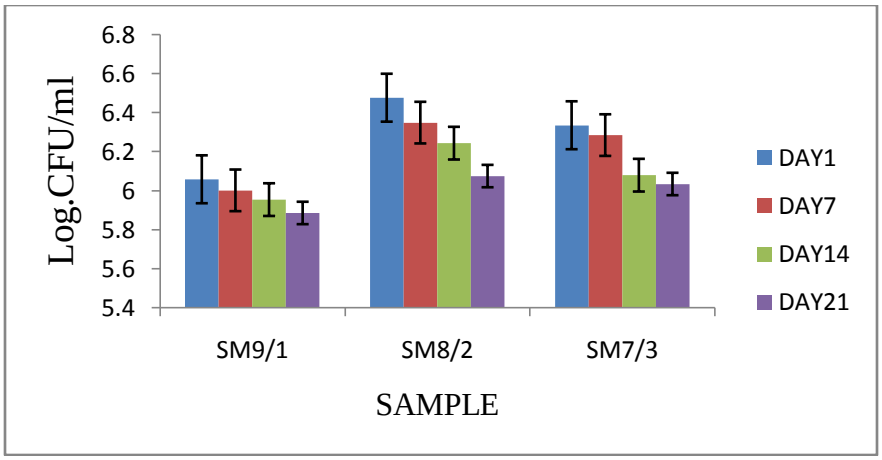

Fig 4.7:- Change in viable count of *S. boulardii* (log.cfu/ml) of yoghurt from soy milk samples during storage.

Discussion:-

Proximate composition of samples

The proximate composition of the soy milk yoghurt were evaluated by their moisture content, ash content, protein content, fat content and carbohydrate content.

The moisture content ranged from 80.32% to 83.42%. SM7/3 had the best moisture content. The moisture content observed in the present study was lower than values of 88.32% observed by Ukwo (2015). The values from the result corresponded with the standard specification of EAS (2006), which stated the maximum moisture content of yoghurt should be 84%. Lots of water in yoghurt makes it less viscous thereby affecting the texture of the yoghurt and moth feel (Bibina*et al*., 2014). A spoonful of yoghurt should be able to maintain its form without displaying sharp edges (USDA, 2001). The addition of *S. boulardii*, besides at SM7/3 decreased the moisture content of the yoghurt samples.

Ash content of the diary samples ranged from 1.2% to 1.6%. The result obtained in the present study was lower than value of 4.57 as reported by Tona (2016) for the same sample. In respect to the result obtained, Ukwo (2015) and Abou-Dobara*et. al.*, (2016) reported a lower value of 0.7% and 0.6% respectively for soy milk yoghurt. The ash content is an index of the mineral content of milk or yoghurt, which is needed for bone devilment, teeth formation and body functions (Bibiana and Joseph, 2014). This result indicates that soy milk is a better source of mineral.

Protein content showed that samples ranged from 7.9% to 9.2%. The differences in the protein content of the samples were observed to be significant ($P \leq 0.05$). In relation to the result, Amanze (2011), Ukwo (2015), Ehirim and Onyeneke (2013), Tang (2013) and Abou-Dobara*etal.*, (2016) reported lower values of 2.0%, 2.5%, 3.22%, 3.91% and 3.54% for soymilk yoghurt. The result obtained shows that the protein content of the treatments were within the CODEX (2003) specification which states that the protein content of yoghurt, Alternate culture yoghurt and Acidophilus milk should be at a minimum of 2.7%. The protein content is an index of amino acid content of the yoghurt which is essential for body building and serves as an important fuel sources for skeletal muscles (Tang, 2013). *S. boulardii* contains higher protein content (Czeruka*et al*., 2007). Addition *S. boulardii* to the yoghurt increased the protein content of the yoghurt samples.

Fat content from the result ranged from 1.4% to 1.2%. The result showed that the treatment decreased in fat content when compared with the control. Relative to the result obtained, similar value, 1.3% for soymilk yoghurt was reported by Amanze (2011) while higher values, 3.4%, 7.4%, 2.6%, and 2.82%, were also reported by Jayalalitha*etal.* (2015), Abou-Dabara*etal.* (2016), and Ukwo (2015) respectively for soymilk yoghurt. According to the International Standard of fat content in yoghurt, USDA (2001) stated a minimum fat content of 3.2%. Fats serve as a vehicle for soluble vitamins Vit A, D and K and promote their absorption. (Jayalalitha*et.al.*, 2015). The inclusion of *S. boulardii*, asides from SM8/2 caused a significant decrease in the fat content of samples.

The carbohydrate content ranged from 7.82% to 4.27%. The treatments were observed to be significantly ($P \leq 0.05$) different. The carbohydrate content obtained from the resultwere similar to that reported by Amanze (2011)for

soymilk yoghurt. The carbohydrate content falls within the range obtained by Osundahuns*et.al.,* (2007) for soymilk yoghurt. The low carbohydrate value is attributed to process of fermentation which converts carbohydrate basically to simple sugar as an energy source for the organisms (Ehirim and Ndimantang, 2004). *S. boulardii* converts carbohydrates in soy milk into simple sugar which aids as a sugar source during the fermentation and storage period.

Shelf life composition of samples

The pH values after 1 day of storage ranged from 4.72 to 4.91. The samples were significantly ($P \leq 0.05$) different after 1 day of storage. After 21 days of storage, significant differences were observed between the samples. The pH values ranged from 4.42 to 4.74. All samples were observed to decline significantly ($P \leq 0.05$) within the 21 days of storage. The addition of *S.boulardii* decreased pH value of the yoghurt samples during the storage time. This might be attributed to the high metabolic activity of *S.boulardii* and yoghurt starter (*L.bulgaricus* and *S.thermophilus*) during the storage (Adhikari*etal.*, 2000).

The Titratable acidity after Day 1 of storage ranged from 7.93 to 8.55. The samples possessed different statistical percentage. From Fig. 4.2, it was observed that a gradual increase in titratable acidity was observed as the days increased. After 21 days of storage. the samples ranged from 9.18 to 10.08. From the result obtained, Ukwo (2015) and Abou-Dobara*etal.*, (2010) reported low values of 1.02 and 0.17 for soy milk yoghurt respectively. The acidity in yoghurt plays an important role in determining attributed to its acidity (Ukwo, 2015). Donkor*etal.*, (2007) observed that the starter culture produce lower amount of organic acids in soy milk than in cow milk even if they grow well.

The water holding capacity (WHC) values after Day 1 of storage ranged from 49% to 57%. The samples were observed to be significantly ($p<0.05$) different. After 21 days of storage, the water holding capacity of the yoghurt samples were observed to have declined significantly ($p<0.05$) from Day 1 of storage. Each of the treatment declined throughout the storage period. The WHC of the samples were affected by the increased percentage of *S. boulardii*. Ukwo (2015) observed a decrease in the WHC of cow yoghurt but with an increase when substituted with soy milk. The water holding capacity is an important parameter in yoghurt production since it is related to syneresis which is due to intrinsic instability in protein gel (Afoakwa*etal.*, 2014). Kumari*et al.*, (2015) reported that the reduction of WHC is due to the irresolute gel network of yoghurt production.

The ability for the strength of coagulum to reduce during storage time was an increase in syneresis of the set yoghurt (Akalin*etal.*, 2012). The syneresis of the samples after Day 1 of storage, samples ranged from 34% to 27%. The samples were observed to be significantly ($p<0.05$) different. After 7, 14 and 21 days of storage, the samples were observed to increase in syneresis. The storage time significantly ($p<0.05$) increased the syneresis of the yoghurt samples. Higher values of syneresis in soymilk yoghurt were reported by Ukwo (2015). Syneresis is an important defect in yoghurt. It is defined as the separation of whey (serum) from the coagulum in yoghurt and is related to shrinkage of the gel (Sahan*etal.*, 2008). Syneresis can limit the shelf life and acceptability of yoghurt because of the undesirable appearance it causes (Obakeng*etal.*, 2018). Yoghurt with high level of syneresis is not liked by consumers.

*L. bulgaricus*at Day1 reached the highest in SM7/3 (7.70) and least in SM10/0(7.59). After 21 days of storage, *L.bulgaricus* cell counts were observed to have decreased. The treatments showed to be statistically significant ($p<0.05$). The decrease in viability was reported to be as a result of the increase in pH and acidity during the storage period (Hattingh and vilijeon, 2001; Kalasapathy, 2006). The addition of *S. boulardii* showed synergistic effect by enhancing the growth and viability of *L. bulgaricus.*

The cell count of *S. thermophiles*after Day 1 of storage was observed to be highest SM7/3 (8.70 Log cfu/ml). The treatments were observed to increase with the increase in percentage of *S. boulardii*. After 21 days of storage, the total cell count was highest in SM7/3 (8.3 Log cfu/ml. The samples were observed to decline during the storage time. The treatments were statistically significantly ($p<0.05$). The results obtained, were similar to observations of Ghoneem*et al.*, (2017) reported a decrease in cell count of *S. thermophilus* in soymilk yoghurt during storage time. The result obtained were satisfactory with the standards of USDA (2001), EAS (2006), CODEX (2003) and Kenya Standard (2011) regulations of yoghurt having a minimum of 10^7cfu/ml cell count per organism. *S. thermophilus* is essential in yoghurt due to its curdling of milk properties. The addition of *S. boulardii* increased the shelf life of *S. thermophilus* with its increase in percentage.

The cell count for *S.boulardii* after Day 1 of storage was observed to be least in SM9/1 (6.058 Log cfu/ml). The treatments were observed to rise with the increased percentage of *S.boulardii*. The result shows that the treatments were significant ($p \leq 0.05$ different). After 21 days of storage, the treatments significantly ($p \leq 0.05$) declined. The samples ranged from 5.8 to 6.0 Log cfu/ml. The results obtained are in accordance with the Codex (2003) standard of minimum of 10^4cfu/ml. Harackova, *et al*., (2015) explained that the growth of *S.boulardii* in soy milk yoghurt was its ability to utilize sucrose, the main sugar in soya milk.

Conclusion:-

The results obtained from the present study show that incorporation of probiotic yeast *Saccharomyces boulardii*was capable of utilizing soya milk constituents and improved the proximate and shelf life of the yoghurt. *Saccharomyces boulardii* showed a synergistic effect by the enhanced growth and cell viability of lactic acid bacteria (*L. bulgaricus and S. thermophilus*) in fermentation which increases its probiotic effectiveness.

References:-

1. Abou-Dobara, M. I., Magdy, M. I &Nawal, M. R. (2016). Chemical composition, sensory evaluation and starter activity in cow, soy, peanut and ricemilk yoghurt. *Journal of Nutritional Health and Food Engineering*. 5(3): 634-640
2. Adhikari, K., Mustapha, A. &Greun, I. (2000). Viability of microencapsulated bifidobacteria in set yogurt during refrigerated storage. *Journal of Dairy Science*, 83, 1946–1951.
3. Afoakwa, E. O., Kwasikpodo, F. M., Amoa, B. B., Badu, A. S. &Saalia, F. K. (2014) Effect of ingredient variation on microbial acidification, susceptibility to syneresis, water holding capacity and viscosity of soy-peanut-cow milk. *Jounal of Nutritional Food Engineering*, 1(2):1-6.
4. Akalin, A. S., Unal, G., Dinkci, N. &Hayaloglu, A. A. (2012). Microstructural, textural and sensory characteristics of probiotic yoghurts fortified with sodium calcium caseinate or whey protein concentrate. *Journal of Dairy Science*, 95(7): 3617-3628.
5. Amanze, K. O. (2011) Quality evaluation of yoghurt from cow milk, soy milk and cow/soy milk. *JORIND*. 9(2): 44-47
6. AOAC (2005) "Official Methods of Analysis" Association of Official and Analytical Chemists Washington DDC (12^{th}ed).
7. Bibiana, I., Joseph, A., and Julius, S. (2014). Physicochemical, microbiological and sensory evaluation of yoghurt sold in makurdi metropolis. African *Journal of Food Science and Technology*, 5 (6): 129–135.
8. Bisson J.F., Hidalgo S., Rozan P., Messaoudi M. (2010) Preventive effects of different probiotic formulations on travelers' diarrhea model in Wister rats: preventive effects of probiotics on TD. *Digestive Diseases and Sciences*, 55: 911–919
9. Buckley, N. D., Champagne, C.P., Mastti, A. I., Wagar, L. E., Tompkins, T. A. & Green J. M. (2011) Harnessing functional food strategies for the health challenges of space ravelFermented soy for astronaut nutrition, *ActaAstronautica*, 68, 731-738.
10. Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. T. &Charalampopoulos, D. (2011) Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices. *International journal of food microbiology*, 149: 185-193.
11. Chandan, R. C. and A., K. (2013). Manufacturing yogurt and fermented milks. (2nd ed.) USA: Wiley-Blackwell
12. Cheng, Y. J. (1988). Comparison of dairy yoghurt and imitation yoghurt fermented by different lactic culture from soyabean milk. Thesis, Texas Technology University. Unpublished. 40-47.
13. Codex (2003). CODEX STAN 243-2003: Standard for fermented milks. In (revised in 2010 ed., Vol. 2012). http://www.fao.org/docrep/015/i2085e/i2085e00.pdf
14. Czerucka, D., Piche, T. &Rampal P. (2007) Review article: yeast as probiotics — *Saccharomyces boulardii*. *Alimentary pharmacology & therapeutics*, 26: 767–778
15. East African Standard (ESA) (2006). Yoghurt specification East Africa Community (1^{st} edition) 1-8
16. Ehirim, F. N &Onyeneke, E. N. (2013). Physico-chemical and organoleptic properties of yoghurt manufactured with cow milk and goat milk. *National and Applied Sciences*. 4(4).
17. Falade, K. O., Ogundele, O. M., Ogunshe, A. O., Fayemi, O.E. &Ocloo, F. C. (2015). Physico-chemical, sensory and microbiological characteristics of plain yoghurt from bambara groundnut (Vignasubterranea) and soybeans (Glycine max). Journal of Food Science and Technology; 52(9):5858-65.
18. Guzmán-González, M., Morais, F., Ramos, M. & Amigo, L. (1999). Influence of skimmed milk concentrate replacement by dry dairy products in a low fat set-type yoghurt model system. I: Use of whey protein

concentrates, milk protein concentrates and skimmed milk powder *Journal of the Science of Food and Agriculture*. 79:1117–1122.
19. Habtegebriel, H. and Admassu, S. (2016). Optimization of the processing conditions of stirred yoghurt from camel milk using linear programming technique. *Food Science and Quality Management*, 53; 33–38.
20. Harackova, S., Slukova, M., Muhlhansova, A., Schulzova, V. &Plockova, M. (2015). Fermentation of soya milk by yoghurt and *Bifidobacteria* strains. *Czech Journal of Food Sciences*, 33(4):313-319.
21. Hasler, C. M. (2002). Functional foods: Benefits, concerns and challenges- a position paper from the American council on Science and Health, *Journal of Nutrition*, 132:3772-3781
22. Hattingh, A. L. & Viljoen, B. C. (2001). Growth and survival of a probiotic yeast in dairy products. *Food Research International*, 34(9): 791-796.
23. Hui, Y. H. (2004) Handbook of Food and Beverage Fermentation Technology. Marcel Decker Inc New York USA.
24. Ihekoronye, A. I. &Ngoddy, P.O. (1985). Integrated food Science and technolgy for the tropics. Macmillan Publishers Ltd., London and Basingstoke, p 137-138.
25. Jayalalitha, V., Manoharan, A. P., Balasundaran, B & Brand E. A (2015) Formulation of value enriched yoghurt with soy milk and mango pulp. *Journal of Nutrition and Food Sciences*, 5(6): 1-12
26. Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt.*LWT-Food Science and Technology*, 39: 1221–1227.
27. Kenya standard (2013). Specification for fermented (cultured) milks. Kenya Bureau of standards (2nd edition) 1-6
28. Kumari, A. G., Ranadheera, C. S, Prasanna, P. H., Senevirathne, N. D. &Vidanarachchi, J. K. (2015). Development of a rice incorporated symbiotic yogurt with low retro gradation properties. *International Food Research Journal*, 22(5): 2032-2040.
29. McFarland, L.V. (2010). Systematic review and meta-analysis of *Saccharomyces boulardii* in adult patients. *World Journal of Gastroenterology*, 16: 2202–2222.
30. Obakeng, G., Eyassu, S. &Sekwatimonang, B. (2018). Production of camel milk yoghurt: physic-chemical and microbial quality and consumer acceptability. *International Journal of Food Science*; 7:51-63.
31. Parnell-clunies, E., Mullen, K., Kakuda, Y. &Arnott, R. (1986). Physical Properties of Yogurt: A Comparison of Vat Versus Continuous Heating Systems of Milk. Journal of Dairy Science, 69(10):2593-2603.
32. Rekha, C. R &Vijayalakshmi, G. (2010). Bioconversion of Isoflavone glycosides to aglycons, mineral availability and vitamin B complex in fermented soy milk by probiotic bacteria and yeast. *Journal of Applied Microbiology*, 109: 1198-1208.
33. Shiby, V. K., & Mishra, H. N. (2013). Fermented milks and milk products as functional foods: A review. *Critical Reviews in Food Science and Nutrition*, 53, 482-496.
34. Tang, J. (2013).Physic-chemical and sensory properties of milk from soybean lines. A thesis to the University of Missouri-Columbia. Unpublished. 1-113.
35. Tona, G, O. (2016). Preparation and quality evaluation of raw milk and yoghurt source from machine milked jersey cows. *Food Science and Quality Management*. 157:1-56.
36. Ukwo, S. P. (2015). Physicochemical profile and sensory attributes of plain yoghurt from cow and soy milk blends. Nigerin Journal of Agriculture, Food and Engineering; 11(2):20-23.
37. USDA (2019). USDA food composition database. https://ndb.nal.usda.gov/ndb/nutrients/index. Reviewed on 23rd May, 2019.