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Microbial evaluation of probiotic beverage from roselle extract

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The study evaluated viability of probiotic lactobacilli in roselle extract at two different temperatures with view to producing acceptable and health beneficial probiotic beverage. *Lactobacillus* species isolated from fermented sorghum, maize grains and yoghurt samples were characterized and then inoculated into sterile roselle juice samples. Samples were stored at ambient (27 ± 2°C) and refrigeration temperatures (4 ± 1°C) over a period of four weeks and the viability of the probiotic isolates investigated by using standard methods. Antimicrobial effect of the probiotic isolates on two selected food borne-borne pathogens (*Escherichia coli* and *Staphylococcus aureus)* was also determined by the broth culture method. Results showed that the strains (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) remained viable in the beverage for 27 days. Except for *L. acidophilus* which decreased gradually after 21 days of storage, other strains (*L. plantarum*) (R_y and R_m) increased from an initial count of 1.0 x 10^6 to 6.4 x 10^6 and 9.8 x 10^6 cfu ml^{-1} respectively in samples kept at ambient temperature. The results further revealed that the probiotic isolates exhibited varying degree of inhibition against the two selected food-borne pathogens with greater inhibition exhibited against *E. coli.*

Key words: Roselle extract, probiotics, viability beverage

INTRODUCTION

In the past decade, health-promoting functional foods have entered the global market with force as a result of awareness on the part of health sensitive consumers. In developed countries, with increases in the ageing population and the increasing prevalence of lifestyle related diseases, many use functional foods and diet to reach and maintain optimal health. In developing countries, similar trend is developing among the higher socio-economic group who now embrace functional foods (World Bank, 2006). Functional or health-enhancing foods are food-type products that influence specific physiological functions in the body, thereby producing benefits to health, well being or performance, beyond regular nutrition and are marketed and consumed for this value added property (Roberfroid, 2002). Consumption of probiotics is a natural way of promoting consumer health. Probiotics are beneficial or 'friendly' bacteria, which when ingested favorably alter the microbial intestinal balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection (Scarpignato and Rampal, 1995). The positive effect of probiotics is not limited solely to the gastro-intestinal tract. Enzymatic hydrolysis with participation of bacteria increases bioaccessibility of lipids and proteins and reduces allergenicity of foodstuffs (Friend and Shahani, 1984). Probiotics can digest lactose and symptoms of lactose intolerance diminish as the lactose level in fermented dairy products decreases. Probiotics also synthesize B- group and K vitamins as well as cytoprotective short-chain fatty acids and polyamines such as putrescine, spermine and spermidine (Buts et al., 1994). Probiotics also function to relieve digestive ailments such as diarrhea, irritable bowel syndrome (IBS), lactose intolerance and also help to improve heart health by lowering serum cholesterol levels and prevention of colon cancer by reducing the activity of enzymes participating in carcinogenesis (Przemyslaw and Piotr, 2003). Probiotics are also involved in the production of several "gut nutrients" such as short-chain fatty acids, omega - 3 unsaturated fatty acids and the amino acids, arginine, cysteine and glutamine (Bengmark, 1998).

Application of probiotic organisms is at present limited to dairy products especially to yoghurt, which can only be

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afforded by the privileged few in the developing countries. Global market for probiotic dairy product was worth $10.2 billion in 2007 led by sales in Asia and Europe, which at present hold 54 and 30% respectively of the overall market. Other fruit juices including mango, rosehips and strawberry are being employed as carriers of probiotic strains (Beverage World, 2009). In Nigeria, roselle juice called “zobo” - a local non-alcoholic beverage obtained from roselle calyces- is consumed widely by different age groups. It contains no artificial flavor or color and it is refreshingly delicious. Consumption of roselle extract, which is already gaining acceptance among the less affluent, can be fortified with probiotic strain. Apart from economic consideration which makes probiotic dairy products inaccessible to many in developing countries, vegetarians are also afforded the opportunity of adhering to a beverage of plant origin without missing out on intake of probiotics (Ouwehand et al., 1999). Incorporation of probiotic *Lactobacilli* isolates, that could survive in the roselle juice samples and also impart bio-preservative functions, would deliver health benefits of both to the consumers.

Roselle calyces are also characterized as highly acidic with low sugar content. Roselle also contains a flavonoid pigment anthocyanin, a non-toxic and natural red colorant with therapeutic properties (Tsai et al., 2002). Probiotic roselle juice will combine the nutritional and therapeutic functions of both probiotics and roselle juice. It also implies that consumers who cannot consume probiotic dairy products due to lactose intolerance (inability to digest lactose) can have an alternative drink in roselle juice. Low pH and presence of antimicrobial substances in roselle extract make it an unfavourable environment for many microbes. It is imperative to screen for probiotic strains than can remain viable in it.

The present study focused on isolation and identification of *Lactobacilli* from yoghurt, fermented sorghum and maize and subsequent inoculation of sterile roselle extract with the strains. The viability of the probiotic strains in the medium was assessed and inhibitory effect of the *Lactobacilli* isolates in the probiotic beverage against two indicator organisms, *Escherichia coli* and *Staphylococcus aureus* was also determined. This was carried out with a view to study the possible probiotic potentiality of the probioticated beverage on selected strains of microbes of health importance.

MATERIALS AND METHODS

Materials

Dry roselle calyces, sorghum and maize grains were obtained from a local market in Ile-Ife, Nigeria. Yoghurt samples were prepared in the laboratory using the method of Robinson (1986) Pure cultures of the pathogenic organisms – *E. coli* (NCIB 86) and *Staphylococcus aureus* (NCIB 8588) were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The media used were de Man, Rogosa and Sharpe (MRS) agar for the cultivation of lactic acid bacteria, Tomato juice agar for identification of lactic acid bacteria, Nutrient agar incorporated with 7.5% NaCl concentration for enumeration of *S. aureus* and MacConkey agar for enumeration of *E. coli*.

Isolation of lactic acid bacteria from fermented sorghum and maize grains

The sorghum and maize grains were cleaned and separately soaked for 72 hours at ambient temperature, rinsed with water, ground and sieved. The fermented sorghum liquor (1.0 ml) was transferred to 9 ml sterile distilled water. The tubes were each shaken well to ensure adequate mixing. Serial dilutions were prepared. One ml each of the dilutions was plated on MRS agar using pour plate method (Harrigan and McCance, 1976). The medium was allowed to set, plates inverted and incubated at 37°C for 48 h.

Isolation of lactic acid bacteria from yoghurt

After serial dilution of yoghurt sample, one ml each of the dilutions was plated on MRS agar using pour plate method (Harrigan and McCance, 1976). The medium was allowed to set, plates inverted and incubated at 37°C for 48 h. Representative colonies of lactic acid bacteria were picked from incubated plates and purified further by repeated streaking on already solidified MRS agar plates. The pure cultures of the isolates were maintained on MRS agar slants in McCartney bottles kept at refrigeration temperature (4 ± 1°C).

Identification of lactic acid bacteria

The pure isolates were identified according to the scheme and procedure described in Bergey’s Manual of Determinative Bacteriology (Holt, 1997) Gram-reaction, catalase test, nitrate reduction, production of gas from glucose and other biochemical tests were performed to identify the isolates (Prescott et al., 2005).

Preparation of sterile probiotic roselle juice samples

The processing method for the production of roselle juice is shown in Figure 1. Roselle juice sample (25 ml) was dispensed into screw-capped tubes and then sterilized at 121°C for 15 min.

Each of the tubes containing cool sterile roselle juice samples was inoculated with 1 ml each (containing about 25.0 x 106 cfu/ml) of the identified lactic acid bacterial suspension (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) culture under aseptic conditions. The tubes were vortexed to ensure thorough mixing and then stored. at both ambient and refrigeration temperatures. Uninoculated sterile roselle juice sample stored at both temperatures served as control.

Determination of the viability of lactic acid bacteria in the roselle juice samples

The microbial counts of the lactics were taken at the onset and then at regular intervals from the probiotic roselle juice samples during storage. Serial dilutions of each of the samples stored at both temperatures were prepared and from each dilution, 1 ml was plated and MRS agar added using the pour plate method. The plates were incubated at 37°C for 48 h. After incubation, plates with colonies between 30 and 300 were counted using a Gallenkamp Colony Counter (CNW-300 Model). The experiment was carried out in triplicate and the mean of the counts was determined.

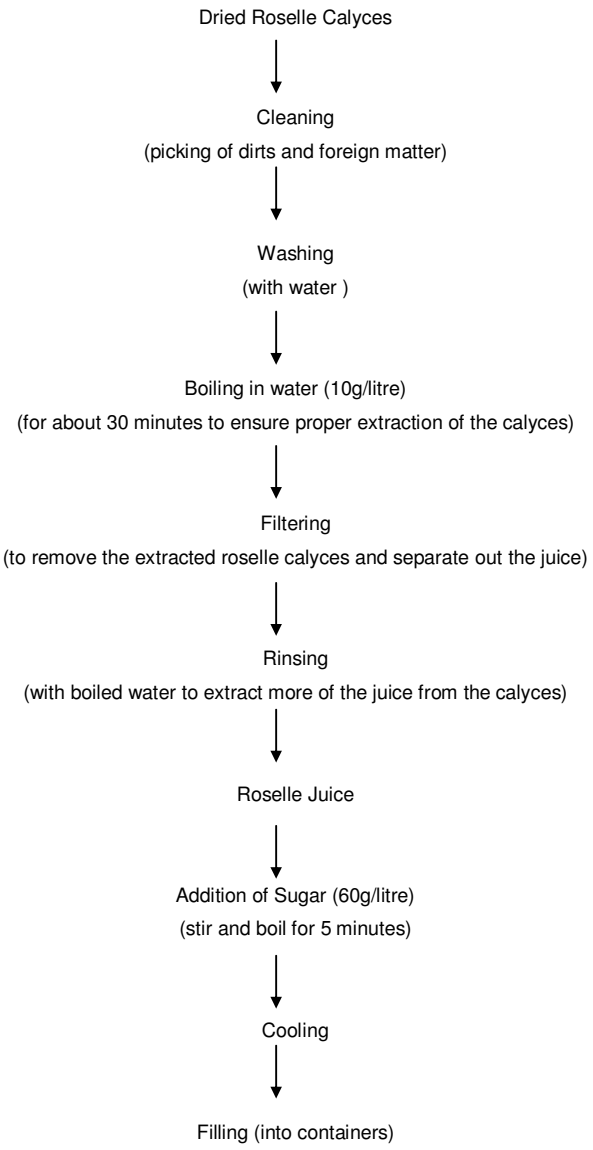

Figure 1. Flow Chart for Production of Roselle Juice.

Inoculation of probiotic roselle juice samples with pathogenic organisms

Each of the tubes containing probiotic roselle juice samples, obtained from procedure described earlier was inoculated with 1 ml each of a 24 h old pure culture of each of the pathogenic organisms, *Escherichia coli* and *Staphylococcus aureus* respectively. Another set of sterile roselle extract (without probiotics) was also inoculated with indicator organisms. In both cases uninoculated samples served as control and samples were stored at both temperatures.

Determination of *In-vitro* antagonism of lactic acid bacteria in roselle juice samples

In-vitro antagonism of the lactic acid bacteria against the pathogenic organisms in the roselle juice samples was determined using the method described by Visser and Holzapfel (1992). Test samples were analysed by determining the microbial counts of *E. coli* and *S. aureus* in the presence or absence of lactic acid bacteria in roselle juice over a period of six weeks.

One ml each of test samples obtained from roselle juice containing *E. coli* and *S. aureus* in the presence or absence of *Lactobacilli* isolates was transferred into each of 9 ml sterile 0.1 peptone water diluent, shaken will to ensure thorough mixing. This was serially diluted and labeled and from each dilution 1 ml was plate using MacConkey agar (for samples containing *E. coli)* or Nutrient Agar containing 7.5% NaCl (for samples containing *S. aureus).* The plates were allowed to set, inverted and incubated at 37°C for 24 and 48 h respectively and the colonies counted afterwards. The experiment was carried out in triplicate and the mean determined.

Statistical analysis

Result obtained as triplicates were subjected to analysis of variance while Duncan's multiple range test was employed to separate the means. The Origin Pro 70 (1992 - 2002) computer package was used for the statistical analysis.

RESULT AND DISCUSSION

Identification of lactic acid bacteria isolates

Bacterial isolates from the fermented sorghum grains, yoghurt and fermented maize grains were identified to be *L. plantarum* and *L. acidophilus* (Table 1). It has been reported by Holt (1997) that the genus *Lactobacillus* can be found in fermenting animal and plant products where carbohydrates are available. *L. plantarum* was also reported to be widely distributed in fermenting plant materials like cereals (such as millet, maize, pickles, sorghum and wheat) and is responsible for the fermentation of pickles and manufacture of sauerkraut (Oyewole, 1987). Fuller (1989) included *L. plantarum* and *L. acidophilus* among the list of microorganisms that can be used in probiotic preparation. Przemyslaw and Piotr (2003) and Savadago et al. (2006) reported that probiotics strains are selected mainly from the genera *Bifidobacterium* and *Lactobacillus.*

Viability of lactobacilli isolates in roselle juice

The Lactic acid bacteria count reflecting the viability of probiotic strains in roselle juice samples stored at both temperatures is shown in Table 2. An increase in Lactic acid bacteria count was observed from initial day of storage to day 12 for samples Rs (roselle juice with *L. plantarum* from sorghum) and Rm (roselle juice with *L. plantarum* from fermented maize) stored at ambient temperature. The count then remained fairly constant until day 18 before a slight drop was observed for the remaining period of storage in those two samples.

Lactic acid bacteria count in the probiotic samples stored at ambient temperature ranged between 1×10^6 and 2.8×10^8 cfu/ml. Sample Rs exhibited a probiotic

Table 1. Characteristics of lactic acid bacteria isolated from yoghurt, fermented sorghum and maize.

Characteristics	Isolates		
	LS	**LY**	**LM**
Cell Morphology	Rods	Rods	Rods
Gram Staining	+	+	+
Catalase Test	–	–	–
Aerobic Growth	+	+	+
Gas Production from Glucose	–	–	–
Ammonia from Arginine	–	–	–
Nitrate Reduction	–	–	–
Growth on MRS Agar	SRC	SRC	SRC
Growth on MRS Broth	UT	UT	UT
Growth at 15°C	+	–	+
Growth at 45°C	–	+	–
Growth at 3.5% NaCl	+	+	+
Growth at 4.0% NaCl	+	+	+
Growth at 4.5% NaCl	+	+	+
Growth at 5.0% NaCl	+	+	+
Sugar Fermentation			
Acid from:			
Glucose	+	+	+
Sucrose	+	+	+
Lactose	+	+	+
Maltose	+	+	+
Trehalose	+	+	+
Arabinose	+	–	+
Salicin	+	+	+
Mannitol	+	–	+
Sorbitol	+	–	+
Galactose	+	+	+
Melibiose	+	+	+
Mannose	+	+	+
Melezitose	+	+	+
Identity of Isolates	*Lactobacillus plantarum*	*Lactobacillus acidophilus*	*Lactobacillus plantarum*

(+) positive, (-) negative, (LS) Isolate from Fermented Sorghum, (LY) Isolate from Yoghurt, (LM) Isolate from Fermented Maize, (SRC) Smooth Round Colonies, (UT) Uniform Turbidity.

effect throughout the period of storage at ambient temperature while it showed a probiotic effect until Day 9 of storage at refrigeration temperature. It can be seen from these results that probiotic sample (roselle juice with *L. acidophilus* from yoghurt) exhibited a probiotic effect until Day 21 when kept at refrigeration temperature while probiotic effect was observed until Day 18 in samples maintained at ambient temperature. Sample R_m maintained probiotic effect throughout the 27 days of storage at ambient temperature while its probiotic effect in refrigerated sample was non-existent. Moreno et al. (2006) reported that a probiotic product will exhibit probiotic effect if it contains at least 10^6 cfu/ml of probiotic organisms.

Antimicrobial effect of probiotic isolates on indicator organisms

The growth pattern of *E. coli* in the roselle juice with or without Lactic acid bacteria during storage is shown in Figure 2. *E. coli* count increased up to the first week of storage and then decreased gradually until the end of the storage period. In sample ERs (roselle juice with *L. plantarum* from fermented sorghum and *E. coli*), there was a decrease in the *E. coli* count for the first three

Table 2. *Lactobacilli* count (cfu/ml) of probiotic roselle juice samples stored at ambient and refrigeration temperatures.

Temperature of Storage	Period of Storage	Lactobacilli count (cfu /ml)		
		Rs	Ry	Rm
Ambient	0	$1.00 \pm 0.20 \times 10^6$	$1.00 \pm 0.10 \times 10^6$	$1.00 \pm 0.20 \times 10^6$
	3	$1.83 \pm 0.15 \times 10^6$	$7.50 \pm 0.15 \times 10^7$	$1.50 \pm 0.25 \times 10^6$
	6	$1.25 \pm 0.10 \times 10^8$	$1.01 \pm 0.10 \times 10^8$	$2.00 \pm 0.17 \times 10^7$
	9	$2.58 \pm 0.15 \times 10^8$	$1.60 \pm 0.20 \times 10^8$	$1.95 \pm 0.23 \times 10^8$
	12	$2.80 \pm 0.18 \times 10^8$	$1.80 \pm 0.22 \times 10^8$	$2.20 \pm 0.27 \times 10^8$
	15	$2.20 \pm 0.27 \times 10^8$	$2.30 \pm 0.14 \times 10^7$	$1.60 \pm 0.20 \times 10^8$
	18	$2.00 \pm 0.16 \times 10^8$	$1.11 \pm 0.12 \times 10^7$	$1.31 \pm 0.15 \times 10^8$
	21	$2.20 \pm 0.18 \times 10^8$	$7.30 \pm 0.10 \times 10^8$	$2.54 \pm 0.17 \times 10^8$
	24	$1.07 \pm 0.12 \times 10^7$	$4.00 \pm 0.16 \times 10^5$	$1.56 \pm 0.26 \times 10^7$
	27	$6.40 \pm 0.10 \times 10^6$	$1.78 \pm 0.23 \times 10^5$	$9.80 \pm 0.18 \times 10^6$
Refrigeration	0	$1.00 \pm 0.18 \times 10^6$	$1.00 \pm 0.21 \times 10^6$	$1.00 \pm 0.10 \times 10^6$
	3	$1.00 \pm 0.10 \times 10^6$	$1.09 \pm 0.14 \times 10^7$	$8.00 \pm 0.19 \times 10^5$
	6	$8.70 \pm 0.14 \times 10^5$	$1.50 \pm 0.20 \times 10^7$	$7.30 \pm 0.22 \times 10^5$
	9	$1.00 \pm 0.20 \times 10^6$	$1.00 \pm 0.15 \times 10^7$	$9.20 \pm 0.15 \times 10^5$
	12	$3.60 \pm 0.12 \times 10^5$	$9.60 \pm 0.16 \times 10^6$	$8.70 \pm 0.10 \times 10^5$
	15	$2.70 \pm 0.17 \times 10^5$	$2.40 \pm 0.10 \times 10^6$	$2.61 \pm 0.14 \times 10^5$
	18	$9.60 \pm 0.23 \times 10^4$	$1.12 \pm 0.10 \times 10^6$	$1.73 \pm 0.17 \times 10^5$
	21	$3.50 \pm 0.20 \times 10^3$	$1.02 \pm 0.15 \times 10^6$	$1.01 \pm 0.20 \times 10^5$
	24	$2.53 \pm 0.10 \times 10^3$	$7.00 \pm 0\ 23 \times 10^5$	$7.50 \pm 0.10 \times 10^4$
	27	$1.76 \pm 0.12 \times 10^3$	$2.53 \pm 0.10 \times 10^5$	$1.82 \pm 0.15 \times 10^4$

Note:
Rs - Sample with *Lactobacillus plantarum* from fermented sorghum.
Ry - Sample with *Lactobacillus acidophilus* from yoghurt.
Rm - Sample with *Lactobacillus plantarum* from fermented maize.

weeks and then extinction of the pathogenic organism was achieved at 4 weeks of storage.

In roselle juice sample ERy (sample with *L. acidophilus* from yoghurt and *E. coli*), there was a reduction in the number of the pathogenic organism till the 4th week and then a subsequent extinction of the pathogenic organism at 5 weeks of storage. A similar trend was observed for sample ERm (roselle juice with *L. plantarum* from fermented maize and *E. coli*). It was observed that the probiotic strain in sample ERs was more effective against *E. coli* than those in ERy and ERm.

Oyetayo (2004) reported that *L. plantarum* strains isolated from fermenting corn slurry and cow milk produced some antimicrobial agents against some major food spoilage and pathogenic bacteria. Perdigon et al. (1995) reported that *Lactobacilli* maintain a healthy balance of intestinal flora by producing organic compounds such as lactic acid, hydrogen peroxide and acetic acid, which increase the acidity of the intestine and inhibit the reproduction of many pathogenic bacteria.

The growth pattern of *S. aureus* in roselle juice inoculated with or without Lactic acid bacteria during storage is shown in Figure 3. In sample SR (sample with *S. aureus* only), *S. aureus* count increased until the 1st week and thereafter decreased during the remaining period of storage. In sample SRs (roselle juice inoculated with *L. plantarum* from fermented sorghum and *S. aureus*), there was a reduction in *S. aureus* count. This implies that the probiotic strain was able to effect inhibition against the pathogenic organism. A similar trend was observed in sample SRy (roselle juice inoculated with *L. acidophilus* from yoghurt and *S. aureus*) and SRm (roselle juice inoculated with *L. plantarum* from fermented maize and *S. aureus*). Itoh et al., (1995) also reported the inhibition of food-borne bacteria by bacteriocins from *L. gasseri*, and observed that several strains of *L. gasseri* showed wide inhibitory activity against *Listeria monocytogenes*, *Bacillus cereus*, *S. aureus* and *E. coli*.

Lactic acid bacteria play an important role in the inhibition of food-borne pathogens and spoilage organisms with the production of antimicrobial metabolites, including acetic acid, lactic acid, other organic acids, hydrogen peroxide, bacteriocins-like substances, diacetyl and reuterin. Incorporation of these probiotics or any of their metabolic products into foods can confer bio-preservative effect on such products (Jin et al. 1996; Cadirci and Citak, 2005). Inclusion of *Lactobacillus* spp in roselle extract can be a way of incorporating functional property

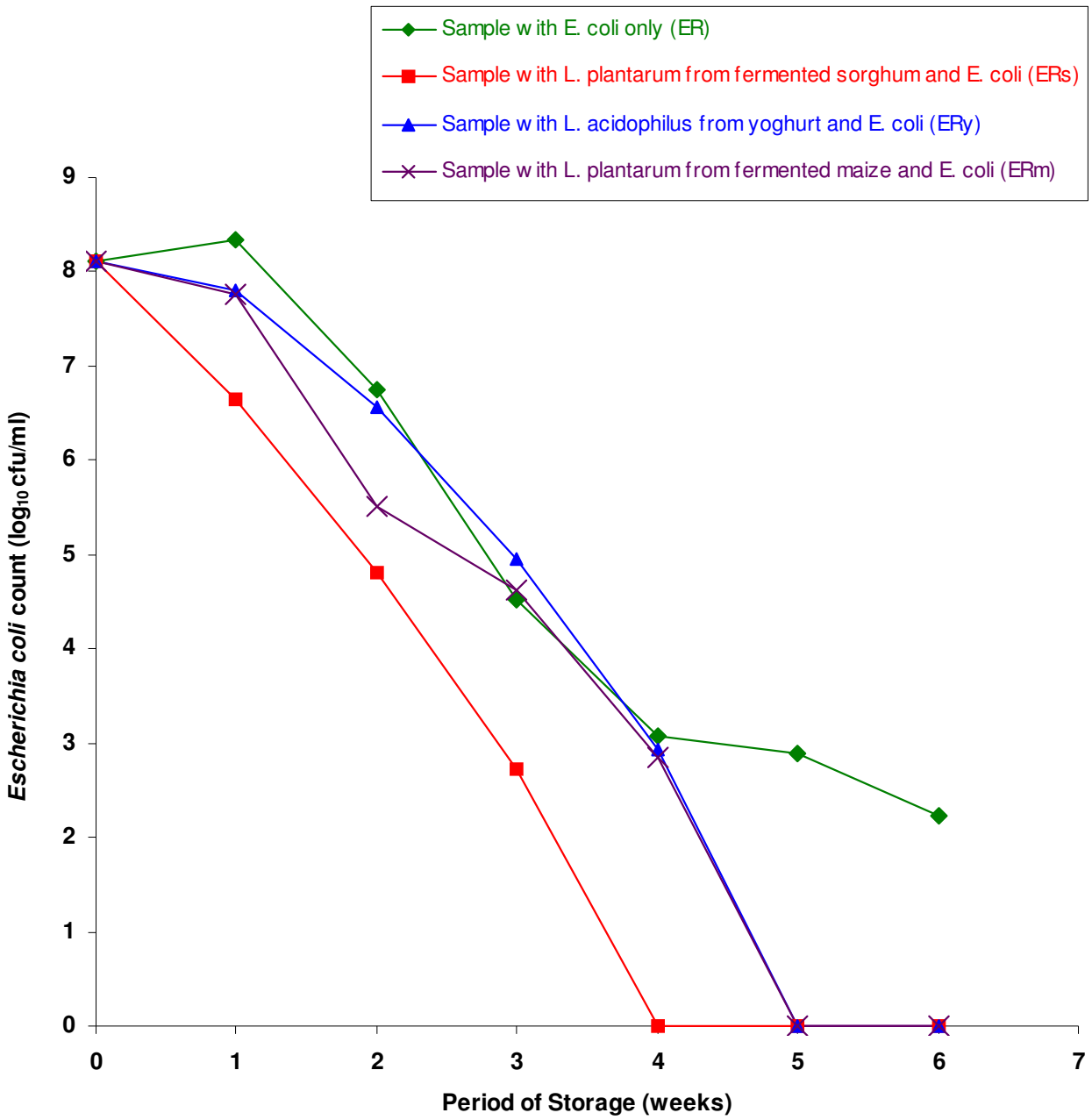

Figure 2. *Escherichia coli* count in Roselle Juice Samples inoculated with *Lactobacillus* spp.

into it just as it is done with other functional food products (Ashwell 2002; Fogliano et al ., 2005).

Conclusion

This study has examined viability of *L. plantarum* and *L. acidophilus* during ambient and refrigerated storage in roselle juice samples. And also investigated was their antagonistic effect on two food borne-pathogens. It was observed in this study that the viability of *L. acidophilus* from yoghurt was greater than that of the *L. plantarum* in roselle extract. The probiotic isolates were found to inhibit the growth of *E. coli* and *S. aureus* - the pathogenic organisms employed in this study. *L. plantarum* from fermented sorghum was more effective against the two pathogenic organisms tested than the other two isolates.

Based on the findings of this study, it can be concluded that probiotic roselle juice can serve as biotherapeutics combining the health and nutritional benefits of the

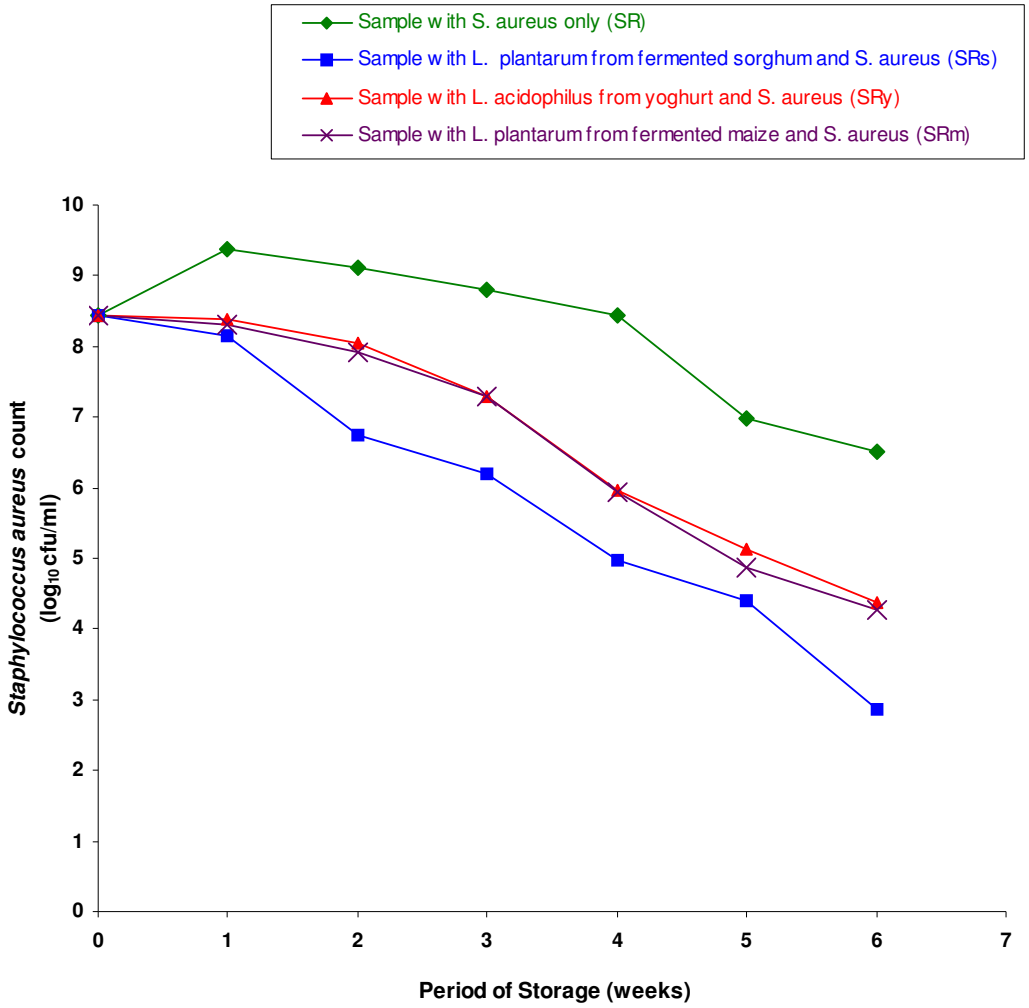

Figure 3. *Staphylococcus aureus* count in Roselle Juice Samples inoculated with *Lactobacillus.*

beverage with that of the probiotics.

REFERENCES

Ashwell M (2002). Concept of Functional Foods. ILSI Europe Concise Monograph Series. International Life Sciences Institute : Brussels. pp. 3-45.

Bengmark S (1998). Immunonutrition: Role of Biosurfactants, Fibre, and Probiotic Bacteria. J. Nutr. 14: 585-594.

Beverage World, 2009. A cultured Trend. www.beverageworld .com. Assessed on May 14, 2009

Buts JP, de Keyser N, de Raedemaeker L (1994). Saccharomyces boulardii enhances rat intestinal enzyme expression by endoluminal release of polyamines. Pediatrics Resource 36: 522-527.

Cadirci BH, Citak S (2005). A Composition of two methods Used for Measuring Antagonistic Activity of Lactic Acid Bacteria. Pak. J. Nutr. 4(4): 237-241.

Fogliano V, Vitaglione P (2005). Functional Foods: Planning and Development. Mol. Nutr. Food Res. 49: 256-262

Friend BA, Shahani, K (1984). Nutritional and Therapeutic Aspects of Lactobacilli. J. Appl. Nutri. 36: 125-153.

Fuller R (1989). A Review: Probiotics in Man and Animals. J. Appl. Bacteriol. 66: 365-378.

Harrigan WF, McCance M (1976). Laboratory Methods in Food and Dairy Microbiology. Academic Press Inc., London, pp. 25-28.

Holt JG (1997). The Shorter Bergey's Manual of Determinative Bacteriology, Bacteria–Classification and Terminology. The Williams and Wilkins Company, Baltimore, U. S. A. pp. 218-221.

Itoh T, Fujimoto Y, Kawai Y, Toba T, Saito T (1995). Inhibition of Food-borne Pathogenic Bacteria by Bacteriocins from *Lactobacillus gasseri*. Lett. Appl. Microbiol. 21:137-141.

Jin LZ, Ho YW, Abdullah N, Ali MA, Jalaludin S (1996). Antagonistic effects of Intestinal *Lactobacilli* Isolates on Pathogens of Chicken. Lett. Appl. Microbiol. 23: 67-71.

Moreno Y, Collando MC, Ferrus MA, Cobo JM, Hernandez E, Hernandez M (2006). Viability Assessment of Lactic Acid Bacteria in Commercial Dairy Products Stored at 40^0C using LIVE/DEAD BacLight TM Staining and Conventional Plate Counts. Int. J. Food Sci. Technol. 4: 275-280.

Ouwehan, AC, Kirjavainen PV, Shortt C, Salminen C (1999). Probiotics: mechanisms and Established Effects. Int. Dairy J. 9: 43-52.

Oyetayo VO (2004). Phenotypic Characterisation and Assessment of the Inhibitory Potential of Lactobacillus Isolates from different sources. Afr. J. Biotechnol. 3(7): 355-357.

Oyewole OB (1987). Lactic Fermented Foods in Africa and their Benefits. Food Control. 8 (51): 280-297.
Perdigon GA, Alwvarez S, Rachid M (1995). Immune System Stimulation by Probiotics. J. Dairy Sci. 78: 1597-1606.
Prescott, LM, Harley JP, Klein DA (2005). Microbiology. McGraw-Hill Book Companies Inc. New York, 6th Edition. pp. 20-45.
Przemyslaw JT, Piotr T (2003). Probiotics and Prebiotics. J. Cereal Chem. 80(2): 113 -117.
Roberfroid MB (2002). Global View on Functional Foods: European Perspectives. Br. J. Nutri. 88(Supp 12): S133-S138.
Robinson RK (1986). Modern Dairy Technology. Elsevier Applied Science. London pp. 13-332
Savadago A, Ouattrara CAT, Bassole IHN, Traore SA, (2006). Bacteriocins and Lactic Acid Bascteria – A Mini Review. Afr. J. Biotechnol. 5(19): 678-684.
Scarpignato CP, Rampal P (1995). Prevention and Treatment of Travellers' Diarrhoea: A Clinical Pharmacological Approach. Chemotherapy 41: 48-81.
Tsai PJ, McIntosh J, Pearse P, Camden B, Jordan TB (2002). Anthocyanin and Antioxidant Capacity in Roselle, *Hibiscus sabdariffa* L. extract. Food Res. International. 35: 351-356.
Visser R, Holzapfel, WH (1992). Lactic Acid Bacteria in the Control of Plant Pathogens. In: The Lactic Acid Bacteria. Vol. 1 Wood, B. J. B. (ed.) Elsevier Science Publisher, London, pp. 193 -210
World Bank (2006). Health Enhancing Foods: Opportunities for Strengthening the Sector in Developing Countries. Agriculture and Rural Development Discussion Paper 30. Kotilainen L, Rajalahti R, Ragasa C, Pehu E (Eds). Washington DC., pp. 1-35.