Full Paper

EVALUATION OF PHYSICO-CHEMICAL PROPERTIES AND PROXIMATE COMPOSITION OF PROBIOTICATED GINGER-BASED BEVERAGES

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ABSTRACT

The study evaluated the effect of *probiotication* on the physico-chemical characteristics and proximate composition of two ginger-based functional drinks. Probiotic ginger and ginger with garlic beverages were produced by inoculating the beverages with Lactic acid bacteria (LAB), *Lactobacillus plantarum* and *Lactobacillus bulgaricus* isolated from fermented sorghum grains and yoghurt, respectively. The beverages were stored at both ambient (27 ± 1 °C) and refrigeration (4 ± 1 °C) temperatures for a period of four weeks. Titratable acidity, pH, reducing sugars and proximate composition of the beverages were determined. Results showed that probiotic isolates reduced the pH of the beverages from 4.17 ± 0.40 to 3.04 ± 0.20 and increased the titratable acidity from 0.35 ± 0.10 to 0.84 ± 0.15. There was a reduction in total reducing sugars and carbohydrate content from 0.65 ± 0.22 mg/ml and 7.88 ± 0.03 % to 0.51 ± 0.15 mg/ml and 6.71 ± 0.10%, respectively. The crude protein and fat content increased from 2.33 ± 0.02 to 2.72 ± 0.01 % and 0.05 ± 0.01 % to 0.29 ± 0.02%, respectively throughout the period of storage. Health promoting drinks with minimal physico-chemical and proximate changes could be produced by inoculating ginger-based beverages with probiotic LAB.

Keywords: Physico-chemical, proximate, probiotic bacteria, ginger beverage.

1. INTRODUCTION

A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well being and/or reduction of risk of disease. Functional food may be considered as a therapeutic aid available without prescription. With functional food, various functions of organisms can be modulated (Gibson and Williams, 2000; Tomasik and Tomasik, 2003)

Japan, the birthplace, of the concept of functional food has adopted Foods for Specified Health Use (FOSHU) since 1991 to describe functional foods. Foods identified as FOSHU are required to provide evidence that the final product, but not isolated individual component(s), is likely to exert a health or physiological effect when consumed as part of an ordinary diet. Moreover, FOSHU products should be in the form of ordinary foods (i.e. not pills or capsules) (Roberfroid, 2000)

Fermented beverages constitute an integral part of the diet in most tropical and sub-tropical countries. The biochemical changes in plant materials during fermentation into beverage products have been investigated (Abiose and Adedeji, 1994)

Ginger beverage is produced with ginger rhizome as the major constituent and other minor constituents such as garlic, aloe vera, fruit juice and sweetened with sucrose or honey while ginger with garlic beverage is produced using ginger, garlic and sweetened with honey.

Ginger (*Zingiber officinale*) along with some 1,400 species of plants is placed in the family Zingiberaceae. Ginger contains volatile oil (inc. borneol, citral), phenols, alkaloid, and mucilage (Foster, 2008)

For ages, it has been embraced by the Chinese in the treatment of elevated blood pressure, elevated body temperature, persistent coughs, all kinds of colds and flu, and other related problems of the respiratory system. Fresh and dried ginger is used as two distinct and different herbal remedies. Fresh ginger has been applied in the treatment of fevers, headaches, and to alleviate the pain and discomfort of aching muscles in the body. Dried ginger remedy is used for the treatment of internal colds, and for physical symptoms such as cold and clammy hands, a weakening of the pulse rate, and a pale or white complexion in patients. Ginger-based preparations are also used in the treatment of joint stiffness, in the topical alleviation of abdominal cramps, in the treatment of kidney stone attacks, to treat stiffness in the neck, to treat neuralgia in different parts of the body. In addition, ginger compresses are employed topically to treat toothache, bladder inflammation, prostatitis and extreme tension in the body. Disorders such as nausea, accumulated intestinal gas, motion sickness, colic can also be treated using herbal remedies made from the ginger.

Garlic (*Allium sativum*) is a member of the Amaryllis family (Amaryllidaceae), which also includes leeks, onions, and shallots. It is a perennial with an underground bulb and is composed of pungent bulblets commonly called cloves (Harris, 1997).

Garlic is made up of several important components. When garlic bulbs are crushed, alliin, an odourless sulphur-containing chemical derived from the amino acid cystein, is converted into another compound called allicin. Allicin appears to be at least one of the primary active compounds that gives garlic its characteristic odor and many of its healing benefits. Allicin confers infection – fighting action, and the ability to lower blood pressure and cholesterol on garlic. Its antimicrobial properties on *Salmonella*, *E. coli*, and some gram-positive bacteria have been documented (Quattara et al., 1997.

Honey is carbohydrate-rich syrup produced by bees, primarily from floral nectars. Besides a high content of a range of saccharides, there are also organic acids, amino acids, mineral matter, colours, aromatic substances and a trace amount of fats. Besides, honey contains very valuable but unstable compounds, such as enzymes, substances of hormonal character, some vitamins and a few minor compounds. Honey has been demonstrated in many studies to have antibacterial effect, on a range of enteropathogenic strains including *Salmonella typhi*, *Vibrio cholerae* and *Yersinia enterocolitica*. It has also been successfully applied in the treatment of abdominal wounds, ulcers, wounds, gangrene and a host of other illnesses (Obi et al., 1994).

The lactic acid bacteria, a group of probiotic microbes, have been found to play a fundamental role in the gut, which enables them to influence the colonization of the gut by other organisms, which are beneficial to the host (Havenaar and Veld, 1992.; Senjong et al, 2000). Other beneficial characteristics that influence their choice include their: easy reproducibility, ability to survive the environmental conditions of the location where they are active, their genetic stability without plasmid transfer, the absence of allergic, toxic, mutagenic or carcinogenic reactions neither its fermentation products nor its cell components being deleterious after consumption by the host, ability to remain viable during processing and, ability to adhere to and colonize the location where they are active (Havenaar, and Veld, 1992; Wolfgand, et al., 1999; Senjong, et al, 2000). For some time now, lactic acid bacteria and bifidobacteria are employed as probiotics in foods especially in fermented dairy products. The new trend is to incorporate probiotics in other foods and drinks as a result of quest of consumers for health promoting functional foods.

There is a rising global demand by consumers for these products due to their health promoting potentials. This work therefore, evaluates the physico-chemical changes associated with ginger-based beverages inoculated with probiotic lactic acid bacteria and stored at both ambient and refrigeration temperatures for a period of four weeks.

2. MATERIALS AND METHODS

2.1. Materials

Ginger Sorghum grains, ginger rhizomes and garlic were obtained from a local market in Ile-Ife, Osun State; pure honey was obtained from a reputable supplier on campus while yoghurt was prepared in the laboratory. The pathogenic organisms, *Escherichia coli* and *Staphylococcus aureus* used were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife.

2.2. Bacterial Isolates For *probiotication*

Standard methods were employed in isolation and characterization of Lactobacilli spp. from steeped water of sorghum and maize grains, and yoghurt (Harrigan, 1998).

They were identified as *Lactobacillus plantarum* and *L. bulgaricus*

2.3. Preparation Of Probiotic Ginger And Ginger With Garlic Beverage

Ginger beverage was prepared using the modified method of (Osuntogun and Aboaba, 2004). This was done by peeling 400 g of wholesome fresh ginger rhizomes and wet milling with 500 ml of water. After wet milling, water (4.5 liters) was added to the smooth paste and subsequently strained through a clean white muslin cloth to obtain the ginger extract. The filtrate was left for 8 hours to sediment with a clear supernatant. The supernatant was sweetened by adding 1000 ml of pure honey. The sweetened ginger extract was kept in the refrigerator for 3 hours for the second sedimentation where the supernatant formed was decanted and dispensed into 500 ml screw cap glass bottles.

The ginger beverage was sterilized by autoclaving at 121°C for 15 minutes, allowed to cool to ambient temperature and finally inoculated separately with 10% probiotic isolates (*Lactobacillus plantarum* and *Lactobacillus bulgaricus*). Ginger with garlic beverage was prepared following the same procedure except that 50 g of garlic was added and milled thoroughly. Inoculated beverages were subsequently stored at ambient and refrigeration temperatures for a period of four weeks. On weekly basis, pH, titratable acidity, reducing sugar content as well as the proximate composition of the *probioticated* ginger, and ginger with garlic beverages were evaluated.

2.4. Titratable Acidity and pH Determination

Titratable acidity was determined according to (Harrigan, 1998) method while pH was measured using a Pye unicam Model 290 pH meter (AOAC, 2000).

2.5. Reducing Sugar Determination

This was determined using the dinitrosalicyclic acid (DNSA) method which involved boiling a known amount of sample with known amount of DNSA and determining its absorbance at 540nm in a spectrophotometer. Absorbances of samples were used as estimates of sugar concentrations when compared with a standard glucose calibration curve. The curve was obtained by plotting absorbances of glucose (of varying concentrations) boiled with DNSA reagent using a Pharmacia Biotech Novaspec 2 model spectrophotometer (Miller, 1959; Adeniran and Abiose, 2009).

2.6. Proximate Composition

Protein, fat, ash, moisture, crude fibre, carbohydrate and total solid content of the probioticated samples over the period of storage to assess the effect of probiotication on the samples (AOAC, 2000).

2.7. Statistical Analysis

The data obtained were subjected to analysis of variance and means of triplicate values were compared using Duncan's multiple range test (Steel et al., 1997).

3. RESULTS AND DISCUSSION

3.1. Titratable Acidity and pH of Probiotic Ginger and Ginger with Garlic beverages

The total titratable acidity values of ginger beverage inoculated with *Lactobacillus plantarum* is shown in Fig.1. At

ambient temperature storage, the total titratable acidity increased from an initial value of 0.40 to 0.84 at the 4th week while at refrigeration temperature it increased from 0.40 to 0.59 at the 4th week of storage. There was an initial increase in total titratable acidity from 0.45 to 0.83 and 0.67 at ambient and refrigeration temperatures respectively in ginger with garlic beverage at the 4th week of storage.

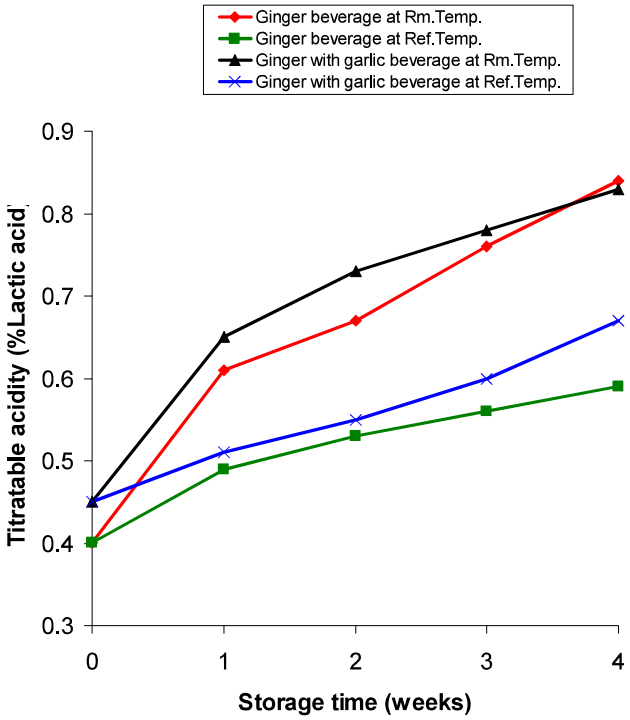

Fig.1: Percentage titratable acidity of ginger and ginger with garlic beverages inoculated with L. plantarum stored at ambient and refrigeration temperatures.

The changes in pH of the beverages inoculated with *Lactobacillus plantarum* is shown in Fig.2. At ambient temperature, the pH declined from an initial value of 3.92 to 3.04 after 4 weeks of storage while during refrigeration storage, the pH ranged between 3.92 and 3.51. A similar trend was observed in ginger with garlic beverage. Ginger is reported to contain 9.11 % wet weight starch (Oti and Mgbolu, 1987). Utilization of the sugars and/or the starch could have caused production of organic acids as end products of fermentation thus lowering the pH of the medium (Schillinger and Lucke, 1987). There was a significant difference ($p < 0.05$) in the change in pH between ginger and ginger with garlic beverages stored at ambient and refrigeration temperatures. The relatively stable pH values of samples stored at refrigeration temperature could be attributed to the effect of cold storage on the microbial cells and its metabolic activities (Yoon et al, 2004).

Ginger beverage that was inoculated with *Lactobacillus bulgaricus* exhibited a similar trend in the increase of titratable acidity and decline in pH values during the period of storage at both storage temperatures (Figs 3 and 4).

3.2. Total Reducing Sugar Content of Probiotic Ginger Beverage

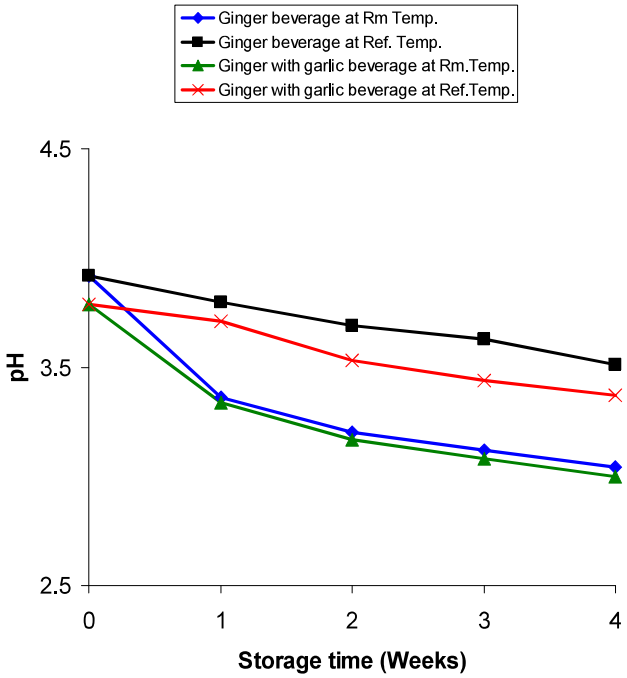

Fig. 2: pH values of ginger and ginger with garlic beverages inoculated with L. plantarum stored at ambient and refrigeration temperatures

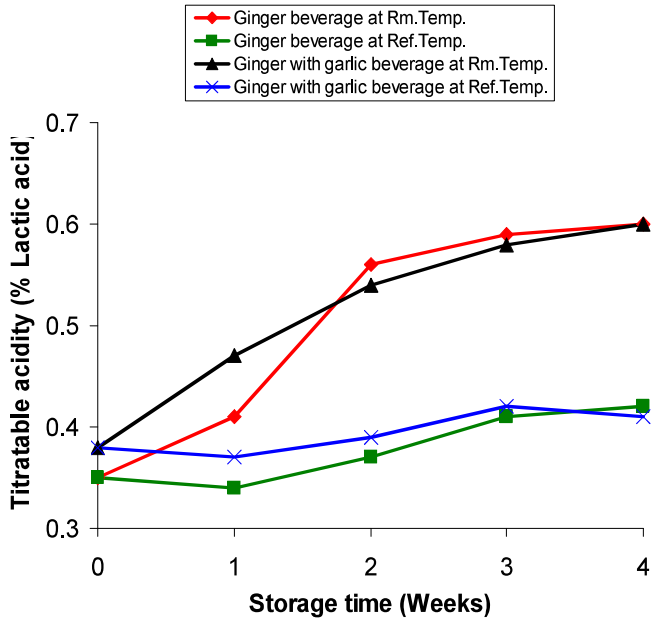

Fig. 3 Percentage titratable acidity of ginger and ginger with Garlic beverages inoculated with L. bulgaricus stored at ambient and refrigeration temperatures

The total reducing sugar content of ginger and ginger with garlic beverages inoculated with *Lactobacillus plantarum* and stored at ambient and refrigeration temperature is presented in Fig 5. The reducing sugar content of ginger samples increased from the initial value of 0.65 mg/ml to 0.70 mg/ml after the first week of storage at ambient temperature. During the remaining period of storage, the sugar content decreased consistently to 0.51 mg/ml at the 4th week of storage. At refrigeration temperature just as in

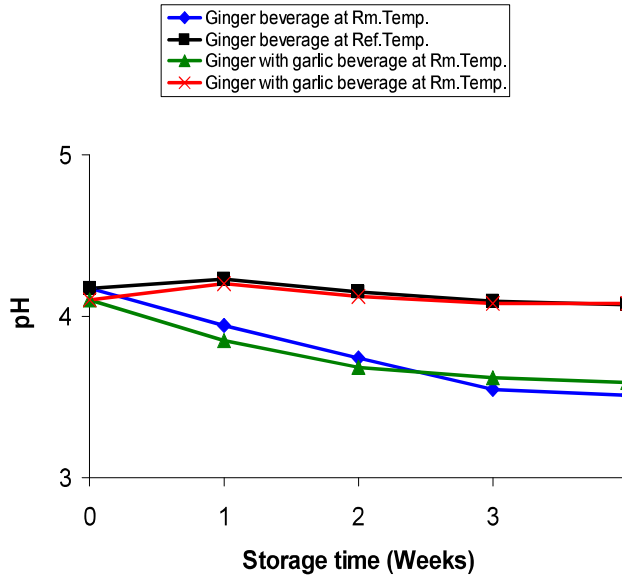

Fig. 4: pH values of ginger and ginger with garlic beverages inoculated with L. bulgaricus stored at ambient and refrigeration temperatures

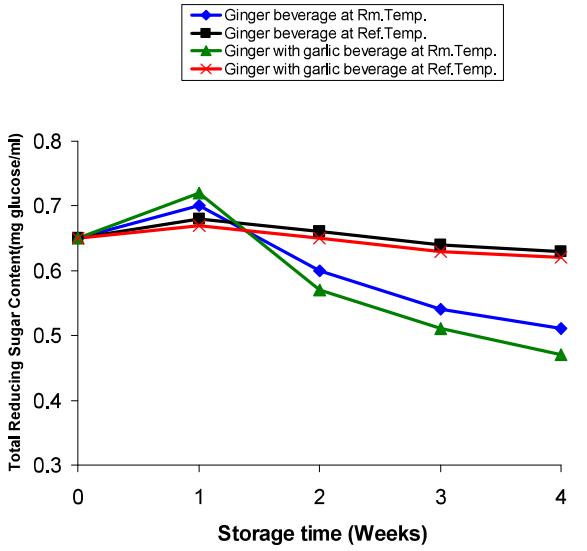

Fig. 5 Total reducing sugar content of ginger and ginger with garlic beverages inoculated with L. plantarum stored at ambient and refrigeration temperatures

ambient temperature storage, a gradual decrease in sugar content was observed in the two samples of ginger and ginger with garlic beverages. There was no significant difference ($p > 0.05$) in the decrease of reducing sugars in ginger and ginger with garlic beverages inoculated with *Lactobacillus plantarum* and stored at ambient and refrigeration temperatures. Samples *probioticated* with *Lactobacillus bulgaricus* displayed a similar trend, the initial sugar content at week zero increased slightly from 0.65 to 0.68 mg/ml at week one and gradually reduced to 0.62 mg/ml at the end of storage period. During refrigeration storage the sugar content was relatively stable throughout the storage period. A similar trend was observed in ginger with garlic beverage as presented in Fig.6.

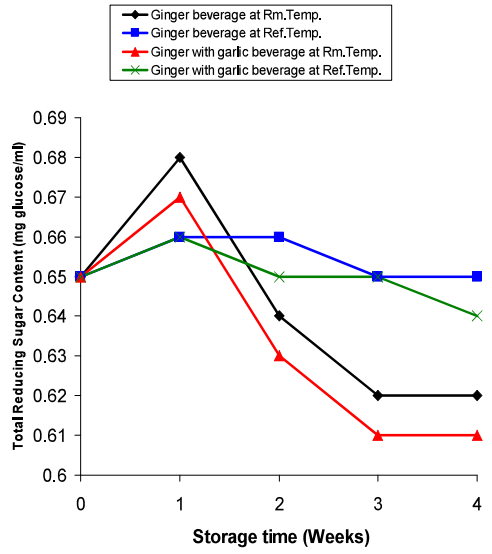

Fig. 6 Total reducing sugar content of ginger and ginger with garlic beverages inoculated with L. bulgaricus stored at ambient and refrigeration temperatures

Also ginger and ginger with garlic beverages containing *Lactobacillus bulgaricus* showed no significant difference ($p > 0.05$) in the decrease in reducing sugars at both ambient and refrigeration temperatures throughout the storage period.

The initial increase in reducing sugar content of all the probiotic beverages within the first week of storage could have been as a result of hydrolytic activities of the probiotic organisms which hydrolyzed the carbohydrate component of the beverages into fermentable sugars. It has been reported (Singleton, 1997) that certain hydrolytic bacteria liberate enzymes which hydrolyze a range of compounds as nutrients. The fermentable sugars are found to be good substrates for the growth of microorganisms (Brankori and Baras, 2001; Ray, 2004). After hydrolysis, the sugars were subsequently utilized by the increased population of LAB leading to its gradual decrease from 10.27 g/100g to 9.64 g/100g and 9.73 g/100g during ambient and refrigeration temperature respectively at the 4th week of storage.

3.3. Carbohydrate Content of Probiotic Ginger Beverage

The carbohydrate content of ginger beverage inoculated with *Lactobacillus plantarum* decreased consistently from the initial value of 7.88 % to 6.41 % and 6.71 % at the 4th week during ambient and refrigeration temperature storage respectively. The consistent decrease in carbohydrate content was also observed in ginger with garlic samples throughout the period of storage at both temperatures. In samples that were inoculated with *Lactobacillus bulgaricus*, a similar trend was noticed as there was a consistent reduction in the carbohydrate content throughout the period of storage as presented in Tables 1- 4.

The reduction of carbohydrate content in ginger and ginger with garlic beverages inoculated with *Lactobacillus plantarum* and *Lactobacillus bulgaricus* showed a significant difference ($p < 0.05$) when stored at ambient and refrigeration temperatures. The consistent reduction in carbohydrate content of all the probiotic samples could have been caused by the activities of the metabolizing organisms (Singleton, 1997; Brankori and Bars, 2001).

Table 1: Proximate analysis of ginger beverage inoculated with L. plantarum stored at ambient and refrigeration temperatures.

Time in weeks	Sample code	% Protein	% Fat	% Ash	% Moisture	% CHO	Total Solids g/100g
0	GLpiRm	2.33 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	89.68 ± 0.01	7.88 ± 0.03	10.32 ± 0.01
	GLpiRe	2.33 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	89.68 ± 0.01	7.88 ± 0.03	10.32 ± 0.01
1	GLp1Rm	2.63 ± 0.03	0.23 ± 0.03	0.24 ± 0.01	90.15 ± 0.01	6.75 ± 0.05	9.85 ± 0.08
	GLp1Re	2.28 ± 0.04	0.21 ± 0.02	0.21 ± 0.01	90.09 ± 0.17	7.21 ± 0.01	9.91 ± 0.10
2	GLp2Rm	2.64 ± 0.01	0.25 ± 0.01	0.23 ± 0.02	90.25 ± 0.27	6.63 ± 0.01	9.75 ± 0.11
	GLp2Re	2.33 ± 0.01	0.28 ± 0.02	0.20 ± 0.02	90.20± 0.13	6.99 ± 0.06	9.80 ± 0.13
3	GLp3Rm	2.67 ± 0.02	0.29 ± 0.01	0.27 ± 0.01	90.32 ± 0.16	6.45 ± 0.07	9.68 ± 0.07
	GLp3Re	2.48 ± 0.06	0.31 ± 0.01	0.24 ± 0.01	90.28 ± 0.12	6.69 ± 0.05	9.72 ± 0.06
4	GLp4Rm	2.72 ± 0.01	0.28 ± 0.03	0.22 ± 0.02	90.37 ± 0.22	6.41 ± 0.03	9.63 ± 0.02
	GLp4Re	2.49 ± 0.01	0.29 ± 0.02	0.18 ± 0.02	90.33 ± 0.10	6.71 ± 0.10	9.67 ± 0.05

GLp = Ginger beverage inoculated with *L. plantarum* i – 4 =Period (weeks) Rm = Room,
Re = Refrigeration Temperature. Values are means ± SE of replicate

Table 2: Proximate analysis of ginger beverage inoculated with L. bulgaricus stored at ambient and refrigeration temperatures.

Time in weeks	Sample code	% Protein	% Fat	% Ash	% Moisture	% CHO	Total Solids g/100g
0	GLbiRm	2.33 ± 0.07	0.05 ± 0.01	0.06 ± 0.01	89.73 ± 0.18	7.83 ± 0.04	10.27 ± 0.05
	GLbiRe	2.33 ± 0.07	0.05 ± 0.01	0.06 ± 0.01	89.73 ± 0.18	7.83 ± 0.04	10.37 ± 0.05
1	GLb1Rm	2.49 ± 0.04	0.15 ± 0.02	0.18 ± 0.03	90.03 ± 0.26	7.15 ± 0.08	9.97 ± 0.02
	GLb1Re	2.47 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	90.12 ± 0.09	7.16 ± 0.07	9.88 ± 0.07
2	GLb2Rm	2.54 ± 0.01	0.20 ± 0.03	0.21 ± 0.01	90.30 ± 0.22	6.65 ± 0.06	9.70 ± 0.05
	GLb2Re	2.50 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	90.14 ± 0.39	7.01 ± 0.05	9.86 ± 0.10
3	GLb3Rm	2.55 ± 0.40	0.21 ± 0.01	0.18 ± 0.01	90.35 ± 0.26	6.76 ± 0.01	9.70 ± 0.02
	GLb3Re	2.52 ± 0.02	0.18 ± 0.01	0.12 ± 0.01	90.26 ± 0.30	6.95 ± 0.05	9.74 ± 0.09
4	GLb4Rm	2.58 ± 0.01	0.21 ± 0.05	0.15 ± 0.02	90.36 ± 0.11	6.70 ± 0.10	9.64 ± 0.06
	GLb4Re	2.53 ± 0.01	0.18 ± 0.04	0.10 ± 0.01	90.27 ± 0.14	6.92 ± 0.09	9.73 ± 0.11

GLb = Ginger beverage inoculated with *L. plantarum* i – 4 =Period (weeks) Rm = Room,
Re = Refrigeration Temperature. Values are means ± SE of replicate.

Table 3: Proximate analysis of ginger with garlic beverage inoculated with L. plantarum stored at ambient and refrigeration temperatures.

Time in weeks	Sample code	% Protein	% Fat	% Ash	% Moisture	% CHO	Total Solids g/100g
0	GGLpiRm	2.34 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	89.64 ± 0.05	7.89 ± 0.05	10.36 ± 0.05
	GGLpiRe	2.34 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	89.64 ± 0.05	7.89 ± 0.05	10.36 ± 0.05
1	GGLp1Rm	2.77 ± 0.06	0.23 ± 0.03	0.27 ± 0.01	90.37 ± 0.11	6.49 ± 0.04	9.76 ± 0.08
	GGLp1Re	2.37 ± 0.05	0.21 ± 0.02	0.23 ± 0.02	90.30 ± 0.13	6.89 ± 0.08	9.70 ± 0.06
2	GGLp2Rm	2.84 ± 0.05	0.30 ± 0.01	0.22 ± 0.01	90.28 ± 0.41	6.36 ± 0.03	9.72 ± 0.12
	GGLp2Re	2.64 ± 0.05	0.28 ± 0.01	0.19 ± 0.02	90.34 ± 0.45	6.55 ± 0.08	9.66 ± 0.10
3	GGLp3Rm	2.86 ± 0.03	0.32 ± 0.01	0.29 ± 0.01	90.36 ± 0.15	6.17 ± 0.05	9.64 ± 0.05
	GGLp3Re	2.67 ± 0.03	0.34 ± 0.01	0.22 ± 0.02	90.38 ± 0.24	6.39 ± 0.10	9.62 ± 0.04
4	GGLp4Rm	2.93 ± 0.01	0.29 ± 0.02	0.28 ± 0.03	90.50 ± 0.10	6.00 ± 0.09	9.50 ± 0.10
	GGLp4Re	2.67 ± 0.01	0.32 ± 0.02	0.24 ± 0.02	90.43 ± 0.26	6.34 ± 0.01	9.57 ± 0.05

GLp = Ginger beverage inoculated with *L. plantarum* i – 4 =Period (weeks) Rm = Room, Re = Refrigeration Temperature. Values are means ± SE of replicate.

Table 4: Proximate analysis of ginger with garlic beverage inoculated with L. bulgaricus stored at ambient and refrigeration temperatures.

Time in weeks	Sample code	% Protein	% Fat	% Ash	% Moisture	% CHO	Total Solids g/100g
0	GGLbiRm	2.40 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	89.85 ± 0.24	7.63 ± 0.05	10.15 ± 0.03
	GGLbiRe	2.40 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	89.85 ± 0.24	7.63 ± 0.05	10.15 ± 0.03
1	GGLb1Rm	2.43 ± 0.03	0.19 ± 0.02	0.20 ± 0.05	90.04 ± 0.15	7.14 ± 0.07	9.96 ± 0.10
	GGLb1Re	2.44 ± 0.02	0.16 ± 0.02	0.15 ± 0.05	90.09 ± 0.15	7.16 ± 0.06	9.91 ± 0.02
2	GGLb2Rm	2.64 ± 0.01	0.21 ± 0.01	0.24 ± 0.01	90.22 ± 0.20	6.69 ± 0.03	9.78 ± 0.02
	GGLb2Re	2.51 ± 0.02	0.18 ± 0.02	0.22 ± 0.15	90.17 ± 0.01	6.93 ± 0.05	9.84 ± 0.01
3	GGLb3Rm	2.66 ± 0.02	0.23 ± 0.01	0.22 ± 0.01	90.30 ± 0.02	6.59 ± 0.06	9.70 ± 0.02
	GGLb3Re	2.42 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	90.27 ± 0.02	6.94 ± 0.03	9.73 ± 0.02
4	GGLb4Rm	2.67 ± 0.01	0.21 ± 0.02	0.19 ± 0.02	90.31 ± 0.02	6.62 ± 0.03	9.69 ± 0.02
	GGLb4Re	2.48 ± 0.02	0.18 ± 0.02	0.16 ± 0.15	90.27 ± 0.01	6.91 ± 0.04	9.73 ± 0.01

GLb = Ginger beverage inoculated with *L. plantarum* i – 4 =Period (weeks) Rm = Room
Re = Refrigeration Temperature. Values are means ± SE of replicate.

3.4. Total Solid Content of Probiotic Ginger based-Beverages

The total solid content of ginger beverage inoculated with *Lactobacillus plantarum* reduced from an initial value of 10.32 g/100 g to 9.63 g/100 g and 9.67/100 g at the 4th week during ambient and refrigeration temperature storage respectively as presented in Table 1. A similar trend was observed in ginger with garlic beverage as the total solid decreased during the storage period as shown in Table 2. There was a significant difference ($p < 0.05$) in the reduction of total solid in both ginger and ginger with garlic beverages when stored at ambient and refrigeration temperatures. When *Lactobacillus bulgaricus* was inoculated into the samples the initial value of total solid reduced from 10.27 g/100g to 9.64 g/100g and 9.73 g/100g during ambient and refrigeration temperature respectively at the 4th week of storage. In *Lactobacillus bulgaricus* inoculated ginger with garlic beverage, a similar trend of reduction in total solids was observed at both ambient and refrigeration temperatures throughout the period of storage as presented in Tables 3 and 4. Also, there was a significant difference ($p < 0.05$) in the decrease of total solid between samples stored at ambient and refrigeration temperatures.

The reduction in total solids of the probiotic samples stored both at ambient and refrigeration temperature was probably due to utilization of nutrients by increasing microbial population.

3.5. Crude Protein Content Of Probiotic Ginger Beverages

The crude protein content of ginger beverage inoculated with Lactobacillus plantarum and stored at ambient and refrigeration temperatures is presented in Table 1. The protein content increased from the initial value of 2.33% to 2.72% and 2.49% at the 4th week during ambient and refrigeration temperature storage respectively. There was also an increase in the protein content of ginger with garlic beverage inoculated with Lactobacillus plantarum (Table 2). The increase in protein content between ambient and refrigeration temperature storage of ginger and ginger with garlic beverages inoculated with *L. plantarum* was significantly different ($p < 0.05$).

When *Lactobacillus bulgaricus* was inoculated into ginger beverage, the protein content increased from the initial value of 2.33 to 2.58% and 2.53% at the 4th week during ambient and refrigeration temperature storage respectively (Table 3). In ginger with garlic beverage inoculated with *Lactobacillus bulgaricus* (Table 4), the protein content similarly, increased over the period of storage at both ambient and refrigeration temperature. There was no significant difference in the crude protein content between ginger beverage stored at ambient and refrigeration temperature($p > 0.05$) but there was significant difference ($p < 0.05$) between ambient and refrigeration temperature storage of ginger with garlic beverage.

The non-reduction in the protein content of all samples may be due probably to protein synthesis and the antioxidant properties of ginger extracts as well as dead probiotic microbes which could have increased its protein content. (Kikuzaki et al, 1994; Belewu et al., 2005) observed that most of the isolated compounds from ginger exhibited very strong antioxidant effect on the metabolism of proteins by microorganisms in ginger extract.

3.6. Crude Fat Content of Probiotic Ginger Beverage

The crude fat content of ginger beverage inoculated with *Lactobacillus plantarum* and stored at ambient and refrigeration temperature is as shown in Table 1. The fat content increased from the initial value of 0.05% to 0.28% and 0.29% during ambient and refrigeration temperature respectively at the 4th week of storage. A similar trend was observed in all the samples as the crude fat content increased both at ambient and refrigeration temperature storage as presented in Tables 2, 3 and 4. There was no significant difference ($p > 0.05$) in the increase of fat content between ginger and ginger with garlic beverages inoculated with *Lactobacillus plantarum* and stored at ambient and refrigerated temperature. However, a significant difference ($p < 0.05$) exists between samples inoculated with *Lactobacillus bulgaricus* and stored at ambient and refrigeration temperature.

The increase in crude fat content over the period of storage in all samples could be due to the effect of antioxidant properties of ginger and garlic on lipid metabolism of probiotic isolates. Krishnakantha and Lokesh, 1993; Belewu et al., 2005 reported that ginger inhibits lipid oxidation and scavenges super-oxide anions.

4. CONCLUSION

This study has shown that two probiotic bacterial isolates (*L. plantarum and L. bulgaricus*) exhibited capability of surviving in ginger and ginger with garlic beverages for a period of four weeks as their metabolic activities led to changes in the physico-chemical parameters of the beverages. The consistent increase in the percentage titratable acidity with a corresponding decrease in pH values, reduction in fermentable sugar content as well as total solid content indicated the viability of the probiotic bacteria isolate in the beverages. These changes are considered desirable as lowered pH and higher titratable acidity play an important role in preservation of fermented drinks. No significant difference was observed between the two beverages that are inclusion of garlic in a set of the beverage did not produce any significant effect on the physic-chemical properties or proximate composition of the beverages ($p > 0.05$). More importantly, presence of probiotic LAB in drinks like ginger-based extracts combines the health promoting potentials of the microbe with that of ginger and/or garlic.

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