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RESEARCH PAPER

Preparation of Probiotic Apple Juice by Lactic Acid Fermentation

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Abstract

Fermentation of apple juice was done for 72 hrs by lactic acid bacteria (*Lactobacillus plantarum* and *Streptococcus thermophilus*) and physico-chemical characteristics of fermented apple juices were recorded at 0. 24, 48 and 72 hrs intervals. On the basis of highest lactic acid bacterial counts, rate of fermentation (°B/24hr), acidity, sugar utilization percentage, retention of ascorbic acid and total phenols, antioxidant activity and antimicrobial activity against pathogenic bacteria such as *E. coli* and *S. aureus*, while lowest pH, coliform, yeast and mold counts, the best treatments *L. plantarum* 10^7cfu/ ml for apple fermented apple juice was selected for storage study viz. During fermentation both vitamin C and total phenols declined, while, the antioxidant activity, antimicrobial activity, viable cell counts and lactic acid bacteria counts were increased. As the antimicrobial activity increased, the counts of coliform, yeast and mold declined. During storage of selected treatment of fermented apple juice for four weeks at 4°C retained physico-chemical parameters above the value of significance. Lactic acid bacteria remained viable in prescribed number ($4.76\text{-}6.00 \times 10^6$ cfu/ml), acid and bile resistance, imparted antimicrobial activity and antioxidant activity to the apple juices during storage and thus, could be called as probiotics juices. Based on the sensory evaluation (visual colour and flavours) of fermented apple juice, it was concluded that lactic acid fermentation did not influence the desirable characteristics of apple juices. So the probiotic juices were acceptable. Identification of lactic acid bacteria by biochemical test concluded that the bacteria were *L. plantarum* in apple juices which were inoculated in juices for fermentation, after storage apple juice were suitable for lactic acid fermentation to produce a probiotic beverage. So, this juice can serve as healthy beverage for vegetarians and lactose allergic consumers.

Keywords: Apple juice, probiotic, lactic acid bacteria, *Lactobacillus plantarum*, *Streptococcus thermophillus*

Food is the most essential requirement of all the living systems and man is no exception. With the advent of agriculture, the man cultivated all types of food crops resulting in a glut during the production season. Consequently, a huge postharvest loss of perishables and economic loss to the farmers took place. According to FAO, postharvest losses of fruit and vegetables range from 20-40% (FAO, 2013). Preservation by fermentation is well-known method and is also one of the oldest methods of food preservation in the world. Fermented foods can generally be described as palatable and wholesome foods, prepared from raw or heated raw materials by microbial fermentation (Holzapfel, 1997). Out of various fermentations, lactic acid fermentation is employed for commercial bulk storage of seasonal fruits and vegetables to increase their availability and to obtain a desired sensory quality of the products (Frazier and Westhoff, 1998). Lactic acid fermentation using LAB (lactic acid bacteria) cultures is used for the preparation of different products such as fermented grape juice, fermented peanut milk, yoghurt, fermented corn meal (*Kuhunzaki*) and fermented beverages from wheat and maize

(Joshi and Thakur, 2000). *Kimchi* and *Sauerkraut* are the well known products made by fermentation of vegetables. Lactic acid fermented foods such as '*Dahi*' is considered as a probiotics. Fermented foods like '*dahi*' are more nutritious than unfermented ones (Pederson, 1963; Joshi *et al*. 1999) and those foods are prepared by using lactic acid bacteria (LAB) have better acceptability (Hang and Jackson, 1967) than raw foods.

In 1900, Metchnikoff discovered the use of fermented milk in the diet for prevention of certain diseases of the gastro-intestinal tract and promotion of healthy day-to-day life. Since then, a number of studies have shown that the fermented food products do have a positive effect on the health of the consumers (Sahlin, 1999). Moreover, LAB has GRAS (generally recognized as safe) status and lactic acid fermented foods constitute 25 per cent of the European diet and 60 per cent of the diet in many developing countries (Stiles, 1996).

The word 'probiotics' originates from the Greek word 'for life' and is currently used to describe the bacteria associated with beneficial affects for human and animals. According to WHO, probiotics are defined as 'live organism which when administered in adequate amount confer a health benefits on the host'. Probiotic bacteria were first studied by Metchinkoff, a Nobel laureate of 1908 in the field of medicine (FAO/WHO, 2003). These bacteria ferment sugar (e.g. lactose) predominantly to lactic acid (Liu, 2003) and have a strong inhibitory effects on the growth and toxin production by other bacteria. Addition of probiotics to food provide several health benefits including reduction in the level of serum cholesterol, improvement of gastrointestinal function, enhancement of immune system and a reduction in risk of colon cancer (Berner and Odonnell, 1998). The health benefits of certain foods had been investigated for several years. Thus, development of foods that promote health and well being is one of the key research priorities of food industry (Klaenhammer and Kullen, 1999). The word 'probiotics' originates from the Greek word 'pro' and 'biotic' which means 'for life'. Probiotics are defined as 'live organism which when administered in adequate amount confer a health benefits on host'. Probiotic bacteria were first studied by Metchinkoff, a Nobel laureate of 1908 in the field of medicine, during 20th century. Most of the presently available probiotics foods are based on milk like fermented milk and yogurt. But these have several problems associated with their consumption such as lactose intolerance and increase in the level of the consumer's cholesterol. So attempt was made if fruit juice could serve as a medium for cultivating probiotics. The use of probiotics in the fruit and vegetable juice industry offer to the consumers of special needs (vegetarian people with allergic reactions to milk proteins), the possibility to experience the positive effect of probiotic bacteria as fruit and vegetables are the richest source of vitamins, minerals, antioxidants, sugars and phytochemicals. There is however, scanty of information on the lactic acid fermentation of fruits and vegetables juices especially with the respect to optimization of fermentation process as probiotic juices. That is why the present investigation has been proposed.

Amongst the fruits, apple (*Malus domestica*) is a prominent fruit and is a member of the rosaceae family, one of the most widely cultivated trees. It can be processed into sauce, slices or juice, wine, cider and vinegar and is favoured for pastries, cakes, tarts and pies (Downing, 1989). Because of its taste and flavor and nutrition apple juice holds promise to be used as a probiotic juice.

Similarly, the storage behavior and safety of such juices have not been documented. Fruit and vegetables are excellent source of minerals, vitamins, sugars, acids besides phyto-chemicals and therefore, consumption of fresh or processed products would be healthful to the consumers. In such products, if probiotic characteristics are also imparted it could add another feather to the already decorated cap of fruits and vegetables.

MATERIALS AND METHODS

Extraction of apple juice and their physico-chemical evaluation

The juice from apple fruits was extracted by following the standard methods (Lal *et al*., 1960; Woodruf and Luh, 1986). The fruits washed followed through a grater and then, extraction of juice was made by using a hydraulic press followed grating by straining.

The fruit juices were pasteurized at 100°C temperature for 10-15 mins immediately followed by hot filling, bottling and pasteurization of filled bottles (Woodruf and Luh, 1986).

Preparation of Probiotic Juices

Preparation of broths cultures: Freeze dried ampoules of cultures viz. *Lactobacillus plantarum* (MTCC 1407) and *Streptococcus thermophillus* (NCDC 074) were purchased from IMTECH, Chandigarh and NDRI, Karnal, respectively. MRS (de Mann Rogousa Sharpe) medium and litmus milk as a growth medium for *Lactobacillus plantarum* (MTCC 1407) and *Streptococcus thermophillus* (NCDC 074), respectively by the institutes. To prepare broth cultures, granules of freeze dried ampoules (*Lactobacillus plantarum* MTCC 1407 and *Streptococcus thermophillus* NCDC 074) were suspended in sterlized broths (MRS (de Mann Rogousa Sharpe) medium and litmus milk, respectively) and were incubated at recommended temperatures (30°C and 37°C, respectively) for recommended incubation period (24-48 hrs and 48hrs, respectively). After completion of incubation period, when colors in MRS broth changed from brownish yellow to whitish yellow and that of litmus milk changed from bluish purple to pinkish red due to growth of bacteria. Broths also when became turbid confirmed the growth of bacteria which was further confirmed by the microscopic examination of the stained smears (Harrigan and McCance, 1966).

Preparation of inocula: Two populationS viz. $10^5 \times 10^7$ cfu/ml of both the bacteria (*Lactobacillus plantarum* and *Streptococcus thermophillus*) were used for fermentation of each fruit and vegetable juice. Then, number of either cultures or bacteria was standardized by pour plate method (Aneja, 2003). One loop inoculation of both bacteria (*Lactobacillus plantarum* and *Streptococcus thermophillus*) was selected for 10^5 cfu/ml while two loops inoculation was selected for 10^7 cfu/ml (1 loop gave equal or more counts of bacteria than 1x 10^5 CFU/ml and 2 loops also gave equal or more counts of bacteria than 1x 10^7 CFU/ ml). After standardization, prepared the stock inocula lactic acid fermentation for apple fruits by inoculation of 1 loop for 10^5 cfu/ml and 2 loops for 10^7 CFU/ ml of both the bacteria (*Lactobacillus plantarum* and *Streptococcus thermophillus*) in separate flask of MRS broth and litmus milk, respectively.

Fermentation of apple fruit juices: To conduct the lactic acid fermentation, 5% inoculum of both bacteria (*Lactobacillus plantarum* and *Streptococcus thermophillus*) at the concentrations of 10^5 cfu/ml and 10^7 CFU/ ml were added in pasteurized apple juice under sterile condition (Yoon *et al.*, 2004). After inoculation of bacteria, fermentation of apple fruit juices was carried out for 72 hrs at the specified temperature in an incubator. On the basis of the physico-chemical and microbiological changes, the strandization of probiotic apple juices was carried out. The best combination used for further cold storage studies was selected.

Effect of cold storage on cell viability and physico-chemical parameters of probiotic apple juice

After completion of 72 h of lactic acid fermentation, the best treatment combination was selected of each probiotic fruit and vegetable juices, and were stored at 4°C for 4 weeks. Samples were taken at weekly interval, and viability of probiotic cultures in probiotic fruit and vegetable juices was determined and expressed as colony forming unit (CFU) (Yoon *et al*., 2006).

Physico-chemical and Microbiological analysis

The juices were analysed for various physico-chemical parameters like TSS, titratable acidity, pH, total sugar, reducing sugar, total phenols, vitamin C, antioxidant activity and juice yield immediately after extraction of juice.

During the fermentation, physico-chemical changes viz., TSS, pH, titratable acidity, total sugar, reducing sugar, total phenols, antioxidant activity, vitamin C, probiotic juice yield and microbiological analysis viz., viable cell counts, antimicrobial activity, lactic acid bacteria counts, coliform counts, detection of *Staphylococcus* and *Salmonella*, yeast, mold, taken at 0, 24, 48 and 72 hrs intervals. The physico-chemical parameters viz., TSS, pH, titratable acidity, total sugar, reducing sugar, total phenols, antioxidant activity, vitamin C (AOAC, 2004) and microbiological analytical parameters viz., viable cell counts, antimicrobial activity, lactic acid bacteria counts, coliform counts, detection of *Staphylococcus* and *Salmonella*, yeast, mold, acid tolerance and bile tolerance (Aneja, 2003) were taken at 0, 1,2,3,4 weeks interval.

Statistical analysis

Statistical analysis of variance of the quantitative data of chemical parameters and microbiological analysis obtained from the experiments was done by Completely Randomized Design (CRD) Factorial as given by Cockrane and Cox, (1963). Where the number of treatments were two, these were compared using't' and the test significance was determined using the statistical tables. The significance of the results were determined as 5% level of significance.

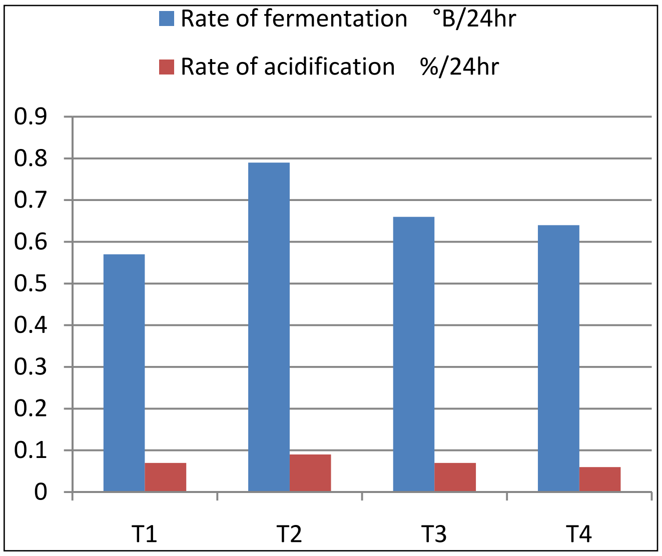
RESULTS AND DISCUSSION

Physico-chemical characteristics of apple juice

Physico-chemical characteristics of fresh apple juice showed that it was a good source of total soluble solids (TSS) (11.4±0.01°B), reducing sugar (6.32±0.14 %), total sugar (8.31±0.01 %), vitamin C (10.28±0.05 mg/100g), antioxidant activity (19.09±0.01%) but had medium acid (0.31±0.01% as malic acid) content, while pH value of the juice was recorded as 4.12±0.20, total phenols of the juice were recorded as 58.25±0.02 mg/100g and juice yield was recorded as 65.8±0.08%. These results of fresh apple juice corrborated the earlier reports of Kaushal and Sharma (1995); Savatovic *et al.* (2009), Chodak *et al.* (2011), Jan and Rab (2012) and Francini and Sebstiani (2013).

Effect of lactic acid fermentation on physico-chemical and microbiological characteristics of apple juice

The highest rate of fermentation (0.79°B//24hr) was recorded in fermentation with *L. plantarum* (10^7cfu/ml), while the lowest rate of fermentation (0.57%/24hr) was recorded in fermentation with *L. plantarum* (10^5cfu/ml) (Fig. 1). Among the four treatments, *L. plantarum* (10^7cfu/ml) gave the highest rate of fermentation (°B/24hr).



T_1 (*L.plantarum* 10^5cfu/ml) T_2 (*L.plantarum* 10^7cfu/ml)
T_3 (*S.thermophilus* 10^5cfu/ml) T_4 (*S.thermophilus* 10^7cfu/ml)

Fig. 1: Effect of lactic acid fermentation on rate of fermentation and rate of acidification

The general trend of change in TSS during lactic acid fermentation is shown in Fig. 2, wherein the TSS decreased with increase in time (0-72hr). Table 1 summarizes the effect of lactic acid fermentation on the TSS of apple juice. The highest TSS (10.24°B) was recorded in fermentation with *L. plantarum* (10^5cfu/ml) and the lowest TSS (9.73°B) was recorded in fermentation with *L. plantarum* (10^7cfu/ml). Among the four treatments, *L. plantarum* (10^7cfu/ml) was the best treatment with the highest rate of fermentation after 72 hr of fermentation. Similar to this, decrease in Brix (TSS) during lactic acid fermentation of red and yellow watermelon has been reported by Alavi *et al.* (2012) and Yoon *et al.* (2004) and that the decrease was due to the utilization of soluble solids (sugar) by lactic acid bacteria to produce lactic acid.

The general trend of change in pH during lactic acid

Table 1: Effect of lactic acid fermentation on TSS, pH and titratable acidity of apple juice

Time interval (hrs)	TSS(°B)				Mean 0	pH				Mean 0	Titratable acidity (% Lactic acid)				Mean
Treatments	**0**	**24**	**48**	**72**		**0**	**24**	**48**	**72**		**0**	**24**	**48**	**72**	
T_1 (*L.plantarum* 10^5cfu/ml)	11.40	10.00	9.87	9.70	10.24	3.24	2.95	2.64	2.56	**2.84**	0.310	0.357	0.403	0.517	**0.397**
T_2(*L.plantarum* 10^7cfu/ml)	11.40	9.30	9.17	9.03	9.73	3.24	2.85	2.61	2.36	**2.77**	0.310	0.383	0.467	0.580	**0.435**
T_3(*S.thermophilus* 10^5cfu/ml)	11.40	9.83	9.72	9.43	10.10	3.24	2.95	2.78	2.42	**2.85**	0.310	0.383	0.417	0.510	**0.397**
T_4(*S.thermophilus* 10^7cfu/ml)	11.40	9.90	9.77	9.47	10.03	3.24	2.80	2.63	2.47	**2.79**	0.310	0.357	0.410	0.500	**0.394**
Mean	11.40	9.76	9.63	9.41		**3.24**	**2.89**	**2.71**	**2.45**		**0.310**	**0.362**	**0.424**	**0.527**	
$CD_{0.05}$															
Treatment (A)					0.046					0.022					0.008
Time Interval (B)					0.046					0.022					0.008
A×B					0.09					0.045					0.016

fermentation of apple is shown in Fig. 2, wherein it is apparent that the pH decreased with increase in time (0-72hr). Table 1 summarizes the effect of lactic acid fermentation on the pH of apple juice, the highest pH (2.86) was recorded in fermentation with *L. plantarum* (10^5cfu/ml) while the lowest pH (2.80) was recorded in fermentation with *L.plantarum* (10^7cfu/ml). Our results are in conformation with those reported earlier for different fruit and vegetable juices during lactic acid fermentation including tomato, cabbage, pomegranate, watermelon and roselle juice (Yoon *et al.*, 2004; Yoon *et al.* 2005; Yoon *et al.* 2006; Tantipaibulvut *et al.* 2008; Guo *et al.* 2009 and Hassan *et al.* 2011).

The general trend of change in titratable acidity during lactic acid fermentation is shown in figure 2 and summarized in Table 1. The titratable acidity increased with increase in time (0-72hr). The highest titratable acidity (0.397%) was recorded in fermentation with *L. plantarum* (10^7cfu/ml) with highest rate of acidification (0.07%/24hr), while the lowest titratable acidity (0.394%) was recorded in fermentation with *S. thermophillus* (10^7cfu/ml) with lowest rate of acidification (0.06%/24hr). Among the four treatments, *L.plantarum* (10^7cfu/ml) was the best treatment with highest lactic acid production after 72 hr of fermentation. Yoon *et al.* (2004); (2005); (2006) have shown that the pH and acidity decreased and increased respectively during lactic acid fermentation of fermented cabbage, beet and roselle juice, respectively. Similar results have been reported by Shah and Jelen (1990) and Joshi and Sharma (2009) for reddish and carrot.

The trend of decrease in total sugars during lactic acid fermentation is shown in Fig. 2 while Table 2 summarizes the effect of lactic acid fermentation on the total sugars of apple juice. The highest total sugar (8.21%) was recorded in fermentation with *L. plantarum* (10^5cfu/ml), while the lowest total sugar (8.19%) was recorded in fermentation with *S. thermophillus* (10^7cfu/ml). Among the four treatments, *L. plantarum* (10^7cfu/ml) was the best treatment with highest sugar utilization (0.10%/24hr) after 72 hr of fermentation. Similar results have been reported by Yoon *et al.* (2004); (2006) and Tantipaibulvut *et al.* (2008) during lactic acid fermentation of various fruits and vegetables.

The trend of change in the reducing sugars during lactic acid fermentation is shown in figure 2 similar to total sugars, wherein the reducing sugar is decreased with increase in time (0-72hr). The highest reducing sugars (6.18%) was recorded in fermentation with *S.*

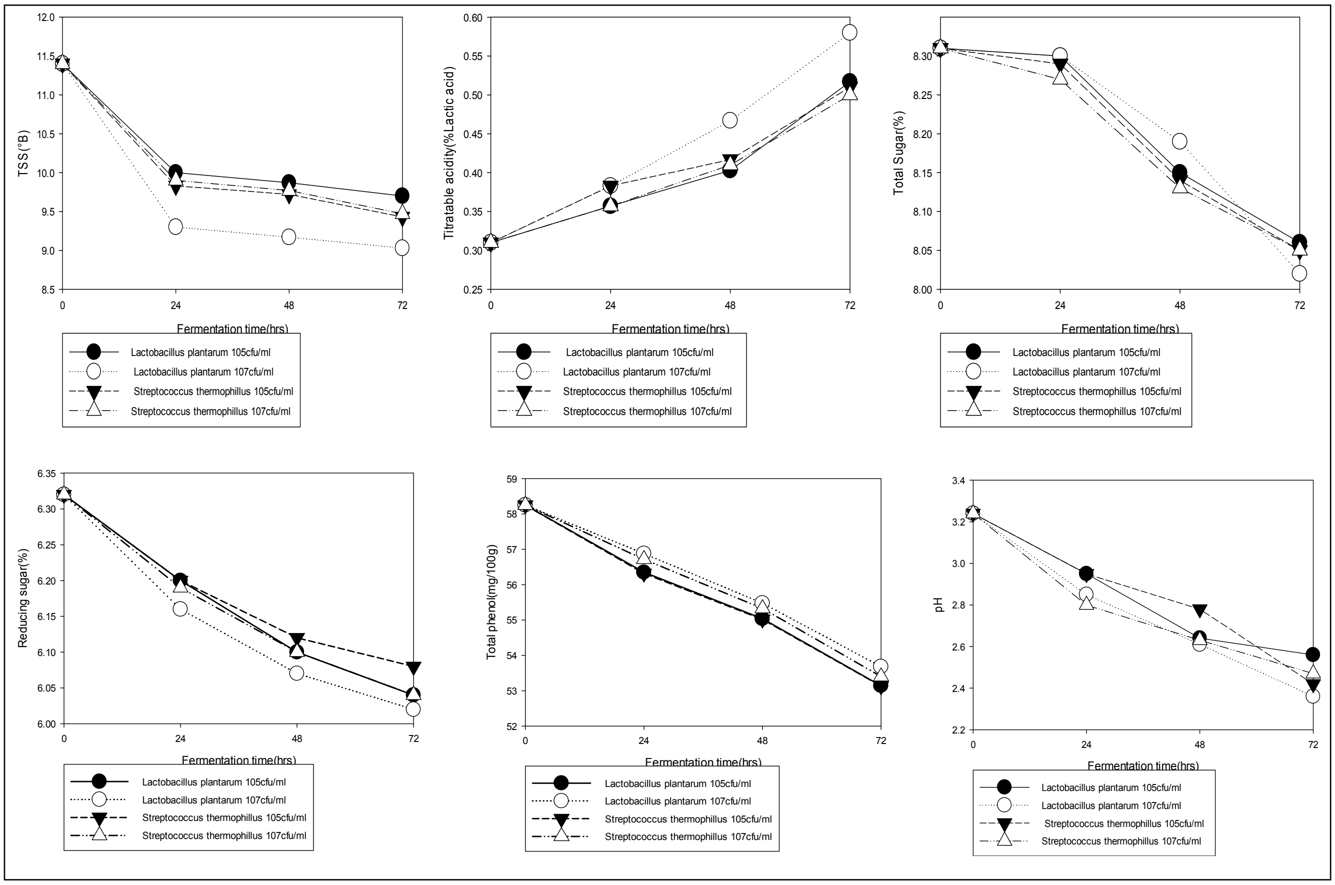


Fig. 2: Changes in TSS, pH, titratable acidity, total sugar, reducing sugar and total phenols during lactic acid fermentation of apple juice

thermophillus (10^5cfu/ml) while the lowest reducing sugars (6.14%) was recorded in fermentation with *L. plantarum* (10^7cfu/ml). Among the four treatments, *L. plantarum* (10^7cfu/ml) was the best treatment with highest sugar utilization (0.10%/24hr) after 72 hr of fermentation.

Babu *et al.* (1992) reported that lactic acid bacteria improved the sugar utilization with more acid production and low pH during lactic acid fermentation of tomato juice as reported by Yoon *et al.* (2004); (2006) and Tantipaibulvut *et al.* (2008) during lactic acid fermentation of various fruits and vegetables juices.

Physico-chemical characterstics

The trend of change in the total phenols during lactic acid fermentation (0-72hrs) is shown in figure 2 and summarized in Table 3. The highest total phenols content (56.07mg/100g) was recorded in fermentation with *L. plantarum* (10^7cfu/ml) while the lowest total phenols (55.68mg/100g) was recorded in fermentation with *S. thermophillus* (10^5cfu/ml). Subrota *et al.* (2015) have been reported that diminishing effect of fermentation on polyphenols was due to the activity of polyphenol oxidase in the food grain or microflora as also been reported by Whiting and Coggins (1974), Whiting (1975), Ciafardini *et al.* (1994) and Marsilio and Lanza (1998). The reduction in polyphenolic content through fermentation improved the digestibility of proteins and carbohydrates. *L.plantarum* was able to degrade some food phenolic compounds and after degradation gave those compounds which influenced the food aroma and ultimately increased the antioxidant activity (Subrota *et al.*, 2015). Results

Table 2: Effect of lactic acid fermentation on reducing sugars, total sugars and total phenols of apple juice

Time interval (hrs) / Treatments	Total Sugar (%)				Mean	Sugar utilization (%/24hrs)	Reducing Sugar (%)				Mean	Sugar utilization (%/24hrs)	Total phenols (mg/100gm)				Mean
	0	24	48	72			0	24	48	72			0	24	48	72	
T_1 (*L.plantarum* 10^5cfu/ml)	8.31	8.30	8.15	8.06	8.21	**0.08**	6.32	6.20	6.10	6.04	6.16	0.09	58.25	56.35	55.04	53.14	55.69
T_2 (*L.plantarum* 10^7cfu/ml)	8.31	8.30	8.19	8.02	8.20	**0.10**	6.32	6.16	6.07	6.02	6.14	0.10	58.25	56.88	55.48	53.68	56.07
T_3(*S. thermophilus* 10^5cfu/ml)	8.31	8.29	8.14	8.05	8.20	**0.09**	6.32	6.20	6.12	6.08	6.18	0.09	58.25	56.32	55.02	53.13	55.68
T_4(*S. thermophilus* 10^7cfu/ml)	8.31	8.27	8.13	8.05	8.19	**0.09**	6.32	6.19	6.10	6.04	6.16	0.09	58.25	56.72	55.33	53.40	55.93
Mean	8.31	8.29	8.15	8.05			6.32	6.19	6.10	6.05			58.25	56.57	55.22	53.34	
$CD_{0.05}$																	
Treatment (A)					0.009						0.007						0.015
Time Interval (B)					0.009						0.007						0.015
A×B					0.018						0.014						0.030

given here showed that even after degradation, a high percentage of total phenols were retained.

The trend of decrease in the vitamin C during lactic acid fermentation (0-72hrs) is shown in Fig. 3 and Table 3. The highest vitamin C (9.22mg/100g) was recorded in fermentation with *L. plantarum* (10^7cfu/ml), while the lowest vitamin C (9.12mg/100g) was recorded in fermentation with *S. thermophillus* (10^5cfu/ml). Among the four treatments, *L.plantarum* (10^7cfu/ml) was the best treatment with the highest retention (76.26%) after 72 hr of fermentation. Similar results have been reported by Kopec (2000) stating that about 20-70% of the initial content of L-ascorbic acid remains preserved in the end products after lactic acid fermentation. However, Kohajdova *et al.* (2006) have also found that at the end of fermentation of tomato and cabbage juice, retention percentage of L-ascorbic acid was 43% and 56% of the original content L-ascorbic acid, respectively.

The antioxidant activity increased with increase in time of fermentation (0-72hr) as revealed by Fig. 3. The highest antioxidant activity (32.79%) was recorded in fermentation with *L. plantarum* (10^7cfu/ml), while the lowest antioxidant activity (29.42%) was recorded in fermentation with *S. thermophillus* (10^5cfu/ml) Table3. Among the four treatments, *L.plantarum* (10^7cfu/ml) was the best treatment with the highest antioxidant activity after 72 hr of fermentation. Hence our results are in conformation with earlier reports for increment of antioxidant activity of soymilk during lactic acid fermentation (Subrota *et al.,* 2015). It was reported that soymilk is fermented with six probiotic lactobacilli (*L. rhamnosus* NCDC 19, *L. rhamnosus* NCDC 24, *L. rhamnosus* C2, *L. rhamnosus* C6 and *L. casei* NCDC 17, *L. casei* NCDC 297). Among these six lactic acid bacteria, *L. rhamnosus* C6 strain showed maximum antioxidant activity in ABTS, DPPH and FRAP method (89.09, 50.09 and 80.25% inhibition, respectively) and proteolytic activity as well as reduced polyphenolic content (29.10mg/100ml to 11.98mg/100ml). Among the six lactobacilli cultures which were incubated for 24hr at 30°C. *L. plantarum* was able to degrade some food phenolic compounds and after degradation gave those compounds which increased the antioxidant activity (Subrota *et al.*, 2015).

Microbiological quality: Table 4 summarizes the effect of lactic acid fermentation on viable cell counts (cfu/ml) of apple juice which show that viable cell counts increased with increase in time (0-72hr). The highest viable cell counts (3.36×10^{9}cfu/ml) were observed in fermentation with *L. plantarum* (10^{7}cfu/ml), while the lowest viable cell counts (2.96×10^{9}cfu/ml) were recorded in fermentation with *S. thermophillus* (10^{7}cfu/ml). Among the four treatments, *L. plantarum* (10^{7}cfu/ml) was the best treatment with highest viable cell counts after 72 hr of fermentation. Increased in viable cell counts during lactic acid fermentation of various fruits and vegetables including tomato, cabbage, beet root, pomegranate and red and yellow watermelon have been reported by Yoon *et al.* (2004); (2005); (2006), Tantipaibulvut *et al.* (2008) and Pereira *et al.* (2010) earlier.

The trend of increase in the lactic acid bacterial counts during lactic acid fermentation (0-72hrs) is shown in Fig. 3. Table 4 summarizes the effect of lactic acid fermentation on the lactic acid bacterial counts (cfu/ml) of apple juice. The highest lactic acid bacteria counts (3.36×10^{8}cfu/ml) were reported in fermentation with *L. plantarum* (10^{7}cfu/ml), while the lowest lactic acid bacteria counts (2.53×10^{8}cfu/ml) were recorded in fermentation with *L. plantarum* (10^{5}cfu/ml). Among the four treatments, *L. plantarum* (10^{7}cfu/ml) was the best treatment with highest lactic acid bacterial counts after 72 hr of fermentation. Similar results have been reported for lactic acid bacterial counts of various fruits and vegetables during lactic acid fermentation including tomato, cabbage, beet root, pomegranate, roselle, peach and red and yellow watermelon by Yoon *et al.* (2004); (2005); (2006), Tantipaibulvut *et al.* (2008) and Pereira *et al.* (2010) earlier.

Results in Table 5 reveal the effect of lactic acid fermentation on the coliform counts of apple juice which were decreased with increase in time of fermentation (0-72hr). This could be due to the production of bacteriocins and antimicrobial substances produced by the lactic acid bacteria. The highest coliform counts (0.64×10^{1}cfu/ml) was recorded in fermentation with *S. thermophillus* (10^{5}cfu/ml), while the lowest coliform count (0.50×10^{1}cfu/ml) was documented in fermentation with *L. plantarum* (10^{7}cfu/ml). Among the four treatments, *L. plantarum* (10^{7}cfu/ml) was the best treatment with the lowest coliform counts after 72 hr of fermentation. Similar results that the bacteriocins produced by *L.plantarum* and *L.fermentum* exhibited a wide spectrum of inhibition to inhibit the food pathogens such as *E.coli*, *Proteus*, *Staphylococcus*, *Pseudomonas* in processed foods have been reported by Saranya and Hemashenpagam (2011) and Lindgren and Dobrogosz (1990).

Table 3: Effect of lactic acid fermentation on antioxidant activity and ascorbic acid of apple juice

Time interval (hrs) Treatments	Antioxidant activity (%)				Mean	Ascorbic acid (mg/100gm)				Mean
	0	24	48	72		0	24	48	72	
T_1 (*L.plantarum* 10^{5}cfu/ml)	19.09	24.47	28.23	30.27	25.51	10.28	9.96	8.54	7.76	9.14
T_2(*L.plantarum* 10^{7}cfu/ml)	19.09	26.78	29.49	32.79	25.65	10.28	10.10	8.66	7.84	9.22
T_3(*S.thermophilus* 10^{5}cfu/ml)	19.09	21.24	25.01	29.42	23.69	10.28	9.92	8.53	7.74	9.12
T_4(*S.thermophilus* 10^{7}cfu/ml)	19.09	22.63	26.39	27.84	23.98	10.28	9.96	8.54	7.73	9.13
Mean	19.09	23.78	27.28	30.08		10.28	9.98	8.57	7.77	
$CD_{0.05}$										
Treatment (A)					0.242				0.027	
Time Interval (B)					0.242				0.027	
A×B					3				0.054	

Table 4: Effect of lactic acid fermentation on viable cell counts and LAB counts of apple juice

Time interval (hrs) / Treatments	Viable cell counts (1×10^9 cfu/ml)				Mean	Lactic acid bacterial counts (1×10^8 cfu/ml)				Mean
	0	**24**	**48**	**72**		**0**	**24**	**48**	**72**	
T$_1$ (*L.plantarum* 10^5 cfu/ml)	0.80	2.10	2.46	3.23	2.15	0.02	2.40	2.93	4.80	2.53
T$_2$(*L.plantarum* 10^{7}cfu/ml)	0.86	2.16	2.46	3.36	2.21	0.11	2.66	4.66	6.00	3.36
T$_3$ (*S.thermophilus* 10^5 cfu/ml)	0.80	1.90	2.10	3.00	1.95	0.01	2.43	3.23	4.76	2.61
T$_4$ (*S.thermophilus* 10^{7}cfu/ml)	0.86	2.16	2.40	2.96	2.10	0.11	2.46	3.16	5.03	2.69
Mean	0.84	2.08	2.35	3.14		0.06	2.49	3.50	5.15	
CD$_{0.05}$										
Treatment (A)					0.092				0.190	
Time Interval (B)					0.092				0.190	
A×B					0.184				0.381	

Table 5: Effect of lactic acid fermentation on coliform counts, yeast and mold counts of apple juice

Time interval (hrs) / Treatments	Coliform counts (1x10^1 cfu/ml)				Mean	Yeast (1×10^1 cfu/ml)				Mean	Mold (1×10^1 cfu/ml)				Mean
	0	**24**	**48**	**72**		**0**	**24**	**48**	**72**		**0**	**24**	**48**	**72**	
T$_1$ (*L.plantarum* 10^{5}cfu/ml)	1.00	0.66	0.40	0.30	0.59	0.90	0.73	0.36	0.06	0.51	0.90	0.50	0.30	0.16	0.46
T$_2$(*L.plantarum* 10^{7}cfu/ml)	1.00	0.63	0.40	0.16	0.50	0.90	0.40	0.06	0.00	0.34	1.00	0.50	0.23	0.10	0.43
T$_3$(*S.thermophilus* 10^{5}cfu/ml)	1.00	0.80	0.50	0.26	0.64	1.00	0.66	0.40	0.03	0.52	1.00	0.50	0.30	0.13	0.48
T$_4$(*S.thermophilus* 10^{5}cfu/ml)	0.93	0.60	0.33	0.16	0.55	0.90	0.43	0.16	0.03	0.38	0.90	0.50	0.20	0.13	0.45
Mean	0.98	0.67	0.40	0.22		0.92	0.55	0.25	0.03		0.95	0.50	0.25	0.13	
CD$_{0.05}$															
Treatment (A)			0.092							0.146					0.082
Time Interval (B)			0.092							0.146					0.082
AxB			NS							NS					NS

Data in Table 5 reveal the effect of lactic acid fermentation on the yeast counts of apple juice which were decreased with increase in time (0-72hr) of fermentation. The highest yeast counts (0.52×10^{1}cfu/ml) were recorded in fermentation with *S.thermophilus* (10^{5}cfu/ml), while the lowest yeast counts (0.34) were recorded in fermentation with *L. plantarum* (10^{7}cfu/ml). Among the four treatments, *L. plantarum* (10^{7}cfu/ml) was the best treatment with the lowest yeast counts after 72 hr of fermentation. Lindgren and Dobrogosz (1990) have reported that lactic acid bacteria produce many antimicrobial substances which include organic acids, diacetyl, hydrogen peroxide and bacteriocins or bactericidal proteins during lactic acid fermentation. Organic acids such as lactic, acetic and propionic acid generally exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting metabolic functions (Ross and Morgan, 2002). Caplice and Fitzerald (1999) have found that antimicrobial substances have a very broad mode of action and inhibit gram positive and gram negative bacteria as well as yeast and mold. These findings are of great significance from public health and food preserving point of view.

It is clear from Table 5 that the after of lactic acid fermentation the mold counts of apple juice which were decreased with increase in time (0-72hr) of fermentation. The highest mold counts (0.48×10^{1}cfu/ml) were recorded in fermentation with *S. thermophillus* (10^{5}cfu/ml), while the lowest yeast counts (0.43×10^{1}cfu/ml)) were recorded in fermentation with *L. plantarum* (10^{7}cfu/ml). Among the four treatments, *L. plantarum* (10^{7}cfu/ml) was the best treatment with the lowest mold counts after 72 hr of fermentation. Similar to these findings, Caplice and Fitzerald (1999) have found that antimicrobial substances have a very broad mode of action and inhibit gram positive and gram negative bacteria as well as yeast and mold.

Staphylococcus and *Salmonella* were however not detected during lactic acid fermentation of apple juice. This could be attributed to the antimicrobial substances which are produced by the lactic acid bacteria. Similar to these findings, Saranya and Hemashenpagam (2011) have reported that the bacteriocins produced by *L.plantarum* and *L.fermentum* exhibited a wide spectrum of inhibition to inhibit the food pathogens such as *E.coli*, *Proteus*, *Staphylococcus*, *Salmonella*, *Pseudomonas* in the processed foods.

Antimicrobial activity

The trend of change in the antimicrobial activity against *S.aureus* and *E.coli* during lactic acid fermentation is shown in Fig. 3, wherein the antimicrobial activity is increased with increase in time (0-72hr) of fermentation. Table 6 summarizes the effect of lactic acid fermentation on the antimicrobial activity against *S.aureus* and *E.coli* of apple juice. Data revealed that initially, antimicrobial activity of apple juice was 8.00mm (diameter the of well), but after completion of fermentation, all the four treatments increased the antimicrobial activity against *S.aureus* and *E.coli*, which ranged from 8-18.4mm and 8-18.0mm, respectively. Among the four treatments, the highest antimicrobial activity was recorded in fermentation with *L. plantarum* (10^{7}cfu/ml) against *S.aureus* and *E.coli* which were ranged from ranged from 8-18.4mm and 8-18mm, respectively. Increased in antimicrobial activity of lactic acid fermented juices due to the production many antimicrobial substances which include organic acids, diacetyl, hydrogen peroxide and bacteriocins or bactericidal proteins during lactic acid fermentation. Similar results have been reported earlier by many research workers (Stevans and Sheldon, 1992; Ogunbanwo *et al.*, 2003; Schillinger and Luke, 1989).

From the result described, it is apparent that TSS declined with passage of time and highest reduction took place in the fermentation with *L. plantarum* (10^{7}cfu/ml), the trend of total sugar and reducing

Table 6: Effect of lactic acid fermentation on antimicrobial activity(mm) of apple juice

Time interval (hrs) **Treatments**	**Test micro-organisms**									
	Staphylococcus aureus				**Mean**	***Escherichia coli***				**Mean**
	0*	**24**	**48**	**72**		**0***	**24**	**48**	**72**	
T$_1$ (*L.plantarum* 10^{5}cfu/ml)	8.0	15.0	17.0	19.0	15.7	8.0	14.0	15.0	17.0	14.5
T$_2$(*L.plantarum* 10^{7}cfu/ml)	8.0	16.6	18.0	20.3	18.4	8.0	12.0	14.6	23.0	18.0
T$_3$(*S.thermophilus* 10^{5}cfu/ml)	8.0	14.0	16.0	19.0	15.3	8.0	12.0	15.3	17.0	13.5
T$_4$(*S.thermophilus* 10^{7}cfu/ml)	8.0	17.0	19.3	22.3	17.0	8.0	16.6	19.0	21.0	14.9
Mean	8.0	15.6	17.5	20.1		8.0	13.6	16.0	19.5	

*:- Diameter of well

CD$_{0.05}$		
Treatment (A)	1.640	1.092
Time Interval (B)	1.640	1.092
AxB	NS	2.184

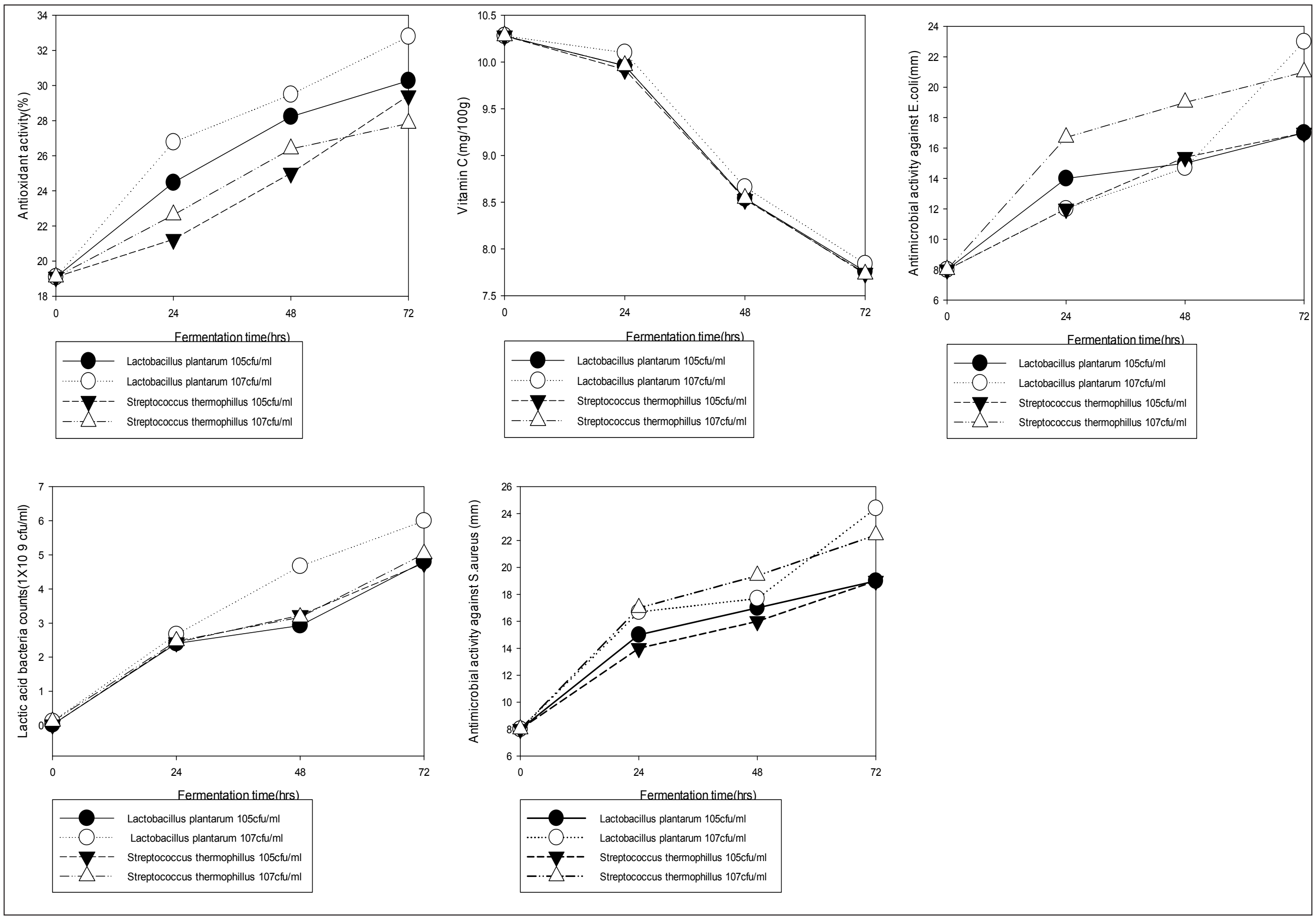


Fig. 3: Changes in antioxidant activity, vitamin C, lactic acid bacteria counts, antimicrobial activity against *S. aureus* and *E.coli* during lactic acid fermentation of apple juice

sugar remained the same as that of TSS, the reduction in pH and increase in acidity corroborated with each other. Among the four treatments, the rate of fermentation was also the highest in apple fermentation with *L. plantarum* inoculated with 10^7cfu/ml during fermentation. Same was true for the rate of acidification. During fermentation both vitamin C and total phenols declined, while, the antioxidant activity, antimicrobial activity, viable cell counts and lactic acid bacteria counts were increased. As the antimicrobial activity increased, the counts of coliform, yeast and mold declined.

Effect of storage on physico-chemical parameters of apple probiotic juice

After completion of lactic acid fermentation, selection of best the treatment on the basis of physico-chemical and microbiological parameters was made. It was the second treatment (*L.plantarum* 10^7cfu/ml) which was used for cold storage studies. The trend of change in TSS during cold storage (4°C) of probiotic juice is shown in Fig. 4, in which TSS is decreased with increase in time (0-4weeks) of storage.

The effect of cold storage (4°C) on TSS of apple probiotic juice (Table 7) showed that initially, TSS value of apple probiotic juice was 9.04°B and during storage, it ranged from 9.04-8.47°B. The trend of change in pH during cold storage (4°C) of probiotic juice is depicted in Fig. 4, in which pH increased with increase time (weeks) of storage. The effect of cold storage (4°C) on the pH value of apple probiotic juice (Table 7) showed that initially, pH value of apple probiotic juice was 2.36 and during storage, it ranged from 2.36-2.71. The trend of change in titratable acidity during cold storage (4°C) of probiotic juice is depicted in Fig. 4, in which titratable acidity is decreasing with increase of time interval (weeks) of storage. The effect of cold storage (4°C) on titratable acidity of apple probiotic juice (Table 7) showed that initially, titratable acidity value of apple probiotic juice was 0.60% which during storage decreased to 0.36%. The trend of change in total sugars during cold storage (4°C) of probiotic juice is shown in figure 4, revealed that total sugars decreased with increase in time (weeks) of storage. The effect of cold storage (4°C) on total sugar of apple probiotic juice (Table 7) showed that initially, total sugars value of apple probiotic juice was 8.06% which during storage, decreased to 7.94%.

The trend of change in reducing sugar during cold storage (4°C) of probiotic juice is shown in Fig. 4, in which reducing sugar is decreased with increase time (weeks) of storage. The effect of cold storage (4°C) on reducing sugar of apple probiotic juice (Table 7) showed that initially, reducing sugar value of apple probiotic juice was 6.02% which during storage, decreased to 5.73%. The trend of change in total phenols during cold storage (4°C) of probiotic juice is shown in Fig. 4, in which total phenols is decreased with increase of time(weeks) of storage. The effect of cold storage (4°C) on total phenols of apple probiotic juice (Table 7) showed that initially, total phenols value of apple probiotic juice was 53.68mg/100g. During storage, it from 53.68-53.23 (mg/100g). The trend of change in vitamin C during cold storage (4°C) of probiotic juice is shown in Fig. 4, in which vitamin C is decreased with increase of time(weeks) of storage. The effect of cold storage (4°C) on vitamin C of apple probiotic juice (Table 7) showed that initially, vitamin C value of apple probiotic juice was 7.84mg/100g. During storage, it ranged from 7.84-7.74(mg/100g).

The trend of change in antioxidant activity during cold storage (4°C) of probiotic juice is shown in Fig. 4, in which antioxidant activity is decreased with increase of time(weeks) of storage. The effect of cold storage (4°C) on antioxidant activity of apple probiotic juice (Table 7) showed that initially, antioxidant activity value of apple probiotic juice was 33.13%. During storage, it ranged from 33.13-30.03%.

Effect of cold storage on microbiological quality of apple probiotic juice

The trend of change in viable cell counts during cold storage (4°C) of probiotic juice is shown in Fig. 5, in which viable cell counts are decreased with increase of time(weeks) of storage. The effect of cold storage (4°C) on viable cell counts of apple probiotic juice (Initially, viable cell counts of apple probiotic juice were 3.40×10^9cfu/ml. During storage, viable cell counts ranged from 3.40-0.20(10^9cfu/ml). Similar observations were reported by Yoon *et al.* (2004), Yanez *et al.* (2008) and Tantipaibulvut *et al.* (2008).

Table 7: Effect cold storage on physico-chemical parameters of apple probiotic juice

Parameters / **Time intervals (weeks)**	**Physico-chemical parameters**							
	TSS	**pH**	**TA (%LA)**	**Total sugars (%)**	**Reducing sugar (%)**	**Total Phenols (mg/100gm)**	**Vitamin C (mg/100gm)**	**Antioxidant activity (%)**
0	9.04	2.36	0.60	8.06	6.02	53.68	7.84	33.13
1	8.94	2.47	0.49	8.04	5.93	53.54	7.80	32.44
2	8.84	2.56	0.44	8.00	5.89	53.45	7.79	31.06
3	8.64	2.61	0.39	7.97	5.82	53.34	7.76	30.24
4	8.47	2.71	0.36	7.94	5.73	53.23	7.74	30.03
Mean	8.79	2.54	0.46	8.00	5.89	53.45	7.79	31.38
SE	0.05	0.01	0.01	0.01	0.008	0.016	0.01	0.22
$CD_{0.05}$	0.158	0.040	0.035	0.008	0.024	0.050	0.025	0.704

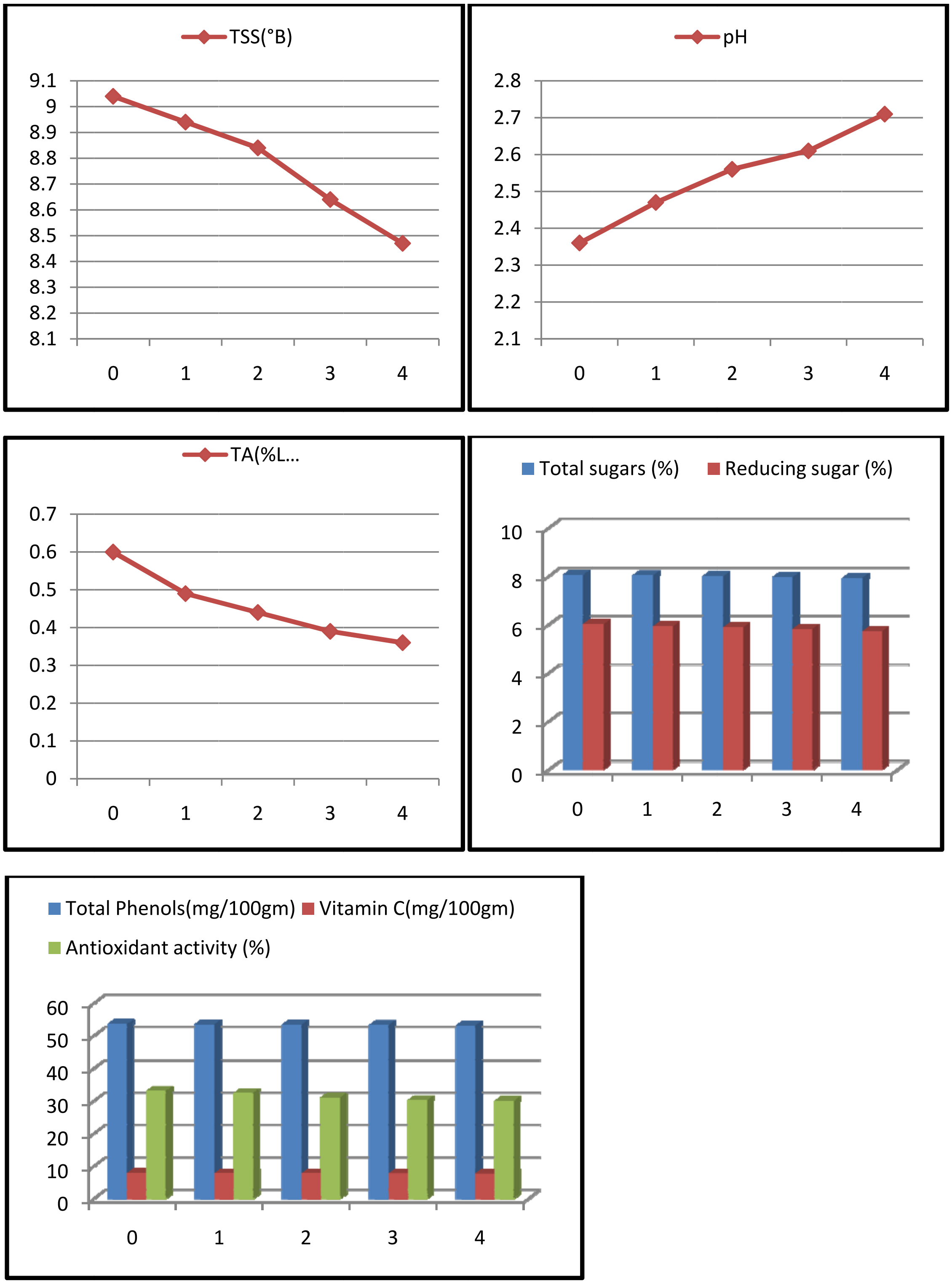


Fig. 4: Changes in TSS(°B), pH, titratable acidity (%), total sugar (%), reducing sugar (%), total phenols (mg/100g), vitamin C (mg/100g) and antioxidant activity (%) during cold storage (4°C) of apple probiotic juice

Table 8: Effect of cold storage on microbiological analysis of apple probiotic juice

Parameters / **Intervals (weeks)**	**Microbiological analysis**					
	Viable cell counts (1×10^9 cfu/ml)	**Lactic acid bacteria counts (1×10^8 cfu/ml)**	**Coliform counts (1×10^1 cfu/ml)**	**Yeast counts (1×10^1 cfu/ml)**	**Mold (1×10^1 cfu/ml)**	**Staphyllo and Salmonella counts (1×10^1 cfu/ml)**
0	3.40	6.00	0.03	0.00	0.00	Not found
1	1.33	5.63	0.06	0.03	0.01	—
2	0.80	5.20	0.13	0.18	0.08	—
3	0.50	4.76	0.23	0.26	0.14	—
4	0.20	2.33	0.40	0.38	0.20	—
Mean	1.24	4.78	0.17	0.17	0.08	
SE	0.09	0.29	0.09	0.04	0.07	
$CD_{0.05}$	0.20	0.66	0.22	0.11	0.18	

Lactic acid bacterial counts: The trend of change in lactic acid bacterial counts during cold storage (4°C) of probiotic juice is shown in Fig. 5, in which lactic acid bacterial counts are decreasing with increase of time(weeks) of storage. The effect of cold storage (4°C) on lactic acid bacterial counts of apple probiotic juice (Table 8) showed taht initially, lactic acid bacterial counts of apple probiotic juice were 6.0×10^8cfu/ml which during storage decreased to 2.33(10^8cfu/ml). Mousavi *et al.* (2011) have reported that microbial population of *L.paracasei* and *L.acidophilus* decreased approximately three logarithmic orders during first week of cold storage of probiotic pomegranate juice and lost their viability after two weeks as reported by many other research workers (Yoon *et al.*, 2004; Yanez *et al.*, 2008; Tantipaibulvut *et al.*, 2008) also. Table 8 reveals the effect of cold storage (4°C) on coliform counts of apple probiotic juice.

Initially, coliform counts of apple probiotic juice were 0.03 which during storage, decreased to 0.40(10^1cfu/ml). Data revealed that coliform counts are increasing with increase of time (weeks). Table 8 revealed the effect of cold storage (4°C) on yeast counts of apple probiotic juice. Initially, yeast counts of apple probiotic juice were 0.00. During storage, yeast counts ranged from 0.00-0.38(10^1cfu/ml). Data revealed that these are increasing with increase of time (weeks). Table 8 reveals the effect of cold storage (4°C) on mold counts of apple probiotic juice. Initially, mold counts of apple probiotic juice were 0.00. During storage, mold counts ranged from 0.00-0.20(10^1cfu/ml). Data revealed that mold counts are increased with increase of time (weeks). *Staphylococcus* and *Salmonella* were not found during cold storage of apple probiotic juices.

Table 9: Effect cold storage on antimicrobial activity (inhibition zone mm) of apple probiotic juice

Time intervals (weeks)	**Test micro-organism**	
	Staphylococcus aureus	***Escherichia coli***
0	20.67	23.00
1	20.00	21.33
2	18.66	19.00
3	17.00	16.00
4	14.66	14.00
Mean	1.18	0.97
SE	1.67	1.38
$CD_{0.05}$	3.77	3.20

Antimicrobial activity: The trend of change in antimicrobial activity during cold storage (4°C) of probiotic juice is shown in Fig. 5, in which antimicrobial activity decreased with increase of time (weeks) of storage. The effect of cold storage (4°C) on antimicrobial activity (Table 9) against *S.aureus* and *E.coli* of apple probiotic juice showed that initially,

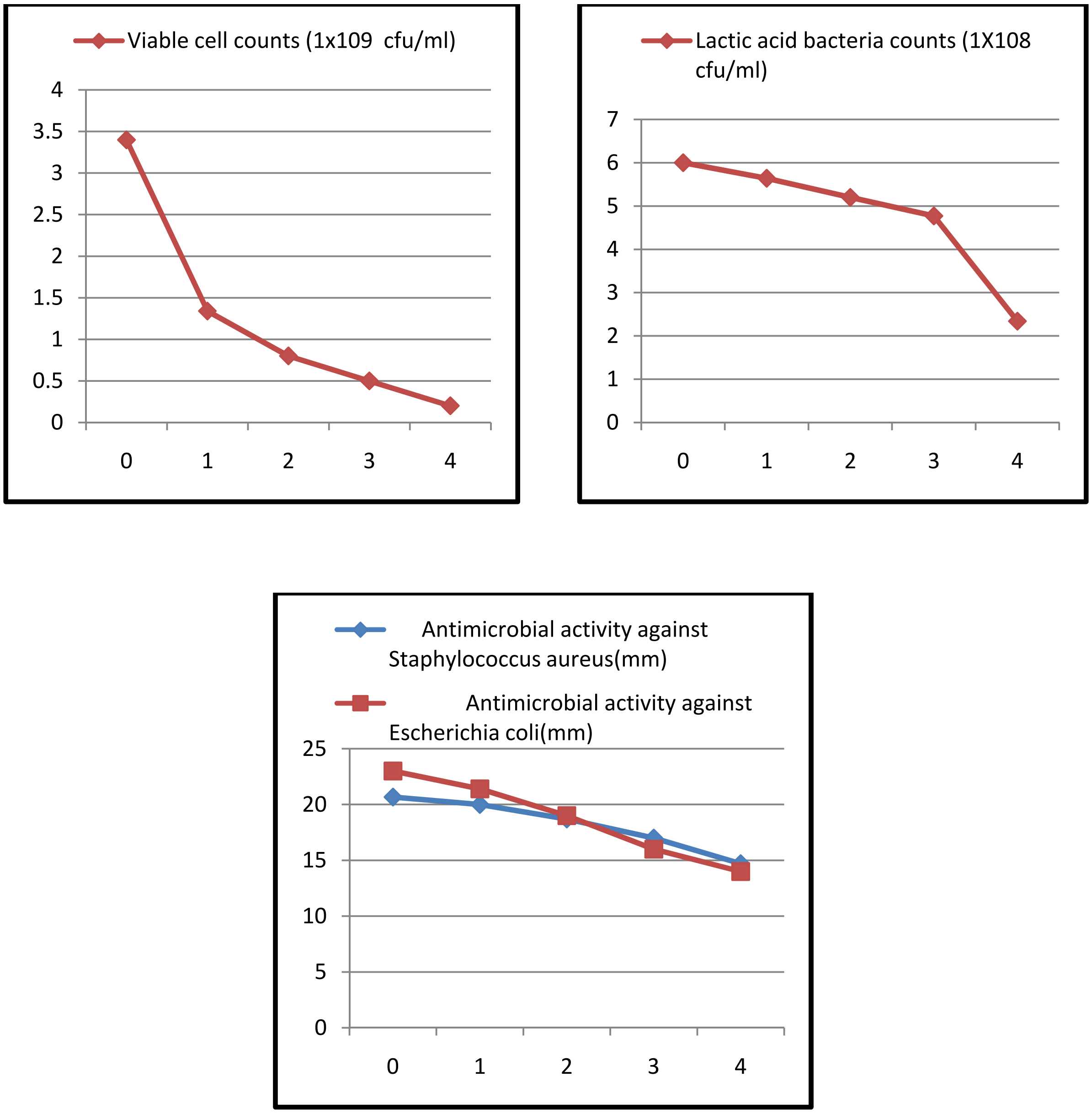


Fig. 5: Changes in viable cell counts (cfu/ml), lactic acid bacteria counts (cfu/ml) and antimicrobial activity against *Staphylococcus aureu*s and *Escherichia coli* during cold storage (4°C) of apple probiotic juice

antimicrobial activity of apple probiotic juice against *S.aureus* and *E.coli* was 20.67mm and 23.0mm, respectively. During storage, antimicrobial activity against *S.aureus* and *E.coli* ranged from 20.67-14.7mm and 23.0-14.0, respectively that means antimicrobial activity against *S.aureus* and *E.coli* were decreased with increase of time (weeks).

Acid tolerance: Table 10 summarizes the effect of cold storage on acid tolerance of apple probiotic juice. Initially, acid tolerance of LAB of apple probiotic juice at 0min of incubation in gastric juice was 4.60 (10^7cfu/ml). But after 4 weeks cold storage (4°C), acid tolerance of LAB remained 0.24(10^7cfu/ml) after 90min of incubation in gastric juice. Percentage of reduction of acid tolerance of LAB from initial week

Table 10: Acid tolerance and bile tolerance of lactic acid bacteria of probiotic apple juice

Period of incubation (min) / Time intervals (weeks)	Acid Tolerance (1×10^7 cfu/ml)					% of reduction	Bile Tolerance (1×10^7 cfu/ml)					% of reduction
	0	30	60	90	Mean	0	0	30	60	90	Mean	
0	4.6	4.54	3.9	3.5	4.25	**23.9**	3.67	3.47	3.04	2.84	2.00	**22.6**
1	3.24	3.34	2.93	2.45	3.14	**24.38**	2.47	2.1	1.84	1.64	1.08	**27.1**
2	1.87	1.67	1.07	1.07	1.54	**42.8**	2.40	2.2	1.94	1.80	1.70	**31.7**
3	1.4	1.17	0.57	0.57	1.03	**59.3**	1.97	1.24	1.57	1.44	1.43	**37.06**
4	0.7	0.77	0.24	0.24	0.65	**65.7**	1.84	1.86	1.34	0.97	0.92	**47.3**
Mean	2.70	2.29	1.91	1.59			1.95	1.78	1.51	1.26		
$CD_{0.05}$												
Treatment (A)					0.060							0.081
Storage Interval (B)					0.068							0.090
A×B					0.135							0.180

to fourth week ranged from 23.9-65.7(%) in gastric juice. Our results on acid tolerance were within the range as reported earlier (Emese *et al.*, 2010; Holzapfel and Schillinger, 2000).

Bile tolerance: Table 10 summarizes the effect of cold storage on bile tolerance of apple probiotic juice. Initially, bile tolerance of LAB of apple probiotic juice at 0 min of incubation in bile juice was 3.67(10^7cfu/ml). But after 4 weeks of cold storage (4°C), bile tolerance of LAB remained 0.97(10^7cfu/ml) after 90min of incubation in bile juice. Per-centage of reduction of bile tolerance of LAB from initial week to fourth week ranged from 22.6-47.3(%) in bile juice. Similar observations were reported by Emese *et al.* (2010).

Identification of lactic acid bacteria of apple probiotic juices after storage

Identification of lactic acid bacteria of best treatment or selected treatment was done by Gram staining and biochemical tests after storage. In case of apple probiotic juices, best treatment was *L.plantarum*. Gram staining and biochemical tests revealed that *L.plantarum* characterized morphologically like colonies were small, circular and white creamy in color, rod shaped bacteria, catalase negative, amylase positive, casein hydrolysis positive, fermentation of sugar positive and microbial reaction in litmus milk was also positive by forming curd and formation of purple ring at the top. Hoque *et al.* (2010) have reported that *Lactobacilli* spp. was isolated from two regional yoghurt samples which were identified and showed the results like gram positive, rod shaped non motile, catalase negative and absence of Endospore and isolates have ability to coagulate in litmus milk.

Sensory evaluation of apple probiotic juices after storage

Sensory characteristics of apple probiotic juices were evaluated by using descriptive analysis without tasting or through nose on scale of 10. Yellow colour of apple probiotic juice scored 6.6±0.3, sedimentation scored 4.0±0.5, apple like scored 6.2±0.7, sweet like scored 5.6±0.3, pungency scored 6.2±0.7, turbidity scored 5.8±0.7 and acid like scored 5.6±0.3. These all scores were given out of 10. Luckow and Delahunty (2004) reported that sensory characteristics of probiotic blackcurrant juice were perfumery and dairy in odour, and sour and savoury in flavour. Similarly, he reported that sensory characteristic of probiotic celery juice were pleasant sweet-sour taste, without serve any acidity. Kohajdova *et al.* (2006) have found that the juices tested ranked as cabbage juice (93.2% of the scale), courgette juice (92% of the scale), tomato juice (79% of the scale) and pumpkin juice (78% of the scale). He also reported that sedimentation occurred in all juices (cabbage, courgette, tomato and pumpkin juice.

CONCLUSION

Two lactic acid bacteria *L. plantarum,* and *S. thermophillus* were examined for their ability to utilize apple juice for cell synthesis and lactic acid production. Out of these two lactic acid cultures *L. plantarum* (10^7cfu/ml) grew well in apple juice at 30°C. During lactic acid fermentation of apple juices, rate of fermentation, titratable acidity, antioxidant activity, lactic acid bacteria counts and antimicrobial activity against *E.coli* and *S. aureus* were increased in fermented apple juices. However, during lactic acid fermentation of apple juices, pH, reducing and total sugars, total phenols and ascorbic acid, yeast, coliform and mold counts were decreased of fermented apple juices. Based on the storage (4°C), it is apparent that the lactic acid bacteria remain in the prescribed number, showed acid and bile resistance, sensory viable and imparted antimicrobial activity and antioxidant activity to the juices thus, quality for the probiotic microorganism. Sensory evaluation of apple probiotic juices concluded that lactic acid fermentation did not influence the sensory characteristics of fruit and vegetable juices adversely and remained desirable. Based on bile resistance, acid resistance and viable counts, it was concluded that apple lactic acid fermented fruits juices can be called as probiotics juices. It is concluded that *L. plantarum* could be used as probiotic culture for production of a healthy beverage from apple for vegetarians or consumers who are allergic to lactose present in probiotic dairy products.

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