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Biochemical, Microbiological and Organoleptic Properties of Probiotic Pineapple Juice Developed by Lactic Acid Bacteria

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Abstract

An experiment was undertaken to determine the suitability of pineapple juices as raw materials for the production of fermented probiotic juice by two lactic acid bacteria, *Lactobacillus plantarum* and *Lactobacillus fermentum*. Three probiotic juice samples inoculated with *L. plantarum* (S-2), *L. fermentum* (S-3) and with both *L. plantarum* and *L. fermentum* (S-4) were compared with that of control sample (S-1) where no *Lactobacillus* strains were used. Fermentation was carried out at 37 °C for 48 h. Then the juice samples were stored at 4 °C and studied the change in their physicochemical properties at day 0, 7, 14 and 21. Both *L. plantarum* and *L. fermentum* were able to withstand and utilize fruit juices for their cell synthesis as indicated by a decrease in fruit sugar and an increase in acidity. Although the lactic cultures in fermented probiotic juices gradually lost their viability, the viable cell counts of these strains remained at 10^7 CFU/100 mL after 14 days in cold storage. From sensory evaluation, the juice sample prepared with *L. plantarum* exhibits the best result during storage time. Hence, pineapple juice might be used as a suitable medium for the production of a healthy probiotic beverage.

Keywords: *Lactobacillus fermentum*; *Lactobacillus plantarum*; Pineapple juice, Probiotification; Quality evaluation.

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1. Introduction

Probiotics are live microbial feed supplements. They can enhance the health of consumer by improving the balance of micro flora in the gut when ingested live in sufficient numbers [1]. The consumption and development of probiotic foods is increasing day by day due to the consumer awareness about functional foods, of which has ability to maintain good health. The role of probiotic organisms as complementary therapy in combating large number of gastrointestinal disorders and their ability to enhance immune response attracts global attention. Probiotic microorganisms are claimed to provide

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several health benefits, including antimicrobial, anti-tumor, anti-cholesterol, immuno-modulation, anti-diabetic, and treatment of diarrhea and lactose intolerance [2-6].

Nowadays probiotics consumption through dairy-based beverages such as milk, yogurt and cheese are very popular, as they are excellent carrier. Because of fear of mycotoxins in fruits and vegetables [7], the use of microorganisms in plant based food is still very low. Consequently, the potential applications of probiotics in non-dairy food products for vegetarian, yet not received formal recognition. On the other hand, consumer demand for non-dairy based probiotic products has increased due to the problems of lactose intolerance and cholesterol content associated with the consumption of fermented dairy products [8]. In this respect, fruit juice offers an alternative to produce probiotic foods using *Lactobacillus* species as some *Lactobacillus* species were found to have the capacity to reduce blood cholesterol levels [9,10].

Different studies have been carried out to explore the suitability of vegetable juices such as tomato, beet and cabbage as raw materials to produce probiotic drinks using *L. plantarum, L. acidophilus* and *L. casei*. Results have indicated that all the strains can grow in the fruit juices and microbial population increases significantly after 48 h of fermentation [11-15]. In this context, an effort was made to develop pineapple juice with potential probiotic *Lactobacillus* isolates and investigate their physicochemical and microbial stability during storage at 4 °C to ensure better health and nutrition of the population upon consumption.

2. Materials and Methods

2.1. *Collection of Lactobacillus strains*

Two *Lactobacillus* isolates viz. *Lactobacillus plantarum* DSM 10492 and *Lactobacillus fermentum* DSM 20052 were collected from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany. Both lactobacillus cultures were kept at -20 °C in MRS medium (Merck, Germany) containing 20 % glycerol. Before probiotic juice preparation, these isolates were selected after performing different potential probiotic confirmation tests like: NaCl tolerance test (1-8 %), pH (2.5 to 6.5) and bile salt tolerance test (0.05-0.25 %) as well as antimicrobial, antibiotic susceptibility and growth test at stressful mixed (low pH, high bile and NaCl) conditions and used for probiotic pineapple fruit juices development.

2.2. *Pineapple juice preparation, inoculation and fermentation*

Fresh pineapple juice was prepared by juice extractor. Extracted juice was pasteurized at 80 °C for 5 min [16]. The lactic cultures were first grown in MRS broth at 37 °C for 48 h. After cooling the pineapple juice at 30 °C they were inoculated by 1 % *L. plantarum* (S-2), 1 % *L, fermentum* (S-3), and their mixed (0.5 % each) culture (S-4). Fresh pineapple juice sample was used as control (S-1).The inoculated samples along with control samples were incubated at 37 °C for 48 h.

2.3. *Physicochemical and microbiological assay*

After fermentation, all the samples were stored at 4 °C. Then physicochemical parameters, such as, titratable acidity (expressed as percent lactic acid), moisture, protein, total soluble solid (TSS) contents, and total sugar content were evaluated to check the physicochemical stability of pineapple juice during storage period. The sugar content was estimated in terms of glucose (mg/mL) by the phenol sulfuric acid method [17]. Protein and moisture content was determined according to the AOAC [18]. Total soluble solid (TSS) were measured by a hand refractometer as percent (%) degree Brix.

Viable cell counts (CFU/mL) was determined by the standard plate count (SPC) method using MRS agar [19]. All Physicochemical and microbiological status of the treated juices were monitored up to 21 days at 7 days interval during storage period.

2.4. *Sensory evaluation*

Thirty tasters were used in this study to assess the sensory properties of produced probiotic juice samples [20]. The panelists were asked to rate different juice samples present in them. Water and bread were provided to panel members to cleanse their palates between samples. In this case, 9 point hedonic rating test was performed to assess the degree of acceptability of these juices as follows: (9=Like extremely, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like or dislike, 4=Dislike slightly, 3=Dislike moderately, 2=Dislike very much, 1=Dislike extremely).

2.5. *Statistical analysis*

All results of this study are reported as mean of three replicates that were analyzed. One way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for all physicochemical analysis were determined by using SPSS (Statistical Package for the Social Science, version 20, copyright of IBM Corporation and others) software [21]. The least significant difference was calculated at 95 % level of significance ($P < 0.05$).

3. Results

3.1. *Physicochemical analysis of juice samples*

The Physiochemical conditions of pineapple juice during storage period are presented in Table 1. In case of control sample (S-1) the values of acidity, moisture, protein content, total sugar content, and TSS were differed slightly throughout the storage days (Table 1). Acidity was increased from 0.191±0.005 to 0.243±0.008 after 21 days of storage. Total soluble solid as indicated by °Bx was reduced from 20.167±0.289 to 18.333±0.289. Total sugar (mg/100 mL) content was also slightly declined during storage.

Table 1. Physiochemical analysis of pineapple juice.

Sample No.	Day	Acidity (% of lactic acid)	Moisture (%)	Protein (%)	Total sugar (mg/100 mL)	Total soluble solid (°Bx)
S-1	0	$0.191\pm0.005^{D*}$	72.680 ± 0.026^{A}	0.432 ± 0.007^{A}	175.237 ± 0.251^{D}	20.167 ± 0.289^{C}
	7	0.203 ± 0.008^{C}	72.973 ± 0.021^{B}	0.430 ± 0.006^{A}	173.500 ± 0.500^{C}	19.167 ± 0.289^{B}
	14	0.227 ± 0.005^{B}	73.200 ± 0.050^{C}	0.432 ± 0.006^{A}	169.403 ± 0.364^{B}	18.333 ± 0.289^{A}
	21	0.243 ± 0.008^{A}	73.637 ± 0.045^{D}	0.415 ± 0.017^{A}	167.006 ± 0.500^{A}	18.333 ± 0.289^{A}
S-2	0	0.316 ± 0.005^{D}	70.843 ± 0.040^{A}	0.453 ± 0.009^{C}	182.037 ± 0.158^{D}	20.167 ± 0.289^{D}
	7	0.379 ± 0.007^{C}	71.040 ± 0.046^{B}	0.434 ± 0.008^{B}	178.387 ± 0.287^{C}	19.000 ± 0.000^{C}
	14	0.423 ± 0.008^{B}	71.400 ± 0.050^{C}	0.424 ± 0.005^{B}	175.170 ± 0.725^{B}	17.333 ± 0.289^{B}
	21	0.442 ± 0.003^{A}	72.047 ± 0.015^{D}	0.410 ± 0.005^{A}	170.390 ± 0.610^{A}	16.167 ± 0.289^{A}
S-3	0	0.212 ± 0.009^{D}	70.543 ± 0.040^{A}	0.443 ± 0.009^{B}	144.500 ± 0.500^{D}	20.167 ± 0.289^{D}
	7	0.299 ± 0.009^{C}	71.057 ± 0.025^{B}	0.426 ± 0.005^{A}	143.537 ± 0.223^{C}	19.333 ± 0.289^{C}
	14	$0.354\pm.004^{B}$	71.663 ± 0.032^{C}	0.424 ± 0.011^{A}	142.380 ± 0.336^{B}	18.000 ± 0.000^{B}
	21	0.389 ± 0.009^{A}	72.127 ± 0.030^{D}	0.423 ± 0.007^{A}	141.493 ± 0.490^{A}	17.333 ± 0.289^{A}
S-4	0	0.282 ± 0.008^{D}	70.550 ± 0.100^{A}	0.445 ± 0.008^{C}	180.173 ± 0.158^{D}	20.167 ± 0.289^{D}
	7	0.322 ± 0.007^{C}	72.623 ± 0.309^{B}	0.434 ± 0.005^{BC}	177.510 ± 0.234^{C}	19.667 ± 0.289^{C}
	14	0.351 ± 0.005^{B}	74.533 ± 0.038^{C}	0.428 ± 0.005^{AB}	173.060 ± 0.010^{B}	19.000 ± 0.000^{B}
	21	0.387 ± 0.005^{A}	75.087 ± 0.040^{D}	0.422 ± 0.010^{A}	168.386 ± 0.280^{A}	18.333 ± 0.289^{A}

*Means and standard deviations for n=3. The experimental values within columns of individual sample that have no common superscript are significantly different ($P< 0.05$) according to Duncan's multiple range test (DMRT).

The physicochemical changes of probiotic pineapple juice prepared by *L. plantarum* (S-2) at chill storage are presented in Table 1 (S-2). There were general increase in the acidity and moisture content throughout the storage days which resulted from the production of lactic acid. However, the value of protein content, total sugar content, and TSS were decreased with time. Though protein content did not show any significant difference ($p < 0.05$), but TSS and total sugar content showed significant difference at different days.

These changes are almost same in case of sample 3 where *L. fermentum* was used as probiotic bacteria. Table 1 (S-3) shows the changes in acidity, moisture, protein content, total sugar content, and TSS of probiotic pineapple juice prepared by *L. fermentum*. It was found that *L. fermentum* produce lower amount of lactic acid (0.212±0.009 % at day 0 to 0.389±0.009 % at day 21) in contrast with *L. plantarum* (0.316±0.005 % at day 0 to 0.442±0.003 % at day 21).

Sample 4 was prepared by equal amount of both *L. plantarum* and *L. fermentum* (0.5 % of each). Like other probiotic juice samples, the protein content, total sugar content and TSS were decreased with time whereas the acidity and moisture percentage showed upward trends at refrigerated storage condition. Interestingly, total sugar content was somewhat higher in sample 4 (180.173±0.158 mg/100 mL at day 0 to 168.386±0.280 mg/100 mL at day 21), but other parameters were almost same as in sample 1, 2 and 3. All parameters showed significant changes ($p < 0.05$) throughout the chilled storage days.

3.2. *Microbiological assay*

The growth and viability of *Lactobacillus* culture in juice samples were studied and presented in Table 2. The lactobacillus counts of 1 mL of starter cultures were measured by growing the pure isolates in MRS broth for 48 h at 37 °C. Initially, *L. fermentum* grows well in broth culture and exhibited the highest count. *L. plantarum* and *L. fermentum* are grown well individually, as well as in mixed state in pasteurized pineapple juice during fermentation without the addition of any other external nutrient. The lactic cultures were able to withstand and reproduce in all three probiotic juice samples. For example, *L. plantarum* (in S-2) count was increased from $5\pm0.13\times10^6$ at starter culture to $1.23\pm0.54\times10^9$ at day 0. This count was increased slightly at day 7 ($1.37\pm0.48\times10^9$). But afterwards, they started losing their viability. The count ($1.07\pm0.39\times10^8$) was well above standard up to day 14. However, the count was reduced to $8.33\pm0.56\times10^6$ at day 21. This trend was almost similar for sample 2 and 3. No *Lactobacillus* strain was found in control sample.

Table 2. Changes in the viability of *L. plantarum* and *L. fermentum*in refrigerated probiotic pineapple juice.

Day	CFU/mL		
	L. plantarum (S-2)	*L. fermentum* (S-3)	Mixed culture of *L. plantarum* and *L. fermentum* (S-4)
Starter culture	$5\pm0.13\times10^6$	$1.63\pm0.41\times10^7$	$1.1\pm0.62\times10^7$
0	$1.23\pm0.54\times10^9$	$1.57\pm0.66\times10^9$	$1.03\pm0.37\times10^9$
7	$1.37\pm0.48\times10^9$	$1.73\pm0.74\times10^9$	$1.17\pm0.54\times10^9$
14	$1.07\pm0.39\times10^8$	$1.23\pm0.72\times10^8$	$1.01\pm0.29\times10^8$
21	$8.33\pm0.56\times10^6$	$9.67\pm0.37\times10^6$	$9.33\pm0.42\times10^6$

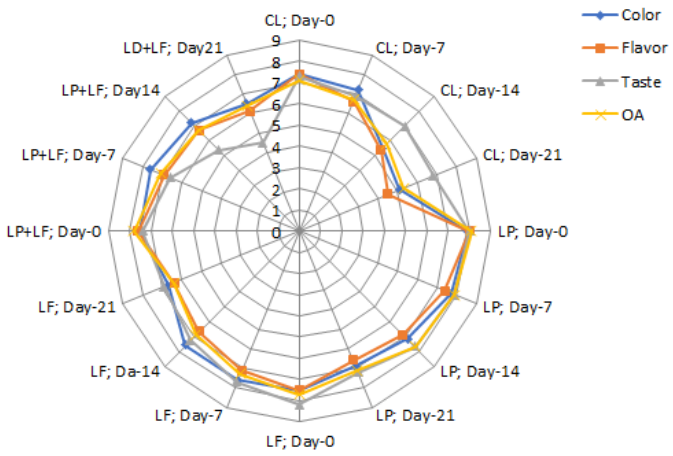

Fig. 1. Sensory profiles of pineapple juice produced by fermentation with *L. plantarum*, *L. fermentum* and their mixed culture. LP = *L. plantarum*, LF = *L. fermentum*, CL = Control, OA = Overall acceptability.

Sensory profiles of pineapple juice produced by fermentation with of *L. plantarum, L. fermentum* and their mixed culture are presented in Fig. 1. The sensory scores of control sample were reduced drastically at long storage. Taste of the mixed culture inoculated juice showed poor results than *L. plantarum* and *L. fermentum* inoculated juice. Color and flavor scores also showed similar results. Overall acceptability of *L. plantarum* (S-2) inoculated juice was quite consistent than others.

4. Discussion

This study was aimed to make probiotic pineapple juice and to check whether probiotic pineapple juice could show a significant difference in physiochemical parameter or in microbial population during storage.

The acidity of all the fermented samples during storage period was low and varied a little, among different days (Table 1). This acidic condition could lead to the decrease in the maximum growth rate and an extended length of the lag phase. During this period metabolic activity and lactic acid production is minor and the bacterial cells tried to adapt with the new conditions. Therefore, in this study, acidity of probiotic juices increases with time. Yoon [22] also found an increased acidity when tomato juice was inoculated with *L. delbrueckii, L. acidophilus, L. plantarum* and *L. casei*.

The moisture content of all the treated samples was slightly increased with time. This may be due to the use of dry matter by bacteria for their metabolism. Increased moisture content reduces the nutritive factors such as fat, protein and carbohydrate, thereby reducing the energy value. It means moisture content and energy values are reversely correlated. Our findings also found similarities with Nazarudeen [23].

In this study, the protein content of pineapple juice was not varied so much (reduced very slowly) but total sugar content and total soluble solid (TSS) were decreased with time. It is anticipated that bacterial cells use those total available sugars and total soluble solid as food. As a result, total sugar and TSS content were decreased gradually [16,22-26]. This may be due to bacterial cell was capable of growing in fruit juice without additional nutrient supplement.

Microbial viability is the most important factor during storage period. Here, *Lactobacillus* culture viability increased with time up to seven days and then decreased gradually (Table 2). The results indicated that both *L. plantarum* and *L. fermentum* were able to withstand and utilized fruit juices for their cell synthesis, as indicated by a decrease in fruit sugar and an increase in acidity. After one week, *Lactobacillus* population started to reduce. Juice inoculated with *L. fermentum* showed the best performance in terms of population density than other samples. The total count of *Lactobacillus* for all probiotic pineapple juice samples were well above the standard value (10^7) up to day 14. This high viable count is important for maximum health benefits by probiotic foods [27]. This result agrees with the report of Hossain *et al*. and Gaanappriya *et al.* [28,29], who stated that probiotic pineapple juice could be considered as a probiotic beverage without any nutritional supplement. The growth of these probiotic bacteria could have been affected by various factors such as availability of oxygen, fermentation time

and storage temperature [30,31]. It has been also reported that the acid production ability of lactic acid bacteria, especially post incubation (post-acidification) affects the cell viability of probiotic bacteria, including *L. acidophilus* and *Bifidobacterium bifidum* [32, 33].

The results of hedonic tests reflect that the color, flavor, taste and overall acceptability of probiotic pineapple juice were preferred by the panelists. Luckow and Delahunty [8] found that for probiotic blackcurrant juice the sensory characteristic was better than the control sample. Some other researchers [28,34] also found similar results in case of mango and pineapple probiotic juice.

5. Conclusion

L. plantarum and *L. fermentum* were used to produce probiotic pineapple juice, and their ability to produce lactic acid and cell synthesis were checked. Both of them were grown well in pineapple juice at 37 °C, and the viable counts reached over 10^9 CFU/mL after 48 h of fermentation at 37 °C. The results of the present study demonstrated that both *L. plantarum* and *L. fermentum* were able to survive in fermented juices with high acidity. Therefore, it could be advocated that pineapple juice could be exploited as a carrier for fermentation and delivery of probiotic lactic acid bacteria, and these probiotic-fortified fruit products could be used as a functional healthy beverage to promote better health and nutrition of the population. However, more extensive *in vitro* and *in vivo* studies are vital in order to authenticate the probiotic potential and safety of such cultures and fruit products based on these beneficial microbes before being endorsed for the better health and nutrition of society.

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