Scientific Study & Research

Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER POTENTIALS OF TWO LACTOBACILLI IN PROBIOTIC FRUIT JUICE DEVELOPMENT AND EVALUATION OF THEIR BIOCHEMICAL AND ORGANOLEPTIC STABILITY DURING REFRIGERATED STORAGE

Mohammad A. Hossain^{1*}, Rana Das², Md. Yasin¹, Humayun Kabir¹, Tanvir Ahmed¹

¹Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh ²Department of Food Engineering and Technology, State University of Bangladesh, Dhaka 1205

*Corresponding Author: mahossain-fet@sust.edu

Received: June, 03, 2021 Accepted: June, 15, 2022

Abstract: In this study, two probiotic lactobacillus strains, such as Lactobacillus fermentum and Lactobacillus plantarum, were inoculated in apple and star fruit juices to develop probiotic juices. The main aim of this study was to test the suitability of these two strains in the fruit juices, and also assess the change of biochemical and sensory properties under chill (4 °C) storage. For each fruit juice, three probiotic juice samples were prepared by inoculating with 1 % L. plantarum, 1 % L. fermentum, and the mixture of both L. fermentum and L. plantarum (0.5 % each) and compared with the control sample (juice with no lactobacillus strain). The changes in pH, acidity, protein, total soluble solid, total cell count, and sensory properties of samples were studied at every seven-day interval at 4 °C for three weeks. The results revealed that the pH, protein, and total soluble solids (TSS) of both probiotic juices, decreased slightly with increasing storage time. At the same time, acidity contents showed a reverse trend. It was found that both L. fermentum and L. plantarum sustained well in fruit juices, though the highest bacterial cell count was found on 7th day of storage for both probiotic juices. Notably fruits juices with L. fermentum supported more cell growth than the juice samples with L. plantarum. Regarding organoleptic analysis, overall sensory scores of fermented apple and star fruit juices including control samples slightly decreased with increasing storage duration. Overall, the study insinuated that two lactobacilli did not put forth any inferior properties of fruit juice samples compared to the control samples regarding physicochemical and sensory properties. Therefore, apple and star fruit juices could be viable media for the growth of L. fermentum and L. plantarum as potential probiotics.

Keywords: *apple, Lactobacillus fermentum, Lactobacillus plantarum, probiotic juice, star fruit*

© 2022 ALMA MATER Publishing House, "VASILE ALECSANDRI" University of Bacău. All rights reserved.

INTRODUCTION

The production of new foods with more functional and nutritional properties is getting attention day by day. With this motive, different kinds of functional foods, which positively impact the intestinal microbial composition and activities, are being developed. The development of probiotic food products is a research priority in this sector, which helps maintain a suitable microbial environment and the supply of essential nutrients [1]. Probiotics are microbial food supplements that improve the consumers' health by increasing microflora balance in the intestine when consumed living in adequate amounts [2]. They have been considered to provide various health benefits, such as antimicrobial and anti-tumor effects, anti-cholesterol, immunomodulation, anti-diabetic, lactose intolerance, and diarrheal treatment [3, 41. Accordingly, there is incessant consumer demand in the food market with such beneficial microbes. Alongside, the physicochemical and sensory properties of processed fruits and vegetables can be improved using lactic acid fermentation [5]. Several Lactobacillus strains are considered to be employed as probiotics providing numerous health-promoting activities, including immunomodulation, reduction of blood cholesterol levels, and resistance against pathogens [6]. The study conducted by Mikelsaar and Zilmer found that L. plantarum and L. fermentum exhibited antioxidative and antimicrobial activity against intestinal pathogens and secured organoleptic and safety of the final product [7].

Highly acceptable food products can be used as a perfect medium for probiotic strains to maintain their viability. Dairy can be an excellent medium for the survival of favorable bacteria, for example, *Lactobacillus* and *Bifidobacterium* species. The addition of probiotics in dairy products, viz. milk, cheese, and yogurt, can improve the stomach's buffer capacity and, consequently, enhance bacterial survival in the intestine [8]. Nevertheless, as the number of vegetarians increases in developed countries, so does the demand for vegetarian probiotic products. Simultaneously, the demand for non-dairy products containing probiotics has been going up because several problems are related to consuming fermented dairy products, including lactose intolerance [9]. In this regard, fruits with their numerous nutritive values provide an alternative method of producing probiotic foods. It is well noted in consumers' perception that fruits contain a high amount of antioxidants, micronutrients, and dietary fibers [10 - 13]. Hence, fruit juices can be a vigorous product with abundant nutrients and could be a viable medium for probiotics [14].

A number of studies were conducted to find out the fitness of fruit juices, for example, pineapple, mango, star fruit, and apple, etc. as raw materials for the development of probiotic drinks, where *L. acidophilus, L. casei,* and *L. plantarum* have been used as probiotic bacteria [15, 16]. Probiotification of vegetable juices, such as cabbage and tomato juice by *Lactobacillus* were studied in several studies [17, 18]. It was found that several fruit juices are rich in vitamins, proteins, carbohydrates (including glucose, fructose, and sucrose), essential omega-3 and omega-6 fatty acid [19]. Apple fruit juice is rich in many potential health-promoting properties, including high phenolic compounds, vitamins, minerals, and good antioxidant capacity, and is widely consumed in many temperate countries [20]. Star fruit is also a tropical fruit containing many beneficial properties such as polyphenols and ascorbic acid, which is helpful in preventing cancer, immune dysfunction, cardiovascular diseases aging, and neural

POTENTIALS OF TWO *LACTOBACILLI* IN PROBIOTIC FRUIT JUICE DEVELOPMENT AND EVALUATION OF THEIR BIOCHEMICAL AND ORGANOLEPTIC STABILITY DURING REFRIGERATED STORAGE

diseases [21]. Thus, with their added nutritional value, apple and star fruit juices are likely to be the food media, where the healthy bacteria can also make their mark on the well-being of the consumers. In this study, *Lactobacillus fermentum*, and *Lactobacillus plantarum* were utilized as potential probiotic strains to develop healthy apple and star fruit juice, and their biochemical and sensory properties were evaluated at refrigerated storage temperature.

MATERIALS AND METHODS

Collection of Lactobacillus strains

Both *Lactobacillus fermentum* DSM 20052, and *Lactobacillus plantarum* DSM 10492 were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Germany). These lactobacillus cultures were preserved in MRS medium (Merck, Germany) containing 20 % glycerol in a refrigerator at -20 °C.

Juice extraction, inoculation and incubation

Fresh apple and star fruits were purchased from the local market of Sylhet, Bangladesh. Two fruit juices, viz. apple and star fruit, were freshly extracted by a juice extractor, and the extracted juices were pasteurized for 5 min at 80 °C. After cooling the pasteurized apple juice samples to 30 °C, they were marked as A1 (control sample with no strain), A2 (inoculated with 1 % *L. plantarum*), A3 (inoculated with 1 % *L. fermentum*), and A4 (1 % mixture of both strains with 0.5 % each). Following the same procedure, star fruit juices were also marked as S1 (Control), S2 (inoculated with 1 % *L. plantarum*), S3 (inoculated with 1 % *L. fermentum*), and S4 (1 % mixture of both strains with 0.5 % each). Fruit juices containing 100 mL in each flask were inoculated with 1 % *Lactobacilli* culture under aseptic conditions. Then, the samples were incubated for 48 h at 37 °C. Both *lactobacilli* strains were previously grown at MRS broth culture for 48 hours. No bacterial culture was used to inoculate the flasks labeled as control, and no sugar or preservatives were used in any of the samples. Each sample type was also subdivided into another four batches based on the physicochemical data taken at different time intervals. All juice samples were stored at 4 °C after fermentation.

Physicochemical analysis

All the samples were stored at 4 °C and analyzed at every seven-day interval. The *p*H, acidity, TSS, and protein content were determined to quantify the changes in fruit juices after microbial inoculation in the juice samples during storage. The *p*H of juice samples was estimated using a benchtop *p*H meter (HI5221, Hanna Instruments, USA), and TSS was measured by a hand refractometer (MASTER-M, ATAGO, Japan) as degree Brix (°Bx). The acidity (%) and protein content (%) were evaluated according to the Official Methods of Analysis of AOAC [22].

Microbial assay

The total number of viable cells (CFU·mL⁻¹) was estimated by the standard plate count (SPC) method using *Lactobacilli* MRS agar (dextrose 20.0 g·L⁻¹, beef extract 10.0 g·L⁻¹, meat peptone 10.0 g·L⁻¹, sodium acetate 5.0 g·L⁻¹, yeast extract 5.0 g·L⁻¹, ammonium citrate 2.0 g·L⁻¹, disodium phosphate 2.0 g·L⁻¹, Tween 80 1.0 g·L⁻¹, manganese sulfate 0.05 g·L⁻¹magnesium sulfate 0.1 g·L⁻¹) after 48 hours of incubation at 37 °C [23]. At first, MRS agar was prepared, autoclaved to sterile, and poured at Petri dishes at an amount of 18 - 20 mL. They were allowed to solidify, followed by inoculation with 1mL juice sample. Then, the plates were incubated at 37 °C for 48 h, and a colony counter was used to measure the total lactobacillus count. The MRS agar media used for microbial assay were of analytical grade and purchased from Merck, Germany.

Sensory Evaluation

In this study, a total of thirty semi-trained tasters were used to analyze the sensory characteristics of the probiotic samples. All the panelists were requested to mark the juice samples for color, taste, flavor, and overall acceptability (OA). They were given bread and water to help them cleanse their palates between samples. A 9-point hedonic rating scale (1= Dislike Extremely to 9 = Like Extremely) was performed to assess the degree of acceptability of probiotic juices [15, 16].

Statistical Analysis

The results were shown as mean \pm standard deviation of three replicates of the analyzed samples. The software package SPSS (Statistical Package for the Social Science, version 20, IBM, USA) software (SPSS, 2011) was used to conduct One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for all physicochemical analysis. The results were considered significant at p-value < 0.05 (95 % level of confidence).

RESULTS AND DISCUSSION

Physicochemical analysis

The results in Table 1 and Table 2 represents the chemical changes in fermented apple and star fruit juice samples under storage conditions at 4 °C. The data exhibits that a higher decrease in pH was found when *lactobacilli* started to inoculate with time compared to that of the control sample. For instance, the pH in the control sample of apple juice was found 4.743, while inoculating with *L. plantarum*, *L. fermentum*, and mixed cultures, it was decreased to 4.263, 4.337, and 4.140, respectively, after 21 days of storage. In fruit juice, *L. plantarum*, *L. fermentum*, and mixed isolates reduced the pH of the control sample (3.345) to 2.950, 2.937, and 2.937, respectively. The pH content of the probiotic juices decreases slowly as the population of the bacteria produces more acid with time. Sharma and Mishra [24] found that pH reduced significantly till 72 hours, and then the rate of reduction starts to lower. Similar results were also found for pineapple and mango fruit juices in previous studies [15, 16]. Yanez *et al.* [25] found

POTENTIALS OF TWO LACTOBACILLI IN PROBIOTIC FRUIT JUICE DEVELOPMENT AND EVALUATION OF THEIR BIOCHEMICAL AND ORGANOLEPTIC STABILITY DURING REFRIGERATED STORAGE

that the reduction in pH of the medium also reduces the growth rate and extends the lag phase of the bacteria culture. At the same time, the acidity content of the probiotic juices also increased with time. As time increases, the population of the bacteria also increases, and they produce more acid, which increases the acidity content of the probiotic juices. The highest acidity content was found for probiotic apple juice as 0.895 % inoculated with *L. fermentum* and for probiotic star fruit juice as 3.64 % after 21 days. Yoon *et al.* [23] found that the acidity in cabbage juice nearly increases 1 % after 72 h of fermentation. Acidity and pH are related to one another, and it is vice versa. With a decrease in pH, the acidity content also increases. It is hypothesized that the *Lactobacillus* bacteria absorbed sugar for their cell synthesis and converted into acid, which causes the increase in acid content [16].

Total soluble solids (TSS) were slightly decreased among different samples for each fruit juice with storage time. On day 0, the TSS value was found 13 °Bx for the fermented samples of apple. The lowest TSS value (10.33 °Bx) was shown on day 21 for sample A3 inoculated with *L. fermentum*, following 10.34 and 10.67 °Bx for sample A2 and A4, respectively. A similar trend was presented in star fruit juice samples, where the lowest TSS value was shown 3.50 °Bx at 21 days for sample S3 inoculated with *L. fermentum*. TSS content was the same among the samples at 0 day, but as the storage time increased, the value decreased. Yoon *et al.* [26] also reported a decline in TSS and *p*H under storage conditions, while probiotic tomato juice was developed using *Lactobacillus delbrueckii, L. plantarum, L. casei,* and *L. acidophilus*. Kumar *et al.* found similar results with mango, grape, cantaloupe, and sapota [27].

	storage condition at 4 C						
Parameters		A1	A2	A3	A4		
	0	4.743±0.037 ^{cA}	$4.633 {\pm} 0.028^{bD}$	$4.543{\pm}0.037^{aC}$	$4.543{\pm}0.038^{aD}$		
pН	7	4.667 ± 0.015^{dA}	4.357 ± 0.02^{aB}	4.410 ± 0.01^{bcB}	4.420 ± 0.026^{cC}		
	14	4.640 ± 0.04^{dA}	4.310±0.02 ^{bA}	4.377 ± 0.006^{cAB}	$4.230{\pm}0.026^{aB}$		
	21	4.593±0.015 ^{dA}	4.303 ± 0.015^{bA}	4.067±0.032 ^{cA}	$4.140{\pm}0.045^{aA}$		
	0	$0.562 \pm .0162^{aA}$	$0.634 \pm .04033^{bA}$	0.700±0.0205 ^{cA}	$0.671 {\pm} 0.0135^{dA}$		
A aidity [0/]	7	$0.565{\pm}0.0075^{aA}$	$0.702 \pm .0205^{bB}$	$0.759 {\pm} 0.0350^{cB}$	0.740±0.0929 ^{cB}		
Acidity [%]	14	$0.570{\pm}0.04471^{\mathrm{aA}}$	0.810 ± 0.01527^{bC}	0.822 ± 0.03245^{cC}	$0.845{\pm}0.0907^{dC}$		
	21	$0.582{\pm}0.0205^{aA}$	0.891 ± 0.0347^{bD}	0.895 ± 10.0527^{cD}	$0.891 {\pm} 0.0347^{cD}$		
Protein [%]	0	0.137 ± 0.005^{bA}	0. 124 ± 0.006^{aC}	$0.120{\pm}0.007^{\mathrm{aB}}$	$0.116{\pm}0.004^{aB}$		
	7	$0.130{\pm}0.010^{bA}$	$0.112{\pm}0.005^{\mathrm{aB}}$	$0.122{\pm}0.005^{\mathrm{aB}}$	0.111 ± 0.007^{aB}		
	14	0.126±0.006 ^{bA}	$0.102{\pm}0.008^{\mathrm{aAB}}$	$0.112{\pm}0.007^{\mathrm{aAB}}$	$0.108{\pm}0.005^{aB}$		
	21	0.125 ± 0.006^{bA}	$0.096{\pm}0.003^{\mathrm{aA}}$	$0.094{\pm}0.008^{\mathrm{aA}}$	$0.100{\pm}0.004^{aA}$		
TSS [°Bx]	0	13.67±0.5 ^{bA}	13 ± 0.5^{abC}	13 ± 0.5^{abC}	12 ± 0.5^{aBC}		
	7	13±0.5ªA	$12{\pm}0.5^{aB}$	$12{\pm}0.5^{aB}$	12.16±0.288 ^{aC}		
	14	13±0.5ªA	11.33 ± 0.577^{bB}	11.33±0.577 ^{bB}	11.33±0.288 ^{bB}		
	21	13±0.5ªA	$10.34{\pm}0.288^{\mathrm{aA}}$	$10.33{\pm}0.288^{aA}$	10.67 ± 0.5^{bA}		

Table 1.	Changes in	chemical	properties	of	apple	juice	samples	under
		storage	condition	at	$4^{\circ}C$			

 abcde Row means containing a common letter are not significantly (at p < 0.05) different, defined by DMRT ABCDE Column means containing a common letter are not significantly (at p < 0.05) different, defined by DMRT

The result revealed that the highest protein content (0.137 %) was shown in control sample A1 at 0 day. There were trivial changes among samples depending upon storage

days. However, the lowest protein content (0.094 %) was found in sample inoculated with *L. fermentum* (A3) on day 21, and a similar trend was found for the protein content of star fruit juice samples. Protein content, total available sugars, and micronutrients are expected to accelerate the growth of probiotic bacteria. With the increase in storage time, bacteria used these nutrients as a source of energy without extra nutrients. In a previous study, the probiotification of tomato pulp by *L. plantarum* and *L. casei* improved protein and starch digestibility, while TSS and protein content decreased with time [28].

under storage condition at 4 C						
Parameters		S1	S2	S3	S4	
	0	3.345±0.01 ^{bA}	$3.127{\pm}0.015^{aD}$	3.187 ± 0.005^{bD}	3.243±0.012 ^{cD}	
pН	7	3.337 ± 0.02^{bA}	$3.067{\pm}0.006^{aC}$	3.060 ± 0.01^{aC}	$3.060{\pm}0.01^{aC}$	
	14	3.333±0.015 ^{bA}	$2.977{\pm}0.005^{aB}$	3±0.01 ^{bB}	3 ± 0.01^{bB}	
	21	3.329±0.021 ^{bA}	$2.950{\pm}0.01^{aA}$	$2.937{\pm}0.006^{\mathrm{aA}}$	$2.947{\pm}0.006^{aA}$	
	0	2.863±0.044 ^{aA}	$3.095{\pm}0.024^{bA}$	$3.095{\pm}0.024^{bA}$	3.167±0.010 ^{cA}	
A aidity [0/]	7	2.895±0.012 ^{aA}	3.134±0.0151 ^{bB}	3.166 ± 0.017^{bB}	3.274 ± 0.0485^{cB}	
Acidity [%]	14	2.895±0.012 ^{aA}	3.238 ± 0.032^{bC}	3.237 ± 0.013^{bC}	3.306±0.015°C	
	21	2.945±0.042 ^{aA}	3.306 ± 0.015^{bD}	3.364 ± 0.058^{bD}	3.307±0.042°D	
Protein [%]	0	1.313±0.015 ^{cA}	$1.005{\pm}0.011^{abB}$	1.006 ± 0.003^{bC}	$0.994{\pm}0.014^{\mathrm{aB}}$	
	7	$1.290{\pm}0.009^{bA}$	$0.992{\pm}0.005^{\mathrm{aAB}}$	$0.999 {\pm} 0.006^{\mathrm{aBC}}$	$0.993{\pm}0.005^{aB}$	
	14	1.289 ± 0.005^{bA}	$0.991{\pm}0.007^{\mathrm{aAB}}$	$0.982{\pm}0.005^{\mathrm{aAB}}$	$0.981{\pm}0.003^{\mathrm{aAB}}$	
	21	1.285 ± 0.010^{bA}	$0.982{\pm}0.004^{\mathrm{aA}}$	$0.966{\pm}0.019^{\mathrm{aA}}$	$0.968{\pm}0.020^{aA}$	
TSS [°Bx]	0	$5.00{\pm}0.50^{aA}$	$4.50{\pm}0.50^{aB}$	4.50 ± 0.50^{aA}	$4.50{\pm}0.50^{aB}$	
	7	5.00±0.426 ^{aA}	$4.00{\pm}0.50^{aAB}$	$4.333{\pm}0.278^{\mathrm{aA}}$	$4.333{\pm}0.278^{aB}$	
	14	4.667±0.325 ^{bA}	$3.667{\pm}0.289^{\mathrm{aA}}$	$4.053{\pm}0.278^{abA}$	4.067 ± 0.289^{aA}	
	21	4.667 ± 0.325^{abA}	$3.667{\pm}0.289^{aA}$	$3.50{\pm}0.50^{abA}$	$3.733{\pm}0.289^{aA}$	

Table 2. Changes in chemical properties of star fruit juice samplesunder storage condition at 4 $^{\circ}$ C

^{abcde}Row means containing a common letter are not significantly (at p < 0.05) different, defined by DMRT ^{ABCDE}Column means containing a common letter are not significantly (at p < 0.05) different, defined by DMRT

Microbial load assessment

The influence of storage time on the cell count of two *lactobacilli* in probiotic apple and star fruit juices has been presented in Table 3 and Table 4. Although both *lactobacilli* culture decreased their viability in the fermented apple and star fruit juices during storage at 4 °C, the cell count of both strains in all apple juice samples ranged from 10^6 to 10^8 CFU/100mL after 21 days of storage. For star fruit juice, the count remained at the standard level (10^6 CFU·mL⁻¹) for up to day 14 and afterward cell counts started to decrease. Overall, the count was higher in apple fruit juice than star fruit juice.

The tables show that cell count was at its peak when counted at day 7 for any of the probiotic juice samples. The highest cell count was found at 2.9×10^8 CFU/100mL for the fermented apple juice sample, which contained *L. fermentum* isolate after 21 days, whereas the fermented star fruit sample containing mixed isolates after 21 days of storage contained the least viable cell of 2.45×10^4 CFU/100mL. The study also found that *L. fermentum* survived more in both apple and star fruit juice representing viable cell count as 2.9×10^8 and 2.33×10^5 , respectively, after 21 days of storage at 4 °C.

POTENTIALS OF TWO *LACTOBACILLI* IN PROBIOTIC FRUIT JUICE DEVELOPMENT AND EVALUATION OF THEIR BIOCHEMICAL AND ORGANOLEPTIC STABILITY DURING REFRIGERATED STORAGE

Cell count in apple juice (CFU/100mL)						
Sample 0 day 7 day 14 day 21 day						
L. plantarum	$(3.6\pm0.54)\times10^8$	$(3.2\pm0.48)\times10^8$	$(2.6\pm0.39)\times10^{8}$	$(1.7\pm0.56)\times10^{8}$		
L. fermentum	$(5.6\pm0.66)\times10^8$	$(5.3\pm0.74)\times10^{8}$	$(3.4\pm0.37)\times10^8$	$(2.9\pm0.72)\times10^{8}$		
Mixed Isolates	$(5.23\pm0.37)\times10^{8}$	$(4.8\pm0.54)\times10^8$	$(3.0\pm0.29)\times10^8$	$(2.4\pm0.42)\times10^{8}$		

 Table 3. Total lactobacillus count in probiotic apple juice during storage

* Apple juice samples were fermented at 37 $^{\circ}$ C for 48 h and then stored at 4 $^{\circ}$ C

The bacterial count was well above the standard level (> 10^6) considered for probiotic foods. It is imperative to retain a significant number of viable *lactobacilli* present in the final product for the consumers' wellbeing [29]. The study also insinuated that several factors, for example, *p*H, acidity, amount of available nutrients, oxygen level, and antimicrobial substances, affect the viability of probiotic cultures in the product. The ability of lactic acid bacteria to produce acid has an impact on probiotic bacteria cell viability, especially after incubation [30]. Researchers are now using microencapsulation technology to coat beneficial bacteria to prolong their shelf life, increase heat and acid resistance [31].

Cell Count in star fruit juice (CFU/100mL)						
Sample	0 day	7 day	14 day	21 day		
L. plantarum	$(3.67\pm0.54)\times10^7$	$(2.67\pm0.48)\times10^7$	$(2.33\pm0.56)\times10^7$	(3.67±0.39)×10 ⁴		
L. fermentum	$(4.53\pm0.66)\times10^8$	$(3.54\pm0.74)\times10^{8}$	$(3.00\pm0.37)\times10^8$	$(2.33\pm0.72)\times10^{5}$		
Mixed Isolates	$(4.40\pm0.37)\times10^7$	$(3.33\pm0.54)\times10^7$	$(2.67\pm0.42)\times10^7$	$(2.45\pm0.29)\times10^4$		

Table 4. Total lactobacillus count in probiotic star fruit juice during storage

* Star fruit juice samples were fermented at 37 $^{\circ}$ C for 48 h and then stored at 4 $^{\circ}$ C

Sensory Evaluation

Figure 1 illustrates that the highest score for apple juice in color was 8.20 for sample A3 at day 0, in flavor as 7.90 for A4 at day 0, and in taste as 7.70 for A2 at day 0. It was also found that panelists gave highest score of 7.70 in overall acceptability (OA) test of apple juice sample A3 inoculating with *L. fermentum*.

Figure 1 and Figure 2 illustrates the sensory properties of fermented apple and star fruit juices during storage for 21 days at 4 °C. The sensory score of star fruit juice during storage at 4 °C was illustrated in Figure 2. Panelists rated S1 and S2 samples as highest (7.60) in color at storage time of day 0; S2 as highest (7.50) in flavor at time 0 day; and S2 as highest (6.50) in taste at time 0 day. In overall acceptability, panelists preferred S4 sample containing mixed isolates to all other star fruit samples It can also be deduced from the data that apple juice samples got a higher score (greater than 7) in the overall acceptability test than star fruit juice samples from the panelists.

HOSSAIN, DAS, YASIN, KABIR and AHMED

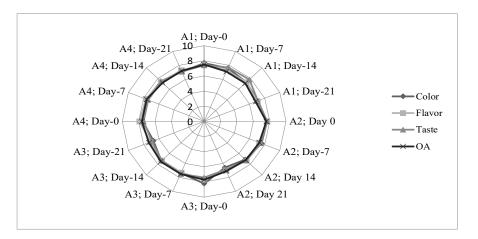


Figure 1. Sensory Evaluation of probiotic apple juice

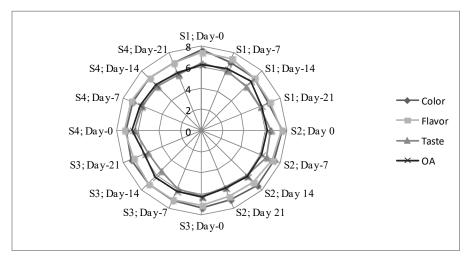


Figure 2. Sensory Evaluation of probiotic star fruit juice

However, the sensory qualities of all the juice samples containing apple and star fruits reduced moderately with the increasing storage time. Luckow and Delahunty (2004) found that the sensory characteristics of probiotic blackcurrant were found to be better than that of the control sample [32]. Some other studies found similar results regarding mango and pineapple fermented juices [33].

CONCLUSION

This study revealed that two lactic acid-forming bacteria, namely *L. plantarum and L. fermentum*, could use apple and star fruit juices for cell synthesis. Changes in *p*H, TSS, ash, and protein content of apple and star fruit juices were observed due to biochemical reactions of the *lactobacilli* under the storage condition at 4 °C. Total cell counts of *lactobacilli* in probiotic fruit juices decreased gradually with storage time. A decline in cell counts was relatively higher in star fruit juices than apple juices due to the low

POTENTIALS OF TWO LACTOBACILLI IN PROBIOTIC FRUIT JUICE DEVELOPMENT AND EVALUATION OF THEIR BIOCHEMICAL AND ORGANOLEPTIC STABILITY DURING REFRIGERATED STORAGE

pH content of star fruit juices. Both probiotic fruit juices appealed more or less similar sensory properties to the consumer compared to control juices. However, the study demonstrated that fermented apple juices were preferable to the consumer than fermented star fruit juices. Hence, it can be concluded that these two fruit juices may be used as a suitable carrier for the growth of probiotic bacteria and thus served as a healthy functional beverage for the consumer to uphold better gastrointestinal health. Further *in vivo* study of these two probiotic juices is recommended to understand the health benefits better.

ACKNOWLEDGEMENT

Authors acknowledge the financial support of the SUST Research Centre, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh to carry out the research works.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- 1. Granato, D., Branco, G.F., Cruz, A.G., Faria, J.d.A.F., Shah, N.P.: Probiotic dairy products as functional foods, *Comprehensive reviews in food science and food safety*, **2010**, <u>9</u>, 455-470;
- 2. Azad, M., Kalam, A., Sarker, M., Li, T., Yin, J.: Probiotic species in the modulation of gut microbiota: an overview, *BioMed research international*, **2018**;
- Kumar, M., Verma, V., Nagpal, R., Kumar, A., Gautam, S.K., Behare, P.V.: Effect of probiotic fermented milk and chlorophyllin on gene expressions and genotoxicity during AFB1-induced hepatocellular carcinoma, *Gene*, 2011, 490, 54-59;
- 4. Azad, M., Kalam, A., Sarker, M., Wan, D.: Immunomodulatory effects of probiotics on cytokine profiles, *BioMed research international*, **2018**;
- 5. Di Cagno, R., Minervini, G., Rizzello, C.G., De Angelis, M., Gobbetti, M.: Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies, *Food microbiology*, **2011**, **28**, 1062-1071;
- 6. Saadat, Y.R., Khosroushahi, A.Y., Gargari, B.P.: A comprehensive review of anticancer, immunomodulatory and health beneficial effects of the lactic acid bacteria exopolysaccharides, *Carbohydrate polymers*, **2019**, <u>217</u>, 79-89;
- 7. Mikelsaar, M., Zilmer, M.: Lactobacillus fermentum ME-3–an antimicrobial and antioxidative probiotic, *Microbial ecology in health and disease*, **2009**, **21**, 1-27;
- Mortazavian, A.M., Mohammadi, R., Sohrabvandi, S.: Delivery of probiotic microorganisms into gastrointestinal tract by food products, *New advances in the basic and clinical gastroenterology*, 2012, <u>10</u>, 47946;
- 9. Panghal, A., Janghu, S., Virkar, K., Gat, Y., Kumar, V., Chhikara, N.: Potential non-dairy probiotic products–A healthy approach, *Food bioscience*, **2018**, <u>**21**</u>, 80-89;
- Hossain, M.A., Dey, P., Joy, R.I.: Effect of Osmotic Pretreatment and Drying Temperature on Drying Kinetics, Antioxidant Activity, and Overall Quality of Taikor (*Garcinia pedunculata* Roxb.) Slices. *Saudi Journal of Biological Sciences*. 2021, <u>28</u>, 7269-7280;
- Hossain, M. A. and Hossain, M.S.: Optimization of Antioxidative Phenolic Compounds Extraction from Freeze-dried Pulp, Peel, and Seed of Burmese grape (*Baccaurea ramiflora* Lour.) by Response Surface Methodology, *Biomass Conversion and Biorefinery*, 2021;

- Rahman, M. M., Khan, F. E., Das, R. and Hossain, M. A.: Antioxidant activity and total phenolic content of some indigenous fruits of Bangladesh, *International Food Research Journal*, 2016, <u>23</u>, 2399-2404;
- 13. Ahmed, S., Jubair, A., Hossain, M. A., Hossain, M. M., Azam, M. S., Biswas, M.: (2021). Free radical-scavenging capacity and HPLC-DAD screening of phenolic compounds from pulp and seed of *Syzygium claviflorum* fruit, *Journal of Agriculture and Food Research*, **2021**, <u>6</u>.
- 14. Pereira, A.L.F., Rodrigues, S.: Turning fruit juice into probiotic beverages, *Fruit juices: Elsevier*, **2018**, 279-287;
- Hossain, M., Hoque, M., Hossain, M., Kabir, M., Yasin, M., Islam, M.: Biochemical, microbiological and organoleptic properties of probiotic pineapple juice developed by lactic acid bacteria, *Journal of Scientific Research*, 2020, <u>12</u> (3), 743-750;
- Hossain, M., Hoque, M., Kabir, M., Yasin, M.: Probiotification of Mango Juice by Lactic Acid Bacteria and Quality Assessment at Refrigerated Storage Condition, *Journal of Engineering Research Innovation and Education*, 2019, <u>1</u>;
- Sharma, V., Mishra, H.N.: Unstructured kinetic modeling of growth and lactic acid production by Lactobacillus plantarum NCDC 414 during fermentation of vegetable juices, LWT-Food Science and Technology, 2014, <u>59</u>, 1123-1128;
- Jaiswal, A.K., Abu-Ghannam, N.: Kinetic studies for the preparation of probiotic cabbage juice: Impact on phytochemicals and bioactivity, *Industrial Crops and Products*, 2013, <u>50</u>, 212-218;
- Batista, C., Barros, L., Carvalho, A.M., Ferreira, I.C.: Nutritional and nutraceutical potential of rape (Brassica napus L. var. napus) and "tronchuda" cabbage (Brassica oleraceae L. var. costata) inflorescences, *Food and chemical toxicology*, **2011**, <u>49</u>, 1208-1214;
- Yazdanshenas, M., Tabatabaee-Nezhad, S.A.R., Soltanieh, M., Roostaazad, R., Khoshfetrat, A.B.: Contribution of fouling and gel polarization during ultrafiltration of raw apple juice at industrial scale, *Desalination*, **2010**, <u>258</u>, 194-200;
- 21. Liu, Z., Ren, Z., Zhang, J., Chuang, C-C., Kandaswamy, E., Zhou, T.: Role of ROS and nutritional antioxidants in human diseases, *Frontiers in physiology*, **2018**, **9**, 477;
- 22. Horwitz, W.: Official methods of analysis of AOAC International, *Volume I, agricultural chemicals, contaminants, drugs/edited by William Horwitz: Gaithersburg (Maryland): AOAC International*, **1997, 2010**;
- Yoon, K.Y., Woodams, E.E., Hang, Y.D.: Fermentation of beet juice by beneficial lactic acid bacteria, *LWT-Food Science and Technology*, 2005, <u>38</u>, 73-75;
- Sharma, V., Mishra, H.: Fermentation of vegetable juice mixture by probiotic lactic acid bacteria, *Nutrafoods*, 2013, <u>12</u>, 17-22;
- Yáñez, R., Marques, S., Gírio, F.M., Roseiro, J.C.: The effect of acid stress on lactate production and growth kinetics in Lactobacillus rhamnosus cultures, *Process Biochemistry*, 2008, <u>43</u>, 356-361;
- Yoon, K.Y., Woodams, E.E., Hang, Y.D.: Probiotication of tomato juice by lactic acid bacteria, Journal of Microbiology, 2004, <u>42</u>, 315-318;
- Vijaya Kumar, B., Sreedharamurthy, M., Reddy, O.: Physicochemical analysis of fresh and probioticated fruit juices with Lactobacillus casei, *International Journal of Applied Science and Biotechnology*, 2013, <u>1</u>, 127-131;
- Sindhu, S.C., Khetarpaul, N.: Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestibility of starch and protein, *Journal of Food Composition and Analysis*, 2001, <u>14</u>, 601-609;
- Shah, N.P.: Functional foods from probiotics and prebiotics: Functional Foods from Probiotics and Prebiotics, *Food Technology* (Chicago), 2001, <u>55</u>, 46-53;
- Faye, T., Tamburello, A., Vegarud, G.E., Skeie, S.: Survival of lactic acid bacteria from fermented milks in an in vitro digestion model exploiting sequential incubation in human gastric and duodenum juice, *Journal of Dairy Science*, 2012, <u>95</u>, 558-566;
- Tripathi, M.K., Giri, S.K.: Probiotic functional foods: Survival of probiotics during processing and storage, *Journal of functional foods*, 2014, <u>9</u>, 225-241;
- Luckow, T., Delahunty, C.: Which juice is 'healthier'? A consumer study of probiotic non-dairy juice drinks, *Food Quality and Preference*, <u>2004</u>, <u>15</u>, 751-759;
- Adebayo Tayo B., Akpeji, S.: Probiotic viability, physicochemical and sensory properties of probiotic pineapple juice, *Fermentation*, 2016, <u>2</u>, 20.