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Probiotic Viability, Qualitative Characteristics, and Sensory Acceptability of Vegetable Juice Mixture Fermented with *Lactobacillus* Strains

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Abstract

The aim of the study was to consider the suitability of a mixture of juices from jicama, winter melon, and carrot as a raw medium for producing probiotic juice by *Lactobacillus* strains (*Lactobacillus plantarum* CICC22696 and *Lactobacillus acidophilus* CICC20710), as well as evaluate changes of physicochemical and microbiological characteristics during fermentation and cold storage (4˚C, 28 days). Both strains grew well in juice mixtures after 24 h of fermentation at 37˚C, reaching nearly 9 and 8 log CFU/mL when inoculated with *L. plantarum* and *L. acidophilus* respectively. The viability of *L. plantarum* was near 8 log CFU/mL at the end of storage whereas viability of *L. acidophilus* only remained 4.57 log CFU/mL. Degradation of total carotenoids was in the range of 12% - 23% in fermentation periods and 16% - 23% during cold storage depending on the strain used. The values of lightness, redness, and yellowness increased during fermentation. However, this tendency was variable during cold storage when the values of redness and yellowness decreased. Sensory acceptability of the products was enhanced by adding sucrose or multi fruit juice (containing mainly tropical fruit juices). The fermented juice with *L. plantarum* is efficient to produce a functional probiotic beverage.

Keywords

Vegetable Juice Mixture, *Lactobacillus* Strains, Fermentation, Probiotic Drink, Acceptability

1. Introduction

Probiotics are defined as live organisms, which provide a benefit to the host

when administered in adequate quantities [1]. Fermented products as a component of a daily diet, may improve the health and life quality of consumers. Beneficial effects of probiotic bacteria in food include reduction in the level of serum cholesterol, improvement in lactose metabolism, enhanced immune system, lower risk of colon cancer, control of gastrointestinal infections, improved antimutagenic properties, and stimulation of anti-diarrheal properties [2] [3]. The genera *Lactobacillus* and *Bifidobacterium* are the most common probiotic microorganisms used commercially in the food industry which comprise more than 90% of probiotic food supplements. Probiotics have been regularly incorporated in yogurt or other fermented dairy products, however, cannot be consumed by humans who are suffering from lactose intolerance and/or allergic to milk proteins. Moreover, consumers tend to prefer the food and beverages that are fresh, highly nutritional, health promoting and ready to consume. Some matrices have been used in the development of non-dairy probiotic products such as fruits, vegetables, legumes, and cereals [4].

Through fermentation, fruit and vegetable juices are preserved and maintained, while improving the nutritive and sensory properties of the products. Various studies have been conducted to investigate the suitability of fruit and vegetable juices as a medium to develop new probiotic beverages from fruits such as pineapple [5], pear [6], grape [7], pomegranate [8] guava [3], fig [2], noni [9], both barberry and black cherry [10] and vegetables such as carrot [11], beet [12], sweet potato [13], potato [14]. However, the survival of probiotics in fruit and vegetable-based matrices is more complex than in dairy products. Probiotic bacteria need protection from the acidic conditions in these media. Moreover, probiotic stability in fruit and vegetable juice products is difficult to maintain during cold storage [15].

Vegetables are rich sources of bioactive compounds which have beneficial effects in prevention of some diseases and certain types of cancer [12]. Jicama (*Pachyrhizus erosus*), also called yam bean, is an edible tuberous root placed in the spotlight as a healthy food ingredient. It is rich in vitamin C, fructooligosaccharides (FOSs), and inulin, a soluble fiber can serve as a potential prebiotic, and appreciable levels of nutraceuticals [16] [17]. Jicama also has other biological benefits such as immunomodulatory activity [18], reducing the risk of colon cancer [19], and anti-diabetic properties [20]. Winter melon (*Benincasa hispida* (*Thunb.*) *Cogn*), a widely used vegetable in India, China and other tropical countries, belongs to the Cucurbitaceous fruit family [21]. It contains carbohydrates, with 8 *g* carbohydrates and 1.5 *g* protein in every 0.5 kg of winter melon [22]. Winter melon has been evaluated to be a potential source of antioxidants for functional drinks and nutraceutical application and has good angiotension-converting enzyme inhibition capacity, which may offer protective effects against cardiovascular diseases, diabetic complications and certain types of cancers [23]. Moreover, benefits of winter melon are used to treat hydrops and turgor, beriberi, stranguria, cough, asthma with rale, fidgets due to summer-heat, diabetes, diarrhea, dysentery, carbuncle, and swelling [24]. Therefore, winter

melon and jicama are ideal substrates to combine with carrots (*Daucus carota* L.), which are rich in carbohydrates, β-carotene, vitamins (A, D, B, E, C, and K), and minerals for the growth of beneficial lactic acid bacteria in the beverages.

To our knowledge, studies dealing with the fermented drink/juice made from jicama and/or winter melon have not been explored. Most fermented juices from fruits and vegetables in past studies used lactic acid bacteria, researchers just limited investigation scope at considering the suitability for producing probiotic juice. Therefore, this study had three main objectives:

1) To investigate the suitability of a vegetable juice medium composed of jicama, winter melon and carrot juices as raw material for production of probiotic vegetable juice by lactic acid bacteria.

2) To determine physicochemical and microbiological changes during fermentation and cold storage of the vegetable juice mixture.

3) To evaluate sensory acceptability of products, furthermore, to confirm whether the addition of sucrose or multi fruit juice (mainly tropical fruit juices) improves sensory quality and hedonics.

2. Materials and Methods

2.1. Preparation of Vegetable Juice Mixture

Jicamas, winter melon with sparser wax covering its surface, and orange carrots were purchased from the supermarket Auchan and local market in Binhu district, kept at 4˚C, and used in the experiment as quickly as possible. Vegetables were careful washed and peeled. The seeds and cavity tissues were removed from the winter melon. Juices were extracted from jicama, winter melon and carrots using a Philips Viva Collection juicer (Shengzheng, China). The extracted juices were filtered separately through a cheesecloth. The clarified juices from jicama, winter melon, carrots were combined in a ratio of 1:1:1 by volume. The juice mixture was analyzed yielding the following characteristics: titratable acidity of 0.09% (expressed as lactic acid), pH of 6.05 and total soluble solid content of 6.0˚Brix. The fresh juice mixture was subjected to pasteurization in a TOMY SX-500-high pressure steam sterilizer (Tomy Kogyo Co. Ltd, Fukushima, Japan) at 80˚C for 15 min [25] in 250 ml Erlenmeyer flasks, then cooled immediately by letting it sit at ice bath to 25˚C. Each of the sealed Erlenmeyer flasks contained 100 mL pasteurized juice mixture without any nutrient supplements or water. The pasteurized vegetable juice mixture was designated as the control formulation (CON).

2.2. Strains and Microbial Culture Stock Preparation

Lactic acid bacteria (*L. plantarum* CICC 22696, *L. acidophilus* CICC 20710) were supplied by China Center of Industrial Culture Collection (CICC), Beijing, China. Both bacterial cultures were stored frozen at −20˚C in MRS (De Man, Rogosa and Sharpe) medium containing 20% glycerol. The strains were reactivated by means of double passage on MRS when needed according to the instructions in the user's manual.

2.3. Fermentation of Vegetable Juice Mixture

Lactobacillus strains were cultivated on MRS broth at 37˚C. When used for fermentation, lactic acid bacteria were cultivated until the late exponential phase of growth was reached. The exponential phase was determined from bacterial growth curve. Erlenmeyer flasks containing 100 mL of pasteurized juice mixture were then inoculated the culture to nearly 7.00 log CFU/mL, this concentration was chosen based on the recommendation for probiotic foods: minimal counts of 7.00 log CFU/mL for better efficacy in regulating beneficial effects [26]. For obtaining an initial cell density of 7.00 log CFU/mL in the final juice, 1.8 mL of cultivated MRS broth of *L. plantarum* and 5 mL of cultivated MRS broth of *L. acidophilus* were centrifuged at 3000 *g* for 5 min. The biomass of the strain was introduced into the juice (100 ml). Fermentations were carried out in an incubator for juices inoculated with *L. plantarum* and in an anaerobic incubator for juices inoculated with *L. acidophilus* at a constant temperature of 37˚C for 48 h. Samples were taken at 0, 24, 48 h for physiochemical and microbiological analysis.

2.4. Effects of Cold Storage on Fermented Vegetable Juice Mixture

To determine the effects of cold storage on cell viability and physicochemical properties after completion of appropriate fermentation time, samples of fermented juice mixture were transfer to storage at 4˚C for 28 days. Viable counts, color and total carotenoids of fermented vegetable juice were recorded at intervals of 7 days, during the 28 days. The microbial population was expressed as log CFU/mL.

2.5. Chemical and Microbiological Analysis

2.5.1. Determination of pH and Total Soluble Solid (TSS) Content

A digital F2-standard pH meter (Mettler-Toledo Instruments (Shanghai) Ltd, China) was used for pH measurements. The total soluble solid (˚Brix) of the juice was measured using a refractometer (Master-20M, ATAGO Co., Ltd., Tokyo, Japan).

2.5.2. Determination of Titratable Acidity and Total Sugar

Total acidity expressed as percent of lactic acid, was determined by titrating the juice sample (10 mL) with 0.1 N NaOH to the end point (pH 8.2 ± 0.1) [27]. The total sugar content was analyzed as glucose using the phenol sulfuric acid method of Dubois, Gilles [28].

2.5.3. Determination of Total Carotenoid Content

The total carotenoid content of the sample was determined by a slightly modified method of Adiamo, Ghafoor [29]. 5 mL of vegetable juice mixture was added to 50 mL of extraction solvent (petroleum ether: methanol 90: 10) in a separatory funnel. The mixture was vigorously mixed by hand and then stood for 5 min to separate solvent phase. The extraction process was repeated with 50 mL

fresh extraction solvent. The aqueous phase was discarded. The solvents were collected together and centrifuged at 900 *g* for 15 min. The absorbance of supernatant was read immediately at 450 nm using a L8 UV-vis spectrophotometer (INESA, China). The concentration of carotenoids was expressed as *β*-carotene (mg/L). The total carotenoid content was calculated using the following formula:

$$\text{Carotenoids content}\left(\text{mg/L}\right)=\frac{A\times V\left(\text{mL}\right)\times 10^4}{E_{1\text{ cm}}^{1\%}\times P}$$

where A = absorbance; V = total extract volume; P = analyzed juice volume (mL); $E_{1\text{ cm}}^{1\%}$ = 2592 (*β*-carotene extinction coefficient in petroleum ether).

2.5.4. Viable Cell Counts

Viable cell counts were obtained by serial dilution with saline until 10^7 dilution was reached. Aliquots of 0.1 ml of dilution were plated in triplicate on petri-dishes containing MRS agar. The petri-dishes were incubated for 48 h (*L. plantarum* CICC22696) and for 72 h (*L. acidophilus* CICC20710) at 37˚C. Plates containing 20 - 350 colonies were measured and recorded as colony forming units (log CFU/mL). *L. acidophilus* appears on agar as irregular light brown colonies ranging in diameter from 0.9 to 1.5 mm [30]. *L. plantarum* characterizes small circular, rough, dull white and convex colonies [31].

2.6. Color Analysis

The juice color was determined by using an UltraScan Pro1166 spectrophotometer (Hunterlab, America), and readings were taken in triplicate. The reflectance instruments determined three color parameters: lightness (L^*), redness (a*), and yellowness (b*). The index of total color difference (ΔE) was calculated by the equation $[(L - L_o)^2 + (a - a_o)^2 + (b - b_o)^2]^{1/2}$. Where L_o, a_o, and b_o are referred to the color reading of control sample. A larger ΔE value indicates the greater color change compared with the control sample.

2.7. Sensory Evaluation

Seven formulations of vegetable juice mixture were prepared for assessment shown as **Table 1**. On day 14 of product storage, three versions of the fermented vegetable juice mixtures were served to judges: juice mixture without any supplements (PFJ and AFJ), juice mixture with added with sucrose to 10˚Brix (SUC-PFJ and SUC-AFJ), or multi fruit juice (10% v/v; MASK-PFJ and MASK-AFJ). The commercial multi fruit juice (Farmerland; e.g., containing orange, grape, mango, tangerine, peach, pineapple, grapefruit, apple, kiwi, and passion fruit juices) of manufacturer (Rea Coop 21200, Argos-Greece) was used for addition based on report of Luckow, Sheehan [32]. The authors emphasized that the tropical fruit juice could positively mask the off-flavors in probiotic juice and contribute to the aroma and flavor of the final product.

The sensory characteristics were evaluated by a panel of 21 judges involved students in faculty of food science and technology who were not familiar with

Table 1. Experimental design of vegetable juice mixture formulations.

Formulations	Fermented juice by *Lactobacillus* strain	Sucrose	Multi fruit juice
CON	-	-	-
PFJ	*L. plantarum*	-	-
AFJ	*L. acidophilus*	-	-
SUC-PFJ	*L. plantarum*	Added to 10˚Brix	-
SUC-AFJ	*L. acidophilus*	Added to 10˚Brix	-
MASK-PFJ	*L. plantarum*	-	10% v/v
MASK-AFJ	*L. acidophilus*	-	10% v/v

characteristics of fermented non-dairy beverage. The judges (13 women, 8 men) ranged from 24 - 35 years old. Two evaluation sessions were conducted in individual booths. All samples of 40 ml were coded with 3-digit random numbers, presented to the panelists at room temperature and evaluated one at a time, in random order. Room temperature spring water was provided to panel members for rinsing the mouth between samples. The acceptance testing of attributes (color, texture, flavor, taste and overall acceptance) used a 9-point hedonic scale, ranging from 1 to 9 where 9 = like extremely and 1 = dislike extremely. The evaluation data were recorded and mean scores for each attribute were calculated to compare with the samples.

2.8. Statistical Analysis

All experimental results were the mean of triplicate. The data were recorded as the mean ± standard deviation (mean ± SD) and the statistical analysis was conducted with IBM SPSS software (version 24, Statsoft, America). Data analysis was done by one-way ANOVA followed by Duncan's post hoc test. Results were regarded as significant differences at $p < 0.05$.

3. Results and Discussion

3.1. Fermentation Characteristics

3.1.1. Growth and Productivity of Lactobacteria

Table 2 and Table 3 present the time courses of lactic acid fermentation of vegetable juice mixture by *L. plantarum* and *L. acidophilus*, respectively. Both *Lactobacillus* strains were found to be capable of rapid growth in the vegetable juice mixture medium with lactic acid production without any nutrient supplementation or altering of the structural characteristics of source matrices. The similarity of pH between the pre-culture of MRS broth (pH = 6.8) and the initial vegetable juice mixture (pH of about 6.0) resulted in the rapid growth rate of *Lactobacillus* strains. Moreover, inulin and FOSs in the juice mixture could be helpful to improve the growth and increase counts of the strains [33]. *L. plantarum* had a greater growth than *L. acidophilus* in the juice. The bacterial population of *L. plantarum* and *L. acidophilus* respectively reached nearly 9 and 8 log

Table 2. Time course of lactic fermentation of vegetable juice mixture by *L. plantarum*.

Time (h)	pH	TTS (Brix)	Titratable acidity (%lactic acid)	Log CFU/mL	Total sugar (mg/mL)	Total carotenoids (mg/L)
0 h	5.97 ± 0.01^{a}	6.4 ± 0.06^{a}	0.09 ± 0.01^{c}	6.80 ± 0.09^{c}	48.76 ± 0.71^{a}	24.22 ± 0.13^{a}
24 h	3.68 ± 0.01^{b}	5.3 ± 0.06^{b}	0.61 ± 0.01^{b}	8.98 ± 0.04^{a}	37.02 ± 1.01^{b}	21.32 ± 0.29^{b}
48 h	3.40 ± 0.01^{c}	5.0 ± 0.00^{c}	0.95 ± 0.05^{a}	8.77 ± 0.11^{b}	34.66 ± 1.04^{c}	19.54 ± 0.14^{c}

Data expressed as means ± standard deviation (n = 3). Values in the same column followed by different superscript letters indicate statistically significant differences at $p < 0.05$. TSS: total soluble solid.

Table 3. Time course of lactic fermentation of vegetable juice mixture by *L.acidophilus*.

Time (h)	pH	TTS (Brix)	Titratable acidity (%lactic acid)	Log CFU/mL	Total sugar (mg/mL)	Total carotenoids (mg/L)
0 h	5.98 ± 0.04^{a}	6.4 ± 0.10^{a}	0.09 ± 0.01^{c}	6.59 ± 0.05^{c}	47.93 ± 1.07^{a}	23.63 ± 0.22^{a}
24 h	3.96 ± 0.01^{b}	5.6 ± 0.06^{b}	0.49 ± 0.02^{b}	7.89 ± 0.01^{a}	40.27 ± 0.89^{b}	20.19 ± 0.24^{b}
48 h	3.83 ± 0.01^{c}	5.4 ± 0.00^{c}	0.59 ± 0.01^{a}	7.42 ± 0.03^{b}	38.67 ± 0.77^{b}	18.25 ± 0.38^{c}

Data expressed as means ± standard deviation (n = 3). Values in the same column followed by different superscript letters indicate statistically significant differences at $p < 0.05$. TSS: total soluble solid.

CFU/mL after 24 h then slightly decreased to 8.77 and 7.42 log CFU/mL after 48h of fermentation at 37˚C. Extending the growth period to 48 h did not increase the number of viable cells of any tested starter but did reduce cell viability. For maximum health benefits, scientists have suggested the minimum probiotic organism level in probiotic food products should be 10^{6} - 10^{7} CFU/mL at the time of consumption [34]. Therefore, to be effective in producing a probiotic beverage, the fermentation time of 24 h was chosen as a proper fermentation period to consider the effect of cold storage on fermented vegetable juice mixture. The initial population of lactic acid bacteria before cold storage at 4˚C could affect the final survival of bacteria [35].

3.1.2. Change of pH, TTS, and Titratable Acidity during Fermentation

The pH and TSS of vegetable juice decreased during the fermentation process (**Table 2** and **Table 3**). For example, *L. plantarum* and *L. acidophilus* reduced the pH from an initial value of 5.97 and 5.98 to 3.40 and 3.83 and the TSS from an initial value of 6.4 to 5.0 and 5.4 after 48 h of fermentation, respectively. The decrease in pH during lactic acid fermentation was due to accumulation of organic acids especially lactic acid. Under the same growth conditions, *L. plantarum* showed a more rapid drop in pH and TSS and produced significantly more lactic acid than *L. acidophilus*. It could be due to the requirement of *L. acidophilus* for essential growth nutrients that are deficient in the vegetable juice mixture. The obtained result agreed with the findings by researchers in tomato, cabbage, and vegetable juice fermented with lactic acid bacteria [36] [37] [38].

3.1.3. Change of Total Sugar and Total Carotenoid Content

The strains rapidly fermented the vegetable juice mixture and resulted the re-

duction of total sugar and carotenoids. The greater growth and metabolism of *L. plantarum* have led to higher sugar consumption than *L. acidophilus*, and decreased the initial sugar content of 48.76 mg/mL to 34.66 mg/mL. There was about 29.70 mg of carotenoids in 1 L of fresh vegetable juice mixture extracted with the Philips Viva Collection juicer. Pasteurizing (80˚C, 15 min) substantially reduced total carotenoid content in fresh juice mixture to ~23 - 24 mg/L (**Table 2** and **Table 3**). This amount decreased after 48 h of fermentation with both strains. When inoculated with *L. plantarum*, about 12% and 19% of carotenoid content were lost after 24 h and 48 h of fermentation, respectively. When inoculated with *L. acidophilus* the reductions were greater, with 15% and 23% lost after 24 h and 48 h of fermentation, respectively. Carotenoid degradation could be due to the metabolism of bacteria and fermentation conditions (temperature, pH, time). The level of effect depends on the strain used, substrate, and factors of fermentation [13] [25] [39].

3.1.4. Effect of Fermentation on Color Change

Table 4 presents the results regarding effect of fermentation on color components (L*, a*, b* and ΔE) in vegetable juice mixture after 24 h and 48 h of fermentation. There was an increase of lightness. This result disagreed with lactic-acid fermented mulberry juice [40] and with cashew apple juice fermented with *Lactobacillus casei* [26]. There was an increase biomass during the fermentation providing a turbidity of juice. However, the increase of lightness was the result of pH reduction in vegetable juice mixture containing carrot juice. According to the investigation of Chen, Peng [41], the lightness (L*) of carrot juice increased after acidification. It has reported that blanching carrots with acid can increase the brightness and decrease the precipitation of carrot juice during processing, improve the color and turbidity of heated or canned juice [42].

The fermentation also resulted an increase in a* and b*, indicating the redness and yellowness of vegetable juice mixture increased. Carotenoids are the main pigments responsible for the color of carrot roots and juices. *Trans* carotenoid isomers are predominant in nature and under extreme pH values (acid and alkali), can be transformed to *cis* carotenoid isomers and cause color change in carrot juice [41]. A similar trend has also been reported by Pereira, Maciel [26]. The ΔE values of fermented vegetable juice mixture with *L. acidophilus* and *L. plantarum* fell within the slightly noticeable range $0.5 < \Delta E < 2$ [43]. The juices inoculated with *L. plantarum* had a greater ΔE value (**Table 4**) compared to the juice inoculated with *L. acidophilus*.

3.2. Storage of the Probiotic Vegetable Juice Mixture

3.2.1. Effect of Cold Storage on Cell Viability

The juice mixtures after 24 h fermentation were stored at 4˚C for 28 days showed the different cell viability between two strains (**Table 5**). *L. plantarum* was highly capable of surviving in the fermented juice mixture until the end of storage, the viable cell count remained at 7.95 log CFU/mL after 28 days of cold

Table 4. Colorimetric properties of vegetable juice mixture during fermentation.

Lactobacillus strain	Time (h)	L*	a*	b*	ΔE
L. plantarum	0	44.57 ± 0.06^{c}	24.38 ± 0.03^{c}	35.72 ± 0.07^{b}	–
	24	45.75 ± 0.07^{b}	24.77 ± 0.10^{b}	35.94 ± 0.04^{a}	1.26 ± 0.07
	48	46.22 ± 0.07^{a}	25.15 ± 0.05^{a}	35.99 ± 0.03^{a}	1.84 ± 0.04
L. acidophilus	0	43.52 ± 0.09^{b}	24.04 ± 0.09^{c}	33.54 ± 0.09^{b}	–
	24	43.67 ± 0.14^{ab}	24.56 ± 0.08^{b}	33.72 ± 0.08^{a}	0.57 ± 0.04
	48	43.92 ± 0.19^{a}	24.87 ± 0.09^{a}	33.88 ± 0.09^{a}	0.98 ± 0.14

Data expressed as means ± standard deviation (n = 3). Values in the same column followed by different superscript letters indicate statistically significant differences at $p < 0.05$. The values within column that do not have a common superscript are also significant different ($p < 0.05$). Unfermented vegetable juice mixture at 0h is used as the control.

Table 5. Effect of cold storage on the viability of lactobacillus bacteria in fermented vegetable juice mixture.

Time (days)	Log CFU/mL	
	L.plantarum	*L.acidophilus*
1	8.88 ± 0.06^{a}	7.57 ± 0.01^{a}
7	8.75 ± 0.04^{b}	6.99 ± 0.04^{b}
14	8.56 ± 0.05^{c}	6.31 ± 0.09^{c}
21	8.27 ± 0.07^{d}	5.47 ± 0.07^{d}
28	7.95 ± 0.08^{e}	4.57 ± 0.07^{e}

Data expressed as means ± standard deviation (n = 3). Values in the same column followed by different superscript letters indicate statistically significant differences at $p < 0.05$.

storage, which is considered a great value for fermented products containing probiotics. In contrast, viable cell count of *L. acidophilus* decreased significantly during cold storage and remained at only 4.57 log CFU/mL after 28 days. Pasteurization for the long period (80˚C, 15 min) could cause nutritional loss in the material which might have contributed to *L. acidophilus* viability loss. *L. plantarum* demonstrates a stronger viability than other *Lactobacillus* strains during cold storage time in fruit and vegetable juices [37] [44]. Generally, the cell viability depends on many factors as the strain used, interaction between species present, culture condition, oxygen content, final acidity of the product, and the concentration of lactic acid and acetic acid [15] [45] [46]. The presence of inulin and FOSs in fermentation medium probably had a positive impact on viability maintenance and improvement of probiotics during cold storage [47] [48]. In this present study, the product containing a combination of prebiotics and probiotics are known as synbiotics. This advantage would improve the survival of bacteria passing the upper part of gastrointestinal tract, improving their effects in the large bowel, and is able to enhance the growth of most positive gut bacteria [49].

3.2.2. Effect of Cold Storage on Total Carotenoid Content and Color of Fermented Vegetable Juice Mixture

After 28 days of cold storage, fermented vegetable juice mixtures with *L. plantarum* and *L. acidophilus* presented a loss about 23% and 16% of their initial total carotenoid content, respectively (Figure 1). The degradation level of carotenoids in juices inoculated with *L. acidophilus* was lower it could be because *L. acidophilus* viability decreased significantly during cold storage periods.

The results of color component (L*, a*, b* and ΔE) in the fermented vegetable juice mixture during storage (4˚C, 28 days) were shown Figure 2. The lightness increased during storage. A decrease in the color components a* and b* were also observed during storage (Figure 2). This implies that the cold storage resulted in a gradual weakening of color. Degradation of total carotenoid content and decrease of color components a* and b* could be related to each other and decrease of one affecting the other (Figure 1 and Figure 2). The total color difference ΔE of fermented vegetable juice mixture increased during the storage. This increase was mainly due to change of all color parameters. Considering a ΔE of 2 represents a noticeable color difference [43], the value reached at 14th day (ΔE = 2.58 ± 0.25) of storage and increased until end storage (ΔE = 4.72 ± 0.29) for fermented vegetable juice mixture with *L. plantarum*. While the vegetable juice mixture fermented with *L. acidophilus* reached (ΔE = 2.00 ± 0.14) at the end of storage time.

3.3. Sensory Evaluation

The sensory assessment of vegetable mixture juices was conducted on 14th day of storage because all formulations (except CON) need to have culture counts high enough to be considered probiotic foods (>6 log CFU/mL) (Table 5). The acceptability of vegetable juice mixture is shown in Figure 3. The judges indicated that they liked the color and texture of juices in all formulations. Supplementation

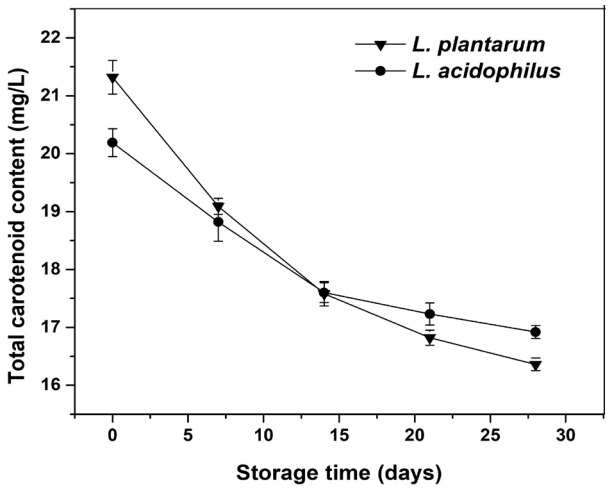

Figure 1. Total carotenoid content (mg/L) of fermented vegetable juice mixture, inoculated with *Lactobacillus plantarum* and *Lactobacillus acidophilus* during storage for 28 days.

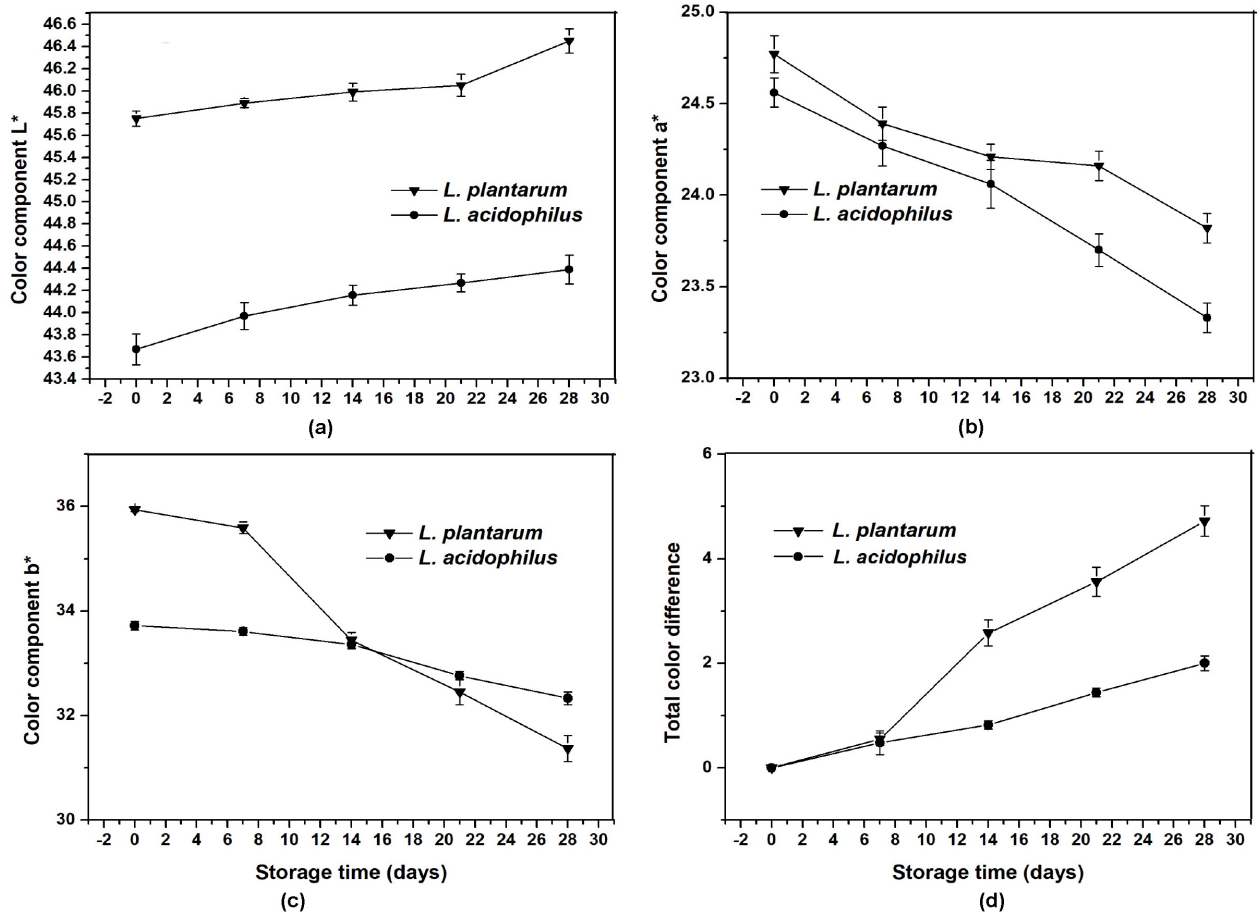

Figure 2. Color component L* (a), a* (b), b* (c), and total color difference ΔE (d) of fermented vegetable juice mixture, inoculated with *Lactobacillus plantarum* and *Lactobacillus acidophilus* during store for 28 days.

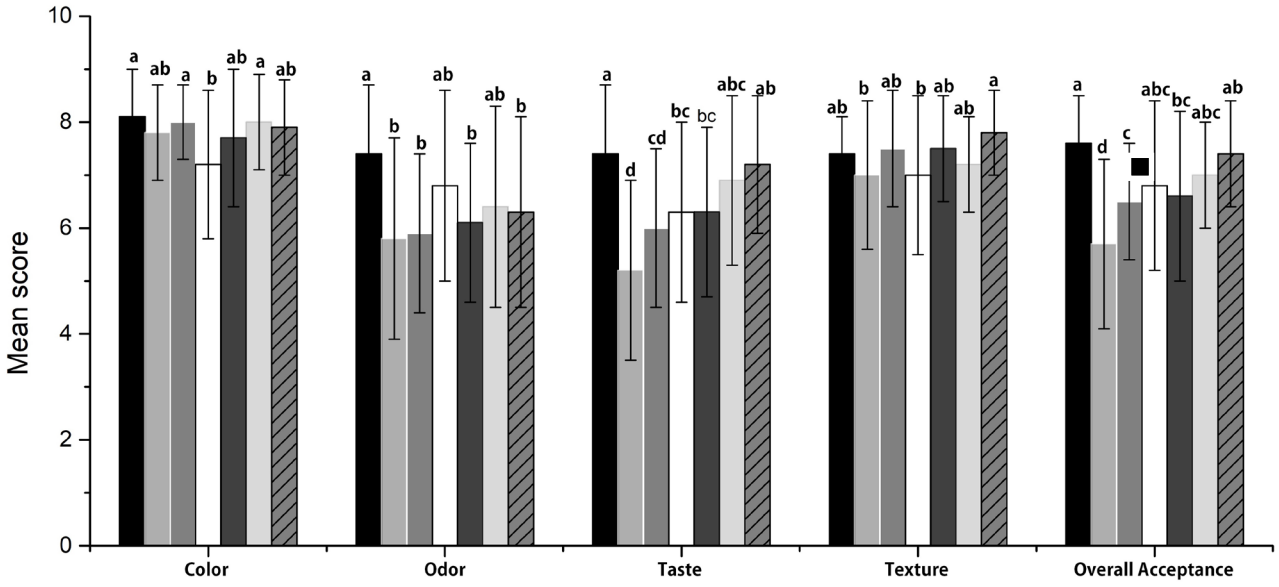

Figure 3. Acceptability of vegetable juice mixtures. Mean score (n = 21), The error bars represent the standard deviation. Hedonic values (color, odor, taste, texture and overall acceptance) are as follows: 1 = dislike extremely; 9 = like extremely. Formations: CON (■), PFJ (■), AFJ (■), MASK-PFJ (■), MASK-AFJ (■), SUC-PFJ (■), SUC-AFJ (■).

with sucrose and multi fruit juice (mainly tropical fruit juice) had no effect ($p > 0.05$) on acceptability of color and texture. The highest acceptability was the CON formulation. The formulations without any supplements got low acceptability (odor, taste, overall acceptance). This is likely because the metabolism of the cultures results changes to the components in the juices that negatively contribute to the aroma and flavor of the final products. Probiotification of juices from fruits and vegetables were characterized as "medicinal", "acid", "bitter", "astringent", "salty" or "dairy". Fermented juices with *L. plantarum* had a stronger sour taste (Table 2) compared to fermented juices with *L. acidophilus* (Table 3), resulting in the PFJ formulation to earn the lowest acceptability. There was a significant increase ($p < 0.05$) of acceptability (taste, overall acceptance).

In vegetable juice mixtures fermented with *L. plantarum* by adding the multi fruit juice (MASK-PFJ) or sucrose (SUC-PFJ) while the vegetable juice mixtures fermented with *L. acidophilus* by only adding sucrose (SUC-AFJ). The results indicated that the preference might be related to sweetness and the addition of pleasant aroma and volatile compounds is able to mask the presence of probiotics. However, the commercial juice was used in adding isn't a good choice to mask unpleasant flavor and change considerably sensory attributes of probiotic juices. A set of tests for mixing the vegetable juice mixture with a wide variety of commercial vegetable juice or fruit juice should be done to find the best option in improving effectively sensory quality. Herein, our study didn't reveal any information about the juices they evaluated which has a significant impact on consumer liking. Exposure and health information have positive effects on the overall liking of juices containing probiotic cultures [32] [50].

4. Conclusion

This research demonstrated both *Lactobacillus* strains were capable of having biochemical activities in vegetable juice mixture without any nutrient supplementation. Change in levels of physicochemical and microbiological characteristics in vegetable juice mixture during fermentation and cold storage depended on the strain used. As sensory analysis, acceptance of the probiotic vegetable juice mixtures was in range of 5 to 8 on a 9-point hedonic scale, which differed between the attribute evaluated and the strain used. Moreover, the sensory quality was improved positively by sucrose addition or adding multi fruit juice (10% v/v, mainly tropical fruit juices). Based on the present findings, *L. plantarum* could be used as a culture for production of probiotic drink from vegetable juice mixture with high nutrient values and health benefits. Optimizing fermentation, improving survival of probiotics during cold storage, as well as enhancement of sensory traits for this vegetable juice mixture require further studies.

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Conflicts of Interest

Authors declare that they have not any conflicts of interest.

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