

Probiotic Beverage from Mangosteen Juice Fermented with *Lactobacillus* Strains

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Abstract

In the present work, a non-dairy fermented beverage was produced from mangosteen juice using *Lactobacillus* strains. Mangosteen juice was fermented with 3 probiotic strains: *Lactobacillus casei* TISTR 390, *L. fermentum* TISTR 391 and *L. plantarum* TISTR 1463 in single and co-cultivations at an incubation temperature of 30°C for 72 h. The changes in chemical and microbiological properties during fermentation at 0, 24, 48 and 72 h were investigated. It was found that the acidity and viable cell count of fermented mangosteen juice increased at 72 h fermentation in all of the treatments, whereas the level of total soluble solid, phenolic compound, and pH decreased. Sensory evaluation showed that mangosteen juice fermented with the combination of *L. casei* TISTR 390, *L. fermentum* TISTR 391, and *L. plantarum* TISTR 1463 had overall the acceptability score in the group of highest score (6.74; moderately like). Furthermore, it showed the highest taste liking score. However, after 4 weeks of cold storage at 4°C, the level of phenolic compound, pH and viable cell count of this fermented juice slightly fell, but the total soluble solid remained the same. Interestingly, the fermented juice had good antioxidant activity compared with the control (without lactic acid bacteria). Moreover, the low concentration of fermented mangosteen juice exhibited to have higher toxicity to colon tumor cell (SW620) than that of the control. This finding suggested that these probiotic mangosteen juice could be served as a healthy alternative functional beverage for general consumers, especially adults and the elderly.

Keywords: Non-dairy, Mangosteen juice, Probiotic, Lactic acid bacteria, Antioxidant activity, Toxicity

Introduction

In recent years, consumers are becoming increasingly aware towards healthy diets resulting in the market demand for new functional foods with a beneficial effect on health has been growing. Several beneficial effects on human health have been reported in probiotics (lactic acid bacteria). These are enhancement of resistance against pathogens, adjustment of the immune response to a desired level and reduction of blood cholesterol levels, cancer prevention, improve the diversity and activity of the gut flora [1-3]. Probiotic microorganisms are also used to treat chronic non-communicable diseases and have been found to reduce the severity of the disease in clinical practice such as, gastrointestinal diseases, obesity, type 2 diabetes, cancer and the novel coronavirus disease 2019 [4,5].

It was found that the market value of probiotic products from 2019 to 2025 trends to increase every year. The value of probiotic products is \$48.38 billion in 2018 and grow to \$77.09 billion in 2025 with a compound annual growth rate (CAGR) of 6.90 % [6]. On the other hand, available probiotic products in the market are usually dairy-based. However, consumers who suffer lactose intolerance and milk protein allergies cannot consume the dairy-based probiotic beverages. Additionally, vegan consumers are increasing as well as the demand for vegan probiotic products [7], leading to development of probiotic products from various food matrices including fruits and vegetables [8-10]. Fruit juices have been reported to be rich in nutrient, sugar and vitamin C which could encourage probiotic growth and are good for health [11]. From the nutritional point of view, a lot of research on fermented fruit juice have already been reported. For example, Dimitrovskiet al. [12] studied the fermentation of apple juice by *Lactobacillus plantarum* PCS26. Mantzourani et al. [13,14] evaluated to produce functional drink from fermentation of *L. plantarum* ATCC 14917 in cornelian cherry juice and

pomegranate juice. Lu *et al.* [15] evaluated star fruit juice fermentation by 3 commercial probiotic strains (*L. helveticus* L10, *L. paracasei* L26 and *L. rhamnosus* HN001). Vieira *et al.* [16] investigated development of probiotic orange juice supplemented with *Pediococcus acidilactici* CE51. Additionally, in the case of litchi juice fermented with *L. casei*, the obtained product showed enhancing immune organs indexes (spleen, thymus) and antioxidant capacity, improving the secretions of cytokines and immunoglobulins and protecting the intestinal tract in mice [17].

Mangosteen (*Garcinia mangostana* Linn.) is a tropical fruit of the Guttiferae family cultivated in Southeast Asia countries including Thailand and others. This fruit is soft, juicy, sweet, slightly acid taste and a pleasant aroma. Therefore, it is very popular which has also been known as the “queen of fruits” and medicinal medicine in Thailand [18,19]. Phytochemicals such as xanthenes, phenolic compound, anthocyanin and procyanidins are found in different parts of mangosteen. Especially α -xanthenes and γ -mangosteen are major bioactive ingredients having anti-proliferative, pro-apoptotic, anti-cancer, anti-diabetes, anti-microbes, anti-inflammatory and protection against damages in various human organs [20,21].

Remarkably, recent reviews have reported that mangosteen juice had high nutritional supplement such as flavonoids, tannins, sugars, dietary fibers and antioxidant vitamins (B₂, B₅ or E) [18]. Furthermore, Anprung and Sangthawan [22] revealed that highest release of bioactive compounds has been resulted by hydrolysis of mangosteen aril for 6 h. Also, the prebiotic activity score of *Lactobacillus acidophilus* and *Bacillus lactis* has been increased by enzymatically depolymerized mangosteen aril. In addition, antioxidant capacity and possesses anti-inflammatory benefits with no side effects on immune and renal function for long-term consumption has been exhibited in mangosteen-based formula [23].

However, no study on probiotic mangosteen juice has yet been found. According to, *Lactobacillus* strain have been deployed extensively as fermentation starter cultures and as probiotics [24]. Since, *L. casei* TISTR 390 and *L. plantarum* TISTR 1463 are facultatively homofermentative microorganism but *L. fermentum* TISTR 391 is obligated heterofermentative microorganism [25]. Thus, co-cultivation could be more produced various of chemical and flavor of the drink. The purpose of this work was to investigate the suitability of mangosteen juice for producing of probiotic beverage by using various strains of *Lactobacillus* sp. such as *L. casei* TISTR 390, *L. fermentum* TISTR 391 and *L. plantarum* TISTR 1463. The fermentability of starter cultures was monitored by the microbial dynamics and metabolites evaluations during the fermentation. Furthermore, phenolic compound, and survivability of *Lactobacillus* sp. during cold storage at 4 °C were investigated. Finally, the antioxidant activity and toxicity to human colon cancer cell (SW620) were evaluated.

Materials and methods

Materials

Maturing mangosteen fruits were purchased from local farmers in Chanthaburi province, Thailand. It was harvested in June which is full blooming season and were then transported to the laboratory.

Strains and culture

Three strains of probiotic lactic acid bacteria such as *L. casei* TISTR 390, *L. fermentum* TISTR 391 and *L. plantarum* TISTR 1463 obtained from the Microbiological Resources Center (Thailand Institute of Scientific and Technological Research in Pathum Thani, Thailand). The 2 loops of cultures were grown in a 10 mL of MRS (de Man Rogosa and Sharpe) broth (dextrose 20.0 g/L, meat peptone 10.0 g/L, beef extract 10.0 g/L, yeast extract 5.0 g/L, sodium acetate 5.0 g/L, disodium phosphate 2.0 g/L, ammonium citrate 2.0 g/L, tween 80 1.0 g/L, magnesium sulphate 0.1 g/L, and manganese sulphate 0.05 g/L) at 37 °C for 24 h under the static condition and were used as an inoculum.

Mangosteen juice preparation

After peeling, the maturing mangosteen fruits were cleaned with distilled water and the juice was prepared. Firstly, the fruit was separated from its pulp (aril) and pericarp. Then, the juice from aril part was extracted by a hydraulic press (Thai sakaya-A2). The obtained juice was mixed with distilled water at a ratio of mangosteen juice to water; 30:70. The initial total soluble solid of the diluted juice was 7 °Brix (data not shown). The juice was adjusted the total soluble solid to 13°Brix with sucrose and 0.3 % (w/v) salt was added. Then, it was pasteurized at 60°C for 30 min and filled in a small glass bottle (50 mL) [26].

Fermentation of mangosteen juice

The starter cultures were prepared in 100 mL of MRS broth and then incubated at 30 °C for 24 h under the static condition. Next, the cultures were centrifuged at 6,682 rpm for 10 min and cell pellet were washed with 0.85 % NaCl for 2 times. The cultures were then continued to starter culture in 100 mL of mangosteen juice at 30 °C for 48 h under the static condition and the final concentration of starter culture was 10⁶ CFU/mL.

The 8 treatments of mangosteen juice fermentation were designed using Completely Randomized Design (CRD) as represented in **Table 1**. Treatment 1 was the control mangosteen juice. Treatment 2-4 was the pure culture of each *Lactobacillus* sp. at the concentration of 5% (w/v). Treatment 5-7 was the combination of 2 strains at the concentration of 2.5% (w/v) per strain and Treatment 8 was the combination of 3 strains at the concentration of 1.67% (w/v) per strain. After inoculation, samples were incubated at 30°C for 72 h under the static condition [27]. The chemical and microbiological properties were investigated every 24 h until 72 h. The final fermented mangosteen juice at 72 h were evaluated for sensory acceptability.

Table 1 Eight treatments of mangosteen juice fermentation.

Treatments	Strains
1(Control)	-
2	<i>L. casei</i> TISTR 390
3	<i>L. fermentum</i> TISTR 391
4	<i>L. plantarum</i> TISTR 1463
5	<i>L. casei</i> TISTR 390 mixed with <i>L. fermentum</i> TISTR 391
6	<i>L. casei</i> TISTR 390 mixed with <i>L. plantarum</i> TISTR 1463
7	<i>L. fermentum</i> TISTR 391 mixed with <i>L. plantarum</i> TISTR 1463
8	<i>L. casei</i> TISTR 390, <i>L. fermentum</i> TISTR 391 and <i>L. plantarum</i> TISTR 1463

Chemical properties determination

Total acidity of samples during fermentation, (expressed as percent lactic acid), was analyzed by titrating with 0.02 N NaOH to pH 8.2 [27]. The total soluble solid content was investigated using hand refractometer (Atago, Japan).

Total phenolic compound content was determined by the method of Iqbal *et al.* [28] with some modification. Briefly, 3 mL of sample was extracted with 30 mL of 80% ethanol and then the solution was shaken for 24 h. The obtained solution was filtrated by Whatman No. 1. Then, 50 µL of filtrated was mixed with 950 µL of distilled water, 2 mL of folin-ciocalteu phenol reagent and 1.6 mL of 7.5% NaCO₃. The mixture was incubated at 37°C for 2 h. The absorbance was measured at 760 nm. Finally, the concentration of phenolic compound was calculated by using standard curve of gallic acid.

Microbiological properties determination

The standard plate count method with *Lactobacilli* MRS medium after 48 h inoculation at 37°C were used for viable cell counts of samples during fermentation and then reported as log CFU/mL [27].

For bacterial cell analysis, the final fermented mangosteen juice inoculated with another *Lactobacillus* sp. were collected from the culture and photographed with a scanning electron microscopy (SEM) (JEOL, model JSM-MEDEL jsm-5410LV, Japan) at a magnification of 20,000× to capture images for the lactic acid bacterial cell grown under the culture condition [29].

Sensory evaluation

The fermented probiotic mangosteen juice with various strains of *Lactobacillus* sp. were evaluated for sensory attributes (color, aroma, taste, texture and overall liking) using a 9-point hedonic scales [30] with 50 untrained panelists from the staffs and students of department of product development and management technology (Faculty of Agro-industrial Technology, Rajamangala University of Technology Tawan-ok, Chanthaburi Campus, Chanthaburi, Thailand).

Storage of the probiotic mangosteen juice

After fermentation at 30°C for 72 h, the fermented mangosteen juice containing probiotics (50 mL) in the glass bottles were stored at 4°C in refrigerator for 4 weeks. Samples were taken at weekly intervals for analyzing the viable cell counts (log CFU/mL) as explained previously. Afterwards, the remaining fermented mangosteen juice was separated for the determination of antioxidant activity (DPPH radical scavenging activity) [31] and human colon cancer cytotoxicity [32].

The DPPH radical scavenging activity was determined at the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI) in Bangkok, Thailand according to the methods of Zhu *et al.* [31]. Briefly, 10 mL of ethanol was mixed with 1 g of sample. Then, the solution was separated by centrifugation at 6,000 rpm. The concentrations of supernatant were adjusted to 10, 20, 30, 40 and 50 µg/mL. The 1 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (0.1 mM in 95% ethanol) was mixed with the sample (1 mL) and then incubated in dark condition for 30 min. The absorbance was measured using a spectrophotometer at 517 nm. The percentage of radical scavenging activity was calculated with the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

when A_0 = the absorbance of control reaction (containing all reagents except the sample)

A_1 = the absorbance of test compound.

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against the sample concentration.

The cytotoxicity of fermented mangosteen juice was determined using MTT assay at the Microbiology Department (Faculty of Science, at Chulalongkorn University in Bangkok, Thailand). This method was performed following the method reported by Senthilraja and Kethiresan [32] with slightly modifications. In brief, seeding cell SW620 was seeded at 1.5×10^4 cell/mL in 96 well plate overnight (total volume 100 µL/well). For the cell treatment, fermented mangosteen juice was prepared by using 6 different concentrations of juice which was diluted in completed media and added to the well that contained the cell (100 µL/well). Supernatant was removed, added complete media that contained juice sample or DMSO (vehicle control) and incubated at 37°C for 24 h. For the measurement of the cell cytotoxicity, MTT solution (concentration of 5 mg/mL) 10 µL/well was added and incubated in CO₂ incubator at 37°C for 4 h. The purple formazan was dissolved by using isopropanol with HCl (100 µL/well) and was mixed subsequently. Finally, the absorbance was measured at 540 nm by micro-plate reader.

Statistical analysis

All experiments were performed in triplicate using different lots of mangosteen juice. Analysis of variance (ANOVA) ($p \leq 0.05$) were proceeded the data. Significant difference among means within each experiment were separated by Duncan's multiple range test (DMRT) at a significance level of $\alpha = 0.05$ by using computer software [33].

Results and discussion

Chemical and microbiological properties of mangosteen juice fermented with and without probiotics

Change in titratable acidity, pH, total soluble solid, total phenolic compound and viable cell count of mangosteen juice samples during fermentation are illustrated in **Figures 1(A) - 1(C)**, **2** and **3**, respectively. The amount of titratable acidity of treatment 2 to 8 significantly ($p \leq 0.05$) increased at longer fermentation time (approximately 0.55% (w/v) at 72 h of fermentation). The *L. casei* TISTR 390 (Treatment 2) produced the highest amounts of lactic acid (**Figure 1(A)**). This result might be due to *L. casei* produced only lactic acid (without or little production of CO₂) [34]. On the other hand, the mixture strains may produce CO₂ is dominant, so the mixed culture showed comparatively lower acidity. The pH values rapidly decreased when the fermentation time increased (**Figure 1(B)**). In addition, using the 3 combination strains of *Lactobacillus* sp. (Treatment 8) tended to decrease pH values more than that of the single strain fermentation ($p \leq 0.05$). It might be due to the 3 strains enhanced the formation of some short-chain fatty acids (SCFA) during the fermentation process, resulting in decreasing pH values [10]. This result was consistent with our previous observation in that

the fermentation of gac(*Momordica cochinchinensis* Spreng) juice using the combination of 3 cultures of *Lactobacillus* sp. showed the lowest pH value [29].

The reduction of total soluble solid of fermented mangosteen juice of Treatments 2-8 were detected when the fermented time was longer ($p \leq 0.05$). After 72 h of fermentation, the levels of total soluble solid was lowest approximately 12.40°Brix in Treatment 2 and 5, corresponding to a greater amount of titratable acidity (**Figure 1 (A)**). According to lactic acid bacteria metabolism, the sugar can be converted to lactic acid and other organic acid by glycolysis partway and relate metabolism which depends on the species of lactic acid bacteria [35]. Chemical properties changing during mangosteen juice fermentation may result from the action of *Lactobacillus* sp. which can metabolize sugar to lactic acid [36] as observed from the downwards of the pH and total soluble solid and the upwards of titratable acidity. Our results were consistent with the previous report for vegetable and fruit juice. They found the decline of pH and the increase of acidity at the end of fermentation of beet root, mango and carrot mixed juice [37,38]. The levels of total phenolic compound of mangosteen juice fermented with various strains of *Lactobacillus* sp. significantly ($p \leq 0.05$) decreased at 72 h fermentation in Treatments 2-8 (**Figure 2**). The change in of total phenolic compound content was probably caused by the pasteurization condition (60 °C for 30 min) during the mangosteen juice preparation which can degrade some phenolic compound. Manurakchinakorn *et al.* [39] revealed that total phenolic compounds of the mangosteen juice progressively decreased after pasteurized at 90 °C for 5 min and stored at 4°C for 5 weeks. Another possible degradation pathways of the phenolic compounds may be related to their oxidation, hydrolysis, or isomerization [40], resulting in the decreased total phenolic compounds in the mangosteen juice during the fermentation. Moreover, this might be due to this fermentation has affect to *p*-hydroxybenzoic acid with the predominant phenolic acid in aril [41].

Regarding change in microbiological cell characters of the fermented mangosteen juice, the numbers of viable cell count were significantly ($p \leq 0.05$) gained when the fermentation time was longer and the highest cell count were obtained at 72 h fermentation in all of the treatments (**Figure 3**). This was likely due to their favorable growth temperature and their growth phase is optimum at this fermentation condition. Therefore, the numbers of viable cell increased from 3 log CFU/mL for 0 h fermentation to approximately 6 log CFU/mL for 72 h fermentation. Similar results were reported on elsewhere [42,43], who founded the numbers of viable cell increased when the fermentation time longer in apricot juice and vegetable juice. Moreover, the higher cell count was observed when using the mixed culture of probiotic *Lactobacillus* sp. This was probably due to the synergistic effect of the 3 strains. In fact, the minimum number of probiotic organisms in a food product should be 6 log CFU/mL in order to promote health benefits [43].

Figure 4 illustrates the bacterial cell as monitored by scanning electron microscope at magnification of 20,000×. The *Lactobacillus* sp. lived by mixing with the polysaccharide of mangosteen juice. The short rod of *L. casei* TISTR 390 was in the polysaccharide portion of the mangosteen juice as shown in **Figure 6 (A)**. While long rod of the *L. fermentum* TISTR 391 and *L. plantarum* TISTR 1463 were also located in polysaccharide of juice similar to the location of the *L. casei* TISTR 390 (**Figure 4(B)** and **4(C)**). Additionally, the combination of 3 stains of lactic acid bacteria were still located in the polysaccharide of juice as represented in **Figure 4(D)**. The polysaccharide in the juice should be the total dietary fiber which can be divide into insoluble and soluble dietary fiber (pectin). Anprung and Sanghawan [22] reported that the soluble dietary fiber of mangosteen aril was 12.91 ± 0.13 %w/w which showed prebiotic activity and bioactive activity. However, our previous study also founded some polysaccharide from gac(*Momordica cochinchinensis* Spreng) juice but difference in size and shape [29].

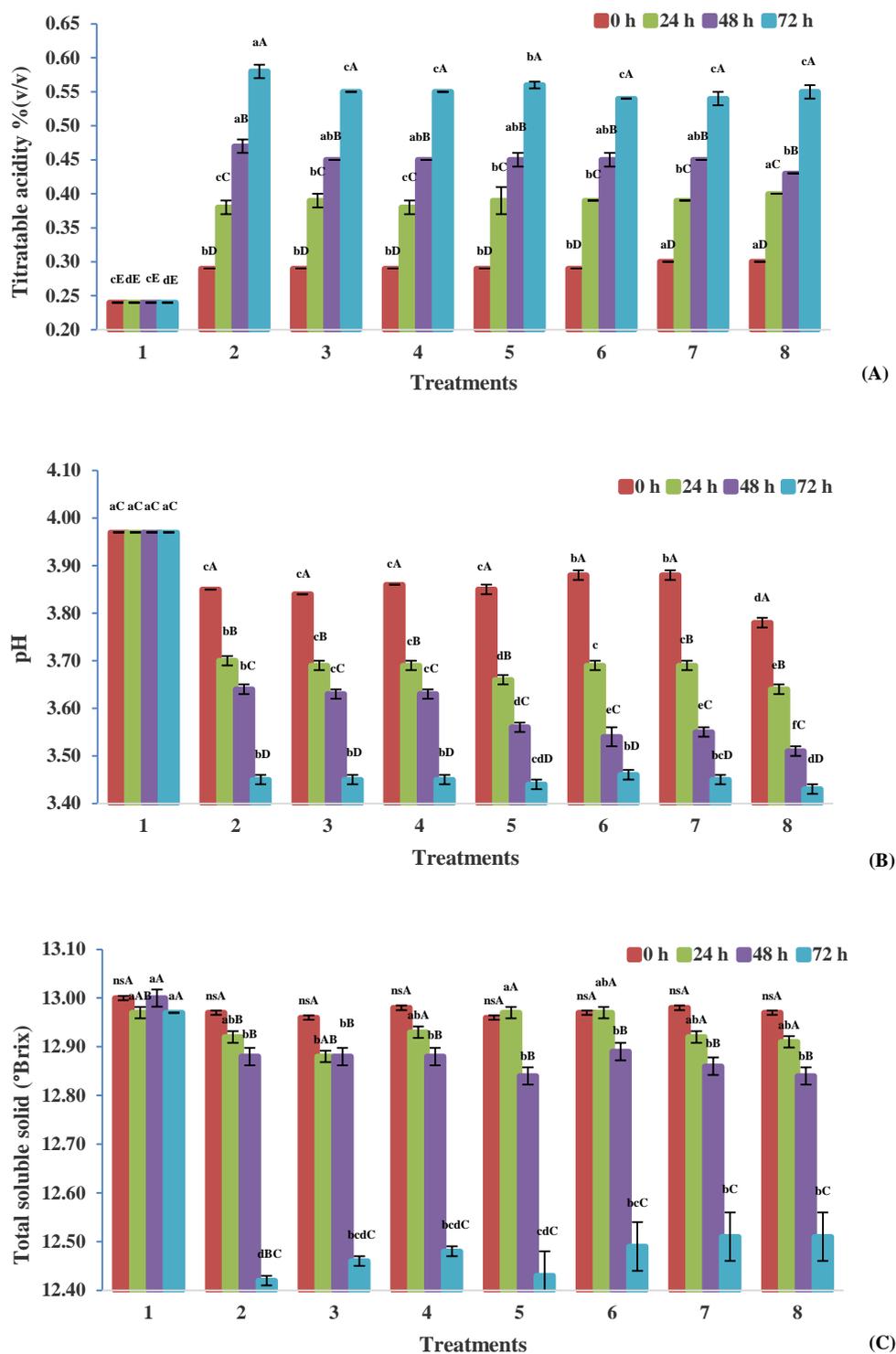


Figure 1 Titratable acidity (A), pH (B) and total soluble solid (C) of mangosteen juice fermented with various strains of *Lactobacillus* sp. at 30°C for 0, 24, 48 and 72 h. Bars represent standard deviation from triplicate determination. ^{a-d}Different small letters above the solid graph at various treatments indicates significantly different ($p \leq 0.05$). ^{A-D}Different capital letters above the solid graph at various fermentation time indicates significantly different ($p \leq 0.05$).

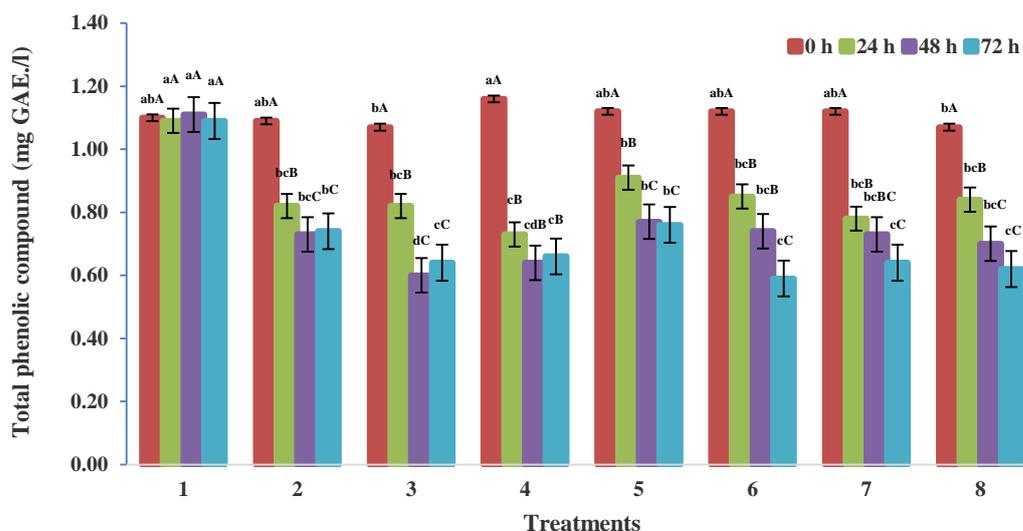


Figure 2 Total phenolic compound content of mangosteen juice fermented with various strains of *Lactobacillus* sp. at 30°C for 0, 24, 48 and 72 h. Bars represent standard deviation from triplicate determination.^{a-d} Different small letters above the solid graph at various treatments indicates significantly different ($p \leq 0.05$). ^{A-D} Different capital letters above the solid graph at various fermentation time indicates significantly different ($p \leq 0.05$).

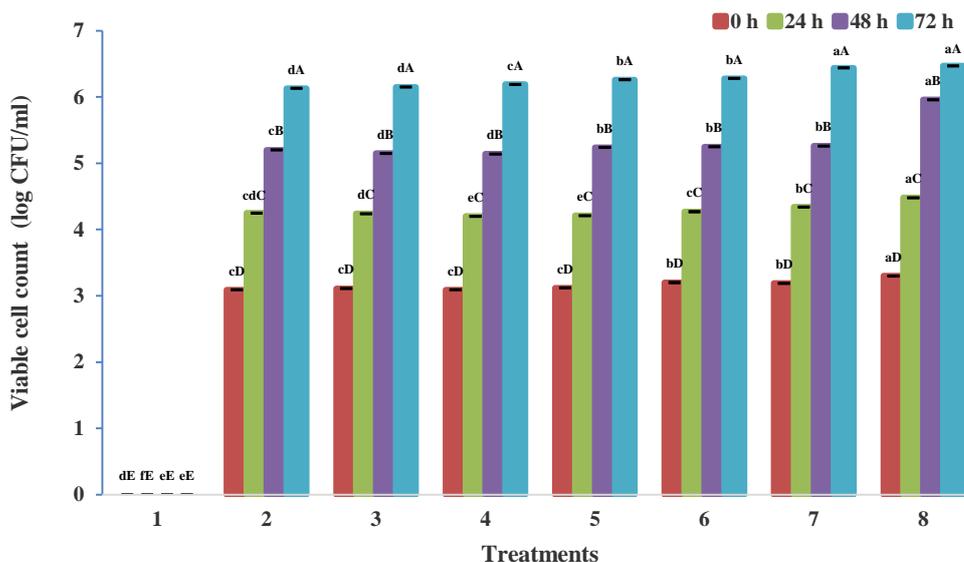


Figure 3 Total viable plate count of mangosteen juice fermented with various strains of *Lactobacillus* sp. at 30°C for 0, 24, 48 and 72 h. Bars represent standard deviation from triplicate determination.^{a-d} Different small letters above the solid graph at various treatments indicates significantly different ($p \leq 0.05$). ^{A-D} Different capital letters above the solid graph at various fermentation time indicates significantly different ($p \leq 0.05$).

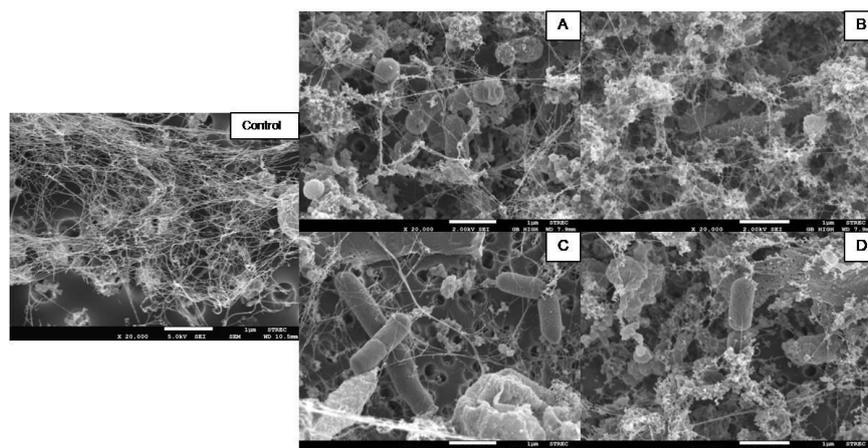


Figure 4 SEM micrographs (20,000 \times magnification) of the mangosteen juice (control) and that fermented with *L. casei* TISTR 390 (A), *L. fermentum* TISTR 391 (B), *L. plantarum* TISTR 1463 (C) and combination of 3 strains of *Lactobacillus* sp. (D) in mangosteen juice after fermentation at 30 $^{\circ}$ C for 72 h.

Sensory evaluation

The sensory attributes of fermented mangosteen juices were shown in **Table 2**. Color, aroma and texture did not differ significantly ($p>0.05$) among the samples. However, the significant difference ($p\leq 0.05$) was found in the attributes of taste and overall liking. Mangosteen juice fermented with the 3 combination of *Lactobacillus* sp. (Treatment 8), the mixture of *L. fermentum* TISTR 391 and *L. plantarum* TISTR 1463 (Treatment 7) and the pure culture of *L. fermentum* TISTR 391 (Treatment 3) are the group having the highest overall liking with a score of 6.74, 6.72 and 6.72 (moderately liked), respectively. Nevertheless, Treatment 8 showed the highest taste liking with a score of 6.84 (moderately liked). Thus, it was chosen for continuing to determine the influence of cold storage (4 $^{\circ}$ C) on the viable microbial cell at 0, 1, 2, 3 and 4 weeks of storage.

Table 2 Sensory attributes of mangosteen juice fermented with various strains of *Lactobacillus* sp. at 30 $^{\circ}$ C for 72 h.

Treatments	Likeness scores				
	Color ^{ns}	Aroma ^{ns}	Taste	Texture ^{ns}	Overall liking
1	6.70 \pm 1.04	6.66 \pm 1.02	6.06 \pm 0.96 ^c	6.52 \pm 0.97	6.32 \pm 1.02 ^{ab}
2	6.68 \pm 0.98	6.20 \pm 1.03	6.24 \pm 1.17 ^{bc}	6.54 \pm 1.03	6.28 \pm 1.21 ^b
3	6.66 \pm 0.98	6.30 \pm 1.30	6.62 \pm 1.23 ^{ab}	6.80 \pm 1.18	6.72 \pm 0.95 ^a
4	6.70 \pm 1.18	6.30 \pm 1.28	6.74 \pm 0.88 ^a	6.56 \pm 1.11	6.64 \pm 0.85 ^{ab}
5	6.90 \pm 0.79	6.24 \pm 1.24	6.46 \pm 0.97 ^{abc}	6.70 \pm 0.93	6.62 \pm 0.97 ^{ab}
6	7.00 \pm 0.93	6.38 \pm 1.18	6.54 \pm 1.28 ^{ab}	6.58 \pm 1.20	6.64 \pm 1.03 ^{ab}
7	6.72 \pm 1.05	6.18 \pm 1.08	6.60 \pm 0.97 ^{ab}	6.70 \pm 1.05	6.72 \pm 0.83 ^a
8	6.92 \pm 0.99	6.44 \pm 1.07	6.84 \pm 0.89 ^a	6.84 \pm 0.89	6.74 \pm 0.69 ^a

Note: Values with a different letter are significantly different ($p\leq 0.05$) according to Duncan's multiple range test. Acceptability was evaluated using a structured hedonic scale of 9 points, from 1 (dislike very much) to 9 (like very much).

Effect of cold storage on the viable cell count of the probiotic mangosteen juice

The probiotic mangosteen juice (Treatment 8) was stored at cold temperature (4 $^{\circ}$ C) for 4 weeks. When compared with **Figure 3**, the amount of viable cell counts reached from 6 log CFU/mL to nearly 8 log CFU/mL in **Figure 5**. It might result from the remaining sugar and some phytochemical of the juice. Due to the lactic acid bacteria capable of growing at the acid condition (pH 3.2-4) as reported in Davis *et al.* [44], they can metabolize some remaining sugar and convert to lactic acid which was

evident from the higher amount of viable cell counts. Nevertheless, when low pH of mangosteen juice generated, long-term utilization and low temperature storage had negative effect on viability of probiotic. The numbers of probiotic bacteria tended to go upwards from the storage beginning to 1 week of storage at 4°C (approximately 6 log CFU/mL to 8 log CFU/mL). However, it was stable at 2 weeks and dramatically decreased at 4 weeks storage (from approximately 8 log CFU/mL to 5 log CFU/mL) as revealed in **Figure 5** ($p \leq 0.05$). This indicates that lactic cultures lost their viability during cold storage which was consistent with previous reports. Our results are in agreement with data reported by Nguyen *et al.* [10] who exhibited that the survival of *Lactobacillus* sp. and *Bifidobacterium longum* tend to be reduced during cold storage when the storage time longer in pineapple juice fermentation. Hence, this fermented mangosteen juice should be consumed for not more than 2 weeks of cold storage in order to be more effective of probiotic. Khurana and Kanawajia [45] have suggested that probiotic products should have a minimum of 6-7 log CFU/mL of probiotic bacteria at the time of consumption.

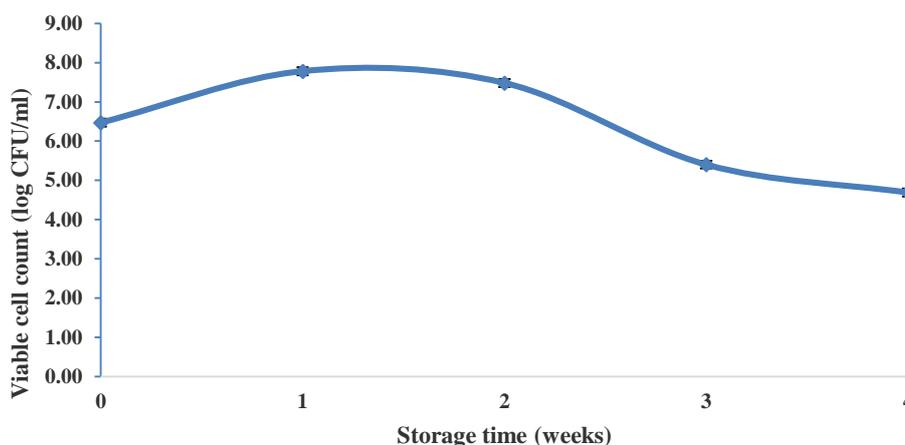


Figure 5 Total viable plate count of mangosteen juice fermented with 3 strains of *Lactobacillus* sp. during cold storage at 4°C for 0, 1, 2, 3 and 4 weeks.

The antioxidant activity and cytotoxicity properties of probiotic mangosteen juice

The antioxidant activity of probiotic mangosteen juice was analyzed by DPPH radical scavenging activity and was expressed as IC_{50} (the half maximal inhibitory concentration) which is a quantitative measurement that indicates that how much of a particular inhibitory substance by 50%. The numbers of IC_{50} of probiotic mangosteen juice and control were 41.62 ± 0.57 and 46.70 ± 0.60 mg/mL, respectively (**Table 3**). Interestingly, the antioxidant activity of probiotic mangosteen juice was higher than those of the control ($p \leq 0.05$) which was not correlated with the levels of total phenolic compound as demonstrated in **Figure 2**. However, it was reported that in addition to anthocyanin and other phenolic compounds containing in the mangosteen pericarp, antioxidant capacity of the mangosteen juice can be mainly influenced by ascorbic acid present in the mangosteen flesh [46]. This finding was in line with the study of Bujna *et al.* [42], Nguyen *et al.* [10] and Mostataet *et al.* [47] who showed that the antioxidant activity of fermented blueberry juice, pineapple juice, date juice by *Serratia raccini*, *Lactobacillus* sp. and *L. Sakei* also slightly increased. This could be probiotic microorganism produced bioactive molecule which related to antioxidant activity and reduce damages caused by oxidation [1]. Regarding cytotoxicity, **Figure 6** showed that the high concentration for both samples (control and probiotic mangosteen juice) tended to be toxic to tumor colon cancer cell SW620. The percentage of viability decreased while the concentration was higher. The lowest percentage of viability was exhibited at 3% (v/v) of fermented mangosteen juice. This result of this research corresponded with the study of Liu *et al.* [48], which revealed that mangosteen xanthones (gartanin and α -mangosteen) inhibited the growth of cancer cell lines from different stage of human urinary bladder cancer. On the other hand, the percentage of viability increased when the concentration added from 0% (v/v) to about 0.03% (v/v) in probiotic mangosteen juice. This might be caused by the vitamins, mineral and sugar in mangosteen juice activated the growth of cell lines. Overall, this cytotoxicity could be related with the antioxidant activity and probiotic microorganisms but not related with total phenolic compound content.

Table 3 Antioxidant activity of mangosteen juice fermented with the combination of 3 strains of *Lactobacillus* sp. at 30°C for 72 h.

Treatments	IC ₅₀ (DPPH assay (mg/mL))
Mangosteen juice (Control, without probiotic microorganism)	46.70±0.60 ^b
Probiotic mangosteen juice	41.62±0.57 ^a

Note: Each data represents mean of 3 replications with standard error. Values with a different letter are significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

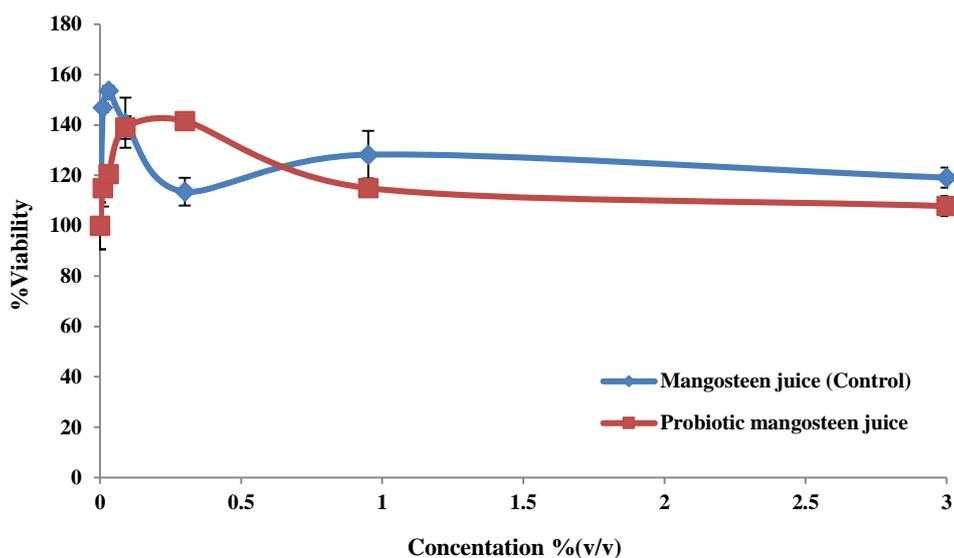


Figure 6 Effect of mangosteen juice fermented with the combination of 3 strains of *Lactobacillus* sp. at 30°C for 72 h to percentage of viability of human colon cancer cell SW 620.

Conclusions

Probiotic mangosteen juice could be produced by fermentation with single and combination of 3 probiotic strains including *L. casei* TISTR 390, *L. fermentum* TISTR 391 and *L. plantarum* TISTR 1463. The chemical and microbiological properties of fermented mangosteen juice varied depending on the fermentation time. The acidity and viable cell count increased at a longer fermentation time of 72 h, whereas the total soluble solid, phenolic compound, and pH tended to decline. Mangosteen juice fermented with the combination of 3 strains had the highest taste liking score. Additionally, it had the highest acceptability score from panelists in the group of highest score. However, after cold storage at 4°C for 4 weeks, the reduction of viable cells of probiotic bacteria was observed, indicating that they lost viability during cold storage. Interestingly, the antioxidant activity of probiotic mangosteen juice was higher than that of the control mangosteen juice, which showed lower IC₅₀ values. Moreover, it exhibited cytotoxicity to the tumor colon cancer cell SW620. These results suggest that the fermented mangosteen juice could be served as an alternative healthy non-dairy probiotic source for vegetarians and milk-allergic consumers in the future. However, the taste could be improved in order to increase the acceptability of the product. Furthermore, the cytotoxicity could be examined by increasing the concentration of fermented mangosteen juice, while the normal cell line could also be tested to compare with the cancer cell.

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