
Fermentation of Gac juice mixture by probiotic lactic acid bacteria

Mongkontanawat, N.^{*}, Laohkitikul, S. and Lertnimitmongkol, W.

Department of Product Development and Management Technology, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-ok Chanthaburi Campus Chanthaburi 22210, Thailand.

Mongkontanawat, N., Laohkitikul, S. and Lertnimitmongkol, W. (2018). Fermentation of Gac juice mixture by probiotic lactic acid bacteria. International Journal of Agricultural Technology 14(7): 1455-1470.

Abstract Probiotic Gac juice was fermented by lactic acid bacteria in this study. From sensory evaluation, treatment 3 (Gac juice fermented by *L. fermentum* TISTR 391) was the highest acceptability with the score 6.07 (moderately like). Changes in pH, acidity, total soluble solid and viable cell counts during fermentation under controlled conditions (30 °C) at 0, 24, 48 and 72 h fermentation were evaluated. Results were found that changing in pH levels and total soluble solid of probiotic Gac juice were significantly ($p \leq 0.05$) decreased at 72 h fermentation. On the other hand, the amount of titratable acidity expressed as lactic acid was significantly ($p \leq 0.05$) increased at fermentation for 72 h. The effect of shelf life on viable cells counted in cold storage (4 °C) was investigated. The result was exhibited that the viable cells were reached at 1 week of storage, then sharply declined when the stored time longer and the lowest was 4 weeks of storage. Finally, the final product was determined the amount of vitamin C, antioxidant activity and cytotoxicity to human colon cancer cell SW620. Interestingly, the amount of vitamin C content exhibited two folds higher than those control. In addition, the free radical scavenging capacity was assayed by DPPH method also shown that IC_{50} values had significantly ($p \leq 0.05$) higher than that those control. However, both fermented Gac juice and control were also tended to be affected to colon cells SW620 when the juice concentration were increased. For the bacterial cell behavior, the scanning electron microscope showed that lactic acid bacteria were grown in an amount the polysaccharide of the juice. These results could be expressed the alternative healthy non-dairy probiotic source for vegetarian and milk allergy consumers in the future.

Keywords: Fermentation, Gac juice, Probiotic lactic acid bacteria

Introduction

Probiotics have several beneficial effects on human health. Lactic acid bacteria have been proven to exert health-promoting activities such as adjustment of the immune response to a desired level, and enhancement of resistance against pathogens and reduction of blood cholesterol levels

^{*} **Coressponding Author:** Mongkontanawat, N. ; **Email:** jeabn2013@gmail.com

(Korovicova and Kohajdova, 2003). Usually, available probiotic product on the market nowadays are dairy-based. It can not be consumed by individuals who suffer lactose intolerance and milk protein allergies. Moreover, the vegan consumers are increasing and also demand for vegan probiotic products (Sharma and Misha, 2008). In addition, fruit juice are reported to be rich of nutrient, high amount of sugar and vitamin C which could encourage probiotic growth and good for health (Ding and Shan, 2008). Gac (Spiny Bitter Cucumber) with the scientific name is *Momordica cochinchinensis* Spreng. This fruit is a Southeast Asia fruit found various region from Southern China to Northeastern Australia, including Thailand, Laos, Myanmar, Cambodia and Vietnam. The local name may be called by different name such as Gac (in Vietnam), Fak kao in Thailand), Bhat kerala (in India), Moc Niet Tu (in China) and Mak kao (in Laos) (Kubola and Siriamornpun, 2011). Phytochemicals such as lycopene, beta-carotene, lutein and phenolic compound have been found in this fruit (Ishida *et al.*, 2004; Kubola and Siriamornpun, 2011). Especially, lycopene and beta-carotene have been found high content in this fruit (Aoki *et al.*, 2002). In addition, these carotenoids are natural anti-oxidants to prevent and treat cancers (Tran, 2007). It may also reduce the oxidative damage to DNA and inhibit the oxidation of LDL cholesterol (Bohm *et al.*, 2003). From the nutritional point of view, there are a lot of researches have been reported. Praychoen *et al.* (2013) studied effect of thermal treatment on phytochemical content and antioxidant activity of Gac fruit. They founded that the quantity of the main of phytochemical compounds significantly increased when the temperature was raised from 60 to 80 °C. However, raising temperature above 80 °C resulted in decreasing of the phytochemical compounds and antioxidant activity in Gac fruit. Overall, the optimum temperature to retain high amount of the phytochemical compound and antioxidant capacity of Gac juice was at 80 °C. Sawaengwutthipan and Siriwanwialichat (2015) reported the effect of Gac fruit preparation addition on characteristics of yoghurt. The results were revealed that addition of Gac fruit preparation at 5 % w/w received the highest linking score but not significantly different from addition of which at 10% w/w ($p \leq 0.05$). The pH of all samples slightly dropped during storage whilst syneresis progressed and viscosity increased with increasing Gac fruit preparation addition. Nevertheless, the viscosity of all yoghurt samples decreased during storage. Furthermore, the increasing amount of Gac fruit preparation influenced on product's color by lowering L* value, increasing a* and b* value. Total plate count of yoghurt samples added with Gac fruit preparation changed slightly as compared to that of control in which more noticeable reduction was monitored during storage. Additionally, Songtummin and Leenanon (2016) observed the survival of probiotic bacteria in Gac ice

cream during storage at -20 °C for 8 weeks was evaluated and it was found that when the storage time increased, the numbers of probiotic bacteria and pH tended downwards while % acidity tended upwards. Finally, by the end of 8 weeks, the probiotic survival numbers in Gac ice cream were significantly different ($p \leq 0.05$) as probiotic Gac ice cream with cryoprotectant had the highest survival of probiotic *L. casei* TISTR 390 with the number of 8.40 log CFU/g. On the other hand, there was no information reported in producing non-dairy probiotic from Gac juice. Therefore, the purpose of this study was to investigate the suitability of Gac juice for production of probiotic juice by various strains of *Lactobacillus* sp. such as *L. casei* TISTR 390, *L. fermentum* TISTR 391 and *L. plantarum* TISTR 1463, respectively. Finally, the amount of vitamin C, antioxidant activity and toxicity to human colon cancer cell (SW620) were compared between fermented and un-fermented Gac juice. Also, bacterial cell behavior was photographed with a scanning electron microscopy (SEM).

Materials and methods

Materials

Maturing Gac fruit was purchased from the local farmer in Chanthaburi province, Thailand and 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 were obtained from the Microbiological Resources Center at the Thailand Institute of Scientific and Technological Research in Pathum Thani, Thailand. The cultures were grown at 37 °C for 24 hours in a 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, manganese sulphate 0.05 g/l) and used as inoculum.

Production of Gac juice fermentation

The maturing of Gac fruit juice was prepared as followed. First, the fruit was separated seed and pulp by using spoon. Then the obtained pulp was filtrated by sieve 3 times. The juice was made by mixing with sugar and distilled water as the ratio of Gac juice: sugar : water; 25:5:70 and salt (0.3 % w/v) was added. From preliminary result, significantly ($p \leq 0.05$) highest overall

acceptability was found in Gac juice mixed with pineapple juice; pH 4.30. Therefore, this formular was used in this futher research. Before fermentation, the Gac juice was produced in the small polypropylene container (50 ml.) which was pasteurized at 80 °C for 10 minutes. The experiment was designed as Randomized Completely Block Design (RCBD) for sensory evaluation and Completely Randomized Design (CRD) for properties determination. The starter cultures were added per various strains of 5 % w/v of *Lactobacillus* sp. (10^6 CFU/ml.) as presented in table1.

Table 1. Eight treatments of fermentation of Gac fruit juice

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

Then, the samples were incubated at 30 °C for 72 hours and were obserbed for change in chemical and microbiological properties which was monitored every 24 hours until 72 hours. Then 72 h fermentation samples was evaluated sensory acceptability.

Change in chemical properties analyses

Samples were taken at 24 hours intervals until 72 hours for chemical and physical analyses. The pH was measured with a pH meter (Subtex, Taiwan). Total acidity expressed as percent lactic acid was determined by titrating with 0.02 N NaOH to pH 8.2. Total soluble solid was analyzed by hand refractometer (Atago, Japan). Lycopene content was evaluated by the method of Fish *el al.* (2002). Brifly, samples (0.50 ml) were mixed with 20 ml of 0.05 % v/v the mixing solution of ethanol:hexan:acetone as the ratio 25:50:25. Then, 3 ml of distilled water was added. The upper part (hexan) was separated and incubated in dark room condition for 5 min. The absorbance was conducted by using spectrophotometer at wavenumber 503 nm.

Change in microbiological properties analyses

For the microbiological, viable cell counts (log CFU/ml) were assessed by the standard plate count method with lactobacilli MRS medium after 48h inoculation at 37 °C and expressed as colony forming unit (CFU).

Sensory evaluation

The non-diary probiotic fermented Gac juice with various strains of *Lactobacillus* sp. for 72 hours at 30 °C were sensory evaluated with 50 untrained panelists from the staff and students of the Department of Product Development and Management Technology at Rajamangala University of Technology Tawan-ok Chanthaburi campus. The panelists evaluated the samples using a nine-point hedonic scales ranging from 1 (extremely disliked) to 9 (extremely liked) (Watts *et al.*, 1989). Each panelist evaluated the samples for colour, aroma, taste, texture, and overall liking.

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

After 72h of fermentation at 30 °C, the fermented samples (25 g) were stored at 4 °C in refrigerator for 4 weeks. Samples were taken at weekly intervals. Then, the fermented samples were analyzed for its microbiological properties. The viable cell counts (log CFU/ml) were evaluated by the standard plate count method with lactobacilli MRS medium after 48h inoculation at 37 °C and expressed as colony forming unit (CFU). Then, the final fermented Gac juice was monitored for vitamin C content, antioxidant activity (DPPH radical scavenging activity), human colon cancer cytotoxicity and bacterial cell behaviour.

Vitamin C content determination

Vitamin C content determination was delivered to analyze by the Institute of Food Research and Product Development in Kasetsart University in Bangkok, Thailand.

DPPH radical scavenging activity determination

The free radical scavenging activity was delivered to analyze by the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI) in Bangkok, Thailand. DPPH radical scavenging activity procedure was performed according to the methods described by Zhu *et al.* (2006). Briefly, one gram of sample was extracted with 10 ml ethanol. The solution was separated by centrifugation at 6,000 rpm. The obtained supernatant was tested by mixing with ethanol at various concentrations of 10, 20, 30, 40, and 50 µg/ml. The sample (1 ml) was mixed with 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in 95% ethanol (1ml) and incubated in dark condition

for 30 minutes. The absorbance was determined using a spectrophotometer at 517 nm. Vitamin C (L-ascorbic acid), Vitamin E (Tocopherol), and BHT (Butylated Hydroxyl Toluene) were used in the reference standard compound. The percentage of radical scavenging activity was calculated with the following equation:

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

A_0 = the absorbance of control reaction

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.

Cytotoxicity of fermented Gac juice

Cytotoxicity using MTT assay was delivered to analyze by the Microbiology department, Faculty of Science, Chulalongkorn University in Bangkok, Thailand. This method was produced following the method reported by Senthilraja and Kethiresom (2015) with the slight modifications. In brief, seeding cell SW620 cell was seeded at 1.5×10^4 in 96 well plate for overnight (total volume 100 μ l/well). For the cell treatment, fermented Gac juice was prepared by using six difference concentrations of bioactive compounds which diluted in completed media and added to the well that contain the cell (100 μ l/well). Supernatant was removed, added complete media that contain with juice sample or DMSO (vehicle control) and incubated for 24 hours. For the measurement the cell cytotoxicity, MTT solution (conc. 5 mg/ml) 10 μ l/well was added and incubated at 37 °C for 4 hours in CO₂ incubator. The purple formazan was dissolved by using isopropanol with HCl (100 μ l/well) and mixed. Finally, the absorbance was measured at wavenumber 540 nm by micro-plate reader.

Bacterial cell behaviour

The final fermented Gac juice and fermented with another *Lactobacillus* sp. were sampled from the culture and photographed with a scanning electron microscopy (SEM) (JEOL, model JSM-MEDEL jsm-5410LV, Japan) at a magnification of 5,000x, 10,000x, 20,000x and 50,000x to capture images for the lactic acid bacterial cell behaviour grown under the culture condition.

Data analysis

Analysis of the above-mentioned properties were carried out in three replicates. The data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$).

Mean with significant differences was separated by Duncan's multiple range test (DMRT) using computer software.

Results

Change in chemical properties analyses of fermented Gac juice

Eight treatments of fermented Gac juice as mention previously were incubated at 30 °C for 72 h. Change in pH, titratable acidity, total soluble solid, lycopene content were determined as shown in figure 1-4. As shown in Figure 1, pH was rapidly decreased when the fermentation time longer in treatment 2-8. In addition, the combination strains of *Lactobacillus* sp. were significantly ($p \leq 0.05$) decreased pH more than those of the single strain fermentation and highest low pH was revealed in the three combination strains of lactic acid bacteria (treatment 8). As presented in figure 2, the numbers of titratable acidity of fermented Gac juice of treatment 2-8 were significantly ($p \leq 0.05$) increased when the fermented time longer and highest at 72 h fermentation (approximately 1 % w/v). Also, the three combination strains (treatment 8) was exhibited to produce highest of lactic acid. From figure 3, the level of total soluble solid of fermented Gac juice of treatments 2-8 were significantly ($p \leq 0.05$) decreased when the fermented time longer and lowest at 72 h fermentation (approximately 8.6 °Brix in treatment 8). As presented in figure 4, the level of lycopene content Gac juice fermented with varios strains of *Lactobacillus* sp. were significantly ($p \leq 0.05$) fluctuated at 24 h fermentation in treatment 2, 3 and 6. The amount of lycopene content of all treatment were tended to be stable at the end of fermentation (72 h).

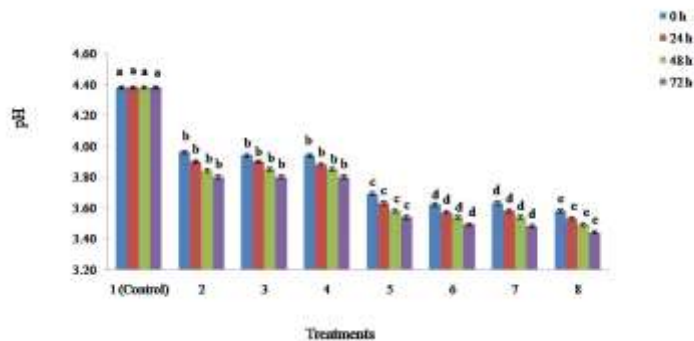


Figure 1. PH of Gac juice fermented with varios strains of *Lactobacillus* sp. for 0, 24, 48 and 72 h at 30 °. Bars represent standard deviation from triplicate determination

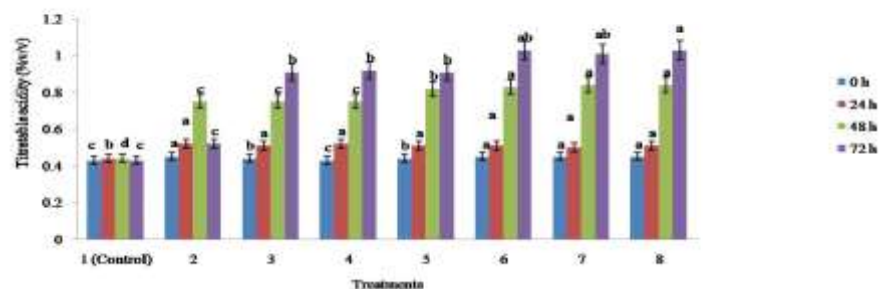


Figure 2. Titratable acidity of Gac juice fermented with various strains of *Lactobacillus* sp. for 0, 24, 48 and 72 h at 30 °C. Bars represent standard deviation from triplicate determination

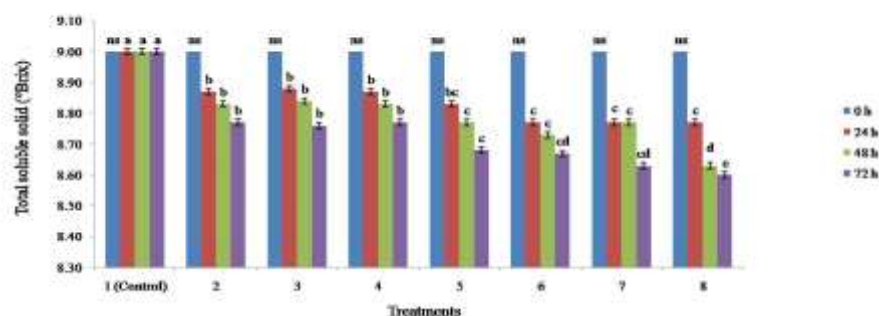


Figure 3. Total soluble solid of Gac juice fermented with various strains of *Lactobacillus* sp. for 0, 24, 48 and 72 h at 30 °C. Bars represent standard deviation from triplicate determination

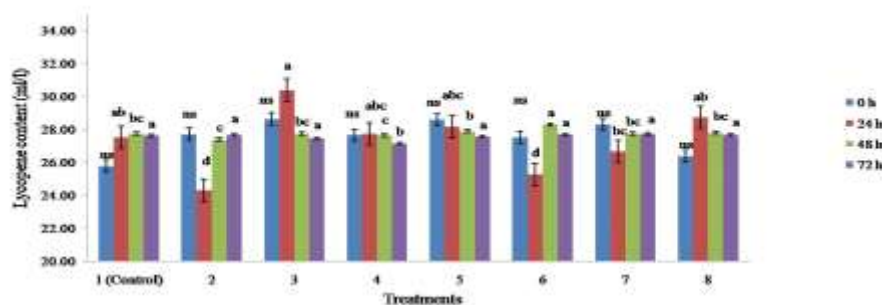


Figure 4. Lycopene content of Gac juice fermented with various strains of *Lactobacillus* sp. for 0, 24, 48 and 72 h at 30 °C. Bars represent standard deviation from triplicate determination

Change in microbiological properties Analyses

Regarding of viable plate count, the numbers of viable cell count were significantly ($p \leq 0.05$) reached when the fermentation time longer and highest (approximately 6 log CFU/ml.) at the end of fermentation (72 h) in all of treatments (2-8) as presented in figure 5. Consequently, all of samples of fermented Gac juice were sensory evaluation with 50 un-trained panelist from our department. It was revealed that treatment 3(fermented with *L. fermentum* TISTR 391) was the highest acceptable for overall liking as presented in table 2 with the score of 6.07 ± 1.36 (moderately like). However, it was not significantly different ($p \leq 0.05$) from treatment 1, 2, 4, 5, 6 and 8. Then, this treatment was further selected to evaluate the influence of cold storage (4 °C) on the viable microbial cell at 0, 1, 2, 3 and 4 weeks of storage. The result was exhibited that the numbers of viable cell count were significantly ($p \leq 0.05$) increased at 1 week cold storage from approximately 6 log CFU/ml to 8 log CFU/ml. After that, the microbial cell were significantly ($p \leq 0.05$) dramatically declined from 6 log CFU/ml to 4 log CFU/ml. at the end of cold stored (4 weeks) as represented in figure 6. Interestingly, the level of vitamin C and antioxidant activity of fermented Gac juice were found higher than that those control samples. The numbers of vitamin C and IC_{50} were 0.08 ± 0.00 , 0.03 ± 0.01 mg/100g and 108.90 ± 0.69 , 131.90 ± 0.48 mg/ml., respectively as shown in table 3.

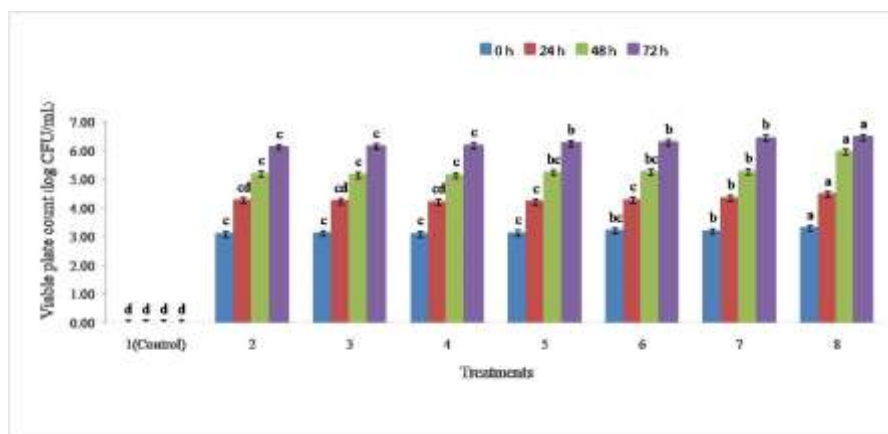


Figure 5. Total viable plate count of Gac juice fermented with various strains of *Lactobacillus* sp. for 0, 24, 48 and 72 h at 30 °C. Bars represent standard deviation from triplicate determination

Table 2. Mean sensory scores of Gac juice fermented with various strains of *Lactobacillus* sp. for 72 h at 30 °

Treatments	Preference scores				
	Colour ^{ns}	Aroma	Taste	Texture ^{ns}	Overall linking
1(Control)	5.83±1.51	5.80±1.54 ^a	5.67±1.54 ^{ab}	5.70±1.49	5.80±1.52 ^{ab}
2	5.80±1.19	4.77±1.52 ^{bcd}	6.47±1.38 ^a	5.50±1.41±	5.33±1.49 ^{ab}
3	5.73±1.46	5.50±1.48 ^{ab}	5.80±1.52 ^{ab}	5.87±1.50	6.07±1.36 ^a
4	5.67±1.42	4.20±1.52 ^d	5.47±1.72 ^b	5.67±1.45	5.23±1.59 ^{ab}
5	5.93±1.44	4.57±1.50 ^{cd}	4.97±1.45 ^b	5.80±1.30	5.50±1.43 ^{ab}
6	5.60±1.48	4.87±1.63 ^{bcd}	5.13±1.68 ^b	5.47±1.17	5.37±1.25 ^{ab}
7	5.37±1.59	5.27±1.55 ^{abc}	5.03±1.61 ^b	5.30±1.47	5.17±1.64 ^b
8	5.60±1.45	5.37±1.33 ^{abc}	5.43±1.63 ^b	5.70±1.56	5.57±1.52 ^{ab}

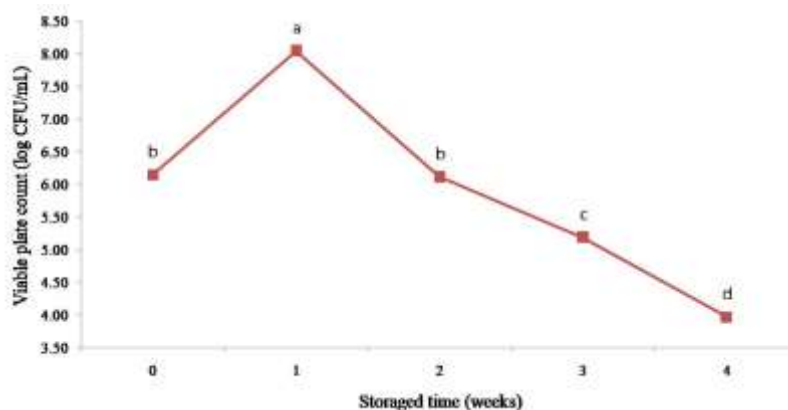


Figure 6. Effect of cold storage (4 °C) on total viable plate count of Gac juice with fermented with *L. fermentum* TISTR 391 for 0, 1, 2, 3 and 4 weeks

Table 3. Vitamin C content and antioxidant activity of Gac juice fermented with *L. fermentum* TISTR 391 for 72 h at 30 °C

Treatments	Vitamin C (mg/100g) ±SD ^{ns}	IC ₅₀ (DPPH assay (mg/ml)) ±SD
Control	0.03±0.01	131.90±0.48 ^b
Fermented Gac juice	0.08±0.00	108.90±0.69 ^a

Regarding the toxicity to the human colon cancer cell SW620, the high level of concentrations were trend to be decreased the numbers of the tumor cell SW620 than that lower concentration in both samples as shown in figure 7. From estimation by linear regression, IC₅₀ of control sample was found 12.06 %

v/v and the equation was $y = -4.95x + 109.70$, $R^2 = 0.77$. Moreover, IC_{50} of fermented sample was found 7.06 % v/v and the equation was $y = -9.66x + 118.20$, $R^2 = 0.52$, respectively. From scanning electron microscope, the result was revealed that the *Lactobacillus* sp. lived by mixing the polysaccharide of Gac juice as shown in figure 8-10. The long rod of *L. fermentum* TISTR 391 with fermented in Gac juice for 72 h at 30 °C was located in polysaccharide of Gac juice as shown in figure 8 (A-D) at magnification 5,000, 10,000, 20,000 and 50,000x, respectively. Moreover, the damage cell was found when the fermented time longer than that of 72 h at 30 °C as presented in figure 8(E-F) at magnification 10,000 and 20,000x, respectively. The short rod of *L. casei* TISTR 390 with cultured in Gac juice for 72 h at 30 °C was also located in polysaccharide of Gac juice as shown in figure 9(A-D) at magnification 5,000, 10,000, 20,000 and 50,000x, respectively. Also, the long rod of *L. plantarum* TISTR 1463 with cultured in Gac juice for 72 h at 30 °C was also located in polysaccharide of Gac juice as shown in figure 10(A-D) at magnification 5,000, 10,000, 20,000 and 50,000x, respectively.

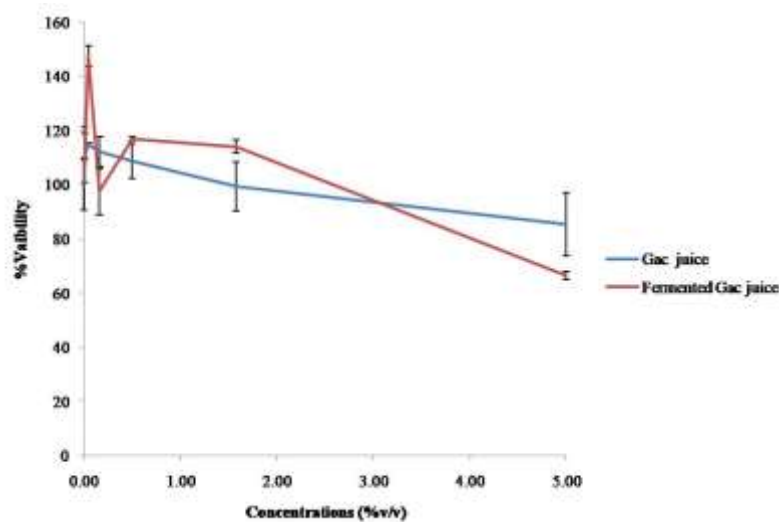


Figure 7. Effect of Gac juice fermented with *L. fermentum* TISTR 391 for 72 h at 30 °C to % viability of human colon cancer cell SW 620

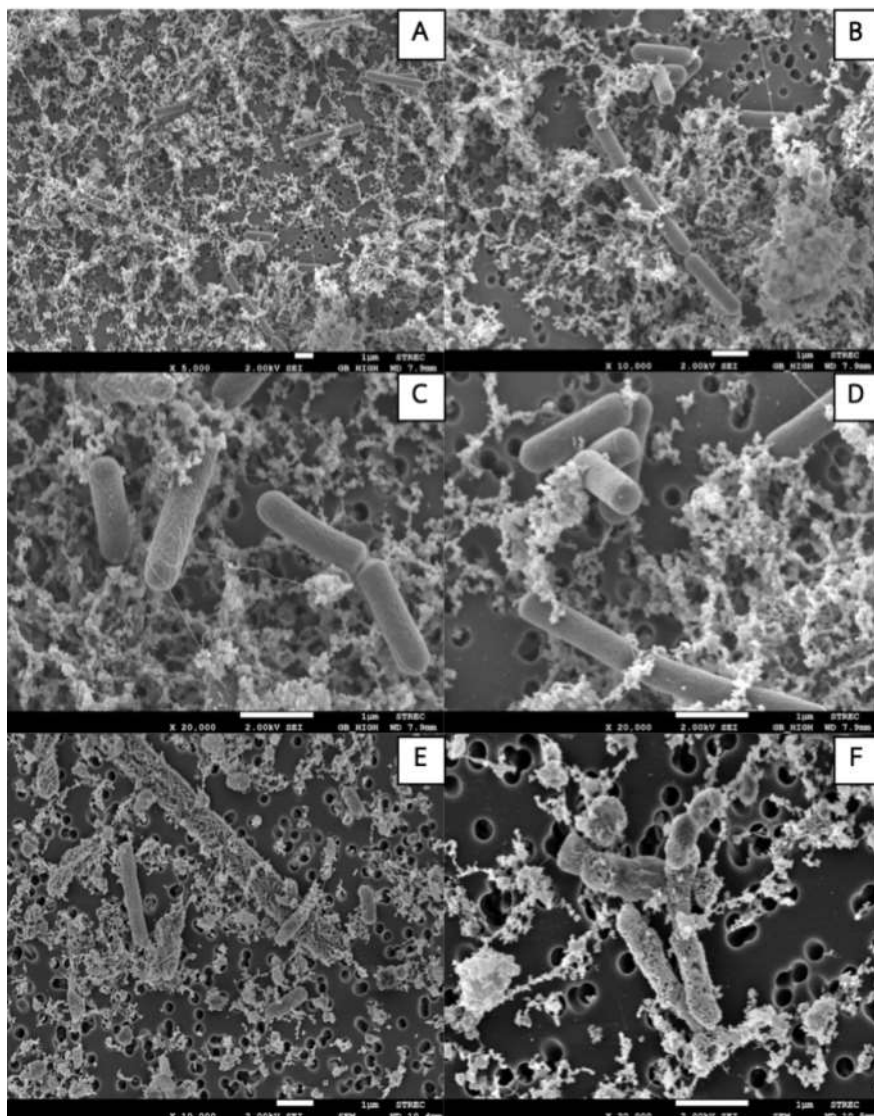


Figure 8. The electron micrograph of *L. fermentum* TISTR 391 grown in fermented Gac juice for 72 h at 30 °C (A-D) and more than 72 h (E-F)

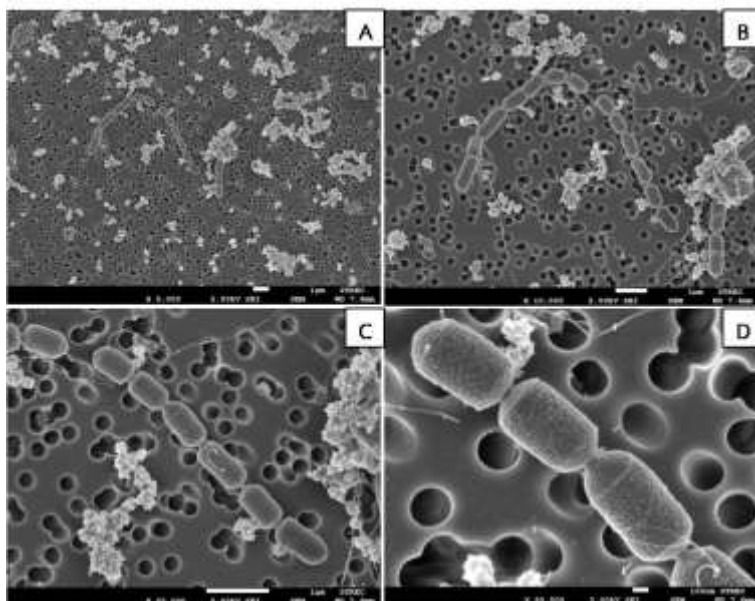


Figure 9. The electron micrograph of *L. casei* TISTR 391 grown in fermented Gac juice for 72 h at 30 °C (A-D)

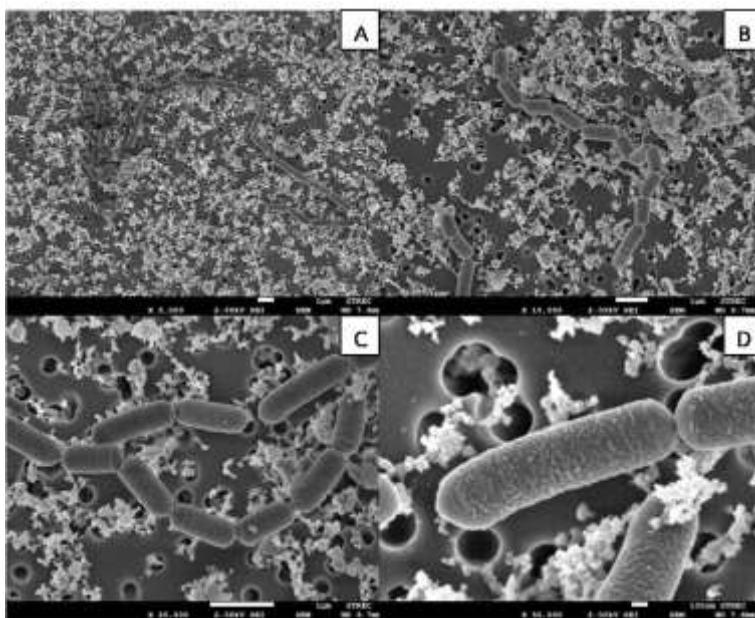


Figure 10. The electron micrograph of with *L. plantarum* TISTR 1463 grown in fermented Gac juice for 72 h at 30 °C (A-D)

Discussion

Regarding change in chemical and microbiological analyses, *Lactobacillus* sp. were metabolized glucose to lactic acid of Gac juice while the fermentation time increased. So, the pH, total soluble solid and titrable acidity were tended downwards and upwards, respectively as exhibited in figure 1-3. Our research was agreed with Sawaengwutthipan and Siriwanwialichat (2015) and Sontummin and Leenanon (2016). They also found the pH was declined and acidity was increased at the end of fermentation. However, the fermentation from *Lactobacillus* sp. did not effect to lycopene content when the fermentation time longer as represented in figure 4. For microbiological analyses, the numbers of viable cell count were significantly ($p \leq 0.05$) reached when the fermentation time longer and highest at 30 °C for 72 h fermentation in all of treatments. This could be because lactic cultures were metabolized utilizing Gac juice for cells synthesis and lactic acid production. Therefore, the numbers of viable cell were increased when the fermentation time longer. For sensory evaluation, non-significantly ($p \leq 0.05$) overall acceptability was found in treatment 3 (fermented with *L. fermentum* TISTR 390). These could be because diffent strains of *Lactobacillus* sp. did not effect to the taste until consumers could be detectable. In contrast, this sample had the highest score of over all acceptability. Thus, it was further determined the survival of probiotic bacteria in cold storage (°C), vitamin C, antioxidant activity and toxicity to tumor cancer cell compared with control sample. The results were revealed that, the survival of probiotic bacteria in Gac juice during cold storage (4 °C) for 4 weeks was declined when the storage time increasing, the numbers of probiotic bacteria were tended upwards at 1 week of storage and then significantly ($p \leq 0.05$) dramatically decreased at the end of cold storage at 4 weeks of storage. This could be because the low pH of Gac juice have negative effect on viability of probiotic. Therefore, lactic cultures were lost their viability during cold storage as found in this study and similar with another previously research (Nematollahi *et al.*, 2016). Interestingly, non-significantly of vitamin C and significantly ($p \leq 0.05$) of antioxidant activity higher than that those control were shown. Regarding cytotoxicity, the high concentration of both samples tended to be toxic to tumor colon cancer cell SW620 cell. Therefore, the %viability were decreased while the concentration higher and lowest % viability was exhibited at 5% v/v of fermented Gac fruit juice. Our result corresponding with Tien *et al.* (2005), they found that the water extract of Gac fruit inhibited the growth of the colon 26-20 adenocarcinoma cell line, transplanted in Balb/c mice, reduce weight by 23.6 %. However, the exactly resulted could be confirm to test cytotoxicity in the higher concentration than that of 5 %v/v. In addition, the cytotoxicity to mormal cell line could be test

for comparing the real properties of fermented Gac juice. For the bacterial cell behavior, rod cell of *Lactobacillus* sp. were lived amount polysaccharide of Gac juice in all of species.

Conclusion

The results indicated that Gac juice fermented with *L. fermentum* TISTR 391 had high acceptability with the score 6.07 (moderately like). Changes in pH, acidity, total soluble solid and viable cell counts during fermentation under controlled conditions at 0, 24, 48 and 72 h fermentation were evaluated. Our results were found that change in pH and total soluble solid of probiotic Gac juice were significantly ($p \leq 0.05$) declined at 72 h fermentation. On the other hand, the amount of titratable acidity (lactic acid) was significantly ($p \leq 0.05$) reached at fermentation for 72 h. The effect of shelf life on viable cell count in cold storage (4 °C) was observed. The result was exhibited that the viable cell was increased at 1 week stored, then decreased when the stored time longer and lowest in 4 weeks storage. Finally, the final product was analyzed the amount of vitamin C, antioxidant activity and cytotoxicity to colon tumor cancer cell. Interestingly, the amount of vitamin C content was exhibited two fold higher than those control. In addition, the free radical scavenging capacity was assayed by DPPH method also shown that IC_{50} values had significantly ($p \leq 0.05$) higher than those control. However, both fermented Gac juice and control were also effected to cell SW620 when the juice concentration was increasing. For the bacterial cell behavior, the scanning electron microscope showed that lactic acid bacteria were grown amount the polysaccharide of the juice. In summary, these results could be served as the alternative healthy non-dairy probiotic source for vegetarians and milk allergy 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 Gac juice and the normal cell line could be tested to compare with the cancer cell.

Acknowledgement

This research was financially supported by Rajamangala University of Technology Tawan-ok. The authors gratefully acknowledge the technical assistance of Sonsri Toikham.

References

- Aoki, H., Kieu, N. T. M., Kuze, N., Tomisaka, K. and Chuyen, N. V. (2002). Carotenoid pigments in GAC fruit (*Momordica cochinchinensis* Spreng). Bioscience Biotechnology and Biochemistry. 66:2479-2482.

- Ding, W. K. and Shah, N. P. (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juice. *International of Food Research Journal*. 15:219-232.
- Fish, W. W., Perkins-Veazie, P. and Collins, J. K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of food composition and analysis*. 15:309-317.
- Ishida, B. K., Turner, C., Chapman, M. H. and Mckeen, T. A. (2004). Fatty acid and carotenoid composition of Gac (*Momordica cochinchinensis* Spreng) fruit. *Journal of Agricultural and Food Chemistry*. 52:274-279.
- Karovicova, J. and Kohajdova, Z. (2003). Lactic acid fermented vegetable juice. *Hort Sci (Prague)*. 30:152-158.
- Kobola, J. and Siriamornpun, S. (2011). Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). *Food Chemistry*. 127:1138-1145.
- Nematollahi, A., Sohrabvandi, S., Mortazavian, A. M. and Jazaeri, S. (2016). Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. *Electronic Journal of Biotechnology*. 21:49-53.
- Praychoen, P., Praydoen, P. and Phongsongpasuk, S. (2013). Effect of thermal treatment on phytochemical content and antioxidant activity of Gac juice. *Burapha Science Journal*. 18:90-96.
- Sawaengwutthipan, W. and Siri Wongwialichat, P. (2015). The effect of gac fruit preparation addition on characteristics of yoghurt. *Journal of Food Scienc and Agricultural Technology*. 1:93-98.
- Senthilraja, P. and Kathiresan, K. (2015). In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. *Journal of Applied Pharmaceutical Science*. 5:080-084.
- Sharma, V. and Mishra, H. N. (2008). Fermentation of vegetable juice mixture by probiotic lactic acid bacteria. *Nutrafoods*. 12:17-22.
- Songtummin, S. and Leenanon, B. (2016). Survival of *Lactobacillus acidophilus* TISTR 1338 and *Lactobacillus casei* TISTR 390 in probiotic gac ice cream. *International Food Research Journal*. 23:790-796.
- Tien, P. G., Kayama, F., Konishi, F., Tamemoto, H., Kasono, K., Hang, N. T. K., Kuroki, M., Ishikawa, S. E., Van, C. N. and Kawakami, M. (2005). Inhibition of tumor growth and angiogenesis by water extract of Gac fruit (*Momordica cochinchinensis* Spreng). *International Journal of Oncology*. 881-889.
- Tran, T. H. (2007). Producing carotenoid rich powder from Gac fruit. Master's thesis. Center for plant and food science. University of Western Synney. Australia.
- Watts, B. M., Yumaki, C. L., Jeffery, L. E. and Elais, L. G. (1989). Basic sensory methods for food evaluation. The International Development Research Centre, Ottawa, Canada. P.159.
- Zhu, K., Zhou, H. and Qian, H. (2006). Antioxidant and free radical scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with catalase. *Process Biochemistry*. 41:1296-1302.

(Received: 12 September 2018, accepted: 31 October 2018)