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Effect of the addition of phytomix-3+ mangosteen on antioxidant activity, viability of lactic acid bacteria, type 2 diabetes key-enzymes, and sensory evaluation of yogurt

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ABSTRACT

Phytomix-3 was prepared from the mixture of *Lycium barbarum, Momordica grosvenori and Psidium guajava* leaves and added together with *Garcinia mangostana* (pulp and pericarp). The effect of phytomix-3+ mangosteen (Ph-3+M) enriched yogurt was studied for peptide content, inhibitory activities on α-amylase, α-glucosidase, the viability of *S. thermophilus* and *Lactobacillus* spp. during 0, 7 and 14 days of storage, both before (pre-) and after (post-) simulated gastrointestinal digestion. Antioxidant activity (DPPH assay), total phenolic content and sensory evaluation were also investigated during the storage period. Ph-3+M yogurt showed significantly higher ($p < 0.05$) antioxidant activity and TPC compared to plain yogurt during storage. The highest peptide concentrations of pre- and post-digested Ph-3+M yogurt was shown on day 7 of storage. Ph-3+M yogurt displayed strong inhibition on α-glucosidase and mild inhibition on α-amylase inhibitory activities. Ph-3+M yogurt enhanced the viability of *S. thermophilus* and *Lactobacillus* spp. In conclusion, Ph-3+M yogurt could provide beneficial effects as a functional food.

1. Introduction

Diabetes is a disease characterized by high blood sugar level (glucose) that result from the failure of the body to produce enough insulin or unable to respond properly to the insulin that had been produced by the pancreas (Shori, 2015a and b). Glucose is an essential nutrient that provides energy for the body to function well. Carbohydrate is broken down into smallest sugar (for example glucose) inside the small intestine and the glucose is absorbed by the intestinal cells into the bloodstream and carried out to all cells in the body that utilized it (Shori, 2015a and b). However, glucose needs insulin to aid in its transport into the cells because it cannot enter cells alone by itself. Consequently, in the absence of insulin, a sudden rise in blood glucose level occurred due to hydrolysis of starch by pancreatic α-amylase and uptake of glucose by α-glucosidase (Shori & Baba, 2013). Thus, an effective strategy for type-2 diabetes management is to inhibit these key enzymes (Baba, Najarian, Shori, Lit, & Keng, 2014).

Yogurt is a dairy product produced from the fermentation of milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Alenisan, Alqattan, Tolbah, & Shori, 2017). It is regarded as a functional food because it contains live LAB, the probiotics that is beneficial for human intestines (Shori, 2016). Yogurt is also rich in calcium, protein, riboflavin, vitamin B6 and vitamin B12 (Muniandy, Shori, & Baba, 2017).

Herbs are normally used for medicine, food flavoring and also as fragrant properties (Emmanuel, Shori, & Baba, 2016). The previous study showed that several dietary herbs, spices, fruits and vegetables which constitute by phenolic phytochemicals can result in high antioxidant activity and possess therapeutic properties which are beneficial for the management of type-2 diabetes (Shetty, Clydesdale, & Vattem, 2005). *Lycium barbarum, Momordica grosvenori, Psidium guajava* leaf and *Garcinia mangostana* have been selected for the present study due to the variety of therapeutic properties in these herbs (Shori, 2015a). *L. barbarum* has shown potential health benefit effects i.e. powerful antioxidant activity, blood glucose levels regulation, blood pressure regulation (Zou, Zhang, Yao, Niu, & Gao, 2010; Baba et al., 2014). *M. grosvenori* has potential against the diabetic effect, anti-carcinogenic and was used as a natural sweetener (Takasaki et al., 2003) while *P. guajava* leaf has a beneficial effect as a powerful antioxidant (Soman, Rajamanickam, Rauf, & Indira, 2013). *P. guajava* contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body and therefore can be expected to prevent various chronic diseases such as diabetes, cancer, and heart-disease (Soman et al., 2013). It was reported that the leaves of *P. guajava* contain an

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essential oil rich in cineol, tannins, and triterpenes (Díaz-de-Cerio, Verardo, Gómez-Caravaca, Fernández-Gutiérrez, & Segura-Carretero, 2017). In addition, three flavonoids (quercetin, avicularin, and guaijaverin) have been isolated from the leaves and showed strong inhibitory activities against α-amylase (Díaz-de-Cerio et al., 2017). *G. mangostana* have dual-purpose values on promoting good health as well as a great flavor enhancer. *G. mangostana* contain xanthone, the most powerful antioxidant especially inside its pericarp (Pothitirat, Mullika, Roongtawan, & Wandee, 2009). Xanthones have the potential to neutralize free radicals, support immune system, maintain intestinal health and promote healthy respiratory system (Andrès et al., 2006).

Phytomix-3 was designed and prepared in the lab by mixing *L. barbarum, M. grosvenori and P. guajava* leaves in the ratio of 3:1:3. Then, phytomix-3 was added together with *G. mangostana* (pulp and pericarp in the ratio of 2:3). The effect of phytomix-3 + mangosteen enriched yogurt was studied for peptide content, inhibitory activities on α-amylase, α-glucosidase, the viability of *S. thermophilus* and *Lactobacillus* spp. during 0, 7 and 14 days of storage, both before (pre-) and after (post-) simulated gastrointestinal digestion. Antioxidant activity, total phenolic content and sensory evaluation were also investigated during the storage period.

2. Materials and methods

2.1. Herbs

Dried leaves of *P. guajava*, *L. barbarum*, and *M. grosvenori* were purchased from local Chinese medicinal store. All the herbs are stored in the dry plastic containers as a powder and kept at room temperature until required.

2.2. Mangosteen

Mangosteen or *G. mangostana* was purchased from local market. The pulp was kept in a plastic container and placed it in the -20 °C fridge until required whereas the pericarp was dried in the oven for at least 2 days before ground to powder form. The powder was kept in the container at room temperature until required.

2.3. Water extraction of herbs

P. guajava, *L. barbarum*, *M. grosvenori*, and *G. mangostana*'s pericarp powder were mixed separately at 20% (w/v) concentration with dH_2O. The mixture was incubated overnight at 70 °C water bath. The mixture then was filtered by using coffee sieve and centrifuge at 2000 rpm for 10 min at 4 °C. The clear supernatant obtained was used as water herbal extract and kept in -20 °C fridge until required.

2.4. Preparation of yogurt

Water herbal extracts of *L. barbarum, P. guajava* and *M. grosvenori* were added using 1:2:3 ratio together with pulp and pericarp using 3:2 ratio (60 ml) into 510 ml of pre-heated full cream milk, followed by addition of 14 g full cream milk powder to adjust the milk solid content. Finally, 30 g of starter culture (Chris-Hansen, Denmark) was added to the milk mixture and mixed thoroughly (Shori & Baba, 2013). The mixture then was put in the container and incubated at 41 °C and stored at 4 °C refrigerator once the pH 4.5 was reached. Plain yogurt was prepared in the same method as the herb's yogurt with the exception that 60 ml of dH_2O were used to replace the water herbal extract. The plain yogurt served as control. All samples were stored at 4 °C refrigerator for 14 days.

2.5. Preparation of yogurt water extract

Yogurt sample was diluted and homogenized with dH_2O in the ratio of 1: 0.25. The pH of the yogurt solution then was adjusted to pH 4.0 with 1.0 M HCl to reduce the solubility of casein in milk. The yogurt was incubated at 45 °C water bath for 10 min. The yogurt was centrifuged at 5000 rpm and 4 °C for 10 min to remove precipitated milk proteins. The pellet was discarded and 0.5 M NaOH was then added to neutralize the supernatant back to pH 7.0 followed by second centrifugation at 5000 rpm for 10 min at 4 °C for further precipitation of proteins and salts. The supernatant from the second centrifugation was used in the analysis of all assays.

2.6. Total phenolic assay

Total phenolics content were determined by applying an assay that had been modified from Muniandy, Shori, and Baba (2016). Yogurt extract (1.0 ml) was transferred to the test tube and 1.0 ml of 95% ethanol together with 5 ml of dH_2O were added. Then, 0.5 ml of 50% (v/v) Folin-Ciocalteu reagent (Sigma Aldrich, USA) was added to the mixture. After 5 min, 1.0 ml of 5% Na_2CO_3 was added and the mixture and was left for 1 h at room temperature. Absorbance was read at 725 nm and the absorbance values were converted to total phenolics by referring to gallic acid standard curve. Gallic acid standard curve was prepared with a various concentration of gallic acid (5-60 μg/ml) in ethanol.

2.7. 1,1-Diphenyl-2-picryhydrazyl (DPPH) assay

The DPPH radical scavenging assay was carried out according to Muniandy et al. (2016) with slight modification. 3 ml of 60 mM DPPH (dissolved in ethanol; Sigma Aldrich, USA) was added with 250 μl of yogurt extracts and allowed to stand at room temperature (25 °C) for 1 h. The absorbance was recorded at 517 nm against control, which contained 250 μl of ethanol instead of the extract. The % of inhibition was calculated by using following formula:

Inhibition % = (AC – AS) / AC X 100%

AC = Absorbance of control
AS = Absorbance of sample

2.8. In vitro gastrointestinal model

2.8.1. Preparation of gastric and duodenum juices

The gastric and duodenum solutions were freshly prepared according to the protocols described by Shori and Baba (2015). To simulate the *in vivo* saliva, 100 ml of a sterile electrolyte solution (6.2 g/l NaCl, 2.2 g/l KCl, 0.22 g/l CaCl2, 1.2 g/l NaHCO3) was mixed with 10 mg lysozyme from chicken egg white (Sigma Aldrich, USA) to obtain a final concentration of 100 ppm. To simulate the stomach environment (gastric juice), 0.3% of the electrolyte solution was added to pepsin (P7000; Sigma Aldrich, USA) and the pH was adjusted to 3 using 5 M HCl. To simulate the intestinal digestion (duodenum juice), the electrolyte solution (6.4 g/l NaHCO3, 0.239 g/l KCl, 1.28 g/l NaCl) mixed with 0.3% bile salts (B8631; Sigma Aldrich, USA) and 0.1% (v/w) pancreatin (P3292; Sigma Aldrich, USA) and adjusted to pH 7.2 by using 5 M NaOH.

2.8.2. Simulation of gastrointestinal digestion

Yogurt samples were taken out and diluted with the artificial saliva solution in the ratio of 1:1 and incubated at 37 °C for 5 min. The samples were further diluted with an artificial gastric fluid solution in the ratio of 3:5 and incubate again at 37 °C for 1 h before 30 ml of samples were taken out for analysis (α-amylase and α -glucosidase inhibition assays). The remaining solutions from stomach section were further diluted with artificial duodenal secretion in the ratio of 1:4 and were incubated again at 37 °C for 2 h where 30 ml of the samples were taken out for analysis (α-amylase and α -glucosidase inhibition assays) after

1 h and 2 h incubation of intestinal digestion. All samples after 3 h gastrointestinal digestion (post-digestion) were analysis for the viability of *S. thermophilus* and *Lactobacillus* spp. and peptide concentration. All samples were manually agitated and stirred intermittently during the incubation time in order to ensure adequate enzymatic digestion to mimic gastrointestinal movement. The samples that were taken out for analysis were extracted by using a similar method as the extraction of yogurt water extract (section 2.3) in order to remove excess proteins, electrolytes and other impurities (Shori & Baba, 2015).

2.9. Microbial viable cell count

2.9.1. Enumeration of Streptococcus thermophilus

S. thermophilus was enumerated using spread plate method. Samples were serially diluted to the desired dilution factor (10^{-7}) using peptone water buffer. Sterilized M17 media then was poured onto a clean sterilized petri dish and this agar was allowed to solidify. Then, 0.1 ml of diluted sample was transferred onto the agar surface and evenly distributed in three different directions using a sterile glass spreader. The plate was then incubated at 37 °C for 2 days. Finally, the number of colonies formed after incubation was expressed as colony forming units per milliliter sample (CFU/ml) using the following formulation:

CFU*/ml = No. of colonies formed X dilution factor of sample/0.1 ml of sample.

*CFU: Colony forming unit

2.9.2. Enumeration of Lactobacillus spp

Lactobacillus spp was enumerated using pour plate method. Samples were serially diluted to the desired dilution factor (10^{-5}) using peptone water buffer. The MRS medium was maintained at molten state (45-50 °C). Then, 1.0 ml of diluted sample was transferred to a sterile petri dish and 10 ml of sterile MRS medium was poured onto the petri dish and this mixture was mixed evenly. Care was taken to ensure that the entire surface of the plate was fully covered by the first layer of medium. The second layer of MRS media was poured on the solidified first layer of MRS agar. The petri dish was sealed with parafilm after the second layer of MRS has solidified and incubation was carried out at 37 °C for 2 days. The number of colonies formed was counted and the viable cell count in the sample was expressed as colony forming units per milliliter sample (CFU/ml) using the following formulation:

CFU/ml = No. of colonies formed X dilution factor of sample/1.0 ml of sample.

2.10. O-phthaldialdehyde (OPA) assay

Yogurt extracts (30 µl, containing 5-100 µg protein) was added with 1.0 ml of OPA reagent (25 ml of 1000 mM sodium tetraborate, 2.5 ml of 20% (w/w) sodium-dodecyl -sulphate, 40 mg OPA dissolved in 1 ml of methanol and 100 µl of β-mercaptoethanol top up with dH_2O until final volume is 50 ml) in 1.5 ml cuvette. The cuvette was then covered with parafilm prior to several times inversion in order to homogenize and facilitate the mixing of OPA reagent and samples. The mixture was then left at room temperature for 2 min. Absorbance was read at 340 nm and the peptide concentration was determined from Tryptone standard curve using different concentrations (Church, Swaisgood, Porter, & Catignani, 1983; Shori, 2013a, pp. 202–208).

2.11. α-Amylase inhibition assay

Yogurt extracts (500 µl) were mixed with 1.0 ml of 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride containing 0.5 mg/ml α-amylase (10080; Sigma Aldrich, USA) solution and this solution were incubated at 37 °C for 10 min (Shori & Baba, 2013). Upon pre-incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer, pH 6.9 with 0.006 M sodium chloride was added to each tube at a pre-determined time interval (every 20 s). The reaction mixtures were then incubated again at 37 °C for 10 min. To terminate the reactions, 1.0 ml of 2% dinitrosalicyclic acid (D0550, Sigma Aldrich, USA) color reagent was added to the mixture. The test tubes were then incubated in a boiling water bath for 8-9 min 1.0 ml of tartrate solution (18.2% w/v; S2377 Sigma Aldrich, USA) was then added into each tube and upon cooling to room temperature (approximately 2–3 min) and the reaction mixture was diluted with 10 ml of dH_2O. Absorbance was read at 540 nm. The readings were compared to a control using 500 µl of buffer solution in place of the extract. The formula to calculate enzyme inhibition is as follows:

α-Amylase inhibition % = (AC – AS) / AC X 100%

AC = Absorbance of control.
AS = Absorbance of sample.

2.12. α -Glucosidase inhibition assay

The α-glucosidase inhibition assay was performed as described by Shori and Baba (2013). Potassium phosphate buffer (1.0 ml, 0.8 M, pH 6.90) containing α-glucosidase (G5003; Sigma Aldrich, USA) solution (0.15 U/ml) was added into 500 µl of sample extract and the solution was incubated in a water bath (37 °C) for 10 min. After incubation, 500 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution (N1377; Sigma Aldrich, USA) in 0.1 M potassium phosphate buffer (pH 6.90) was added to each tube at pre-determined time intervals (every 30 s), and the absorbance at 405 nm was taken (time = 0 min). The reaction mixtures were further incubated at 37 °C for 10 min. After 10 min of incubation, absorbance readings were taken again at 405 nm. The readings were compared to a control using 500 µl of buffer solution in place of the extract. Readings for both control and samples at 10 min were deducted from the readings at 0 min. The α-glucosidase inhibitory activity was expressed as follows:

α- Glucosidase inhibition % = (AC – AS) / AC X 100%

AC = Absorbance of control.
AS = Absorbance of sample.

2.13. Sensory evaluation

0, 7, and 14 days yogurt samples were assessed by 30 untrained assessors (Shori & Baba, 2012). Sensory parameters which include texture (presence of whey), consistency (graininess, lumpiness, and firmness), sourness, sweetness, bitterness, overall aroma and overall preference were evaluated using a score rating of 1–10 points. The assessors were briefed on how to rate the yogurt samples before the assessment session to give a better understanding of the parameters which were being assessed. All the sensory parameters were analyzed with respect to a reference sample which includes the plain and phytomix-3+ mangosteen yogurt.

2.14. Statistical analysis

The experiment was carried out using three different batches of yogurt (n = 3). Data were expressed as mean ± SE. The statistical analysis was performed using one-way analysis of variance (ANOVA, SPSS 14.0), followed by Duncan's post hoc test for mean comparison. The criterion for statistical significance was $p < 0.05$.

3. Results and discussion

3.1. Effect of phytomix-3+ mangosteen on total phenolic content (TPC) of yogurt

The presence of phytomix-3+ mangosteen increased significantly ($p < 0.05$) TPC in yogurt ranged from 40 to 41 µg/g compared to plain

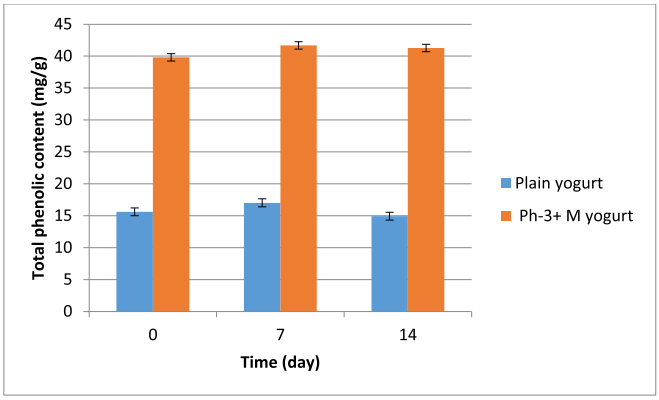

Fig. 1. Total phenolic content (μg/g) of phytomix-3+ mangosteen (Ph-3+M) yogurt and plain yogurt during 14 days of storage. Value are presented as mean value ± SE (n = 3). The level of significant was present at $p < 0.05$.

yogurt (15-17 μg/g) during 14 days of storage Fig. 1. The combination of phytomix-3+ mangosteen contributed to a high amount of phenolic compounds. This could be due to the presence of phenolic phytochemicals in these herbs (Shetty et al., 2005). This result was similar to the TPC of *Allium sativum*, white tea, and soybean yogurt during 14 days of storage which related to active compounds derived from *Allium sativum* (Muniandy et al., 2016; Shori et al., 2013\; Shori & Baba, 2014b). Further study is needed to characterize the phenolic profiles of phytomix-3+ mangosteen yogurt. Phenolic phytochemicals are plant secondary metabolites which constitute one of the most abundant groups or natural metabolites. The plants synthesize these compounds for their protection against biological and environmental stress (Shetty et al., 2005). Elevation of TPC in food is commonly associated with increased antioxidant activities (Shori & Baba, 2013).

The degradation of milk proteins during milk fermentation resulted in an increase in TPC. This is because the release of phenolic amino acids and non-phenolic compounds such as sugars and proteins may interfere during the total phenolic assessment (Ainsworth & Gillespie, 2007).

3.2. Effect of phytomix-3+ mangosteen on antioxidant activity of yogurt

Plain yogurt showed antioxidant activity ranged between 17 and 19% during 14 days of storage (Fig. 2). However, the presence of phytomix-3+ mangosteen increased ($p < 0.05$) antioxidant activity in yogurt. The antioxidant activity was ranged from 60 to 62% in the first week of storage followed by reduction ($p < 0.05$) to 54% on day 14 of storage (Fig. 2). Previous studies reported lower DPPH values ranged

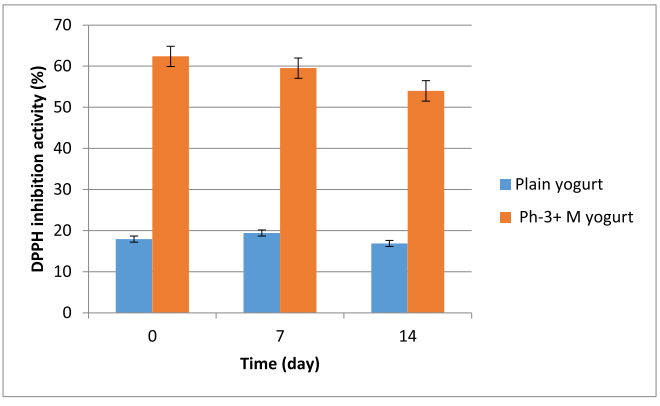

Fig. 2. Total antioxidant activity (DPPH %) of phytomix-3+ mangosteen (Ph-3+M) yogurt and plain yogurt during 14 days of storage. Value are presented as mean value ± SE (n = 3). The level of significant was present at $p < 0.05$.

Table 1
Viability of *S. thermophilus* and *Lactobacillus* spp. before (pre-) and after (post-) gastrointestinal digestion of phytomix-3+ mangosteen and plain yogurt over 14 days of storage.

Viability of *S. thermophilus* (log_{10} CFU/ml)			Sections	Yogurt Samples
14 day	**7 day**	**0 day**		
8.9 ± 0.07	9.2 ± 0.09	8.5 ± 0.07	**Pre**	Phytomix-3+
6.2 ± 0.06	7.1 ± 0.12	5.0 ± 0.09	**Post**	Mangosteen
				Plain
8.1 ± 0.06	8.4 ± 0.06	7.9 ± 0.12	**Pre**	
5.3 ± 0.09	6.1 ± 0.06	4.2 ± 0.06	**Post**	
Viability of *Lactobacillus* spp. (log_{10} CFU/ml)				
6.9 ± 0.03	7.4 ± 0.06	6.5 ± 0.12	**Pre**	Phytomix-3+
4.9 ± 0.03	5.5 ± 0.06	4.8 ± 0.06	**Post**	Mangosteen
6.5 ± 0.03	7.0 ± 0.03	6.9 ± 0.06	**Pre**	Plain
4.3 ± 0.09	5.1 ± 0.06	4.0 ± 0.09	**Post**	

*Before (pre-) gastrointestinal digestion = during storage at 0, 7, & 14 days. After (post-) gastrointestinal digestion = during 3 h of digestion (gastric digestion = 1 h; intestinal digestion = 2 h).

from 35% to 22% and 30%-50% for *Cinnamomum verum* and *Azadirachta indica* yogurt, respectively during 7 days of storage (Shori and Baba, 2011, 2013). The DPPH inhibition activity is closely related to the phenolic content (Baba et al., 2014). Therefore, antioxidants in phytomix-3+ mangosteen yogurt were found to be positively correlated with TPC ($R^2 = 0.977$; data not shown). The present study gives rise to the possibility of the prolonged shelf life of yogurt in the presence of phytomix-3+ mangosteen as it recorded high and retained antioxidant capacity throughout the 14 days of storage.

3.3. Effect of phytomix-3+ mangosteen on the viability of S. thermophilus and Lactobacillus spp of yogurt

There was no significant difference in the viable cell counts of *S. thermophilus* of pre-digested yogurt during 14 days of storage which ranged from 8.5 to 9.2 $long_{10}$ CFU/ml and 7.9–8.4 $long_{10}$ CFU/ml for phytomix-3+ mangosteen and plain yogurt respectively (Table 1). A similar result was almost observed for the viability of *Lactobacillus* spp for both types of yogurt ranged from 6.5- 7.4 $long_{10}$ CFU/ml during 14 days of storage (Table 1). Post-digested yogurt reduced the viability of *S. thermophilus* and *Lactobacillus* spp in both types of yogurts (Tables 1 and 2). However, the presence of phytomix-3+ mangosteen significantly increased (1 $long_{10}$ CFU/ml; $p < 0.05$) the viability of *S. thermophilus* in post-digested yogurt compared to plain yogurt during 14 days of storage. The highest viability of *S. thermophilus* and *Lactobacillus* spp was found on day 7 of storage for both plain and phytomix-3+ mangosteen yogurt (Tables 1 and 2).

Plant ingredients such as *Azadirachta indica*, *Cinnamomum verum*, soybean; chickpea; *Lycium barbarum*; *Allium sativum* were found to enhance the viability of LAB in yogurt (Baba et al., 2014; Shori, 2013a, pp. 202–208, b; Shori & Baba, 2011, 2013; Shori & Baba, 2014b). In the

Table 2
Peptide concentration before (pre-) and after (post-) gastrointestinal digestion of phytomix-3+ mangosteen and plain yogurt over 14 days of storage.

Peptide concentration (mg/ml)			Sections	Yogurt Samples
14 day	7 day	0 day		
33.66 ± 0.52	36.14 ± 0.32	27.28 ± 1.35	Pre	Phytomix-3+
57.79 ± 1.40	89.03 ± 1.94	55.41 ± 1.79	Post	Mangosteen
27.73 ± 1.12	28.13 ± 1.29	24.20 ± 0.48	Pre	Plain
57.73 ± 2.52	64.23 ± 0.81	54.39 ± 2.12	Post	

*Before (pre-) gastrointestinal digestion = during storage at 0, 7, & 14 days. After (post-) gastrointestinal digestion = during 3 h of digestion (gastric digestion = 1 h; intestinal digestion = 2 h).

Table 3
α-Glucosidase and α-Amylase inhibition (%) before (pre-) and after (post-) gastrointestinal digestion of phytomix-3+ mangosteen and plain yogurt over 14 days of storage.

α-Glucosidase Inhibition %			GI sections	Yogurt samples
14 day	7 day	0 day		
9.22 ± 0.63	16.32 ± 1.32	15.20 ± 0.60	Pre-digested	Phytomix-3+ Mangosteen
5.88 ± 0.60	5.38 ± 0.11	4.88 ± 0.26	Gastric digestion	
53.30 ± 1.86	72.55 ± 1.05	51.73 ± 1.94	Intestinal digestion (1st hour)	
66.67 ± 1.03	50.68 ± 0.54	58.21 ± 1.04	Intestinal digestion (2nd hour)	
6.21 ± 0.47	11.51 ± 0.63	11.29 ± 0.79	Pre-digested	Plain
2.20 ± 0.44	3.84 ± 0.65	5.16 ± 0.22	Gastric digestion	
39.22 ± 1.03	55.68 ± 0.83	48.53 ± 0.26	Intestinal digestion (1st hour)	
59.78 ± 1.82	34.16 ± 0.18	49.17 ± 2.10	Intestinal digestion (2nd hour)	
α-Amylase inhibition				
50.51 ± 2.13	53.31 ± 0.63	49.97 ± 2.19	Pre-digested	Phytomix-3+ Mangosteen
34.53 ± 2.89	39.04 ± 1.38	39.99 ± 1.49	Gastric digestion	
25.19 ± 1.70	29.57 ± 0.94	32.97 ± 1.50	Intestinal digestion (1st hour)	
23.93 ± 0.54	27.29 ± 0.52	33.94 ± 1.93	Intestinal digestion (2nd hour)	
45.59 ± 2.74	49.62 ± 0.39	48.36 ± 1.91	Pre-digested	Plain
29.97 ± 2.55	33.64 ± 1.63	31.99 ± 0.48	Gastric digestion	
20.82 ± 1.20	24.64 ± 1.50	28.92 ± 2.51	Intestinal digestion (1st hour)	
18.72 ± 0.78	22.79 ± 0.62	28.73 ± 3.08	Intestinal digestion (2nd hour)	

*Before (pre-) gastrointestinal digestion = during storage at 0, 7, & 14 days.
After (post-) gastrointestinal digestion (Gastric digestion = 1 h; Intestinal digestion = 1 & 2 h).

present study, the presence of phytomix-3+ mangosteen in yogurt increased *S. thermophilus* and *Lactobacillus* spp. counts compared to plain yogurt. However, the viability of both *S. thermophilus* and *Lactobacillus* spp. was drastically reduced as yogurt subjected to gastrointestinal digestion compared to undigested yogurt. This could be due to the nature of *S. themophilus* which is acid sensitive (Shori & Baba, 2012) and unable to resist the bile salts that cause the low rate of viability. *S. themophillus* can grow well at pH range 5.5–6.2 but decreased as pH approaches 4.1 (Shori & Baba, 2015). The normal yogurt cultures (*Lactobacillus* spp. and *S. thermophilus*) are not bile resistant or acid tolerant and thus cannot survive in the intestinal tract (Shori & Baba, 2012).

3.4. Effect of phytomix-3+ mangosteen on peptide concentration of yogurt

Peptide concentrations for pre-digested fresh (0 day) plain and phytomix-3+ mangosteen yogurt were 24.20 ± 0.48 and 27.28 ± 1.35 mg/ml, respectively (Table 2). The highest peptide concentrations of pre-digested phytomix-3+ mangosteen and plain yogurts were shown on day 7 of storage (36.14 ± 0.32 and 28.13 ± 1.29 mg/ml; respectively). These followed by a non-significant decline on last day of storage (Table 2). There were no significant differences in peptide concentration of post-digested fresh yogurt in both presence and absence of phytomix-3+ mangosteen (Table 2). However, post-digested phytomix-3+ mangosteen yogurt showed the highest peptide concentrations (89.03 ± 1.94 mg/ml) on day 7 of storage while plain yogurt was 64.23 ± 0.81 mg/ml. Prolonged storage for another week reduced significantly ($p < 0.05$) peptide concentrations to a similar number for both types of post-digested yogurt (Table 2).

OPA has been widely used for the assay of amines group of amino acid, peptides, and protein (Medina Hernandez, 1990). It can detect the smallest amount of protein and peptide that may be formed during the proteolysis. The proteolytic activity was largely carried out by *L. bulgaricus* (Shori & Baba, 2012). The highest peptide concentrations of phytomix-3+ mangosteen yogurt both pre- and post-digested on day 7 of storage could be due to the greater viability of LAB which enhanced the proteolytic activity by the LAB in yogurt. This assumption was supported by higher viable cell counts of *Lactobacillus* spp. in phytomix-3+ mangosteen yogurt (Table 1). Extension of the storage to 14 days resulted in a decrease in peptide concentrations of pre- and post-digested yogurt possibly due to the low viability of LAB (Tables 1 and 2). Lower peptide concentrations (22-17 mg/g) have been shown in *Azadirachta indica* yogurt pre-digested (Shori & Baba, 2013). Similarly, the peptide concentrations pre-digested of *A. sativum* and *C. verum* yogurt were ranged between 0.24 and 0.26 mg/g and 0.14–0.17 mg/g, respectively. *A. sativum*-fish collagen-yogurt and fish collagen-yogurt were shown highest peptide concentrations (0.34 ± 5.3 and 0.28 ± 2.0 mg/g; respectively) on day 7 of storage (Shori, Baba, & Chuah, 2013).

The increasing number of peptide has been identified in milk protein hydrolysates and also in fermented dairy products such as yogurt (Kilara & Panyam, 2003). Bioactive peptides can be released from their parent protein via three ways, (1) enzymatic hydrolysis by digestive enzyme, (2) fermentation of milk with proteolytic starter cultures and (3) proteolysis by the enzyme derived from microorganism or plants. Each or combination of this steps has been reportedly shown to affect the production of short functional peptides (Korhonen & Pihlanto, 2006).

In comparison to post-digestion yogurt, the peptide concentration was greater than pre-digest. This suggests the release of some of the bioactive peptides by the enzymic hydrolysis of the proteolytic enzyme (Korhonen, 2009). Proteolysis of milk protein by proteolytic LAB usually associated with the degradation of the protein by proteinases into polypeptides, which can be further degraded to small molecular weights peptides and free amino acids by the peptidases (Christensen, Dudley, Pederson, & Steele, 1999). Besides, the presence of enzyme protease inside the pancreatin of the digestive system enhanced the proteolysis of the peptide. This observation is in agreement with our findings (Table 2) since the amount of peptide was greatly increased after simulated gastrointestinal digestion.

3.5. Effects of phytomix-3+ mangosteen on α-glucosidase and α -amylase inhibition of yogurt

The presence of phytomix-3+ mangosteen in yogurt (pre-digested) enhanced ($p < 0.05$) the inhibition of α-glucosidase and α-amylase activity compared to plain yogurt during 14 days of storage (Table 3). The highest α-glucosidase inhibition was shown on day 7 of storage for both phytomix-3+ mangosteen and plain yogurts (16.32 ± 1.32% and 11.51 ± 0.63%; respectively). Similarly, phytomix-3+ mangosteen yogurt showed the highest α-amylase inhibitory activity

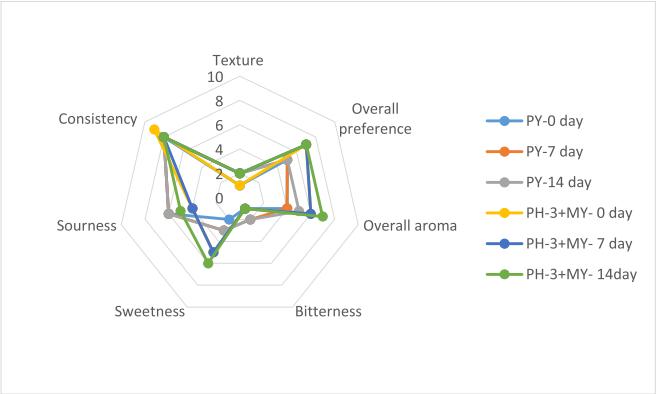

Fig. 3. Sensory evaluation of phytomix-3+ mangosteen (Ph-3+M) yogurt and plain yogurt during 14 days of storage.

(53.31 ± 0.63%) on day 7 of storage whereas plain yogurt showed no significant difference of α-amylase inhibitory activity on day 0 and 7 of storage (Table 3).

The gastric digestion of both phytomix-3+ mangosteen and plain yogurts showed lower ($p < 0.05$) α-glucosidase inhibition than pre-digested yogurt during the 2 weeks of storage (Table 3). Inhibition of α-glucosidase activity was further enhanced after undergoing the intestinal digestion. The presence of phytomix-3+ mangosteen increased significantly ($p < 0.05$) the inhibition of α-glucosidase activity compared to plain yogurt during 14 days of storage. The highest inhibitory effect on α-glucosidase activity has occurred at the 2nd hour of intestinal digestion. However, yogurt at 7 days showed the highest inhibitory effect at the 1st hour of intestinal digestion (72.55 ± 1.05% and 55.68 ± 0.83%) for phytomix-3+ mangosteen and plain yogurt respectively. Gastrointestinal digestion decreased ($p < 0.05$) α-amylase inhibitory activity for both types of yogurt overall storage period. The highest α-amylase inhibitory activity was shown at the 2nd hours of intestinal digestion for fresh yogurt (Table 3).

The present study has discovered the possibility of enhancing natural yogurt ability to inhibit diabetic enzymes by adding phytomix-3+ mangosteen water extract. In fact, the effects of herbal extracts addition to yogurt on α-glucosidase and α-amylase inhibitory activities have been previously reported for *Azadirachta indica* (Shori & Baba, 2013), *Cinnamomum verum* (Shori & Baba, 2011); *Lycium barbarum* (Baba et al., 2014); *Allium sativum* (Shori & Baba, 2014b). Such an effect is not only attributed to these herbs natural ability to inhibit α-glucosidase and α-amylase activities (Baba et al., 2014; Pothitirat et al., 2009; Takeo et al., 2002) but also possibly due to bioactive peptides released by the proteolytic activity of starter culture to hydrolyse milk proteins particularly caseins into peptides and amino acids (FitzGerald & Meisel, 2003, pp. 675–698). This assumption was supported by a positive concentration between peptide concentrations and α-glucosidase inhibitory activity ($R^2 = 0.591$; data not shown). Furthermore, excessive inhibition of α-amylase is not desirable because the bacterial fermentation of undigested carbohydrate in the colon will take place. For this reason, natural α-amylase and α-glucosidase inhibitors with lower inhibitory effect would be better to use as an effective therapy for post-prandial hyperglycemia with minimal side effects (Apostolidis, Kwon, & Shetty, 2006).

3.6. Sensory evaluation

Sensory evaluation is important in order to evaluate the consumer preference for the products and to ensure that the products have high chances to be accepted by consumers. In the present study, phytomix-3+ mangosteen yogurt showed higher overall preference compared to plain yogurt. In addition, the storage days have no significant effects ($p < 0.05$) on the overall preference for both types of yogurt (Fig. 3). Phytomix-3+ mangosteen yogurt showed higher score in term of sweetness (5-6) and aroma (6-7) compared to plain-yogurt (2-3 and 4-5; for sweetness and aroma respectively). However, the texture and consistency had almost the same score for both types of yogurt (Fig. 3). Moreover, sourness and bitterness were lower in phytomix-3+ mangosteen yogurt compared to plain yogurt; indicated that the taste was considered more acceptable for phytomix-3+ mangosteen yogurt.

Food additives are commonly added into dairy products (Shori and Baba, 2012, 2013; Shori, Baba, & Solear, 2016) because they attributed important sensory characteristics such as taste, appearance, consistency and prolong the shelf life of the dairy products (Nakazawa et al., 1992). The addition of phytomix-3+ mangosteen into yogurt showed greater preference by consumers than plain yogurt. However, since phytomix-3+ mangosteen yogurt has potential to be used as suitable health supporting dietary product, several important characteristics such as texture and the shelf life of this yogurt need to be improved in order to fulfill consumer acceptance.

4. Conclusion

The present study showed that yogurt enriched with phytomix-3+ mangosteen can inhibited over 50% of free radical scavenging-linked antioxidant activity. In addition, phytomix-3+ mangosteen yogurt displayed strong inhibition toward α-glucosidase and mild inhibition of α-amylase inhibitory activities after exposure to simulated gastrointestinal digestion. Phytomix-3+ mangosteen yogurt (pre- and post-digested) enhanced the viability of *S. thermophilus* and *Lactobacillus* spp. compared to plain yogurt during 14 days of storage. The presence of phytomix-3+ mangosteen in yogurt has some influence on the sensory characteristics. Thus, phytomix-3+ mangosteen yogurt has great potential to be suitable functional food rich in antioxidant and to help manage type-2 diabetes by inhibiting the key enzymes linked to this disease. Further studies are needed to evaluate phenolic compounds and antioxidant activity of phytomix-3+ mangosteen yogurt during 0, 7 and 14 days of storage, both before (pre-) and after (post-) simulated gastrointestinal digestion as well as characterize the phenolic and antioxidant profiles of the sample.

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