Effects of formulation on characteristics of probiotic yogurt enriched by Gac and Passion fruits

Nafisah Eka Puteri[1], Filli Pratama[1], and Visaka Anantawat[2]

ABSTRACT: The objective of this research was to analyze the physicochemical, microbiological, and sensory characteristics of Gac and passion fruits enriched-probiotic yogurt. The products were formulated by different formulations of skim milk powder and sucrose. The research was designed as Complete Randomized Design (CRD) with 2 treatments, which were consisted of skim milk powder (4%, 6% and 8%) and sucrose (4%, 6% and 8%) concentration. Parameters were titrable acidity, pH, texture, syneresis, viscosity, colour, microbiological characteristics, and sensory test. The results showed that the concentration of skim milk powder and sucrose significantly affected ($P<0.05$) the titrable acidity, pH, syneresis, viscosity, texture (hardness and cohesiveness), colour (L^* and a^*) and total yogurt bacteria counts. The yogurt that was formulated by 8% skim milk powder and 4%, 6%, or 8% sucrose (A3B1, A3B2, and A3B3) resulted in relatively good quality yogurt based on its physicochemical and microbiological properties. However, sensory analysis showed that the treatment of A3B2 (skim milk powder 8% and sucrose 6%) resulted in the most preferred yogurt based on the average of hedonic score. It had the characteristics of 13.23% for the titrable acidity, 3.43 for pH value, 2.76 N for hardness, 0.35 for cohesiveness, 3.86 for syneresis, 221.74 mPas for viscosity, 7.2 log CFU/g of yogurt bacteria counts and 6.6 log CFU/g of *Lactobacillus acidophilus* counts.

Keywords: probiotic, yogurt, Gac fruit

Introduction

Most people are aware of healthy foods due to their benefits to health. This condition encourages the development of healthy foods such as functional foods. Functional foods are foods that are specifically processed to give physiological benefits or reduce the risk of disease (Wildman, 2007). Functional food was firstly introduced as the concept of foods for specific health used (FOSHU). It should include the key concepts of health benefits, the nature of the food, level of function, and consumption pattern (Doyon and Labracque, 2008.). In other words, functional food should contain biologically active substances, probiotic organism, specific macronutrients or micronutrients.

Probiotic food which is included as f unctional foods contains live and active bacteria cultures or food supplements that beneficially affect a host organism by improving its microbial balance; therefore, probiotics contribute the positive effect on gastrointestinal system (Neha et al., 2012). The microorganisms in probiotic food might also produce beneficial substances that prevent health problem in human digestive tract.

One of the probiotic products is yogurt. Yogurt has long been recognized as functional food due to its contribution to health benefit by maintaining healthy digestive system. Yogurt is

[1] Technology of Agricultural Product, Faculty of Agriculture, Sriwijaya University, Indralaya 30662, Indonesia

[2] School of Agricultural Technology, Walailak University, 222 Thaiburi, Thasala, Nakhon Si Thammarat, 80161, Thailand

* Corresponding author: nafisahekaputeri093013@gmail.com; filipatama@hotmail.com ; anvisaka@gmail.com

the most popular and preferred vehicle for probiotic culture. As the popularity of probiotic yogurt continues to rise, dairy based food manufacturers are continuously looking into value-added ingredients. The main ingredient in yogurt is milk. Milk is pasteurized and added with probiotic yogurt starter. The starter will produce lactic acid that gives the sour taste in yogurt. Lactic acid reacts with protein in milk and results in texture that is similar to soft cooked agar in set yogurt, and creamy texture comes out when yogurt was stirred. The whole milk in yogurt is sometimes replaced by skim milk in order to reduce the fat content of the product (Edwards, 2000).

Yogurt consists of a casein network aggregated through iso-electric precipitation by lactic acid bacteria. Yogurt has been modified to enhance its health effect and consumers' interests. Fruit juice or certain substance might be added into yogurt in order to enrich the vitamins and minerals as well as its fun;ctionality. Sometimes, flavourant or colorant is added in order to enhance the flavour or colour of yogurt. A research was modifying yogurt by adding 500 ppm. of polyphenols that were extracted from olives (Petrotos, et al., 2012). The polyphenols were first encapsulated in modified starch before being added into yogurt. Encapsulation could mask off the undesired color, bitterness effect, and improve the functionality.

One of fruit that has potential to be added in yogurt is Gac fruit (*Momordica cochinchinensis*). Gac fruit aril contains substantial amount of lycopene and β-carotene (Kubola and Siriamonpurn, 2011), and its addition into yogurt could increase the functionality. However, Gac fruit arils taste slightly bitter; therefore, passion fruit (*Passiflora edulis*) is also added to mask off the unpleasant taste and flavour of Gac fruit. Passion fruit, which has pleasant aroma and flavor, is widely used to enhance the aroma in syrups and juices.

The characteristics of plain yogurt highly depend on the formulation of milk and sucrose (Nifea et al., 2012). The addition of Gac fruit aril and passion fruit puree in yogurt would affect the formulation of milk and sugar, and as a result, it could affect the characteristics of yogurt. This research focused on formulating the skim milk powder (SMP), sucrose, Gac and passion fruits puree for yogurt making. Gac and passion fruit-enriched yogurt was analyzed for its physical, chemical, microbiological and sensory characteristics. The main aim of the present study was to produce probiotic-yogurt containing Gac and passion fruits with acceptable sensory properties and to investigate the effect of these fruits on the viability of the chosen probiotics in yogurt.

Materials and methods

Experimental method

This research was designed as Complete Randomized Design (CRD) with 2 treatments. The treatments are percentage of SMP (A) with 3 factors (4 %, 6 %, and 8 %) and percentage of sucrose (B) with 3 factors (4 %, 6 %, and 8 %). Treatments were performed in triplicate. The parameter of the research was measured every week for 4 weeks.

Preparation of starter culture and probiotics

Freeze-dried (FD) granules of Direct Vat Set® probiotic cultures (ABY-3) were obtained from Chr. Hansen Pty., Ltd., and Australia. As per the recommendation from the manufacturer, 1 units of FD-DVS culture was used for each trial (5 L of milk, preheated to fermentation temperature at 43 °C).

Processing procedure of plain yogurt

Plain probiotic yogurt was made of commercial pasteurized milk, with the fat content (3.5%) (Dutch Mill, Nakhon Pha Thom) purchased from Thasala, Nakhon Si Tammarat, Thailand. The milk was heated at least till 50 °C, mixed with sucrose (Mitr Phol Pure Refined Sugar, Singburi Sugar Co., Ltd., Singburi, Thailand) and SMP (NZ milk product, New Zealand) based on the treatment. The mixture was then heated at 80 °C for 20 mins. in a control water bath. The mixture was cooled to the fermentation temperature of 43 °C and incubated with probiotic FD-DVS yogurt starter culture. The inoculated mixture was incubated at 43 °C in incubator (Elecrem, Type Y300, France) until the pH reached 4.5 (approximately 6 h). The yogurt was cooled and stored at below 8 °C.

Gac puree preparation

Fresh ripe Gac fruit was purchased from Nakhon Si Thammarat, Thailand. The aril was manually separated from the seed, mesocarp, and rind by using knife. The obtained aril was mixed with water (1:1) (w/w) and blended by using juice extractor (Tefal, BL3101, China). Gac fruit puree was then filtered by using stainless sieve (50 mesh).

Processing procedure of probiotic yogurt enriched by Gac and passion fruits

The mixture of Gac fruit puree and passion fruit flesh (1:5 (w/w)) was mixed with salt and sugar (1:19 (w/w)) with the comparison of 9:1 (w/w). The puree was filtered by using stainless sieve (50 mesh) and pasteurized at 80 °C for 20 minutes. The pasteurized puree (20 % w/w) was stirred with plain yogurt (80 % w/w) and stored at approximately 4 °C.

Physical and chemical analysis

Colour

The colour of sample (10 g) was measured based on CIE L^*, a^*, b^* colour space by using colorimeter (HunterLab, Colorflex 45°/0°, Reston, VA) which was standardized by using black and white tile before used.

Texture

The texture analyzer (Lloyd, Model LR-5 K, Lloyd Instruments Ltd., Hampshire, UK) with cylindrical probe was combined with texture analysis software (Nexigen Plus, Ver. V4.0, Hampshire, UK) to measure the texture of plain yogurt. The analysis was carried out directly on the samples set in the sample container (43 mm in diameter and 37 mm in sample height). A disc probe with a diameter of 1 cm and thickness of 1 mm was used. The crosshead speed during measurements was set at 60 mm/min to the distance of 25 mm for the yogurt surface. A load cell of 5 N was used and the trigger force was set to 0.3 N. The test was carried out in five replicate on individual yogurt pots for each formulation. Texture properties such as firmness, consistency and cohesiveness were considered. As yogurt

presents a pseudoplastic behavior and exhibits partial thixotropy, firmness was measured as the force requited to break the structure formed after the cessation of stirring and during the cold storage of the yogurt. Consistency refers to the property by which a material (yogurt) resists to a change in shape and cohesiveness as the extent to which the yogurt could be deformed before it ruptures. The peak stress at the fracture was calculated as the stress required fracturing the gel, which corresponds to the first peak in the stress-strain curve

Viscosity

Measurement of viscosity (mPas) was done by using viscosimeter (SV-10, A&D Co., Japan) combined with viscosity analysis software (Rs-Visco, Ver.1.11V, A&D Instruments, Oxfordshire, UK). Sample was placed in the container 10 mL and adjusted to 5 °C before being analyzed.

Syneresis

Sample (30 g) was prepared in the centrifuge tube and analyzed by using centrifuge (Biofuge Stratos, Kendro Laboratory Products, Germany). The sample was centrifuged in 2400 rpm at 5 °C for 10 mins. The percentage of syneresis was calculated using Eq.(1).

$$\text{Percentage of syneresis} = B/A \times 100\% \quad (1)$$

Where A = weight of initial sample (g);
and B = weight of the water (g)

pH

Yogurt sample (10 g) was mixed with distilled water (1:1) and the pH was measured using a pH meter (Orion, model 420A), calibrated routine with pH 4.0 and 7.0 standard buffers. Yogurt sample was monitored for pH during the fermentation process and during storage for 1, 7, 14 and 28 days at 4 °C.

Titrable acidity

Measurement of acidity was carried out by the method of AOAC 942.15 (AOAC, 2005). Sample (10 g) was placed on Erlenmeyer 250 mL and diluted into 10 mL aquadest. Indicator phenolphthalein (3 drops) was added to sample solution. Sample solution was then titrated against NaOH 0.1 N until reached the endpoint pH 8.1 which was signed by red colour. Titrable acidity (%) was performed as lactic acid percentage using Eq.(2).

$$\text{Acidity (\%)} = (V \times N \times MW \times DF)/(\text{sample weight (g)} \times 10) \times 100\% \quad (2)$$

Where V = volume of NaOH (mL); N = normality of NaOH (0.1 N); MW = molecular weight of lactic acid (90); and DF = dilution factor

Microbiological analysis

Microbiological analysis was done to enumerate total bacteria and *Lactobacillus acidophilus* in yogurt. Probiotic yogurt (10 g) was diluted in 90 g of buffered peptone water (Oxoid, UK) and homogenized by using vortex (Vortex-Genie 2, Model G560E, Scientific Industries Inc., USA). Sample (1 mL) was obtained from primary dilution and pipetted into test tube containing 9 mL sterile diluent (peptone water). The dilution of sample was then homogenized by using vortex and diluted up to 10^{-6} (200 CFU/mL to 500 CFU/mL) with sterile diluent (NaCl 1%). The dilution of sample was plated on appropriate media in Petri dish by pour plate technique. Total bacteria was enumerated by using media PCA (Plate Count Agar), while *L. acidophilus* was enumerated by using media MRS (de Man, Rogosa, Sharpe) agar.

Sample was incubated under anaerobic condition in incubator at 37 °C as long as 48 – 72 hours for yogurt bacteria count and 42 °C as long as 24 hours for *L. acidophilus* count. The obtained result will be expressed as log CFU/g.

Sensory analysis

Sensory analysis has been done to determine the consumer acceptance by using 5-points hedonic scale. Three treatments were taken as samples by comparing the quality of yogurt based on the characteristics. They were coded as 257, 162, and 304. The samples were served in small cups 3 oz and evaluated by 30 untrained panellists. The panellists were student of Food Technology Division, School of Agricultural Technology, Walailak University. The observed parameters were viscosity, colour, and flavour. Consumer acceptance of the experimental products was evaluated using a hedonic scale of 1 – 5 where 1 corresponds with "dislike extremely" and 5 corresponds "like extremely". The sensory tastings were conducted in a specially designed sensory evaluation laboratory illuminated with natural fluorescent lights.

The yogurt samples were stored at 4 °C and approximately 15 g of yogurt samples were portioned in uniform plastic cups with lid labelled with 3-digited random codes just before the arrival of participants. Participants were initially asked to complete a brief questionnaire in order to classify them according to age and gender. Participants were then presented with three samples along with water. Panellists were instructed to rinse their mouths with water between sample testing. Panellists were instructed to evaluate each attribute separately to overcome the halo effect. Panellists evaluated the appearance of the samples first, then aroma, texture and taste, respectively. Finally, the overall liking of each sample was evaluated.

Statistical analysis

All data analyses were performed using the Statistical Package for Social Science software 18 (SPSS, ver.18, Chicago, IL). A one-way analysis of variance (ANOVA) was used to evaluate the difference between the yogurts with respect to the five sensory attributes. Duncan's Multiple Range Test was used to compare the mean value between samples and the data was significant if the P-value was found to be < 0.05. Results were presented as the mean and the standard error of the mean (± SE). The source, which was statistically significant, was analyzed by using Duncan's Multiple Range Test to find out which means are significantly different from one another. Significance was set as $p \leq 0.05$. The non-parametric Friedman Test was applied to analyze the data of sensory analysis. Wilcoxon Signed Rank Test was matched as post hoc test to find out which pair was significantly different (Bonferroni – Holm adjusted $\alpha = 0.017$).

Results and discussion

Colour

L^*

The averages of L^* value on the yogurt are reported in **Figure 1(a)**. The statistical analysis showed that SMP and sucrose percentage were statistically significant ($P<0.05$) on the L^* value of the yogurt. There was no significant effect ($P>0.05$) for the interaction of treatment on L^*

value. The results of DMR Test are shown in **Tables** 1 and 2.

The addition of dried dairy ingredient increased the density and reduced the pore size of the yogurt (Stefanakis et al., 2011). The increase of density and reduction of pore size resulted in the lower L^* value due to increase of darkness. The reduction of L^* value was due to the increase of SMP might also relate to the colour compound in SMP. SMP has colour compound that produced by Maillard reaction during heat treatment (Fayle and Gerrard, 2002).

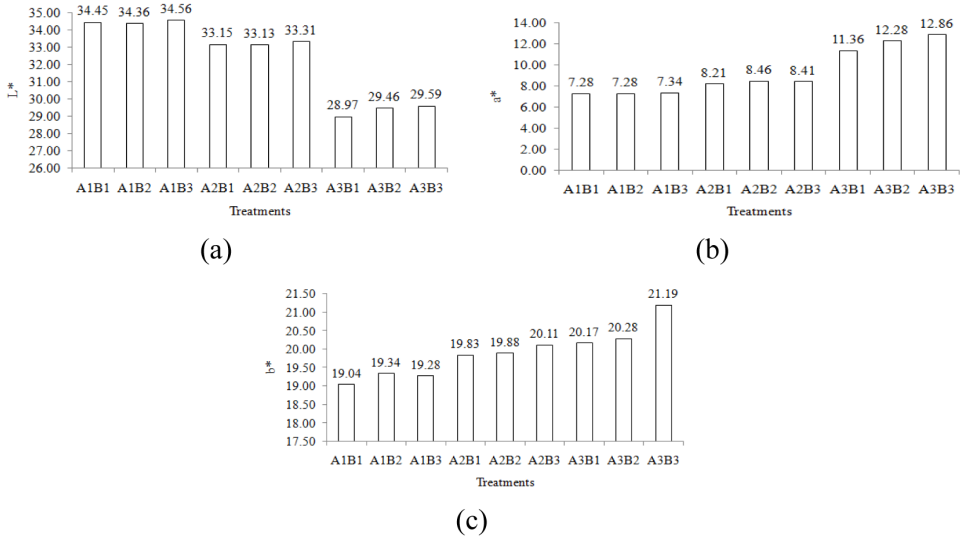

Figure 1 The (a) L^*; (b) a^*; and (c) b^* values of yogurt for all treatments

*a**

The averages of a^* value from all treatments are presented in **Figure 1(b)**. The statistical analysis showed that the increase of total solids and protein content resulted in the increase of a^* value. Similarly, the addition of dried dairy ingredient caused the increasing of total solids and protein content and resulted in non-fat yogurt with higher L^*, a^*, and b^* values (Nouri et al., 2011).

The statistical analysis showed that the percentage of SMP, sucrose, and their interaction significantly affected ($P<0.05$) the a^* value of probiotic yogurt enriched by Gac and passion fruits. The DMR Test showed the significance of SMP and sucrose percentages, which are reported in **Tables 1** and 2, respectively. The effect of interaction between the treatments on a^* value is shown in **Table 3**.

*b**

The averages of b^* value from all treatments are reported in **Figure 1(c)**. The result of statistical analysis showed that SMP percentage was significantly affected ($P<0.05$) the b^* value of yogurt. There were no significant effect ($P>0.05$) of sucrose percentage and interaction between treatments on the b^* value.

The result of post hoc test (**Table 1**) showed that A1 was significantly different with A2 and A3, while there was no significant difference between A2 and A3. The increase of b^* value might be due to the increase of protein content (Nouri et al., 2011).

Texture

Hardness

Hardness is the force which is applied to deform a material (Yang and Li, 2010). The average of texture in yogurt is presented in **Figure 2(a)**. There was significant effect ($P<0.05$) for the SMP, sucrose, and their interactions on the hardness (N) of the yogurt based on statistical analysis. The effects of SMP, sucrose, and their interaction on the hardness (N) of yogurt are shown in **Tables 4**, **5**, and **6**, respectively.

Table 1 Effect of skim milk powder based on DMR Test

Treatment	*L**	*a**	*b**
A1 (skim milk powder 4 %)	34.46^{c} ± 0.47	7.30^{a} ± 1.04	19.22^{a} ± 1.06
A2 (skim milk powder 6 %)	33.20^{b} ± 1.71	8.36^{b} ± 1.90	19.94^{b} ± 1.18
A3 (skim milk powder 8 %)	29.34^{a} ± 1.29	12.17^{c} ± 1.26	20.55^{b} ± 2.83

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

Table 2 Effect of sucrose based on DMR Test

Treatment	*L**	a*
B1 (sucrose 4 %)	32.19^{a} ± 2.68	8.95^{a} ± 2.22
B2 (sucrose 6 %)	32.32^{ab} ± 2.45	9.34^{b} ± 2.68
B3 (sucrose 8 %)	32.49^{b} ± 2.49	9.54^{b} ± 2.73

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

The result showed that the higher SMP percentage, the higher hardness value of yogurt. The addition of SMP may increase the hardness of yogurt. The increase of hardness was affected by the increase of protein content from SMP. Protein content significantly increased ($P<0.05$) in yogurt with higher level of dried dairy ingredients. The increase of protein content by addition of SMP affected the formation of protein matrix that related to hardness (Supavititpatana et al., 2009). Hardness might be affected by the EPS (exopolysaccharides) that produced by the starter culture. Exopolysaccharides were responsible for the texture of yogurt (Feldmane et al., 2013). The increase of SMP might increase the viability of starter culture that produced the exopolysaccharides. Increase sucrose content significantly decreased the hardness (N) of yogurt. It might be affected by the viability of starter culture that produced exopolysaccharides (Nifea et al., 2012). The higher sucrose content might produce lower exopolysaccharides in yogurt, and resulted in the lower value of hardness (N).

Table 3 Effect of SMP and sucrose formulation based on DMR Test

Treatment	a*
A1B1	$7.28^{a} \pm 1.02$
A1B2	$7.28^{a} \pm 1.02$
A1B3	$7.34^{a} \pm 1.17$
A2B1	$8.21^{b} \pm 1.80$
A2B2	$8.46^{b} \pm 2.42$
A2B3	$8.41^{b} \pm 1.54$
A3B1	$11.36^{c} \pm 1.21$
A3B2	$12.28^{d} \pm 1.01$
A3B3	$12.86^{e} \pm 1.16$

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

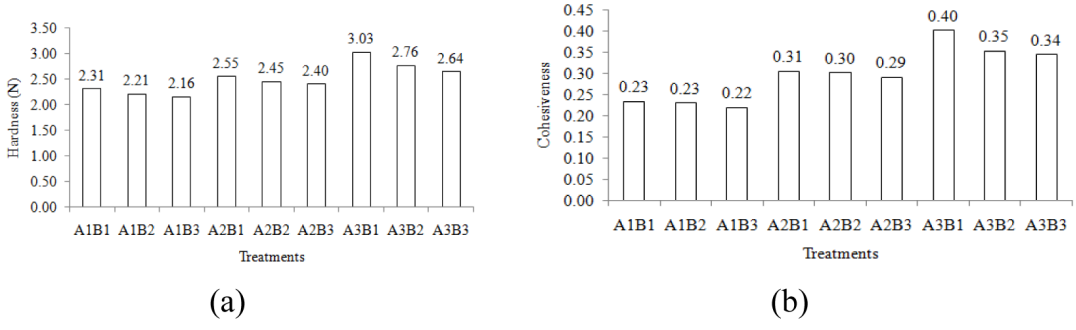

Figure 2 The (a) hardness (N) and (b) cohessiveness of yogurt for all treatments

Table 4 Effect of SMP based on DMR Test

Treatment	Hardness (N)	Cohesiveness
A1 (SMP 4 %)	$2.22^{a} \pm 0.21$	$0.23^{a} \pm 0.12$
A2 (SMP 6 %)	$2.47^{b} \pm 0.21$	$0.30^{b} \pm 0.12$
A3 (SMP 8 %)	$2.81^{c} \pm 0.25$	$0.37^{c} \pm 0.09$

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

Table 5 Effect of sucrose based on DMR Test

Treatment	Hardness (N)	Cohesiveness
B1 (sucrose 4 %)	$2.63^{c} \pm 0.36$	$0.31^{b} \pm 0.13$
B2 (sucrose 6 %)	$2.47^{b} \pm 0.31$	$0.30^{a} \pm 0.13$
B3 (sucrose 8 %)	$2.40^{a} \pm 0.27$	$0.28^{a} \pm 0.12$

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

Table 6 Effect of SMP and sucrose formulation based on DMR Test

Treatment	Hardness (N)
A1B1	$2.31^{a} \pm 0.22$
A1B2	$2.21^{a} \pm 0.19$
A1B3	$2.16^{a} \pm 0.22$
A2B1	$2.55^{ab} \pm 0.22$
A2B2	$2.40^{ab} \pm 0.19$
A2B3	$2.40^{ab} \pm 0.22$
A3B1	$3.03^{b} \pm 0.19$
A3B2	$2.76^{ab} \pm 0.26$
A3B3	$2.64^{ab} \pm 0.11$

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

There were significant effects ($P<0.05$) among SMP, sucrose, and their interaction on viscosity (mPas) of yogurt based on statistical analysis. The effect of SMP, sucrose, and their interaction on the viscosity (mPas) of yogurt were shown in **Tables 7**, **8**, and **9**, respectively. There was correlation between syneresis and viscosity. The increase of syneresis that caused by increasing of sucrose resulted in the higher viscosity. The higher viscosity in yogurt might also be due to the viability of starter culture that produced EPS. The excessive addition of sucrose affected the mortality of lactic acid bacteria that produced EPS and resulted in the lower viscosity of yogurt (Walstra, et al., 1999).

Syneresis

Syneresis will increase the moisture content of yogurt. This condition results in the enhancement of microbiological infection and the reduction of nutritive value (Stefanakis et al., 2011). Therefore, the quality of yogurt is related to the resistance on syneresis.

The syneresis (%) of all treatment is reported in **Figure 3(b)**. The higher SMP percentage, the lower syneresis occured in yogurt. The syneresis in yogurt made of 9 % SMP was significantly higher than yogurt with 14 % of SMP (Amatayakul, et al., 2006). Higher protein content resulted in an increase in density and reduction of pore size of protein matrix in yogurt gel (Stefanakis et al.,

2011). The reduction of pore size in protein matrix of yogurt gel was due to the increase of SMP percentage that was added. The smaller pore size in protein matrix, the lower of the occurence of syneresis in yogurt.

The statistical analysis showed that the SMP and sucrose percentage significant affected ($P<0.05$) the syneresis (%) of the yogurt, while the interaction had no significant difference ($p > 0.05$). The effects of SMP, sucrose, and their interaction on the syneresis (%) are shown in **Tables 7**, **8**, and **9**, respectively. There were significant differences among the effect of interaction between treatment on syneresis (**Table 9**). The increase of sucorse resulted in the increasing of syneresis in yogurt. It might be due to the unstable gel. The excessive amount of sucrose might contribute the unstable gel in yogurt (Walstra et al., 1999).

pH

The averages of pH value for all treatment are shown in **Figure 3(c)**. The statistical analysis showed that the SMP and sucrose that was added in yogurt had significant effect ($P<0.05$) on pH value; on the other hand, the interaction between SMP and sucrose percentage had no significant effect ($P>0.05$) on pH value. The DMR Test on the effect of the addition of SMP and sucrose content on pH value is shown in **Tables 7** and **8**, respectively.

The pH value correlated to the titrable acidity. The increase of SMP increased the viable counts of lactic acid bacteria which produced lactic acid (Pham and Shah, 2009). The increase of sucrose might reduce the viability of starter culture and lactic acid produced (Early, 1998), and resulted in the lower titrable acidity.

Titrable acidity

The averages of titrable acidity (%) for all treatments are shown in **Figure 3(d)**. The statistical analysis showed that the SMP and sucrose that were added in yogurt had significant effect ($P<0.05$) on titrable acidity (%); on the other hand, the interaction between SMP and sucrose percentage had no significant effect ($P>0.05$) on titrable acidity (%). The DMR Test on the effect of the addition of SMP and sucrose content on titrable acidity (%) is shown in **Tables 7** and **8**, respectively.

Lactic acid is produced by the starter culture as the result of fermentation (Walstra et al., 1999). *Lactobacillus bulgaricus* produces amino acids that are used by *Streptococcus thermopilus* to produce lactic acid and formic acid. Formic acid stimulates the *L. bulgaricus* to produce lactic acid. The increase of titrable acidity might be due to the increase of *L. bulgaricus* and *S. thermopilus* that actively produced lactic acid, during fermentation. SMP has an effect on the viability of *L. bulgaricus* and *S. thermopilus*. Viable counts of *L. bulgaricus* and *S. thermopilus* in yogurt were significantly affected ($P<0.05$) by the increase of SMP (Pham and Shah, 2009).

The addition of excessive sucrose might reduce the viability of starter culture which is related to the decrease of lactic acid production in yogurt during fermentation. Sucrose might bind and reduce the available water for the growth of starter culture (Buckle et al., 2009). The low availability of water might reduce the viability of starter culture. Addition of sucrose might increase the lactose content in yogurt drink which indicated the decrease of viable counts of starter culture Hartati et al., 2012).

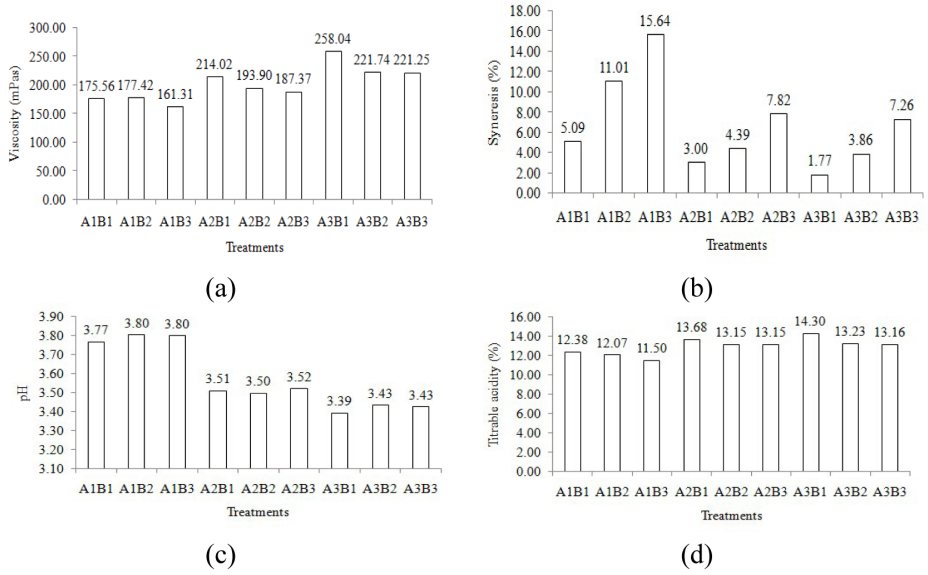

Figure 3 The (a) viscosity (mPas); (b) syneresis (%); (c) pH; and (d) titrable acidity (%) of yogurt for all treatments

Table 7 Effect of SMP based on DMR Test

Treatment	Viscosity (mPas)	Syneresis (%)	pH	Acidity (%)
A1 (SMP 4 %)	$171.43^{a} \pm 15.57$	$10.58^{c} \pm 4.54$	$3.79^{c} \pm 0.10$	$11.99^{a} \pm 0.89$
A2 (SMP 6 %)	$198.43^{b} \pm 24.96$	$5.0^{b} \pm 2.31$	$3.51^{b} \pm 0.05$	$13.33^{b} \pm 1.64$
A3 (SMP 8 %)	$233.67^{c} \pm 40.63$	$4.3^{a} \pm 2.43$	$3.42^{a} \pm 0.14$	$13.56^{b} \pm 1.31$

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

Table 8 Effect of sucrose based on DMR Test

Treatment	Viscosity (mPas)	Syneresis (%)	pH	Acidity (%)
B1 (sucrose 4 %)	$215.87^{c} \pm 45.89$	$3.29^{a} \pm 1.72$	$3.56^{a} \pm 0.20$	$13.45^{b} \pm 1.49$
B2 (sucrose 6 %)	$197.69^{b} \pm 27.35$	$6.42^{b} \pm 3.38$	$3.58^{b} \pm 0.18$	$12.82^{a} \pm 1.26$
B3 (sucrose 8 %)	$189.98^{a} \pm 36.10$	$10.24^{b} \pm 4.09$	$3.58^{b} \pm 0.19$	$12.61^{a} \pm 1.58$

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

Table 9 Effect of SMP and sucrose formulation based on DMR Test

Treatment	Viscosity (mPas)	Syneresis (%)
A1B1	$175.56^{b} \pm 6.43$	$5.09^{e} \pm 1.39$
A1B2	$177.42^{c} \pm 9.77$	$11.01^{h} \pm 0.82$
A1B3	$161.31^{a} \pm 21.57$	$15.64^{i} \pm 1.38$
A2B1	$214.02^{f} \pm 32.07$	$3.00^{b} \pm 0.95$
A2B2	$193.90^{e} \pm 18.80$	$4.39^{d} \pm 0.83$
A2B3	$187.37^{d} \pm 13.39$	$7.82^{g} \pm 1.40$
A3B1	$258.04^{i} \pm 43.76$	$1.77^{a} \pm 0.68$
A3B2	$221.74^{h} \pm 28.91$	$3.86^{c} \pm 0.52$
A3B3	$221.25^{g} \pm 39.13$	$7.26^{f} \pm 1.18$

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

Microbiological analysis

Yogurt bacteria counts

S. thermophilus produces lactic acid and adjusts the pH value to the certain acidity for *L. bulgaricus* (Walstra et al., 1999). *L. bulgaricus* is grown in low pH to actively produce lactic acid. The increase of SMP as material might increase the production of lactic acid. This condition might lower the pH value rapidly and reduced the viability of *L. acidophillus* and *B. bifidum*. *L. acidophillus* and *B. bifidum* were sensitive of over-acidification (Hattingh and Viljoen, 2001). Hydrogen peroxide that is produced by *L. bulgaricus* might also reduce the viable counts of *L. acidophillus*.

The increase of bacteria counts by the increase of sucrose might relate to the syneresis in yogurt. Syneresis could be increased by the increasing of sucrose percentage. Syneresis allowed the microbiological infection by increasing the moisture content of yogurt (Amatayakul et al., 2006; Pham and Shah, 2009). The increase of yeast and molds affected the bacteria counts in yogurt.

The averages of yogurt bacteria counts are shown in **Figure 4(a)**. The statistical analysis showed that SMP and sucrose percentage significantly affected ($P<0.05$) the yogurt bacteria counts while the interaction between SMP and sucrose was not significant different ($p > 0.05$). The effect SMP percentage on the yogurt bacteria counts (log CFU/g) is shown in **Table 10** while effect of sucrose percentage is shown in **Table 11**.

Lactobacillus acidophilus counts

The averages of *L. acidophilus* counts are reported in **Figure 4(b)**. There was significant effect ($P<0.05$) of SMP percentage on *L. acidophilus* counts based on statistical analysis. The increase of SMP showed significant effect on the increase of lactic acid content which is shown in **Table 10**. The reduction of *L. acidophilus* might be caused by the activity of *L. bulgaricus* that excessively produced lactic acid and hydrogen peroxide which can inhibit the growth of *L. acidophilus* (Hattingh and Viljoen, 2001). In addition, the reduction of *L. acidophilus* by the increase of sucrose was related to osmotic stress. Excessive amount of sucrose caused the reduction of avai-

ble water for growth of starter culture and resulted in the mortality of starter culture.

Sensory Analysis

Viscosity

The result showed that the viscosity of A3B2 (SMP 8 % and sucrose 6 %) was most preferred while the viscosity of A3B1 (SMP 8 % and sucrose 4 %) was the least preferred by the panellists. Friedman test showed that there was no significant effect (P>0.05) of SMP and sucrose formulation on the panellists acceptance for viscosity of A3B1 (SMP 8 % and sucrose 4 %), A3B2 (SMP 8 % and sucrose 6 %), and A3B3 (SMP 8 % and sucrose 8 %).

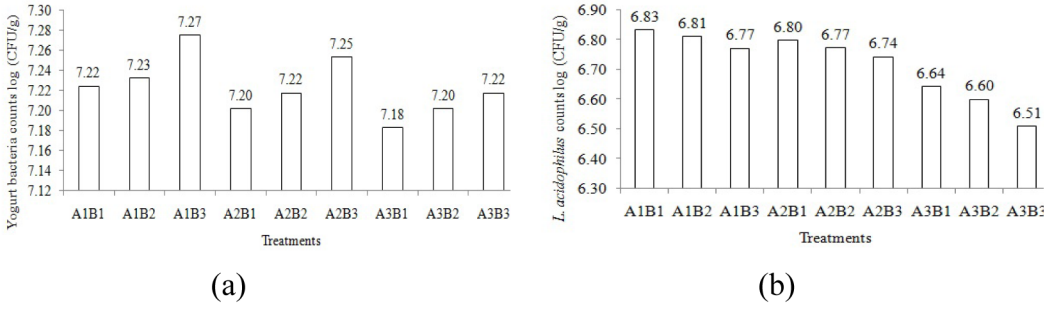

Figure 4 (a) Yogurt bacteria counts (log CFU/g); (b) *L. acidophilus* counts (log CFU/g) of yogurt for all treatments

Table 10 Effect of SMP based on DMR Test

Treatment	Yogurt bacteria counts (log CFU/g)	*L. acidophilus* counts (log CFU/g)
A1 (SMP 4 %)	7.24^{c} ± 0.12	6.8^{b} ± 0.13
A2 (SMP 6 %)	7.22^{b} ± 0.11	6.77^{b} ± 0.10
A3 (SMP 8 %)	7.20^{a} ± 0.12	6.58^{a} ± 0.08

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at p ≤ 0.05 by Duncan's Multiple Range Test.

Table 11 Effect of sugar based on DMR Test

Treatment	Yogurt bacteria counts (log CFU/g)
B1 (sucrose 4 %)	7.20^{a} ± 0.12
B2 (sucrose 6 %)	7.22^{a} ± 0.11
B3 (sucrose 8 %)	7.25^{b} ± 0.12

Notes: Value are average ± standard deviation. Within the same column, the values with different letters are significantly different at p ≤ 0.05 by Duncan's Multiple Range Test.

Colour

The average of hedonic rating on the colour of yogurt is reported in **Figure 5(a)**. Friedman test showed that there was significant effect (P>0.05) of SMP and sucrose formulation on the panelists acceptance for colour. The result of post hoc test is shown in **Table 12**. The result indicated the significant difference between the colour of A3B2 (SMP 8 % and sucrose 6 %) and A3B1 (SMP 8 % and sucrose 4 %) based on panellists acceptance.

Flavour

The averages of hedonic rating on the flavour of yogurt are reported in **Figure 5(b)**. Friedman test showed that SMP and sucrose formulation significantly affected the panellists acceptance on flavours. The result of Wilcoxon Signed Ranks Test is presented in **Table 12**. There was significant difference between the flavour of A3B2 (SMP 8 % and sucrose 6 %) and A3B1 (SMP 8 % and sucrose 4 %) based on panellists acceptance. The flavours of A3B3 (SMP 8 % and sucrose 8 %) and A3B1 (SMP 8 % and sucrose 4 %) were also significantly different one another.

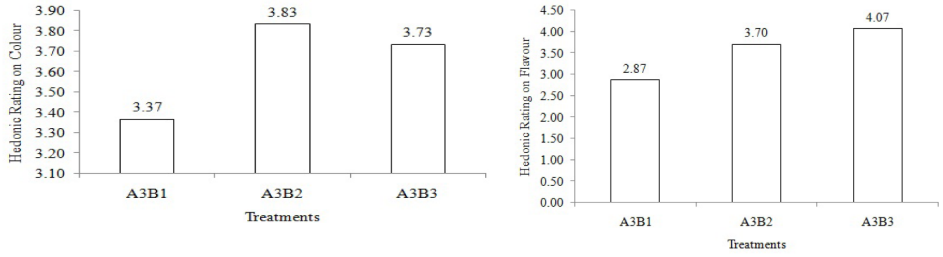

Figure 5 The hedonic rating on the (a) colour and (b) flavour of yogurt for all treatments

Table 12 The p value of the treatment pair based on Wilcoxon Signed Ranks Test

Treatments	Colour ($p \leq 0.017$)	Flavour ($p \leq 0.017$)
A3B2 - A3B1	0.001	0.002
A3B3 - A3B2	0.499	0.224
A3B3 - A3B1	0.040	0.000

Notes : There was significant difference between the pair based on Wilcoxon Signed Ranks Test, if the value was ≤ 0.017 (Boferroni-Holm).

Most of the flavour in dairy product are produced by the degradation of fat, protein, and carbohydrate in milk. Milk powder has a specific flavour that mainly generated from methyl ketones and lactones by heating of the fat and Maillard reaction (Walstra et al., 1999). Acetaldehyde, lactic acid, acetoin, and diacetyl are flavour components that produced by microbiological fermentation on cow milk (Ashurts, 1999). Acetaldehyde is the most responsible compound for yogurt flavour. Most of acetaldehyde are formed by the *L. bulgaricus*.

Conclusion

In conclusion, probiotic yogurt enriched with Gac and passion fruits was successfully manufactured with acceptable sensory properties with viable probiotic counts up to the acceptable range at 28 days. Skim milk powder percentage significantly affected ($P<0.05$) the titrable acidity, pH, syneresis, viscosity, texture (hardness and cohesiveness), colour (*L*, a*, b**), yogurt bacteria counts, and *L. acidophilus* counts. There were significant effects of sucrose percentage on titrable acidity, pH, syneresis, viscosity, texture (hardness and cohesiveness), colour (*L** and *a**), and yogurt bacteria counts. Formulation of 8 % SMP with 4 %, 6 %, and 8 % sucrose (A3B1, A3B2, A3B3) resulted in yogurt with good quality based on physical, chemical, and microbiological properties. Sensory profile showed that A3B2 (SMP 8 % and sucrose 6 %) had the highest hedonic score on viscosity and colour while A3B3 (SMP 8 % and sucrose 8 %) resulted in the highest hedonic score on flavour.

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