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Improvement of Syneresis and Viscosity of Yoghurt with The Addition of Psyllium Husk (*Plantago ovata*) and Full Cream Milk with Response Surface Method Approach

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Abstract

Yogurt is considered as functional food due to its nutritional and functional properties. In the manufacture of yogurt, there are often several problems that have an impact on yogurt products, including low viscosity and the onset of syneresis during the storage process. To overcome these problems, a hydrocolloid, such as psyllium husk (*Plantago ovata*), used as stabilizer which can improve texture, consistency, and give a solid appearance to the product. The present study was directed to determine the concentration of full cream milk and psyllium husk to obtain good physicochemical and organoleptic characteristics of yogurt. Fresh milk was fermented using *Lactobacillus bulgaricus* with the addition of varying concentrations of psyllium husk (0.05-2%) and full cream milk (2-5%). The viscosity and syneresis of the product were then recorded. The data obtained were then processed using the Minitab 20. The yoghurt was prepared at optimum conditions and organoleptic tests were carried out by semi-trained panelists and the differences between the data was analyzed using the Tukey post-hoc test. The lowest syneresis value (28.28%) and the highest viscosity (1933 mPas) was achieved at 0.16% and 5.62% of psyllium husk and full cream milk, respectively. The results of the organoleptic test showed a significant effect of adding psyllium and full cream milk on texture and overall appearance. In conclusion, psyllium husk and full cream milk can improve syneresis dan viscosity property of yoghurt.

Keywords: yoghurt, psyllium husk, full cream milk, response surface method

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1. Introduction

Milk has a high nutritional value that plays a significant role in improving the nutrition of the community. To improve the efficiency in its utilization, this animal origin product is also easily processed into various dairy products and combined with other food products for consumption. The utilization of technology in its processing is very diverse, from simple contemporary methods to modern processing. One of the milk processing efforts is fermentation. Fermentation is a simple milk processing method and has been widely recognized, one of which is yogurt [1].

Chemically, yogurt is a complex gel system consisting of proteins, polysaccharides, and lipids in its structure. Yogurt is generally made by fermenting cow's milk using symbiotic cultures of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* under controlled temperature and environmental conditions. The role of these two bacterial starters can acidify milk and also produce aroma in yogurt. Acidification of milk results in denatured casein micelles resulting in gel clumping. Various types of milk can be used to make yogurt, such as fresh cow or goat milk, skim milk (nonfat milk), soy milk, and a combination of these milks [2]. In addition, whey proteins incorporated in the gel contribute to yogurt texture and tangy flavor characteristics [3]. The nutritional content of yogurt is higher than whole milk due to the addition of nutrients from microbial synthesis and the nutritional content of the microbes themselves. Yogurt becomes richer in protein, vitamins, folic acid, riboflavin, and phosphorus [4].

Yogurt is a functional food, which is a food that not only fulfills nutritional needs but also has beneficial health effect on body. It has been shown that this dairy product has many health benefits such as anticarcinogenic effects, growth stimulation, cholesterol reduction, hypoallergenic effects, increased availability of calcium and other nutrients, improvement of lactose intolerance, control of

gastrointestinal infections and also stimulation of the immunological system [5].

One of the most important quality characteristics of yogurt is the texture and overall appearance that corresponds to a low level of syneresis [5]. The things that must be considered in making yogurt are consumer acceptance and good quality yogurt quality with viscosity that is not too thick or runny and smooth texture [6]. Problems that often occur in yogurt making are low viscosity (texture instability) and the onset of syneresis during storage. This can be occurred since during the process of making yogurt, there is an increase in the amount of total acid that triggers a decrease in pH to around the isoelectric pH of casein so that there is a decrease in water binding capacity, which makes yogurt prone to syneresis [1].

Several studies have shown that the way to improve the physical state, texture, and rheology of yogurt is to add various stabilizers to the milk. There are many types of stabilizers, among which is psyllium husk. Psyllium (*Plantago ovata*) is an excellent source of natural fiber and has more than 8% more soluble fiber than wheat germ per basic weight. In addition, psyllium seeds have high hydrocolloids so they can absorb water well [7]. Psyllium is used as a stabilizer because the polysaccharides in psyllium can improve the consistency and stability of the system through the formation of a strong gel [5]. Previous research has been carried out making yogurt using stabilizers. Everett & Mcleod [8] used five of stabilizers (LM-pectin,λ-carrageenan, locust bean gum, guar gum, and xanthan gum) added to yogurt and the results showed an effect on the viscosity of the yogurt. In addition, Ladjevardi *et al*. [5] reported that the use of psyllium gum combined with the fat content of skim milk had an effect on the texture (firmness and syneresis) and rheology (viscosity) of the yogurt. Considering the above description, a study was conducted on the optimization of yogurt quality with the addition of psyllium husk and full cream milk fermented with *L. bulgaricus* using the response surface method.

2. Materials and methods

2.1. Preparation of Starter Culture

Solution of 10% (w/v) skim milk was prepared by dissolving skimmed milk powder with hot distilled water and put into an Erlenmeyer. The skim milk solution was pasteurized at 75°C for 30 minutes. *L. bulgaricus* culture was introduced to the liquid media of skim milk solution at 37°C Then incubated for 24 hours at 37°C in an incubator. The culture was streaked in a slanted agar medium containing bacto agar (1.5%), yeast extract (1%), and lactose (1.5%). The *L. bulgaricus* starter can be used for fermentation.

2.2. Preparation of Psyllium Husk

Psyllium was blended and ground until smooth. Then the psyllium was filtered using 100 mesh filter. Psyllium in powder form can be used.

2.3. Yogurt Optimization using RSM-CCD

A total of 13 experiments (Table 1) were conducted with various combinations of psyllium husk concentration (x_1, 0.05-2%) and full cream milk concentration (x_2, 2-5%). Based on the experimental design, a quadratic model can be generated as shown in Equation (1).

$$y = \beta_0 + \sum_{i=1}^{k}\beta_i x_i + \sum_{i=1}^{k}\beta_{ii} x_i^2 + \sum\sum_{i<j}\beta_{ij} x_i x_j + \in \ \ldots (1)$$

Where y is the response (y_1=viscosity, y_2=syneresis), β_0 is the intercept coefficient, βi the linear effect coefficient, βii is the quadratic effect coefficient, βij is the interaction effect coefficient, x_i and x_j (psyllium husk concentration and full cream milk). The regression coefficient (R^2) was obtained from the accuracy analysis of the equation model. The optimum levels for psyllium and full cream milk were determined from the analysis of regression equation and contour plot.

2.4. Yogurt Fermentation

Yogurt fermentation was carried out based on the method of Phadungath [9] A total of 250 mL of milk was pasteurized at 75℃ for 30 minutes. When the temperature reached 50℃, full cream milk and psyllium husk were added to the milk according to Table 1. The pasteurized milk was cooled until the temperature reached 37°C. Then 5% *L. bulgaricus* starter was added and incubated at 37°C for 20 hours. After that, the yogurt product was subjected to further analysis.

2.5. Syneresis

Syneresis of yogurt was determined by the centrifugation as described by Zainoldin & Baba [10]. Briefly, 20 g of yogurt was centrifuged (640 *g*, 20 min, 4°C). The supernatant was taken, weighed, and recorded as (%) syneresis.

2.6. Viscosity

Viscosity determination was measured using a Raypa RP1 Viscometer. One hundred mL yogurt sample was stirred with a rotation speed of 3 rpm using spindle no.2. Viscosity measurements were taken at 25°C (room temperature). The measured viscosity value was recorded.

2.7. Organoleptic Test

The method used to determine the sensory characteristics of yogurt in accordance with Tavakolipour [11] is hedonic test. The yogurt used in this organoleptic test was added 10% (b/v) sucrose added to 500 mL of yogurt. Hedonic test was based on product preferences which is assessed based on color, aroma, texture, taste, and overall appearance. Hedonic evaluation was conducted using a five-point hedonic scale (1 = dislike, 2 = somewhat dislike, 3 = average, 4 = somewhat like, 5 = like) by 15 semi-trained panelists.

3. Results and Discussions

3.1. Preliminary Experiment

Yogurt was prepared by preliminary determination of the minimum and maximum concentrations of psyllium husk and full cream milk. Determination of concentration using RSM experimental design by setting the lower limit and upper limit of concentration refers to previous article [5]. This study used of 0.05-2% psyllium husk and 1-10% full cream milk. However, the results of preliminary experiment showed that addition of more than 6% full cream milk produced a very viscous yoghurt so that the viscosity of the product cannot be accurately determined. Therefore, full cream milk concentration was lowered to a minimum of 2% and a maximum of 5%, while the concentration of psyllium husk was maintained at the same range. The concentration variation is presented in Table 1.

Table 1. Variation of concentration of psyllium husk and full cream milk

No.	Concentration (%w/v)	
	Psyllium Husk	Full Cream Milk
1	0.05	2
2	0.2	2
3	0.05	5
4	0.2	5
5	0.0189	3.5
6	0.2311	3.5
7	0.125	1.379
8	0.125	5.621
9	0.125	3.5
10	0.125	3.5
11	0.125	3.5
12	0.125	3.5
13	0.125	3.5

Table 2. Syneresis results on yogurt with varying concentrations of psyllium husk and full cream milk

No	Concentration (%w/v)		Experimental Syneresis (%)	Predicted Syneresis (%)
	Psyllium Husk	Full Cream Milk		
1	0.05	2	40.46	41.05
2	0.2	2	38.61	35.86
3	0.05	5	33.32	34.27
4	0.2	5	34.16	31.78
5	0.019	3.5	43.34	41.86
6	0.231	3.5	33.15	36.42
7	0.125	1.379	35.03	36.18
8	0.125	5.621	27.86	28.50
9	0.125	3.5	36.45	36.91
10	0.125	3.5	36.65	36.91
11	0.125	3.5	37.14	36.91
12	0.125	3.5	36.89	36.91
13	0.125	3.5	37.46	36.91

Table 3. ANOVA of the effect of full cream milk and psyllium husk on yoghurt syneresis

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Model	5	141.073	28.2146	6.64	0.014
Linear	2	88.749	44.3745	10.44	0.008
Psyllium	1	29.725	29.7253	6.99	0.033
Full Cream Milk	1	59.024	59.0236	13.88	0.007
Square	2	50.515	25.2576	5.94	0.031
Psyllium*Psyllium	1	8.581	8.5808	2.02	0.198
Full Cream Milk*Full Cream Milk	1	36.461	36.4608	8.58	0.022
2-Way Interaction	1	1.809	1.8090	0.43	0.535
Psyllium*Full Cream Milk	1	1.809	1.8090	0.43	0.535
Error	7	29.762	4.2517		
Lack-of-Fit	3	29.137	9.7123	62.12	0.001
Pure Error	4	0.625	0.1564		
Total	12	170.835			

Table 4. Validation of syneresis values from optimization results

Condition	Experiment Variable		Response
	Psyllium Concentration (%w/v)	**Full Cream Milk Concentration (%w/v)**	**Syneresis (%)**
Center point	0.125	3.5	36.91
Optimum	0.158	5.621	28.28
Experiment 1	0.158	5.621	28.29
Experiment 2	0.158	5.621	29.11
Experiment 3	0.158	5.621	28.74
		Average	28.71 ± 0.41

Table 5. Results of yogurt viscosity values at varying concentrations of psyllium husk and full cream milk

No.	Concentration (%w/v)		Experimental Viscosity (mPas)	Predicted Viscosity (mPas)
	Psyllium Husk	Full Cream Milk		
1	0.05	2	1530	1344
2	0.2	2	1858	1649
3	0.05	5	1740	1438
4	0.2	5	2128	1803
5	0.019	3.5	844	1083
6	0.231	3.5	1285	1556
7	0.125	1.379	1536	1709
8	0.125	5.621	1549	1885
9	0.125	3.5	1633	1693
10	0.125	3.5	1605	1693
11	0.125	3.5	1694	1693
12	0.125	3.5	1819	1693
13	0.125	3.5	1717	1693

Table 6. ANOVA table of the effect of full cream milk and psyllium husk on yoghurt viscosity

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Model	5	540724	108145	1.31	0.358
Linear	2	255387	127694	1.55	0.277
Psyllium	1	224339	224339	2.72	0.143
Full Cream Milk	1	31048	31048	0.38	0.559
Square	2	284436	142218	1.73	0.246
Psyllium*Psyllium	1	243068	243068	2.95	0.130
Full Cream Milk*Full Cream Milk	1	18865	18865	0.23	0.647
2-Way Interaction	1	900	900	0.01	0.920
Psyllium*Full Cream Milk	1	900	900	0.01	0.920
Error	7	576717	82388		
Lack-of-Fit	3	548922	182974	26.33	0.004
Pure Error	4	27795	6949		
Total	12	1117441			

Table 7. Validation of viscosity values from optimization results

Condition	Experiment Variable		Response
	Psyllium Concentration (%w/v)	**Full Cream Milk Concentration (%w/v)**	**Viscosity (mPas)**
Center point	0.125	3.5	1693
Optimum	0.162	5.621	1933
Experiment 1	0.162	5.621	2068
Experiment 2	0.162	5.621	2423
Experiment 3	0.162	5.261	2022
		Average	2171 ± 219

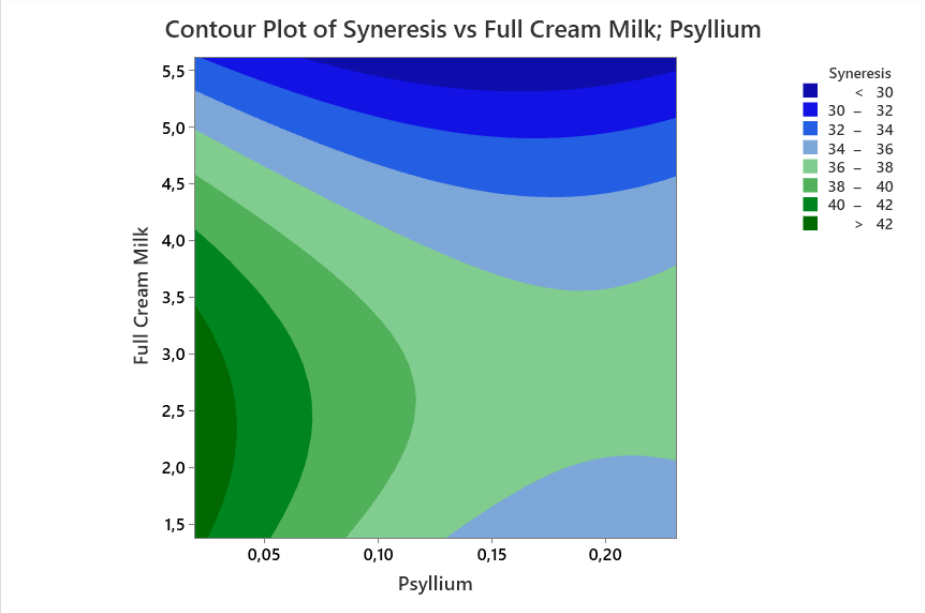

Figure 1. Contour plot between the concentration of psyllium husk and full cream milk on yogurt syneresis value

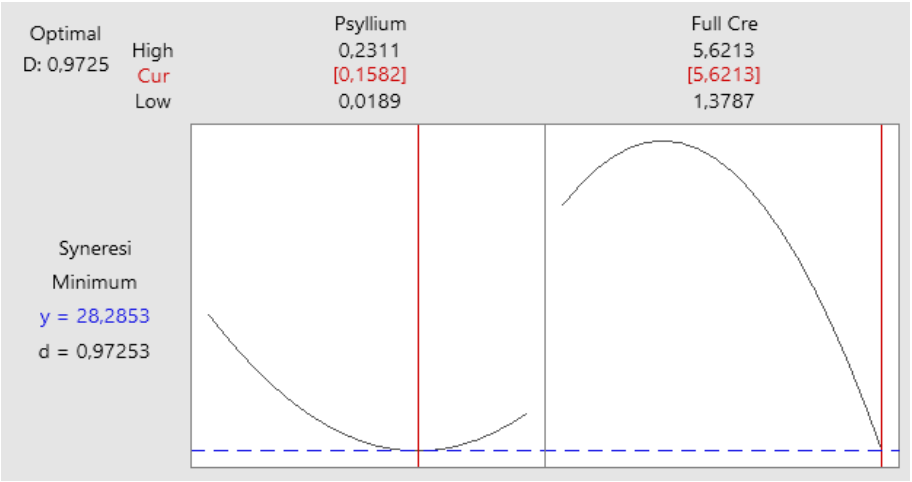

Figure 2. Graph of optimum concentration of psyllium and full cream milk for minimum syneresis

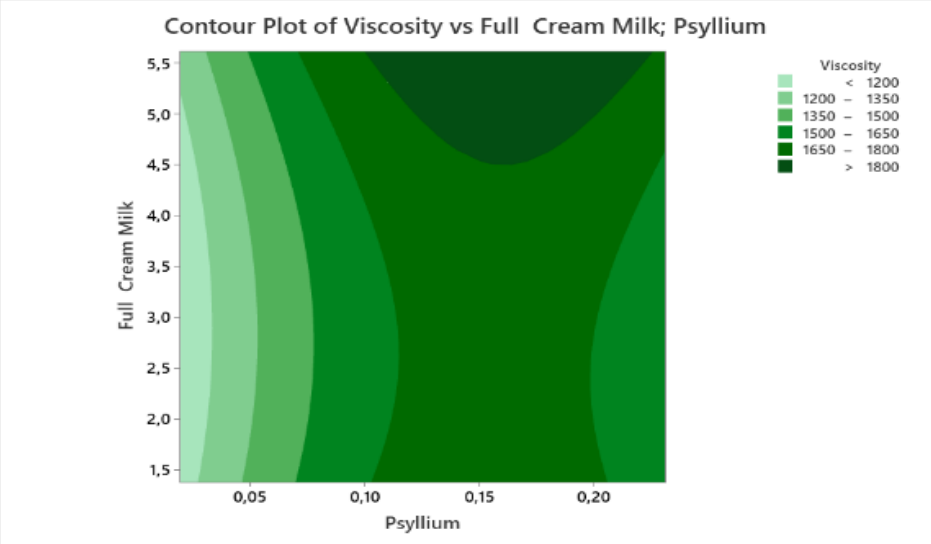

Figure 3. Contour plot between the concentration of psyllium husk and full cream milk on yogurt viscosity value

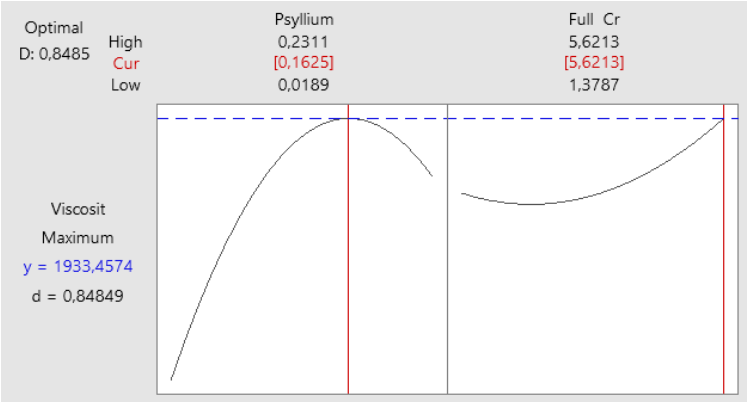

Figure 4. Graph of optimum concentration of psyllium and full cream milk for maximum viscosity

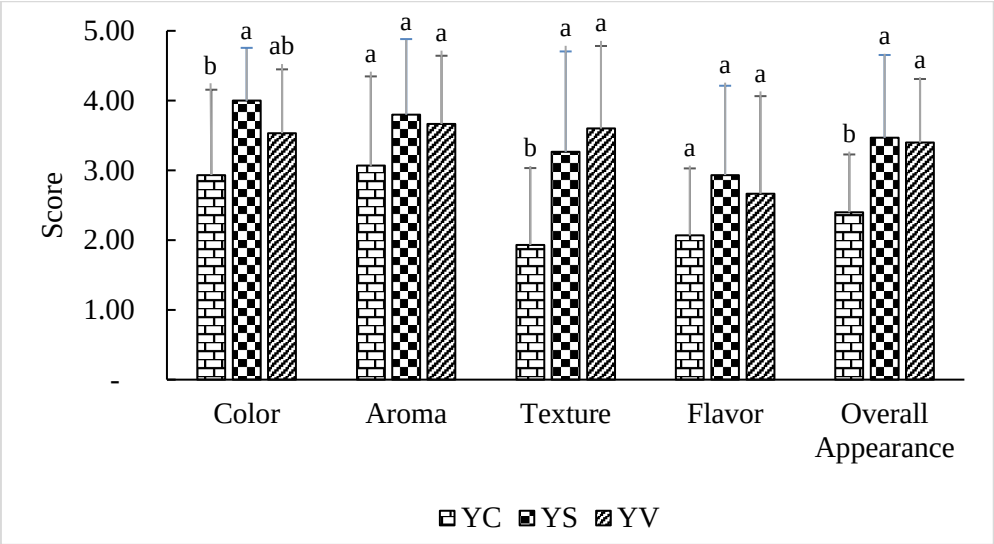

Figure 5. Hedonic test results. The data shown is the average of the ratings of 15 panelists. Error bars indicate standard deviation and different letter above the bars indicate significant differences based on Tukey HSD test with α = 5%

3.2. Yogurt Syneresis

The result of the syneresis analysis is presented in Table 2. ANOVA results showed that of psyllium husk (P=0.033) and full cream milk (P=0.007) had significant effect on syneresis (Table 3). In this case, psyllium can bind water well so that it can reduce the syneresis of yogurt. Previous studies have shown that increasing the concentration of added stabilizer increases the water-holding capacity and thus reduces the level of syneresis produced. Ladjevardi *et al*. [5] found that increasing psyllium gum concentration can reduce whey separation (syneresis). Mahdian & Tehrani [12] also stated that increasing the amounts of total solids in yogurt (in this case full cream milk) can reduce the level of syneresis. Increasing the total solids content in the gel structure of yogurt may be able to form a stable network matrix to avoid gel shrinkage through stronger intermolecular interactions between the branches on the psyllium and fat globulins [13].

Experiments to optimize syneresis was conducted according to the experimental design shown in Table 1 and the design results is shown in Table 2. The data were processed to get a mathematical equation which describe the effect of variables on syneresis. The mathematical equation can be used as a model of the desired response in this experiment. The model equation can then be used to predict syneresis. The second-order equation for the syneresis value of the results is presented to Equation (2).

$$\begin{aligned}\text{Syneresis} &= 39.71 - 96.0\ \text{Psyllium} + 4.56\ \text{Full Cream Milk} + \\ &\quad 197\text{Psyllium*Psyllium} \\ &\quad - 1.018\ \text{Full Cream Milk*Full Cream Milk} + \\ &\quad 5.98\ \text{Psyllium*Full Cream Milk} \ldots (2)\end{aligned}$$

The response of the equation model obtained can be plotted as contour plot (Figure 1). From these results, the optimum level of psyllium husk and full cream milk concentration can be predicted using model Equation (2). Figure 1 shows that as the concentration of psyllium husk and full cream milk to yogurt increases, the syneresis value decreases. To get optimum conditions for the syneresis value, optimization is necessary. Optimum condition of syneresis was achieved when the value achieve the lowest value because good yoghurt has a low syneresis. The optimum condition was obtained at psyllium husk and full cream milk of 0.16% and 5.62%, respectively (Figure 2). At this condition. the syneresis value reached 28.28% according to the model. The model of Equation (2) for syneresis as a function of psyllium husk and full cream milk was used to

find the optimum point of syneresis. Based on Table 4, the calculation of the syneresis value at the center point is 36.91% at concentration of psyllium husk and full cream milk of 0.125% and 3.5%, respectively. The lowest value of syneresis based on the model Equation (2) is achieved at a concentration of psyllium full cream milk of 0.158% and 5.621%, respectively. At the optimum condition, the syneresis value reach 28.28% according to the equation. Validation of optimum conditions carried out with three repetitions resulting a syneresis of values 28.71 ± 0.41%. The experimental value is 0.15% higher than the model prediction. This result show that the model and experimental result are in a good agreement.

3.3. Optimum Condition for Highest Viscosity

Yogurt with good characteristics have a stable viscosity. Viscosity affects the texture and appearance of the yogurt produced and affect consumer acceptance of the product. Based on the results of the present study, the viscosity of yogurt obtained for various concentrations is presented in Table 5 and the ANOVA is presented in Table 6. The results of ANOVA (Table 6) showed that the addition of psyllium husk (P=0.143) and full cream milk (P=0.559) had no significant effect on the viscosity of yogurt. There are also no significant effect of quadratic and interaction between the factors in the present study as indicated by high p value (P>0.05). This experiment was conducted according to the experimental design shown in Table 1 and the design results is shown in Table 6. The data were processed to get a mathematical equation which explains the effect of variables on the desired response, in this case the viscosity of yogurt. The mathematical equation, if appropriate and statistically qualified, can be used as a model of the desired response in this experiment. The model equation can then be used to predict the response of interest. The second-order equation for the viscosity value of the results is shown in Equation (3).

Viscosity = 1092 + 10074 Psyllium - 137 Full Cream Milk –
33231 Psyllium*Psyllium +
23.1 Full Cream Milk*Full Cream Milk+
133 Psyllium*Full Cream Milk … (3)

Ladjevardi *et al*. [5] reported that the use of psyllium husk gum combined with fat content has an influence on yogurt viscosity. Psyllium gum concentration (0.0072-0.128%) and fat content (0.29-1.71%) combined using RSM showed an influence on yogurt viscosity when higher concentrations of psyllium were added to the yogurt. However, in this study, these parameters did not have a significant effect, this could be due to the determination of the psyllium concentration used which was too high compared to that used by Ladjevardi *et al*. [5], so the concentration of psyllium used in this yogurt research needs to be lowered. The response of the model equation obtained can be plotted with a contour plot (Figure 3) to see more clearly the shape of the response surface method of the experimental unit points. From these results, the optimum level of the concentration of psyllium rind and full cream milk can be predicted in the model Equation (3).

Although it was previously stated that the parameters of psyllium and full cream milk had no significant effect, the RSM plot in Figure 3 indicate that the use of higher concentrations of psyllium and full cream milk tend to lead to higher viscosity value. This is in agreement with Ladjevardi *et al*. [5] who showed that with the addition of higher concentration of psyllium gum into yogurt, it will increase the viscosity value of yogurt. This is due to the carboxyl group contained in the arabinoxylan structure of psyllium which able to bind hydrogen ions in water. In addition, Ca^{2+} ions present in yogurt can also covalently form crosslinks between free carboxyl and amino groups between polymer side chains [7]. In addition to psyllium, it has also been shown that increasing the amount of total solids (full cream milk) in yogurt can improve the textural properties of yogurt [11]. The optimum condition to get the highest viscosity can be achieved by using condition as indicated on Figure 4. Optimization was carried out in the maximum state because good yogurt has a texture with a thick viscosity level resulting in an optimum concentration of 0.16% psyllium husk and 5.62% full cream milk with an optimum viscosity value of 1933 mPas. The conformity of the model with reality was tested to determine the correctness of the model. The model validation test was conducted under optimum conditions with three replications. The model validation for viscosity is presented in Table 7. The model of Equation (3) for viscosity as a function of psyllium husk and full cream milk. was used to find the optimum point of viscosity. Based on Table 7, the calculation of the viscosity value at the center point is 1693 mPas at concentration of psyllium husk and full cream milk of 0.125% and 3.5%, respectively. The highest value of viscosity in the model Equation (3) is achieved at a concentration of psyllium full cream milk of 0.162% and 5.621%, respectively. which can reach viscosity value of 1933 mPas. Validation experiment on optimum conditions carried out with three repetitions resulting a viscosity of 2171 ± 219 mPas. The experimental value is 238 mPas higher than the model prediction. The optimization results of viscosity were actually set according to the target viscosity of commercial yogurt which is 5121 mPas, but the optimization results obtained did not reach this target because the maximum viscosity conditions during the study only reached 2128 mPas.

3.4. Panelist Preference

Organoleptic and acceptability assessments were conducted by 15 semi-trained panelists who asses three type yoghurt samples, namely control yogurt (YC), yogurt optimized for its viscosity (YV), and yogurt optimized for its syneresis (YS). Organoleptic analysis used in this study is hedonic test (preferences).

The hedonic test is a preferences test with panelists to assess whether the product is liked or disliked. The test result is presented in Figure 5. The hedonic test assessment of the color, aroma, texture, taste and overall appearance of the yogurt showed that texture, and overall appearance are a significantly different (P≤0.05) with control yogurt. Hedonic assessment of color result indicates there is a significant difference in the panelist preference between the samples (P=0.003). According to Figure 5, in term of color, YS is the most preferred but YV is not significantly different with YC and YS. For texture there is also a significant difference of panelist preference (P=0.002) where YC is the least preferred and YS and YV are not significantly different. Likewise, the overall appearance (P=0.007) was favored for YS and YV and had a better overall appearance than YC. However, the aroma (P= 0.273) and taste (P=0.184) of yogurt tested did not

show any significant differences on panelists' preferences. In brief, addition of psyllium and full cream milk significantly affect panelist preference in term of color, texture and overall appearance, but did not give any significant effect on aroma and flavor

4. Conclusions

The optimum condition for the lowest syneresis and the highest viscosity achieved when psyllium husk and full cream milk was adjusted to 0.16% and 5.62%. This condition, the syneresis can reach 28.28% according to the mathematical model, and validation of the experiments give 28.71%. While for viscosity according to the mathematical model the highest value can reach 1933 mPas, and the validation of the model give 2171 mPas. Both validations indicate that the mathematical model are in good agreement with experimental value. According to hedonic test, the optimized yoghurt has better color, texture and overall appearance compared to control yoghurt.

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