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Optimation Formula of Goat Milk Yoghurt and White Oyster Mushroom Powder with Mixture Design Methods

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Abstract: Yoghurt is a product of fermented milk using lactic acid bacteria as a starter. As a probiotics, Lactobacillus achidophillus was applied in the making of symbiotic yoghurt with white oyster mushroom powder as a prebiotic source. The objective of this research was to optimize goat milk yoghurt formula by mixture design with three component (white oyster mushroom, skim milk and skim goat milk) and four responses (pH, viscosity, total titrated acid and lactid acid bacteria). The result indicated that the optimum formula was a mixture of 0.5% white oyster mushroom, 3% skim milk and 96% goat skim milk. The optimum formula low pH, high viscosity and high total lactic acid bacteria. Lastly, the optimum formula was further modified by adding spices syrup as a topping (made from ginger extract, mixture extract of cloves-cardamom-anise and mixture extract of cloves-cardamom-anise) in a different volume of addition (2, 4 and 6 mL) with serving size of 100 mL goat milk yoghurt. These hedonic test result showed that the highest score of the taste properties were 4.37 for 4 mL addition of ginger syrup; 4.13 for 6 mL addition of mixture of cloves-cardamom-anise syrup and 4.23 for 6 mL addition of mixture of cloves-cardamom-cinnamon syrup. Based on these results, the consumer' preference was still at the level of netral rather like.

Key words: Lactid acid bacteria, yoghurt, mushroom, goat milk, mixture design

INTRODUCTION

Etawah Breed goat milk has not been widely consumed in Indonesia because it has distinctive characteristic of goaty smell so that it is not appreciated by the public and the lack of public knowledge concerning the benefits of goat's milk. The feature possessed by goat milk is that it can increase the absorption of Fe (Barrionuevo *et al*., 2002), is useful as a natural antiseptic and helps suppressing bacteria breeding in the body, contains milk protein, casein, milk fat, minerals and vitamins A higher than cow's milk (Hanlein, 2004). Goat's milk has a great potential as a probiotic carrier (Cahyanti, 2011). The high content of medium-chain fatty acids content (C11-C17) is known to have a bacteriostatic effect (Boycheva *et al*., 2011).

Milk fermentation process causes the formation of distinctive flavor as the result of activities from lactic acid bacteria, such as acetaldehyde and diacetyl compounds that can reduce or even eliminate the goaty smell. Conventionally, the bacteria commonly used in the formulation of yoghurt were *Lactobacillus bulgaricus* and *Streptococcus thermophillus*. However, it was later discovered that the bacteria are not sufficient to maintain the health of digestive tract because they cannot survive in the digestive tract (Lourens-Hattingh and Viljoen 2001). To increase the functional value of the yoghurt product, probiotic bacteria should be added. *Lactobacillus achidophillus* is one of probiotic bacteria that can be added in the making of yoghurt and has the ability to pass through the barriers to the intestine alive, such as the presence of gastric acid and bile acids (Aswal *et al*., 2012). Probiotic bacteria is expected to survive in the digestive tract and to achieve that, it is required nutrients in the form of prebiotics.

White oyster mushrooms and shiitake mushrooms are two types of mushrooms well known by the people of Indonesia with affordable prices and potential prebiotic. The fiber content in these two mushrooms especially the soluble fiber, makes the mushrooms as potential prebiotic. The component of oyster mushrooms polysaccharide dietary fiber can stimulate the growth of intestinal microorganisms (Synytsya *et al*., 2009). The results by Indratiningsih *et al*. (2004) show that the addition of shiitake powder into yoghurt can improve the viability of probiotic and yoghurt bacteria of *L.casei*. The use of white oyster mushroom powder as much as 0.3% can increase the viability of *Lactobacillus bulgaricus* and *Streptococcus thermophillus* yoghurt bacteria (Padli, 2010). Probiotic yoghurt enriched with the addition of prebiotics produces the product known as symbiotic yoghurt. The formulation of yoghurt can be performed by adding full cream and skim milk. Skim milk contains all nutrients of milk except the fat and fat-soluble vitamins. The fat content in skim milk is approximately 1%. Based on these researches instead of facts, the formulation of yoghurt is conducted using skim milk ingredients.

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Indonesia has a huge wealth of biodiversity, including medicinal plants and spices. The spices that have been known for a long time in Indonesia are ginger, cloves, anise and cinnamon. For yoghurt, there is an alternative to add flavors in the form of spices syrup, not only creating new flavor varieties but also the spices has known for their aromatic compounds and have antimicrobial compounds. This research aims to assess the formulation of goat milk yoghurt in determining the optimum formula using mixture design and to examine the effects of spices syrup addition on the preference of the yoghurt.

MATERIALS AND METHODS

Materials: The materials used in this study is Etawah breed goat milk, skim powder milk merk "Sunlac", MRS agar (Oxoid), MRS broth (Oxoid). The bacterial strains used were *L.bulgaricus* FNCC 004P, *S. thermophilus* FNCC 1.9.0.3, *L. achidophillus* FNCC 0051 obtained from Laboratory of Microbiology, Gadjah Mada University, Indonesia. White oyster mushrooms and shiitake mushrooms were obtained from mushroom growers in Ciomas Bogor, Indonesia.

The chemicals used in this study included chemicals for protein analysis, fat, ash, water, total dietary fiber (TDF) and soluble fiber (SDF). The equipments used laminar airflow, vortex, incubator, pH meter, autoclave and Brookfield viscometer.

Analysis of chemical constituents and nutrients of white oyster and shiitake mushrooms: For chemical and nutritional analysis of white oyster and shiitake mushrooms, the previous mushroom was made into powder. The making of mushroom powder has been conducted using a drum dryer at instead of with 80°C temperature. Then the powder was analyzed for the content of protein, fat, ash, water, SDF and TDF. The analysis of protein, fat, ash and water content were conducted using AOAC method (1999).

Yoghurt formulation process: Yoghurt formulation process was started by refreshing pure culture to MRSB, the refreshed culture was inoculated into 10% skim milk solution and incubated at 37°C for 24 h (starter culture). The next stage, mushrooms powder mixing was carried out with cow milk skim and goat milk skim, then yoghurt starter culture was inoculated, consisting of *L.bulgaricus, S.thermophilus* and *L.achidophilus* with 1:1:2 ratio as much as 2% into the yoghurt.

Optimation of goat milk yoghurt with mixture design: The optimization was performed using mixture technique with D-Optimal Design. The components were mushroom powder (0.3-0.5%), skim milk (3-5%) and goat skim milk (95-97%) and the measured quality respons of yoghurt were pH, viscosity, total titrated acid (TTA) and lactid acid bacteria (LAB). The optimation process was performed to find a formula that has optimal responses in accordance to the desirable optimization targets. Optimation target value that could be achieved, known as desirability,is in the range 0-1. The higher desirability value indicated the higher achievement for yoghurt suitability formula to the expected response variables. Optimum process was set based on pH constraints, TTA and LAB in range, while the viscosity was maximize. The program of Design Expert 7 was used to design and process the data obtained. Having obtained the optimum conditions, the study was continued by doing the verification.

Sensory analysis of optimum formula with spices syrup topping addition: The optimum formula of yoghurt has been added by spice syrup topping in the hedonic test. Thirty panelists has been asked to score the preference of taste attribute. The panelists were asked to score the samples for preference of goat milk yoghurt characteristic on a scale of 1 to 7, where scale 7 = very like, 6 = like, 5 = rather like, 4 = neutral, 3 = rather dislike, 2 = dislike and 1 = very dislike.

Statistical analysis: The program of Design Expert 7 was used to process data obtained to optimum formulation. The sensory analysis scores were subjected to one-way analysis of variance and the significant different detected was evaluated by Duncan Multiple range test using SPSS Statistic version 20 software.

RESULTS AND DISCUSSION

Chemical and nutritional content of white oyster and shiitake mushrooms: The result of this analysis revealed that the shiitake mushroom showed high protein and fat while SDF and IDF was better than white oyster mushroom. Shiitake mushroom showed that protein 22.70%, fat 10.85%, SDF 11.66%, IDF 8.08% and white oyster mushroom showed that protein 16.43%, fat 1.34%, SDF 9.89%, IDF 6.86% (Table 1).

As the material to be added in the dairy food products, then the information concerning the chemical and nutritional content of white oyster and shiitake mushroom powder is important. The nutritional contents available in both mushrooms are suitable for consumption as food. Components of dietary fiber of both soluble dietary fiber and insoluble dietary fiber can act as a health food. The soluble dietary fiber can lower total cholesterol level and insoluble dietary fiber can shorten the length of stay of food in the digestive system, so that it can reduce the chances of colon cancer (Wirawati and Nirmagustina, 2009). Polysaccharides component of oyster mushrooms can stimulate the growth of intestinal microorganisms (Synytsya *et al.*, 2009).

Table 1: Result of chemical and nutritional content of white oyster and shiitake mushroom powder

Analysis	White oyster mushroom powder (g)	Shitake mushroom powder (g)
Protein	16.43	22.7
Fat	1.34	10.85
Water	9.87	3.57
Ash	4.52	7.95
Crude fiber	91.06	31.83
Soluble dietary fiber (SDF)	9.89	11.66
Insoluble dietary fiber (IDF)	6.86	8.08

Table 2: Result of four responses of sixteen formula

	Composition			Respons			
Formula	Mushroom powder (%)	Skim milk (%)	Goat skim milk (%)	pH	Viscosity (cP)	Total titrated acid (%)	Lactid acid bacteria (LAB) (log10 cfu/mL)
1	0.5	5	95	4.53	4450	0.62	9.3
2	0.5	3	97	4.49	6450	0.58	10.3
3	0.4	3	96	4.51	3050	0.54	9.3
4	0.4	4	95	4.55	3100	1.29	9.0
5	0.3	3	97	4.26	6650	1.33	9.8
6	0.3	3	97	4.25	5000	1.29	9.8
7	0.3	5	95	4.55	5800	1.50	9.1
8	0.5	3	97	4.47	6200	0.58	10.1
9	0.5	5	95	4.52	3100	0.58	9.8
10	0.4	4	95	4.54	3800	1.20	9.4
11	0.5	4	96	4.38	7300	0.75	10.4
12	0.3	5	95	4.47	4900	1.50	9.4
13	0.3	5	95	4.54	5600	1.54	9.2
14	0.5	5	95	4.55	4000	0.66	9.2
15	0.5	4	95	4.31	6900	1.29	9.9
16	0.3	4	96	4.32	5100	1.20	9.8

Optimation of goat milk yoghurt with mixture design: Based on D-Optimal Mixture Design, it is 16 trial formulas. The results show response values in pH 4.25-4.55, viscosity 3050-7300 cP, TTA 0.54-1.54 % and LAB 1.10 x 10^9 -2.79 x 10^{10} cfu/mL. The higher pH value of 4.55 and low pH of 4.25 (Fig. 1), the higher viscosity value of 7300 cP and low viscosity of 3050 cP (Fig. 2) and the TTA higher value 1.54% and low value of 0.54% (Fig. 3) and then LAB higher value of 2.79 x 10^{10} cfu/mL and low value of 1.10 x 10^9 cfu/mL (Fig. 4). The result of four responses for each formula are presented in Table 2. Mathematical models of four responses show pH, viscosity and LAB are special cubic and TTA is quadratic. The results of Variance analysis (ANOVA) at 5% significance level indicate that all the models are significant. From equations (Table 3) shows that the four responses are influenced by the components and the interactions among these three components. For the optimum formula of goat milk yoghurt, criteria of four responses should be established. This criteria are presented in Table 4 and verification result of four responses from optimum goat milk yoghurt is presented in Table 5.

Analysis of variance show that the response of pH, viscosity, TAT and LAB are significant. Values of lack of fit, predicted R-squared and adjusted R-Squared and Adequate precision for each response meets the requirements for a good model. The model is a good if it has not a significant value of lack of fit, reasonable agreement of the predicted R-squared and adjusted R-Squared (the difference is smaller than 0.2) and adequate precision value is greater than 4.

Mushroom powder acting as prebiotics that stimulate the growth of yoghurt bacteria to produce lactic acid resulting in pH decrease, high viscosity, TAT and LAB. Prebiotics stimulate the growth of good bacteria in the digestive tract and during milk fermentation process (Chen *et al*., 2003). Aryana and McGrew (2007), yoghurt made with *L.casei* bacteria with the addition of different types of inulin produces pH 4.32-4.60. As a prebiotic, soluble fiber which can spur the growth of yoghurt bacteria that will produce lactic acid as a result of its metabolism so that the resulted lactic acid is increased. As a result, the increased milk protein lactic acid, that is casein, will be coagulated to form yoghurt gel that ultimately lead yoghurt to be viscous so that the viscosity of the yoghurt increases. The lower the pH value indicates the increased acid production and results in the formation of more coagulant, thereby, increasing the viscosity. Low pH will cause the formation of more coagulant, thus increasing the value of viscosity (Zurriati *et al*., 2011). The higher the acidity level of a product, the more viscous the viscosity will be. Concentration of fat and protein, temperature, pH and the shelf life of products influenced viscosity (Park, 2007). The addition of 0.3% mushroom powder can increase the viscosity of yoghurt Kurnia (2010).

Table 3: Mathematical models of four responses in goat milk yoghurt formula optimation

		p-value				
Response	Equation	Model	Lack of fit	Adjusted R^2	Predicted R^2	Adequate precision
pH	-1849.38A-7.65B-0.04C+04.50AB+8.81AC +0.30BC-0.94ABC	0.0003 (sig)	0.0731 (not sig)	0.8508	0.6879	10.890
Viscosity	2.83 x 10^7A+2.85 x 10^6B+880.35C-1.13 x 10^6AB -2.86 x 10^6AC-3085.89BC+9147.84ABC	0.0132 (sig)	0.1640 (not sig)	0.6337	0.4363	6.846
TTA	2058.41A-30.08B -0.01C-21.34AB-20.74AC +0.33BC	0.0090 (sig)	<0.0001 (sig)	0.6160	0.3768	6.769
LAB	6159.59A+46.20B+0.26C-235.04AB-62.32AC -0.50BC+1.87ABC	0.0008 (sig)	0.3015 (not sig)	0.8147	0.6209	9.275
Remarks	A: mushroom powder	B: skim milk	C: goat milk skim			

Table 4: Criteria of three component and four response for optimation processing

Components of responses	Goal	Lower constraint	Upper constraint	Importance
Mushroom powder	in range	0.3	0.5	-
Skim milk	in range	3	5	-
Goat milk skim	in range	95	97	-
pH	in range	4	4.5	(++++)
Viscosity (cP)	maximize	-	7300	(++++)
TTA (%)	in range	0.5	2	(+++)
LAB (log_{10}cfu/mL)	in range	9.0294	10.4456	(+++++)

Table 5: Verification results of four responses from the optimum goat milk yoghurt

Responses	Verification results	95% confident interval	95% prediction interval
pH	4.30	4.31-4.43	4.26-4.48
Viscosity (cP)	6200	5911-8230	4818-9322
TTA (%)	1.08	0.54-1.16	0.23-1.47
LAB (log_{10}cfu/mL)	10.04	10.09-10.60	9.85-10.84

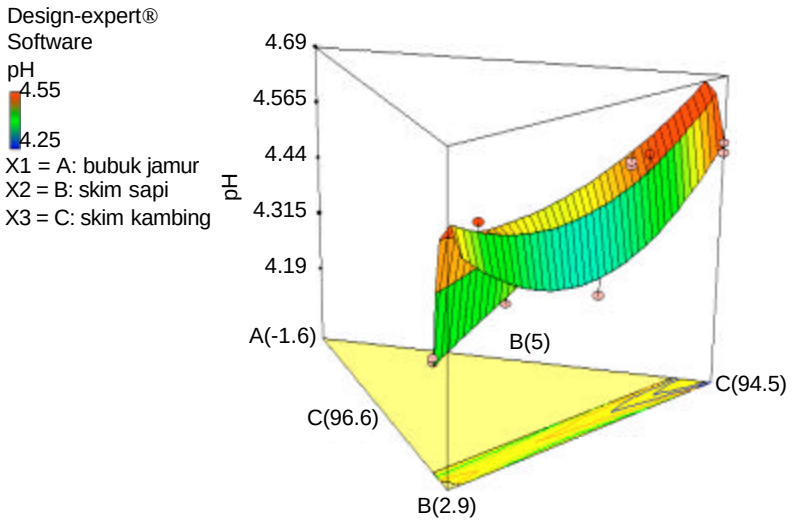

Fig. 1: Three-dimensional graph in pH response

The total response of titrated acid is expressed by lactic acid percentage. Lactic acid is the largest component formed from the fermentation of milk into yoghurt. The increasing amount of lactic acid is in line with the decreased pH value. The lower the pH value indicates the increased acid production. The TAT response value is inversely proportional to the pH value (Usmiati *et al*., 2011). The study results show that the resulted TAT response values have already met the TAT response values based on yoghurt quality standard of SNI 01-2981-2009 that is ranged between 0.5-2%. White oyster mushroom increased total LAB of yoghurt produced, because the mushroom powder contains soluble fiber which acts as a prebiotic which is a carbohydrate not digested that can increase the viability of yoghurt bacteria, so that the total colony of LAB will increase. The component of polysaccharides dietary fiber of oyster mushrooms can stimulate the growth of intestinal microorganisms (Synytsya *et al*., 2009).

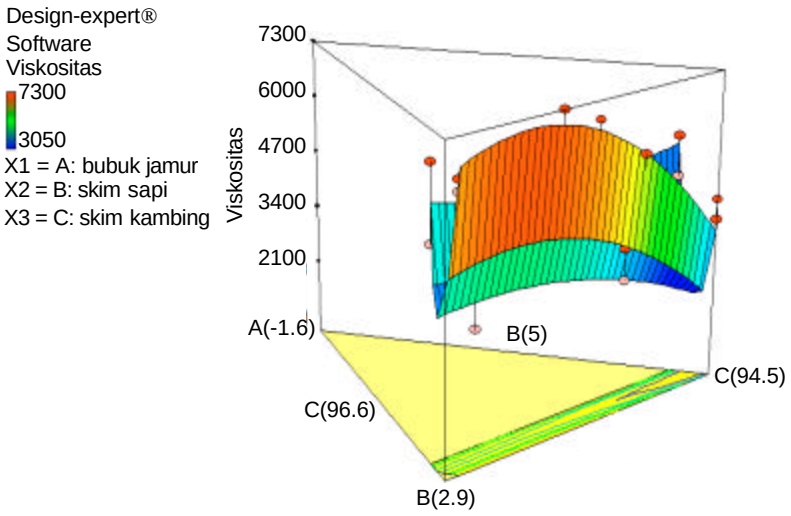

Fig. 2: Three-dimensional graph in viscosity response

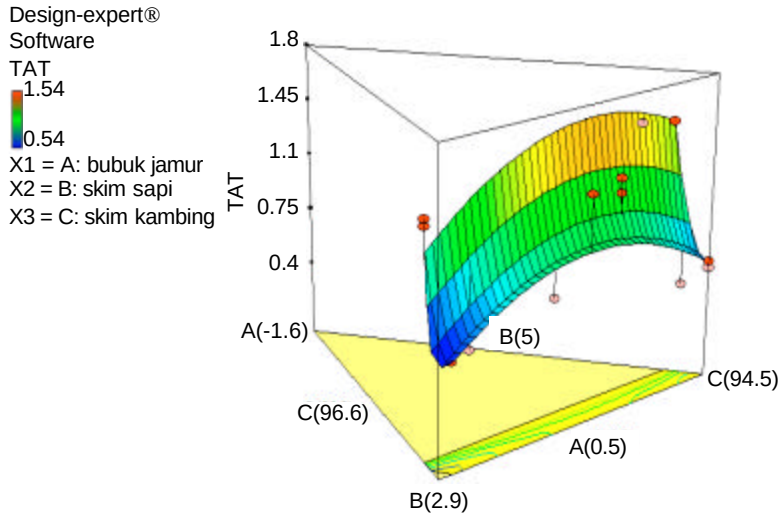

Fig. 3: Three-dimensional graph in TTA response

Optimation process is performed to get a formula with optimal responses. The most optimal response is obtained if the desirability value is close to one. Each optimized component optimized is set with importance weigh to achieve the expected goal. This importance weigh is ranging from 1 (+) to 5 (+ + + + +) depending on the response variable concerned. The more positive signs indicate the higher level of response variable importance.

The pH and viscosity responses are targeted to be in range with importance of 4 (+ + + +), conducted as pH and viscosity measurements are performed using the instrument. The responses are measured to estimate the characteristics of the formulas and to keep the formula not to deviate from the expected characteristics of the formula. LAB response the goal is in range and importance 5 (+ + + + +). This is because the number of LAB is one of the important functional properties of yoghurt. Therefore, LAB total response is the response that should be considered in its formulation in yoghurt formulation.

The optimation target value that can be achieved is known as the desirability value indicated by the value of 0-1. The higher the desirability value indicates the higher the suitability of yoghurt formula to achieve optimal formula with the expected response variables. Formula 1 has desirability value of 0.946, formula 2 of 0.629, formula 3 of 0.562. Of the three optimum formula solution resulted from the optimization process, formula 1 has the highest desirability value so that it is recommended by the program of Design Expert 7.0® (selected).

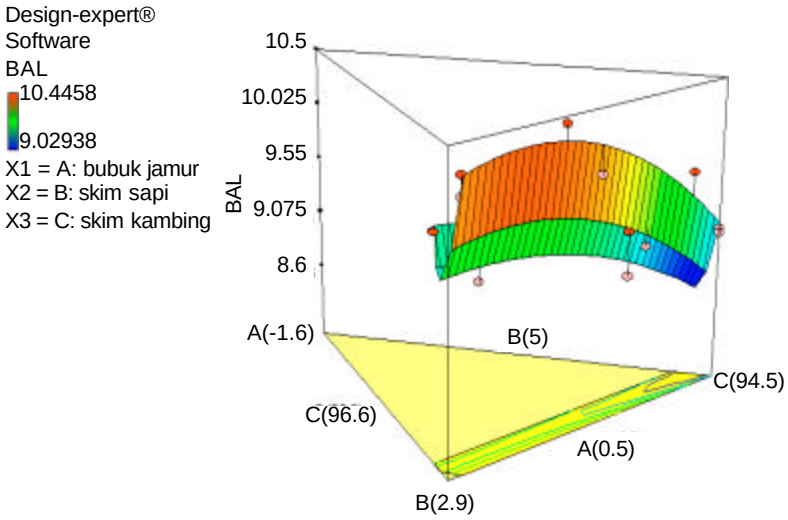

Fig. 4: Three-dimensional graph in LAB response

The optimum formula solution, that is formula 1 has mushroom powder of 0.5%, skim milk 3% and goat skim milk of 96%. Verification is performed after optimation step. Verification step aims to prove the prediction of the response value of optimum formula given by the program of Design Expert 7.0®, the actual response value will be compared with the predictions of the response generated by Design Expert 7.0®. In addition to the prediction of response value of each optimum formula solution, the program of Design Expert 7.0® also provides confident interval and prediction interval for each response predictive value at 5% significance level. From the comparison of verification result data with the predictions made by the program of Design Expert 7.0®, it is found that the predictions of equation for solution of formula1 is in accordance with the test results obtained. pH and LAB responses meet 95% of Prediction Interval which has been predicted by the program of Design Expert 7.0®, while the viscosity and TAT meet 95% of Confident Interval. Therefore, the equation obtained is considered good for determining the optimum formula and the response obtained.

Sensory analysis of optimum formula with spices syrup topping addition: Sensory test is conducted to determine the level of preference (hedonic) of goat milk yoghurt with spices-based syrup topping for ginger syrup, mixture syrup of clove-cardamom- cinnamon and mixture syrup of clove-cardamom-anise. The tasted yoghurt for sensory analysis is the optimum formula yoghurt resulted from the optimation process using mixture design. The high score of the sensory are given below: 4 mL ginger syrup had score 4.37, 6 mL mixture of cloves-cardamom-anise had score 4.13 and 6 mL mixture of cloves-cardamom-cinnamon had score

Table 6: Result of hedonic test for three different of concentration and types of topping syrup

Formula	Scor of taste
JHE 2 mL	3.60[a]
JHE 4 mL	4.37[b]
JHE 6 mL	4.60[b]
CKK 2 mL	3.47[a]
CKK 4 mL	3.43[a]
CKK 6 mL	4.23[b]
CKA 2 mL	3.23[a]
CKA 4 mL	3.53[a]
CKA 6 mL	4.13[b]

Same letter (s) indicated not significant difference on p<0.05 in the same colom. Remarks:
JHE 2: ginger 2 mL
JHE 4: ginger 4 mL
JHE 6: ginger 6 mL
CKK 2: cloves-cardamom-cinnamon 2 mL
CKK 4: cloves-cardamom-cinnamon 4 mL
CKK 6: cloves-cardamom-cinnamon 6 mL
CKA 2: cloves-cardamom-anise 2 mL
CKA 4: cloves-cardamom-anise 4 mL
CKA 6: cloves-cardamom-anise 6 mL

4.23. ANOVA results of hedonic test for three different concentrations and types of syrup topping are presented in Table 6.

Sensory analysis of optimum formula with spices syrup topping addition: The optimum formula of goat milk yoghurt with the addition of three type of syrups (ginger/JHE, cloves-cardamom-anise/CKA, cloves-cardamom-cinnamon/CKK) with concentrations 2, 4 and 6 ml, sensory analysis showed that the yoghurt provided by ginger syrup topping of 4 and 6 ml was not significant for the taste score, CKK syrup topping of 6 ml with 4 and 2 ml was not significant, the high score CKK syrup topping of 6 ml was most preferred. CKA syrup topping of 6 ml with 4 and 2 ml was not significant for taste score, the high score CKA syrup topping of 6 ml was most preferred by the panelists. This mean, adding

spices syrup as a topping into goat milk yoghurt can influence the judgment for taste by panelist.

Conclusion: Optimization-based formula goat milk yoghurt using mixture design is based on the response of pH, viscosity and total titrated acid according to the optimization criteria for each response produces formula goat's milk yoghurt optimum was a mixture of 0.5% white oyster mushroom, 3% skim milk, and 96% goat skim milk. These hedonic test result showed that the highest score of the taste properties for 4 ml addition of ginger syrup; 6 ml addition of mixture of cloves-cardamom-anise syrup, and 6 ml addition of mixture of cloves-cardamom-cinnamon syrup. Based on these results, the consumer preference was still at the level of netral rather like.

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