Anti–odor activity of milk kefir on organosulphur polysulfide cyclic compounds in petai (parkia speciosa hassk)

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Anti–odor activity of milk kefir on organosulphur polysulfide cyclic compounds in petai (parkia speciosa hassk)

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Abstract. This study aims to assess the activity of milk kefir whey in neutralizing odor-causing cyclic polysulfide compounds in petai (*Parkia speciosa* Hassk.). RAL designs used to determine the optimum fermentation conditions. The data obtained were processed using SPSS 20. Results showed the characteristics of the microbes in the kefir grains include lactic acid bacteria consisting of genus *Lactobacillus* and yeast of the genus *Candida* and *Saccharomyces*. The optimum fermentation conditions using cow's milk kefir grain starter obtained in the fermentation time of 24 hours at a concentration of 5% kefir grain. Whey kefir which is produced have high levels of fat, protein, carbohydrates, fiber and lactic acid respectively 1.81; 4.35; 5.59; 0.26 and 0.16%, pH 4.4; a density of 1.0628 g/mL and 7.9368 cP viscosity. Kefir milk whey actively reduced the level of petai smell significantly different at the level of $\alpha = 0.05$.

1. Introduction

Parkia is a genus of plants in the family Fabaceae, Mimosoide (*petai-petai*an), has 77 species [1], including species *Parkia speciosa* Hassk. *P. speciosa* is growing endemic in Southeast Asia, are found in Indonesia, Malaysia, Thailand, and the Philippines. *P. speciosa* known locally *peuteuy* (Sunda), *pete* (Betawi), *petai* (Indonesia, Singapore, Malaysia), *sataw* (Thailand) and *u'pang* (Philippines). *P. speciosa* is known to have a number of important chemical compounds and medicinal properties such as organo polysulfide cyclic compound which is used as an antibacterial infection of the kidneys, ureters and bladder. *P. speciosa* has also been reported to have various benefits and the potential for human health are as antihypertensives [2] antioxidants [3], antiangiogenic [4] and even has a hypoglycemic effect for their secondary metabolite β-sitosterol action in synergism with stigmasterol [5]. Recent reports suggest the organo polysulfide compounds contained in *P. speciosa* play an important in the regulation of cardiovascular [6].

Even for good efficacy for patients with diabetes mellitus type 2, Burning and Berg (2013) has patented a way to make the *petai* extract preparations [7]. However, the weakness of grains/fruit *petai* (*P. speciosa*) is that the seeds of this plant has a bad odor, which is often the reason why *petai* has earned the nickname 'stinky bean' because it smells so pervasive strong and remain in the mouth and excretion systems of the human body, which after eating *petai* seeds, smell still wafted in the urine of a person up to two days. In addition, *petai* fruit can also cause bloating and flatulence pungent [8]. The odor of *petai* is because these plants produce a cyclic polysulfide organosulfur compounds such as 1,2,4-trithiolan, 1,3,5-trithian, 1,2,4,6-tetrathiepan, 1,2,3,5, 6-pentathiepan (lenthionin), and 1,2,4,5,7,8-heksathionin [6]. Whey acidic milk kefir is expected to reduce the release of compounds organosulfur of *petai*. It is based on the nature of the organosulfur compound itself is very influenced by pH or acidity [6]. Therefore, whey activity studies are necessary to overcome the smell of milk kefir *petai* (*P. speciosa* Hassk.). The purpose of this study is to develop the production process, analyze activity and beverage applications anti-odor *petai* (*P. speciosa*) whey-based milk kefir primarily to 1) analyze the characteristics of the microbes in the kefir grain is used as a starter, 2) determine the optimum fermentation conditions by using cow's milk kefir starter grain based on dose and time, 3) analyzing the characteristics of kefir whey which is produced at optimum fermentation conditions and 4) determining the activity of whey of cow's milk kefir in lowering the level of *petai* odor.The results of this study are expected to provide an important contribution towards enhancing the quality of human life through the use and exploitation of biological resources from the surrounding environment so that more valuable and useful.

2. Methods

Identification of microbes found in kefir grain is done by diluting the storied and calculated by plate count or viable count. Then staining and identification of microbial colonies formed. The next method is experimental fermentation stage with experiments using a completely randomized design (CRD) factorial design is the first factor concentration (d), and the second factor of time (w). The pattern of 3 × 3 with 3 repetitions, with a concentration mokulum (d) respectively d1 = 25 g, d2 = 50 g, and d3 = 75 g and fermentation time (w) respectively w1 = 24 hours, w2 = 48 and w3 = 72 hours. Kefir was prepared by incubating milk with kefir grains to form two layers. Then do the filtering to be produced whey kefir. Analysis of nutrient content were analyzed by proximate based on a modified method of AOAC (Association of Official Agricultural Chemists) (1990) based on the concentration and time to determine the nutrient content, especially protein, fat, carbohydrates, fiber, and lactic acid found in kefir whey [19][9]. Based on data the nutrient content of the proximate analysis statistically tested by analysis of variance (ANOVA) and if there are differences, was tested further by Duncan Multiple Range Test with significance level of 5% [9,10]. Testing the activity of milk kefir whey as anti-odor petai performed in vitro and in vivo using quasi-experimental method with respondents as many as 34 people consisting of 17 people with each experimental group and control group. On testing in vitro, briefly made by reacting fruit extracts petai fresh milk kefir whey then measured levels of odor using the tool breath checker Tanita HC-212M after incubation 5, 10, 15, 30 and 60 minutes respectively performed triplo. In in vivo testing, any type and number of respondents consumed the same food in the form of lunch menu 12-13 spoon coupled with petai seed of the board, either the experimental group or the control group. In the experimental group were given further treatment in the form of whey drink kefir dairy in the fifth minute after eating petai fruit. Measuring the level of bad breath is done at minute 0, 5, 10, 15, 30, 60, and 120 after drinking kefir whey repetitions three times using breath checker Tanita HC-212M. The level of bad breath is recorded and then recorded on observation sheets. The same measurements were also conducted on the control group. The data were then processed using the SPSS 20.0 for paired t-test at level $\alpha = 0.05$.

3. Results and Discussion

3.1. Results identification of microorganisms on grain kefir

Kefir grain inoculum containing microorganisms 1.0×10^6 cfu/g. Types of microorganisms found in kefir grain isolates are some *Lactobacillus* genus of gram-positive and some types of yeast.

3.2. Optimum conditions fermented milk cow with kefir grain

Fermented cow's milk with kefir grain on the dose and time of fermentation obtained the data volume and clarity whey obtained, as presented in Table 1.

Table 1. The volume and clarity Whey on Concentration and Fermentation Time

Fermentation Time	Volume *Whey* (mL) in Concentration of Grain				whey position
(hour)	2.5%	5%	7.5%	Assertion.	
24	96.33	188.33	138.67	clear	under
48	208.67	145.33	139.00	rather muddy	under
72	211.67	177.00	168.67	muddy	amid

The data in Table 1 shows that the average volume of whey produced the most numerous and clear on a 24-hour fermentation, kefir grain concentrations of 5% and 48-hour fermentation with kefir grain concentration of 2.5%.

3.3. Proximate test results of kefir whey

Based on starter concentration and fermentation time was obtained of the following data in figure 1.

3.3.1. Total protein. Protein test is performed to determine the protein content of milk whey based kefir starter concentration and fermentation time, are presented in Table 2.

Table 2. Dwi Directions Concentration (d) and Time (w) Protein

treatment	w_1	w_2	w_3	total	average
d_1	1.66	1.81	1.95	5.42	0.60
d_2	1.90	1.93	2.14	5.97	0.66
d_3	2.75	2.84	3.20	8.79	0.98
Total	6.31	6.58	7.29	20.18	
Average	0.70	0.73	0.81		

The treatment resulted in the highest protein concentration of 75 g starter (d3) and fermentation time 72 hours (w3) significantly (α <0.05) higher protein content than other treatments. Results of analysis of variance showed starter concentration of 75 g (d3) and kefir whey fermentation time of 72 hours (w3) significantly (α <0.05) on protein content, but there is no interaction between the concentration of starter fermentation time.

3.3.2. Total fat. Fat testing was conducted to determine the fat content of milk whey based kefir starter concentration and fermentation time, are presented in Table 3.

Table 3. Dwi Directions Concentration (d) and Time (w) Fat

treatment	w1	w2	w3	total	average
d1	3.89	4.35	3.56	11.80	1.31
d2	3.83	3.57	3.59	10.99	1.22
d3	3.22	3.14	2.52	8.88	0.99
total	10.94	11.06	9.67	31.67	
average	1.22	1.23	1.07		

The treatment resulted in the highest fat concentration of 25 g starter (d1) and fermentation time of 48 hours (w2) significantly (α <0.05) higher fat content than other treatments. Figure 3 shows that the results of analysis of variance showed starter concentration of 25 g (d1) and kefir whey fermentation time of 48 hours (w2) significantly (α <0.05) on the fat content, and there is interaction between the concentration of starter fermentation time.

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3.3.3. Total carbohydrate. Carbohydrate testing was conducted to determine the carbohydrate content milk kefir whey starter based concentration and fermentation time, are presented in Table 4.

Table 4. Dwi Directions Concentration (d) and Time (w) Carbohydrate

treatment	w1	w2	w3	total	average
d1	5.96	17.08	19.44	42.48	4.72
d2	7.14	16.43	22.11	45.68	5.08
d3	12.16	17.60	23.88	53.64	5.96
total	25.26	51.11	65.43	141.80	
average	2.81	5.68	7.27		

The treatment resulted in the highest fat concentration of 75 g starter (d3) and fermentation time 72 hours (w3) significantly ($\alpha < 0.05$) higher fat content than other treatments. Results of analysis of variance showed starter concentration of 75 g (d3) and kefir whey fermentation time of 72 hours (w3) significantly ($\alpha < 0.05$) against the carbohydrate content and there is interaction between the concentration of starter fermentation time.

3.3.3.1. Total fiber. Fiber test is performed to determine the fiber content based on the concentration of milk kefir whey starter and fermentation time, are presented in Table 5.

Table 5. Dwi Directions Concentration (d) and Time (w) Fiber

treatment	w1	w2	w3	total	average
d_1	1.71	0.79	0.86	3.36	0.37
d_2	0.34	0.58	0.45	1.37	0.15
d_3	0.51	0.27	0.20	0.98	0.11
total	2.56	1.64	1.51	5.71	
average	0.28	0.18	0.17		

The treatment resulted in the highest fiber concentration of 25 g starter (d1) and fermentation time of 24 hours (w1) significantly ($\alpha < 0.05$) higher fiber content than other treatments . Results of analysis of variance showed starter concentration of 25 g (d1) and kefir whey fermentation time of 24 hours (w1) significantly ($\alpha < 0.05$) on the fiber content, and there is interaction between the concentration of starter fermentation time.

3.4. Physico chemical test results whey kefir

Psikokimia Tests performed on kefir whey milk produced in the fermentation process includes testing the degree of acidity (pH), the determination of lactic acid levels, testing the thickness (viscosity), and density.

Table 6. Test Results Psychochemical Milk kefir whey by Dose Starter and fermentation time

Fermentation time (w)		Dose *Starter* (d) (%)		
		2.5 (d_1)	5.0 (d_2)	7.5 (d_3)
24 hours(w_1)	pH	4.107	4.409	5.207
	Lactic acid (%)	0.17	0.16	0.28
	Density (gr/m)	1.0869	1.0628	1.1038
	viscosity (cP)	7.9999	7.9362	8.232
48 hours (w_2)	pH	3.95	3.84	3.83
	Lactic acid (%)	0.27	0.31	0.33
	Density (gr/mL)	1.0430	1.0798	1.0498
	viscosity (cP)	7.19357	7.5584	7.3488
72 hours (w_3)	pH	3.94	3.88	3.91
	Lactic acid (%)	0,31	0,36	0,36
	Density (gr/mL)	1,0748	1,0669	1,0500
	viscosity (cP)	7,2009	7,5980	6,7889

3.5. Test result milk kefir whey as anti-odor petai

Testing milk kefir whey as anti-oder *petai* done both *in vitro* and *in vivo*. In *in vitro* testing, fresh extract of 0.5, 1, and 1.5 reacted *petai* grains 50 mL milk kefir whey, then shaken and incubated. We then measured the level of odor by taking 5 mL steam banana and whey extract mixture using syringe directed at Tanita breath checker HC-212M, so the smell of *petai* known level generated. The following in vitro test data is presented by the graph in Figure 6

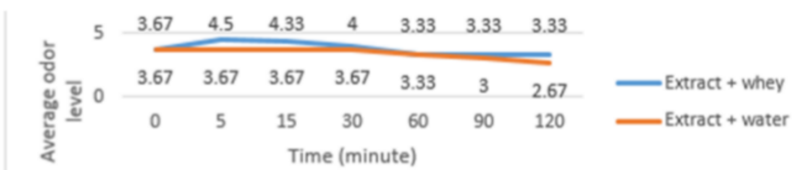

Figure 1. Graph the results of testing the level of *petai* smell in vitro on *petai* + whey extract and extract of *Petai* + water (control)

The graph in Figure shows the level of petai smell + whey extract experienced an increase in the measurement of the 5th minute after the addition of whey, but decreases gradually each time measurement start the 15th minute, 30.60, 90, until the end of the measurement minute -120 indicates the average level of odor 2.67. The average data rate measurement results petai odor in the mouth for both groups of respondents is presented in Figure 6 below. It shows the pattern of a declining trend in the level of bad breath measurement of the experimental class after eating petai grains. In the minute-0 shortly after drinking the whey, bad breath rate reached an average of 1.92, then except in the 30th minute, a decrease in the level of bad breath occurs since the 15th minute to 120, where the level of bad breath only average 0.21 range. Meanwhile, the control class, the level of bad breath at the beginning of the measurement below class average experiment, which stands at an average of 1.15 and experience the pattern of a declining trend since the minute 15 to 90, until the end of the measurement reaches the level of odor an average of 0.51, two times higher than the average level of bad breath after 120 minutes in the experimental class. In statistical data processing using paired t-test for average data rate of mouth before and after drinking milk kefir whey showed highly significant differences at the level of α 0.05. While the comparison of the level of bad breath experimental class control class shows significant value 0.079, it means very different with the confidence level α of 0.05.

Inoculum kefir grains have fulfilled the requirements as a starter, which contained microorganisms 1.0×10^6 cfu/g. In order to produce lactic acid fermentation goes well, the number of lactic acid bacteria that is needed more than 10^6 cfu/mL. The population of yeast that is needed for inoculation was 106-107 cfu/mL [11]. mLactic acid is produced from the breakdown of lactose and sucrose through carbohydrate metabolism. Under optimal conditions Lactic Acid Bacteria (LAB) at room temperature is able to change the 95% lactose into lactic acid [11]. The process of yeast fermentation to produce ethanol and CO2 increases, stimulating the growth of *L. bulgaricus* which produce proteolytic transform proteins into peptides and amino acids [12]. In addition, the enzyme protease causes the degradation of proteins into amino acids [13]. While whey kefir made from raw material whey (WK) to produce a final pH of 4.00 [14], of soya ranged from 4.58 to 4.65 [14]. While the raw material palm, soya and whey are respectively 3.21; 3.11 and 3.01 [14]. The test data rate of bad breath after drinking milk kefir whey, decreased so that only an average of 0.21 after 120 minutes of application. Bad breath after eating petai prevalent even until two days later [7]. A very distinctive smell on banana caused organo sulphur polysulfide compounds that are volatile (VSC, volatile sulphur compounds) [7]. In addition to coming from the grains of petai, VSC also commonly found in people with halitosis as a result of the growth of pathogenic bacteria in the mouth that cause an imbalance of micro flora in the mouth [7]. This is overcome by using probiotics to suppress the growth of odor-producing bacteria. Results of a study of halitosis patients who showed a decrease in VSC levels after consuming candy containing probiotics *S. salivarius* K12. [7]. It can be understood why milk kefir whey is able to reduce the level of bad breath is caused by VSC on petai grain. Special characteristics VSC on petai grain of release is determined by the pH, which at pH 3.0 VSC concentration produced only 152 ppm, almost twice lower than at pH 7 (243 ppm) [6]. This proves the role of milk kefir whey having a pH between 3.8 to 5.2 is able to reduce the level of bad breath due to the VSC derived from the petai seed. Not just the smell of banana in his mouth, even some respondents no longer find the smell of petai seed on the first urine the next day.

4. Conclusion

Based on these results a number of conclusions that the optimum conditions cow milk fermented using kefir grain starter obtained in the fermentation time of 24 hours at a concentration of 5% kefir grain. Characteristics of microbes on kefir grains include lactic acid bacteria consisting of genus *Lactobacillus* and yeast of the genus *Candida* and *Saccharomyces*. Characteristics of milk kefir whey produced at optimum fermentation condition has high levels of fat, protein, carbohydrates, fiber and lactic acid respectively 1.81; 4.35; 5.59; 0.26 and 0.16%, pH 4.4; a density of 1.0628 g / mL and 7.9368 cP viscosity. Milk kefir whey actively reduce the level of *petai* smell significantly different at the level of $\alpha = 0.05$. Further studies are recommended to analyze the changes that occur in a cyclic polysulfide organo secondary metabolites after incubation with whey milk applicate milk kefir whey as an alternative way to overcome of bad breath halitosis sufferers to determine the effective dose of milk kefir whey as anti-odor *petai*.

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