"Bioteknologi, Perubahan, dan Masa Depan"

CropLife

Program Studi S2/S3 Bioteknologi
Sekolah Pascasarjana UGM

PROSIDING

Seminar Nasional Bioteknologi IV
Universitas Gadjah Mada

BIOTEKNOLOGI, PERUBAHAN, DAN MASA DEPAN

Sekolah Pasca Sarjana UGM, 29 Oktober 2016

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Tema

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KATA PENGANTAR

Puji syukur kehadirat Tuhan Yang Maha Esa sehingga peny usunan pro sidin g semina r dapat ter selesaik an. Prosiding ini merupakan media komunikasi hasil penelitian yang telah disajikan dalam Seminar Nasional Bioteknologi IV Universitas Gadjah Mada tahun 2016. Semoga selanjutnya terwujud komunikasi yang bersinergi antara peneliti untuk memberikan sumbangsih dalam mewujudkan masa depan Indonesia yang lebih baik.

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TOTAL ASAM LAKTAT, PROTEIN, LEMAK, KARBOHIDRAT, DAN SERAT *WHEY KEFIR* SUSU SAPI BERDASARKAN KONSENTRASI *STARTER* DAN WAKTU FERMENTASI

THE TOTAL OF LACTIC ACID, PROTEIN, FAT, CARBS, AND FIBER WHEY OF COW'S MILK KEFIR STARTER BASED ON CONCENTRATION AND TIME FERMENTATION

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Abstrak

Whey kefir merupakan produk dari pembuatan kefir yang memiliki banyak manfaat karena mengandung zat-zat makanan yang mudah diserap oleh tubuh. Penelitian ini bertujuan untuk mengetahui total asam laktat, protein, lemak, karbohidrat, dan serat whey kefir susu sapi berdasarkan konsentrasi starter dan waktu fermentasi. Data eksperimen dikumpulkan dari sejumlah percobaan pada suhu kamar 25^{o}C. Metode yang digunakan metode eksperimental RAL 3 x 3 dengan 3 kali ulangan, data dianalisis dengan menggunakan analisis variansi. Hasil penelitian menunjukkan kandungan asam laktat tertinggi pada konsentrasi starter 75 g dan waktu fermentasi 72 jam (d_3w_3) sebesar 0,36 unit/ml, protein tertinggi pada konsentrasi starter 75 g dan waktu fermentasi 72 jam (d_3w_3) sebesar 1,07 unit/ml, lemak tertinggi pada konsentrasi starter 25 g dan waktu fermentasi 48 jam (d_1w_2) sebesar 1,45 unit/ml, karbohidrat tertinggi pada konsentrasi starter 75 g dan waktu fermentasi 72 jam (d_3w_3) sebesar 7,96 unit/ml, dan serat tertinggi pada konsentrasi starter 25 g dan waktu fermentasi 24 jam (d_1w_1) sebesar 0,57 unit/ml. Dapat disimpulkan bahwa konsentrasi starter dan waktu fermentasi whey kefir berpengaruh nyata ($\alpha<0,05$) pada asam laktat, lemak, karbohidrat, dan serat, serta terdapat interaksi. Sedangkan konsentrasi starter dan waktu fermentasi berpengaruh nyata ($\alpha<0,05$) pada protein, tetapi tidak terdapat interaksi.

***Kata Kunci**: whey kefir, asam laktat, protein, lemak, karbohidrat, serat*

Abstract

Whey kefir has many benefits that contains nutrients easily absorbed by the body. This study was to determine the total of lactic acid, protein, fat, carbohydrates, and fiber whey of cow's milk kefir starter based on concentration and fermentation time. Experimental designed was RAL 3 x 3 with 3 repetitions in room temperature 25^{0} C. The result showed that the highest content of lactic acid at a concentration of 75 g starter and fermentation time of 72 hours (d_3w_3) was 0.36 unit/ml, the highest protein at a concentration of 75 g starter and fermentation time of 72 hours (d_3w_3) was 1.07 unit/ml, the highest fat at a concentration of 25 g starter and fermentation time of 48 hours (d_1w_2) was 1.45 unit/ml, the highest carbohydrate at a concentration of 75 g starter and fermentation time of 72 hours (d_3w_3) was 7.96 unit/ml, and the highest fiber at a concentration of 25 g starter and fermentation time of 24 hours (d_1w_1) was 0.57 unit/ml It concludes there was an interaction and influence significantly of the concentration of whey starter and kefir fermentation time on lactic acid, fat, carbohydrate ($\alpha <0.05$), while there was no interaction between the starter concentration and fermentation time ($\alpha <0.05$).

***Keywords:** kefir whey, lactic acid, protein, fat, carbohydrates, fiber*

Introduction

Kefir is a fermented dairy product using Lactic Acid Bacteria (LAB) such as *Lactobacillus lactis, Lactobacillus sp delbrueckiisub*. *Bulgaricus* together yeast and produce acid and ethanol. Kefir grains shaped like a bunch of tiny cauliflower, a length of about 1-3 cm, lobe-shaped, irregular blobs with white or yellowish white color and the texture is slimy but chewy. Kefir grain must be kept alive and grow by moving into fresh milk every day and allow it to grow about 20 hours, during which time period kefir grains will grow 25% more (Farnworth, 2005).

Lactic acid bacteria (LAB) plays produce lactic acid and flavor components, whereas yeast produces carbon dioxide and alcohol. That is why in addition to taste, kefir is not only sour but also there is little flavor of soda, which makes the taste more fresh (Usmiati, 2007).

Kefir has good effects on health, such as controlling cholesterol metabolism, as probiotics for animal antitumor, antibacterial, antifungal, and others (Farnworth, 2003).

Kefir contains 0.65 to 1.33 kg CO2 per liter, 3.16 to 3.18% protein, fat 3.07 to 3.175, 1.8 to 3.8 percent lactose, ethanol 0.5-1.5% and 0.7-1.0% lactic acid (Ide, 2008)

Kefir whey formed during the process of making kefir, kefir microbes produce acids and enzymes that make the milk is separated into crud and whey the clear liquid. Protein and milk fat contained in the crud while the remaining substance contained in a solution of whey kefir fractions.

Whey kefir contains 320 K Call kefir and whey kefir protein 0.8% - 1%. In addition, whey kefir containing 65% beta-lactoglobulin, alpha lakalbumin 25%, bolvine serumalbumin 8%, and imonuglobulin.

Methodology

1.Tools and materials

The tools used are: petri dish, Erlenmeyer flask, flask, test tube rack, test tubes, pipettes volumetric, pipette, an analytical balance, a measuring cylinder 100 cc, incubators, refrigerators, burette, light spirits, electric cookers, cotton, lighters, label paper, scissors, pH meter, magic com, the volume of 1000 ml plastic jar, funnel, stirrer, alcohol 70%, rubber, plastic hose diameter of 0.5 cm. As for the material used as follow.

a. Materials used in the research are pasteurized cow's milk from Lembang
b. Isolates Lactic acid bacteria (LAB) in the form of home of kefir of kefir grain.

2. Research methods

Making kefir by way of incubating milk with kefir grains to form two layers. Then do the filtering kefir to kefir whey kefir produced were then tested proximate based on the concentration and time to determine the nutrient content, especially protein, fat, carbohydrates, fiber, and lactic acid found in kefir whey kefir. Method is experimental stage with trials using Completely Randomized Design (CRD - in English abbreviation) factorial design is the first factor concentration d, and the second factor of time w. The pattern of 3 x 3 with three 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. The parameters measured were the nutritional content of kefir. While whey kefir fermentation product is lactic acid, protein, fat, carbohydrates, and fiber.

3. Data analysis

This nutrient content data based on the proximate analysis were statistically tested by analysis of variance ANAVA and if there are differences, tested further by Duncan Multiple Range Test with significance level of 5% Sudjana, 1995).

Table 1. Combination Treatment Kefir Fermented Milk of *Whey kefir* Based *Starter* of concentration and Fermentation Time

Concentration *Starter* (d) / Time of Permentation (w)	2,5 % (d_1)			5 % (d_2)			7,5 % (d3)		
24 hours (w_1)	$1d_1w_1$	$2d_1w_1$	$3d_1w_1$	$1d_2w_1$	$2d_2w_1$	$3d_2w_1$	$1d_3w_1$	$2d_3w_1$	$3d_3w_1$
48 (w_2)	$1d_1w_2$	$2d_1w_2$	$3d_1w_2$	$1d_2w_2$	$2d_2w_2$	$3d_2w_2$	$1d_3w_2$	$2d_3w_2$	$3d_3w_2$
72 hours (w_3)	$1d_1w_3$	$2d_1w_3$	$3d_1w_3$	$1d_2w_3$	$2d_2w_3$	$3d_2w_3$	$1d_3w_3$	$2d_3w_3$	$3d_3w_3$

Further testing is about proteins, fats, carbohydrates, and fiber in *whey kefir* through proximate analysis. Analysis of nutrient content were analyzed by proximate based on a modified method of (AOAC) Association of Official Agricultural Chemists (1990) for protein content, fat content, carbohydrate content, fiber content, and the content of lactic acid.

4. Place and time of Research

Research conducted at the Laboratory of Biology and Chemistry Laboratory in State Islamic University Sunan Gunung Djati.

Results and Discussion

1. Results

a. Total Lactic Acid

Lactic acid test is performed to determine the content of lactic acid whey kafir in milk based starter concentration and fermentation time, presented in Table 2 and Figure 1.

Table 2. Statistical Analysis Lactic Acid

doses	time	examination			number	mean	average	total
		1	2	3				
d1	w1	0,16	0,17	0,18	0,51	0,17		
d1	w2	0,28	0,26	0,28	0,82	0,27	0,25	2,25
d1	w3	0,26	0,36	0,30	0,92	0,31		
d2	w1	0,15	0,17	0,16	0,48	0,16		
d2	w2	0,32	0,28	0,32	0,92	0,31	0,27	2,46
d2	w3	0,35	0,37	0,34	1,06	0,35		
d3	w1	0,29	0,28	0,27	0,84	0,28		
d3	w2	0,35	0,31	0,33	0,99	0,33	0,3244	2,92
d3	w3	0,39	0,34	0,36	1,09	0,36		

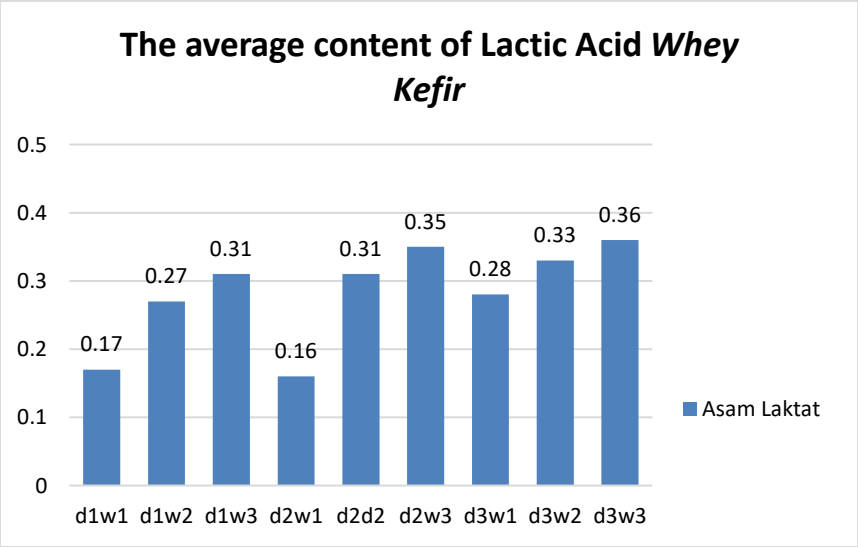

Figure1. Bar Graph Showing the Average Content of Lactic Acid *Whey Kefir* Starter Based Concentration and Fermentation Time

Table 3. Dual Direction Concentration (d) and Time (w) Lactic Acid

Treatment	w1	w2	w3	Amount	Average
d1	0,51	0,82	0,92	2,25	0,25
d2	0,48	0,92	1,06	2,46	0,27
d3	0,84	0,99	1,09	2,92	0,32
total	1,83	2,73	3,07	7,63	
average	0,20	0,30	0,34		

Treatment that produces lactic acid *starter* is the concentration of 75 g (d3) and incubated for 72 hours (w3) significantly, d3w3 is higher lactic acid content compared to other treatments.

Table 4. Analysis of Variance

source of diversity	db	JK	KT	Fhit	F Table 0,05	F Table 0,01	Information.
treatments	8	0,12819					
concentration (d)	2	0,02610	0,01305	24,63636	3,55	6,01	Sig. different
time (w)	2	0,09123	0,04561	86,12587	3,55	6,01	Sig. different
interaction (dw)	4	0,01086	0,00271	5,12587	2,93	4,58	Sig. different
errors	18	0,00953	0,00053				
total	26	0,14					

Results of analysis of variance shows a concentration of 75 g starter (d3) and whey kefir fermentation time of 72 hours (w3) are significant ($\alpha < 0.05$) the content of lactic acid, and there are interactions between starter concentration and
Fermentation time

b. Total Protein

Tests conducted to determine the protein content of *whey kefir* milk which is based on the concentration of *starter* and fermentation time, presented in Table 5 and Figure 2.

Table 5. Statistical Analysis of Protein

doses	time	examination			amount	mean	average	total
		1	2	3				
d1	w1	0,54	0,61	0,51	1,66	0,55		
d1	w2	0,62	0,65	0,54	1,81	0,60	0,60	5,42
d1	w3	0,64	0,76	0,55	1,95	0,65		
d2	w1	0,75	0,63	0,52	1,9	0,63		
d2	w2	0,69	0,61	0,63	1,93	0,64	0,66	5,97
d2	w3	0,77	0,68	0,69	2,14	0,71		
d3	w1	0,91	0,89	0,95	2,75	0,92		
d3	w2	0,89	0,97	0,98	2,84	0,95	0,98	8,79
d3	w3	0,99	1,12	1,09	3,2	1,07		

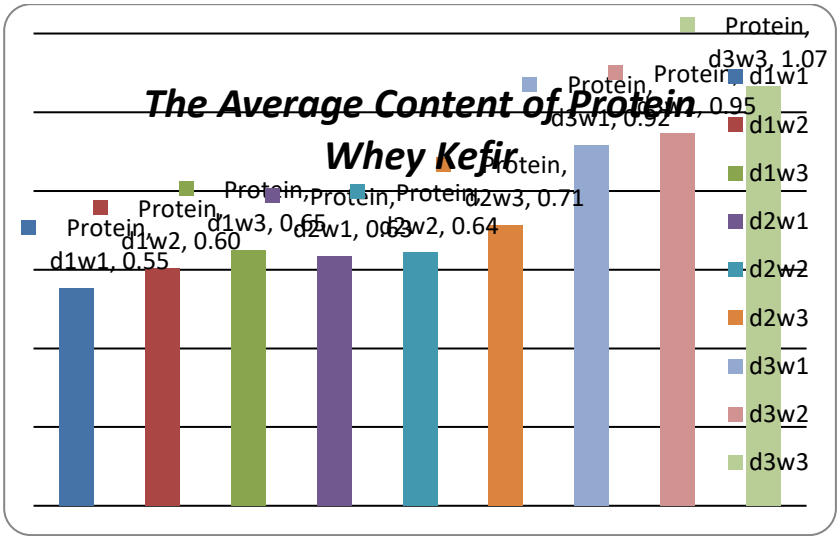

Figure 2. Graph that shows average *Whey kefir* on content of protein
Based on the concentration of *Starter* and Fermentation Time

Table 6. Dual Direction of Concentration (d) and Time (w) Protein

treatment	w_1	w_2	w_3	amount	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		

Treatment that produces the highest protein concentration starter is 75 g (d_3) and fermentation time 72 hours (w_3) significantly, d3w3 is higher protein content than other treatments.

Table 7. Analysis of Variance

source of diversity	Db	JK	KT	Fhit	F Table 0,05	F Table 0,01	information
treatment	8	0,79					
concentration(d)	2	0,73	0,36	77,15	3,55	6,01	Sig. different
time (w)	2	0,06	0,03	6,05	3,55	6,01	Sig. different
Interaction (dw)	4	0,01	0,00	0,33	2,93	4,58	Not sig. different
errors	18	0,08	0,00				
total	26	0,87					

Results of analysis of variance show a concentration of 75 g starter (d3) and whey kefir 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.

c. Total Fat

Fat test is performed to determine the fat content in milk of *whey kefir* which is based on the concentration of *starter* and fermentation time, presented in table 8 and Figure 3.

Table 8. Statistical analysis Fat

doses	time	experiment 1	2	3	amount	mean	average	total
d1	w1	1,33	1,3	1,26	3,89	1,30		
d1	w2	1,46	1,42	1,47	4,35	1,45	1,31	11,8
d1	w3	1,21	1,18	1,17	3,56	1,19		
d2	w1	1,27	1,22	1,34	3,83	1,28		
d2	w2	1,16	1,18	1,23	3,57	1,19	1,22	10,99
d2	w3	1,17	1,19	1,23	3,59	1,20		
d3	w1	1,02	1,09	1,11	3,22	1,07		
d3	w2	1,03	1,05	1,06	3,14	1,05	0,99	8,88
d3	w3	0,84	0,83	0,85	2,52	0,84		

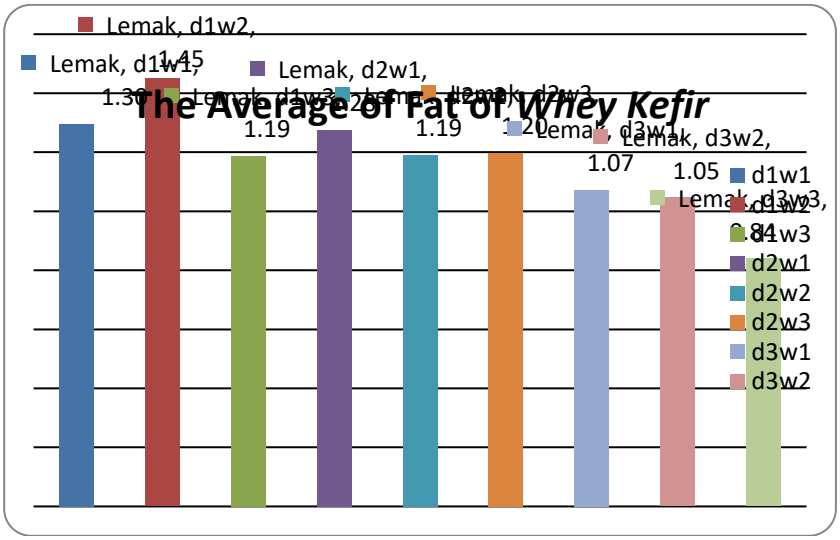

Figure 3. Graph that shows average Fat Whey Kefir Based on the concentration of Starter and Fermentation Time

Table 9. Dual Direction Concentration (d) and time (w) Fat

treatment	w1	w2	w3	amount	average
d_1	3,89	4,35	3,56	11,80	1,31
d_2	3,83	3,57	3,59	10,99	1,22
d_3	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 concentration of fat 25 g starter (d1) and fermentation time of 48 hours (w2) which is significantly d1w2 higher fat content than other treatments.

Table 10. ***Sidik Ragam***

source of diversity	db	JK	KT	Fhit	F Table		information
					0,05	0,01	
treatment	8	0,72					
concentration (d)	2	0,50	0,25	210,41	3,55	6,01	Sig. different
time (w)	2	0,13	0,07	54,93	3,55	6,01	Sig. different
interaction (dw)	4	0,08	0,02	17,70	2,93	4,58	Sig. different
errors	18	0,02	0,00				
Total	26	0,74					

The analysis of variance shows a concentration of 25 g *starter* (d1) and whey kefir fermentation time of 48 hours (w2) significant effects to (α <0.05) fat content, and there is interaction between the concentration of *starter* fermentation time.

d. Total Carbohydrates

Tests of carbohidrates conducted to determine the content of carbohydrates in milk whey kefir based on *starter* concentration and fermentation time, presented in Table 11 and Figure 4.

Table 11. Statistical Analysis of Carbohydrates

doses	time	experiment			number	main	average	total
		1	2	3				
d1	w1	1,76	1,88	2,32	5,96	1,99		
d1	w2	5,69	5,68	5,71	17,08	5,69	4,72	42,48
d1	w3	6,43	6,49	6,52	19,44	6,48		
d2	w1	2,29	2,42	2,43	7,14	2,38		
d2	w2	5,35	5,57	5,51	16,43	5,48	5,08	45,68
d2	w3	7,34	7,45	7,32	22,11	7,37		
d3	w1	4,01	3,83	4,32	12,16	4,05		
d3	w2	5,72	5,92	5,96	17,6	5,87	5,96	53,64
d3	w3	7,91	7,98	7,99	23,88	7,96		

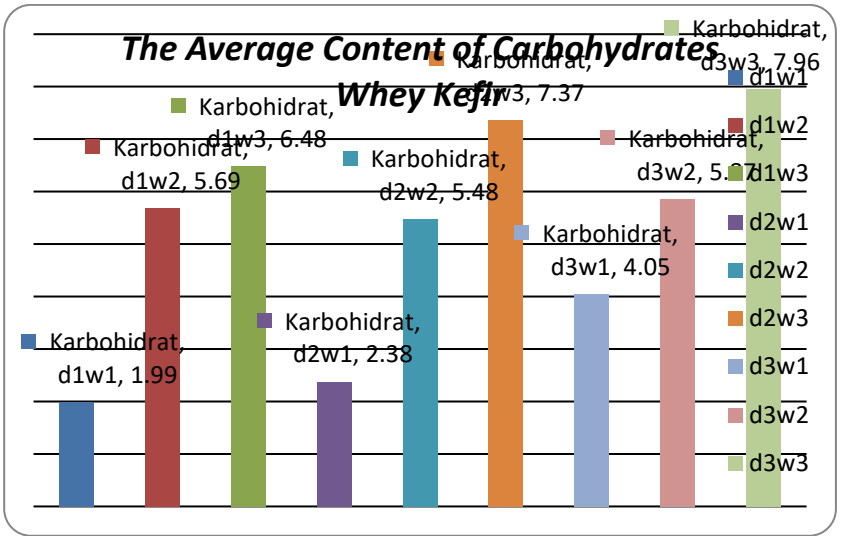

Figure 4. Chart showing Carbohydrate
Whey Kefir *Starter* Based on Concentration and Fermentation Time

Table 12. Dual Direction Concentration (d) and time (w) Carbohydrates

treatment	w_1	w_2	w_3	number	average
d_1	5,96	17,08	19,44	42,48	4,72
d_2	7,14	16,43	22,11	45,68	5,08
d_3	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 produces the highest concentration of carbohydrates 75 g *starter* (D3) and fermentation time of 72 hours (w3) d3w3 significant effect to higher carbohydrate content than other treatments.

Table 13. *Sidik Ragam*

source of diversity	db	JK	KT	Fhit	F Table 0,05	F Table 0,01	information
treatment	8	102,89					
concentration (d)	2	7,34	3,67	171,05	3,55	6,01	Sig. different
Time (w)	2	92,11	46,05	2146,85	3,55	6,01	Sig. different
interaction (dw)	4	3,45	0,86	40,17	2,93	4,58	Sig. different
errors	18	0,39	0,02				
Total	26	103,28					

The analysis of variance shows a concentration of 75 g starter (D3) and *whey kefir* fermentation time of 72 hours (w3) significant effect to (α <0.05) against the carbohydrate content and the interaction between the concentration of *starter* fermentationtime.

e. Total Fiber

Testing of fiber is performed to determine the fiber content of *whey kefir* in milk *starter* based on concentration and fermentation time, presented in Table 14 and figure 5.Table 14. Statistical Analysis of Fiber

doses	time	experiment			number	main	average	total
		1	2	3				
d1	w1	0,57	0,56	0,58	1,71	0,57		
d1	w2	0,29	0,24	0,26	0,79	0,26	0,37	3,36
d1	w3	0,27	0,28	0,31	0,86	0,29		
d2	w1	0,09	0,14	0,11	0,34	0,11		
d2	w2	0,21	0,18	0,19	0,58	0,19	0,15	1,37
d2	w3	0,14	0,16	0,15	0,45	0,15		
d3	w1	0,16	0,14	0,21	0,51	0,17		
d3	w2	0,07	0,12	0,08	0,27	0,09	0,11	0,98
d3	w3	0,06	0,07	0,07	0,2	0,07		

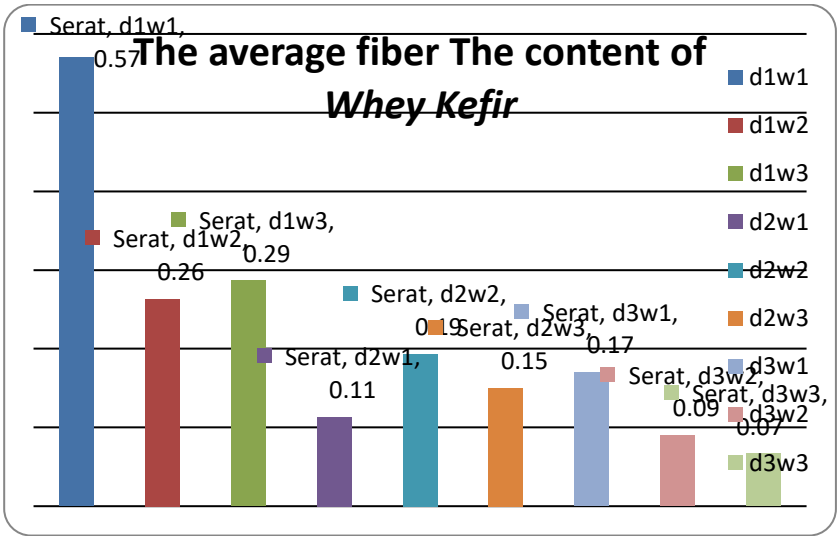

Figure 5. Graph showing Fibre *Whey kefir* Based on concentration and time *starter* Fermentation

Tabel 15. Dual Direction Concentration (d) and time (w) Fiber

treatment	w1	w2	w3	number	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 produces the highest concentration of fiber 25 g starter (d1) and fermentation time with 24 hours (w1) d1w1 significant effect to higher fiber content than other treatments.

Table 16. ***Sidik Ragam***

source of diversity	db	JK	KT	Fhit	F Table 0,05	F Table 0,01	Ket.
treatment	8	0,56					
consentration (d)	2	0,36	0,18	391,06	3,55	6,01	Sig. different
Time (w)	2	0,07	0,04	78,63	3,55	6,01	Sig. different
interaction (dw)	4	0,13	0,03	69,82	2,93	4,58	Sig. different
errors	18	0,01	0,00				
total	26	0,57					

The analysis of variance shows a concentration of 25 g *starter* (d1) and *whey kefir* fermentation time of 24 hours (w1) significant effect to (α <0.05) on the fiber content, and there is interaction between the concentration of *starter* fermentation time.

2. Discussion

The content of lactic acid in the milk whey kefir relatively rise (Figure 1). It is caused by acid-producing microbial activity that converts carbohydrates (lactose) into lactic acid. According to Magalhaes et al. (2011), lactic acid is produced from the breakdown of lactose and sucrose metabolism of carbohydrates. Under optimal conditions lactic acid bacteria (LAB) at room temperature is able to change 95% of glucose into lactic acid.

According to Ide (2008), kefir has the acidity range of 0.85% to 1%. The increase in total acid kefir is caused by BAL and mutually beneficial yeast. During the fermentation of lactose BAL changes into lactic acid, to produce ethanol.

The process of fermentation of yeast to produce ethanol and CO2 increases, stimulated the growth of L. bulgaricus which produce proteolytic change proteins into peptides and amino acids (R., et al., 1992). In addition, the enzyme causes the degradation of proteins into amino acids (Cahyadi, 2006). This causes the protein content of the whey milk kefir relative increase (Figure 2). According to Buckle et al., (2007) the contents of protein is dissolvedin whey ranges between 0.5-0.7%. Bahar (2008) states that kefir seeds containing khamir with protein content of about 40-60%.

Then the fermentation process causes the fat content decreased (Figure 3). Savitri (1996) states that the longer the fermentation, the proliferation of BAL will rise and cause lipase produce more fat until the hydrolysed also more and more, it could lead to a decrease in fat content.

Unlike fat, carbohydrate content is relatively increased. It is caused by the activity of the yeast enzyme that converts maltose carbohydrates into maltose. In the process of fermentation kefir, yeasts/ khamir and LAB change of complex organic molecules such as proteins, carbohydrates, and fats into simpler compounds that are soluble and high digestibility.

Conclusion

The content of lactic acid starter highest concentration of 75 g and fermentation time 72 hours (d3w3) of 0.36 units / ml. The highest protein contents of 75 g starter concentration and fermentation time of 72 hours (d3w3) of 1.07 units / ml. The highest fat contents at a concentration of 25 g starter and fermentation time of 48 hours (d1w2) was 1.45 units / ml. The highest concentration of carbohydrate is 75 g starter and fermentation time of 72 hours (d3w3) of 7.96 units / ml. The highest fiber contents of 25 g starter concentration and fermentation time of 24 hours (d1w1) of 0.57 units / ml. The

concentration of starter and fermentation time on whey kefir significantly effects (α <0.05) in lactic acid, fat, carbohydrates, and fiber, and there is interactions. The starter concentration and fermentation time significantly effects (α <0.05) in protein, but there is no interaction.

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