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# Quality of Kefir Combination Between Soy Milk and Skim Milk On Variation of Sugar and Fermentation Duration

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#### **ABSTRACT**

#### **KEYWORDS:**

Fermentation duration Kefir Organoleptic Skim Milk Soy Milk

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Kefir is one of the functional beverages that has an acidic taste due to the activity of lactic acid during the fermentation process. One of the ingredient innovations for kefir was soy milk, skim milk and variations of sugar types. Soy milk protein has an amino acid structure similar to cow's milk, so it is very good for people with lactose intolerance. The aim of this study is to know the quality (total of lactic acid and the organoleptic quality) of the kefir combination between soy milk and skim milk on the variation of sugar and fermentation duration. The study used experimental methods and complete random design (CRD) with two factors. Factor I was the sugar variation (N) palm sugar and white sugar. Factor II was the variation of fermentation duration (K) 24 hours, 36 hours, and 48 hours. The results of the study showed that the lowest lactic acid of kefir combination between soy milk and skim milk was 0.896 % on N2K1 treatment (white sugar + 24-hour fermentation duration). The best organoleptic quality of kefir combination between soy milk and skim milk was N2K1 white sugar + 24-hour fermentation duration) in white color, sour taste, and soft texture.

# 1. INTRODUCTION

Functional beverages are one of the types of beverages that give a positive influence on the health of the body for those who consume them because they contain elements of nutrients and non nutrients (Al Fath, 2021). Functional beverages are supplemented with tertiary functions such as probiotics, adding to the intake of certain vitamins and minerals, increasing the body's stamina, and reducing the risk of certain diseases (Widyantari, 2020). Functional beverages should fulfill two main food functions, namely to provide nutritional intake and sensory loading such as good taste and texture (Herawati, 2012). One of the functional beverage products is kefir. Kefir is one of the functional beverages with the basic ingredients of animal milk that has an acidic taste due to the activity of lactic acids formed during the fermentation process to preserve products and give a taste or flavor to certain food products that are expected to improve the nutritional quality of the products (Aryanta, 2021). Kefir contains proteins with complete essential amino acids, vitamin A, vitamin B1, vitamin B2, vitamin B5, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin K, potassium, calcium, phosphorus, magnesium, iron, zinc, copper, and manganese (Lararenjana, 2020). Kefir has a total acidity of about 0.5 % to 2.0 %, a pH of 4.6, and a protein content of 3.2 % (SNI, 2009). Kefir can be made from natural materials that are easily obtained in everyday life, such as plant and animal materials (Fortin, 2021).

Soy milk is a beverage made from yellow beans that are yellow-white and similar to milk (Arianty, 2019). The nutritional content of soy milk per 100 g of soybeans is 44 calories, 3.6 g of protein, 2 g of fat, and 2.9 g of carbohydrates (Sugiarsih, 2022). Soy milk protein has an amino acid structure similar to cow's milk, so it is very good to consume for those who are allergic to dairy, lactose intolerance (Alfiyah, 2017). Soy milk contains carbohydrates including oligosaccharides that can be indirectly digested into kefir because it is difficult to be used by starter cultures as an energy source and a source of carbon. Therefore, it is necessary to add other sources of carbs such as lactose that will be utilized by the Lactate Acid Bacteria (BAL) as a source of energy and nutrients during its growth in the kefir fermentation process (Rossi, 2016).

Skim milk is milk made by reducing the amount of water and existing fat. The low fat content of skim milk can be replaced by its deficiency because it has a high enough protein content, lactose, and minerals. (Afrizal, 2019). According to Mandei (2019) skim milk has a fat content of 0.50 %, protein of 1.10 %, ashes of 0.68 %, acidity of 0.8 %, and is a source of nitrogen to carry out the growth of microorganisms during fermentation. The use of skim milk plays an important role in the manufacture of probiotic beverages in order to obtain the amount of bacteria that meets the standards (Hakiki, 2022). Adding skim milk can increase acidity and lower pH (Septiani, 2013). The Lestari study (2021) also stated that the addition of skim milk is aimed at increasing the protein and carbohydrate substrate, thus supporting the growth of kefir microorganisms.

Factors affecting the quality of kefir are kefir seeds, milk types, sugar types, time, temperature, and fermentation duration (Gamba, 2020). Sugar is one of the simple carbohydrates that is a major source of energy and a commodity. Sugar is used to change the taste into sweet and the condition of food or beverage (Mahfud, 2018). Sugar can be in the form of particles, flour, liquid, and solid. The color can be white or brown with a sweet taste. The types of sugar circulating in society are white sugar, brown sugar, and liquid sugar (Rahmi, 2020). Palm sugar contains approximately 84 % more sucrose compared to cane sugar and beet sugar which are only 20 % and 17 % respectively so palm sugar is able to provide higher energy than beet sugar and cane sugar (Lempang, 2020). In the fermentation of kefir, sugar is used as an energy source and a source of carbon for cell biosynthesis especially for glycolysis cycles (Gunawan et al., 2015). Fermentation time is one of the factors in the process of making kefir. As the fermentation duration increases, there is more and more time available for lactic acid bacteria to refine the nutrients contained in the substrate which can allow the accumulation of organic acids such as lactic acids in more amounts (Pangaribuan, 2022). Lestari study (2018) found that different fermentation duration influenced the levels of acidity, viscosity, and alcohol levels in which the yield rate increased as the fermention duration increased. Therefore, this study aims was to determine the Quality of Kefir Combination Between Soy Milk and Skim Milk On Variation of Sugar and Fermentation Duration.

## 2. MATERIALS AND METHOD

The study was conducted in February – June 2023 at the Biology Laboratory of the Faculty of Keguruan and Education Sciences of the Muhammadiyah University of Surakarta. Testing of pH levels and organoleptic quality (taste, colour, flavour, texture, and acceptivity) at the Muhammadiyah Surakarta University Biological Laboratory, and total of lactic acid testing was carried out at the Pratama Yogyakarta Chem Mix Laboratory.

# 2.1. Materials

The ingredients used in the manufacture of kefir combination between soy milk and skim milk is namely soymilk, skim milk (greenfields), palm sugar, white sugar, and kefir grain.

#### 2.2. Research Procedure

The research process includes: 1. The preparatory stage by preparing the tools and materials to be used for research 2. Sterilize equipment using hot water for 10 minutes 3. The stage of aplication first, mixing soy milk and skim milk then pasteurized, then put the white sugar and palm sugar into the mixture of soy milk and skim milk while stired until evenly mixed, then put the mixture of ingredients into the jar in a cold state, then put the grain kefir into the jar according to the measure, then close the jar and store at room temperature, then kefir harvesting is adjusted to the fermentation duration, and then the harvested kefir was put into the refrigerator to further test the organoleptic quality of the 15 panelists.

# 2.3. Research Design

The study used experimental methods and complete random design (CRD) with two factors. Factor 1 was the type of sugar (N1= palm sugar 16 g, N2= white sugar 30 g) and factor 2 was the fermentation duration (K1 = 24 hours, K2= 36 hours, K3= 48 hours). Testing the total of lactid acid content by titration method and presenting it in the form of a table with the final result in percentage form and performing organoleptic tests (taste, colour, flavour, texture, and acceptivity) with a questionnaire sheet of 15 panelists.

# 2.4. Data Analysis

Research data were presented in the form of tables. Total of lactid acid testing analysis using quantitative descriptive methods. During organoleptic testing, taste, colour, flavour, texture, and acceptivity. Quantitative test data analysis using a two-way variance analysis test (Two Way ANOVA) and qualitative test data analysis using Excel.

## 3. RESULTS AND DISCUSSION

#### 3.1. Result

Based on the study of pH and total of lactid acid kefir combination between soy milk and skim milk on variation of sugar and fermentation duration that have been performed from six samples obtained the following results:

**Table 1.** Results of pH and total of lactid acid kefir combination between soy milk and skim milk on variation of sugar and fermentation duration.

	Treatment	pН	Total of lastid	
Number		Before the fermentation	After the fermentation	Total of lactid acid (%)
1	N1K1 palm sugar 24 hours	6.55	3.83	1.079
2	N1K2 palm sugar 36 hours	6.65	3.74	1.192
3	N1K3 palm sugar 48 hours	6.68	3.62	1.337
4	N2K1 white sugar 24 hours	6.88	4.48	0.896
5	N2K2 white sugar 36 hours	6.86	4.25	1.057
6	N2K3 white sugar 48 hours	6.71	3.68	1.151

Based on table 1, the highest total acid content in kefir combination between soy milk and skim milk in the N1K3 treatment showed an average of 1.337 %, while the lowest total acid content

in kefir in the N2K1 treatment showed an average of 0.896 %. Based on the results of the Two Way Annova statistical test, which showed sig 0.001 <0.05 that there was a significant effect on the total sugar variation and fermentation time. The results of Duncan's further test showed that the fermentation time of 48 hours had a significant effect on the total acidity of the kefir combination of soy milk and skim milk.

Based on the research of organoleptic quality kefir combination between soy milk and skim milk on variation of sugar and fermentation duration that has been performed from six samples obtained the following results:

**Table 2.** Result of organoleptic quality kefir combination between soy milk and skim milk on variation of sugar and fermentation duration.

No	Treatment	Organoleptic Quality					
		Taste	Color	Flavour	Texture	Acceptivity	
1	N1K1 palm sugar 24 hours	Sour	Young Chocolate	Less Tasteful	Softly	Less Like	
2	N1K2 palm sugar 36 hours	Sour	Young Chocolate	Tasty	Softly	Less Like	
3	N1K3 palm sugar 48 hours	Sour	Young Chocolate	Less Tasteful	Softly	Less Like	
4	N2K1 white sugar 24 hours	Sour	White	Tasty	Softly	Like	
5	N2K2 white sugar 36 hours	Sour	White	Tasty	Softly	Like	
6	N2K3 white sugar 48 hours	Sour	White	Less Tasteful	Softly	Less Like	

Based on table 2, the organoleptic quality of kefir combination between soy milk and skim milk showed a sour taste in all treatments, the color of kefir in treatment N1 was light brown and in treatment N2 it was white. The aroma of kefir in the N1K1, N1K3, and N2K3 treatments was not pleasant, while the N1K2, N2K1, and N2K2 treatments were pleasant. The texture of kefir on all treatments was soft, while the panelists acceptability on all treatments did not like it except for the N2K1 and N2K2 treatments, they liked it.

# 3.2. Discussion

# 3.2.1. Total of Lactid Acid

Table 1 showed best total of lactid acid value on N2K1 treatment (white sugar + 24 hours fermentation duration) was 0.89 %. The total result of the total of lactid acid treatment of N2K1 in accordance with SNI (2009) was 0.5 - 2.0 %. The decrease in total of lactid acid output was affected by bacterial activity. Bacterial activity is influenced by the available energy sources. The more energy sources available, the more bacterial activity will increase, so that the acid produced also greatly affects the decrease in the pH of kefir. In Table 1, the total acidity of palm sugar is higher than that of white sugar. This is because the content of palm sugar is higher than white sugar, so the bacterial activity in the kefir with added palm sugar was higher than white sugar. The highest sucrose content was found in palm sugar of 84.31% compared to cane sugar of 71.89 % (Yudho, 2021). In the study Widya et al (2018) also stated palm sugar contains higher sucrose 84 % compared to cane sugar which is 20 %. Palm sugar contains approximately 84% more sucrose compared to cane sugar and beet sugar, so palm sugar is able to provide more energy than cane sugar and beet sugar (Lempang, 2020). The increase in the amount of BAL occurs because the availability of substrate in the fermentation media increases with the increase in sucrose concentration. The more the amount of LAB, the more the total acid produced (Indriasari, 2022). In the study Nisa et al (2018) also stated that lactic acid bacteria use sugar as a source of growth energy and produce metabolites in the form of lactic acids during fermentation. In the study Pangaribuan (2022) it was stated that the length of fermentation also affects the resulting pH. As the fermentation time increases, more and more time is available for lactic acid bacteria to refine the nutrients contained in the substrate which can allow more organic acids such as lactic acids to accumulate. This is demonstrated in the long-term treatment of the 48 – hour fermentation which produced the highest total acid compared to the 24 – hour and 36 – hour processings.

# 3.2.2. Organoleptic Quality

Based on Table 2 (results), it shows that in terms of flavor, the kefir combination between soy milk and skim on treatment N1K1 (palm sugar + 24 hours), N2K2 (palm sugar + 36 hours), N3K3 (palm sugar + 48 hours), N2K1 (white sugar + 24 hours), N2K2 (white sugar + 36 hours) and N2K3 (white sugar + 48 hours) have a sour taste. The results of organoleptic quality treatment N2K1 in accordance with SNI (2009) indicate that the kefir beverage should have a distinctive acidic flavor, a fluid appearance, and a normal flavour. The kefir combination between soy milk and skim milk has a characteristic acidic taste. The feeling of sour in kefir is caused by the activity of lactic acid bacteria which produce primary metabolites and lower the pH (Rahmiati & Mumpuni, 2017). The sensory value of the flavor of the panels is related to the pH produced by kefir, the more acidic it is. The lower the pH of kefir, the more sour the panelis feel. The color of kefir combination between soy milk and skim on the treatment N1K1 (palm sugar + 24 hours), N1K2 (palm sugar + 36 hours) and N1K3 (palm sugar + 48 hours) have the same color which is brown white. Brown-white can appear due to the basic ingredients of palm sugar. At the treatment N2K1 (white sugar + 24 hours), N2K2 (white sugar + 36 hours) and N2K3 (white sugar + 48 hours) have the color white. This is in line with Mandang study (2016) stated that the difference in color kefir soy milk is due to the presence of fat and solid content in milk. Research by Jaya et al (2017) also revealed that the type of sugar will affect the color of kefir. When whey kefir milk is added, a combination of types of sugar with different concentrations will produce a brown color. The brown color appears because of the base ingredients of the palm sugar used, so the color of whey kefir tends to be brown.

Based on Table 2 (result) shows that in terms of flavour, the average kefir combination between soy milk and skim milk has a tasty flavour. According to a Medi study (2023) it is stated that the interaction of the type of sugar affects the aroma of whey because during the fermentation of the kefir it will produce the smell of alcohol. The flavor that resembles tape is due to the presence of high alcohol and esters, so both palm and tea sugar will produce an acidic flavour and cover the characteristic flavour of raw materials before fermentation. The acid flavour produced by kefir is due to the presence of lactic acid present in kefir. This is consistent with the statement of Musdholifah et al (2016) that the flavour in kefir is caused by the formation of volatile compounds during the fermentation process, thus generating a characteristic acid flavour. The volatile compounds found in kefir are lactic acid, acetic acid, and alcohol. This is supported by a statement by Lestari et al (2018) that stated that the volatile compounds in kefir affect the sharpness of the flavour of kefir where the higher the content of the volatile compound in the food, the sharper the flavour of the kefir. The kefir combination between soy milk and skim milk on treatments N1K1 (palm sugar + 24 hours), N2K2 (palm sugar + 36 hours), N3K3 (palm sugar + 48 hours), N2K1 (white sugar + 24 hours), N2K2 (white sugar + 36 hours) and N2K3 (white sugar + 48 hours) the same texture is soft. Kefir composition of soy milk and skim milk has a soft texture due to its low flavor content, which is consistent with the results of the Nurkhoeriyati (2017) study which stated that the sourness flavor of soy kefir is lower compared to commercial kefir. This is because soy milk have a lower fat content than animal milk. Skim milk also affects the texture produced by kefir. This is in line with Lestari's study (2021) which states that the higher the total kefir solidity of corn milk, the thicker the texture of the kefir milk products produced. The kefir combination between soy milk and skim milk on treatment N1K1 (palm sugar + 24 hours), N2K2 (white sugar + 36 hours) and N2K3 (white sugar + 48 hours) have less like acceptifity. This was caused by a too acidic taste and a too sharp smell. Kefir combination between soy milk and skim on treatment N2K1 (white sugar + 24 hours), N2K2 (white sugar + 36 hours) has a acceptifity like.

## 4. CONCLUSION

It can be concluded the lowest lactic acid and best organoleptic quality of kefir combination between soy milk and skim milk was 0.896 % in white color, sour taste, and soft texture on N2K1 treatment (white sugar + 24-hour fermentation duration) where according to SNI 2009.

#### 5. ACKNOWLEDGMENTS

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