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Chemical Composition and Sensory Characteristics of Skimmed Milk Kefir Sensory on Variation of Concentration of Light Coconut Extract (Cocos Nucifera L.)

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Abstract: Kefir is a fermented milk product that is processed through fermentation of kefir granules. The objectives of this study are: 1. To analyse the effect of young coconut extract concentration on total microbes, ethanol and pH value of skimmed milk kefir. 2. To analyse the sensory test results of skimmed milk kefir with young coconut extract concentration. The method used in this research is Completely Randomised Design (CRD) with the treatment of variations in the percentage of skimmed milk and young coconut extract, namely: 100% skimmed milk: 0% young coconut extract; 95% skimmed milk: 5% young coconut extract; 90% skimmed milk: 10% young coconut extract; 85% skimmed milk: 15% young coconut extract; 80% skimmed milk: 20% young coconut extract with 3 repetitions and obtained 15 experimental units. The data obtained were analysed by ANOVA (Analysis of Variant). The results showed that increasing the concentration of young coconut extract increased the total number of microbes. Total microbes ranged from 1.48×10^8 to 2.17×10^{10}. Increasing the extract concentration from 15% to 20% increased the alcohol content from 0.07% to 0.20. Increasing the concentration of young coconut extract also significantly decreased the pH value of kefir after fermentation, with the lowest pH value (3.18) at 20% concentration. The concentration of young coconut extract in skimmed milk kefir significantly affected colour, texture and taste.

Keywords: Kefir; Light coconut extract; Skimmed milk

Introduction

Indonesia is one of the largest producers of food commodities in the world. The country has abundant natural resources and a tropical climate that favours agriculture. Indonesia is also one of the largest producers of coconut commodities in the world. Data from the Central Statistics Agency (BPS) recorded that coconut production in 2021 reached 2,853,399 tonnes. National superior plantation statistics for the period 2002-2022 reveal that the total area of coconut plantations in Indonesia reached 3,391,933 hectares, with 99.90% of them being smallholder holdings that still adopt traditional processing methods, without the application of existing innovative technologies. This condition has an impact on coconut productivity. With the adoption of innovative technologies, coconut productivity can be increased, and diversification of coconut-based products such as kefir can be a solution to increase the added value of agricultural products. Coconut has several varieties, one of which is early maturing coconut, which is commonly processed into coconut sugar using nira as the main ingredient. However, the use of water and flesh of early maturing coconuts is still limited, and most of them are only used as coconut ice. In the context of technological development and innovation, early maturing coconut is diversified into water kefir due to its sugar content. This carbohydrate content makes young coconuts have great

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potential to be utilised in microbial fermentation processes.

Previous studies on the use of young coconut as a substrate for fermentative bacteria have shown significant potential in functional food production. Young coconut water contains sugar components such as glucose, fructose, and sucrose which are ideal fermentation media for microbial growth (Rohman et al., 2019). The high sugar content in coconut makes coconut a suitable substrate for the production of fermented products such as water kefir. Research by Prasetyo & Rahayu (2021) showed that young coconut water has a total sugar content of about 2.08%, which consists of glucose, fructose, and sucrose. This composition provides a potential source of energy for fermentative microbes to produce products with probiotic content.

The use of young coconut as a fermentation raw material is not only limited to its water, but also involves coconut meat which is rich in nutrients. According to research by Isma & Fitriani (2020), young coconut meat contains 85.26% water content, 6.16% fat content, 1.60% protein content, and 3.39% carbohydrate content. This nutritional content is a medium for fermentative microbes to produce various bioactive compounds that are beneficial to human health.

Kefir is a fermented milk product that is processed through fermentation of kefir granules. The probiotic content in kefir, which comes from lactic acid bacteria such as Lactobacillus sp. and yeast contained in kefir granules, makes kefir a nutritious drink. Kefir, with its sour flavour and distinctive aroma created by lactic acid bacteria, has been recognised as a fermented product that provides functional benefits to the human body (Rohman et al., 2019). Cow's milk is a commonly used raw material in the manufacture of kefir. Lactose contained in cow's milk will be broken down by microbes to produce lactic acid and other compounds used for microbial growth (Tania & Parhusip, 2022). Various types of cow's milk, such as whole milk, UHT milk, and skimmed milk, can be used in the kefir fermentation process. Skimmed milk contains lactose which plays a role in forming lactic acid, ethanol and CO_2 (Liputo et al., 2019). In addition to milk kefir, there is also water kefir which uses water as a fermentation medium. Unlike milk kefir, water kefir uses a sucrose solution which is then added to dried fruit or fresh fruit as a source of nutrients. Coconut fruit is one of the commodities that shows potential to be used as raw material in kefir production. Based on the description above, research is needed on the effect of young coconut extract concentration (Cocos Nucifera L.) on chemical composition and also sensory tests with testing parameters of colour, aroma, taste and texture of skim milk kefir. The objectives of this research are: 1) Analyse the effect of young coconut extract concentration on total microbes, ethanol and pH value of skimmed milk kefir; 2) Analyse the sensory test results of skimmed milk kefir with young coconut extract concentration.

Method

This research was conducted at the Food Technology Laboratory and Organoleptic Laboratory, Department of Agricultural Technology, Sam Ratulangi University Manado, and the Standardisation and Industrial Services Agency (BSPJI) Manado, during February - March 2024.

The tools used include blender, spoon, pot, stove, measuring cup, analytical balance, sieve, stirrer/spatula, glass jar, bowl, thermometer, oven, flask, petri dish, incubator, pH meter, drop pipette, micropipette, autoclave, bunsen, magnetic stirrer hotplate, erlenmeyer, measuring flask, beverage cup, small spoon, aluminium foil.

The materials used included 8-month-old GTT (Genjah Tebing Tinggi) coconut obtained from BSIP Tanaman Palma, kefir granules obtained from e-commerce, greenfield brand liquid skim milk, sucrose, Plate Count Agar (PCA) media, distilled water, 0.1 NaOH, and 0.1% Buffered Peptone Water (BPW).

The research method used in this study is a Completely Randomised Design (CRD) with 5 treatments and 3 repetitions and obtained 15 experimental units with the formulation (Yelnetty et al., 2023 and (Ginting et al., 2019) as follows:

A = 100% liquid skimmed milk: 0% light coconut extract
B = 95% liquid skimmed milk: 5% light coconut extract
C = 90% liquid skimmed milk: 10% light coconut extract
D = 85% liquid skimmed milk: 15% light coconut extract
E = 80% liquid skimmed milk: 20% light coconut extract

Table 1. Kefir Beverage Manufacturing Formulation

Ingredients	A_0	A_1	A_2	A_3	A_4
Skimmed Milk (ml)	1000	950	900	850	800
Light Coconut Extract (ml)	0	50	100	150	200
Kefir Granules (gr)	50	50	50	50	50
Sucrose (gr)	10	10	10	10	10

Preparation of Young Coconut Extract (Jannah et al., 2014)

Liquid skimmed milk and young coconut extract were homogenised, 10% sucrose was added and pasteurised at 85°C for 15 minutes. After the pasteurisation process, the sample was cooled to room temperature and then 5% kefir granules were added to each formula and incubated or fermented at room temperature for 24 hours. After the fermentation process was stopped, the kefir was then filtered to separate it from the granules. The flowchart of making coconut juice kefir is presented in Figure 2.

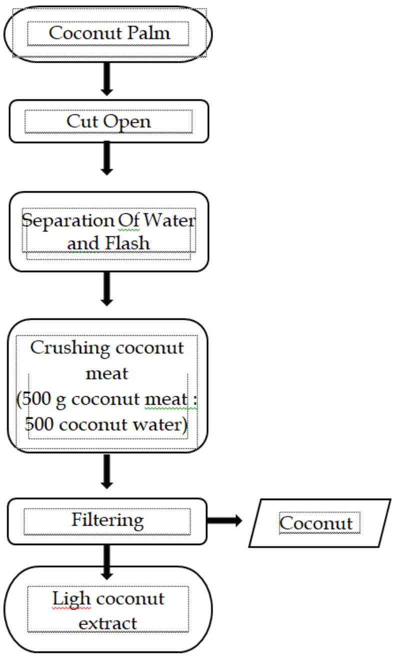

Figure 1. Kefir preparation (Yelnetty et al., 2023)

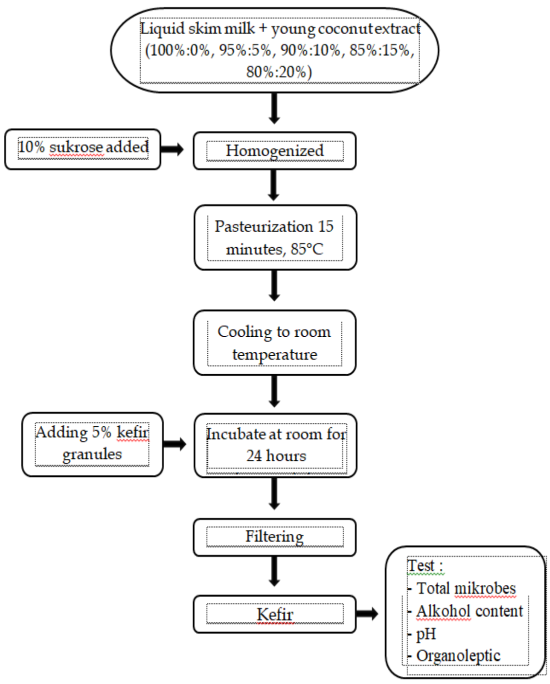

Figure 2. Flowchart of Kefir Preparation (Source: Yelnetty et al., 2023)

Observation Variables

Observations that will be made in this study are total microbial test, acidity test, alcohol content test, pH test, total soluble solids test and organoleptic test. Organoleptic test or the level of panelist preference for colour, aroma, taste and texture.

Total Microbial Test

Total microbial testing using Total Plate Count (TPC) is done to determine the total bacteria in kefir. 1 ml of sample was taken and then put into a flask containing 9 ml of distilled water and diluted to 10^{-8} dilution. From dilutions 10^{-6}, 10^{-7}, 10^{-8}, 1 ml of each dilution was taken and put into a sterilised petri dish. Sterilised Plate Count Agar (PCA) medium was poured into the petri dish containing the sample. Petri dishes containing samples and PCA were incubated in an incubator for 24 hours at 37°C with the dish inverted. The number of colonies that grow can be calculated using the Standard Plate Count (SPC) method and expressed in units of CFU/ml or log CFU/ml. the minimum limit of total yoghurt colonies is $1x10^{7}$ CFU/ml as the standard for total yoghurt bacteria in SNI 2981: 2009.

Ethanol Test (SNI 4019:2013)

The pycnometer is rinsed with alcohol then allowed to dry completely and then placed at room temperature, covered and weighed. The pycnometer was then filled with distilled water and immersed in a thermostat to a temperature of 20°C with the pycnometer line submerged in water. After 30 minutes, open the pycnometer and adjust the contents of the pycnometer to the mark with water using a capillary tube. The surface of the pycnometer was dried using a roll of filter paper and then left for 15 minutes at room temperature and weighed. A sample of 100 ml and 50 ml distilled water was added and then distilled in a distillation tube. Destillate

Then put into a pycnometer and immersed in a thermostat at 20 °C with the pycnometer border submerged in water. After 30 minutes open the pycnometer and adjust the contents of the pycnometer to the water mark using a capillary pipe. Dry the neck of the pycnometer using a roll of filter paper and leave at room temperature for 15 minutes and then weigh. Calculate the specific gravity of alcohol (20/20°C) using Formula 1.

$$\text{BJ alkohol} = \frac{S}{W} \quad (1)$$

Description:
S is the weight of the test sample
W is the weight of water

pH test

Measurement of the acidity or pH value is carried out using a pH meter. The pH meter is initially calibrated using a pH 7 buffer solution and a pH 4 buffer. The pH meter that has been calibrated with acidic pH buffer is then measured on the sample. The pH meter is immersed in 20 ml of a homogeneous sample and then waited for a while until a stable pH value is read (Barus et al., 2019).

Panelist Preference Test

Organoleptic test or sensory test is a test that uses the senses. The organoleptic test used in this study is the hedonic test or favourability test. The liking level test was used to analyse the level of acceptance of panelists towards the texture of skimmed milk kefir with young coconut extract concentration. This test required 25 untrained panelists who were asked personally to conduct the test with testing parameters including colour, aroma, taste and texture of skimmed milk kefir with young coconut extract concentration. There are 7 rating scales, namely:

1) Strongly dislike
2) Dislike
3) Somewhat dislike
4) Neutral
5) Somewhat like
6) Like

Data Analysis

The data later obtained then continued with ANOVA (Analysis of Variant) data analysis to determine the effect of young coconut extract concentration on liquid skim milk kefir.

Result and Discussion

Total Microbial Test

Table 2. Average Total Microbes of Kefir Skimmed Milk and Light Coconut Extract

Treatment	Concentration	Average Total Microbes
A_0	100 : 0	1,48 x 10^{8} [a] Coloni/g
A_1	95 : 5	3,31 x 10^{9} [ab] Coloni/g
A_2	90 : 10	6,80 x 10^{9} [b c] Coloni/g
A_3	85 : 15	1,14 x 10^{10} [c] Coloni/g
A_4	80 : 20	2,17 x 10^{10} [d] Coloni/g

*Different lowercase superscript symbols indicate significant difference based on one-way ANOVA test.

Total microbes in each treatment were 1.48 x 10^{8}, 3.31 x 10^{9}, 6.80 x 10^{9}, 1.14 x 10^{10} and 2.17 x 10^{10} respectively, indicating an increase in total microbes along with the treatment of young coconut extract given. The results of One Way ANOVA statistical analysis (P < 5%) showed that the concentration of young coconut extract in skimmed milk kefir had a significant effect.

The increase in the total number of microbes in Table 2 is due to the availability of sufficient substrate for microbes to multiply. In other words, sufficient substrate for microbes during fermentation can be fulfilled properly so that microbes are able to grow and develop optimally. This is in accordance with (Kinteki et al., 2019) the adequacy of energy sources in microbes can affect the growth of these microbes.

Based on the results of the average number of microbial colonies, the higher the concentration of coconut extract, the more microbes grow. This is thought to be because young coconut extract as a substrate can increase microbial activity, both in developing and in the process of forming organic compounds. The use of young coconut as a microbial substrate in fermentation is based on its nutritional composition that supports microbial growth. These nutrients in young coconut not only provide a source of energy for microbes but also support more optimal microbial growth. The sugar content in young coconut, such as glucose, fructose and sucrose, is an ideal fermentation medium that acts as an energy source for microbial growth (Rohman et al., 2019). This is in accordance with the results of research by (Andarti & Wardani, 2015) which showed that coconut water in kefir fermentation can significantly increase the number of fermented microbes due to the sugar content in coconut water. (Andarti & Wardani, 2015) also argued that microbes will utilise carbohydrates as nutrients that will be broken down into simple sugars for growth so that the number of microbes increases. The increase in the number of microbes indicates that the ability of microbes to break down substrates into organic acids and alcohol is also increasing (Barus et al., 2019). This is reinforced by the statement of (Liputo et al., 2019) during the fermentation process, LAB will break down glucose and other carbohydrates into organic compounds such as lactic acid and yeast will break down carbohydrates into alcohol and CO_2.

Ethanol Test

Table 3. Average Total Alcohol of Skimmed Milk Kefir and Light Coconut Extract

Treatment	Concentration	Average Total Alcohol
A_0	100 : 0	0,07%
A_1	95 : 5	0,07%
A_2	90 : 10	0,07%
A_3	85 : 15	0,20%
A_4	80 : 20	0,20%

*Different lowercase superscript symbols indicate significant difference based on one-way ANOVA test.

The results showed an increase in alcohol content by 0.13% to 0.20% in the treatment of 85% skimmed milk: 15% young coconut extract and 80% skimmed milk: 20% young coconut extract from 0.07% alcohol content in the other 3 treatments. This is directly proportional to the increase in total microbes that each treatment has increased because the more concentration of young coconut extract. Alcohol formation in kefir is a fermentation activity carried out by yeast in the fermentation process. During the fermentation process, yeast will metabolise glucose and other simple sugars in the substrate into ethanol and carbon dioxide. The sugar content in young coconut extract is utilised by microbes to produce alcohol. The more the concentration of young coconut extract, the sugar that becomes a substrate for microbes increases and the alcohol content in kefir also increases. This is in accordance with the statement of (Insani et al., 2018) that the formation of alcohol in the solution occurs due to microbial activity in the solution. This is supported by the statement of Julianto et al (2016) that yeast is able to convert pyruvic acid obtained from the glycolysis process and reduced to alcohol under anaerobic conditions. According to Insani et al (2018) under anaerobic conditions pyruvic acid will be converted into CO_2. In contrast to aerobic conditions, pyruvic acid will be converted in the oxidative decarboxylation stage which is then continued with the Krebs cycle and produces energy.

The resulting alcohol content was <1%, indicating that the skimmed milk kefir drink with young coconut extract is still safe for consumption. Based on the decree of the Indonesian Ulema Council (MUI), the alcohol content allowed in consumed beverages is 1%. The maximum alcohol content in cow's milk kefir is 1%. This shows that although there is an increase in alcohol content in skimmed milk kefir with young coconut extract, these levels are still within the established safe limits.

pH test

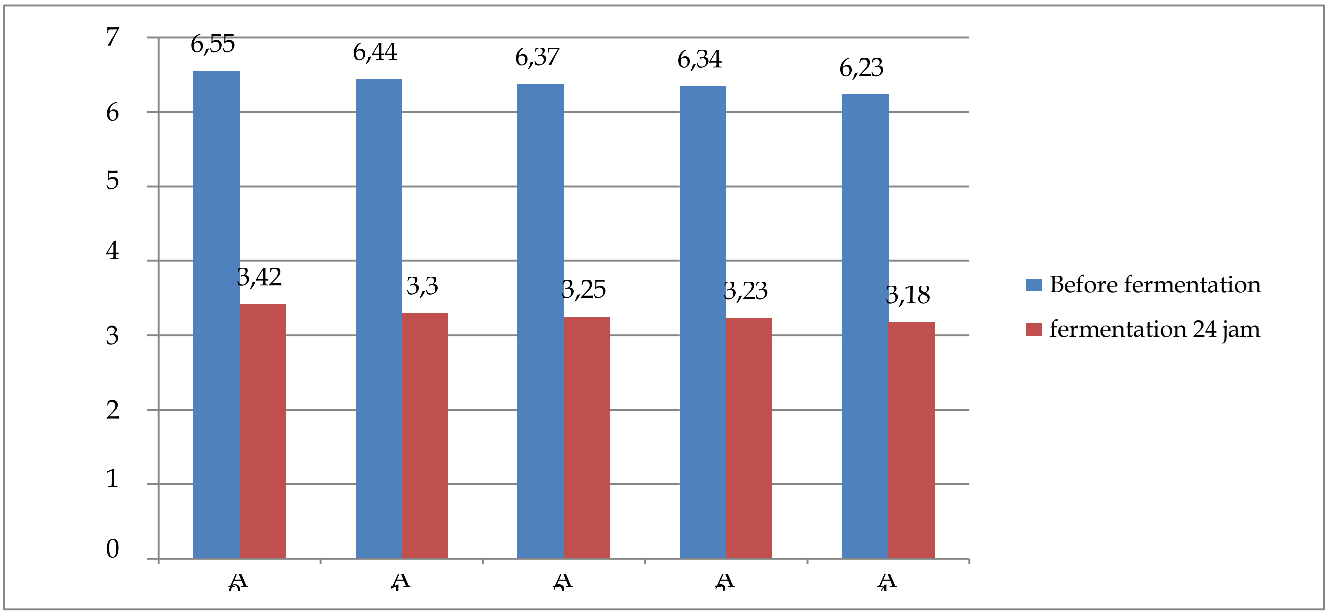

Figure 3. Kefir pH Value, Before and After Fermentation

Based on Figure 3, it can be seen that there is a decrease in pH in skimmed milk kefir with young coconut extract after the fermentation process. The decrease in pH in kefir in each treatment is thought to be caused by the increasing number of microbes. The higher microbial population is influenced by the concentration of young coconut extract. The need for susbtrate derived from young coconut extract is indicated by the increasing number of microbial colonies in each treatment. In each treatment, the concentration of young coconut extract increased from 5% to 20%. Microbes will break down the carbohydrates available in the fermentation medium and convert them into acidic compounds so that the microbial environmental conditions will be more acidic according to the number of microbial populations that exist based on their needs in the process of glycolysis and fermentation. The higher the microbial population, the lower the pH value produced. This is supported by the opinion of (Dante et al., 2017) that the more sugar added, the more substrate availability for microbes, the activity also increases. The increase in lactic acid is directly proportional to the total number of microbes in each treatment. The more sugar sources can be metabolised by bacteria, the greater the amount of acid produced and the lower the pH (Jannah et al., 2014).

Organoleptic Test

Table 4. Colour of Skimmed Milk Kefir and Light Coconut extract

Treatment	Concentration	Average	Criteria
A_0	100 : 0	4,00 ± 1,291[a]	Neutral
A_1	95 : 5	4,04 ± 1,020[a]	Neutral
A_2	90 : 10	6,04 ± 0,889[c]	Like
A_3	85 : 15	4,80 ± 0,913[b]	Somewhat Like
A_4	80 : 20	4,28 ± 1,208[ab]	Neutral

*Different lowercase superscript symbols indicate significant difference based on one-way ANOVA test.

The average test results of panelists' level of liking for colour sensory attributes obtained ranged from 4.00 to 6.04 with neutral to like rating criteria. Panelists' comments stated that the colour of kefir products tested tended to look bright. The more young coconut extract given, the brighter the colour. This is because the colour of the coconut extract itself is white, so any treatment that increases the concentration of coconut extract will produce a brighter colour.

The fermentation process can cause chemical reactions that result in colour changes, especially when there is interaction between compounds in milk and fat in coconut extract with active microbes. Microbes in the fermentation process produce compounds that affect milk pigments (carotenoids) and fat content in young coconut extracts. This agrees with (Martharini & Indratiningsih, 2017) who state that increased microbial proliferation will cause the lipase enzyme produced to be directly proportional to fermentative microbes so that more fat is hydrolysed and reduce fat content. This is supported by the statement of (Muizuddin & Zubaidah, 2015) which states that a decrease in pH during the fermentation process causes the resulting colour to become brighter.

Aroma

Table 5. Average Aroma of Skimmed Milk Kefir and Light Coconut Extract

Treatment	Concentration	Mean	Criteria
A_0	100 : 0	4,00 ± 1,258	Neutral
A_1	95 : 5	4,36 ± 1,551	Neutral
A_2	90 : 10	4,88 ± 1,166	Somewhat like
A_3	85 : 15	4,56 ± 1,356	Somewhat like
A_4	80 : 20	4,24 ± 1,363	Neutral

Based on the results of One Way ANOVA statistical analysis, the treatment of young coconut extract given to skimmed milk kefir after the fermentation process did not have a significant effect ($P > 5\%$) on the aroma produced.

Organic compounds that affect the aroma of kefir are volatile compounds (Ginting et al., 2019) stated that lactic acid, acetic acid and also alcohol are volatile compounds contained in kefir. This is reinforced by Muizuddin & Zubaidah (2015) who stated that the distinctive aroma generated by kefir comes from volatile compounds formed during the fermentation process.

Texture

Table 6. Average Texture of Skimmed Milk Kefir and Light Coconut Extract

Treatment	Concentration	Mean	Criteria
A_0	100 : 0	4,08 ± 1,077[a]	Neutral
A_1	95 : 5	4,12 ± 1,054[a]	Neutral
A_2	90 : 10	4,96 ± 1,306[b]	Somewhat Like
A_3	85 : 15	4,76 ± 1,012[b]	Somewhat Like
A_4	80 : 20	4,08 ± 0,997[a]	Neutral

*Different lowercase superscript symbols indicate significant difference based on one-way ANOVA test.

The slightly liquid texture of kefir is thought to be because kefir has a different microbial mixture from yoghurt. Young coconut extract also plays a role in giving texture to kefir. The slightly thick texture of young coconut extract affects the final texture of kefir. The increasing total microbial count due to the increasing concentration of young coconut extract results in a low pH value or high acidic conditions that can cause coagulation of proteins present in skimmed milk and in young coconut extract to form a dense texture. This is in accordance with the statement of Setioningsih et al (2004) in (Pratiwi et al., 2021) that proteins will coagulate due to changes in acidity by lactic acid produced by LAB during the fermentation process.

Taste

Table 7. Average Flavour of Skimmed Milk Kefir and Light Coconut Extract

Treatment	Concentration	Mean	Criteria
A_0	100 : 0	3,64 ± 1,221[ab]	Neutral
A_1	95 : 5	4,28 ± 1,208[b]	Neutral
A_2	90 : 10	5,12 ± 0,927[c]	Somewhat like
A_3	85 : 15	3,72 ± 1,458[ab]	Neutral
A_4	80 : 20	3,36 ± 0,952[a]	Somewhat like

*Different lowercase superscript symbols indicate significant difference based on one-way ANOVA test.

Based on the results of One Way ANOVA statistical analysis, the treatment of young coconut extract given to skim milk kefir after the fermentation process had a significant effect ($P < 5\%$) on the flavour produced.

The increasing young coconut extract in each treatment causes the total microbes to increase and produces a lower pH value which affects the taste produced. This agrees with (Jannah et al., 2014) that the flavour produced in kefir is related to the resulting pH and acidity. The sour taste is influenced by the total number of microbes that ferment carbohydrates in milk and coconut extract into lactic acid, and yeasts that also ferment carbohydrates into alcohol.

Conclusion

Skimmed milk kefir with young coconut extract on total microbes and ethanol increased based on the concentration of coconut extract. Apart from that, the pH value showed a decrease and became more acidic due to the young coconut extract. The results showed that young coconut extract is an optimal substrate for kefir fermentation.The panellists' liking level of the combination of skimmed milk with young coconut extract with the proportion of 90% skimmed milk and 10% young coconut extract was the most preferred. This is indicated by sensory attributes such as colour, aroma, texture and taste which obtained the highest mean scores.

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Author Contributions
YYOE: Developing ideas, analyzing, writing, reviewing, responding to reviewers' comments; LCM, CAF: analyzing data, overseeing data collection, reviewing scripts, and writing.

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Conflicts of Interest
The authors declare no conflict of interest.

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