

**ANTIOXIDANT ACTIVITY TEST ON KEFIR WHEY WITH TURMERIC
NATURAL DYE
(*Curcuma longa* L.)**

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ABSTRACT

Excess free radicals in a person's body to exceed the body's ability to manage them can cause a condition called oxidative stress. Oxidative stress is a condition of imbalance between the amount of free radicals present and the amount of antioxidants in the body. One of the Prevention of oxidative stress is by increasing the intake of antioxidants. During this time whey in most cheese companies just becomes a waste. As we know that the Whey contains high nutrients and has health benefits and contains antioxidants. The purpose of this study was to determine the use of cheese whey in kefir whey beverage processed products with turmeric natural dyes that are rich in antioxidants. This study was conducted by an experimental method consisting of 5 (five) stages, namely the first stage of making kefir whey drink with turmeric natural dyes. The second stage is testing pH, the third stage is testing water content, the fourth stage is antioxidant activity testing, the fifth is testing hedonic (liking). The results showed that the variant concentration of cheese whey significantly affect the water content, antioxidant activity and preferences (color, flavor, scent). But there is no real effect on the pH value because the average pH value is the same. Variable pH value, water content and hedonic (preference) does not affect antioxidant activity. So it can be concluded that kefir whey with turmeric natural dye has the potential as a drink that contains antioxidants that can reduce levels and can prevent oxidative stress.

Keywords: *Antioxsidant, Kefir, Whey, Turmeric*

1. BACKGROUND

In the world of Medicine and health, there is a lot of talk about free radicals and antioxidants. Free radicals have accumulated in the body. This is due to air pollution, water pollution, food, beverages, pesticides, drugs, cigarette smoke, radiation, sunlight, and electromagnetic waves of electronic equipment. As a result, degenerative diseases such as coronary heart, rheumatism, cataracts, cancer, and stroke.¹²

Antioxidants stabilize free radicals by supplementing the lack of electrons possessed by free radicals and inhibit the occurrence of chain reactions from free radical formation.⁹ Antioxidants are needed to prevent oxidative stress. Oxidative stress is a condition of imbalance between the amounts of free radicals present with the amount of antioxidants in the body.²¹

During this time the *whey* in most cheese companies only manjadi waste and has not been used optimally.¹⁴ In Indonesia, *whey* has not been widely used because the cheese processing industry is still focused on its main product, namely cheese, even though the composition of *whey* still contains 50% of milk nutrients. The largest component of *whey* is lactose (4.5-5%). *Whey* has a concentration of dissolved organic matter such as protein. *Whey* contains lactoferrin and vitamin C, which are sources of antioxidants.⁵

One type of plant that is rich in antioxidants is turmeric. Turmeric is one of the herbs that are easily found in Indonesia.¹⁸ This herb is used as a food coloring, medicine, preservative, and flavoring.¹⁹ Turmeric contains curcumin compounds as a very powerful anti-inflammatory and antioxidant.¹⁷

The purpose of this research is make formula and antioxidant activity test on kefir *whey* with natural dyes turmeric (*Curcuma longa* L.)

2. METHODOLOGY

This study used a complete randomized design (RAL) with a single factor that is the variation of the use of cheese whey by 200 mL, 150 mL, 100 mL, and 50 mL. The method used is an experimental method consisting of 5 (five) stages, namely the first stage of making kefir whey drink with turmeric natural dye. The second stage is pH testing, the third stage is water content testing, the fourth stage is antioxidant activity testing hedonic testing (liking), the fifth stage is hedonic testing(liking).

a. Tools and Material

The tools used in this study are glassware (Beaker), stirring rod, spatula, analytical scales (DRAWEL FA2204), bottle, dropper, incubator (IN30), measuring flask, thermometer, UV-Vis spectrophotometer (Shimadzu UV-1900), pH meter (Ohaus St300 Portable), petri dish, micropipette, blue tip, stove, jerry can, spoon, test tube, erlenmeyer flask, measuring cup, stove, freezer, showcase, italic filters.

Materials used in this study are starter kefir, whey cheese, whole cow's milk, turmeric powder, inulin, sucrose, DPPH(2-2Diphenyl-1-Picryhidrazyl), methanol for analysis, aluminum foil, ascorbic acid for analysis, aquadest, buffer 7, buffer 4.

b. Manufacture of Kefir *Whey* Turmeric

In the manufacture of kefir *whey* required pure cow's milk obtained from the center of production of cow's milk Boyolali and *whey* used is the result of processing in Boyolali cheese industry and Yogyakarta cheese industry. Kefir making procedure refers to the Indonesian milk Kefir Association (2016) which has been modified. At the first stage, cow's milk is heated at a temperature of 85°C for 15 seconds. Then a decrease in temperature until it reaches room temperature. The next step is to measure the concentration of cheese *whey* to be used in each experiment, the concentration of cheese *whey* used is 200 mL, 150 mL, 100 mL and 50 mL. Then inoculated with 5% kefir grains per 200 mL, fermented at room temperature (25-30°C) for 24 hours. Kefir harvesting is done using an italic sieve that aims to separate kefir grains with milk that has been harvested.⁴ *Kefir grains* obtained from home kefir Yogyakarta. Kefir grains are used as starter bacteria and yeast in the manufacture of kefir.

Then *whey* kefir is added with turmeric powder that has been determined as much as 2 grams and stirred using a stirring rod for 3 minutes in a beaker. The addition of 2 grams refers to the guidelines for the use of food additives by BPOM in the food category, namely milk-flavored drinks with a maximum limit of sufficient which means that food additives can be used in sufficient quantities as needed to produce the desired effect. Turmeric powder obtained from the East Java provincial Health Office UPT Herb Materia Medika Batu Malang. Then turmeric *whey* kefir added granulated sugar as much as 2.5 grams as an additional sweet flavor of sweetness and added inulin as much as 0.5 grams. The addition of inulin as a water binder thus preventing the formation of 2 layers. Turmeric *whey* kefir is stored on showcase with temperature less than 4°C for 1 day.

c. pH Test

Kefir *whye* turmeric pH test analysis is measured using a pH meter device. Before the measurement, the pH meter must be calibrated using a buffer solution of 7.0 and 4.0. Then the measurement of the pH of the sample by means of electrodes dipped into the sample and wait a while until a stable reading is obtained. Each change of electrode sample should be rinsed with aqueduct and dried, then put into another sample.¹¹

d. Water Content Test

The water content test was done by gravimetric method. The principle of the gravimetric method is to remove the water content in the sample by heating using an oven at a temperature of 105°C so that the water physically bound to the sample can be evaporated so that a constant weight is obtained.¹ The water content test refers to AOAC (2005) which has been by means of an empty cup dried in an oven with a temperature of 105°C for 1 hour. After an hour, the saucer is taken out and cooled in a desiccator for 5 minutes, then the saucer is weighed to obtain the empty (constant) weight of the saucer (W0). Furthermore, the sample weighed as much as 5 grams (W1) put into an empty cup. Then put in the oven with a temperature of 105°C for 3 hours. A cup containing a sample that does not contain water content is taken and placed in the desiccator for 5 minutes, then weighed (W2).² Percentage of water content is calculated by the formula:

$$\text{Water Content (\%)} = \frac{(W1 - W2)}{(W1 - W0)} \times 100\%$$

Description:

W0 = Weight of empty cup

W1 = Weight of the cup + sample before oozing

W2 = Weight of the cup + sample after oozing

e. Preparation of 100 ppm DPPH solution

A total of 0.0025 g of DPPH was put into a 25 mL measuring flask and then methanol was added to the limit mark. The solution is incubated for 30 minutes and protected from light.

f. Determination of the maximum wavelength of DPPH

¹ Syamsul, Eka Siswanto, *et al.* 2019. Penerapan Kadar Flavonoid Ekstrak Daun Kelakai (*Stenochlaena palustri* (Burm. F.) Dengan Metode Spektrofotometri UV-Vis. *Jurnal Riset Kefarmasian Indonesia*, 1 (1).

How to determine the maximum wavelength of DPPH is done by observing the absorption of wavelengths in the range of 400-800 nm.

g. Determination of antioxidant activity of kefir whey turmeric

Antioxidant activity was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, a concentration series of 20 ppm, 30 ppm, 40 ppm, and 50 ppm was created. Each concentration taken 8 mL added 2 mL DPPH in methanol. Homogeneous mixture and put into UV-Vis spectrophotometry. The absorption of the solution was measured at a wavelength of 516 nm. Testing was done with 5 times replication. Next, the IC₅₀ value is calculated based on the regression equation obtained. IC₅₀ is a cheese whey concentration that inhibits DPPH activity by 50%. The more kesil IC₅₀ value the higher the antioxidant activity.

h. Hedonic Test (Liking)

The preferred testing method is scoring. Test preferences assessed include color, taste, and aroma. Each respondent gave a favorite score. The Score Scale is made of five levels of preferences, namely: (1) very like, (2) like, (3) rather like, (4) dislike, (5) very dislike.⁸

i. Analisis Data

The Data obtained from the analysis of pH, water content, was analyzed by analysis of variation (Anava) version 26.0 with a confidence level of 96% to determine the effect of treatment then continued with Duncan test to determine the real difference between treatments. Antioxidant activity test Data were analyzed by calculating the value of IC₅₀ in each treatment to determine the category of antioxidant activity level of antioxidant activity is very strong (<50), strong (50-100), moderate (100-150), and weak (151-200) ppm.

For hedonic data processing in stages starting from the data collected until ready for analysis. Against the data from the collection is done editing, coding and data entry into excel. Furthermore, data cleaning by looking at the distribution and frequency. The data obtained from the research results are tabulated, then analyzed. Hedonic test data processing performed with non-parametric test *Kruskal Wallis*.

3. RESULT AND DISCUSSION

a. pH Test

The pH test performed showed that the variation of cheese whey concentration did not have a significant difference as in table 1.

Table 1. pH Value

Sample	pH Value	Standard
F 1	4,18	Maximum 4,5 (Australian Food Standard Code)
F 2	3,92	
F 3	4,3	
F 4	4,14	
F 5	4,22	

The pH value of the five formulas of kefir *whey* turmeric drink with the lowest pH is formula 2 at 3.92 and the highest pH value is formula 3 at 4.3. Formula 2 with a concentration of whey cheese as much as 200 mL has the lowest pH due to pH levels of whey cheese before fermentation of 4.6 (acid).¹⁴ After fermented using kefir grains, the pH level decreases. This is in accordance with the opinion of Wulandari *et al.* (2014) stating that microorganisms in kefir thrive and increase total acid during fermentation.²² Formulas 3, 4 and 5 have a higher pH value compared to Formula 2. Based on these data, it is shown that variations in the concentration of cheese whey affect the pH value. The more cheese *whey* concentration, the lower the pH value. However, at F3 there is an increase in pH value. This is because there is a possibility of a decrease in the ability of kefir grains in converting lactose into lactic acid. In research Meilan (2021) states that the production of organic acids by lactic acid bacteria and yeast is one of the factors that cause a decrease in the pH value.³ The control treatment (F1) produced a pH of 4.18. The pH value corresponds to the range of pH values of cow's milk kefir stated by Herly (2020), namely with a pH value of 3.90.⁶ The quality of fermented milk based on good pH according to Adriani (2005) is 3.8-4.6.¹

b. Water Content Test

Water content test conducted showed that variations in the concentration of whey cheese does not have a noticeable difference as in table 2.

Tabel 2. Kadar air

Sampel	Nilai kadar air	Standar
F 1	93,44	83% - 84% (SNI Yoghurt)
F 2	95,36	
F 3	96,92	

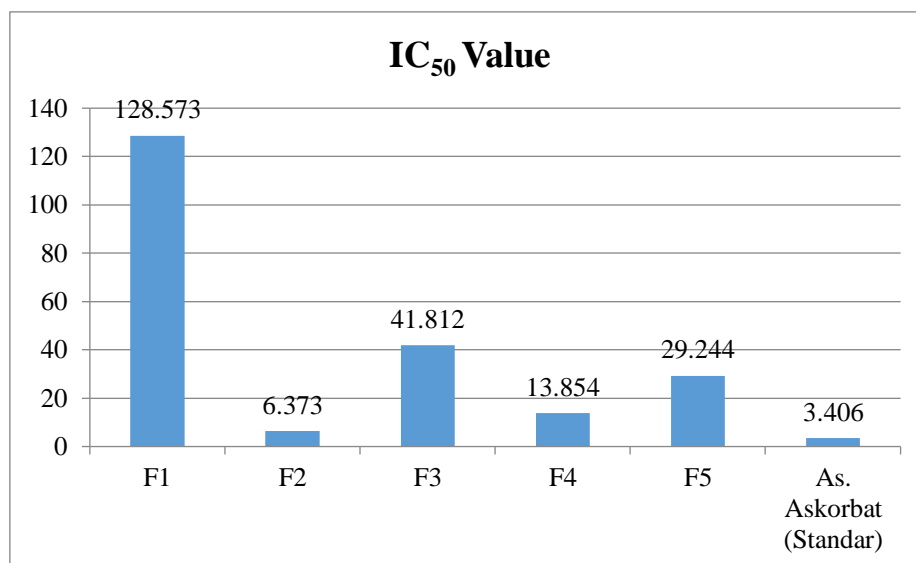
F 4	94,72
F 5	94,1

The calculation of the water content in kefir is aimed at finding out how sticky a product is, and also to avoid microbial activity that can damage the product.²⁰ In addition, the water content is the percentage of water contained in a material also greatly affects the shelf life of a material.⁷ SNI water content in kefir has not been determined, then the water content of kefir is adjusted to other fermented milk products are almost the same, namely yoghurt. In Indonesia national standards on yogurt for water content of 83% - 84%. According to Handayani *et al* (2007) said that the water content of a product can be influenced by the total amount of solids in the dough of a product, so we can know that the water content in yogurt is less because the product form of yogurt is thicker while kefir has a higher water content because it is thinner.¹³ In this study the highest water content in F3 is 96.92% with a concentration of whey cheese as much as 150 mL. F3 has the highest moisture content because the cheese whey moisture content ranges from 93.42%.¹⁴ This is because the concentration of cheese whey is more than F4 and F5. So that the water content of F3 is higher than F4 and F5. Based on these data, it is shown that variations in the concentration of cheese whey affect the value of water content. The more the concentration of cheese whey, the higher the water content value. However, F2 decreased the value of water content. This is because during the fermentation process there is an increase in other ingredients in kefir, such as protein.¹⁶ One of the functions of protein is as a water binder. The control treatment (F1) has the lowest moisture content of 93.44%. This is because the protein content in cow's milk is lower than the protein content in cheese whey. Thus, the ability of water binding to proteins is getting stronger.

c. Antioxidant Activity Test

Antioxidant activity tests performed showed that variations in the concentration of whey cheese has a marked difference as in Graph 1.

Grafik 1. IC₅₀ Value



Based on the data obtained obtained linear regression equation of the standard solution (ascorbic acid) is $y = 7.6022 x + 40.683$ and obtained IC₅₀ value of the standard solution (ascorbic acid) of 3.406 ppm. This indicates a very strong antioxidant activity (<50 ppm). Formula 1 with linear regression equation is $y = -10.878 x + 102.83$ with IC₅₀ value of 128.573 ppm. This indicates that the antioxidant activity of F1 enters the medium category (100-500 ppm). Formulation 2 with linear regression equation is $y = -15.368 x + 78.464$ with IC₅₀ value of 6.373 ppm, Formula 3 with linear regression equation is $y = 19.749 x + -23.728$ with IC₅₀ value of 41.812 ppm, Formula 4 with linear regression equation is $y = 17.798 x + 3.2147$ with IC₅₀ value of 13.854 ppm and Formula 5 $y = 43.084 X + -95.442$ with an IC₅₀ value of 29.244 ppm. Formulas 3, 4 and 5 have very strong antioxidant activity due to the average IC₅₀ of each formula < 50 ppm.

IC₅₀ value on kefir *whey* with turmeric natural dye is the highest F2 of 6,373 ppm with a variant concentration of whey cheese 200 mL. At F3, F4 and F5 have lower antioxidant activity compared to F2. The Data stated that the variant concentration of cheese *whey* affects antioxidant activity. The higher the variant of cheese *whey* concentration, the antioxidant activity of semankin increases. However, F4 has increased antioxidant activity. This is because the ratio of cheese *whey* to cow's milk is the same or comparable. So, with the same ratio, it will produce higher antioxidant activity. The control treatment (F1) had the lowest antioxidant activity. This is because in F1 there is no addition of cheese *whey*. On

the data obtained from the whole Formula, it can be concluded that the formula of kefir *whey* with turmeric natural dye contains antioxidant activity.

d. Uji Hedonik (Kesukaan)

Tabel 3. Hasil Uji Hedonik (Kesukaan)

Variabel	Perlakuan				
	F1	F2	F3	F4	F5
Warna	1,50	2,20	2,37	2,20	2,23
Rasa	2,57	2,17	3,17	3,13	3,37
Aroma	2,43	2,77	2,87	2,77	2,87

Keterangan : (Skor 1) Sangat suka, (Skor 2) Suka, (Skor 3) Agak suka, (Skor 4)

Tidak suka, (Skor 5) Sangat tidak suka.

1) Color Test

Setyaningsih (2010) said that color is the most important quality attribute, although a food product with high nutritional value, good taste and good texture, but if the color displayed is less attractive, it will cause food products to be less attractive to consumers. Testing with the sense of sight is still very decisive in color sensory testing on food products.¹⁵ Assessment of the level of color preference to the overall formulation shows the average score on F1 is a score of 1.50 means in the criteria really like. In F2, F3, F4, and F5 have an average score that is not much different, namely 2.20; 2.37; 2.20 and 2.23 means in the criteria like. F1 (200 mL of milk) has a white color. Because in F1 there is no addition of cheese *whey*. F2 with a variant of cheese *whey* concentration of 200 mL has a pale yellow color. Because basically cheese *whey* is clear yellow. F3, F4 and F5 have the same color, which is whitish yellow. The color is due to the addition of turmeric dye. Based on the overall data of the formula, it can be concluded that the variant of cheese *whey* concentration and the addition of turmeric dye affect the level of panelists ' preference for color.

2) Taste Test

Taste is one of the important factors in food products. The taste of kefir in general is sour. This is in accordance with the opinion of Mahdiana et al., (2015) which states that the sour taste in kefir is caused from kefir seed starter consisting of lactic acid bacteria and yeast that converts lactose to form flavor. During the fermentation process takes place lactic acid

bacteria will produce lactic acid from the activity of enzymes that remodel lactose to produce a distinctive flavor of kefir.¹⁰

Based on the results of hedonic test observations taste value at F1 and F2 preferred by panelists than F3, F4 and F5. Because F1 and F2 have a score of 2.57 and 2.17 which means like. F1 and F2 are original samples without the addition of turmeric powder . So it still has the characteristic sour taste of kefir. While F3, F4 and F5 have a score of 3.17; 3.13 and 3.37 which means rather like. Due to the addition of turmeric powder in the sample so as to produce a slightly bitter and bitter taste due to the sensation of turmeric powder. It can be concluded that the variant concentration of cheese whey and the addition of turmeric dyes affect the level of preference of panelists to taste.

3) Scent Test

Aroma is the smell that the sense of smell can observe. Odor or aroma testing is one of the important tests because it can provide an assessment of the acceptability of the product.⁸ Aroma can be used as an indicator of the occurrence of damage to food products. Kefir that smells rancid is no longer suitable for consumption. Or kefir that is too acidic can be dangerous for consumers who have a history of magh/ gerd disease.

The overall scent of the formula stated that the panelists liked the scent of all five formulas. F1, F2, F3, F4 and F5 have the same score of 2 which means like. Because kefir has a distinctive aroma of yeasty (like tape).⁸ It can be concluded that the variant concentration of cheese *whey* and the addition of turmeric dye does not affect the level of preference of panelists to the scent.

4. CONCLUSION

The conclusion of this study is:

- a. Kefir whey Formula with turmeric natural dye has the potential as a functional food. Because the results of testing data pH, moisture content, antioxidant activity and hedonic have met the testing standards.
- b. F1 has antioxidant activity with medium category (100-150 ppm) with IC50 value of 128.573 ppm. While F2, F3, F4 and F5 indicate the range of IC50 values with very

strong Category (<50 ppm) , namely F2 of 6,373 ppm, F3 of 41,812 ppm, F4 of 13,854 ppm, F5 of 29,244 ppm.

- c. The best Formula of kefir whey with turmeric natural dye that produces the highest antioxidant activity is F2 with a concentration of 200 mL cheese whey which has an IC50 value of 6.373 ppm. Category very strong (<50 ppm).

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