The Effect of Kecombrang (*Etlingera elatior*) Extract Addition on Characteristics of Kefir

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Abstract. This study aimed to investigate the characteristics of cow's milk kefir using different types of kecombrang extracts (leaves, flowers, and stems) and varying extract concentrations to identify the optimal combination of extract types and concentrations to achieve the highest antioxidant activity in kefir. A completely randomized design (CRD) was employed, factoring the kecombrang extracts (leaf, flower, and stem) and the concentrations (2.5%, 5%, and 7.5%). The kefir characteristic variables were analyzed using ANOVA, followed by Duncan's multiple range test at a significance level of α=5%. The results indicated that the type of kecombrang extract influenced viscosity, color value, antioxidant content, and sensory properties of taste and overall acceptability. Increasing the extract concentration enhanced the color value and viscosity. The optimal kefir formulation was achieved with the addition of 7.5% kecombrang flower extract that resulted in the highest antioxidant content of 74.83%. Cow's milk kefir incorporated with 7.5% kecombrang flower extract contained 90.6% water, 0.99% ash, 2.19% fat, 6.82% protein, and 0.6% carbohydrates, all of which comply with CODEX STAN 243-2003.

Keywords: Kecombrang, extract, Kefir, Antioxidant Activity

Abstrak. Penelitian ini bertujuan untuk mengkaji karakteristik kefir susu sapi dengan penambahan berbagai jenis ekstrak kecombrang (daun, bunga, dan batang) serta variasi konsentrasi ekstrak. Selain itu, penelitian ini juga bertujuan untuk menentukan jenis dan konsentrasi ekstrak kecombrang yang terbaik untuk menghasilkan kefir dengan aktivitas antioksidan tertinggi. Penelitian ini menggunakan rancangan acak lengkap (RAL) dengan faktor yang diteliti, yaitu jenis ekstrak kecombrang (ekstrak daun, ekstrak bunga, dan ekstrak batang) serta konsentrasi ekstrak (2,5%, 5%, dan 7,5%). Hasil uji variabel karakteristik kefir dianalisis menggunakan ANOVA dan uji lanjut Duncan's advanced multiple range test pada taraf signifikansi α=5%. Hasil penelitian menunjukkan bahwa jenis ekstrak kecombrang berpengaruh signifikan terhadap viskositas, warna, kandungan antioksidan, serta sifat sensorik yang mencakup rasa dan kualitas keseluruhan kefir. Konsentrasi ekstrak kecombrang meningkatkan warna dan viskositas kefir. Formulasi kefir terbaik diperoleh dari penambahan ekstrak bunga kecombrang sebesar 7,5%, yang memiliki kandungan antioksidan tertinggi yaitu 74,83%. Penambahan ekstrak bunga kecombrang 7,5% pada kefir susu sapi mengandung 90,6% air, 0,99% abu, 2,19% lemak, 6,82% protein, dan 0,6% karbohidrat, semuanya sesuai dengan CODEX STAN 243-2003.

Kata kunci: Kecombrang, Ekstrak, Kefir, Aktivitas Antioksidan

Introduction

Consumers seek foods that not only please their palate but also contribute to their health and well-being. This demand boosts the development of functional foods, including fermented food, to meet this demand (Melini *et al,* 2019). The trend of functional food is on the rise, and it includes kefir, milk or water fermented with kefir grain that produce beverage with minimal alcohol content and distinctive acidic taste and carbonation (Garofalo *et al.,* 2020). Kefir can be made of milk from goats, buffaloes, cows, camels, and soybeans, and the kefir grain as a starter contains lactic acid bacteria (LAB) and yeast bound in a polysaccharide matrix (O'Brien *et al.,* 2016).

Kecombrang (*Etlingera elatior*) is a spice and medicinal plant that with potential bioactive components of antibacterial and antioxidant for functional food According to Bahari et al. (2019) all parts of the kecombrang plant have antioxidant activity from the rhizome, leaves, flowers and stems, but it's strongest in the flower. The antioxidant activity of kecombrang flowers was 61.61% - 83.17%, the stems was 57.43% - 84.65%, the leaves was 40.64% - 60.40%, and the rhizomes was 58.40% - 69.66% (Naufalin *et al.* 2021). The antioxidant compounds found in kecombrang include phenolics, flavonoids, triterpenes, saponins, tannins, steroids, alkaloids, and glycosides. (Nuryanti *et al.,* 2021). The total phenols in parts of kecombrang plants are 484.59 – 959.73 mg/100g in the flower, 462.92 – 1,205.47 mg/100g in the stem, 1,338.06 – 8,636.15 mg/100 g in the leaves, and 510 – 2,453.41 mg/100 g in the rhizome.

There has been growing interest in using plants for preventive and therapeutic phytotherapy, as they contain high concentrations of antioxidant compounds such as carotenoid, phenolic, flavonoid, and anthocyanin derivatives, alongside vitamins, enzymes, and cofactors that can neutralize free radicals (Munteanu and Apetrei, 2021). These properties are important in molecular biology where degenerative processes are linked to an overproduction of free radicals, which drive harmful oxidative reactions within the organism.

The effect of increasing the concentration of kecombrang stems, leaves and flowers can also be an alternative to increase the nutritional value, color, taste, aroma, and especially the antioxidant content (Naufalin *et al.,* 2021). This research was conducted to identify the optimal combination of kecombrang extracts and concentrations to achieve the highest antioxidant activity in kefir.

Materials and Methods

This research was carried out at the Agricultural Technology Laboratory, Jendral Soedirman University, Purwokerto, Central Java, Indonesia from September 2021 to January 2022.

Research Materials

The materials for this study were kecombrang purchased from a traditional Market in Purwokerto, milk from the Experimental Farm of Universitas Jenderal Soedirman, Aqua dest, grain kefir, skim milk, plastic wrap, cotton, aluminum foil, pro-analysis (buffer solvent, diethyl ether, K_2SO_4, CuSO4, NaOH, boric acid, HCl, methanol, MRS agar media, and DPPH). The tools used in this study included a pH meter (Mediatech), color reader (CR10 plus Konica Minolta), viscometer (brookfield DVE), pipette, sensory test form, UV-Vis spectrophotometer UV-Vis (Thermo Scientific genesys 150), Kjeldahl flask, extractor, cabinet dryer, incubator, distillation apparatus, and furnace.

Experimental Design

The research was carried out experimentally in a factorial completely randomized design (CRD) with two factors and three replications. The factors were kecombrang extract and its concentration at the level of 2.5%, 5%, and 7.5%. Variables observed in this study included pH values, viscosity, color value, antioxidant activity, and lactic acid bacteria (LAB), as well as sensory variables which included aroma, sour taste, texture and preferences, chemical properties including moisture content, ash content, fat content, protein content, and carbohydrate content. The data gathered from the research were analyzed using Analysis of Variance (ANOVA) at a 5% significance level. If the analysis indicated a significant effect, it was further examined with Duncan's multiple range test.

The Preparation of the kecombrang extract

Kecombrang extract was made from simplicial powder and then extracted by maceration and water solvent. The powder of kecombrang leaf, flower, and stem was measured individually using 60 mesh, then introduced into the extractor and mixed with water solvent at 50°C through the extractor's cover pipe. The powder-to-water ratio was maintained at 1:14. The extractor was then sealed, activated, and set to an agitator speed of

60 rpm for 3 hours. Once the extraction was complete, the mixture was left to stand in a closed container in a dark condition for 19 to 24 h. Following this, the sample was filtered to separate the powder from the filtrate, then the powder was dehydrated in a cabinet dryer at 50°C for 2 to 3 hours. After drying, the initially extracted powder followed the second extraction at the same ratio of 1:14, using the same temperature and duration. The results from both extractions were combined until homogeneous (Fatimah et.al., 2019).

The Preparation of Kefir

Kefir was made by adding kecombrang extract into milk. First, 650 ml of fresh cow milk and 2% skim milk was poured into a container, then stirred and pasteurized in a water bath for 30 min at 60^{o}C, and cooled until the temperature was ± 28^{o}C. Once cool, the milk was inoculated with 5% kefir grains in a sterile glass bottle, and incubated at room temperature about 28^{o}C for 24 hours and then added with kecombrang extract (Yelnetty et. al., 2016).

Analysis Method

The pH value was measured using a pH meter, color analysis using a color reader, viscosity using a Brookfield viscometer, and total LAB using the cup count method. The next measurement was antioxidant activity (DPPH method), water content (moisture analyzer), ash content analysis (AOAC, 2005), lipid content analysis (Soxhlet method), protein content analysis (Kjeldahl method, AOAC, 1990), and carbohydrate content analysis (by difference). The sensory characteristics were measured through a hedonic test with 20 panelists.

Data Analysis

The data obtained from the research was analyzed statistically using the Analysis of variance (ANOVA) with a level of 95% and further testing was carried out with use the Duncan Multiple Range Test (DMRT) if the test results show real difference.

Results and Discussion

pH Value

The pH value of kefir after treatment was in the range of 4.2 - 4.5. Farnworth (2008) stated that kefir pH is generally 4.2 - 4.6, therefore, the addition of kecombrang bioactive components did not significantly affect the pH value. The pH or acidity of beverage products can be influenced by the presence of organic acids such as acetic acid and pyruvic acid formed during fermentation (Bahari et al., 2019). The formed microbial activity can lower kefir pH and inhibit the growth of spoilage microbes with the formation of compounds such as alcohol and bacteriocin.

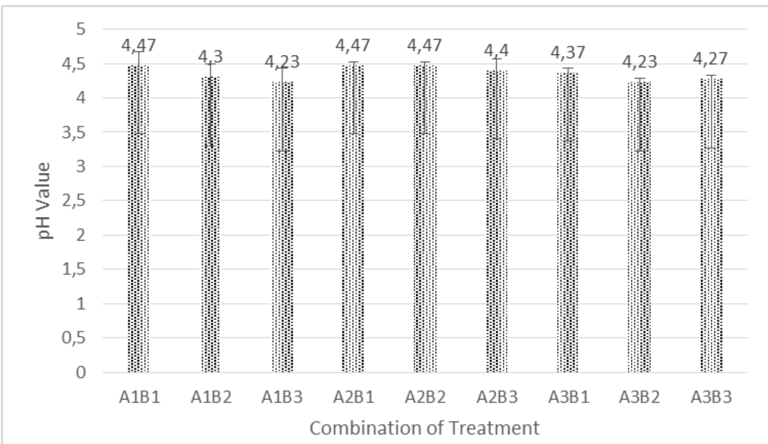


Figure 1. The average pH value of kefir with kecombrang extract

Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

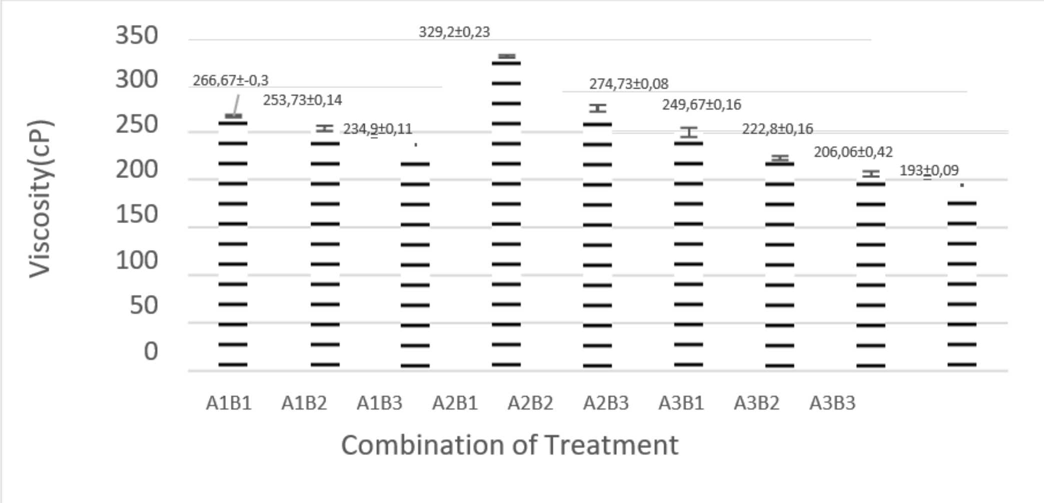


Figure 2. The average viscosity value of kefir with kecombrang extract

Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

Viscosity

The Analysis of Variance results indicated that the addition of kecombrang extract had a significantly (P<0.05) increased the viscosity of kefir. The addition of different concentrations also significantly affected the viscosity of kefir. Differences in particle size in each part of the plant before the extraction process can affect the dispersed and dispersing phases, resulting in differences in the extract produced, thus affecting the viscosity of kefir. The higher the extract concentration, the lower the viscosity because the added extract was in a liquid form liquid which increased water content and reduced viscosity (Sutrisno *et al.* 2019).

Color (*Lightness*)

The results showed that the lowest L* was observed in the addition of 7.5% kecombrang extracted from flowers(A3B3). It was tannins that gave the brown color and the total anthocyanins gave the red color to the extract which combinedly affected the white color of the kefir produced (Kumalasari, 2016).

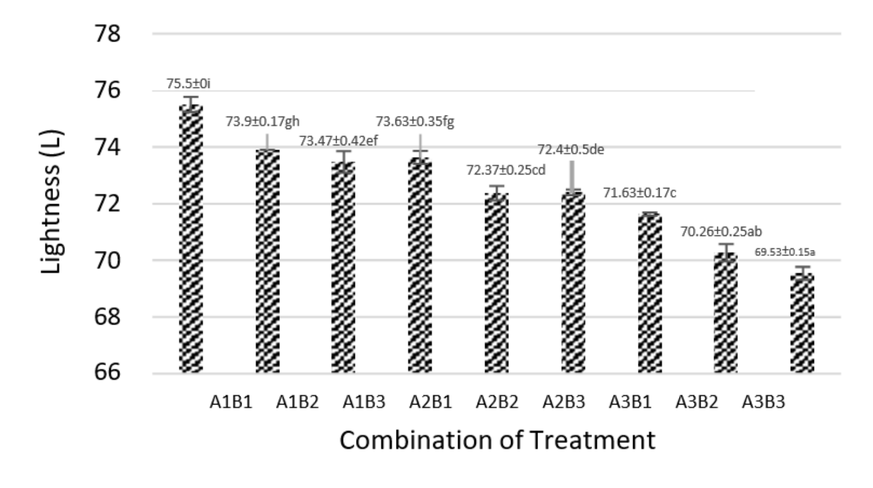


Figure 3. The average lightness value of kefir with kecombrang extract

Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%)

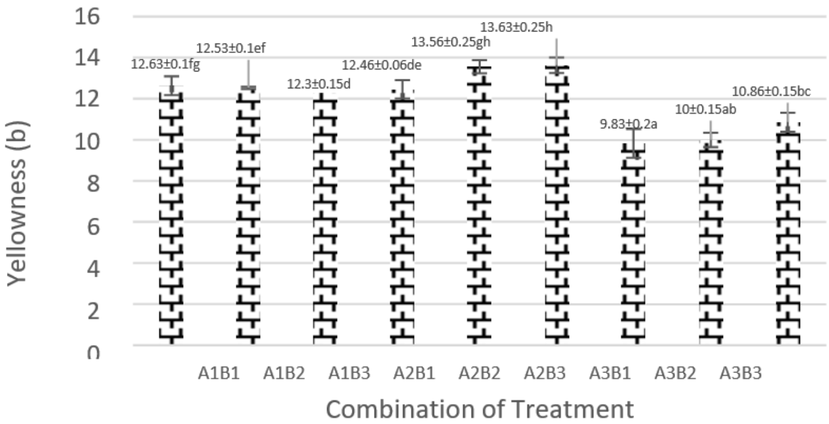


Figure 4. The average yellowness value of kefir with kecombrang extract

Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

Color (*Yellowness*)

The results showed that b* value in the color reader serves to determine the blue (-60) to yellow (+60) color in kefir. The results indicated that the addition of kecombrang extract (A) and extract (B), also the interaction between the two has a significant effect. Leaf is a bioactive component of kecombrang with the highest number, especially at a concentration of 7.5%.

Color (*Redness*)

Redness (a*) value represents green (-60) to red (+60). Kecombrang flowers had the smallest minus value close to 0 especially at the highest concentration of 7.5% because flower anthocyanin compounds are natural coloring pigments. The treatment that was close to -60 in the study was A2B3 product (combination treatment of kecombrang leaf bioactive components with a concentration of 7.5%) (Kumalasari, 2016). Leaves contain chlorophyll or leaf green substances that can affect the color of kefir.

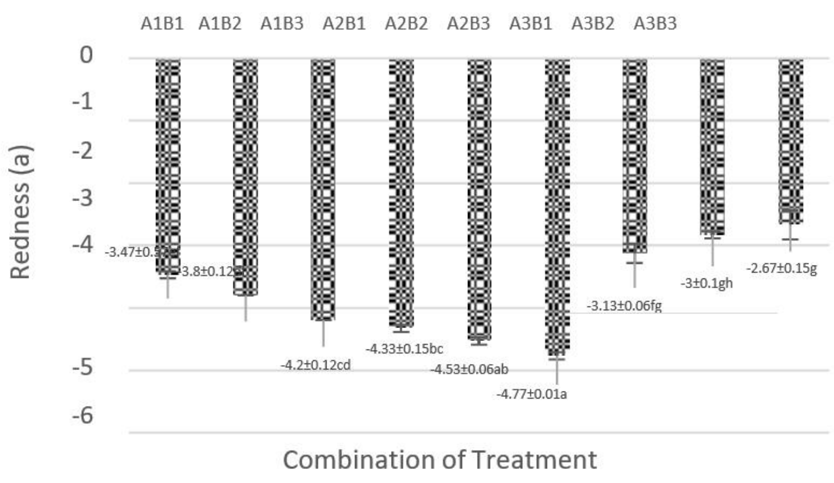


Figure 5. The average redness value of kefir with kecombrang extract

Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

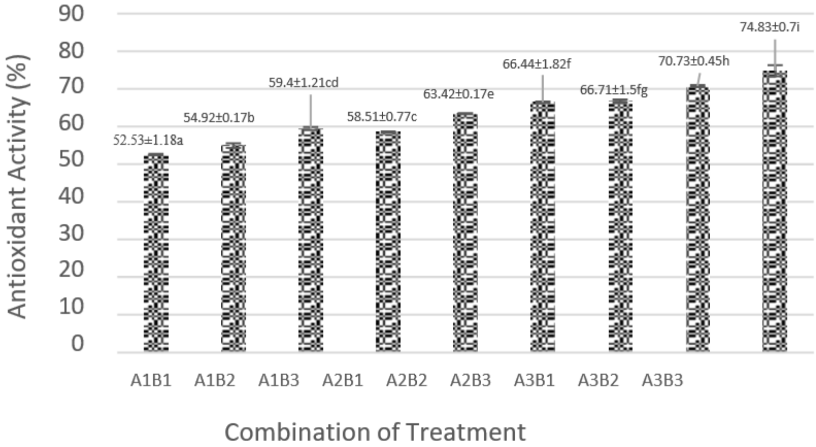


Figure 6. The average antioxidant activity value of kefir with kecombrang extract
Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

Antioxidant Activity

The results showed that the most effective treatment, exhibiting the highest antioxidant activity, was kefir supplemented with kecombrang flower extract at a concentration of 7.5% (A3B3) with a value of 74.83%. The results of this research on antioxidant activity matched the range reported by Naufalin and Rukmini (2010) of antioxidant activity in macerated kecombrang plant (Etlingera elatior) 61.61% - 83.17% in flowers, 40.64% - 60.40% in leaves, and 57.42% - 84.65% in stems.

Lactic Acid Bacteria (LAB)

The type of kecombrang extract, the concentration of the extract, and the combined treatment gave a significant effect ($p<0.05$) on the total LAB on kefir. The results of the DMRT further test showed that each treatment combination was significantly different from one another. It is evident that the combination of A2B3 treatments gave the highest total LAB yield of 1.33×10^{12} CFU/ml, while A1B3 had the lowest total LAB of 4×10^{11} CFU/ml. The physicochemical and microbial characteristics of kefir fermented milk are affected by factors like the type of milk, the ratio of grains to milk, fermentation time and temperature, and storage conditions (*Hecer et al.,* 2019).

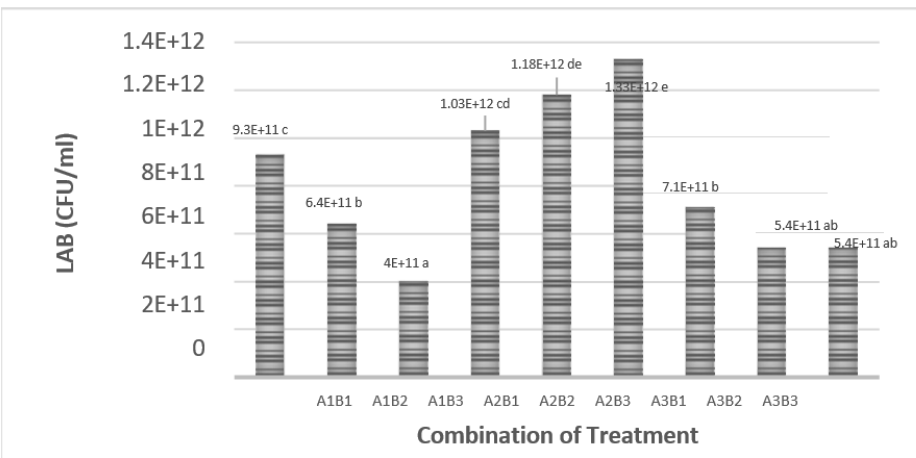


Figure 7. The average Lactic Acid Bacterial value of kefir with kecombrang extract
Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

The extracted kecombrang leaves had a higher total LAB than the other parts of kecombrang plant. This is closely related to the pH value of each combination treatment that added kecombrang extract from stems, leaves and kecombrang flowers with various concentrations. Total LAB of kefir with the addition of kecombrang extract with various concentration in this study was in the range of $4x10^{11}$ CFU/ml - $1.33x10^{12}$ CFU/ml. Based on CODEX STAN 243-2003 of the total LAB standard in fermented milk, which is at least 10^7 CFU/ml, it can be concluded that each treatment combination has met the total LAB standard in fermented milk. In addition, the total LAB value of kefir with the addition of bioactive compounds with various concentration. The total LAB in kefir in this study was in accordance with SNI (Indonesian National Standard) 2981:2009, namely 10^7 CFU/ml.

Syneresis

Syneresis analysis on kefir with the addition of kecombrang extract in various concentrations did not have significant effect because the two factors did not directly affect the percentage of kefir syneresis. This is due to the presence of intervening variables. Intervening variables (or mediators) are variables that, in theory, influence the relationship between independent variables (type of kecombrang extract with various concentrations) and dependent variables (syneresis) create an indirect relationship that cannot be directly observed or measured.

Sensory Characteristics

Aroma

Around 60% of panelists tended to give an assessment of the uniqueness of kefir with the addition of kecombrang leaf bioactive extract with a concentration of 2.5% (A2B1) and samples of kefir with the addition of kecombrang stem extract with a concentration of 2.5% (A1B1). The sour aroma of kefir is influenced by the dominant acid content, namely lactic acid. This is in accordance with Andaru *et al.* (2019) that the taste and aroma of kefir are the result of acid compounds, especially lactic acid and a combination of alcohol compounds resulting from yeast activity. Other factors that influenced aroma are the quality of the aroma components, temperature, aroma composition, food viscosity, natural interactions between components and nutritional components in the food such as protein, fat and carbohydrates (Musdholifah & Zubaidah, 2016).

Texture

Based on the results of descriptive analysis, panelists tend to give an assessment of having a distinctive texture on kefir with the addition of kecombrang stem bioactive extract with a concentration of 2.5% (A1B1), samples of kefir with the addition of kecombrang leaf extract with a concentration of 2.5% (A2B1), and samples of

	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3
Score 5	6.67%	0.00%	0.00%	6.67%	0.00%	0.00%	0.00%	0.00%	0.00%
Score 4	53.33%	26.67%	6.67%	60.00%	6.67%	6.67%	60.00%	13.33%	6.67%
Score 3	33.33%	53.33%	53.33%	20.00%	66.67%	40.00%	26.67%	40.00%	33.33%
Score 2	6.67%	13.33%	20.00%	13.33%	20.00%	33.33%	13.33%	33.33%	33.33%
Score 1	0.00%	6.67%	20.00%	0.00%	6.67%	20.00%	0.00%	13.33%	26.67%

Score 1 Score 2 Score 3 Score 4 Score 5

Figure 8. The average aroma value of kefir with kecombrang extract

Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

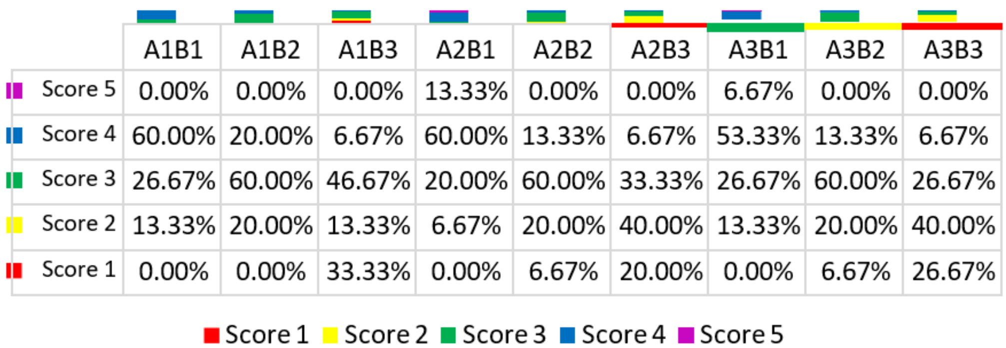
kefir with the addition of kecombrang extract of kecombrang flower extract at a concentration of 2.5, namely 60% each of the panelists.

Taste

Based on the result, it can be concluded that the panelists assessed the uniqueness of kefir by adding kecombrang extract extracts of kecombrang stems and leaves and the higher the concentration used, the less distinctive the taste of milk kefir. This is in accordance with the literature that kecombrang stems produce delicious and fresh, so the addition of kecombrang stems was more preferred. Kecombrang stems have a fresh sour taste so the addition of kecombrang stems from organic acids gives a dominant sour taste. The fresh sour taste of *kecombrang* stems can affect the product to taste sour due toorganic acids (Naibaho *et al.,* 2020).

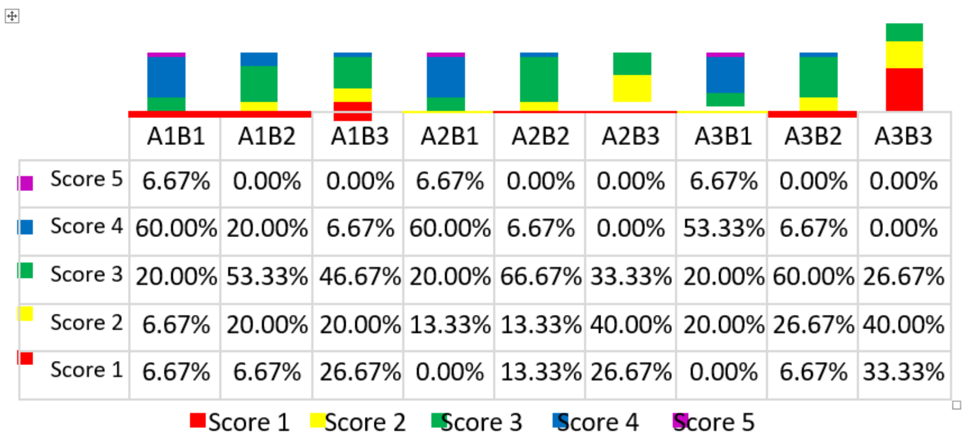
Overall Assessment

The results of the descriptive analysis revealed that panelists expressed a range of preferences, from strong dislike to strong like for each treatment combination of kefir. They showed a preference for kefir with a 2.5% concentration of *kecombrang* stem bioactive extract (A1B1), kefir with a 2.5% concentration of *kecombrang* leaf extract (A2B1), and kefir with a 2.5% concentration of *kecombrang* flower extract. Approximately 60% of the panelists favored each of these samples. The



	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3
Score 5	0.00%	0.00%	0.00%	13.33%	0.00%	0.00%	6.67%	0.00%	0.00%
Score 4	60.00%	20.00%	6.67%	60.00%	13.33%	6.67%	53.33%	13.33%	6.67%
Score 3	26.67%	60.00%	46.67%	20.00%	60.00%	33.33%	26.67%	60.00%	26.67%
Score 2	13.33%	20.00%	13.33%	6.67%	20.00%	40.00%	13.33%	20.00%	40.00%
Score 1	0.00%	0.00%	33.33%	0.00%	6.67%	20.00%	0.00%	6.67%	26.67%

Figure 9. The average Texture value of kefir with kecombrang extract
Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).



	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3
Score 5	6.67%	0.00%	0.00%	6.67%	0.00%	0.00%	6.67%	0.00%	0.00%
Score 4	60.00%	20.00%	6.67%	60.00%	6.67%	0.00%	53.33%	6.67%	0.00%
Score 3	20.00%	53.33%	46.67%	20.00%	66.67%	33.33%	20.00%	60.00%	26.67%
Score 2	6.67%	20.00%	20.00%	13.33%	13.33%	40.00%	20.00%	26.67%	40.00%
Score 1	6.67%	6.67%	26.67%	0.00%	13.33%	26.67%	0.00%	6.67%	33.33%

Figure 10. The average taste value of kefir with kecombrang extract
Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

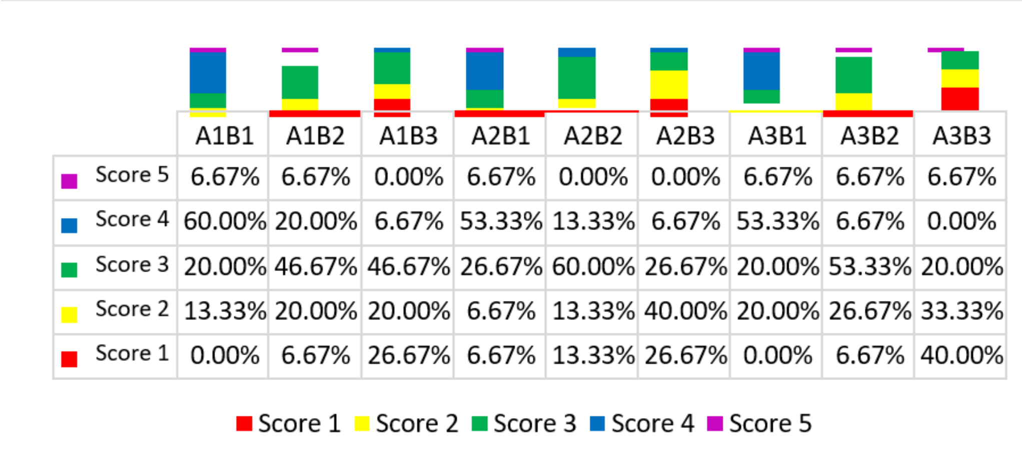


Figure 11. The average Overall value of kefir with kecombrang extract

Notes: A = Type of kecombrang extract (A1 = Kecombrang stem extract; A2 = Kecombrang leaf extract; A3 = Kecombrang flower extract); B = Extract concentration (B1 = 2.5%; B2 = 5%; B3 = 7%).

sample that the panelists did not like was kefir with the addition of kecombrang extract (stems, leaves, and kecombrang flowers) with a concentration of 7.5%, which were 26.67% - 40% respectively.

Chemical Characteristics

Water Content

In the control study, the water content of the control had an average value of 87.8%,while for the best treatment, kefir with the highest antioxidant content (A3B3) had a water content of 90.6%.This increase in water content could be due to a decrease in the viscosity value of kefir due to the addition of kecombrang flower extract which was considered quite high, namely 7.5%, so that there was an increase between control kefir and kefir that had been added with kecombrang extract.

Ash Content

Ash content is an inorganic substance produced from the combustion of an organic material. Ash content is related to the mineral content of a material; the higher the ash content, the higher the minerals contained in the material. The standard of yogurt ash content according to SNI 2981:2009 is a maximum of 1%. Kefir in this study was in accordance with the corresponding SNI standard at an average of 0.85%, while in the best treatment (A3B3) was 0.99%. A difference was evident between the untreated kefir and fermented kefir because there was an additional raw material in the form of liquid extract of kecombrang flowers in the manufacture of kefir.

Fat Content

According to CODEX STAN 243-2003, the standard fat content in kefir was less than 10%.

Table 1. Proximate Analysis of Kefir

Proximate Analysis	Sample	
	Control	A3B3
Water Content (%)	87,87	90,60
Ash Content (%)	0,85	0,99
Fat Content (%)	2,76	2,19
Protein Content (%)	6,65	6,83
Carbohydrate Content (%)	1,87	0,61

Notes = Control = without the addition of extracts; A3B3 = 7% kecombrang flower extract

The difference between the best kecombrang and control treatments was due to the fat contained in the kecombrang extract. According to Naufalin *et al.,* (2005) kecombrang flower has a very high fat content (10,81%), while based on the results of research by Wijekoon *et al.,* (2011) stated that the fat content in kecombrang flowers was 18.6%.

Protein Content

One of the important ingredients in probiotic drinks is protein. The results showed that the control kefir and the combination of factors with different kecombrang extract and varied concentrations have met the requirements for protein content according to international standards (CODEX STAND 243-2003), namely a minimum of 2.7%. The protein content in kefir is derived from the protein content of the milk and the kefir grains as the starter. Muawanah *et al.* (2012) reported that jelly candy incorporated with *kecombrang* extract contained 7.34% protein, which was higher than that of jelly candy without *kecombrang* extract, namely 5.95%.

Carbohydrat Content

Kefir with the addition of *kecombrang* extract contained 0.6% carbohydrate, while kefir without kecombrang extract had 1.8% carbohydrate. The carbohydrate levels were determined by subtracting the amounts of other components, such as fat, protein, ash, and water content. When the levels of these other proximate components are low, the carbohydrate content in kefir tends to be higher, and vice versa (Makanjuola et al., 2012).

Conclusions

The type of kecombrang extracts influences viscosity, heat, antioxidant levels, and lactic acid bacteria of the kefir product. Kecombrang flower extract is the optimal treatment as it exhibits the highest antioxidant activity of all other parts of kecombrang plant. This study showed that the higher the kecombrang extract concentration, the higher the viscosity and antioxidant content. In sensory testing, kefir added with 2.5% kecombrang extract in the form of sticks received a more favorable overall evaluation from panelists compared to other treatments. The most effective kefir formulation was achieved with the addition of 7.5% kecombrang flower extract, which demonstrated the highest antioxidant content at 74.83%. Kefir added with 7.5% kecombrang flower extract had 90.6% water content, 0.99% ash, 2.19% fat, 6.82% protein, and 0.6% carbohydrate, all in accordance with CODEX STAN 243-2003.

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