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The potential of the addition of *Secang* wood extract on physico-chemical properties, antioxidant and antibacterial activities of goat-milk kefir as a functional food product

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KEYWORDS

Antimicrobial activity
Antioxidant activity
Kefir
Goat milk
Sappan wood

ABSTRACT

This study aimed to improve the functional quality of goat-milk kefir by adding *secang* wood extract. The initial stage of the research was the extraction of *secang* wood using distilled water. Kefir is made by mixing *secang* wood extract and pasteurized goat milk with 5% kefir grains, then incubated at 25̊C for 20 hours. Goat-milk kefir was then tested for physicochemical quality (i.e., total solids, water content, pH value, acidity value, viscosity, and alcohol content), microbiological quality (i.e., total lactic acid bacteria (LAB) and total yeast), organoleptic properties, antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, and antioxidant activity. The results showed that the physicochemical quality of kefir with the addition of *secang* wood extract had an effect ($p<0.05$) on total solids, water content, pH value, acidity value, and viscosity. The results showed that the addition of *secang* wood extract 8% to goat-milk kefir has an antioxidant activity of 12.18% and an antibacterial activity by inhibiting *Escherichia coli* and *Staphylococcus aureus* 24.81mm and 18.95mm, respectively. These findings confirmed that goat-milk kefir, with the addition of *secang* wood extract, has the potential as a functional food.

Introduction

The increasing interest in functional foods has led to high interest in developing effective functional foods, especially from natural sources because they are safer and non-toxic (Yadav et al., 2016; Flieger et al., 2021). Consumers have become more interested in goat milk and its derivatives in recent years due to its nutritional and bio-functional characteristics. Compared to cow milk, goat milk contains a higher amount of dry matter, total protein and casein, milk fat, and mineral compounds. Small-sized fatty acid globules, high quantities of medium-chain fatty acids, and short-chain fatty acids such as caproic, caprylic, and capric acid define the fatty acid content of goat milk (Yilmaz-Ersan et al., 2016). In addition, due to the molecular components of the fat granules in goat milk are smaller than those in cow', it has a more uniform combination and is easier to digest (Turkmen, 2017; Nayik et al., 2022). However, goat milk has a disadvantage in that it has a distinct goaty flavor. According to previous research, kefir manufactured from goat milk no longer has the distinctive goaty flavor owing to the fermenting process (Huang et al., 2020; Muelas et al., 2022).

Kefir is known as a dairy product that can be produced using pasteurized milk from cows, goats, or sheep (Farag et al., 2020). Kefir is also a probiotic drink product that can balance bacteria in the digestive tract (Karkadova and Ghoddusi, 2018). In addition, Suhartanti (2014) found that kefir manufactured from goat milk has antibacterial activity towards *Staphylococcus aureus* with an inhibition zone of 5.67 mm. Many research has been done on kefir based on the origin of kefir grain, such as Thailand kefir (Luang-In and Deeseenthum, 2016); and milk type, such as goat-milk kefir (Martharini and Indratiningsih, 2017; Endah et al., 2022). Therefore, innovation is required in establishing kefir as a functional food by integrating commonly accessible and inexpensive components. Adding natural substances with functionality features is one way to improve the functional properties of goat-milk kefir.

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Secang wood, known as *Caesalpinia sappan*, is a plant that can be easily found in Indonesia at an affordable price. The active ingredients widely known in *secang* wood are brazilin, sappanin, brazilein, essential oils, and gallic acid (Vardhani, 2019). Chu et al. (2013) demonstrated that the content of these active compounds has antioxidant activity comparable to commercial antioxidants such as BHT and BHA. In addition, the brazilin content in *secang* wood may reflect its ability to counteract the activity of pathogenic bacteria, both from Gram-negative and Gram-positive compounds (Safitri et al., 2022).

The potential of *secang* wood extract as a functional food has been widely studied. Previous studies have proven that components of *secang* wood extract have activity as antioxidants (Suwan et al., 2018), antibacterial (Kondo et al., 2017), anti-inflammatory (Nirmal and Panichayupakaranant, 2015), anti-atherosclerotic, and anticarcinogenic (Vardhani, 2019; Flieger et al., 2021). *Secang* wood has also been applied to several food products, such as pasteurized milk (Kimestri et al., 2018), traditional beverages (Fadhilah et al., 2022), and added to herbal drinks (Raharjo et al., 2016; Vardhani, 2019). However, producing kefir using goat milk with the addition of *secang* wood extract has never been done before. Therefore, in this study, the potential of *secang* wood extract in goat-milk kefir as a functional food (antioxidant and antibacterial activity) and changes that occur in the physicochemical properties were investigated.

Research Methods

Secang wood extraction

Secang wood was obtained from West Imogiri, Bantul, Yogyakarta (3 kg), dried under the hot sun for 4 hours, and ground to obtain a dry powder with a 60 mesh sieve. *Secang* wood powder is extracted using water. A 100 g of *secang* powder is added to 1.5 L of distilled water and then boiled for 3 hours. The filtrate is obtained by filtering the extraction results using a filter cloth (Xu and Lee, 2004).

Raw material analysis

Raw material analysis was carried out on pasteurized goat milk and *secang* wood extract. The tests on both raw materials were water content and total solids testing using the oven method (AOAC, 1995), pH test using pH meter (Hanna-HI 98103), viscosity measurement using a viscometer (Brookfield DV-II+ Pro, USA), antioxidant activity testing using the DPPH method (Payet et al., 2005), antibacterial activity using the well diffusion agar method against *Escherichia coli* and *Staphylococcus aureus* (Sulmiyati et al., 2019).

The process of kefir production

Kefir was made using the modified Martharini and Indratiningsih (2017) method. A sterile glass jar was prepared and filled with 200 ml of goat's milk. Fresh goat milk was treated with 0%, 4%, and 8% *secang* wood extract (v/v), respectively, and pasteurized at 80 to 85 °C for 30 minutes, then cooled to 25°C. Afterward, the milk mixture was inoculated with 5% kefir grains (w/v), followed by incubation at 25°C for 20 hours. Following incubation, kefir grains was separated from the jar using a filter. Kefir was then cooled to 4-6° C until further analysis. All goat-milk kefir products were tested for physicochemical properties, as well as antioxidant and antibacterial activities.

Microbiological analysis

The total lactic acid bacteria (LAB) test was carried out by calculating the total LAB growing on the MRS Agar culture medium. About 0.1 mL of diluted sample was put into a petri dish and then poured with MRS agar. The sample was then incubated at 37°C for 24 hours. The incubated samples were counted for the number of bacteria by counting the number of colonies (Nurliyani et al., 2014).

The calculation of total yeast began with diluting the sample. The 0.1 mL of diluted samples, sterile Malt Extract Agar (MEA) media, and chloramphenicol were added to the petri dish. The petri dish was incubated with reversed conditions at 37°C for 24 hours to count the number of colonies (Nurliyani et al., 2014).

Physico-chemical analysis

Physico-chemical tests carried out in this study were pH value, total acid, water content, total solids, viscosity, and alcohol content. The pH of goat-milk kefir with the addition of *secang* wood extract was measured using a pH meter (Hanna-HI 98103). The total acid test was carried out by calculating the acid content equivalent to lactic acid using the titration method. Acid content is calculated by Eq. 1:

$$Acid\ content\ (\%) = \frac{ml\ NaOH\ x\ N\ NaOH\ x\ 0.09}{sample\ weight\ (g)}\ x\ 100\% \quad \text{......} (1)$$

Water content and total solids were measured using the AOAC (1995). Viscosity was measured using a viscometer (Brookfield DV-II+ Pro, USA). Spindle 63 was chosen and connected to a sample in a container. The viscosity test on the kefir sample was conducted twice.

The alcohol content of the samples was tested using the Micro Conway Diffusion method (Nurliyani et al., 2014) with modifications. Ethanol solution was used to make a standard curve (y= -4.88x+0.686).

Organoleptic test

The organoleptic assessment criteria tested were color, aroma, taste, consistency, alcoholism, and acceptability. The organoleptic test consisted of 15 untrained panelists filling out a questionnaire. The tests were conducted to examine the attributes of color, aroma, texture, taste, alcoholism, and acceptability overall preference for goat-milk kefir with a range of 1-5. Each sample was given a code of two-digit numbers randomly.

Antioxidant activity

Antioxidant activity was analyzed based on its ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the method of Payet et al. (2005) with modifications. Prior to measurement, the samples were diluted first. A spectrophotometer observed the absorbance value at a wavelength of 517 nm. Antioxidant activity is expressed in Eq. 2:

$$Antioxidant(\%) = \frac{\text{DPPH absorbance} - \text{Sample absorbance}}{\text{DPPH absorbance}} \text{ X } 100\% \quad (2)$$

Antibacterial activity

Antibacterial activity was detected using the well diffusion agar method, according to Sulmiyati et al. (2019). Antibacterial activity testing was tested using two bacteria, *Escherichia coli* and *Staphylococcus aureus*. Sterilized nutrient agar (NA) was cooled at 50°C in a water bath. Two wells were made in the agar with a diameter of 9.5 mm. In each well, 200 µl of goat milk kefir at various levels of *secang* wood was added and incubated at 37°C for 24-48 hours. The diameter of the inhibition zone was observed using a micrometer and stated in mm. Clear zones around the well show the ability of goat-milk kefir to inhibit *Escherichia coli* and *Staphylococcus aureus*. Antibacterial activity was calculated by measuring the diameter of clear zones (mm) minus the well diameter (mm). The antibacterial activity test was done in duplicate.

Data analysis

The data from antioxidant activity testing, microbial inhibition activity, microbiological testing (i.e., total LAB and total yeast), and physicochemical testing were analyzed statistically using one-way ANOVA (α=5%) and continued with the Duncan Multiple Range Test (DMRT) using IBM SPSS 24 software. Data organoleptic test results were analyzed using non-parametric analysis, the Kruskal-Wallis hedonic test, followed by the Mann Whitney test using IBM SPSS 24 software.

Results and Discussion

Table 1 depicted of the characteristics of raw material, pasteurized goat milk, and *secang* wood extract, used in making goat-milk kefir.

This study uses a water extraction method to obtain *secang* wood extract. Hence, the *secang* wood extract obtained in this study has a high water content of 98.93% with a total solid value of 1.07% and a viscosity value of 3.95 cP. It was previously reported that water extraction with the ratio of secang powder to water at 1:15 (w/v) at 100°C has the ability to effectively extract active compounds in *secang* wood (Palasuwan et al., 2005). Therefore, the water content was considerably high, with a lower total solid, creating less viscous extract (Utari et al., 2017). The yellowish-red color was obtained because of the characteristics of the active compound brazilin from *secang* wood, which has a pH range of 4,5-5,5 (Rina et al., 2017). The *secang* wood extract used is known to have an antioxidant activity of 87.98%.

Table 1. The test result of raw materials of kefir production

Parameters	*Secang* Wood Extract	Goat Milk
Total solids (%)	1.07	14.07
Water content (%)	98.93	85.93
Viscosity (cP)	3.95	4.45
pH	5.83	6.5
Antioxidant activity (%DPPH)	87.98	0.96
Antibacterial activity		
- *Escherichia coli* (mm)	33.15	0
- *Staphylococcus aureus* (mm)	37.88	0

The extraction temperature influences the value of the antioxidant activity. Increasing the extraction temperature showed a higher total antioxidant activity. This is due to the extraction of the active substance brazilin, a potent antioxidant compound inside the *secang* wood's heart (Djaeni et al., 2021). A previous study found that the bioactive compounds present in *secang* wood water extract at 100ºC were phenol, flavonoid, tannin (Kimestri et al., 2018); and quinone (Adirestuti et al., 2018). *Secang* wood extract can also inhibit the activity of *Escherichia coli* and *Staphylococcus aureus* bacteria with inhibition zones of 33.15 mm and 37.88 mm, respectively. These findings are in line with previous research, which stated that the inhibition zone of *secang* wood extract with water at 100ºC showed more significant inhibition of *Staphylococcus aureus* (37.88 mm) compared to *Escherichia coli* (33.15 mm) (Kimestri et al., 2018).

The pasteurized goat milk has a total solids value of 14.07% with a water content value of 85.93%. Goat milk has a viscosity of 4.45 and a pH of 6.5. This result is in accordance with previous results that reported the moisture content, total solids, and pH of pasteurized goat milk were 88%, 12%, and 6.42, respectively (Rahmawati and Suntornsuk, 2016). Goat milk has an antioxidant activity of 0.96%. The lower antioxidant activity is due to decreased concentration of antioxidant vitamins and enzymes during pasteurization (Andrei et al., 2015). In addition, goat milk did not show the ability to inhibit *Escherichia coli* and *Staphylococcus aureus*. In a previous study, raw and pasteurized goat milk have no inhibition towards *S. aureus* and *E. coli* because of the small amount of antimicrobial agent and were still not activated nor excreted before the fermentation process (Rahmawati and Suntornsuk, 2016).

Microbiological quality of kefir

The results of the total LAB and yeast on goat-milk kefir with the addition of *secang* wood extract can be seen in Table 2. The statistical analysis results showed that goat-milk kefir with the addition of *secang* wood extract had a significant effect ($p<0.05$) in elevating the total number of LAB. A higher concentration of *secang* wood extract added to goat-milk kefir resulted in higher total LAB values (Table 2). Adding 8% *secang* wood extract can produce kefir with the highest total LAB value of 9.99 logs CFU/mL. This condition may be due to the presence of a growth factor compound in *secang* wood extract, namely quinone (Sinsawasdi, 2012).

The *secang* wood extract did not inhibit yeast growth in this study. This phenomenon was also reported by the research of Mazaya et al. (2020), that no inhibition zones were formed by antibacterial compounds against yeast. This could be due to yeast having biochemical stability, the ability to spread quickly, resist autotoxicity (Periadnadi et al., 2018), and tolerate low pH between 3.5 and 4.5 (Mazaya et al., 2020). The analysis showed that goat-milk kefir with the addition of *secang* wood extract had a significant effect ($p<0.05$) in elevating the total number of total yeast. The more the *secang* wood extract was added, the higher the yeast in kefir. Kefir made without adding *secang* wood extract has a total yeast value of 6.11 log CFU/mL. Adding 4% *secang* wood extract may produce a total yeast of 6.43 log CFU/mL. Adding 8% *secang* wood extract will produce a total yeast value of 6.97 logs CFU/mL. The increase in the number of yeast and LAB simultaneously is caused by a symbiosis between the two microorganisms through the sharing of bioproducts as a source of energy and growth factors (Stadie et al., 2013; Bengoa et al., 2019).

In this study, the higher concentration of *secang* wood extract added to goat-milk kefir resulted in a significant increase in the total LAB (Table 2). This increase in the total LAB can be caused by quinone compounds in *secang* wood extract (Sinsawasdi, 2012). Therefore, increasing the total LAB can also result in more energy and growth factors forming during fermentation and can be used for yeast growth. Thus, a higher total of yeast was found in this study when a higher concentration of *secang* wood extract was added to goat-milk kefir (Table 2). The symbiotic interaction between yeast and LAB was also reported by research conducted by Andrei et al. (2015). They reported that yeast (*S. cereviceae*) provided amino acids for the growth of LAB (*L. nagelii* and *L. hordei*), and the LAB produced side products that became growth factors for yeast growth. Similar study regarding a mutually beneficial symbiotic interaction between yeast and LAB have also been reported (Ponomarova et al., 2017). From their findings, it is known that in a nitrogen-rich environment, *S. cereviceae* produces amino acids that can be used for the growth of *L. lactis*. Furthermore, *L. lactis* provides carbon sources such as glucose and galactose for the growth of *S. cereviceae*.

Table 2. Total LAB and yeast of goat-milk kefir with the addition of different concentration of *secang* wood extract

The Concentration of *Secang* Wood Extract	Total LAB (log CFU/mL)	Total Yeast (log CFU/mL)
0%	9.10 ± 0.12[a]	6.11 ± 0.03[a]
4%	9.73 ± 0.09[b]	6.43 ± 0.10[b]
8%	9.99 ± 0.06[c]	6.97 ± 0.03[c]

Note: Different letters in the same column indicate a significant difference ($p<0.05$).

Table 3. The pyscho-chemical characteristics of goat-milk kefir with adding different concentration of *secang* wood extract

The Concentration of *Secang* Wood Extract	Total Solid (%)	Water Content (%)	pH Value	Acidity Value (%)	Viscosity (%)	Alcohol Content (%)
0%	13.1 ± 0.63[b]	86.19 ± 0.63[b]	3.83 ± 0.11[a]	1.34 ± 0.04[c]	765.82 ± 60.97[b]	0.11 ± 0.03[a]
4%	12.26 ± 0.70[a]	87.74 ± 0.70[a]	4.08 ± 0.07[b]	0.73± 0.01[b]	708.02 ± 18.86[b]	0.13± 0.00[a]
8%	11.47 ± 0.78[a]	88,53 ± 0.78[a]	4.73 ± 0.05[c]	0.53 ± 0.01[a]	236.27 ± 64.48[a]	0.12 ± 0.03[a]

Note: Different letters in the same column indicate a significant difference ($p<0.05$).

Physico-chemical quality of kefir

In the physicochemical test of kefir with the addition of *secang* wood extract, it was found that increasing the concentration of *secang* wood extract causes a decrease in the total solids of kefir. On the other hand, the decrease in total solid content was followed by an increase in the water content of kefir, as shown in Table 3. The *secang* wood extract used in this study was obtained from an extraction process using pure sterile distilled water. The use of distilled water on this *secang* wood extract caused a change in the ratio of total solids and water content in goat-milk kefir. In a study by Öner et al. (2010), the resulting goat-milk kefir had a total solid content of 13.02 ± 2.92%. While, in this study, the total solid content in kefir with the highest *secang* wood extract (8% v/v) was 11.47 ± 0.78 %. These findings illustrate that adding *secang* wood extracts up to 8% (v/v) did not significantly change the total solid content of goat-milk kefir. The total solids of goat-milk kefir with the addition of 4% *secang* wood extract approached the solids values of kefir in other similar studies (Setyawardani et al., 2014; Moreno and Emata, 2020).

In making goat-milk kefir, LAB bacteria have an essential role in lowering the pH value of the product. This is because LAB bacteria decompose lactose in milk into lactic acid through a fermentation process (Chen et al., 2017; Bintsis, 2018; Hendarto et al., 2019). The lactic acid produced from the fermentation process causes kefir products to taste sour (Suriasih et al., 2012; Sulmiyati et al., 2019).

In this study, all goat-milk kefir samples met the standard kefir acidity value in Codex STAN 243-2003 (minimum 0.6%). The addition of *secang* wood extract increased in the pH value and decreased in the acidity of the goat-milk kefir sample (Table 3). Goat-milk kefir without *secang* wood extract has a lower pH and a higher acidity than kefir with *secang* wood extract. Increasing the concentration of *secang* wood extract added to goat-milk kefir resulted in an increase in the pH value and a decrease in the product's acidity, as shown in Table 3. The increase in the pH value was because the *secang* wood extract obtained from water extraction had a pH value of 5.83 (Table 1). Thus, the increasing concentration of *secang* wood extract increases the pH value and decreases the acidity level of goat-milk kefir.

The lactic acid produced during the fermentation process by LAB contributed to an increase in the viscosity value of kefir due to the increased amount of casein, which coagulates during fermentation. In the milk fermentation process, the acid produced by LAB causes a decrease in pH. This decrease in pH resulted in protein destabilization in milk, resulting in milk casein clumping (Badem and Uçar, 2017).

In this study, there was an increase significantly in the viscosity value of kefir samples when compared to the raw goat milk used (Table 1 and Table 3). However, adding *secang* wood extract resulted in a lower viscosity value of the goat-milk kefir samples. The lowest viscosity value (236.27 ± 64.48 %) was recorded for the kefir sample with the addition of 8% (v/v) of *secang* wood extract compared to other goat-milk kefir samples. With 8% *secang* wood extract added, goat-milk kefir has less casein composition than the other samples. This results in a small amount of casein clumping in the goat-milk kefir fermentation process, which lowers the viscosity value. In addition, the pH value of the goat-milk kefir sample with the addition of 0% and 4% was lower than that of 8% *secang* wood extract. According to research conducted by Husnaeni et al. (2019), the amount of acid produced during the fermentation process may cause a decrease in the pH value, which then causes protein (casein) destabilization. The protein destabilization could cause casein clumping and increase the kefir viscosity.

One of the characteristics of kefir is that it has an alcoholic taste. The values of the alcohol content of goat milk added with different concentrations of *secang* wood extract are presented in Table 3. The statistical analysis results showed that goat-milk kefir with the addition of *secang* wood extract did not affect ($p>0.05$) the alcohol content of kefir. The alcohol value with the addition of *secang* wood extract ranges from 0.12% to 0.13%. These results follow other studies, which has an alcohol content value of 0.01% to 1% (Setyawardani et al., 2014; Kesenkaş et al., 2015). According to Moreno and Emata (2020), the alcohol content in kefir is influenced by the metabolism of yeast and heterofermentative bacteria that produce ethanol. During fermentation, the lactose in milk is converted by the LAB into glucose and galactose. This glucose is then hydrolyzed by yeast and homofermentative bacteria in kefir into alcohol (Leite et al., 2013; Listiawati et al., 2019).

The sensory attributes quality

The organoleptic properties of goat-milk kefir with the addition of *secang* wood extract are described in Table 4. The statistical analysis showed that goat-milk kefir with the addition of *secang* wood extract showed an effect ($p<0.05$) on the color of the kefir. Before fermentation, the mixture between goat's milk and the *secang* wood extract had a pink colour. After fermentation, the kefir made without adding *secang* wood extract showed a white color. While kefir with the addition of *secang* wood extract, the color was a more yellow color. Kefir, with 4% *secang* wood extract, scored 3.13. According to the assessment score, it can be seen that kefir is slightly yellow. Kefir with 8% *secang* wood extract has the highest score of 4.07, which means that the kefir is yellow. The addition of *secang* wood extract to goat-milk kefir causes a color change due to biochemical changes in the oxidation of tannin compounds contained in *secang* wood extract (*Caesalpinia sappan* L.). Oxidation of the active substance in *secang*, namely brazilin, produces a brownish-yellow compound (Ngamwonglumlert et al., 2020).

The organoleptic result shows that the addition of *secang* wood extract had an effect ($p<0.05$) on the aroma of kefir. The aroma of kefir without *secang* wood extract showed the highest result, 4.27 (typical for kefir) while adding *secang wood* extract tends to lower the kefir's aroma. Yeasty unique aroma of kefir is produced during the fermentation, which involves kefir grain that consists of a mixture of yeast and bacteria. Furthermore, *secang wood* extract also contributes to the specific aroma of *secang* wood that covers the kefir's aroma. The flavonoid content (i.e., acid gallates, tannins, and brasilin) and essential oils in *secang* wood give a distinctive aroma (Irawan et al., 2022).

Kefir made without *secang* wood extract has the highest score in texture. The texture of kefir produced using higher concentrations of *secang* wood extract addition generates a lower consistency of goat-milk kefir. The panelists suggest that using *secang* wood extract and goat-milk kefir significantly ($p<0.05$) influences the texture of kefir. The texture of fermented products such as kefir is influenced by the acidity during fermentation. Increasing the concentration of *secang* wood extract added to goat milk resulted in an increase in the pH value and a decrease in the acidity of the resulting kefir (Tabel 3), thus, decreasing the consistency of kefir. A previous study reported that raw materials and ingredients influenced the product's texture (Setyawardani et al., 2019).

Table 4. Organoleptic properties of goat-milk kefir products with the addition of *secang* wood extract

The Concentration of *Secang* Wood Extract	Organoleptic Properties					
	Color	**Aroma**	**Taste**	**Alcoholism**	**Consistency**	**Acceptability**
0%	1.13 ± 0.36^{a}	4.27 ± 0.59^{c}	4.47 ± 0.64^{b}	3.13 ± 1.13^{ns}	4.33 ± 0.49^{b}	3.53 ± 0.83^{ns}
4%	3.13 ± 0.35^{b}	3.07 ± 0.70^{b}	3.47 ± 0.74^{a}	2.80 ± 0.77^{ns}	3.60 ± 0.63^{a}	2.87 ± 0.99^{ns}
8%	4.07 ± 0.45^{c}	2.47 ± 0.91^{a}	3.00 ± 0.85^{a}	2.67 ± 0.90^{ns}	3.20 ± 0.86^{a}	2.80 ± 1.20^{ns}

Note: Different letters in the same column indicate a significant difference ($p<0.05$).

Statistical analysis showed that goat-milk kefir with the addition of *secang* wood extract showed an effect on the taste ($p<0.05$). The average taste of kefir without adding *secang* wood extract showed the highest result, 4.47 (typical for kefir). Adding 4% and 8% *secang* wood extract showed lower average values of 3.47 and 3.00 (slightly acidic). These results are in agreement with the pH value of kefir with the addition of *secang* wood extract (Table 3). The taste of kefir was attributed to the addition of *secang* wood extract due to the presence of tannins (Widowati, 2011). Furthermore, the addition of *secang* wood extract did not affect the alcoholic taste of kefir. These results are in line with the alcohol content of the samples using the Conway method (Table 3). The statistical analysis results showed that goat-milk kefir with the addition of *secang* wood extract did not affect the acceptability of kefir ($p>0.05$). About 27% of the panelists had never consumed kefir, and all panelists only consumed kefir once a year.

Antioxidant activity

Antioxidants are compounds that can counteract the formation of radicals by giving electrons (electron donors) (Adi et al., 2022). One of the methods to determine antioxidant activity is by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The antioxidant activity of goat-milk kefir with the addition of *secang* wood extract can be seen in Figure 1.

The analysis showed that goat-milk kefir with the addition of *secang* wood extract had an effect ($p<0.05$) on antioxidant activity. The increased amount of *secang* wood extract produces kefir with higher antioxidant activity. The highest antioxidant activity was obtained by adding 8% *secang* wood extract (12.18%).

Adding *secang* wood extract during fermentation can increase antioxidant activity. This result is due to the active compounds contained in *secang* wood extract. Kimestri et al. (2018) stated that the active compounds found in *secang* wood extracted using distilled water andhave antioxidant activity are phenols and flavonoids. Furthermore, Ngamwonglumlert et al. (2020) and Nirmal and Panichayupakaranant (2015) stated that brazilin is one of the main phenolic compounds in *secang* wood, which can produce antioxidant activity. Flavonoid, phenol, and brazilin compounds in *secang* wood act as antioxidants by donating their hydrogen atoms to free radicals, making them non-radical.

Fermentation during kefir production can also increase antioxidant activity. This result can be seen from the antioxidant activity of pasteurized goat milk before fermentation, which has a value of 0.96% (Table 1). After being fermented for 20 hours, the value of the antioxidant activity increased to 2.24% (Figure 1). This result is supported by (Liu et al., 2005), who stated that goat-milk kefir could donate more protons than unfermented milk. Nurliyani et al. (2015) also stated that fermentation could enable proton donation process.

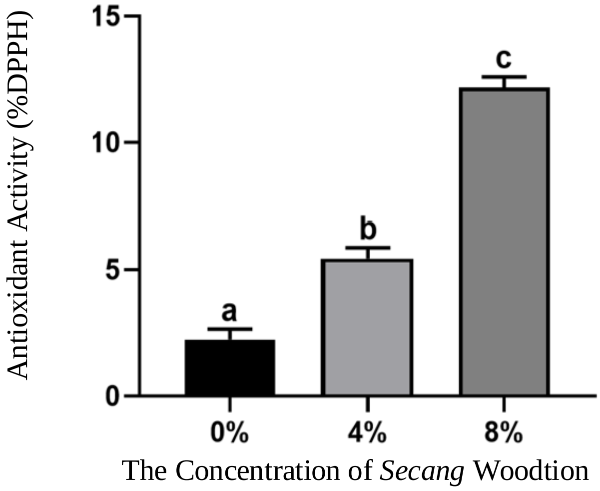

Figure 1. Antioxidant activity of goat-milk kefir with different concentration of *secang* wood extract. The difference between superscripts letters (a, b, c) in the same column shows significant differences ($p<0.05$).

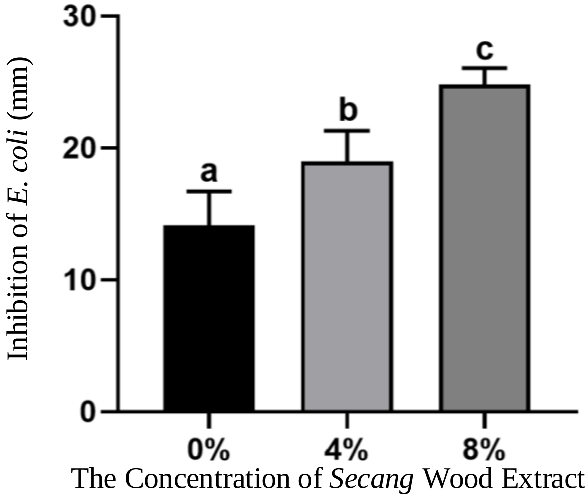

Figure 2. Antibacterial activity of goat's milk kefir with the addition of different concentrations of *secang* wood extract against *Escerichia coli* bacteria. The difference between superscripts (a, b, c) in the same column shows significant differences ($p<0.05$).

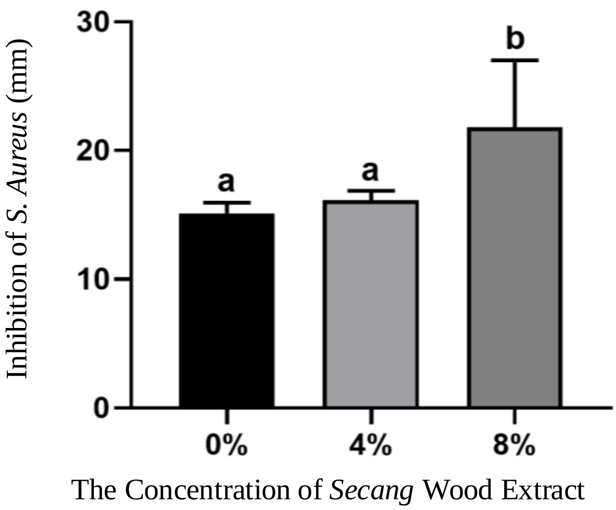

Figure 3. Antibacterial activity of goat-milk kefir with the addition of different concentrations of *secang* wood extract against *Staphylococcus aureus* bacteria. The difference between superscripts letters (a, b, c) in the same column shows significant differences ($p<0.05$).

Antibacterial activity

The antibacterial activity of goat-milk kefir added with *secang* wood extract was tested against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria (Figures 2 and 3).

The results showed that goat-milk kefir added with *secang* wood extract affected the antibacterial activity against *E. coli* bacteria ($p<0.05$). Adding 8% *secang* wood extract had the most significant inhibition of 24.81 mm compared to that of 0% and 4% with a value of 14.13 mm and 18.99 mm, respectively.

Based on the criteria for strong antibacterial power (Kimestri et al., 2018), an inhibition zone is categorized into weak (diameter of 5 mm or less), moderate (diameter of 5 to 10 mm), strong (diameter of 10 to 20 mm), and very strong (diameter of 20 mm or more). It can be seen that kefir samples from without and with the addition of 4% *secang* wood extract were categorized as strong. While adding 8% *secang* wood extract produced kefir with a very strong antibacterial activity.

The addition of *secang* wood extract increased the antibacterial effect of goat-milk kefir against *E. coli* bacteria. Antibacterial compounds of *secang* wood extract, such as tannins and brazilin (both are polar), caused the elevation of this antibacterial effect (Srinivasan et al., 2012; Nirmal and Panichayupakaranant, 2015). Galdiero et al. (2012)

stated that Gram-negative bacteria are sensitive to polar antibacterial compounds. This bacteria's cell wall is polar, so it will be easier for polar antibacterial compounds to pass. Therefore, *secang* wood extract may easily damage the outer membrane structure of Gram-negative bacteria. This result aligns with a previous study, which states that *secang* wood extract can inhibit *E.coli* bacteria by 20.3 mm in pasteurized milk (Kimestri et al., 2018),.

Figure 3 shows the antibacterial activity of goat-milk kefir with the addition of *secang* wood extract against *Staphylococcus aureus* bacteria. The results of statistical analysis showed that goat-milk kefir added with *secang* wood extract had an effect on antibacterial activity against *Staphylococcus aureus* bacteria ($p<0.05$). Antibacterial activity in kefir with 8% *secang* wood extract had the largest inhibition zone diameter (18.95 mm) than, with 4% or without *secang* wood extract of 16.14 mm and 15.12 mm, respectively. According to Kimestri et al. (2018), a compound is categorized as having robust antibacterial activity if it has an inhibition zone diameter of 10 - 20 mm. Thus, antibacterial activity in kefir without the addition of *secang* wood extract is classified as having a robust antibacterial activity. The addition of 8% *secang* wood extract could significantly elevate the antibacterial activity of goat-milk kefir against S*taphylococcus aureus* bacteria.

The higher the addition level of *secang* wood extract may produce a more significant inhibition zone. This significance is due to the content of flavonoids and tannins in *secang* wood extract. Carbon dioxide, hydrogen peroxide, diacetyl ethanol, and peptides (bacteriocins),produced during fermentation, also play a role in inhibiting the growth of pathogenic bacteria (Kesenkaş et al., 2015; Karkadova and Ghoddusi, 2018). The addition of *secang* wood extract in the production of goat-milk kefir can inhibit both Gram-positive and Gram-negative pathogenic bacteria. However, adding 4% *secang* wood extract did not significantly increase the antibacterial activity of goat-milk kefir (Figure 3) against Gram-positive bacteria such as *Staphylococcus aureus*. Moreover, the antibacterial activity of goat-milk kefir with the addition of *secang* wood extract against *Staphylococcus aureus* was lower than that against *Escherichia coli*. The peptidoglycan layer of Gram-positive bacteria caused these results. The thick peptidoglycan layer on Gram-positive bacteria cell walls causes antibacterial compounds hard to penetrate, resulting in lower antibacterial activity.

Conclusions

The addition of *secang* wood extract to goat milk kefir affects the microbiological and physicochemical quality of goat milk kefir. The increasing addition of *secang* wood extract concentration can increase the amount of LAB and yeast contained in goat milk kefir. In addition, increasing the concentration of *secang* wood extract had no effect on the total solid content of the resulting goat milk kefir. However, the addition of the concentration of *secang* wood extract in goat milk kefir can increase the pH value, decrease the acidity level and product viscosity. Furthermore, the addition of *secang* wood extract with various concentrations did not affect the alcohol content of goat's milk kefir. The addition of *secang* wood extract also could increase the antioxidant and antibacterial activity of goat's milk kefir against pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*).

Declarations

Conflict of interests The authors declare no competing interests.

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