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STUDY ON THE ADDITION OF HONEY AS A NATURAL ANTIMICROBIAL AGENTS IN AVOCADO JUICE (*Persea americana* Mill)

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Abstract. *Unpasteurized avocado juice is a popular food product, considered natural, fresh, and healthy. Microbial contamination in unpasteurized juice is high and can pose health risks to humans. Such processing technology is applied to reduce the level of contamination, such as pasteurization, adding preservatives, cooling/freezing temperature, or adding antimicrobial compounds. Honey has a high sugar content, low pH, contains hydrogen peroxide, and organic acid that can inhibit the growth of pathogenic bacteria. This research aims to determine the antibacterial activity of honey in avocado juice using different types of honey types including honey produced by Apis dorsata (AD), Apis mellifera from wildflowers (AMW), Trigona sp (TG) and A. mellifera from kapok (Ceiba pentandra) (AMC) flowers). The second factor was honey concentration (5, 10, and 15%). The results showed that after 2h of incubation, AD honey inhibited the total coliform bacteria by 98.84% at a concentration of 10%, AMW honey effectively inhibited total Staphylococci by 99.04% at a concentration of 15%, and TG honey successfully inhibited total Salmonella-Shigella bacteria at a concentration of 15%. Therefore, using honey in avocado juice can inhibit the growth of pathogenic bacteria, although it has not reached the minimal limits of fruit juice quality standards. Further research is needed to determine the minimum inhibitory concentration of honey required to inhibit pathogenic bacteria in avocado juice.*
Keywords: *antimicrobial; avocado; honey; juice*

1. Introduction

Many people consume avocados for health reasons, such as preventing cardiovascular disease and coronary heart disease (Pacheco *et al*., 2022) or therapy for people who are obese and insulin resistant (Zhang *et al*., 2022), support babies health (de Melo *et al*., 2019), and adult physical and cognitive development (Edwards *et al*., 2020). Apart from that, avocados are also believed to have an antimicrobial effect (Jesus *et al*., 2015), amylase inhibitor activities (Abd-Elkader *et al*., 2022), antioxidant and anti-inflammatory properties (Nguyen *et al*., 2021; Pătruică *et al*., 2022). Avocados are also a source of fiber and can lower cholesterol. Two tablespoons of avocado per serving contain 2 g of fiber, while the entire avocado fruit flesh contains 10 g of fiber (Wijayanti *et al*., 2014).

Avocados are consumed in various ways, starting from consuming them directly, processing them to sauce, toppings for eating bread, or in the form of juice. Most Indonesians consume avocados either by consuming them directly or in the form of juice. Avocados are even one of the

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fruits that must be available when someone sells fruit juice. However, unpasteurized fruit juice is more susceptible to bacterial contamination, which can affect its microbiological quality and shorten its shelf life compared to pasteurized fruit juice. The previous research conducted by Astuti *et al*. (2020) showed high levels of microbial contamination in fruit juice sold in Surakarta (Middle Java, Indonesia). Total number of aerobic microbes reached 6.07-8.95 $\log_{10}$ CFU/ml, total coliform 3.28-4,60 $\log_{10}$ CFU/ml, Total Staphylococci 4.47-7.35 $\log_{10}$ CFU/ml, and total Salmonella-Shigella reached 2,34-4,80 $\log_{10}$ CFU/ml. Similar results were also found in research conducted in Ethiopia, where 33% of juice taken on the market was contaminated with *Escherichia coli* and exceeded the predetermined standard (Mengistu *et al*., 2022). Research conducted by Lestari *et al*. (2015) found contamination in 13 out of 25 fruit juice samples in the Tembalang (Middle Java, Indonesia), with a count of 10^3 CFU/ml. Consuming food containing contaminants such as *E. coli* and *Salmonella* sp can cause illnesses such as diarrhea, stomach cramps, fever, vomiting, and even bloody diarrhea (Esmael *et al*., 2023). The symptoms are believed to be related to microbiological contamination in fruit juice stemming from several factors, including unhygienic conditions during household preparation, poor personal hygiene, and an unsanitary environment (Senbeta & Beyene, 2017).

Processes that can be applied to control the growth of microbial contaminants in fruit juice are cold storage, pasteurization, adding preservatives, or adding natural antimicrobial compounds. Mustofa *et al*. (2020) evaluated the effectiveness of yogurt as a natural antimicrobial in avocado juice. The addition of up to 20% yogurt can reduce the number of microbes, although the total microbe is still above the minimum allowable threshold. In addition, Astuti *et al*. (2021) reported that the addition of mint leaves (*Menta piperita*) was able to suppress the growth of *Salmonella-Shigella* and *Staphylococcus* sp. However, alternatives need to be found to reduce the level of contamination in minimally processed food such as fruit juice. The processing technology applied should be safe and not require additional processes that could result in a decrease in nutritional value. Exploring natural potential which is rich in antimicrobial compounds needs to be done to obtain natural products.

One natural ingredient that also contains antimicrobial compounds is honey (Suhartatik *et al*., 2023; Maimunah *et al*., 2021; Stagos *et al*., 2018). Honey is a product that has a sweet taste from flower nectar collected by bees. Types of honey can be differentiated based on the type of bee collecting it or the nectar source. The antimicrobial components in honey include its low water activity, high sugar content which creates high osmotic pressure, and the presence of organic acids, and also hydrogen peroxide (Albaridi, 2019). Besides its antimicrobial compound, honey also has anticancer and immunomodulatory effects (Ghramh *et al*., 2020). Research that states the

differences between honey is still limited. For example, research conducted by Prasetyo *et al.* (2014), analyzed the levels of reducing and non-reducing sugars in *Apis mallifera* honey from rubber and rambutan trees. Comparative data on the composition of honey with that used in this study is still lacking. Scripcă *et al.* (2019) said that each honey has a different composition, starting from the content of sugars, hydrogen peroxide, organic acids, phenolic acids, or other phenolic compounds. Even in the same season, honey collected in the hives and honey from flower nectar has a different composition (Sawicki *et al.*, 2020). These differences were more likely to affect the antimicrobial activity of honey.

Research about honey as a natural antimicrobial compound in avocado juice has never been done before. Avocado juice has characteristics that are slightly different from other fruit juices because it contains more complex proteins, fats, and sugars (Ford *et al*. 2023). The addition of honey when making fruit juice is often done to replace sweeteners with lower calorific value. To what extent the addition of honey can reduce natural contaminants in avocado juice needs to be studied to find out how safe it is to consume avocado juice. The addition of honey may also affect the number of microbes in avocado juice because naturally, honey also contains yeast and mold (Scripcă *et al.*, 2019). Meanwhile, honey can affect the microbiological quality of beef by suppressing the growth of *Escherichia coli* bacteria through the marination process (Ismail *et al*., 2015). Lan *et al*. (2021) reported that non-industrial mango fruit juice is safe for direct consumption within 2, 4, and 8 hours, at storage temperatures of 37, 25, and 4°C. This research aims to determine the antimicrobial activity of several types of honey with different concentrations and incubation periods in avocado juice. Providing healthy and safe food is the responsibility of the government.

2. Methods

2.1.Research methods

This research was an exploratory study that tested honey's ability as a natural antimicrobial using avocado juice as a food system. The experiment used 2 factors, namely the type of honey and honey concentration. Types of honey include honey produced by *Apis dorsata* (AD) bee, *Apis mellifera* bee from wildflowers nectars (AMW), nectar collected by *Trigona* sp (TG), and form *A. mellifera* from kapok (*Ceiba pentandra*) (AMC) flowers nectars. The second factor was honey concentration, which is 5, 10, and 15%. Statistical analyses were done using SPSS (Version 19.0). One-way ANOVA using the Duncan Multiple Range (DMR) test was used. Data from microbial analyses were done using Microsoft Excel and expressed as log 10 CFU/ml.

2.2.Avocado Juice Preparation

The process of making avocado juice refers to the procedure presented by Astuti *et al*. (2021) with modifications. Avocado juice was made by weighing 68.04 grams of avocado flesh, 128.19 ml of distilled water, and 27.21 grams of sugarcane. Honey was added according to the treatment

per total weight of the mixture. This research was an exploratory study that tested honey's ability as a natural antimicrobial using avocado juice as a food system. The materials and tools used did not go through a sterilization process to provide conditions that are suitable for the conditions when people make avocado juice in general. The mixture was then incubated in a refrigerator at 4-5°C. Samples were taken before and after incubation for 2 hours.

2.3.Determination of pH

Three grams of honey was dissolved in 30 mL of water and mixed with a stirrer at room temperature. pH meter inoLab pH 730 (Xylem Analytics, Germany) was used to determine the pH. Working areas were having 25-26 ^{o}C.

2.4.Determination of Titratable Acidity

Ten grams of honey were dissolved in 50 mL of aquadest and mixed with a magnetic stirrer at room temperature. Two drops of phenolphthalein were added to each sample. After dissolution, the samples were titrated with sodium hydroxide 0.1 N solution until the pink color persisted for 30 s. The titratable acidity was expressed meq of NaOH/mL using (1).

$$\text{Acidity} = \big((V\ x\ 0.1)x\ 10\big)x100\ (ml\ NaOH\ 0.1\ N/100\ g\ honey \quad (1)$$

where:

V represents the volume of sodium hydroxide solution used in titration (mL)

0.1 represents the normality of sodium hydroxide solution used for titration

2.5.Enumeration of Bacteria

Spread plate count was used for enumeration of the bacteria from avocado juice. Samples were taken before and after incubation. Ten-fold serial dilutions of homogenized suspensions were prepared aseptically using 9 mL of sodium chloride 0.85% as a hypotonic solution. All suspensions were prepared aseptically using Laminar airflow by transferring 1 mL of samples into 9 mL of hypotonic sterile solution. The analysis carried out included total plate count using nutrient agar (NA) medium (Oxoid), total Salmonella-Shigella using SSA (Salmonella-Shigella agar), total staphylococci using Staph 110 (Oxoid) medium, total coliforms using violet red bile agar (VRBA, Merck), and total yeast/mold using PDA (potato dextrose agar) media. Test standards are carried out based on the method carried out by Astuti *et al*. (2021).

3. Results and Discussion

The addition of honey to avocado fruit juice can fundamentally affect the microbiological quality of the fruit juice by suppressing or inhibiting the growth of pathogenic bacteria. Honey contains macro and micro compositions in the form of compounds that can act as antimicrobial agents. Some of the components of honey used in this research can be seen in Table 1. Each honey has a different pH value due to the bee species, environmental conditions, geographic location,

and nectar source. The differences in pH can be attributed to the acid and mineral content in each honey (Sawicki *et al*., 2020). According to Adalina *et al*. (2020), the pH values of honey from *A. dorsata*, *A. mellifera*, *Trigona* sp, and honey from *Ceiba pentandra* flower are 4.00, 4.50, 3.80, and 3.80, respectively, which are within a relatively close range to the measured pH values (Table 1). Honey contains various organic acids that are responsible for the low pH and acidity. Honey pH ranged from 3.4-6.1 and the maximum titratable acidity was 50 ml. eq/kg. Higher titratable acidity may indicate honey fermentation (Pătruică *et al*., 2022). Honey fermentation occurs due to the natural presence of yeast in honey. Sugar degradation by yeast will produce alcohol, organic acids, and gas. The accordance of honey fermentation is undesirable and is thought to be an indication of honey's freshness.

The titratable acidity of various types of honey yielded the lowest result of 90.5 ml NaOH/Kg for AMW honey and the highest result of 251 ml NaOH/Kg for TG honey. This acidity indicates the total amount of organic acids contained in honey. High titratable acidity indicates unwanted fermentation due to high water content, which leads to the fermentation process. Honey fermentation transforms glucose and fructose in honey into carbon dioxide and alcohol due to the osmophilic yeast activity, particularly *Zygosaccharomyces* species (Fatma *et al*., 2017; Ahmed *et al*., 2015). Furthermore, high titratable acidity can be influenced by differences in honeybee food sources (nectar), cultivation locations, harvest and post-harvest handling, harvesting seasons, and storage management (Sawicki *et al*., 2020). According to Adalina *et al*. (2020), the acidity in *Trigona* sp honey is not due to yeast-induced fermentation but rather the content of free acids, minerals, and amino acids in honey.

Table 1. Chemical Analysis Results of Various Types of Honey

Parameter	Honey				SNI 8664-2018
	AD	AMW	TG	AMC	
pH	3.70	4.25	3.45	3.95	-
Total Titratable Acidity	246	90.5	251	92	max 50 ml NaOH/kg max 200 ml NaOH/kg
Reducing Sugar (%) (Suhartatik *et al*., 2023)	69.98	34.23	64.18	41.32	min 65%

Note: Honey from *A. dorsata* bee (AD), *A. mellifera* from wildflowers nectars (MAW), honey from *Trigona* sp bee (TG), and *A. mellifera* from *Ceiba pentranda* nectars flower (AMC)

According to Indonesian National Standards (SNI) number 8664 (2018), the minimum standard for reducing sugar in honey was 65%. AMW honey had the lowest reducing sugar content compared to other types of honey (34.23%), while the highest was found in *A. dorsata* honey at 69.98% (AD). Two varieties of the honey were matched with INS. The low amount of reducing sugar in honey is suspected to be caused by the decomposition of sugars into acetic acid due to the fermentation process caused by yeast, as well as the presence of organic acids and minerals

naturally present in the honey. Differences in sugar content occur due to geographical factors, leading to variations in climate conditions, cultivation environment, nectar quality, and storage conditions (Fatma *et al*., 2017).

Total Plate Count is a quantitative test used to determine the number of microorganisms in a test sample, specifically the total count of aerobic mesophilic or anaerobic mesophilic microorganisms. This is typically done using solid media such as Nutrient Agar (NA), and the result is expressed as the number of colonies per ml/g in CFU (Colony-Forming Units). As seen in Table 2, the total plate count test results for avocado juice range from 5.07±0.59 $\log_{10}$ CFU/ml to 7.97±0.00 $\log_{10}$ CFU/ml. AMC honey, in particular, had the lowest TPC at a 15% honey concentration within 2 hours of incubation and the highest TPC at a 10% concentration before incubation. According to the fruit juice quality standards set by the Indonesian National Standard in 2014, the maximum allowable total plate count is 4.0 $\log_{10}$ CFU/ml. Based on the research results, it can be observed that none of the honey treatments were able to inhibit bacterial growth to the minimum limit set. According to Bucekova *et al*. (2018), the factor that greatly influences honey's antibacterial activity is its hydrogen peroxide content. But apart from that, the moisture content of the ingredients also greatly influences honey's ability to act as an antibacterial. Moreover, in the process of making avocado juice, the water used is not sterile.

Table 2. Total Plate Count (log CFU/ml) of Avocado Juice with Addition of Various Types of Honey and Incubation Periods

Honey Percentage	Incubation	Honey Type			
		AD	AMW	TG	AMC
5%	before	6.05±0.65^{bcdef}	5.36±0.00^{abc}	5.75±0.01^{abcde}	7.85±0.00^{i}
	after	5.15±0.29^{ab}	5.27±0.28^{ab}	6.61±0.38^{ef}	6.80±0.00^{fgh}
10%	before	5.78±0.55^{abcde}	6.05±0.53^{bcdef}	6.67±0.00^{efg}	7.97±0.00^{i}
	after	5.23±0.49^{ab}	5.30±0.00^{abc}	5.64±0.00^{abcd}	6.55±0.00^{def}
15%	before	6.58±0.00^{ef}	7.52±0.37^{ghi}	7.64±0.00^{hi}	5.36±0.00^{abc}
	after	6.23±0.00^{cdef}	6.41±0.24^{def}	6.32±0.00^{def}	5.07±0.59^{a}

Note: Values followed by the same letter are not significantly different according to Duncan's test at a significance level of 5%

The addition of honey can inhibit the total aerobic bacteria growth, mainly because the temperature conditions are not optimal for growth (refrigerated). Additionally, the addition of honey concentration has a significant effect on reducing the bacterial count because honey contains compounds that act as antimicrobial agents depending on the bee species, geographical region, post-harvest treatment, and storage management. AMC honey is the most effective in reducing the total plate count by 96.24%. The inhibition of bacteria by AMC honey is believed to be due to the presence of non-enzymatic antioxidants in the form of phenolic acid. According to Ustadi *et al*. (2017), AMC honey has a phenolic content of 309.12 mg GAE/100g. Furthermore, AMC honey has a titratable acidity value of 92 ml NaOH/kg, indicating that there is likely less fermentation of

sugar into alcohol and carbon dioxide, which could otherwise reduce honey quality. Honey also contains sugar that can support the growth of bacteria in avocado juice. Nutrients in avocado juice also can support the growth of bacteria. Avocado juice with honey has complex systems which can support the growth of bacteria. Besides the acid and hydrogen peroxide, lower temperatures could suppress bacterial growth. Adding honey to avocado juice can maintain the number of aerobic microbes during cold storage.

Naturally, honey contains yeast/osmotolerant yeast that can initiate the fermentation process in honey, resulting in the production of alcohol and carbon dioxide through the conversion of glucose and fructose. Wen *et al.* (2017) reported that some genera of fungal were found in honey such as *Metschnikowia*, *Cladosporium*, *Phoma*, *Alternaria*, *Aurebasidium*, and *Candida* sp. According to the results (Table 3), the yeast count in avocado juice treated with honey ranged from 3.12±0.00 $\log_{10}$ CFU/ml to 6.23±0.00 $\log_{10}$ CFU/ml, with the lowest value found in *Trigona* sp honey among the AMC honey. Based on the quality standards for unpasteurized fruit juice set by Indonesian National Standard number 3719 year 2014, the maximum allowable yeast count is 2.0 $\log_{10}$ CFU/ml. The results (Table 3) showed relatively high counts, which are suspected to be due to the presence of yeast in the honey apart from the avocado juice. According to Prihatini and Ilmi (2018), yeast can be found in flowers, fruits, and flower nectar.

AMC honey is a type of honey that significantly reduces the yeast count, with an inhibition percentage of 97.62% (Table 3). This is believed to be caused by the presence of antimicrobial agent compounds in AMC honey. Additionally, the total titratable acidity of AMC honey is relatively low at 92 ml NaOH/Kg, which indicates that Zygosaccharomyces may not have caused much fermentation.

Table 3. Total Yeast ($\log_{10}$ CFU/ml) in Avocado Juice with Addition of Various Types of Honey and Different Incubation

Honey Percentage	Incubation (h, hour)	Honey Type			
		AD	AMW	TG	AMC
5%	before	4.46 ± 0.00^{bcd}	5.52 ± 0.29^{ghi}	4.65 ± 0.27^{bcdef}	3.89 ± 0.00^{b}
	after	5.52 ± 0.22^{ghi}	5.44 ± 0.29^{fghi}	6.12 ± 0.00^{hi}	5.11 ± 0.37^{cdefg}
10%	before	4.61 ± 0.29^{bcdef}	4.13 ± 0.30^{b}	4.27 ± 0.30^{bc}	6.23 ± 0.00^{i}
	after	4.22 ± 0.18^{b}	4.32 ± 0.38^{bcd}	4.36 ± 0.00^{bcd}	4.50 ± 0.32^{bcde}
15%	before	5.18 ± 0.00^{defg}	4.67 ± 0.33^{bcdef}	4.45 ± 0.40^{bcd}	4.41 ± 0.37^{bcd}
	after	4.58 ± 0.31^{bcde}	5.34 ± 0.00^{efgh}	3.12 ± 0.00^{a}	4.45 ± 0.27^{bcd}

Note: Values followed by the same letter are not significantly different according to Duncan's test at a significance level of 5%

Total coliform testing aims to identify the presence of coliform bacteria, such as *Escherichia coli*, in a food product due to fecal contamination. The presence of coliform is considered a fecal contamination and can cause severe problems to the consumer's health. The presence of this bacteria group in food can be used as an indicator that there are other pathogens. The presence of

coliforms is also used as an indicator of poor hygiene and sanitation practices. To determine the presence of such pathogens, further tests need to be carried out to confirm certain pathogens. The quantity of coliform bacteria in avocado juice treated with honey can be seen in Table 4. The results show that total coliform ranges from 2.65±0.00 log CFU/ml to 5.46±0.26 log CFU/ml, with the lowest value found in *Trigona* sp honey with a 15% concentration. Each treatment has varying and relatively high counts, which are suspected to be influenced by the quality of the avocado juice in terms of coliform count. According to the Indonesian National Standard No. 3719 in 2014 for unpasteurized fruit juice, the maximum allowable coliform count is 2.0 log CFU/ml. The high coliform is suspected to originate from the cleanliness of the equipment used. Food handlers who use improperly cleaned utensils repeatedly and store them in open spaces can lead to cross-contamination (Laluraa *et al.*, 2014). Such factors as natural contamination in raw materials, environment, equipment, water supply, water quality, personal hygiene, and implementation of standards for processing food have a huge impact on food quality (Pires *et al.*, 2019).

A. dorsata honey significantly influences the reduction in the total coliform bacteria with an inhibition percentage reaching 98.84% (Table 4). This can be attributed to its high content of reducing sugars compared to other types of honey (69.98%). High sugar content can lead to osmotic activity, leaving fewer water molecules available for bacterial growth, causing bacterial dehydration and inability to survive (Huda, 2013; Nadhilla, 2014). Additionally, the inhibition of coliform counts is believed to occur because the honey contains organic acids and polyphenol content which provide antibacterial effectiveness against the coliform bacteria in avocado juice (Sawicki *et al*., 2022).

Table 4. Total Coliform (log CFU/ml) of Avocado Juice with the Addition of Honey and Different Incubation Periods

Honey Percentage	Incubation	Honey Type			
		AD	AMW	TG	AMC
5%	before	3.70±0.34^{bcde}	5.46±0.26^{i}	4.50±0.20^{efgh}	4.48±0.25^{efgh}
	after	3.48±0.44^{abcd}	4.32±0.00^{cdefg}	4.73±0.55^{ghi}	4.37±0.40^{cdefgh}
10%	before	4.61±0.38^{efghi}	4.42±0.28^{defgh}	3.45±0.00^{abc}	4.71±0.50^{fghi}
	after	2.82±0.00^{ab}	4.30±0.18^{cdefg}	4.04±0.00^{cdef}	3.49±0.29^{abcd}
15%	before	4.93±0.00^{ghi}	5.30±0.19^{hi}	2.96±0.00^{ab}	4.07±0.35^{cdefg}
	after	3.74±0.00^{bcdef}	4.85±0.00^{ghi}	2.65±0.00^{a}	4.22±0.52^{cdefg}

Note: Values with the same letter are not significantly different according to Duncan's test at a significance level of 5%.

Bacteria of the Staphylococci group are a type of bacteria that indirectly affect health, as some genera must colonize first to cause a health problem. The number of Staphylococci bacteria in avocado juice can be seen in Table 5. The range of Staphylococci in avocado juice with the addition of honey varies from 4.25±0.00 log CFU/ml to 6.98±0.00 log CFU/ml, with the lowest value found in *A.mellifera* honey at a concentration of 5%. According to the Indonesian National

Standard number 3719 in 2014, there should be no *Staphylococcus aureus* in 1 ml of the sample. The relatively high number of Staphylococci in avocado juice is believed to be due to the presence of these bacteria in the fruit used.

The addition of different concentrations of honey types significantly reduces the number of Staphylococci in avocado juice. This is likely due to the compounds in honey that work together to inhibit bacterial growth. *A.mellifera* honey is a type that significantly reduces the number of Staphylococci bacteria, by 99.04%. This honey type has a low total titratable acidity of 90.5 ml NaOH/Kg, which is believed to be one of the factors inhibiting bacteria. Moreover, honey also contains inhibited compounds in the form of hydrogen peroxide (H_2O_2), which is known as an antibiotic. Peroxide itself is a major antibacterial component in some penicillins, such as notanin. Honey also contains phenolic compound which has antimicrobial activity. Inhibitory mechanisms include membrane damage, inhibition of virulence factors such as enzymes and toxins, and suppression of the formation of bacterial biofilm layers (Miklasińska-Majdanik *et al*., 2018). High levels of Staphylococci in fruit juice were also reported by Iqbal *et al.* (2015). Fifteen percent of fruit juice samples were contaminated with *Staphylococcus aureus* with the mean value reaching 5.45 $\log_{10}$ CFU/ml.

Table 5. Total Staphylococci (log CFU/ml) in Avocado Juice with the Addition of Various Types of Honey and Different Incubation Periods

Percentage of Honey	Incubation	Honey Type			
		AD	AMW	TG	AMC
5%	before	6.38 ± 0.00^{efg}	5.46 ± 0.00^{bc}	5.86 ± 0.34^{bcde}	6.82 ± 0.00^{fgh}
	after	5.23 ± 0.54^{b}	4.25 ± 0.00^{a}	6.31 ± 0.23^{defg}	5.73 ± 0.00^{bcde}
10%	before	5.89 ± 0.51^{bcde}	6.66 ± 0.00^{fgh}	6.73 ± 0.00^{fgh}	7.56 ± 0.00^{i}
	after	5.73 ± 0.53^{bcde}	5.62 ± 0.00^{bcd}	5.52 ± 0.00^{bc}	6.98 ± 0.00^{ghi}
15%	before	6.84 ± 0.00^{fgh}	6.11 ± 0.44^{cdef}	7.38 ± 0.00^{hi}	6.30 ± 0.00^{defg}
	after	6.38 ± 0.00^{efg}	4.28 ± 0.00^{a}	6.72 ± 0.00^{fgh}	5.46 ± 0.00^{bc}

Note: Values followed by the same letter are not significantly different according to Duncan's test at a significance level of 5%.

Salmonella-Shigella is a group of microorganisms that belong to pathogenic bacteria, which can cause health issues in the human body. The quantity of Salmonella-Shigella bacteria in avocado juice with added honey can be observed in Table 6. According to the research results, the amount of Salmonella-Shigella bacteria ranged from 1.77±0.00 log CFU/ml to 5.53±0.42 log CFU/ml, with the lowest value found in *Trigona* sp honey at a concentration of 15%. Based on the quality standards for unpasteurized fruit juice set by the Indonesian National Standard number 3719 in 2014, there should be no Salmonella colonies per 25 ml. The presence of Salmonella-Shigella is suspected to occur due to the components in honey that can support some species of Salmonella growth. Naturally, honey contains organic acids such as gluconic acid, citric acid,

lactic acid, and formic acid. These acids result from the enzymatic reaction between glucose oxidase and honey sugars (Albaridi, 2019). Some *Salmonella* species, except *S. typhi*, produce gas during the fermentation process (Clark & Cronan, 2005). This could be the reason for the high quantity of Salmonella-Shigella in avocado juice with honey.

Table 6. Total *Salmonella-Shigella* (log CFU/ml) in Avocado Juice with the Addition of Honey and Different Incubation Periods

Percentage of Honey	Incubation Period	Honey Type			
		AD	AMW	TG	AMC
5%	before	3.59 ± 0.45^{cdefgh}	4.08 ± 0.00^{ghi}	3.98 ± 0.00^{fghi}	4.38 ± 0.00^{i}
	after	3.26 ± 0.00^{bcdef}	4.15 ± 0.00^{ghi}	4.12 ± 0.00^{ghi}	4.28 ± 0.00^{ghi}
10%	before	3.58 ± 0.31^{cdefg}	4.23 ± 0.00^{ghi}	3.30 ± 0.22^{bcdef}	3.68 ± 0.30^{defghi}
	after	4.08 ± 0.00^{ghi}	3.58 ± 0.33^{cdefg}	3.32 ± 0.28^{bcdef}	2.91 ± 0.00^{bc}
15%	before	2.96 ± 0.00^{bcd}	3.73 ± 0.58^{efghi}	2.80 ± 0.00^{b}	5.12 ± 0.00^{j}
	after	3.23 ± 0.00^{bcde}	5.53 ± 0.42^{j}	1.77 ± 0.00^{a}	4.32 ± 0.00^{hi}

Note: Values followed by the same letter indicate no significant difference according to Duncan's test at a significance level of 5%; (+) indicates an increase in bacteria; (-) indicates a decrease in bacteria.

Adding honey to avocado juice has varying effects. The addition of 15% TG and 10% of AMC was able to suppress the growth of Salmonella-Shigella. The same results were also shown by Pătruică *et al*., 2022 where several types of honey positively inhibited *Salmonella* sp. Apart from that Pătruică *et al*., 2022 also added that the inhibitory effect of honey was not as great as inhibiting other test bacteria such as *E. coli* (26.93%), *Candida albicans* (44,61%), *Listeria monocitogenes* (32.93%), and *Bacillus cereus* (21.28%). *Trigona* sp honey is a type of honey that significantly reduces the quantity of Salmonella-Shigella bacteria in avocado juice, with an inhibition percentage of 90.79% (Table 6). *Trigona* sp honey has a reducing sugar of 64.88%, which is close to the applicable quality standards. The high content of reduced sugar can lead to osmotic activity, creating a strong interaction between sugar molecules and water molecules, resulting in limited water availability to support bacterial growth. Bacteria will undergo dehydration and cannot increase in quantity (Albaridi, 2019). Additionally, the incubation process at refrigerator temperatures (4-5°C) can be one of the supporting factors in inhibiting the growth of *Salmonella-Shigella* bacteria since this temperature is not optimal for their growth. This aligns with research that utilized refrigerator temperatures for storing meat, which affected the growth of *Salmonella* sp (Edi & Rahmah, 2018).

The composition of avocado juice used in this study is slightly different from that presented by Jobil *et al*. (2021). In general, the amount of avocado added was less, and more honey was added. However, the composition will determine the total contamination and the development of microbiological contamination in avocado juice during storage. Further research needs to be carried out to study how the composition of honey avocado juice can influence its microbiological quality. It is also possible to add citric acid or other organic acid to suppress the growth of

microbiological contamination in juice.

4. Conclusions

The honey that has the greatest ability to inhibit the growth of coliform bacteria in avocado juice is honey from *Apis dorsata* bee (AD) and TG (*Trigon*a sp) types. In contrast, the honey that inhibits the growth of pathogenic bacteria such as Salmonella-Shigella is TG followed by AD. The concentration of honey that can be added to get the lowest total Salmonella-Shigella is by adding 15% of honey to avocado juice. Further research is needed on the composition of honey harvested in Indonesia, especially based on the region, season, type of nectar, and type of bee. Bioactive compounds in honey can be developed as antimicrobials in avocado juice.

References

Abd-Elkader, A. M., Labib, S., Taha, T. F., Althobaiti, F., Aldhahrani, A., Salem, H. M., ..., & Ibrahim, F. M. (2022). Phytogenic Compounds from Avocado (*Persea americana* L.) Extracts; Antioxidant Activity, Amylase Inhibitory Activity, Therapeutic Potential of Type 2 Diabetes. *Saudi Journal of Biological Sciences*, 29(3), 1428–1433. https://doi.org/10.1016/j.sjbs.2021.11.031.

Adalina, Y., Kusmiati, E., & Pudjiani, M. (2020). Phytochemical Test and Physical Chemical Properties of Rubber Honey from Three Types of Bees (*Apis mellifera*, *Apis dorsata,* and *Trigona itama*). *IOP Conference Series: Materials Science and Engineering*, 935(1), 1-9. https://doi.org/10.1088/1757-899X/935/1/012007

Ahmed, M., Djebli, N., Aissat, S., Meslem, A., & Bacha, S. (2015). The Influence of Botanical Origin and Physico-Chemical Parameters on the Antifungal Activity of Algerian Honey. *Journal of Plant Pathology & Microbiology*, 3(5), 1000132. https://doi.org/10.4172/2157-7471.1000132

Albaridi, N. A. (2019). Antibacterial Potency of Honey. *International Journal of Microbiology*, 2019, 1-11. https://doi.org/10.1155/2019/2464507

Astuti, B. C., Yuliastuti, E., Mustofa, A., Mardiyah, A., & Suhartatik, N. (2020). Cemaran Mikrobiologis Jus Alpukat Yang Dijual di Jalanan Kota Surakarta. *Agrointek*, 14(2), 315–322. https://doi.org/10.21107/agrointek.v14i2.6374

Astuti, B. C., Yuliastuti, E., Mustofa, A., Suhartatik, N., & Aditya, I. B. (2021). Pemanfaatan Daun Mint (*Menta piperita*) sebagai Antimikrobia Alami untuk Menghambat Pertumbuhan Patogen pada Jus Buah Alpukat. *Agrointek*, 15(3), 728–35. https://doi.org/10.21107/agrointek.v15i3.9032

Bucekova, M., Buriova, M., Pekarik, L., Majtan, V., & Majtan, J. (2018). Phytochemicals-Mediated Production of Hydrogen Peroxide Is Crucial for High Antibacterial Activity of Honeydew Honey. *Scientific Reports*, 8(1), 1–9. https://doi.org/10.1038/s41598-018-27449-3

Clark, D. P., & Cronan, J. E. (2005). *Two-Carbon Compounds and Fatty Acids as Carbon Source. Cellular and Molecular Biology of E.coli, Salmonella, and the Enterobacteriaceae. EcoSalPlus*. Retrieved from: Two-Carbon Compounds and Fatty Acids as Carbon Sources (asm.org)

de Melo, M. F. F. T., Pereira, D. E., Moura, R. L., da Silva, E. B., de Melo, F. A. L. T., Dias, C. C. Q., …, & Soares, J. K. B. (2019). Maternal Supplementation with Avocado (*Persea americana* Mill.) Pulp and Oil Alters Reflex Maturation, Physical Development, and Offspring Memory in Rats. *Frontiers in Neuroscience*, 13, 1–16. https://doi.org/ 10.3389/fnins.2019.00009.

https://www.frontiersin.org/articles/10.3389/fnins.2019.00009/full
Edi, S., & Rahmah, R. S. N. (2018). Pengaruh Lama Penyimpanan Daging Ayam pada Suhu Ruang dan Refrigerator terhadap Angka Lempeng Total Bakteri dan Adanya Bakteri *Salmonella* sp.. *Jurnal Biosains*, 4(1), 23–31. https://doi.org/10.24114/jbio.v4i1.9452
Edwards, C. G., Walk, A. M., Thompson, S. V., Reeser, G. E., Erdman, J. W., Burd, N. A., Holscher, H. D., & Khan, N. A. (2020). Effects of 12-Week Avocado Consumption on Cognitive Function among Adults with Overweight and Obesity. *International Journal of Psychophysiology*, 148(July 2019), 13–24. https://doi.org/10.1016/j.ijpsycho.2019.12.006
Esmael, A., Al-Hindi, R. R., Albiheyri, R. S., Alharbi, M. G., Filimban, A. A. R., Alseghayer, M. S., …, & Teklemariam, A. D. (2023). Fresh Produce as a Potential Vector and Reservoir for Human Bacterial Pathogens: Revealing the Ambiguity of Interaction and Transmission. *Microorganisms*, 11(3). https://doi.org/10.3390/microorganisms11030753 https://www.mdpi.com/2076-2607/11/3/753
Fatma, I. I., Haryanti, S., & Suedy, S. W. A. (2017). Uji Kualitas Madu pada Beberapa Wilayah Budidaya Lebah Madu di Kabupaten Pati. *Jurnal Biologi*, 6(2), 58–65. https://ejournal3.undip.ac.id/index.php/biologi/article/view/19538.
Ford, N. A., Spagnuolo, P., Kraft, J., & Bauer, E. (2023). Nutritional Composition of Hass Avocado Pulp. *Foods*, 12(13). https://doi.org/10.3390/foods12132516.
Ghramh, H. A., Ibrahim, E. H., & Kilany, M. (2020). Study of Anticancer, Antimicrobial, immunomodulatory, and Silver Nanoparticles Production by Sidr Honey from Three Different Sources. *Food Sci Nutr*., 8(1), 445-455. https://doi.org/10.1002/fsn3.1328.
Huda, M. (2013). Pengaruh Madu terhadap Pertumbuhan Bakteri Gram Positif (*Staphylococcus aureus)* dengan Bakteri Gram Negatif (*Eschericia coli*). *Jurnal Analis Kesehatan*, 2(2), 250-259. http://dx.doi.org/10.26630/jak.v2i2.437 https://www.ejurnal.poltekkes-tjk.ac.id/index.php/JANALISKES/article/view/437
Ismail, Y. S., Yulvizar, C., Sugiarti, & Misrahanum. (2015). Pengaruh marinasi madu terhadap kualitas mikrobiologis daging sapi (*Boss* Sp.). in P*rosiding Seminar Nasional Biotik 2015*. Pp. 396–400. https://jurnal.ar-raniry.ac.id/index.php/PBiotik/article/download/2710/1967
Iqbal, M. N., Anjum, A. A., Ali, M. A., Hussain, F., Ali, S., Muhammad, A., Irfan, M., Ahmad, A., Irfan, M. & Shabbir, A. (2015). Assessment of Microbial Load of Un-pasteurized Fruit Juices and in vitro Antibacterial Potential of Honey Against Bacterial Isolates. *The Open Microbiological Journal*, 9, 26-32. https://doi.org/10.2174/1874285801509010026
Jesus, D., Oliveira, J. R., Oliveira, F. E., Higa, K. C., Junqueira, J. C., Jorge, A. O. C., Back-Brito, G. N., & Oliveira, L. D. (2015). Persea Americana Glycolic Extract: In Vitro Study of Antimicrobial Activity against *Candida albicans* Biofilm and Cytotoxicity Evaluation. *Scientific World Journal*, 2015, 1–5. https://doi.org/10.1155/2015/531972.
Jobil, A. J., Parameshwari, S., Husain, F. M., Alomar, S. Y., Ahmad, N., Albalawi, F., & Alam, P. (2021). Scientifically formulated avocado fruit juice: phytochemical analysis, assessment of its antioxidant potential and consumer perception. *Molecules*, 26(24). https://doi.org/10.3390/molecules26247424.
Laluraa, L. F. H., Lohoo, H. J., & Mewengkang, H. W. (2014). Identifikasi Bakteri *Escherichia* pada Ikan Selar (*Selaroides* sp.) Bakar di Beberapa Resto di Kota Manado. *Jurnal Media Teknologi Hasil Pertanian*, 2(1), 5-8. https://doi.org/10.35800/mthp.2.1.2014.5359.
Lan, T., Bao, S., Wang, J., Ge, Q., Zhang, H., Yang, W., Sun, X., & Ma, T. (2021). Shelf Life of Non-Industrial Fresh Mango Juice: Microbial Safety, Nutritional and Sensory Characteristics. *Food Bioscience*, 42, 101060. https://doi.org/10.1016/j.fbio.2021.101060.
Lestari, D. P., Nurjazuli, & Hanani, Y. D. (2015). Hubungan Higiene Penjamah dengan Keberadaan Bakteri *Escherichia coli* pada Minuman Jus Buah di Tembalang. *Jurnal Kesehatan Lingkungan Indonesia*, 14(1), 14–20. https://doi.org/10.14710/jkli.14.1.14%20-%2020.
Maimunah, S., Supartiningsih, Marpaung, J. K., Silalahi, Y. C. E., & Turnip, I. W. (2021). Uji

Aktivitas Antibakteri Madu Hutan terhadap Bakteri *Bacillus cereus*. *Farmanesia*, 8(1), 9–15. Retrieved from: http://e-journal.sari-mutiara.ac.id/index.php/2/article/view/2780/1891.

Mengistu, D. A., Mulugeta, Y., Mekbib, D., Baraki, N., & Gobena, T. (2022). Bacteriological Quality of Locally Prepared Fresh Fruit Juice Sold in Juice Houses of Eastern Ethiopia. *Environmental Health Insights*, 16. https://doi.org/10.1177/11786302211072949.

Miklasińska-Majdanik, M., Kępa, M., Wojtyczka, R. D., Idzik, D., & Wąsik, T. J. (2018). Phenolic Compounds Diminish Antibiotic Resistance of Staphylococcus Aureus Clinical Strains. *International Journal of Environmental Research and Public Health*, 15(10). https://doi.org/10.3390/ijerph15102321.

Mustofa, A., Suhartatik, N., Saraswati, A. M., Astuti, B. C., & Yuliastuti, E. (2020). Utilization of Yogurt As a Natural Antimicrobial Agent To Inhibit the Growth of Pathogens in an Avocado Juice. *International Conference on Technology, Education, and Social Science*, 3, 348–60. https://ejurnal.unisri.ac.id/index.php/proictss/article/download/5046/3766.

Nadhilla, N. F. (2014). The Activity of Antibacterial Agent of Honey against *Staphylococcus aureus*. *J Majority*, 3(7), 94–101. https://juke.kedokteran.unila.ac.id/index.php/majority/article/download/484/485

Nguyen, T. V. L., Nguyen, Q. D., Nguyen, N. N., & Nguyen, T. T. D. (2021). Comparison of Phytochemical Contents, Antioxidant and Antibacterial Activities of Various Solvent Extracts Obtained from 'Maluma' Avocado Pulp Powder. *Molecules*, 26(24). https://doi.org/10.3390/molecules26247693.

Pacheco, L. S., Li, Y., Rimm, E. B., Manson, J. A. E., Sun, Q., Rexrode, K., Hu, F.B., & Guasch-Ferré, M. (2022). Avocado Consumption and Risk of Cardiovascular Disease in US Adults. *Journal of the American Heart Association*, 11(7). https://doi.org/10.1161/JAHA.121.024014.

Pătruică, S., Alexa, E., Obiștioiu, D., Cocan, I., Radulov, I., Berbecea, A., …, & Moraru, D. (2022). Chemical Composition, Antioxidant and Antimicrobial Activity of Some Types of Honey from Banat Region, Romania. *Molecules*, 27(13), 1–24. https://doi.org/10.3390/molecules27134179.

Pires, A. M., Majowicz, S., Gill, A., & Devleesschauwer, B. (2019). Global and Regional Source Attribution of Shiga Toxin-producing *Escherichia coli* Infections Using Analysis of Outbreak Surveillance Data. Epidemiology and Infection 147, e236, 1-9. https://doi.org/10/1017/S095026881900116X

Prasetyo, B. A., Minarti, S., & Cholis, N. (2014). *Perbandingan Mutu Madu Lebah Apis mellifera Berdasarkan Kandungan Gula Pereduksi dan Non Pereduksi Di Kawasan Karet (Hevea brasiliensis) Dan Rambutan (Nephelium lappaceum)*. Retrieved from https://fapet.ub.ac.id/wp-content/uploads/2014/06/JURNAL2.pdf

Prihatini, M., & Ilmi, M. (2018). Karakterisasi dan Klasifikasi NUmerik Khamir dari Madu Hutan Sulawesi Tengah. *Jurnal Mikologi Indonesia*, 2(2), 112–127. http://doi.org/10.46638/jmi.v2i2.41.

Sawicki, T., Bączek, N., & Starowicz, M. (2020). Characterization of the Total Phenolic, Vitamins C and E Content and Antioxidant Properties of the Beebread and Honey from the Same Batch. *Czech Journal of Food Sciences*, 38(3), 158–63. https://doi.org/10.17221/312/2019-CJFS.

Sawicki, T., Starowicz, M., Kłębukowska, L., & Hanus, P. (2022). The Profile of Polyphenolic Compounds, Contents of Total Phenolics and Flavonoids, and Antioxidant and Antimicrobial Properties of Bee Products. *Molecules*, 27(4). https://doi.org/10.3390/molecules27041301.

SNI number 8664. (2018). Retrieved from https://jogja.bsilhk.menlhk.go.id/?page_id=1467

Scripcă, L. A., Norocel, L., & Amariei, S. (2019). Comparison of Physicochemical, Microbiological Properties and Bioactive Compounds Content of Grassland Honey and Other Floral Origin Honeys. *Molecules* 24(16). https://doi.org/10.3390/molecules24162932.

Senbeta, D., & Beyene, M. (2017). Bacteriological Assessment of Avocado Juice Vended in Cafeteria and Restaurants in Adigrat Town, Northern Ethiopia. *International Journal of Advanced Technology and Innovative Research,* 09(04), 593–596. http://www.ijatir.org/uploads/461325IJATIR14133-116.pdf.

Stagos, D., Soulitsiotis, N., Tsadila, C., Papaeconomou, S., Arvanitis, C., Ntontos, A., …, & Mossialos, D. (2018). Antibacterial and Antioxidant Activity of Different Types of Honey Derived from Mount Olympus in Greece. *International Journal of Molecular Medicine,* 42(2), 726–34. https://doi.org/10.3892/ijmm.2018.3656.

Suhartatik, N., Karyantina, M., & Lestari, E. (2023). The Minimum Inhibitory Concentrations (MICs) of Four Types of Honey against Several Bacteria. *IOP Conference Series: Earth and Environmental Science,* 1177(1), 1-8. https://doi.org/10.1088/1755-1315/1177/1/012031.

Ustadi, Radiati, L. E., & Thohari, I. (2017). Komponen Bioaktif pada Madu Karet (*Hevea brasiliensis*) Madu Kaliandra (*Calliandra callothyrsus*) dan Madu Randu (*Ceiba pentandra*). *Jurnal Ilmu dan Teknologi Hasil Ternak,* 12(2), 97–102. https://doi.org/10.21776/ub.jitek.2017.012.02.6

Wen, Y., Wang, L., Jin, Y., Zhang, J., Su, L., Zhang, X., ..., & Li, Y. (2017). The Microbial Community Dynamics during the Vitex Honey Ripening Process in the Honeycomb. *Front. Microbiol.*, 8, 1649. https://doi.10.3389/fmicb.2017.01649

Wijayanti, Yulina, & Elliya, R. (2014). Pengaruh Pemberian Jus Alpukat (*Persea americana* Mill) terhadap Penurunan Kolestrol Tikus Putih Jantan (*Rattus novergicus*) Galur Wistar Kota Bandar Lampung Tahun 2014. *Jurnal Kesehatan Holistik,* 8(3), 147–152. https://ejurnalmalahayati.ac.id/index.php/holistik/article/download/198/138.

Zhang, X., Xiao, D., Guzman, G., Edirisinghe, I., & Burton-Freeman, B. (2022). Avocado Consumption for 12 Weeks and Cardiometabolic Risk Factors: A Randomized Controlled Trial in Adults with Overweight or Obesity and Insulin Resistance. *The Journal of Nutrition,* 152(8), 1851–1861. https://doi.org/10.1093/jn/nxac126.