

CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY TEST OF HONEY, LEMON, GINGER FERMENTATION AGAINST BACTERIA CAUSING ACUTE RESPIRATORY INFECTIONS (ARI)

Andi Dian Astriani^{1*}, Muhammad Iqbal¹

¹*S1 Farmasi, Fakultas MIPA, Universitas Islam Makassar, Indonesia*

**Email Corresponding: andidianastriani.dty@uim-makassar.ac.id*

Submitted: October 13, 2023 Revised: May 20, 2024 Accepted: May 20, 2024

ABSTRACT

Acute respiratory infection (ARI) is a disease that attacks one or more parts of the airway, from the nose to the alveoli. The most common bacteria found in the samples of patients with ARI were *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Klebsilia pneumonia*. Honey contains flavonoids, amino acids and potassium as antibiotics that are bactericidal (kill bacteria) and bacteriostatic (inhibit bacterial growth). Gingerol in ginger (*Zingiber officinale*) and lemon (*Citrus limon*) has antibacterial activity. Honey fermentation is believed to have great potential as an antibacterial compound. This study aimed to determine the inhibitory activity and physical characteristics of fermented honey, lemons, and ginger against ARI bacteria. The test bacteria used were *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Klebsilia pneumonia*. Fermentation of honey, lemon, and ginger was performed with variations in fermentation time (days 1, 3, 5, and 7) and honey without fermentation to determine the effect of fermentation time on inhibitory activity. The inhibition activity was tested using the agar diffusion method. Measurement of inhibition diameter using a caliper. Determination of ginger lemon fermented honey characteristics, including water content, sucrose content, and total sugar content, using gravimetric and titrimetric methods. Based on the activity test, honey fermented for 1 day had the greatest inhibitory activity of 9.71 mm; 9.20 mm; and 9.86 mm against *Staphylococcus aureus*, *Staphylococcus pneumonia*, *Klebsilia pneumonia* compared to honey fermented for 3 days, 5 days, 7 days and honey without fermentation. The test results were compared with the day 1 activity test, negative control (non-fermented honey), and positive control (amoxicillin). The test results showed that day 1 ginger lemon fermented honey had an inhibition diameter against *Staphylococcus aureus*, *Staphylococcus pneumonia*, and *Klebsilia pneumonia* of 27.70 mm (very strong category); 14.25 mm (strong category); 10.69 mm (medium category), respectively. Based on the one-way ANOVA statistical test, the inhibitory activity of fermented ginger lemon honey against acute respiratory tract infection bacteria was significantly different from that of non-fermented honey. Ginger lemon fermented honey are 33.38 a water content of 6.57% sucrose content, and total sugar content of 56.86 %. Based on these results, it can be concluded that lemon fermented honey from ginger shows increased inhibitory activity against ARI bacteria compared to non-fermented honey.

Keywords : *Fermentation, Honey, Lime, Ginger, ARI*

INTRODUCTION

Acute Respiratory Tract Infection (ARI) is a transient infectious disease that affects several components of the respiratory system, including the sinuses, middle ear canal, and pleura (Pakadang & Salim, 2020). According to Sultana et al., (2022), Acute Respiratory Tract Infection (ARI) can be categorized into two types, namely upper ARI (non-pneumonia

ARI) and lower ARI (pneumonia ARI). Based on data from the World Health Organization (WHO), an estimated 13 million children under the age of five die each year worldwide. The majority of these deaths occur in developing countries, where Acute Respiratory Tract Infection (ARI) is identified as the leading cause of death in four million children under the age of five each year (Dongky & Kadrianti, 2016). According to the routine report of the ISPA Sub-directorate in 2017, the incidence of ARI in Indonesia in 2017 was recorded at 20.54 per 1000 children under five. According to a report from the Ministry of Health of the Republic of Indonesia in 2018, the mortality rate in infants with pneumonia was found to be 0.56%, while in children aged 1-4 years it was 0.23% (Ode Umi Kalsum et al., 2020).

According to Pakadang & Salim (2020), the most prevalent bacterial species detected in samples collected from individuals with acute respiratory infections (ARI) consisted of *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Staphylococcus epidermidis*. In medical practice, it is common to administer multiple antibiotics to treat and control various diseases. Based on research released in 2016 by the Directorate General of Pharmaceuticals and Medical Devices, Indonesian Ministry of Health, there is a high prevalence of antibiotic use in health centers, indicating a high level of antibiotic consumption (Ambarwati et al., 2018). However, on the other hand, inappropriate antibiotic administration is also one of the causes of antibiotic resistance (Little et al., 2013). Antibiotic administration has the potential to cause side effects, including gastrointestinal disorders such as diarrhea, in addition to manifestations of nausea, vomiting, and skin responses (Utami F, 2013).

Many cases of failure in the use of antibiotics have caused some people to change their mindset and lifestyle to use drugs derived from natural ingredients; however, more scientific research is needed to determine the effectiveness of these natural ingredients. In Islam, Allah prepared bee liquid (honey) as a cure for various diseases found in QS An Nahl: 68-69. Honey contains flavonoids, amino acids, and potassium, which are bactericidal (killing bacteria) and bacteriostatic (inhibiting bacterial growth) (Angela et al., 2022). Honey has a naturally sweet flavor derived from plant nectar (floral nectar). Cultured honey is a natural liquid that generally has a sweet taste and is produced by *Apis mellifera*, *Apis cerana*, and *Trigona sp.* from plant flower juice (floral nectar). Honey can contain (lactic acid bacteria (LAB) up to 108cfu/g fresh honey from the genus *Lactobacillus* and *Bifidobacterium*, which are known as probiotic bacteria.

Ginger rhizomes contain active ingredients, including terpenes and an oleoresin called ginger oil. The main identified terpenes are sesquiterpene hydrocarbons and phenolic compounds, gingerol, and shogaol. Gingerol has antibacterial activity against periodontal bacteria and is also an active inhibitor of *Mycobacterium tuberculosis* (Rahmani et al., 2014). Lemons (*Citrus limon*) contain active ingredients, including flavonoids, carotenoids, limonoids, tannins, and terpenoids. These components are antibacterial agents. Other lemon ingredients included vitamin C and citric acid. The content of vitamin C and citric acid lowers the acid (pH) (Ekawati & Darmanto, 2019). These three components have potential as antibacterial agents.

Fermentation is a biological process that involves enzymatic degradation of complex organic compounds into simpler substances through the participation of microbes. According to Pamungkas (2014), the fermentation process requires the presence of a substrate that serves as a medium for microbial growth and provides the necessary nutrients for the fermentation process. The duration of fermentation is an important element in the overall fermentation procedure, as highlighted by Hamzah & Fifi Nofiyana (2013), the utilization of fermentation in the exploration of new compounds offers important advantages over extraction procedures, largely due to the substantial reduction in raw material requirements.

According to the description given above, research has been conducted to determine how well honey, ginger rhizome extract, and lemon extract suppress bacterial growth. However, as many raw materials are used in the extraction process, they are inefficient. By effectively using natural components through the fermentation process, the potency of these ingredients can be increased. It is commonly known that fermented honey has the potential

to be converted into an antibacterial chemical. The goal of this study was to ferment honey with ginger and lemon to boost its potential efficacy and efficiency.

These studies used honey produced by *Apis mellivera bees*. Although fermented honey has been combined with ginger and lemon for a long time, its effectiveness has not been scientifically proven. This study investigated the characteristics and antibacterial activities of fermented honey, lemon, and ginger against bacteria that cause acute respiratory tract infections (ARI): *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Klebsiella pneumonia*. Different fermentation times were considered as variables when applying the agar diffusion method. Determining the special qualities of ARI bacterial inhibitors, which are easy to synthesize, do not create resistance, and are safe for children, remains a critical goal of this research.

RESEARCH METHODE

Tools and Materials

The tools used in this research include, autoclave, incubator (Mettler®), Laminar air flow, oven (Mettler®), petri dishes, pH meter (Milwaukee®). The ingredients used were nutrient agar, alcohol, distilled water, honey (*Apis mellivera*), lemon (*Citrus limon*), ginger (*Zingiber officinale*), *Staphylococcus aureus* bacteria, *Streptococcus pneumonia*, *Klebsiella pneumonia*, and 70% alcohol.

Research Procedures

1. Tools sterilization

All tools used for testing were sterilized. The glassware was sterilized by autoclaving at 120°C for 15 minutes. Petri dishes were wrapped and sterilized in an oven at 180°C for 2 hours.

2. Bacterial rejuvenation

The test tube containing the corresponding test bacterial cultures of *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Klebsiella pneumonia* was streaked on NA media in a zigzag pattern and then incubated at 37°C for 24 hours. The tube needle was flame-lit until the tip became incandescent.

3. Ginger lemon honey fermentation

First, the fermentation container was sterilized. The ginger was thoroughly washed under running water, cut into thin slices, and stored in a jar. After giving the lemon a salt rub and giving it a quick rinse under running water, it was thinly sliced and placed in a jar. The honey from *Apis mellifera* was placed in a jar. The container was kept away from direct sunlight, securely closed, and kept at room temperature. Days 1, 3, 5, and 7 of fermentation were examined for activity.

4. Antibacterial activity test

Ten milliliters of nutritional medium were added to a Petri dish, and the mixture was allowed to solidify. Following solidification, 20 µL of the test bacterial suspension was applied to the surface of the medium on a petri dish and incubated at 37°C. Activity tests were conducted on days 1, 3, 5, and 7 of fermentation. The creation of a clear region surrounding the disc indicated activity test findings.

5. Physical characteristics test

Fermentation, which has the greatest resistance value, continues with characteristic tests, including water content, glucose content, and sucrose content (SNI Honey).

a. Water content test

The porcelain cup was dried for one hour in an oven at 105°C. Subsequently, the sample was placed in a porcelain cup and dried in an oven at 105°C until the weight of the honey remained constant (Departemen Kesehatan Republik Indonesia, 1979).

$$\text{Water content} = \frac{\text{Material weight (before - after)}}{\text{Material weight before}} \times 100\%$$

b. Sucrose Content Determination (Depkes RI, 1995)

Testing the accuracy of Luff Schoorl's solution

Add 3 grammes of KI and 25 millilitres of 3M H₂SO₄ to 25 millilitres of Schoorl's Luff solution. Use a 0.1 N Na₂S₂O₃ solution and a 0.5% starch indicator for titration. A Na₂S₂O₃ solution (25 mL) was used. The pH of the Luff Schoorl solution must be between 9.3 and 9.4.

Test of sugar content reduction

2 g sample was placed in a 250 ml volumetric flask, followed by the addition of distilled water and vigorous shaking. Ten milliliters of the solution were pipetted and placed in an Erlenmeyer. The sample solution was added to 15 ml of distilled water and 25 ml of Luff Schoorl solution, which was connected to an upright cooler, heated on an electric heater, and allowed to boil within 3 minutes. The sample solution was continuously heated for ten minutes, after which it was removed and cooled in a cold bath. After the mixture had cooled, 10 ml of 20% KI solution and 25 ml of H₂SO₄ solution were added. The solution was then titrated with a 0.1 N sodium thiosulfate solution containing an indicator solution containing 0.5% starch (V1). A sample containing 25 ml of water and 25 ml of Luff Schoorl solution was used to conduct blank determination (V2).

c. Analysis of Total Sugar Content (Depkes RI, 1995)

5 grams of the sample was dissolved in 100 ml of distilled water in a 250 ml erlenmeyer. Added 15 ml of distilled water, some boiling stones and 25 ml of Luff Schoorl solution, then refluxed for 10 minutes. Add 2 drops of methyl red indicator and titrate with 0.1N sodium thiosulfate until the color of the solution turned yellow. The volume of sodium thiosulfate used was recorded.

$$\text{Total Sugar Content (\%)} = \frac{V \times N \times 0,9 \times 100}{b}$$

Data analysis

The test resistance was determined by measuring the diameter of the transparent zone surrounding a paper disc. Observations were made after 24 hours of incubation using a caliper.

RESULT AND DISCUSSION

In this study, lemon ginger honey was fermented on days 1, 3, 5, and 7 using the disc diffusion method.

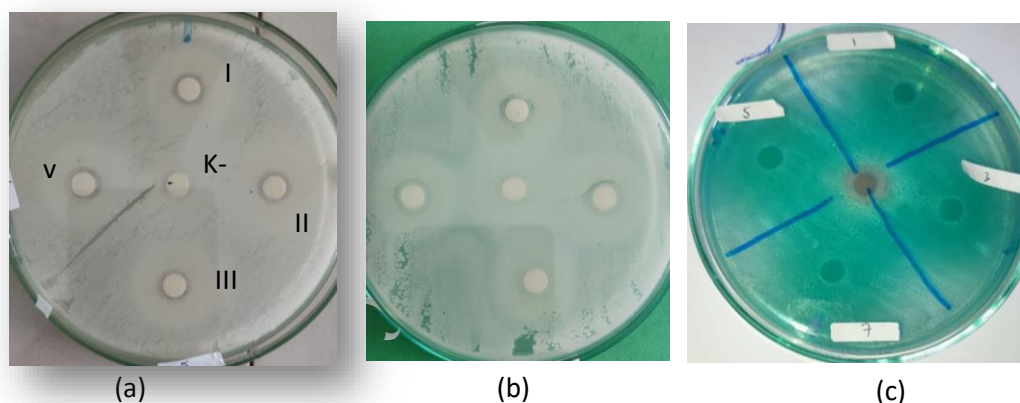


Figure 1. Inhibition test results resulting from fermentation of honey, lemon and ginger

Information :

a : inhibition test against *Staphylococcus aureus* bacteria

b : inhibitory power test against *Streptococcus pneumonia* bacteria

c: inhibitory power test against *Klebsilla pneumonia* bacteria

Based on the inhibition test results from honey fermentation, lemon ginger showed a clear zone formed from fermentation results on days 1, 3, 5, and 7. As shown in **Figure 1**, there was no significant difference in the inhibition diameter between the first, third, fifth, and seventh days of fermentation.

Tabel I. Activity Test Results of Fermented Honey

Bacteria	Resistance diameter (mm)				Honey
	Day of fermentation				
	1 st	3 rd	5 th	7 th	
<i>Staphylococcus aureus</i>	9,71	9,39	9,23	9,41	7,78
<i>Streptococcus pneumonia</i>	9,20	8,52	8,81	9,65	8,12
<i>Klebisilia pneumonia</i>	9,86	8,71	8,74	9,60	8,70

The development of lactic acid bacteria has a substantial impact on the fermentation process. Salt level, temperature, pH, and the availability of carbohydrates as a source of nutrients are all environmental factors that influence the growth of lactic acid. The BAL development phase comprises four phases: lag, exponential, stationary, and death. During the lag phase, the number of microbes increases slowly because they adapt to the environmental conditions (pH, temperature, and nutrients). The latency phase of the research potential BAL takes place between the 0th and 4th hours. The subsequent phase is the exponential phase, which is characterized by rapid bacterial proliferation. The subsequent phase is the stationary phase, during which there is no addition of bacteria, because the number of growing cells is equal to the number of dying cells. The stationary growth phase occurs between the eighth and twenty-fourth hour (Mardalena, 2016).

According to **Tabel I**, fermented honey on day 1 had the greatest barrier diameter against *S. aureus*, *Klebsilia pneumonia*, and *Streptococcus pneumonia* when compared to days 3, 5, and 7. This corresponds to the stationary phase of BAL, which occurs at 24 hours or one day. The stationary phase is characterized by nutrient limitations and the accumulation of toxic products, which causes microbial growth to slow or stop completely. During this phase, the ratio of primary to secondary metabolism increases, and the unique metabolite products are referred to as secondary metabolites (Astriani and Dwijayanti, 2022).

As shown in **Table Tabel I**, fermented honey had a larger resistance diameter than unfermented honey. This demonstrates that fermentation technology is more active than other alternatives. Fermentation is a microorganism-mediated process of decomposing organic compounds into simpler compounds (Pamungkas, 2014). Yulia (2014) discovered that ripe fruit fermentation generates LAB, which can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. Since fermentation produces optimal conditions for LAB growth, it is possible to increase the desired number of LAB through the fermentation process (Utami F, 2013).

The research data was carried out a One Way Anova test statistical test, before the test was carried out, a normality test was carried out to ensure that the data was normally distributed and a variance test because the data had to be homogeneous. Based on the normality test, $p > 0.432$ indicated that the data were normally distributed. Normally distributed data were required for homogeneity and one-way ANOVA analysis.

Tabel II. One Way Anova Analisis

One Way Anova Analisis	Sig
Length of Fermentation	0,004

Table II shows that the results of the one-way ANOVA test on the length of fermentation had a p-value = 0.004. Because the p value was <0.05, the average value between the treatment groups was significantly different. To determine significant differences, further post-hoc analysis was performed.

Tabel III. Post-Hoc Analysis

	1 st	3 rd	5 th	7 th	Non
1 st	-	0,033*	0,045*	0,902	0,001*
3 rd	0,033*	-	0,858	0,041*	0,043*
5 th	0,45*	0,858	-	0,056	0,031*
7 th	0,902	0,041*	0,056*	-	0,001*
Non	0,001*	0,043*	0,031*	0,001*	-

Post-hoc test results showed that the data had a p value <0.05, indicating that the data were significant or significantly different from other concentrations. In **Tabel III**, it can be seen that day 1 fermentation was significantly different from days 3 and 5. Non-fermented honey looks significantly different from fermented honey (days 1, 3, 5, and 7).

The duration of bacterial growth fermentation is significantly affected by the pH of the medium. The use of an optimal pH can enhance the rate of sucrose consumption, thereby promoting accelerated bacterial development. The sucrose present in the medium is converted into lactic acid through the metabolic activity of lactic acid bacteria. However, as time progressed, the concentration of sucrose was expected to decrease. The occurrence of bacteria that lead to degradation decreases as the fermentation period increases (Perdana M et al., 2021).

The results of honey fermentation on day 1, followed by activity tests, were compared with those of the positive control (amoxicillin) and negative control (non-fermented honey).

Tabel IV. Honey Activity Test Results from Day 1 Fermentation

Bacteria	Resistance diameter (mm)		
	Honey fermentation	Negative control	Positif control (Amoxicilin)
<i>Staphylococcus aureus</i>	27,70	11,00	42,86
<i>Streptococcus pneumonia</i>	14,25	0,00	16,93
<i>Klebisilia pneumonia</i>	10,69	10,53	12,57

According to the data presented in **Tabel IV**, fermented honey exhibits a higher degree of inhibitory efficiency compared to non-fermented honey, which serves as the negative control. The use of amoxicillin as a comparative agent implied that the microorganisms employed in this study exhibited no resistance. Amoxicillin is a first-line antibiotic that is generally administered to patients with ARI (Karangayu et al., 2019). Amoxicillin is a beta-lactam category of penicillin derivative antibiotic that inhibits gram-negative and gram-positive bacteria (Pratiwi Mida et al., 2023).

The barrier diameters of fermented honey against *S. aureus*, *S. pneumonia*, *K. pneumonia* were 27.70 mm, 14.25 mm, and 10.69 mm, respectively. The inhibitory power can be classified into different categories based on the diameter of the inhibitory zone. The

very strong category is characterized by an inhibitory zone diameter greater than 20 mm, while the strong category corresponds to inhibitory zone diameters ranging from 10 to 20 mm. The medium category was defined by inhibitory zone diameters between 5 and 10 mm, and the weak category was associated with inhibitory zone diameters of less than 5 mm. Fermented honey exhibits significant inhibition against *S. aureus*, substantial inhibition against *S. pneumonia*, and moderate inhibition against *K. pneumonia*. Research of [Pakadang & Salim, \(2020\)](#), The bitter melon leaf extract (*Momordica charantia* L.) exhibited an inhibitory effect against *S. aureus*, *S. pneumonia*, and *K. pneumonia*, with average inhibitory diameters of 22.24 mm, 13.53 mm, and 14.70 mm, respectively, when used at a concentration of 15,000 ppm. Based on these findings, it is evident that the fermentation process involving honey, lemon, and ginger exhibits a higher degree of inhibitory efficacy than bitter melon leaf extract (*Momordica charantia* L.). Consequently, it can be inferred that the fermentation of honey, lemons, and ginger holds significant promise in terms of its ability to inhibit bacteria associated with Acute Respiratory Infections (ARI).

Based on **Tabel IV**, non-fermented honey has the ability to inhibit ARI bacteria.) This is based on the ability of lactic acid bacteria to ferment carbohydrates such as glucose or sucrose to produce bacteriocins, organic acids, and hydrogen peroxide. Bacteriocins are proteins that suppress the growth of various pathogens that compete in the same ecology. Lactic acid is formed through a fermentation process that takes place in the presence of lactic acid bacteria, namely *Lactobacillus*, which takes place in an anaerobic environment. Bacteriocins are a class of proteins that can inhibit the proliferation of diverse pathogenic microorganisms ([Perdana M et al., 2021](#)).

The inhibitory activity of honey increased after fermentation with lemons and ginger. This is because of the synergistic action of the compounds contained in lemons and ginger with bacteriocins. Bacteriocins can inhibit the growth of resistant bacteria and do not trigger the body's immune reaction, so they have great potential as antibacterial agents for therapeutic purposes. However, bacteriocins are sensitive to protease enzymes, so their use may affect their effectiveness ([Shavira et al., 2022](#)). Bacteriocins are protein compounds, so their antibacterial activity will be lost or inactive if they interact with protease enzymes ([Shavira et al., 2022](#)). Ginger contains gingerol, based on in silico tests, and has inhibitory activity against protease enzymes ([Promdam and Panichayupakaranant, 2022](#)). Lemons contain ascorbic acid and citric acid ([Ekawati & Darmanto, 2019](#)). Hydrogen peroxide is an important compound responsible for the antibacterial activity of honey peroxide. This compound is aerobically produced from glucose via the activity of glucose oxidase. The function of H₂O₂ in honey is to prevent the breakdown of raw honey, where the sugar concentration is insufficient to prevent microbial growth. A study found that a mixture of hydrogen peroxide and ascorbic acid produced an antibacterial mechanism, resulting in increased lysozyme lysis and bacterial death ([Ratna Fadhillah et al., 2020](#)). Based on the results of the activity test, ginger lemon honey fermentation showed potential

Tabel V. Physical characteristics of honey, lemon and ginger fermentation results on day 1

Characteristic	Honey	Honey fermentation	Method
Water content	16,94 %	33,38 %	Gravimetric
Sucrosa content	7,09 %	6,57 %	Titrimetric
Total sugar content	55,52 %	56,86 %	Titrimetric

The physical parameters examined in this study were the moisture content, sucrose sugar content, and total sugar content. Forest honey must not exceed a moisture content of

22%, as specified in the SNI 8664:2018. Moisture affects the moisture content of honey because of its hygroscopic qualities (Betha Nanda et al., 2017). The moisture percentage of honey varies between 15-25% and is influenced by the nectar source and meteorological conditions (Hakim et al., 2021). The extended shelf life of honey is attributed to its low moisture content (Wulandari, 2017). The investigation found that The moisture level of the honey tested was 16.94%. This indicates that honey meets the Indonesian National Standard (SNI) and does not undergo fermentation. Tosi et al. (2004) found that honey with a moisture content below 17% does not ferment, whereas Snowdon and Cliver (1996) stated that honey with a moisture content above 17% is prone to fermentation (Baloš et al., 2019).

The elevated water content in 33.38% fermented honey **Tabel V** suggests that the honey had undergone fermentation. Chasanah in Betha Nanda et al. (2017) stated that high water content boosts yeast activity, leading to fermentation. Osmophilic yeast and *Zygosaccharomyces* are yeasts that are resistant to high sugar levels and are responsible for fermentation in honey (Betha Nanda et al., 2017). Fermentation transforms sugar into organic acids, leading to sourness of honey (A. Lastriyanto & A. I. Aulia, 2021).

Honey is a natural liquid that generally has a sweet taste and is produced by honey bees from plant flower juice (floral nectar) (Wulandari Devyana Dyah, 2017). Sucrose is a sugar derived from sugar cane and beets. Sucrose hydrolysis results in a mixture of glucose and fructose, called invert sugar (Lubis et al., 2022). According to SNI 2004, the maximum sucrose content is 5%. As shown in **Tabel III**, the sucrose content of honey before fermentation was 7.09%. In some cases, the sucrose content exceeds the requirements owing to the addition of granulated sugar and artificial sweeteners, so that the honey may have been modified. Sucrose levels in honey are influenced by the presence of invertase, an enzyme that converts sucrose into glucose and fructose. Fermented honey resulted in a 6.57% decrease in sucrose content. The high sucrose content in honey can help in the fermentation process because sucrose is more easily broken down by yeast than glucose (Wulandari, 2017).

The total sugar content in honey is the combined amount of fructose and glucose present in honey. Forest honey must contain a minimum of 65% total sugar content, whereas stingless bee honey must have at least 55% sugar content. High sugar levels in honey indicate good maturation and stability of the honey. According to **Tabel III**, the initial sugar concentration of honey was 55.52%, and it rose during fermentation. Honey, which has a high concentration of total sugar, can impede yeast growth. Honey with low sugar content can enhance fermentation owing to the presence of a diluted sugar solution, which facilitates the breakdown of sugar by yeast (Adityarini et al., 2020).

CONCLUSION

The study found that honey, lemon, and ginger fermentation had water, sucrose, and total sugar contents of 33.38 %, 6.57%, and 56.86 %, respectively. The fermentation of honey, ginger, and lemon for 1 day exhibits the highest inhibitory effect against ARI bacteria, with inhibition zone diameters against *S. aureus*, *S. pneumonia*, and *K. pneumonia* measuring 27.70 mm (very strong category), 14.25 mm (strong category), and 10.69 mm (medium category) respectively. Based on the one-way ANOVA statistical test, the inhibitory activity of fermented ginger lemon honey against acute respiratory tract infection bacteria was significantly different from that of non-fermented honey.

REFERENCES

- A. Lastriyanto, & A. I. Aulia. (2021). Analisa Kualitas Madu Singkong (Gula Pereduksi, Kadar Air, dan Total Padatan Terlarut) Pasca Proses Pengolahan dengan Vacuum Cooling. *Jurnal Ilmu Produksi Dan Teknologi Hasil Peternakan*, 9(2), 110–114. <https://doi.org/10.29244/jipthp.9.2.110-114>
- Adityarini, D., Suedy, A. W., & Darmanti, S. (2020). *Kualitas Madu Lokal Berdasarkan Kadar Air, Gula Total dan Keasaman dari Kabupaten Magelang*.

- Ambarwati, W., Setiawaty, V., & Wibowo, A. (2018). Antibiotics Used for Upper Respiratory Tract Infection: a Case Study at a Primary Health Center Bogor Indonesia. *Global Medical & Health Communication (GMHC)*, 6(3). <https://doi.org/10.29313/gmhc.v6i3.3618>
- Angela, E., Kunaedi, A., Suharyani, I., & Tinggi Farmasi Muhammadiyah Cirebon, S. (2022). Pengaruh Waktu Fermentasi Madu Dengan Bawang Putih Tunggal (*Allium sativum* L.) Terhadap Daya Hambat Bakteri *Propionibacterium acnes*. In *Open Journal Systems STF Muhammadiyah Cirebon : ojs.stfmuhammadiyahcirebon.ac.id* (Vol. 7, Issue 3).
- Astriani, A. D., & Dwijayanti, E. (2022). Uji Aktivitas Antibakteri Mikroba Endofit Dari Buah Merah (*Pandanus conoideus* Lam.) terhadap Bakteri Patogen. *Journal Syifa Sciences and Clinical Research*, 4(2). <https://doi.org/10.37311/jsscr.v4i2.14369>
- Baloš, M. Ž., Jakšić, S., Popov, N., Mihaljev, Ž., & Pelić, D. L. (2019). Comparative Study of Water Content in Honey Produced in Different Years. *Archives of Veterinary Medicine*, 12(1). <https://doi.org/10.46784/e-avm.v12i1.42>
- Betha Nanda, P., Eka Radiati, L., & Rosyidi, D. (2017). *Perbedaan Kadar Air, Gulosa Dan Fruktosa Pada Madu Karet dan Madu Sonokelling*.
- Departemen Kesehatan Republik Indonesia. (1979). *Farmakope Indonesia*. Edisi Ketiga. Jakarta.
- Depkes RI. (1995). *FARMAKOPE IV. Farmakope Indonesia Ed IV*.
- Dongky, P., & Kadrianti Universitas Al Asyariah Mandar, dan. (2016). 324 *UJPH 5 (4) (2016) Faktor Risiko Lingkungan Fisik Rumah Dengan Kejadian ISPA Balita Di Kelurahan Takatidung Polewali Mandar*. <http://journal.unnes.ac.id/sju/index.php/ujph>
- Ekawati, E. R., & Darmanto, W. (2019). Lemon (Citrus limon) Juice Has Antibacterial Potential against Diarrhea-Causing Pathogen. *IOP Conference Series: Earth and Environmental Science*, 217(1). <https://doi.org/10.1088/1755-1315/217/1/012023>
- Hakim, S. S., Wahyuningtyas, R. S., Rahmanto, B., Halwany, W., Lestari Balai Penelitian dan Pengembangan Lingkungan Hidup dan Kehutanan Banjarbaru Jl Ahmad Yani Km, F., & Selatan, K. (2021). Sifat Fisikokimia dan Kandungan Mikronutrien Pada Madu Kelulut (*Heterotrigona itama*) Dengan Warna Berbeda (Physico-chemistry and Micronutrient Contents of Different Colour Kelulut Honey Bee (*Heterotrigona itama*)). *Jurnal Penelitian Hasil Hutan*, 39(1). <https://doi.org/10.20886/jphh.2021.39.3.1-12>
- Hamzah, F., & Fifin Nofiyana, D. (2013). Penambahan Madu dan Lama Fermentasi Terhadap Kualitas Susu Fermentasi Kacang Merah (*Phaseolus vulgaris* L.]. 12(1), 25–33.
- Karangayu, P., Ovikariani, S., Saptawat, T., & Rahma, F. A. (2019). Evaluasi Rasionalitas Penggunaan Antibiotik Pada Pasien ISPA. *Jurnal Ilmu Keperawatan Dan Kebidanan STIKES Telogorejo*, 2.
- Little, P., Stuart, B., Moore, M., Coenen, S., Butler, C. C., Godycki-Cwirko, M., Mierzecki, A., Chlabicz, S., Torres, A., Almirall, J., Davies, M., Schaberg, T., Mölstad, S., Blasi, F., De Sutter, A., Kersnik, J., Hupkova, H., Touboul, P., Hood, K., ... Verheij, T. (2013). Amoxicillin for acute lower-respiratory-tract infection in primary care when pneumonia is not suspected: a 12-country, randomised, placebo-controlled trial. *The*

- Lancet Infectious Diseases*, 13(2), 123–129. [https://doi.org/10.1016/S1473-3099\(12\)70300-6](https://doi.org/10.1016/S1473-3099(12)70300-6)
- Lubis, N., Sofiyani, S., & Junaedi, E. C. (2022). Penentuan Kualitas Madu Ditinjau dari Kadar Sukrosa dengan Metode Luff Schoorl. *Jurnal Sains Dan Kesehatan*, 4(3), 290–297. <https://doi.org/10.25026/jsk.v4i3.1050>
- Mardalena. (2016). Fase Pertumbuhan Isolat Bakteri Asam Laktat (BAL) Tempoyak Asal Jambi yang Disimpan Pada Suhu Kamar. *Jurnal Sain Peternakan Indonesia*, 11(1), 58–66. <https://media.neliti.com/media/publications/225882-fase-pertumbuhan-isolat-bakteri-asam-lak-59f543d1.pdf>
- Ode Umi Kalsum, W., Ode Nur Isnah Sabriyati, W., & Utami, N. (2020). TRIO DISPA: EFFORT TO ESTABLISH FAMILY HEALTH EXPERTS IN ACUTE RESPIRATORY INFECTIONS. In *Indonesian Contemporary Nursing Journal* (Vol. 4, Issue 2).
- Pakadang, S. R., & Salim, H. (2020). Pengaruh Ekstrak Daun Pare (*Momordica charantia* L.) Terhadap Pertumbuhan *Streptococcus pneumonia*, *Staphylococcus epidermidis*, *Staphylococcus aureus* Dan *Klebsiella pneumonia* Penyebab Infeksi Saluran Pernapasan Akut. *Media Farmasi*, 16(2), 207. <https://doi.org/10.32382/mf.v16i2.1802>
- Pamungkas, W. (2014.). Teknologi Fermentasi, Alternatif Solusi Dalam Upaya Pemanfaatan Bahan Pakan Lokal.
- Perdana M, Sastyarina Y, & Arifuddin M. (2021). *Pengaruh Waktu Fermentasi Bakteri Asam Laktat Dari Sari Kulit Buah Naga Merah (Hylocereus Polyrhizus) Terhadap Aktivitas Bakteri Propionibacterium acne*. Proceeding Mulawarman Pharmaceutical Conferences. <https://prosiding.farmasi.unmul.ac.id/index.php/mpc/article/view/548/501>
- Pratiwi Mida, Nabila Novrilia Atika, & Sutomo Ahmad. (2023). Efektivita Penggunaan Obat Antibiotik Pada Pasien ISPA. *Jurnal Farmasi*. <https://journal.aisyahuniversity.ac.id/index.php/JFA/article/view/EFEKTIVITASMIDA/462>
- Promdam, N., & Panichayupakaranant, P. (2022). [6]-Gingerol: A narrative review of its beneficial effect on human health. *Food Chemistry Advances*, 1, 100043. <https://doi.org/10.1016/J.FOCHA.2022.100043>
- Rahmani, A. H., Al Shabrmi, F. M., & Aly, S. M. (2014). Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. In *Int J Physiol Pathophysiol Pharmacol* (Vol. 6, Issue 2). www.ijppp.org
- Ratna Fadhilla, R., Wahyu Indriati, D., & Siti Sundari, A. (2020). Antibacterial Potency of Indonesian Randu Honey Against *Staphylococcus* sp. *Malaysian Journal of Medicine and Health Sciences*, 16(SUPP16), 2636–9346.
- Shavira, A., Cahyadi, A. I., & Windria, S. (2022). KAJIAN PUSTAKA: AKTIVITAS ANTIBAKTERI DARI BAKTERIOSIN *Lactobacillus* spp. TERHADAP BAKTERI RESISTAN. *Jurnal Sain Veteriner*, 40(1), 60. <https://doi.org/10.22146/jsv.67927>
- Snowdon, J. A., & Cliver, D. O. (1996). Microorganisms in honey. *International Journal of Food Microbiology*, 31(1–3), 1–26. [https://doi.org/10.1016/0168-1605\(96\)00970-1](https://doi.org/10.1016/0168-1605(96)00970-1)

- Sultana, N., Mollah, M. A. H., Kabir, A. L., Saha, B. R., Sarker, A., Roy, S., & Jahan Kabir, S. M. E. (2022). Recovery of Children from Acute Lower Respiratory Tract Infection(Pneumonia and Bronchiolitis) : In a tertiary Care Hospital. *Sir Salimullah Medical College Journal*, 29(2). <https://doi.org/10.3329/ssmcj.v29i2.58969>
- Tosi, E. A., Ré, E., Lucero, H., & Bulacio, L. (2004). Effect of honey high-temperature short-time heating on parameters related to quality, crystallisation phenomena and fungal inhibition. *LWT - Food Science and Technology*, 37(6), 669–678. <https://doi.org/10.1016/J.LWT.2004.02.005>
- Utami F. (2013). Pengaruh Suhu Terhadap Daya Tahan Hidup Bakteri Pada Sediaan Probiotik.
- Wulandari, D. D. (2017). Kualitas Madu (Keasaman, Kadar Air dan Kadar Gula Pereduksi) Berdasarkan Perbedaan Suhu Penyimpanan. *Jurnal Riset Kimia*, 2(1).
- Wulandari Devyana Dyah. (2017, June). *View of Analisa Kualitas Madu (Keasaman, Kadar Air, dan Kadar Gula Pereduksi) Berdasarkan Perbedaan Suhu Penyimpanan*. *Jurnal Kimia Riset*. <https://e-journal.unair.ac.id/JKR/article/view/3768/3135>
- Yulia. (2014). Isolasi Bakteri Asam Laktat Dari Fermentasi Buah Sirsak (*Annona muricata* L.) Dan Penentuan Aktivitas Antimikrobanya. Tesis. <https://onsearch.id/Record/IOS3630.22350/Details>

