Malaysian Journal of Chemistry, 2023, Vol. 25(5), 230-238
(Special Issue: *The 4th International Conference on Chemistry and Material Sciences 2022*)

Effect of Additional Red Dragon Fruit Peel and Lemon Peel in the Development of Coconut Water Fermented Beverage Products using Lactic Acid Bacteria to Increase Antibacterial Activity

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This study aims to develop a fermented drink with the main ingredient of coconut water which is processed with the addition of mass variants of dragon fruit peel and lemon peel to increase antibacterial activity. Coconut water is one part found inside mature coconuts that contains bioactive compounds that have the potential as antibacterial such as catechin compounds. Catechin compounds are major components is reported to have antimicrobial activities. In this case, the fermentation process using lactic acid bacteria is known to affect the bio-access ability to degrade catechins into catechol and gallic acid which is supported by the addition of dragon fruit peel and lemon peel to increase antibacterial activity. The stages of this research include, the first stage is determining the optimum conditions which include determining the fermentation time (0, 12, 24, 36, 48, and 60 hours) and the composition of variations in the ratio of the addition of dragon fruit peels and lemon peels (3:0, 1:2 and 2:1) based on total acid produced from the fermentation process. Then, continued on the second stage is the identification of the content of organic acid compounds formed using the HPLC method and the antibacterial activity test using the "The disc diffusion" method. Based on the results showed the optimization of fermentation time that produces the optimum total acid is 48 hours. Product characteristics test showed that the addition of dragon fruit peels and lemon peels can increase to produce metabolic (organic acid components) which has a positive correlation antibacterial activity. The antibacterial activity from fermented coconut water using lactic acid bacteria started with 48 hours incubation period against *Escherichia coli* was higher (inhibitory zones of 4.6 mm) than *Staphylococcus aureus* (inhibitory zones of 3.24 mm). This study confirmed that the addition of dragon fruit peel and lemon peel can increase antibacterial activity during coconut water fermentation using lactic acid bacteria.

Keywords: Coconut water; fermentation; organic acids; antibacterial

Received: September 2022; Accepted: November 2022

Based on the top ten the most coconut producing in countries in 2019, Indonesia site in the first rank. Indonesia is the world's largest coconut producing countries reaching 14 million coconuts [1]. This high production rate is not followed by maximum product development, such as processing coconut water which is often disposed of directly in large quantities as waste. Several research groups have revealed that coconut water contains high levels of nutrients that can be attributed to the presence of sugars such as glucose and fructose, with existing nutrients consisting of vitamins, minerals, proteins, free amino acids and growth promoting factors for microorganisms [2, 3]. In other side, coconut water also contains unique compounds called catechin. Catechins are secondary metabolite that are naturally produced by coconut water with concentration of 0.344 g/mL [4]. Catechins are flavan-3-ols have high antibacterial activity against pathogenic bacteria. Many microbes have a demonstrated ability to degraded catechin such as lactic acid bacteria (*Lactobacillus sp.*)[5]. As well as gut microbiome such as *Lactobacillus plantarum* IFPL935 utilized catechin as the carbon source and catalyzed the conversion of catechin into catechol and other phenols such as gallic acid [5].

Several studies have also shown that the use of lactic acid bacteria in the fermentation process can degrade tannin. In addition to catechin compounds, coconut water also has other bioactive compounds, namely tannin compounds which are quite high. Biochemical pathways for tannin degradation by *Lactobacillus plantarum* involves enzymes hydrolyzing galloyl ester bonds from tannins and produce gallic acid and glucose [6]. Catechol and gallic acid are included in phenolic compounds. These polyphenols are known to have antibacterial activity. Their anti-bacterial activity was investigated on food borne pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*).

†Paper presented at the 4th International Conference on Chemistry and Material Sciences 2022 (IC2MS2022)

Food borne pathogenic microbes that contaminate food have become a concern for the potential risk of synthetic food additives to human health, thus prompting renewed interest in exploring methods of food preservation by fermentation. The fermentation process using lactic acid bacteria will have a health effect when consumed. Based on this, the coconut water fermentation process with the help of lactic acid bacteria has been shown to be able to degrade catechin compounds and tannin compounds through the activity of the cellular tanase enzyme produced. The fermentation process using lactic acid bacteria is also known to be able to hydrolyze the glucose content of the substrate to produce organic acid compounds and produce a distinctive taste [7]. The longer the fermentation time causes the increase in sour taste in coconut water fermentation. This is supported by Sofi's research which shows that the hedonic test of fermented coconut water using *Lactobacillus plantarum FNCC 0027* produces a sour taste [7]. The sour taste in coconut water fermentation is caused by the presence of acidic components formed during the fermentation process. Based on Cholida, said that the longer the fermentation process, the more the bacterial activity increased so that it was to break down the substrate and the greater the effect on the antibacterial activity. Based on the description, the organic acids formed will dissociate with water to produce H^+ which causes a decrease in the pH of the media. Possible organic acids formed in this process are lactic acid, acetic acid, and gallic acid. Organic acid compounds produced by metabolic enzymes in lactic acid bacteria have the potential to inhibit the growth of pathogenic bacteria. This is supported by Ziska who reported that the use of lactic acid bacteria cultures *Lactobacillus plantarum* and *Lactobacillus casei* was able to increase antibacterial activity [8].

During the fermentation process, the growth conditions of lactic acid bacteria to produce secondary metabolites remains a problem that needs to be solved. So that during the fermentation process, the activity of lactic acid bacteria to produce secondary metabolites is influenced by the addition of other ingredients. The ingredients to be used are dragon fruit peel and lemon peel which also have polyphenols compounds to increase antibacterial activity. The increase in total phenol was caused by the fermentation process of lactic acid bacteria which has enzymes to degrade the content of phenolic compounds in the fruit peels. This is supported by Alberto's research which showed polyphenols from apples peels can inhibit pathogenic bacteria in human such as *Escherichia coli* and *Staphylococcus aureus* [9].

Dragon fruit peel is a fruit waste that is rarely used. Red dragon fruit peel contains vitamin C, vitamin E, and contains betacyanin, flavonoid and phenol can also be potential for antimicrobial activities [10]. From previous studies, the red dragon fruit possessed antimicrobial activities against pathogenic microbial. This is in accordance with the analysis which showed that dragon fruit peel had inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* using the microdilution method. Lemon peel is also one of the most garbage consist bioactive compounds, such as polyphenols, the most important being vitamin C can be antimicrobial activity. The studies showed that the antibacterial activity of lemon peel is attributed to the content of flavonoids and essential oils such as monoterpenes. Monoterpenes have higher antimicrobial activity than hydrocarbons [11]. Based on the research, showed the activity of the inhibitory zone on *Staphylococcus aureus ATCC 12600* with an inhibition zone diameter of 21 mm [12]. Based on the description above, the addition of red dragon fruit peel and lemon peel in coconut water encourage the development of new antimicrobial substances.

TOOL AND MATERIAL

The instrument used in the research was HPLC (*High Performance Liquid Chromatography*). The reagents were oxalic acid, phenolphthalein, $CaCO_3$, NaOH, *de Man Rogosa Sharpe Broth* (MRSB), *de Man Rogosa Sharpe Agar* (MRSA), *Nutrient Agar* (NA), and *Nutrient Broth* (NB). The material used were coconut water, red dragon fruit peels, lemon peels from Pandaan, East Java, Pasuruan. The plant specimen was identified and authenticated by plant taxonomist of the Biology Laboratory, Department of Biology, Brawijaya University. In the study commercial probiotic Yakult was used. The pathogenic bacteria was investigated (*Staphylococcus aureus* and *Escherichia coli*).

METHODS

Microorganism and Inoculums Preparation

Lactic acid bacteria obtained commercially, *Escherichia coli* and *Staphylococcus aureus* were collected from Airlangga University. Inoculum preparation was cultured in 100 g of coconut water had been sterilized at 70°C for 10 minutes. Then the sterile coconut water was cooled to 40°C and added 10% of commercially obtained lactic acid bacteria. then immediately cover with plastic and incubation at room temperature for 24 hours. After 24 hours, the starter culture of lactic acid bacteria is ready to be used as a starter culture for further fermentation.

Fermentation of Coconut Water with Addition of Dragon Fruit Peel and Lemon Peel

To make coconut water fermented drink. Coconut water sterilized at 70°C for 10 minutes. after sterilization, coconut water was inoculated with 10 % (v/v) lactic acid bacteria and divided into 5 fermentation treatments, namely:

P1 : Coconut water without fruit peels
P2 : Coconut water + red dragon peels (3:0)
P3: Coconut water + lemon peels (3:0)

P4 : Coconut water + red dragon fruit peel and lemon peel (2:1)
P5 : Coconut water + red dragon fruit peel and lemon peel (1:2)

Fermentation was carried out at room temperature for 48 hours in anaerobic condition.

The Effect of Fermentation Time in The Optimum Time of Lactic Acid Bacteria

To make coconut water fermented drink. Coconut water sterilized at 70°C for 10 minutes. after sterilization, coconut water was inoculated with 10 % (v/v) lactic acid bacteria. Furthermore, coconut water was fermented with a fermentation time (0, 12, 24, 36, 48 and 60 hours). Then, each sample at the interval of fermentation is calculated total acid titrated with 0.1 N NaOH.

Total Acid Analysis

The total acid test was carried out using the titration method which was expressed as a percentage of lactic acid which refers to fermented sample of 10 ml taken, then melted and put into an Erlenmeyer. After that phenolphthalein indicator was added as much as 2-3 drops then titrated using 0.1 N NaOH. Titration is stopped until a color change occurs constantly pink. Calculation of total acid can use the following formula :

$$\frac{V1 \times N \times B \times 100\%}{V2 \times 1000} \qquad \text{Eq 1}$$

Medium pH Measurement

Measurement of the sample solution is carried out using a pH meter that has been calibrated later dip the pH meter electrode into the sample solution and leave it for a while until a stable reading is obtained [6].

Identification of Organic Acids by HPLC

Identification of organic acid from fermented coconut water using HPLC. The fermented filtrate was centrifuged at a speed of 9000 rpm for 10 minutes. Furthermore, as much as 10 mL of the supernatant put into a test tube and add 2.5 grams of NaCl. The mixture was vortexed until all of the NaCl solution. After that, the solution added with 1 mL of diethyl ether and vortexed for 5 minutes.

Then, the solution was centrifuged at 9000 rpm for 15 minutes. The organic phase (above) is separated and added with Na_2SO_4 to remove excess water. After that, the solution was filtered using 0.22 m filter paper and ready to be analyzed using HPLC. Sample analysis was carried out using UHPLC, which was generally carried out in the C18 stationary phase, while the mobile phase was generally a mixture of acetonitrile and phosphoric acid aqueous solution. In the determination of organic acids (ascorbic acid, lactic acid, acetic acid) direct injection is used. Ascorbic acid used a C18 column and 3 mM phosphoric acid as the mobile phase, and two organic acids (lactic acid and acetic acid) used an analytical column and a 50 mM potassium dihydrogen phosphate solution. The mobile phase velocity is 1.5 mL/min, the running time is 5 minutes, temperature of 30 °C, injection volume of 15 L, and a wavelength of 210 nm. Mobile phase velocity 1.5 mL/min, time running 5 minutes, temperature 30°C, injection volume 15 µL, and wavelength 210 nm.

Antibacterial Activity

The antibacterial activity of fermented products was tested against *Escherichia coli* and *Staphylococcus aureus* by disc method [13]. All fermentation treatments were tested and the control was positive (*Streptomycin*). Briefly, preculture of the tested microorganisms were prepared by inoculation of 25 mL of medium and incubated for 24 hours at 37°C. then the cell density is adjusted to 10 [7] CFU/mL. Sterile disc paper was immersed in 30 µL of the test solution for 30 minutes. in this case the test solution is a cell-free supernatant obtained from the centrifugation of the fermentation filtrate. The paper discs were then placed sterilely on Nutrient Agar Medium which had previously been inoculated with 1 mL of *Staphylococcus aureus* and 1 mL of *Escherichia coli*. Then incubated at 37°C for 24 hours. The clear zone formed around the paper disc is measured in diameter using a caliper.

Analysis Statistic

The research data was analyzed by ANOVA One Way. When there is a significant difference between treatment, it will be continued with Tukey test at $\alpha = 0.05$.

RESULT AND DISCCUSION

Effect of Fermentation Time in The Optimum Time of Lactic Acid Bacteria

The duration of fermentation affects the total acid, because the longer the fermentation, the lactic acid bacteria used in the coconut water fermentation process are more active, multiply, so that the ability to break down substrates is increasing and producing lactic acid is increasing. This measurement of total acid shows the effect of the growth of lactic acid bacteria on metabolic production.

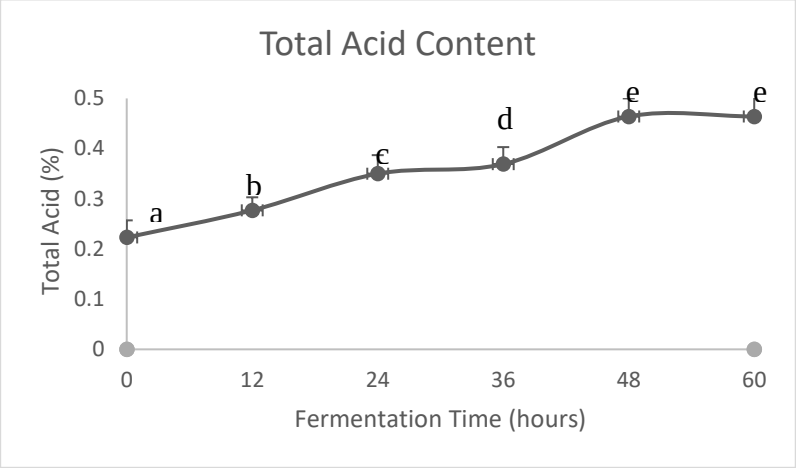


Figure 1. Effect of Fermentation Time on the Total Acid Content Formed during the Fermentation Process with Lactic Acid Bacteria.

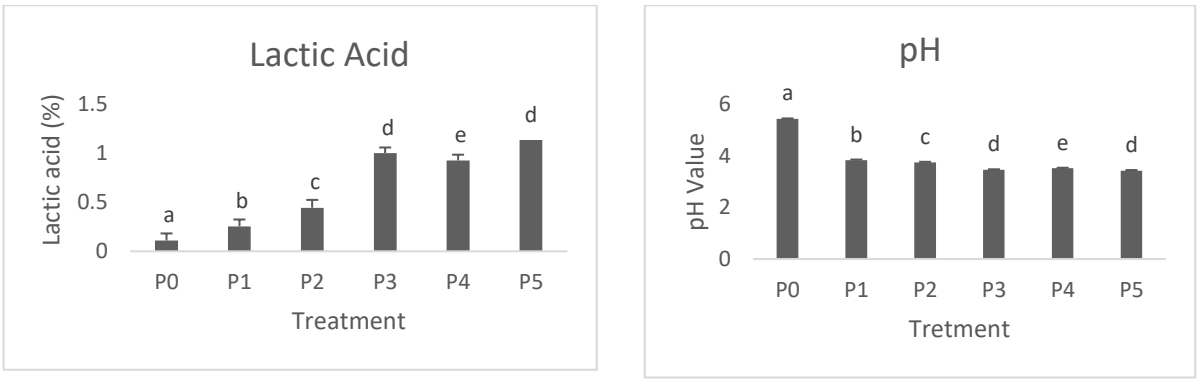
Based on Figure 1, it shows the value of the total acid level formed is increasing. The average value of total acid content in this study ranged from 0.223-0.464%. The increase in total acid formed is caused by the metabolism of glucose from coconut water by lactic acid bacteria which is converted into lactic acid through the glycolysis process [14]. This is supported by Nurdyansyah, who said that the production of lactic acid using *Lactobacillus plantarum* produce lactic acid of 4.68 g/L [15].

In addition, the fermentation process using lactic acid bacteria is also able to increase the secondary metabolites contained in coconut water. The content of bioactive compounds in coconut water are catechins and tannins. In this study, the coconut water fermentation process using lactic acid bacteria can degrade tannins and catechins into compounds that provide antibacterial potential [16]. based on previous research, it is known that the lactic acid bacteria of the *Lactobacillus sp* group are able to produce tanase enzymes [6]. This tanase enzyme has the ability to hydrolyze tannins and catechins into gallic acid, catechol, and glucose. Catechol is an organic compound that is inherently antimicrobial [17]. The presence of these organic acids accumulates in the form of total acids. Acid concentration in coconut water fermentation only increased to a certain extent, namely at 12-48 hours, then decreased at 60 hours (Figure 1). This can happen because the carbon source used by Lactic acid bacteria in the coconut water medium has been finished.

Determination of the optimization of the variable total acid content shows the length of time 48 hours of fermentation is considered the optimum time to produce lactic acid in the coconut water fermentation process using lactic acid bacteria. Determination optimization of total acid based on coconut water fermentation using lactic acid bacteria reached the optimum value of 0.464% at 48 hours of fermentation. This is supported by Sofi's research, which shows that the optimum growth of lactic acid bacteria occurred at 48 hours of fermentation [7]. Determination of optimization of total acid content based on (Figure 1) is used to determine the optimum point of production lactic acid from coconut water fermentation using lactic acid bacteria in the next fermentation process.

Effect of Addition of Dragon Fruit Peel and Lemon Peel to Total Acid content and pH Value

The pH value and titratable acidity are relative with the quality of taste in fermented foods. As shown in Figure 2, the changes in pH and acidity content in during process fermented coconut water. In this research, dragon fruit peels and lemon peels was added to increase antibacterial activity.



(a) (b)

Figure 2. Effect of Addition of Dragon Fruit Peel and Lemon Peel to (a) Total Acid Content, (b) pH Value.

Table 1. Effect of Addition Dragon Fruit Peel and Lemon Peel to Total Acid Content and pH Value.

Treatment Fermented Coconut	Parameter	
	Total Acid (%)	pH Value
P_0	0.11 ± 0.040^a	5.42 ± 0.019^a
P_1	0.25 ± 0.072^b	3.83 ± 0.021^b
P_2	0.44 ± 0.069^c	3.74 ± 0.019^c
P_3	1.00 ± 0.08^d	3.46 ± 0.016^d
P_4	0.92 ± 0.056^e	3.52 ± 0.016^e
P_5	1.13 ± 0.058^d	3.46 ± 0.021^d

Information:
Data shown as mean ± standard deviation. Different superscripts in the same column show very significant effect.

P_0 = without fermentation
P_1 = without fruit peel
P_2 = with addition dragon fruit peels
P_3 = with addition lemon peels
P_4 = with addition dragon fruit peels and lemon peels (2:1)
P_5 = with addition dragon fruit peels and lemon peels (1:2)

Based on table 1 shows that the results of the study of pH values and total lactic acid have shown the characteristics of fermented drinks based on SNI. During the fermentation process, the addition of dragon fruit peel and lemon peel changes the degree of acidity and the total acid formed. This fermentation process will utilize the glucose contained in coconut water and fruit skins change peels to produce metabolites. The greater the concentration of sugar in the substrate medium, the greater the activity of lactic acid bacteria so that it affects the increase in total acid and decrease the pH of the media [18].

Based on Figure 2 (b), the pH value of the fermented fruit with the addition of fruit peel shows a decreasing trend, where before fermentation, coconut water has an initial pH of 5.42. then after the fermentation process and the addition of fruit peel, the pH value decreased ranging from 3.83-3.46. The decrease in pH occurs due to the high metabolic activity of bacteria with glucose consumption and lactic acid production that occurs due to the addition of other ingredients that can increase the viability of lactic acid bacteria [19]. During the fermentation process, lactic acid is formed as a result of glucose metabolism of coconut water by bacteria starter into glucose or galactose-6-phosphate. Furthermore, through the glycolysis chain, glucose is converted into lactic acid through the Kreb cycle. The lactic acid produced may also be influenced by the fruit skin which has the potential as a carbon source for microorganisms.

Based on Table 2 shows that the total acid content of coconut water before fermentation is 0.11%, and this acidity value increases after the fermentation process and the addition of dragon fruit peels and lemon peels, the acidity values ranged from 0.25-1.13%. The increase in the amount of this acid indicates the activity of lactic acid bacteria through metabolic system that utilizes the glucose content in the substrate as a carbon source for lactic acid bacteria [20]. Total acid analysis has an inverse relationship with the pH value of the medium. More lactic acid bacteria that grow, the total acid increases, this causes the fermented product to have a sour taste with an increasing pH value low[21]. Therefore, based on this description, the best composition of fermentation of coconut water with the addition of fruit peel as seen

from the analysis lactic acid productivity, the resulting pH value is on the composition of fermented coconut water with the addition of dragon fruit peel and lemon peel in a ratio (1:2). The results of the analysis of the best composition are used for knowing the potential for adding fruit peels to be used as a carbon source in the coconut water fermentation process in producing organic acid compounds. The growth of lactic acid bacteria will increase secondary metabolites in the form of lactic acid compounds and other organic acids [22].

Effect of Addition of Dragon Fruit Peel and Lemon Peel to Identification of Organic Acid Compounds

Based on this assay to identification of organic acid compound. In this research, dragon fruit peels and lemon peels was added to increase antibacterial activity. Based on Table 1, it is known that the addition of dragon fruit peel and lemon has an effect on the total acid content formed. The organic acid composition formed can be analyzed using HPLC. This organic acid formed is an intermediate compound produced from the overhaul of the substrate medium by lactic acid bacteria. The presence of this organic acid have both bacteriostatic and bactericidal effects which hamper growth of bacteria and leading loss of viability [23]. The activity of organic acid can act as antimicrobial agents at pH less than 5. Some examples of organic acids that are often found include lactic acid, acetic acid, ascorbic acid, and oxalic acid [24]. Based on the results of HPLC (Figure 3), the filtrate from fermented coconut water with the addition of dragon fruit peel and lemon peel contains lactic acid, acetic acid and ascorbic acid.

Based on Figure 2 shows that the filtrate from fermented coconut water contains organic acid compounds consisting of lactic acid, acetic acid and ascorbic acid. It is based on the correspondence of the peaks of organic acids at a given retention time in the sample chromatogram when compared to the standard chromatogram. The retention time (RT) of the mixture of organic acids is shown in Table 2. In addition to standard organic acids, the potential of Lactic acid bacteria as a biological catalyst in the coconut water fermentation process to produce different organic acids. Organic acids from lactic acid bacteria have the potential to prevent spoilage and improve the taste of food which increases consumer appeal. Organic acids can be useful as starting materials in a variety of applications. Gallic acid is the result of the degradation of tannins and catechins produced from the tanase enzyme from fruit peel waste [25].

Based on Table 2 shows that the concentration of lactic acid is formed with a fairly high concentration of 884.62 g/mL. This is due to the activity of lactic acid bacteria which are able to hydrolyze glucose contained in the substrate so that the ability to produce lactic acid is higher. Lactic acid is one of the primary metabolites produced during the fermentation process.

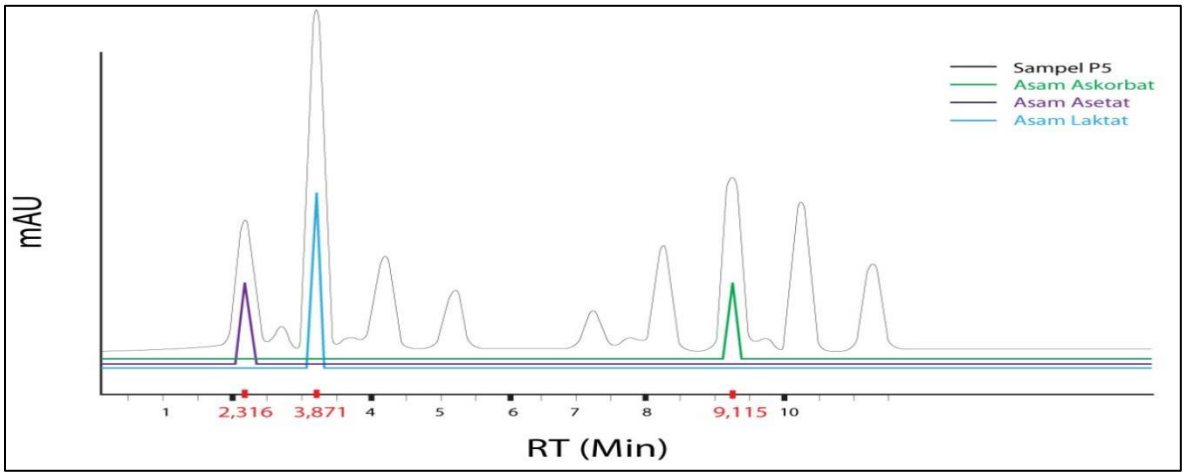


Figure 3. Chromatogram of Organic Acid Fermented by Coconut Water with the Addition of Dragon Fruit Peel and Lemon Peel.

Table 2. The Retention Time of Organic Acid Analyzed by HPLC.

RT (standard)	RT (sample)	Concentration (µg/mL)	Component
2.316	2.316	324.69	Acetic Acid
3.871	3.871	884.62	Lactic Acid
9.115	9.115	447.38	Ascorbic Acid

This primary metabolite plays a role in the growth of lactic acid bacteria. The presence of viability of lactic acid bacteria in fermented coconut water has an important role in inhibiting enteric pathogenic bacteria related to the amount of lactic acid bacteria activity in fermented coconut water [26]. In addition, as a comparison, other organic acids produced in hetero-fermentative metabolism as a by product of lactic acid will also be explained, it also produce acetic acid. This is because the commercial bacteria used in this study are not a group of homofermentative bacteria, so it is suspected that acetate compounds are formed as a result of the fermentation pathway of the hetero-fermentative group of bacteria. Likewise with the formation of ascorbic acid which is produced based on the metabolic production of lactic acid bacteria in utilizing dragon fruit peel and lemon peel. Several studies have reported that ascorbic acid has a synergistic interaction with a spectrum of antibiotic activity [27]. Recently, high doses of ascorbic acid combined with antibiotics have been shown to inhibit the growth of Helicobacter pylori in vitro as well as in vivo [27]. Ascorbic acid showed a statistically significant synergistic interaction with *Staphylococcus aureus* and *Escherichia coli*. This is used as a support to study the effect of organic acids on pathogenic bacteria with the aim of increasing their antibacterial activity.

Effect of Addition of Dragon Fruit Peel and Lemon Peel to Antibacterial Activity

In general, dragon fruit peels and lemon peels have antimicrobial compounds. Antimicrobial studies on coconut water fermentation to the addition of dragon fruit peel and lemon peel of mass variants are mentioned in Table 2. This research was conducted on Gram-positive and Gram-negative bacteria to see the antimicrobial activity. The antimicrobial activity against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* by using disc method. This method was used due to it is having advantages such as more accuracy and high sensitivity [28]. In this research, dragon fruit peels and lemon peels was added to increase antibacterial activity.

Information:

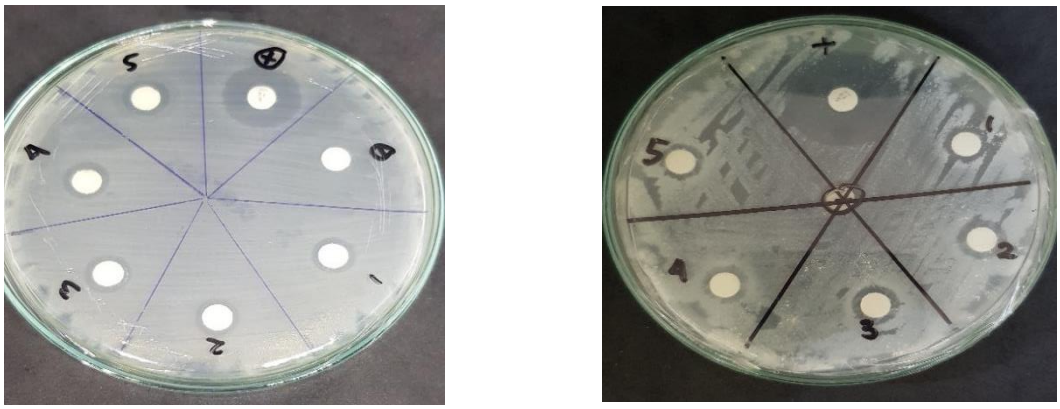
P_1 = without fruit peel

P_2 = with addition dragon fruit peels

P_3 = with addition lemon peels

P_4 = with addition dragon fruit peels and lemon peels (2:1)

P_5 = with addition dragon fruit peels and lemon peels (1:2)



(a) (b)

Figure 4. Antimicrobial Activity from Coconut Water Fermentation with Addition of Dragon Fruit Peels and Lemon Peels using Lactic Acid Bacteria (a) *Staphylococcus aureus*, (b) *Escherichia coli*.

Table 3. Antimicrobial Activity from Coconut Water Fermentation with Addition of Dragon Fruit Peels and Lemon Peels using Lactic Acid Bacteria

Treatment Fermented Coconut	Antibacterial activity diameter of inhibitor zone (mm)	
	Escherichia coli	***Staphylococcus aureus***
P_1	1.86	1.81
P_2	4.14	2.84
P_3	4.66	2.97
P_4	2.93	3.24
P_5	1.95	3.13
Control (+) *Streptomycin*	10.4	8.33
Control (-) *Nutrient Broth*	-	-

Table 2 shows that fermentation of coconut water using lactic acid bacteria and the addition of fruit peel can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*, with the inhibition zone of *Escherichia coli* being greater than that of *Staphylococcus aureus*. The antimicrobial activity of lactic acid bacteria is caused by the production of antimicrobial compounds which include the production of organic acids and a decrease in pH [29]. During the fermentation process, lactic acid bacteria will hydrolyze the glucose present in coconut water into organic acid compounds. The result supported by data identified organic acid compound consist lactic acid, acetic acid and ascorbic acid can be correlated with their potential as a source of antibacterial. In other side, coconut water itself contains catechins compound that are able to synthesize antibacterial peptides to fight pathogenic bacteria [30]. Several studies have also shown that the use of lactic acid bacteria in the fermentation process can degrade catechin. As well as gut microbiome such as *Lactobacillus plantarum* IFPL935 utilized catechin as the carbon source and catalyzed the conversion of catechin to catechol and other phenols such as gallic acid [5]. The result showed that the fermentation treatment with the addition of lemon peels had a maximum inhibition zone of 4.66 mm against *Escherichia coli* bacteria. This is due to the phytochemical content of lemon peels such as phenolic compounds which are secondary metabolites that can be used in defense mechanisms against pathogenic bacteria. Based on the research said that fermented with using lactic acid bacteria shows an increase in total phenol. The increase in total phenol may be due to activity of lactic acid bacteria. Based on research, showed that the use of lemon peel resulted in antimicrobial activity against *Escherichia coli* bacteria with an inhibition zone of 19.5 mm [11].

In other side, the inhibitory activity of the lactic acid bacteria from coconut water fermentation also showed antibacterial activity against *Staphylococcus aureus* bacteria. From this data, it was show that the fermentation treatment with addition of dragon fruit peels and lemon peels with ratio (2:1) had a maximum inhibition zone of 3.24 mm. This finding is in linier which has been published that Staphylococcus aureus is sensitive to the antibacterial activity of dragon fruit peel extract. From previous studies, the red dragon fruit possessed antimicrobial activities against pathogenic microbial. This is in accordance with the analysis which showed that dragon fruit peel had inhibitory activity against *Escherichia coli* and *Staphylococcus aureus* [28].

CONCLUSION

Based on the research that has been done, that it can be concluded that the addition of mass variants of dragon fruit peel and lemon peel can affect the total acid and pH value formed during the fermentation process. Then, the study showed that addition fruit peels can be classified as a good source of potential natural antibacterial agents.

REFERENCES

1. Maulana, R. (2019) Comparative Study Perkebunan Kelapa (Coconut) Di Indonesia Dan Otomatisasi Pengeringan (Oven Drier) Pada Industri Briket. *ResearchgateNet*., January 2019. doi:10.13140/RG.2.2.14736.05127.

2. Kantachote, D., Ratanaburee, A., Hayisama-ae, W., Sukhoom, A., Nunkaew, T. (2017) The use of potential probiotic Lactobacillus plantarum DW12 for producing a novel functional beverage from mature coconut water. *J. Funct. Foods*, **32**, 401–408. doi:10.1016/j.jff.2017. 03.018.

3. Ramamurthy, V. (2022) Production & Characterisation of Fermented Coconut Water and a Report on Its Health Benefits Food Science & Nutrition Technology. *Published online*, 1–5. doi:10.23880/fsnt-16000278.

4. Chang, C. L., Wu, R. T. (2011) Erratum: Quantification of (+)-catechin and (-)-epicatechin in coconut water by LC-MS (Food Chemistry (2010) 126: 2 (710-717)). *Food Chem.*, **129(2)**. e1. doi:10.1016/j.foodchem.2011.04.051.

5. Knezevic, S., Ghafoor, A., Mehri, S., et al. (2021) Catechin and other catechol-containing secondary metabolites: Bacterial biotransformation and regulation of carbohydrate metabolism. *Pharma Nutrition*, **17**, 100273 (May). doi:10.1016/j.phanu.2021.100273.

6. Ananda, T. D., Srihardyastutie, A., Prasetyawan, S., Safitri, A. (2019) Effect of mixed inoculums volume and ph on anti nutritional level in cabbage fermentation using saccharomyces cerevisiae and lactobacillus plantarum. In: *IOP Conference Series: Materials Science and Engineering*, *Institute of Physics Publishing*, **546**. doi:10.1088/1757-899X/546/6/062004.

7. Nabila, S. (2017) Pengaruh Lama Fermentasi Air Kelapa Dengan Starter Lactobacillus Plantarum FNCC 0027 Terhadap Mutu Produk.

8. Ziska, R. A. T. (2017) Uji Aktivitas Antimikroba dan Antioksidan dari Minuman Probiotik Hasil Fermentasi Air Kelapa (Cocos nucifera), **4(1)**, 14–19.

9. Kunaepah, U. (2008) Pengaruh lama fermentasi dan konsentrasi glukosa terhadap aktivitas anti-bakteri, polifenol total dan mutu kimia kefir susu kacang merah. *Univ Diponegoro, Published online*, 1–90.

10. Hendra, R., Masdeatresa, L., Abdulah, R., Haryani, Y. (2019) Antibacterial Activity of Red Dragon Peel (Hylocereus polyrhizus) Pigment. In: *Journal of Physics: Conference Series, Institute of Physics Publishing*, **1351**. doi:10.1088/1742-6596/1351/1/012042.

11. Ali, J., Das, B., Saikia, T. (2017) Antimicrobial Activity of Lemon Peel (Citrus limon) Ectract. *Int. J. Curr. Pharm. Res*., **9(4)**, 79. doi:10.22159/ijcpr.2017v9i4.20962.

12. Ifeanyi Okeke, M., Pharm Sci PJ, Stanley Okoli, A., et al. (2015) Antibacterial Activity of Citrus Limonum Fruit Juice Extract, **28**. https://www.researchgate.net/publication/281861543.

13. Hasan, A., Wikandari, P. R. (2018) Determination of Optimum Bacteriosin Production Time from Lactobacillus Plantarum B1765 Based on Antimicrobial Activity Against Staphylococcus Aureus, **7**.

14. Mulyani, S., Sunarko, K. M. F., Setiani, B. E. (2021) Pengaruh Lama Fermentasi terhadap Total Asam, Total Bakteri Asam Laktat dan Warna Kefir Belimbing Manis (Averrhoa carambola). *J Ilm. Sains*, **21(2)**, 113. doi:10.35799/jis.21.2.2021.31416.

15. Nurdyansyah, F., Widyastuti, D. A. (2017) Pengolahan Limbah Air Kelapa Menjadi Nata De Coco oleh Ibu Kelompok Tani, **21(XI)**, 22–30.

16. Fekadu Gemede, H. (2014) Antinutritional Factors in Plant Foods: Potential Health Benefits and Adverse Effects. *Int. J. Nutr. Food Sci*., **3(4)**, 284. doi:10.11648/j.ijnfs.20140304.18.

17. Razaviamri, S., Wang, K., Liu, B., Lee, B. P. (2021) Catechol-based antimicrobial polymers. *Molecules*, **26(3)**. doi:10.3390/molecules26030559.

18. Parasthi, L. Y. E., Afifah, D. N., Nissa, C., Panunggal, B. (2020) Total Lactic Acid Bacteria and Antibacterial Activity in Yoghurt with Addition of Ananas comosus Merr. and Cinnamomum burmannii. *Amerta Nutr*., **4(4)**, 257. doi:10.20473/amnt.v4i4.2020.257-264.

19. Ulva Dianasaril, R. M., Maruddin, F. (2018) The Values of Ph, Lactid Acid and Color of the Fermented Milk Added with Red Dragon. *Hylocereus*, March 2019.

20. Adi Wira Kusuma, G. P., Ayu Nocianitri, K, Kartika Pratiwi, I. D. P. (2020) Pengaruh Lama Fermentasi Terhadap Karakteristik Fermented Rice Drink Sebagai Minuman Probiotik Dengan Isolat Lactobacillus sp. F213. *J. Ilmu dan Teknol. Pangan*., **9(2)**, 181. doi:10.24843/itepa.2020.v09.i02.p08.

21. Fazriyah, N. (2015) Pengaruh Perbedaan Konsentrasi Madu dan Lama Fermentasi terhadap pH, Total Assam, Gula Reduksi dan Potensi Aantibakteri Kefir Air Leri. *J. Geotech. Geoenvironmental Eng. ASCE*., **120(11)**, 259.

22. Nurdyansyah, F., Hafidz, U., Hasbullah, A. (2018) Optimization of Lactid Acid Fermentation by Lactobacillus casei on Fermentation Medium with Banana Peel Flour Subtitution. *J. Biol*., **11(1)**, 64–71. http://dx.doi.org/10.15408/kauniyah.v11i1.

23. Ahmed, M., Muhammad, D., Qadir, A. (2013) Organic Acids Effective Antimicrobial Agents against Escherichia Coli, Staphylococcus Aureus and Pseudomonas Aeruginosa at Ambient Temperature. *An Int J*., **1(11)**, 983–987. www.jpronline.info.

24. Sitorus, L., Pontoh, J., Kamu, V. (2015) Analisis Beberapa Asam Organik dengan Metode High Performance Liquid Chromatography (HPLC) Grace Smart Rp 18 5µ. *J MIPA*., **4(2)**, 148. doi:10.35799/jm.4.2.2015.9113.

25. Junaidi, E., Anwar, Y. A. S. (2018) Aktivitas Antibakteri dan Antioksidan Asam Galat dari Kulit Buah Lokal yang Diproduksi dengan Tanase. *ALCHEMY J. Penelit Kim*., **14(1)**, 131. doi:10.20961/alchemy.14.1.11300.131-142.

26. Purwijantiningsih, E. (2014) Viabilitas Bakteri Asam Laktat dan Aktivitas Antibakteri Produk Susu Fermentasi Komersial terhadap Beberapa Bakteri Patogen Enterik. *Biota J. Ilm Ilmu-Ilmu Hayati*, 15–21 (June, 2014). doi:10.24002/biota.v19i1.450.

27. Srividya, G., Deepthi, B., Lakshminarasaiah, S. (2017) Ascorbic acid enhances ciprofloxacin antibacterial activity in vitro against isolates of Escherichia coli from subclinical mastitis cases of buffaloes, **2(5)**, 21–24.

28. Hendra, R., Masdeatresa, L., Abdulah, R., Haryani, Y. (2019) Antibacterial Activity of Red Dragon Peel (Hylocereus polyrhizus) Pigment. *J. Phys. Conf. Ser*., **1351(1)**. doi:10.1088/1742-6596/1351/1/012042.

29. Fachrial, E., Harmileni, H. (2018) Isolasi Dan Aktivitas Anti Mikroba Bakteri Asam Laktat Dari Fermentasi Nira Kelapa Sawit. *BIOLINK (Jurnal Biol Lingkungan, Ind Kesehatan)*, **5(1)**, 51. doi:10.31289/biolink.v5i1.1707.

30. Camargo Prado, F., De Dea Lindner, J., Inaba, J., Thomaz-Soccol, V., Kaur Brar, S., Soccol, C. R. (2015) Development and evaluation of a fermented coconut water beverage with potential health benefits. *J. Funct. Foods*, **12**, 489–497. doi:10.1016/j.jff.2014.12.020.