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Comparison of Ethanol Levels in Yellow and Red Watermelon Wine

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Abstract
Watermelon is one of the tropical fruits that has high water and sugar content. However, the selling price of watermelon is still relatively low, and there is limited processing variation. By utilizing the high water and sugar content in watermelon to create an alcoholic beverage, wine is one promising form of innovation. The aim of this research is to determine the difference in alcohol content of wine made from yellow and red watermelon. The samples used in this study were yellow and red watermelons. Yellow and red watermelons fermented with Saccharomyces cerevisiae yeast to transform them into wine. The ethanol content in wine and red watermelons was analyzed using GC-MS instruments. Subsequently, the soluble sugar content, pH, IR spectra, as well as the color, taste, and aroma of the wine also analyzed. The research results show that the average ethanol content of yellow and red watermelon wines is 7.59% and 6.61% respectively. The average soluble sugar content in yellow and red watermelon wines is 8.6% Brix and 12.64% Brix, respectively. The pH value of each wine sample is 4, and the IR spectra of each wine sample indicate the presence of stretching OH groups. In conclusion, red watermelon wine has a lower ethanol content than yellow watermelon wine.

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INTRODUCTION

As a nation characterized by a tropical climate situated along the equatorial belt, Indonesia boast an exceptionally rich biodiversity. This is partly evidenced by the wide variety of fruit species within its borders. Among these, watermelon stands out as a particularly popular tropical fruit among the populace. Watermelon is very popular among people because this fruit has a sweet tasting pulp and contains a lot of water (Kuswandi & Marta, 2021).

Further elucidating the nature of watermelon, taxonomically it belongs to the Cucurbitaceae family, which includes cucumbers. In particular, watermelon has a large water content in its flesh. In this regard, Ibrohim (2023) explains that the water content of watermelons can reach an impressive 92% of the total fruit composition. Aside from its high waters content, watermelon is known for its sugar content, which contributes to its characteristic sweetness. Amin et al. (2014) report that the sugar content of watermelon typically ranges between 9-10% of the total fruit composition. These sugars come in various forms, including monosaccharides such as glucose and fructose, alongside disaccharides like sucrose. Overall, these three sugars represent the main component of watermelon sugars. Indeed, the total amount of these sugars together can account for 20-50% of the total sugar content (Yativ et al., 2010).

Although watermelon is known for its delicious taste and refreshing properties, it is generally underutilized in culinary applications. Typically, watermelons are consumed as is or occasionally incorporated into fruit salads or beverages. Due to the lack of innovative processing methods, watermelon tends to have relatively low market value. Current data from 2023 shows that watermelons are typically sold for approximately IDR 3,000.00 per kilogram. The highest selling price for watermelons ranges IDR Rp6,000.00 to IDR 8,000.00, while during periods of excess supply, prices can drop to as low as IDR 2,000.00 to IDR 3,000.00 (Hanani, 2023; Rina, 2023; Rumpakaadi, 2023). These figures underscore the modest market value of watermelons. In addition, its relatively short post-harvest shelf life poses a challenge for their commercialization. Watermelon that has been peeled cut and store for three days at room temperature will rot (Annisa Kirana et al., 2020).

A solution to increase the sales value and storage time of watermelons. Watermelons is made into wine through a fermentation process. Wine is an alcoholic beverage produced through the fermentation process carried out by microorganisms. During fermentation, specific microorganisms, including yeast or bacteria, convert carbohydrates (sugars) into alcohol or organic acids (Maicas, 2020). *Saccharomyces cerevisiae*, a fungus, is commonly used in wine production. Under anaerobic conditions (without oxygen), such as those present during fermentation, microorganisms metabolize glucose into alcohol and CO_2 gas (Walker & Stewart, 2016). Sugar containing substrates are suitable for carrying out the fermentation process. Sugar is a carbon and energy source that can be easily utilized by microorganism (Stanbury et al., 2016).

The idea of converting watermelon into wine is an innovative approach to increasing both the sales value and the shelf life of the fruit. Wine production through fermentation allows for value-added processing, transforming a perishable product into a more stable one with longer shelf life and higher market potential (Maicas, 2021; Pinto et al., 2015). Fermentation is a biological process in which microorganisms, such as yeast or bacteria, convert organic compounds (typically sugars) into alcohol or acids. In wine production, the primary microorganism involved is a species of yeast known as *Saccharomyces cerevisiae* (Ciani & Comitini, 2015). This species is favored because it efficiently ferments sugars and produces alcohol, which is the key element in wine. Saccharomyces cerevisiae is widely used in winemaking due to its ability to thrive under anaerobic conditions environments without oxygen (Albergaria & Arneborg, 2016; Ogodo et al., 2015). During fermentation, the yeast metabolizes sugars present in the watermelon juice, converting them into ethanol and carbon dioxide (CO_2). This process takes place in a controlled environment, where temperature, oxygen levels, and nutrient availability are carefully managed to ensure optimal fermentation.

Watermelon contains a high amount of water and natural sugars, particularly fructose and glucose. These sugars are ideal substrates for fermentation because they serve as carbon and energy sources for the microorganisms. The high sugar content allows for efficient alcohol production, making watermelon an excellent candidate for winemaking (Endoh et al., 2021; Guo et al., 2021; Wang et al., 2022). In addition, since watermelon has a naturally sweet taste and a unique flavor, it can produce a distinctive type of fruit wine that may appeal to niche markets. This could increase the fruit's commercial potential beyond its traditional fresh consumption.

The watermelon is crushed or juiced to obtain the must, the liquid that will be fermented. In some cases, additional sugar may be added to increase alcohol content, depending on the natural sugar levels in the fruit. Saccharomyces cerevisiae is introduced into the must. This yeast begins metabolizing the sugars under anaerobic conditions (Albergaria & Arneborg, 2016). During this stage, the majority of sugar is converted into alcohol. The process typically lasts between a few days to several weeks, depending on the desired alcohol content and flavor profile. After the primary fermentation is complete, the wine is transferred to another container to allow secondary fermentation and aging. This process helps refine the flavors and develop the desired characteristics of the wine (Maicas, 2021).

Bioethanol is becoming an increasingly important renewable energy source due to its potential to reduce greenhouse gas emissions, diversify energy supplies, and stimulate rural economies. It is produced from biomass, primarily from crops like corn, sugarcane, wheat, and other agricultural residues, making it a sustainable alternative to fossil fuels (Tse et al., 2021). Bioethanol burns cleaner than traditional fossil fuels, leading to a significant reduction in greenhouse gas emissions. Since the CO_2 emitted during its combustion is absorbed by plants during their growth cycle, bioethanol is considered to be carbon-neutral (Park et al., 2022; Ru Fang et al., 2022). When blended with gasoline, bioethanol reduces harmful emissions like carbon monoxide, particulate matter, and other pollutants, improving air quality.

Bioethanol can be blended with gasoline to reduce dependence on fossil fuels, which are finite and concentrated in certain regions of the world. Using domestically produced bioethanol helps countries reduce their reliance on imported oil, increasing energy security (Meloni et al., 2022). Since bioethanol is produced from renewable biomass sources, it offers a more sustainable option compared to petroleum, which is depleting.

Bioethanol can also be continuously replenished through agricultural processes. (Melendez et al., 2022; Parascanu et al., 2021) report production of bioethanol creates new markets for agricultural products and by-products, benefiting farmers and rural communities. It stimulates local economies by providing employment opportunities in farming, processing, and distribution. It can be blended with gasoline at various levels, such as E10 (10% ethanol) and E85 (85% ethanol), to optimize fuel efficiency in vehicles. Ethanol blends like E10 (10% ethanol, 90% gasoline) are widely used around the world, directly reducing the amount of gasoline needed for fuel. This, in turn, decreases the extraction and consumption of fossil fuels, lessening the environmental impact of oil drilling and refining.

In conclusion, the fermentation of watermelon into wine is an effective strategy to increase the fruit's commercial value while offering an innovative product that can capture new markets. The ethanol content in the wine was analyzed using infrared spectroscopy instrument (IR). Analysis using GC-MS instrument is faster and easier (Stupak et al., 2017). The advantage of ethanol analysis using IR instrument is more accurate and reliable (Korban et al., 2021). One use of the GC-MS instrument is to measurement of ethanol content in various types of wine (Stupak et al., 2017). The use of IR instrument in fermentation process is to determine the ethanol content in wine made from watermelon (Hulyadi et al., 2023).

The selling value and storage time of watermelon can be increased by processing watermelon into wine through a fermentation process. Moreover, upon transformation into wine, watermelon's low market value can be elevated, and its limited shelf life can be extended. Therefore, the aim of this study is to investigate the ethanol level of watermelon wine. This research aims to identify the alcohol content in watermelon wine using GC-MS. Furthermore, compare the alcohol content resulting from fermenting two different varieties of watermelon red and yellow.

METHOD

Tools and Materials

The tools used in this research were glassware, digital scales, electric stove, pans, bottles for the fermentation process, FT-IR, GC-MS, and sugar refractometer. The materials used consist of red and yellow watermelon, instant yeast (*Saccharomyces cerevisiae*), sugar, and water.

Preparation and Fermentation Process

Red and yellow watermelons were prepared and crushed. The crushed watermelons were sieved to separate the pulp from the juice. The juice of red and yellow watermelon is collected, a total of 500 grams each. Each 500 grams of watermelon juice is mixed with 1 liter of clean water and stirred well. Sugar is added, totaling 225 grams per watermelon juice. The juice of red and yellow watermelons are boiled until all the sugar is dissolved and left for 10 minutes. Then, they are allowed to cool to around 27-30°C. Each juice is divided into 3 portions, each portion having a volume of 300 mL. Thus, there are 3x300 mL portions of red and yellow watermelon juice. Each portion of watermelon juice is poured into bottles for fermentation, and Saccharomyces cerevisiae yeast is added at a rate of 0.3 grams per bottle, resulting in a yeast concentration of 1 gram per liter. The watermelon juice bottles are tightly sealed and stirred to evenly distribute the yeast. The fermentation process is allowed to proceed for 1 week. Then, the fermentation process is halted by placing the fermentation bottles in the refrigerator (at a temperature of 3°C) for 1 week. The preparation and fermentation process of red and yellow watermelon are adapted from the study conducted by Darman et al., (2010).

Determination of Dissolved Sugar Content in Wine

The determination of soluble sugar content accurs after the fermentation process. The sugar level in the wine solution from each fermentation bottle (K01, K02, K03, M01, M02, and M03) is analyzed using a wine refractometer in %Brix at a temperature of 26°C. The sugar level in the wine solution is analyzed using a wine refractometer (Misto et al., 2020).

Analysis of Ethanol Concentration in The Wine

Compound analysis was carried out using IR. The device was set to an injection temperature of 150ºC, a column temperature of 115 ºC, and a detector temperature of 200 ºC. During the analysis process the injection temperature is maintained for 3 minutes. The temperature then increases by 2 ºC per minute until it reaches 185 ºC and is held for 2 minutes. The identified compounds were compared with data in the literature. The detected alcohol content in terms of percentage area from the IR instrument is compared for each type of watermelon (Budiono et al., 2023).

IR Spectra Analysis of Watermelon Wine Samples

The IR spectra of each watermelon wine sample (M01, M02, M03, K01, K02, and K03) were analyzed with an FT-IR instrument. The compound in the wine sample was analyzed using Shimadzu Spirit 8000 Infrared Spectrometer (Liu et al., 2021).

Determination of Wine pH

Each watermelon wine sample (M01, M02, M03, K01, K02, and K03) had its pH value measured using universal indicator paper.

Organoleptic Test

Five rater rated the wine products made from watermelon. The obtained average is then presented in the result. This organoleptic test includes taste, color and smell test (Madhumita Barooah et al., 2017).

1. **Taste test:**
   Wine samples are poured into small glasses and tested by rater.
2. **Color test:**
   Wine samples are poured into small glasses and placed on a piece of white paper. The wine is observed.
3. **Odor test:**
   Wine is poured into a glass and the aroma it produces is smelled.

Sugar is the main ingredient in the fermentation process to produce alcohol. Theoretically, yeast uses sugar as a carbon source to ferment. Common sugars that can be fermented into ethanol and carbon dioxide include glucose, fructose, sucrose, mannose, galactose, maltose, and maltotriose (Maicas, 2020). When these sugars present, the yeast begins converting glucose into pyruvate. Pyruvate is then converted into ethanol with an intermediate compound called acetaldehyde (Walker & Stewart, 2016). To explain futher: the conversion of pyruvate to ethanol involves two reaction steps. First, when CO_2 is released from pyruvate, acetaldehyde is formed. Second, acetaldehyde is reduced by NADH to produce ethanol. This process creates two fermentation products: ethanol and CO_2 gas (Urry et al., 2016).

$$C_6H_{12}O_6\ (l) \xrightarrow{\text{Saccharomyces cerevisiae}} 2C_2H_5OH\ (l) + 2CO_2\ (g)$$

As theoretically expected, watermelon, known for its high sugar content, has significant potential to be processed into an alcoholic beverage, namely wine. This is supported by the presence of glucose, fructose, and sucrose in watermelon, which collectively account for 30-50% of its total sugar content (Yativ et al., 2010). In this study, the alcohol fermentation process was carried out using these sugars presents in watermelon, which produces ethanol and CO_2 gas.

Analysis of Ethanol Concentration in The Wine

The ethanol content produced from each fermentation process was analyzed using a GC-MS instrument. In this analysis, the ethanol content is expressed as the percentage of the area under the ethanol peak to the total area of the chromatogram of the sample analyzed.

Table 1. Ethanol content from fermentation process

Sample	Ethanol concentration (%)	Average of Ethanol concentration (%)
M01*	3,89	
M02*	7,36	6,61
M03*	8,57	
K01*	3,12	
K02*	9,38	7,59
K03*	10,28	

*M = Red watermelon; K = Yellow watermelon

Based on Table 1, it can be seen that on average, the ethanol content produced from the fermentation of yellow watermelon is higher than the ethanol content produced by the fermentation of red watermelon. However, generally speaking, the ethanol content of both types of watermelon is usually not at least 10%. Although each replicate was examined separately, samples M01 and K01 only produced ethanol at around 3-4% after 1 week of fermentation. In samples M02, M03, K02, and K02, ethanol production ranged from 7-10%. The fermentation time for winemaking is only a week, resulting in low alcohol level. The longer the fermentation process lasts, the higher the alcohol content in the wine(Ezemba et al., 2022). The content of yellow watermelon wine is already in the theoretical ethanol content. The alcohol in the obtained wine was confirmed by the results of analytical tests using FT-IR spectroscopy in Figure 2. From the IR spectral pattern obtained, it was clear that OH groups were present in each wine sample. The peak resulting from OH groups in each sample is visible in the wavelength 3400-3100 cm^{-1} (Sigmaaldrich, 2023).

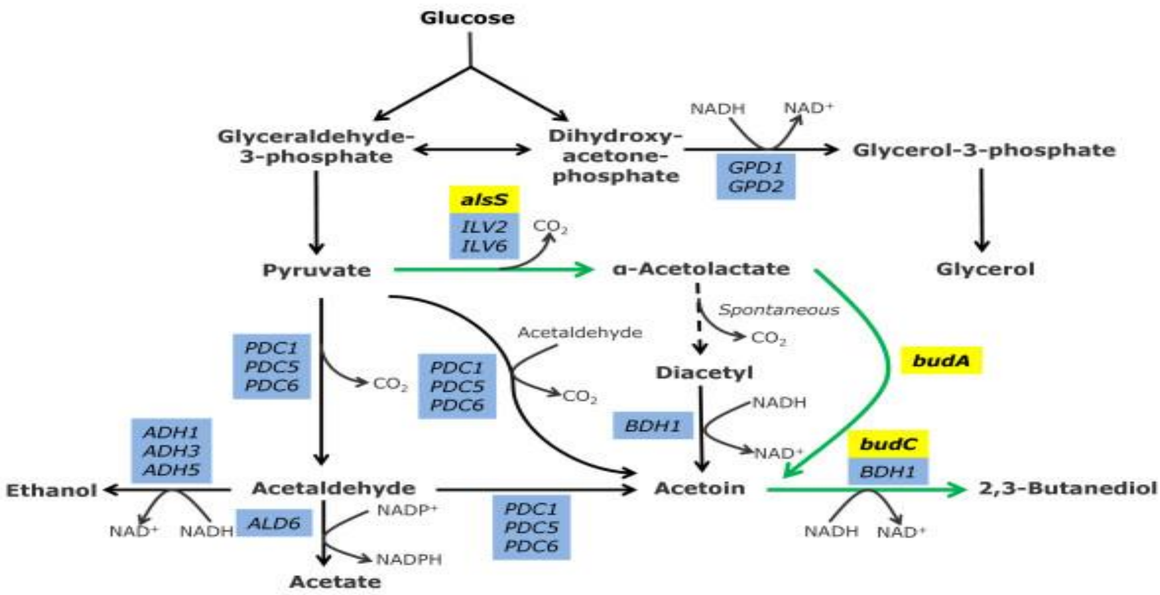

Figure 1. The metabolic pathway for the biosynthesis of 2,3-Butanediol compound and the pyruvate metabolism in S. Cerevisiae (Ng et al., 2012).

Due to the low content of ethanol produced, the GC-MS analysis results also show that ethanol is not the compound with the highest content in each wine analyzed. Compounds with relatively high levels in red and yellow watermelon wines based on GC-MS analysis are glycerin and 2,3-butanediol. The glycerin content was highest in sample M02, at 20.17%. Meanwhile, the highest content of 2,3-butanediol compound was found in sample M01, at 64.02%. The presence of these two compounds is not new in the process of alcohol fermentation. The available information suggests that the alcohol formation reaction reaction using the yeast *Saccharomyces cerevisiae*, the formation of glycerin and 2,3-butanediol compounds is highly likely to occur. The reaction pathways for the formation of these two compounds in the fermentation process can be seen in Figure 1 (Ng et al., 2012).

Figure 1 shows that the compound 2,3-Butanediol can be synthesized when pyruvate, which should normally become acetaldehyde, is converted to α-acetolactate by acetolactate synthase. Subsequently, the α-acetolactate compound further converts into acetoin using catalysis by α-acetolactate decarboxylase (under anaerobic conditions). Finally, the compound 2,3-Butanediol can be synthesized when butanediol dehydrogenase reduces acetoin (Ng et al., 2012). However, apart from this pathway, acetoin which is converted into 2,3-Butanediol can also be synthesized through the condensation process of active acetaldehyde by pyruvate decarboxylase. This condition tends to be more dominant during the fermentation process with Saccharomyces cerevisiae as this microorganism mostly lacks α-acetolactate decarboxylase (Ng et al., 2012).

Furthermore, in terms of the formation of products such as glycerol, also known as glycerin, Figure 1 also shows the formation path of this compound. Whether or not glycerolis is formed in the fermentation process is influenced by the water activity in the reaction taking place. This is due to the important role of water in the physiological function of *Saccharomyces cerevisiae* cells, especially with regard to the high or low sugar content during fermentation. When the sugar used is excessive or has a high sugar content, the sugar in the solution excerts osmotic pressure on the cells, disrupting the physiology of the microorganisms used. The relationship between high sugar content and glycerol production is that excessive glycerol production is one of the responses of yeast cells when experiencing water shortage (Walker & Stewart, 2016).

In this study, the comparison between sugar and the fermented watermelon juice solution is 225 grams of sugar: 1500 grams of total watermelon juice volume. This value corresponds to a sugar content of 15% (w/w). This sugar content value does not yet include the pure sugar already contained in the watermelon. From the total 500 grams of watermelon used in this fermentation process, the estimated pure sugar content is approximately 5%. Therefore, the estimated total sugar content contained in the watermelon juice solution before the fermentation process begins is 20%. This value can be considered quite high for alcoholic fermentation processes. The reason is that in wine production, the maximum sugar content used is 20% (Arif et al., 2016). Information suggest that when the sugar content used for fermentation exceeds 20%, yeast activity in producing ethanol is inhibited, requiring a longer fermentation time and not all sugars present can be converted into ethanol. Therefore, this explains why the ethanol content produced in this study is relatively low.

Consistent with the lower ethanol content in the red watermelon wine samples compared to the yellow watermelon samples, determination of the soluble sugar content in the wine using a wine refractometer revealed the average sugar content in the red watermelon was 12.64% Brix (1%Brix = 1 gram of sugar in 100 grams of solution). The average soluble sugar content in the yellow watermelon was 8.6% Brix. Data on the soluble sugar content of each sample can be seen in Table 2. The data shows that when after the fermentation process is completed, the sugar content remaining in the red watermelon wine is still quite significant compared to the initial estimate of 20%. In other words, only about 7.36% of the sugar is converted into ethanol. Since the average sugar content after fermentation is 8.6% Brix, this suggests that approximately 11.4% of the sugar is converted into ethanol.

Table 2. Sugar content in wine after fermentation process

Sample	**Sugar content after fermentation (%Brix)**	**Average of sugar content (%Brix)**
M01	13,3	
M02	11,7	12,64
M03	12,9	
K01	8,4	
K02	8,6	8,6
K03	8,8	

IR Spectra Analysis of Watermelon Wine Samples

The results of the IR spectrum analysis of each sample of red and yellow watermelon wine can be seen in Figure 2. The IR spectra obtained from the analysis using the FT-IR instrument show that, in general, all wine samples have similar IR spectral patterns, which are distinguished by their %transmittance values. From the obtained IR spectral patterns, it can be clearly seen that the OH groups are present in each wine sample. The peaks resulting from the presence of OH groups in each sample can be seen in the wavelength range of 3400-3100 cm^{-1} (Sigmaaldrich, 2023). However, despite the clear presence of OH groups, the presence of other groups that should be visible is less evident in the obtained IR spectra. These other groups that should be visible include the stretching and bending of C-H alkane bonds in the wavelength range of

3000-2840 cm^{-1} (stretching) and 1450-1375 cm^{-1} (bending); C-O bonds in the wavelength range of 1085-1050 cm^{-1} (Sigmaaldrich, 2023).

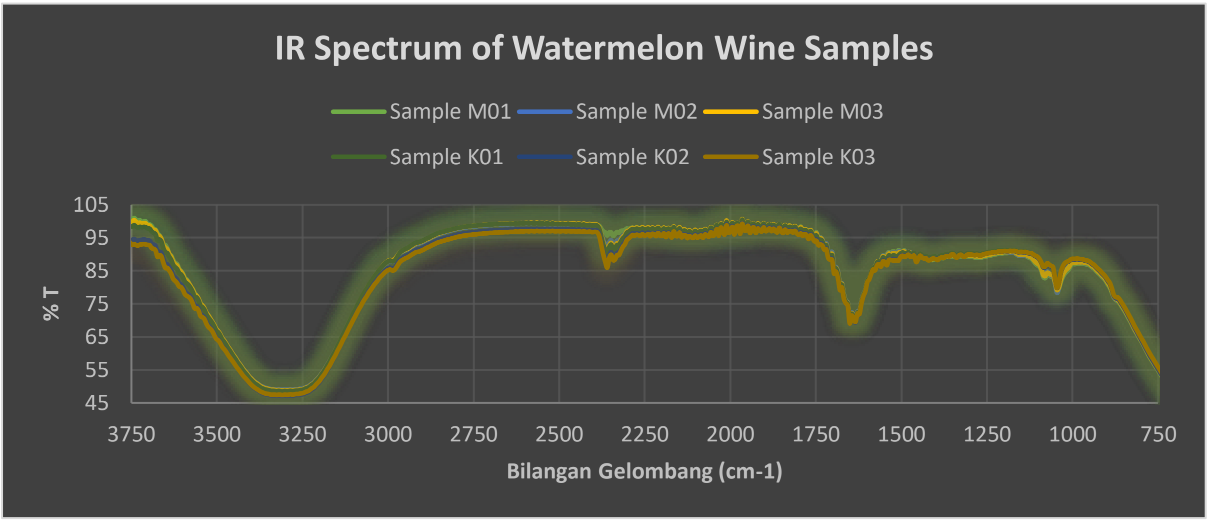

Figure 2. IR spectra of each watermelon wine sample

The absence of other types of groups that should be present in alcohol compounds, especially ethanol, may be due to the fact that the samples analyzed by the FT-IR instrument are directly wine solutions. To obtain pure compounds in wine fermented from fruit, a distillation process must be carried out (Reyes, 2020).

Determination of the pH of Watermelon Wine and Organoleptic Test Results

Table 3. Results of determining pH values and organoleptic tests

Sample	pH	Taste*	Color*	Odor*
M01	4	++	+++	++
M02	4	+++	++	+
M03	4	++	++	+
K01	4	+	+	+
K02	4	+	+	++
K03	4	+	++	+

*The symbol (+) indicates the level of sweetness, clarity, and pungent aroma in each sample. The more symbols (+), the sweeter the taste, the clearer the color, and the more pungent the aroma.

As seen in Table 3, the result of pH determination of each sample using universal indicator paper show that all wine samples have a pH value of 4. Comparing to existing theories, wine enthusiasts generally prefer wines with pH values around 3.6-4.5 (Satav & Pethe, 2016). Thus, it can be said that the pH of the wine produced from red and yellow watermelon falls within the desired range. In addition, this is also related to the pH, which can optimize yeast fermentation processes. The optimal pH value of the fermentation medium is around 4.5. When the pH significantly lower or higher, the yeast's performance in the fermentation process will decrease and affect the quality of the wine produced. In the fermentation process, the pH of the solution generally decreases. This is due to the formation of various acidic compounds such as lactic acid and pyruvate during fermentation (Satav & Pethe, 2016).

Organoleptic tests were also conducted in this study. This test includes taste, color, and aroma of each wine sample. The summarized results of the organoleptic test can be seen in Table 3.

The test results show that red watermelon wine, generally has a sweeter taste than yellow watermelon wine. This is consistent with the results of the test for soluble sugar content, which suggests that the sugar content in red watermelon wine is indeed higher. However, even though it tastes sweeter, the sweetness level of the watermelon wine can still be enjoyed like regular wine. The sample with the sweetest taste is sample M02. In terms of color, all red watermelon wines have a clear yellow color, while yellow watermelon wine has a more yellowish color, but not too dark. This is due to the basic ingredients of the two types of wine. However, in terms of clarity, red watermelon wine appears clearer than yellow watermelon wine. The clearest red watermelon is sample M01, while the darkest is samples K01 and K02. Finally, as for the aroma produced by each sample of red and yellow watermelon wine, the aroma produced by all wine samples is general the typical aroma of wine. The aroma of each sample is quite pungent and has a characteristic aroma of alcoholic beverages. The sample with the strongest wine aroma is samples M01 and K02. Based on the results described above, including taste, color, and odor wine have good quality (Reyes, 2020).

CONCLUSION

Based on the research results, red watermelon and yellow watermelon can be used as the basic ingredients in making wine for alcoholic beverage. According the analysis by GC-MS, the ethanol content in each sample was on average higher for yellow watermelon wine (7,59%) than for red watermelon wine (6,61%). The ethanol content obtained also corresponds to the measurement results of soluble sugar content, which show a higher sugar content in red watermelon compared to yellow watermelon. In addition, both yellow and red watermelon wines generally exhibit acidity, color, taste, and aroma that are consistent with typical wines. Making wine from yellow and red watermelon can be done through a simple fermentation process. In order to achieve higher ethanol content in wine production, a distillation process must be carried out.

RECOMMENDATIONS

Since the results of this study show that watermelon can be processed into wine as an alcoholic beverage product in this study, the research team hopes that this can inspire the many people to innovate further in processing and possibly increasing its market value. For future similar research, the watermelon fermentation results in this study were, as mentioned not perfect. One of the issues is attributed to the water and sugar content in the fermentation process, which was not optimal and significantly affected the fermentation process. Therefore, the research team suggests that future similar studies optimize the sugar and water content to provide accurate information about the sugar and water content suitable for fermentation, especially for watermelon.

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