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The Effect of Lemon (*Citrus Limon* [L.] Osbeck) and Cinnamon (*Cinnamomum* Spp.) Addition towards Antioxidant Activity and Physicochemical Properties of Honey Wine

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ABSTRACT: The characteristics of honey wine can be improved by the addition of fruits and herbs. Lemon and cinnamon contain phenolic and flavonoid compounds as antioxidant activities. This research aims to determine the effect of adding lemon and cinnamon towards the antioxidant activity and physicochemical properties of honey wine. The lemons used in this research are local and imported lemon juice, with citric acid as control. The cinnamon used in this research are cassia and ceylon cinnamon. The honey wine is analyzed based on its antioxidant activities (IC_{50}, phenolic and flavonoids) and physicochemical properties (alcohol content, density, acidity (pH), total dissolved solids and color). Imported lemon juice and cassia cinnamon are chosen as the best formulation due to the highest antioxidant activity and physicochemical properties of the honey wine as a result. This formulation has alcohol content 13.93%, antioxidant activity (IC_{50} 1.81%, total phenolic 794.61 mg GAE/L, total flavonoid 23.45 mg QE/L), density 0.98 g/mL, pH 3.28, total dissolved solids 9.42 ºBrix, color lightness 53.83 and ºHue 86.26º. In conclusion, the addition of imported lemon juice and cassia cinnamon in making honey wine increases the IC_{50}, total phenolic, total flavonoids, alcohol content, pH, while decreases the ºHue color of wine.

KEYWORDS: alcohol, antioxidant, cinnamon, honey wine, lemon

I. INTRODUCTION

Alcoholic fermentation is one of the processes in food processing that converts simple sugars to alcohol and carbon dioxide with the help of yeast as the microorganism in the fermentation. Honey wine also often called mead, is an alcoholic beverage obtained by fermenting a mixture of honey and water. Honey contains phenolic and flavonoid compounds that act as antioxidant. The antioxidant activity of honey is classified as low activity. In the process of making honey wine, ingredients such as fruits, herbs, and spices can also be added to the must (unfermented mixture of honey and water) to stimulate fermentation and improve the characteristics of honey wine.

Lemon contains phenolics, flavonoids, and ascorbic acid as antioxidants (Ghasemi et al., 2009). According to Krisnawan et al. (2017), IC_{50} of the imported (Australian) lemon juice is 5388.58 ppm (0.54%) and local (Indonesian) lemon juice is 1905.96 ppm (0.19%). The organic acids that contained in lemons are citric acid, malic acid, tartaric acid, ascorbic acid, lactic acid (Nour et al., 2010).

Cinnamon contains phenolics and flavonoids that also act as antioxidants (Latief et al., 2013). Based Ying et al. (2015) the antioxidant activity of nine spices from China, the ethanol extract of cassia cinnamon spice gave the highest antioxidant activity with an IC_{50} value of 171.39 µg/mL (0.017%). This research, it is expected that the addition of lemon and cinnamon can increase the antioxidant activity of honey wine.

II. MAKING OF HONEY WINE

(Gupta and Rajesh, 2009; Iglesias et al., 2014; Morales et al., 2013 with Modification)

The honey used for honey wine was weighed and mixed with water with 1:2 ratio. A total of 0.08% diammonium phosphate was then added to the honey mixture. The acidity of the honey mixture was adjusted to pH of 3.7 with the addition of lemon juice according to the treatment. Different types of cinnamon pieces was added to the honey mixture according to the treatment with a total of 2% to obtain must. A total of 55% dehydrated yeast was added to the must and fermented for 4 weeks at room temperature. After the fermentation process was completed, the honey wine is separated from the sediment using the decantation method. The honey wine was then analyzed for IC_{50} (DPPH-Radical-Scavenging Activity), total phenolic, total flavonoid, alcohol content, density, acidity (pH), total dissolved solids, and color. The best formulation for honey wine (lemon and cinnamon type) was determined based on the highest antioxidant activity of honey wine.

Analysis

Analysis conducted on this research are IC_{50} and total phenolic (Czabaj et al., 2017 with modification), total

flavonoid (Pontis et al., 2014 with modification), alcohol content (OIV-MA-AS312-01A, 2016 with modification), density, pH, titratable acidity (AOAC, 2005 with modification), total dissolved solids (ISO 2173, 2003), and color (Ariadi et al., 2015 with modification).

Experimental Design

The experimental design used in this study was a completely randomized factorial design with two factors. The first factor was the type of lemon. Type of lemon used in this research are local lemon, imported lemon, and citric acid as control factor. The second factor was the type of cinnamon. Type of cinnamon used are cassia cinnamon, ceylon cinnamon and without the addition of cinnamon as the control factor. This research was repeated 3 times with a total of 27 experimental units. The data obtained will be subjected to statistical analysis of Univariate Two-Way ANOVA and Duncan Multiple Range Test (DMRT) further test. The correlation between antioxidant activity parameters (DPPH Radical-Scavenging Activity, total phenolic, and total flavonoid) and alcohol content was analyzed using Pearson correlation.

III.RESULT AND DISCUSSION

Preliminary Research

TABLE 1. HONEY ANTIOXIDANT ACTIVITY

Parameters	Honey±SD
IC_{50}	32374.33±1533.74 ppm*)
Total phenolic	788.04±9.12 mg GAE/kg
Total flavonoid	19.32±0.31 mg QE/kg

*)Note: 1%=10000 ppm

Based on Table 1., the IC_{50} value of the honey analyzed was 32374.33±1533.74 ppm which is greater than 150 ppm, therefore the antioxidant activity is categorized as weak (Sukweenadhi et al., 2020). The composition of honey is strongly affected by plant sources, seasons, and environmental factors. Honey from different types or batches will have different chemical compositions (Dzugan et al., 2018). causes the antioxidant activity to vary. According to the reasearch by Sumarlin et al. (2014), the antioxidant activity of several honeys in the local Indonesian market had IC_{50} value of 5453.75-58507.5 ppm.

The total phenolic and total flavonoid analyzed from the honey sample were 788.04±9.12 mg GAE/kg and 19.32±0.31 mg QE/kg, respectively. Honey contained phenolic compounds such as flavonoids, phenolic acids, and phenolic acid derivatives. These phenolic compounds provided antioxidant activity in honey. According to Saputri and Yolli's research (2017) regarding the antioxidant activity of forest honey from several sub-districts in Sumbawa Besar district, the samples analyzed gave a total phenolic yield of 63.33-387.50 mg/kg. Research conducted by Lianda et al. (2012) on 9 types of honey with different plant sources, yielded total phenolic and total flavonoid of 340-782 mg GAE/kg and 1.7-42.7 mg QE/kg respectively. High total phenolic and total flavonoid in multiflora honey can be caused by the combination of nectar sources from different plant species.

Main Research

The main research is conducted by making honey wine with lemon and cinnamon which then was analyzed for antioxidant activity and physicochemical properties. The best formulation was chosen based on the highest antioxidant activity of the final product. Based on Figure 1., the IC50 values analyzed from all honey wine formulations were greater than 150 ppm, meaning all honey wine samples had weak antioxidant activity (Sukweenadhi et al., 2020). Weak antioxidant activity of the honey wine samples can be caused by the weak antioxidant activity of the raw materials used in the making of honey wine and the addition of water twice the weight of honey which causes the concentration of antioxidant compounds to decrease. According to Socha et al. (2015), the antioxidant activity of honey wine with a water:honey ratio of 1:1 is higher than that with a water:honey ratio of 1:2.

The addition of lemon juice in the making of honey wine increased the antioxidant activity of honey wine.

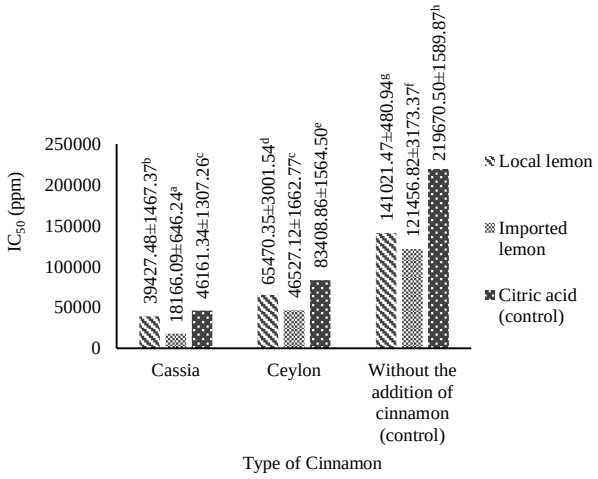

Figure 1. Interaction effect of lemon and cinnamon types towards IC_{50} value of honey wine

The addition of imported lemon juice gives higher antioxidant activity results in honey wine than the ones with the addition of local lemon juice. This could be due to the concentration of imported lemon added to achieve the pH of 3.7 on the must is higher than the concentration of local lemon juice. A total of 2.4% of imported lemon juice was added while only 2% of local lemon juice was added to achieve the pH value of 3.7. The differences in the chemical composition of types of lemons also caused differences in the antioxidant activity of of lemon. According to Krisnawan et al. (2017), imported lemon juice showed antioxidant activity IC_{50} of 5388.58 ppm and local lemon juice resulted an IC_{50} value of

1905.96 ppm. The addition of cinnamon in the making of honey wine increased the antioxidant activity of honey wine. Cinnamon contains compounds that could act as antioxidants such as phenolics, terpenoids, and saponins, therefore when it is added to honey wine it could increase the antioxidant activity of honey wine (Latief et al., 2013). Honey wine with the addition of cassia cinnamon resulted in higher antioxidant activity than honey wine with the addition of ceylon cinnamon. Cassia cinnamon aqueous extract showed an IC50 value of 3.45-20.53 ppm (Ervina et al., 2019) which is lower than ceylon cinnamon aqueous extract with the IC50 of 21.25 ppm (Gulcin et al., 2019).

Effect of Lemon and Cinnamon type on Total Phenolic of Honey Wine

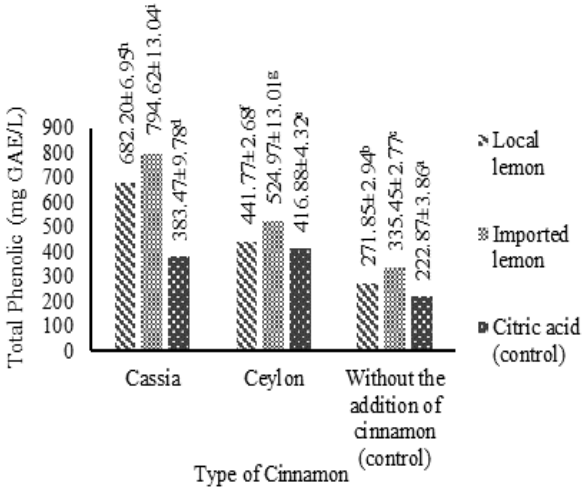

Figure 2. Interaction effect of lemon and cinnamon types towards total phenolic compound of honey wine

Cinnamon contains phenolic compounds and when added to honey wine it could increase the total phenolic characteristics of honey wine. The result obtained is similar with the results of the research by Sivapriya and Sheila (2020) and Ervina et al. (2019), where the results of a qualitative test of phytochemical compounds in ceylon and cassia cinnamon extracts showed the presence of flavonoids and tannins which are phenolic compounds.

Honey wine with the addition of cassia cinnamon gave a higher total phenolic yield compared to honey wine with the addition of ceylon cinnamon. Cassia cinnamon aqueous extract showed total phenolic of 259.08 mg GAE/g (Ervina et al., 2019) which is higher than the total phenolic of ceylon cinnamon aqueous extract of 153.5 mg GAE/g (Gulcin et al., 2019).

Effect of Lemon and Cinnamon Type on Total Flavonoid of Honey Wine

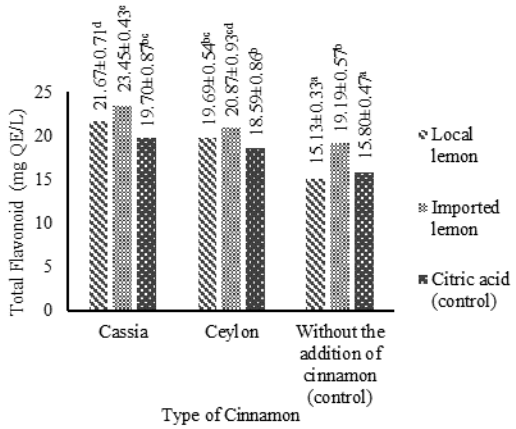

Figure 3. Interaction effect of lemon and cinnamon types towards total flavonoid of honey wine

Cinnamon contains flavonoids, therefore when added to honey wine it increased the total flavonoid of honey wine. based on the qualitative test results of phytochemical compounds in ceylon and cassia cinnamon extracts, it was found that they contained alkaloids, flavonoids, terpenoids, coumarins, tannins, glycosides, and saponins.

Honey wine with the addition of cassia cinnamon gave a higher total flavonoid yield than honey wine with the addition of ceylon cinnamon. Cassia cinnamon ethanol extract showed a total flavonoid of 20.30 mg QE/g (Yang et al., 2012) which is higher than the total flavonoid of Ceylon cinnamon ethanol extract with 11.25 mg QE/g (Gulcin et al., 2019).

Effect of Lemon and Cinnamon Type on Alcohol Content of Honey Wine

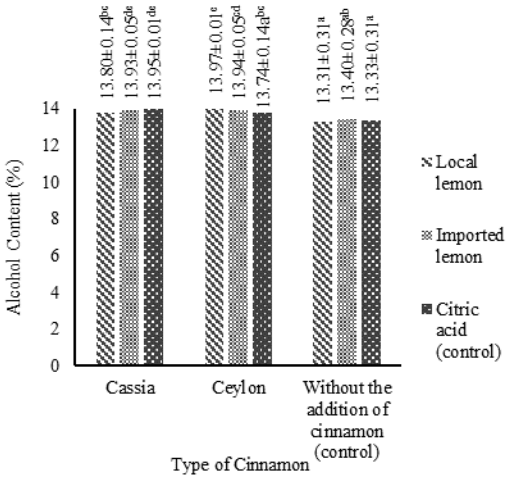

Figure 4. Interaction effect of lemon and cinnamon types on alcohol content of honey wine

The alcohol content of honey wine were calculated based on the density of the honey wine distillate which was measured by pycnometer. The standard alcohol content of

honey wine based on the Brazilian Decree 410/74 (2008) is 4-14%. Based on the Official Journal of the European Union (2019), the standard for alcohol content is at least 22%, and based on the Alcohol and Tobacco Tax and Trade Bureau (2020) the alcohol content needs to be at least 7%.

Based on Figure 4., the highest alcohol content was obtained from honey wine treated with the addition of local lemon and ceylon cinnamon with a value of 13.97±0.01%. The lowest alcohol content was obtained from honey wine with the addition of imported lemon and without the addition of cinnamon (control) with a value of 13.31±0.31%. Lemon and cinnamon contain minerals, vitamins, and nitrogen that could increase the activity of fermentation done by yeast and could affect the alcohol content of the honey wine final product.

According to Pereira et al. (2015), the addition of minerals such as magnesium, calcium, and zinc affects the rate of sugar converted by yeast and are required by yeast as a cofactor in several metabolic pathways.

Effect of Lemon and Cinnnamon Type on Honey Wine Acidity (pH Value)

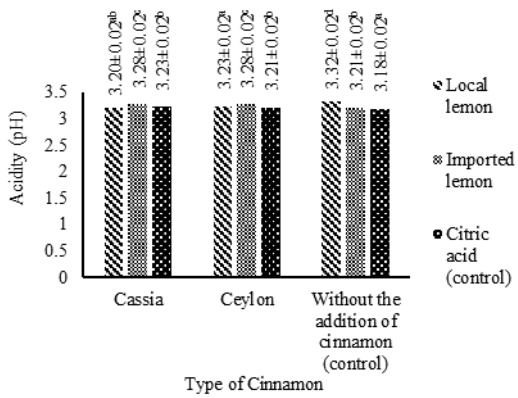

Figure 5. Interaction effect of lemon and cinnamon types on honey wine acidity (pH)

Based on Figure 5., the highest pH value was obtained from the honey wine made with the addition of local lemon and without the addition of cinnamon (control) with pH value of 3.32±0.02. The lowest pH value was obtained from honey wine made with the addition of citric acid (control) and without the addition of cinnamon (control) with a pH value of 3.18±0.02.

Effect of Lemon and Cinnnamon Type on Honey Wine Color

TABLE 2.EFFECT OF LEMON AND CINNAMON TYPE ON °HUE

Lemon	Cinnamon	°Hue±SD	Color
Local	Cassia	87.61±0.10	Yellow-red
Imported		86.26±0.11	
Control		87.75±0.10	
Local	Ceylon	88.25±0.04	
Imported		87.02±0.14	
Control		88.16±0.07	
Local	Control	87.85±0.10	
Imported		87.09±0.12	
Control		86.77±0.09	

°Hue is a measurement of color angle that can be converted into a color description that represents the color of the sample. Based on Table 2., the °Hue of all honey wine formulations analyzed are in the range of 86.26-88.25°. A °Hue value between 54-90° meaning the product analyzed have a yellow-red color (Ariadi et al., 2015).

Correlation of Antioxidant Activity (DPPH Radical-Scavenging Activity, Total Phenolic, and Total Flavonoid) and Alcohol Content of Honey Wine

TABLE 3. PEARSON CORRELATION

	IC_{50}	TF1	TF2	KA
IC_{50}	1	-0.822**	-0.855**	-0.766**
TF1	-0.822**	1	0.898**	0.595**
TF2	-0.855**	0.898**	1	0.683**
KA	-0.766**	0.595**	0.683**	1

Note:
** significant correlation at 0.01 significance level
TF1 (Total Phenolic), TF2 (Total Flavonoid), KA (Alcohol Content)

Based on Table 3., IC_{50}, total phenolic, total flavonoid, and alcohol content each have a Pearson correlation value of more than 0.5, indicating a strong correlation between the four variables. Alcohol content has a negative correlation with the IC_{50} value and a positive correlation with total phenolic and total flavonoid.

Determination of Best Honey Wine Formulation

The addition of imported lemon juice and cassia cinnamon in the production of honey wine (best formulation) produced a final product with the highest antioxidant activity due to the phenolic compounds and flavonoid compounds contained in imported lemon juice and cassia cinnamon that could act as antioxidant. The IC50 value of the best honey wine formulation is higher than 150 ppm, meaning the best honey wine formulation produced had weak antioxidant activity (Sukweenadhi et al., 2020).

CONCLUSIONS

The addition of local lemon juice and also imported lemon juice increased antioxidant activity, total phenolic, total flavonoid, and pH value, as well as lowering the titratable acidity and °Hue of the honey wine made. The addition of cassia and Ceylon cinnamon increased antioxidant activity,

total phenolic, total flavonoid, alcohol content, and decreased the density and total soluble solids of honey wine. The best honey wine formulation was obtained from honey wine made with the addition of imported lemon juice and cassia cinnamon.

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