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Effect of Processing Temperature and Liquid Palm Sugar Concentration on the Physicochemical Characteristics of Adulterated Multifloral Honey

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ABSTRACT

The high demand for honey in Indonesia has triggered counterfeiting practices combined with thermal processes, decreasing its quality. While studies on honey adulteration and heating exist, the specific combination using liquid palm sugar remains unexamined. This study aims to analyze the effect of adding liquid palm sugar (0-30% w/w) and heating temperatures (control, 50-90°C) on hydroxymethylfurfural (HMF) levels, water content, and color in multiflora honey. The research used a Randomized Block Design (RBD) with two replications. Data were analyzed statistically using ANOVA, followed by DMRT tests at the $\alpha = 5\%$ level if significant effects occurred. The results showed that increasing liquid palm sugar concentration and heating temperature significantly ($p < 0.05$) reduced honey quality. The HMF content rose from 8.77±0.26 mg/kg in pure honey to 54.25±0.15 mg/kg, exceeding the maximum SNI 8664:2018 limit of 40 mg/kg. Higher heating temperatures significantly decreased water content ($p<0.05$), whereas liquid palm sugar addition increased the initial water content. Furthermore, honey lightness (L*) decreased with higher temperatures and sugar concentrations; the lowest lightness (L* = 44.75±0.54) and a shifted hue value (from 57.15±1.33° to 54.10±2.32°) occurred at 30% concentration and 90°C, producing darker color. In conclusion, adulteration with liquid palm sugar and thermal treatment significantly affect the quality parameters of multiflora honey.

INTRODUCTION

Honey is a natural food product produced by honeybees from flower nectar or secretions of other parts of plants [1]. Honey contains various bioactive compounds, including phenolics, flavonoids, vitamins, organic acids, and volatile compounds. Therefore, honey is considered a functional food because it provides functional properties, including antioxidant, antibacterial, and anti-inflammatory activities, and can also improve the immune system [2]. In Indonesia, high public awareness of the health benefits of honey has led to consistenly growing demand for honey every year. Based on data from the State Forestry Company (Perum Perhutani) [3], the annual demand for honey consumption by the Indonesian population ranges from 15,000 to 150,000 tons. However, this demand cannot be met by honey production in Indonesia, which only reaches around 9,430.62 tons [4]. This imbalance is exacerbated by the decline in bee populations due to environmental damage to honey bee habitats [5].

The imbalance between honey production and demand in Indonesia increases the risk of honey adulteration. This reduces product quality and harms authentic honey producers. According to Fakhlei [6], adulteration is often done by adding coconut sugar, corn syrup, rice syrup, cane sugar, or invert sugar, either directly or via bee feeding. Among the various types of adulterants, liquid palm sugar has the potential to be an adulterant due to its physical properties including a yellowish-brown to dark brown color, a thick consistensy, and a relatively high viscosity [7]. Honey quality may also decline during post-harvest handling. In commercial honey processing, heating reduces viscosity, aids bottling, lowers moisture to prevent fermentation, slows granulation, and standardizes color for consumers. However, heating may negatively affect honey quality, especially by increasing hydroxymethylfurfural (HMF) levels [8]. HMF forms when simple sugars in honey (glucose and fructose) undergo transformation under acidic conditions and high temperatures. HMF levels above 40 mg/kg indicate honey damage from excessive heating or the addition of invert sugar (a glucose-fructose mixture from sucrose hydrolysis) [9].

Research by Zarei [8], showed that prolonged heating time significantly increased the HMF content beyond the permitted standard (<40 mg/kg). In addition, prolonged heating also reduced the total phenolic content, decreased antioxidant activity, and changed the color of honey. Similarly, Damto [10] reported that the addition of sugar syrup-based adulterants significantly increased the HMF content. Based on previous research, the influence of heating and honey adulteration factors in reducing honey quality has been known, but has not shown how the effect when both factors are combined. In addition, there has been no specific research on the use of liquid palm sugar as an adulterant. Therefore, this study aims to determine the effect of liquid palm sugar adulteration and heating temperature on the physicochemical characteristics of multiflora honey.

MATERIALS AND METHODS

Materials

The materials used in this study included multifloral honey obtained from *Apis mellifera*, liquid palm sugar, distilled water, potassium ferricyanide, zinc acetate, sodium bisulfite, and filter paper. The equipment used in this research consisted of a hot plate, thermometer, overhead stirrer, analytical balance, UV–Vis spectrophotometer, vortex mixer, laboratory glassware, Abbe refractometer, and color reader. All chemicals used in this study were of Pro Analysis (PA) grade. The honey samples were obtained from the Prawita Garden honey farm located in Banyumas, Central Java, Indonesia.

Experimental Design

This study used a Randomized Block Design (RBD) consisting of two factors. The first factor was the addition of liquid palm sugar (P), which consisted of four levels: P0 (0% w/w or control), P1 (10% w/w), P2 (20% w/w), and P3 (30% w/w). The second factor was heating temperature (T), which consisted of six levels: T0 (normal temperature or control), T1 (50°C), T2 (60°C), T3 (70°C), T4 (80°C), and T5 (90°C). The study was replicated twice, resulting in 48 experimental units.

Sampel preparation

A 25 g honey sample was weighed for each experimental unit. The sample was then mixed with liquid palm sugar according to the treatment concentration (0%-30% w/w) until homogeneous using an overhead stirrer. All samples were then heated at 50, 60, 70, 80, and 90°C for 20 minutes using an electric stove. After heating, the samples were then tested for HMF content, water content, and color intensity.

Hydroxymethylfurfural (HMF)

HMF analysis was conducted using a UV-Vis spectrophotometer. The testing procedure refers to the SNI 8664:2018 method by measuring the absorbance difference between the honey solution and the reference solution of the same honey at wavelengths of 284 nm and 336 nm [11]. The HMF content of honey can be calculated using the following equation:

$$\text{HMF (mg/kg)} = \frac{A_{284} - A_{336} \times 14{,}97 \times 5}{W\ (g) \times 10} \qquad (1)$$

Keterangan:

A_{284}, A_{336}	: Sampel absorbance;	5	: Sampel weight in gram;
14,97	: Constant value;	W	: Sampel weight (gram)

Moisture content

The moisture content analysis was conducted using an Abbe refractometer for refractive index. The testing procedure refers to the SNI 8664:2018 method. The refractometer will determine the amount of dissolved substance in the solution by passing light through it. The obtained scale is then converted into a standard table of moisture content values referring to SNI 8664:2018 [11].

Color intensity

Color intensity testing is conducted based on Turkut [9]& Fadhlurrohman [12] using a color reader with the principle of measuring the light absorbed or retained on the colored sample. The results are in the form of L* (value from dark to bright), a* (value from red to green), and b* (value from yellow to blue). Then the color is measured using the following psychometric parameter formula:

$$\textit{Hue} = h^* = \tan^{-1}\left(\frac{a*}{b*}\right) \qquad (2)$$

Data analysis

All test data are expressed as the mean ± standard deviation (2 replicates). Then, the data obtained were analyzed using a two-way analysis of variance (ANOVA) at a 95% confidence level (α = 0.05) using Microsoft Excel software. If there was a significant difference (α = 0.05), the analysis was continued with the Duncan test or Duncan's Multiple Range Test (DMRT) at a 95% confidence level (α = 0.05) using SPSS software version 22.

RESULTS

Based on the ANOVA analysis in **Table 1**, it shows a significant effect ($P<0.05$) on the honey samples after adding liquid palm sugar and heating on increasing HMF levels. All samples were then further tested using DMRT (α = 5%), the results showed that there was a significant difference between treatments. The average value of HMF levels in honey is presented in **Table 1**.

Table 1. The impact of HMF levels on the adulterated honey

Parameter	Heating temperature (˚C)	Concentration of liquid palm sugar (%)			
		0	10	20	30
HMF* (mg/kg)	Control	$8,77^{a}\pm0,26$	$10,16^{b}\pm0,07$	$18,90^{h}\pm0,50$	$35,50^{k}\pm0,67$
	50	$9,82^{b}\pm0,45$	$13,33^{d}\pm0,30$	$28,45^{j}\pm0,31$	$37,65^{l}\pm0,01$
	60	$11,18^{c}\pm0,16$	$15,30^{e}\pm0,16$	$39,25^{m}\pm0,07$	$40,44^{n}\pm0,17$
	70	$12,72^{d}\pm0,63$	$16,64^{f}\pm0,39$	$41,19^{o}\pm0,13$	$44,74^{q}\pm0,03$
	80	$15,37^{e}\pm0,42$	$17,67^{g}\pm0,12$	$42,42^{p}\pm0,05$	$49,00^{r}\pm0,64$
	90	$19,49^{h}\pm0,42$	$21,32^{i}\pm0,05$	$50,87^{s}\pm0,01$	$54,25^{t}\pm0,15$

Note: *shows the ANOVA results are significantly different. The subset differences in the table show that the average between treatments is significantly different (5%) after the DMRT test.

The increase in HMF levels is also seen in the graph presented in **Figure 1**. The figure shows that the largest increase in HMF levels was seen with the addition of 30% liquid palm sugar, from 8.77 mg/kg to 35.5 mg/kg. The HMF levels continued to increase after being combined with a heating temperature of 90°C.

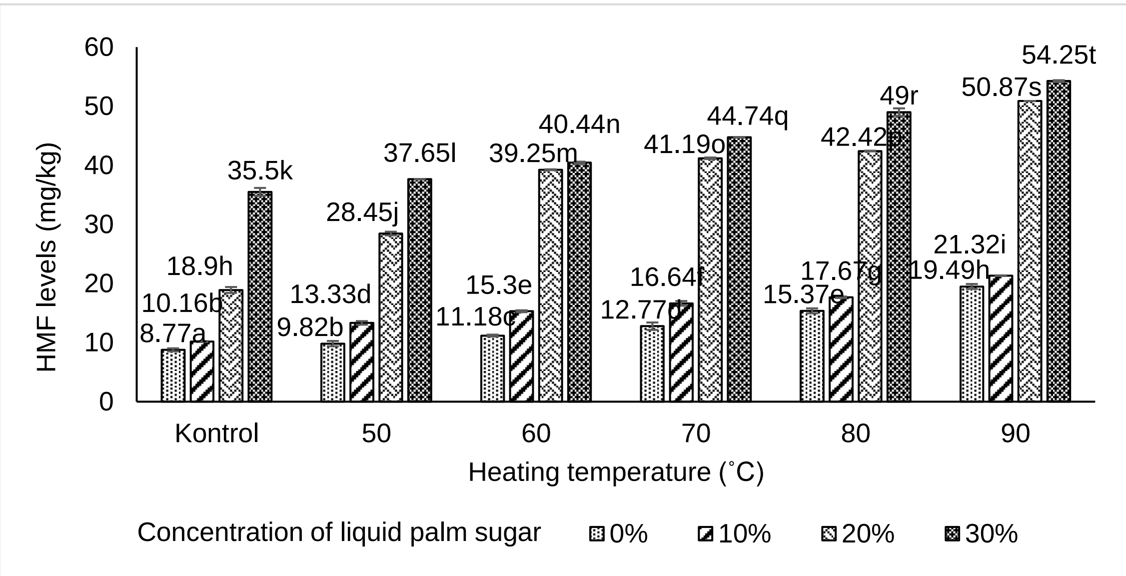

Figure 1. Diagram of increasing honey HMF levels.

The adulteration treatment of liquid palm sugar and heating on multiflora honey each had a significant effect ($P<0.05$) on changes in water content. However, there was no interaction between the two factors. After further DMRT testing (α = 5%), the results showed that there was a significant difference between the treatments. The average water content of the honey samples is presented in **Table 2**. The addition of liquid palm sugar to honey caused the honey to experience an increase in water content. The largest increase occurred in the honey sample with the addition of 30% liquid palm sugar from 20.93% to 22.07%. After being combined with the heating process, the water content of the honey decreased to 16% in the control honey and to 18.60% in the honey added with 30% liquid palm sugar.

Table 2. The impact of moisture content levels on the adulterated honey

Parameter	Heating temperature (˚C)	Concentration of liquid palm sugar (%)			
		0	10	20	30
Moisture content (%)	Control	20,93^{jklm}±0,28	21,50^{lmn}±0,05	21,67^{mn}±0,09	22,07^{n}±0,09
	50	20,10^{fghi}±0,24	20,60^{hijk}±0,75	21,27^{klmn}±0,09	21,50^{lmn}±0,24
	60	19,87^{efgh}±0,19	20,03^{fghi}±0,14	20,47^{ghijk}±0,38	20,83^{ijkl}±0,24
	70	19,13^{de}±0,00	19,40^{ef}±0,09	19,77^{efg}±0,42	20,13^{fghij}±0,00
	80	17,70^{b}±0,42	18,13^{bc}±0,47	18,47^{bcd}±0,19	19,37^{ef}±0,33
	90	16,00^{a}±0,75	16,57^{a}±0,52	17,83^{bc}±0,24	18,60^{cd}±0,57

Note: * shows the ANOVA results are significantly different. The subset differences in the table show that the average between treatments is significantly different (5%) after the DMRT test

The addition of liquid palm sugar adulterant and heating treatment each had a significant effect (P<0.05) on the decrease in honey lightness. However, there was no interaction between the two factors. After further DMRT testing (α = 5%), the results showed that there was a significant difference between the treatments. In this study, honey color intensity was assessed based on the lightness value (L*) of the honey after testing using a color reader and is presented in **Table 3**.

Table 3. The impact of Lightness (L*) levels levels on the adulterated honey

Parameter	Heating temperature (˚C)	Concentration of liquid palm sugar (%)			
		0	10	20	30
Lightness (L*)	Control	77,37^{w}±0,28	66,83^{r}±0,42	56,48^{l}±0,16	50,22^{f}±0,12
	50	76,37^{v}±0,05	65,52^{q}±0,40	55,27^{k}±0,14	49,30^{e}±0,24
	60	75,22^{u}±0,02	64,47^{p}±0,38	54,42^{j}±0,02	48,35^{d}±0,12
	70	74,62^{u}±0,12	63,65^{o}±0,40	53,50^{i}±0,14	47,42^{c}±0,07
	80	73,43^{t}±0,00	62,58^{n}±0,31	52,32^{h}±0,21	46,37^{b}±0,09
	90	71,83^{s}±0,52	60,20^{m}±0,85	51,32^{g}±0,12	44,75^{a}±0,54

Note: * shows the ANOVA results are significantly different. The subset differences in the table show that the average between treatments is significantly different (5%) after the DMRT test.

Besides lightness, honey color intensity is also assessed based on the resulting hue value. The hue value indicates the honey's color tendency, indicating wavelengths for the red, yellow, green, blue, and violet color ranges. After ANOVA analysis, in general, the addition of liquid palm sugar and heating each showed a significant effect (P<0.05) in reducing the hue value in honey samples. However, there was no interaction between the two factors. All samples were then further tested using DMRT (α = 5%), the results showed that there was a significant difference between the treatments.

The average HMF content of honey is presented in **Table 4.**

Table 4. The impact of hue (h*) levels levels on the adulterated honey

Parameter	Heating temperature (˚C)	Concentration of liquid palm sugar (%)			
		0	10	20	30
Hue (h*)	Control	90,93^{i}±0,49	75,64^{h}±1,93	63,83^{ef}±3,48	57,15^{abc}±1,33
	50	90,97^{i}±0,08	72,39^{gh}±2,08	64,44^{f}±2,93	58,97^{bcd}±0,58
	60	90,80^{i}±0,22	73,64^{gh}±1,35	63,84^{ef}±1,26	55,71^{ab}±0,96
	70	90,74^{i}±0,17	72,28^{gh}±1,52	63,91^{ef}±1,70	54,83^{a}±0,70
	80	90,81^{i}±0,02	70,80^{g}±3,96	62,29^{def}±0,50	56,03^{ab}±2,62
	90	90,29^{i}±0,07	71,09^{g}±1,31	60,31^{cde}±1,54	54,10^{a}±2,32

Note: * shows the ANOVA results are significantly different. The subset differences in the table show that the average between treatments is significantly different (5%) after the DMRT test.

DISCUSSION

Effect of Adulterant Addition and Heating on the HMF Content of Honey

Hydroxymethylfurfural (HMF) is an important parameter used as an indicator of honey freshness and quality deterioration. Pure honey naturally contains small amounts of HMF formed through the decomposition of monosaccharides under acidic conditions. During this process, monosaccharides undergo dehydration reactions involving the removal of three water molecules, resulting in the formation of HMF compounds. In addition, non-enzymatic browning reactions, such as the Maillard reaction, may also contribute to HMF formation, particularly through interactions between reducing sugars and amino acids [13]. The natural HMF content in freshly harvested honey is generally less than 1 mg/kg or approximately 0.06–0.2 mg per 100 g of honey [14]. However, the HMF content in honey can increase during post-harvest handling due to several factors, including temperature fluctuations, storage duration, storage conditions, and the pH level of the honey [8].

Based on the HMF content in Table 1, control honey had an HMF content of 8.77±0.26 mg/kg. The HMF content increased to 19.49±0.42 mg/kg after heating at 90°C. This is because heating honey degrades the glucose and fructose content in honey. The conversion of glucose to fructose due to heating temperature causes the HMF content to increase because fructose is more reactive in forming HMF compounds [15]. According to Rababah [16], HMF levels increase after heating because heat energy accelerates the cleavage of glycosidic bonds in sugar molecules which triggers the formation of HMF. In honey samples, after adding liquid palm sugar, the HMF levels increase. Honey added with 30% liquid palm sugar experienced an increase in HMF levels of 35.50 mg/kg. HMF levels continued to increase in honey samples that were adulterated with liquid palm sugar concentrations of 10-30%, respectively, to 21.32±0.05 mg/kg, 50.87±0.01 mg/kg, and 54.25±0.15 mg/kg after being combined with heating at 90˚C. This increase was caused by liquid palm sugar changing the ratio of glucose and fructose in honey, so that more glucose was degraded into fructose due to the heating temperature and increasing HMF levels [17].

Based on the results shown in **Figure 1**, sample containing 30% liquid palm sugar showed an HMF content of 35.50 ± 0.67 mg/kg. Samples containing 20% and 30% liquid palm sugar heated at temperatures of 70 °C, 80 °C, and 90°C showed HMF levels exceeding the maximum limit specified in SNI 8664:2018 [11]. The highest HMF content was observed in the sample containing 30% liquid palm sugar heated at 90 °C, reaching 54.25 ± 0.15 mg/kg. According to Shapla [13], the rate of HMF formation is influenced not only by temperature but also by sugar composition and the fructose-to-glucose ratio in honey. The addition of sugar adulterants, such as liquid palm sugar, can accelerate HMF formation due to the increased availability of reducing sugars that are susceptible to dehydration reactions. Furthermore, HMF has a negative correlation with decreased water content. This is because the formation of HMF occurs through a Maillard reaction involving ketone or aldehyde groups with free amino groups of amino acids. The Maillard reaction occurs at temperatures ranging from 68 to 100°C with a water content of around 15%. Therefore, the higher the HMF formation, the lower the water content in honey [18]. Therefore, higher adulterant concentrations combined with higher heating temperatures accelerate the deterioration of honey quality, as indicated by the significant increase in HMF levels. Based on these results, honey with an HMF content of >40 mg/kg is categorized as low-quality honey due to indications of heating and adulteration. According to Pereria [19], the water content in honey ranges from 15 to 20%. Although the maximum standard of SNI 8664:2018 states a maximum water content of 22%, a water content of >20% can Therefore, honey samples with an HMF content of >40 mg/kg and a water content of >20% indicate a decrease in honey quality.

Effect of Adulterant Addition and Heating on the Moisture Content of Honey

The moisture content of honey is an important indicator of its quality, stability, and resistance to microbiological fermentation processes [8]. According to SNI 8664:2018 [11], the maximum permissible moisture content in honey is 22%. The reduction in moisture content in honey is strongly influenced by several factors, including environmental conditions, the initial moisture content of the honey, and its viscosity [20].

Based on the results presented in **Table 2**, the untreated honey (control) had a moisture content of 20.93 ± 0.28%. After the addition of liquid palm sugar at concentrations of 10%, 20%, and 30%, the moisture content increased to 21.50 ± 0.05%, 21.67 ± 0.09%, and 22.07 ± 0.09%, respectively. This increase indicates that the addition of the liquid phase from liquid palm sugar contributes to a higher moisture content in honey. In the sample adulterated with 30% liquid palm sugar, the moisture content reached 22.07 ± 0.09%, exceeding the maximum limit specified in SNI 8664:2018 [11]. The increase in water content is caused by honey being hygroscopic so it can absorb water from the environment. According to Soeswanto, the water content of liquid palm sugar ranges from 11.41% to 20%, depending on the heating time. This water content influences the increase in the water content of honey because honey is hygroscopic, meaning it readily absorbs water from the environment [21]. These findings are consistent with the study conducted by Tomczyk [22], which reported that untreated honey (control) experienced an increase in moisture content from 17.9% to 21% after adulteration with 30% sugar syrup. The increase in moisture content indicates that honey adulteration can make honey more diluted and more susceptible to fermentation by yeasts, which may affect its stability and reduce overall honey quality.

The greatest reduction in moisture content for all samples occurred after heating at 90 °C, where the values decreased from 20.93 ± 0.28%, 21.50 ± 0.05%, 21.67 ± 0.09%, and 22.07 ± 0.09% to 16.00 ± 0.75%, 16.57 ± 0.52%, 17.83 ± 0.24%, and 18.60 ± 0.57%, respectively. These results are consistent with the findings of Rababah [16], who reported that moisture content decreases with increasing heating temperature. This reduction in moisture content occurs because the heating process increases the kinetic energy of water molecules, thereby promoting evaporation, which involves the phase change of water from liquid to vapor [18]. The increased water content indicates that honey adulteration can make it more liquid and susceptible to fermentation by yeast, which affects stability and reduces honey quality. The water content of adulterated honey decreases after being combined with a heating treatment. The heating process causes a decrease in water content. Although the water content decreases, it needs to be correlated with the HMF content.

Effect of Adulterant Addition and Heating on the Color Intensity of Honey

The L* (lightness) parameter represents the lightness level of a sample; the lower the L* value, the darker the color of the sample. The color characteristics of honey are influenced by several factors, including the floral source, the content of phenolic compounds and minerals, and the presence of Maillard reaction products such as hydroxymethylfurfural (HMF) [9]. The effect of liquid palm sugar addition and heating temperature on the lightness of honey is presented in **Table 3.** The untreated honey (control) had an L* value of 77.37 ± 0.28. However, after the addition of liquid palm sugar at concentrations of 10%, 20%, and 30%, the L* values decreased to 66.83 ± 0.42, 56.48 ± 0.16, and 50.22 ± 0.12, respectively. The reduction in lightness can be attributed to the darker color of liquid palm sugar compared with the original color of pure honey. Control honey has an L* value of 25.63, while control honey has an L* value of 77.37±0.28. Therefore, the decrease in the lightness of the honey color is caused by the color of the liquid palm sugar adulterant being darker than the color of the control honey. According to Kristanti [23], substitution with glucose may increase honey lightness because glucose has a lighter initial color than honey. These findings indicate that changes in the L* value of honey depend on the initial color of the honey and the color characteristics of the adulterant used.

In addition to adulteration, increasing heating temperature also led to a decrease in L* values in all samples. The lowest L* value was observed in the sample containing 30% liquid palm sugar heated at 90 °C, where the value decreased from 50.22 ± 0.12 to 44.75 ± 0.54. The decrease in the lightness of the honey color is caused by the evaporation process that occurs during heating, which causes an increase in the concentration of dissolved dyes. According to Turkut [9], honey lightness and hydroxymethylfurfural (HMF) content after heating show a negative correlation. Higher HMF levels resulting from heat treatment correspond to lower L* values. This negative correlation is associated with non-enzymatic browning reactions, particularly the Maillard reaction and caramelization. High temperatures accelerate the condensation reaction between the carbonyl groups of reducing sugars and the amino groups of proteins in honey, leading to the formation of brown macromolecular pigments known as melanoidins. The greater the formation of melanoidins, the lower the resulting L* value [24]. In addition, color darkening may also result from an increased concentration of dissolved coloring compounds, such as minerals, which occurs as a consequence of moisture evaporation during heating that reduces the water content of honey [8].

The intensity of honey color can also be expressed through the hue value. Hue represents the dominant wavelength corresponding to color ranges such as red, yellow, green, blue, and violet [12]. The effect of liquid palm sugar addition and heating temperature on the hue value of honey is presented in **Table 4.** In the control sample (0% concentration), the hue value was 90.93 ± 0.49°. After the addition of liquid palm sugar at concentrations of 10%, 20%, and 30%, the hue values decreased to 75.64 ± 1.93°, 63.83 ± 3.48°, and 57.15 ± 1.33°, respectively. This decrease indicates that the color of the honey, which initially fell within the yellow–green range, gradually shifted toward the red region. The change in hue value is likely due to the presence of additional colored compounds introduced by liquid palm sugar that contribute to the overall color of the honey. Furthermore, the color of honey can also be influenced by the content of hydroxymethylfurfural (HMF), which may oxidize in the presence of oxygen and lead to a darker color in honey [25].

Heating treatment generally resulted in a further decrease in hue value. The greatest reduction occurred at the 30% liquid palm sugar concentration, where the hue value decreased from 57.15 ± 1.33° to 54.10 ± 2.32° after heating at 90°C. This reduction indicates that increasing heating temperatures tend to shift the honey color toward a darker brown tone. The decline in hue value may be attributed to the degradation of natural pigments in honey and the formation of brown melanoidin compounds through Maillard reactions or caramelization processes [9].

CONCLUSION

Based on the research results, it can be concluded that the addition of liquid palm sugar as an adulterant and variations in heating temperature significantly affect the physicochemical characteristics of multiflora honey. Increasing the concentration of liquid palm sugar and heating temperature causes an increase in HMF content, while the water content, lightness (L*), and color value decrease. The most significant changes were observed in the treatment with a liquid palm sugar concentration of 30% and a heating temperature of 90°C. Under these conditions, the HMF content exceeded the national quality standard, reaching 54.25 ± 0.15 mg/kg. In addition, the water content, lightness, and hue value decreased from 22.07 ± 0.09%, 50.22 ± 0.12, and 57.15 ± 1.33° to 18.60 ± 0.57%, 44.75 ± 0.54, and 54.10 ± 2.32°, respectively. These results indicate that adulteration with liquid palm sugar and thermal treatment contribute to significant changes in the quality parameters of multiflora honey. Based on the results of the study, heating is recommended for honey producers at temperatures below 60°C because HMF levels do not increase significantly and do not reduce water content to the point of changing viscosity. Further research on reducing sugar levels, sucrose levels, and antioxidant activity is needed to determine changes in the chemical profile due to adulteration and heating. Furthermore, non-destructive FTIR testing is needed to identify the chemical groups of honey after adulteration and heating.

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