THE TOTAL PHENOLIC, TOTAL FLAVONOID, AND BROWN PIGMENT IN HONEY BEFORE AND AFTER HEATING

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Abstract: Honey has unique components, a characteristic that makes it a valuable food for consumers. It is known that UV radiation and heating impact the quality of honey's bioactive compounds, including total phenolic, total flavonoid, and brown pigment contents. The absorbance of brown pigment at a wavelength of 420 nm, total phenolic at a wavelength of 733 nm, and total flavonoid at a wavelength of 430 nm was measured using a UV-Vis spectrophotometer. The method used in the total phenolic test was the Folin-Ciocalteu method with gallic acid standard (mgGAE/g sample). In contrast, for flavonoids, the method used was the colorimetric method with quercetin standard (mg QE/g sample). The qualitative test results showed that all honey samples contained phenolic and flavonoid compounds. Total phenolic and flavonoid levels showed a decrease in several samples, including TR SLT (Trigona Southeast Sulawesi), TRG BGR (Trigona Bogor), and TR SLS (Trigona *Genotrigona Indica* South Sulawesi) in the range of 11.8–57.6%. However, most of the total phenolic and flavonoid levels increased after the heating process, *i.e*., in the samples of AP LMB (apis North Lombok) (25.3% and 88.8%), AP MG (apis mango) (73.1% and 114%), AP MAC (Aceh honey, Buloh Seuma) (8.8% and 199%), TR BIR (Trigona *tetroginola biroi* South Sulawesi) (58.8% and 146%), and TR LMB (Trigona North Lombok) (44.3% and 84.9%). In contrast, for the formation of brown pigment, there was an increase in all honey samples between 32 and 1.428%. The presence of brown pigment at the end of the heating process of honey samples is thought to have the same role as phenolic compounds and flavonoids, which change the heating process, especially in antioxidant activities and other bioactivities. However, further analysis is needed to prove the conjecture.

Keywords: brown pigment; honey; total phenolic; total flavonoid; UV-Vis spectrophotometer

Abstrak: Madu memiliki komponen yang unik, yang membuatnya menjadi makanan yang berharga bagi konsumen. Telah diketahui bahwa radiasi UV dan pemanasan berdampak pada kualitas senyawa bioaktif madu, di antaranya kandungan total fenolik, total flavonoid, dan pigmen coklat. Pengukuran absorbansi pigmen coklat pada panjang gelombang 420 nm, pengukuran total fenolik pada panjang gelombang 733 nm dan total flavonoid pada panjang gelombang 430 nm dilakukan menggunakan spektrofotometer UV-Vis. Metode yang digunakan untuk uji total fenolik adalah metode Folin-Ciocalteu dengan standar asam Galat (mgGAE/g sampel), uji flavonoid menggunakan metode Kolorimetri dengan standar kuersetin (mg QE/g sampel). Hasil

uji kualitatif menunjukan bahwa semua sampel madu terdapat senyawa fenolik dan flavonoid. Kadar total fenolik dan flavonoid menunjukkan penurunan pada beberapa sampel, di antaranya TR SLT (Trigona Sulawesi Tenggara, TRG BGR (Trigona Bogor), TR SLS (Trigona *Genotrigona Insica* Sulawesi Selatan) pada kisaran 11,8 – 57,6%. Namun, sebagian besar kadar total fenolik dan kadar flavonoid mengalami kenaikan setelah proses pemanasan, yaitu pada sampel AP LMB (apis Lombok Utara) (25,3% dan 88,8%), AP MG (apis mangga) (73,1% dan 114%), AP MAC (madu Aceh, Buloh Seuma) (8,8% dan 199%), TR BIR (Trigona *Tetroginola biroi* sulawesi selatan) (58,8% dan 146%), TR LMB (Trigona North Lombok) (44,3% dan 84,9), sedangkan untuk pembentukan pigmen coklat, terjadi peningkatan pada semua sampel madu di antara 32 – 1.428%. Keberadaan pigmen coklat pada akhir proses pemanasan, diduga memiliki peran yang sama dengan senyawa fenolik dan flavonoid yang mengalami perubahan selama proses pemanasan, terutama pada aktivitas antioksidan dan bioaktivitas lainnya. Namun diperlukan analisis lebih lanjut untuk membuktikan dugaan tersebut.

Kata Kunci: madu; pigmen coklat; spektrofotometer UV-Vis; total fenolik; total flavonoid.

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Introduction

Honey has a unique combination of components, a characteristic that makes it a valuable food for consumers. Honey is also a natural product that contains nutrients and beneficial compounds such as flavonoids, carotenoids, and phenolic acids produced by bees. The nutritional content contained in honey includes carbohydrates, protein, and several types of minerals (Putu et al., 2017). Local Indonesian honey, namely trigona honey, calliandra honey, rambutan honey, and longan honey, can be used as an additional supplement for people with laryngeal cancer (Sumarlin et al., 2019).

Honey, the main component is sugar, is a good source of various molecules, flavonoids, and phenolic acids, with high biological and antioxidant activity (Alqarni et al., 2014). Honey is also presented as a “reaction pot" with the main constituents of honey, such as sugars, amino acids/proteins, and polyphenols—a substrate and a reactant in the Maillard reaction. Several bioactive molecules, modified proteins, and protein-polyphenol complexes are formed during the process. As a result, melanoidin, a chocolate polymer, is formed, increasing and decreasing honey's antioxidant capacity and antibacterial function (Brudzynski & Mioto, 2011). In addition, cysteine, which reacts with glucose, produces pyrolothiazolate compounds, one of the brown pigments that are claimed to have antioxidant activity (Noda et al., 2016).

As a commercial product, producers and consumers have also considered the adverse effects of sunlight and temperature on the antioxidant quality of honey

(Yalcin, 2021). Yalcin's (2021) study also stated that ten days of sun exposure could reduce the total phenolic content and flavonoids, and the antioxidant capacity decreases after six days. In addition, the browning variables and Total Phenolic Content, TPC, ACW, and FRAP (Ferric Reducing Antioxidant Power) were positively correlated. It can be concluded that the browning index is very influential on the parameters of honey appearance, the content of bioactive compounds, and antioxidant activity (Starowicz et al., 2021).

UV radiation and heating harm the food quality (Huvaere & Skibsted, 2014), but information about the effect of heating on the total phenolic content, flavonoids, and brown honey pigments in local honey in Indonesia is still very scarce. Thus, this research is essential because of the health benefits and demand for high-quality honey. The preservation and enhancement of the properties of its bioactive compounds during processing and storage should be a significant concern.

Methods

Chemicals dan Instrumentations

The materials used in this research include aquadest, DPPH (2,2-diphenyl-1-pikrilhidrazil) (Sigma), filter paper, methanol, quercetin (Sigma), gallic acid (Merck), Na_2CO_3, Folin Ciocalteu (Merck), $AlCl_3$ (Merck), aluminum foil. Used in this study were ten samples of liquid honey (consisting of Apis honey and trigona honey) from various parts of Indonesia taken from honey farmers in the region and had not undergone the heating process (Table 1).

Table 1. Sample code and description

Code	Description	No	Code	Description
AP LMB	apis North Lombok	6.	AP MG	apis mangga
TR LMB	trigona North Lombok	7.	TR SLS	trigona Genotrigona Insica South Sulawesi
TRG BGR	trigona Bogor	8.	TR BIR	rigona tetroginola biroi South Sulawesi
AP LK	apis Klengkeng	9.	TR SLT	trigona South East Sulawesi
MG KLS	apis mangrove South Kalimantan	10.	AP MAC	Honey from Aceh, Buloh Seuma

The tools used in this study consisted of commonly used glassware. Other instruments are an oven (MEMMERT), a UV-Vis spectrophotometer (GENESYS S101), and a hand refractometer Brix ATC 0% – 80% (YIERYI).

Honey Preparation Before Heating

A total of 2 grams of the sample was placed in a test tube, then dissolved in 10 ml of distilled water and shaken using a vortex. After that, the solution was filtered with Whatman filter paper no. 1 and carried out the phytochemical tests.

Total phenolic and total flavonoid tests (Turkmen et al., 2006) and a brown pigment test were done.

Honey Preparation by Heating

The honey sample was placed in a 2 g vial and tightly closed. Then, put in the oven at a temperature of 50, 70, and 80 °C and left for 1 to 10 days. Afterward, total phenolic, flavonoid, and brown pigments were tested. Each temperature parameter was tested three times (Triplo).

Phenolic and Flavonoid Qualitative Test

Phenolic

About three drops of the sample were pipetted into the drip plate, and then two drops of 5% $FeCl_3$ were added. The sample is positive if a solid green, bluish black, or black color is formed (Idris, 2016).

Flavonoids

A total of three drops of the sample were put into a test tube, then 1 ml of methanol was added, then heated to boiling, shaken, then added magnesium powder, and concentrated HCl was added. The test is positive if a red color appears (Agustina et al., 2017).

Total Phenolic and Flavonoid Level Test

Total Phenolic

About 80 mg of each honey sample was weighed and dissolved with methanol in a 10 mL volumetric flask. The honey sample solution in the methanol was pipetted 0.1 mL, added with 7.9 mL of distilled water, 1.5 mL of 10% Folin-Ciocalteu reagent, and vortexed for 1 minute, then allowed to stand for 5 minutes. The 20% Na_2CO_3 solution was added by 1.5 mL, then incubated for 90 minutes at room temperature, and the absorbance was measured at the maximum wavelength (733 nm) using a UV-Vis spectrophotometer. This test is carried out on the sample before heating and at the optimum temperature and heating time. Total phenolic content (TFN) was calculated as (mg Gallic Acid Equivalent /GAE)/g sample)) with the formula (Krishnan, 2020).

$$\text{TFN} = \frac{\text{concentration}\left(\frac{\text{mg}}{\text{ml}}\right)\text{-sample volume(mL)}}{\text{g sample}} \text{ X FP}$$

Flavonoids

The honey sample was weighed at 80 mg. Then it dissolved with methanol in a 10 mL volumetric flask to the limit mark. 2 mL of the sample was pipetted into a test tube, then 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M CH3COONa, and 2.8 mL of distilled water were added, then incubated at room temperature for 40 minutes. The absorbance of the sample solution was measured at a maximum wavelength of 430 nm using a UV-Vis spectrophotometer. The flavonoid

concentration was calculated from the calibration plot and expressed in QE (Quercetin equivalent) as the equivalent number of milligrams of quercetin in 1 gram of sample. This test is carried out on the sample before heating and at the optimum temperature and heating time. The following formula calculated total flavonoid content (TFL) (mg QE/g sample) (Krishnan, 2020).

$$TFL = \frac{\text{concentration}\left(\frac{mg}{ml}\right)\text{-samplevolume (mL)}}{\text{g sampel}} \text{ X FP}$$

Brown Pigment Level Test

The brown pigment test measured the absorbance of the extract dissolved in aquadest of 1 Brix. One degree Brix is equivalent to 1 gram of sucrose in 100 grams solution. However, if the solution contains dissolved solids other than sucrose, the degree of Brix can only be estimated close to the content of other dissolved solids. At a wavelength of 420 nm using a UV-Vis spectrophotometer, every 24-hour interval on the sample before and after heating for ten days (Turkmen et al., 2006).

Results and Discussion

Qualitative Content of Flavonoids and Phenolics

Qualitative testing of flavonoids and phenolics aims to determine the presence of bioactive compounds in various honey types. Meanwhile, the analysis of total phenolic and flavonoid levels in all honey samples (apis and Trigona) showed a positive reaction. These common compounds are present in the tested samples (Table 2). The positive flavonoid test results were indicated by the formation of a brownish red color when the sample was reacted with Mg and dripped with HCl (Figures 1a and 1b). The more the red color formed, the higher the flavonoid content in the honey sample (Parbuntari et al., 2018). The most concentrated red color in the flavonoid test was found in trigona honey, namely TR SLS and TR LMB honey, while in apis honey, such as AP LK, AP MG, and MG KLS honey, a faint yellow color was produced (Figure 1a).

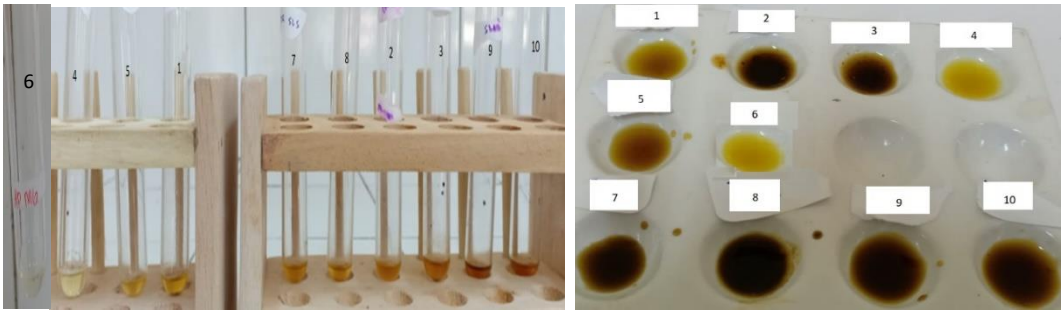
Table 2. Qualitative Data of Flavonoids and Phenolics

Sample	Flavonoid	Phenolic
APIS		
AP LK	+	+
AP LMB	+	+
AP MG	+	+
AP MAC	+	+
MG KLS	+	+

Sample	Flavonoid	Phenolic
TRIGONA		
TR SLT	+	+
TR BIR	+	+
TRG BGR	+	+
TR LMB	+	+
TR SLS	+	+

Note: + (contain phenolic/flavonoid compound)

AP LK (apis Klengkeng); AP LMB (apis North Lombok); AP MG (apis mangga); AP MAC (honey from Aceh, Buloh Seuma); MG KLS (apis mangrove South Kalimantan), TR SLT (trigona South East Sulawesi); TR BIR (trigona tetroginola biroi South Sulawesi); TRG BGR (trigona Bogor); TR LMB (trigona North Lombok); TR SLS (trigona Genotrigona Insica South Sulawesi).



a **b**

Figure 1. The results of the qualitative test of flavonoids (a) and phenolics (b)

Note : (1) AP LMB; (2) TR LMB; (3) TRG BGR; (4) AP LK; (5) MG KLS; (6) AP MG; (7) TR SLS; (8) TR BIR; (9) TR SLT; (10) AP MAC.

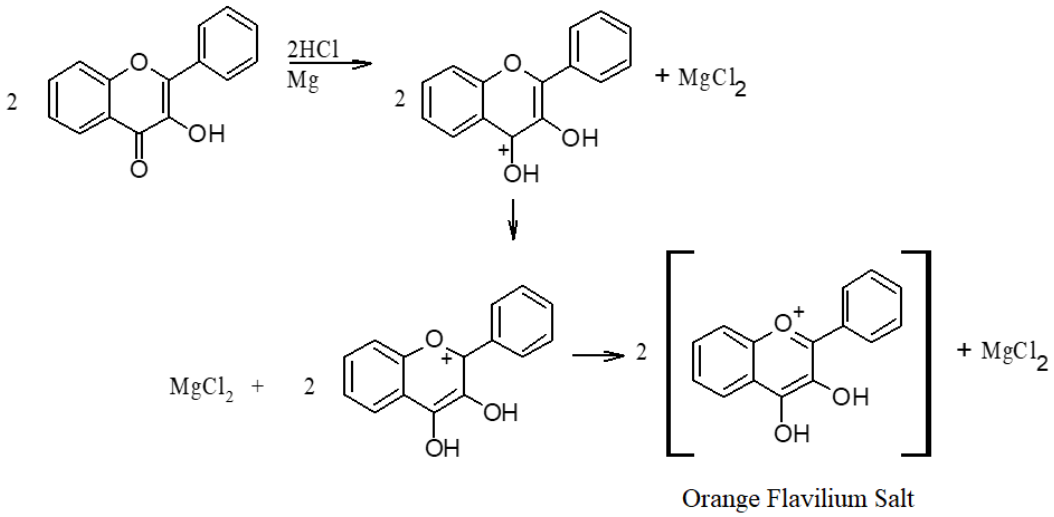


Figure 2. Flavonoid Qualitative Test Reaction (Tandi et al., 2020)

According to Ergina et al. (2014), the orange to dark red color is formed in the flavonoid test because Mg and HCl will reduce benzopyrone contained in the flavonoid structure (Figure 2). The benzopyrone reduction process occurs due to the donation of the H atom. Then electron delocalization occurs, forming a complex compound colored orange to brick red which is the color of the flavilium salt (Mukhriani et al., 2019).

Meanwhile, qualitative phenolic analysis was carried out by adding 5% FeCl3, which produced a greenish solid yellow, bluish green, or black color (Idris, 2016). The darkest black color was found in samples of trigona honey. Namely, TR SLS (Trigona Sulawesi) and the faintest color was found in samples of Apis AP LK honey (Apis Longan), AP MG (Apis mangrove), and MG KLS (Apis Kalimantan) (Figure 1b). The reaction in the phenolic qualitative test was caused by FeCl3 reacting with phenolic compounds to produce a greenish-yellow color (Figure 3) (Putri et al., 2018). Fe3+ ions form complex compounds with phenol, causing color changes (Mukhriani et al., 2019).

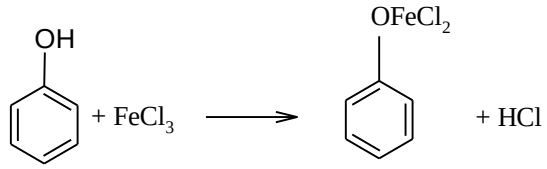


Figure 3. Phenolic qualitative test reaction (Almey et al., 2010)

Total Phenolic and Flavonoid Levels.

The colorimetric method is used at a temperature of 50^{o}C (optimum temperature). Gallic acid is a standard because it is an example of a simple phenolic compound. The reaction of gallic acid with Folin-Ciocalteu under alkaline conditions will produce a green color which indicates the presence of phenolic compounds. Adding Na_2CO_3 as a base will change the color to blue, which can be measured at a maximum wavelength of 733 nm because it produces the largest absorbance in the sample incubated for nine days at 90 minutes. The resulting blue color is a complex of molybdenum-blue compounds (Figure 4) (Bensalah et al., 2018).

Based on the measurement of the total phenolic of honey heated at the optimum temperature and time, each sample increases in all apis honey and two trigona honey types. While three samples of trigona honey, namely TR SLT, TRG BGR, and TR SLS, decreased. In addition, it also shows that the average total phenolic in trigona honey is higher than that of apis honey (Table 3)—the highest total phenolic after heating TR LMB with an increase of 44.3% compared to before heating. However, the sample with the largest percentage increase was AP MG at 73.1% (Table 3).

The increase in total phenolics can be caused by the release of phenolic compounds previously bound to other compounds in food so that the total phenolics can increase (Jeong et al., 2004). Heating can increase free phenolic compounds while phenol compounds in esters and glycosides are reduced (Bensalah et al., 2018).

After heating, the increased total phenolic content in foodstuffs occurs in roasted quinoa seeds. It is due to the release of phenolic compounds from esterified and glycosylated bonds (Carciochi et al., 2016). The increase in total phenolic heated also occurred in jujube honey from Iran, which has an increase in total phenolic after heating for ten days (Molaveisi et al., 2019).

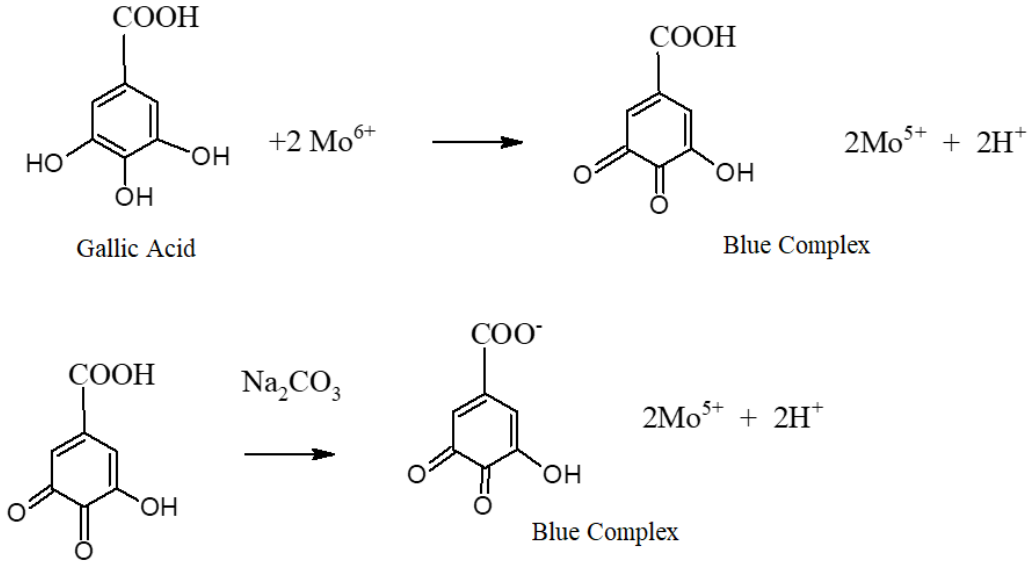


Figure 4. Total phenolic Test Reaction (Martono et al., 2019)

The form of phenolic compounds in nature is rarely found in free form. Examples of phenolic compounds in honey can be found as tannins. Tannins in honey are the most common type of phenolic acid, around 4.14 mg/100g sample in honey compared to other phenolic acids (Imtara et al., 2019). Tannins will be degraded into glucose and gallic acid. Gallic acid will react more quickly with Folin-Ciocalteu (Figure 5) compared to tannins and then absorbed at a wavelength of 733 nm so that the absorbance can increase in 5 samples of apis honey and two samples of trigona honey (Figure 5). The degradation of heated tannin compounds has been carried out by other researchers at a temperature of 60 °C and obtained a decrease in tannin levels of 3.14% (Rakic et al., 2004). Tannin compounds heated at a temperature of 65 to 150 °C can hydrolyze tannins into gallic acid and pyrogallol (Kim et al., 2011). Thermal decarboxylation of gallic acid in a copper autoclave produces pyrogallol (Kambourakis et al., 2000). The interaction

between phenolic compounds and Folin-Ciocalteu is influenced by the type of structure of the phenolic compounds. The simpler the phenolic compound, the easier it will be to react with Folin-Ciocalteu because it is not influenced by large substitution groups, electron interactions, and steric stress (Blainski et al., 2013).

Table 3. Total phenolic compound before and after heating

Sample	Before heating (mg GAE/ g sample)	After heating (mg GAE/ g sample)	Increase (%)	Decrease (%)
		APIS		
AP LK	2,303 ± 0,115	3,340 ± 0,121	45	-
AP LMB	4,847 ± 0,012	6,493 ± 0,012	25,3	-
AP MG	2,717 ± 0,127	4,703 ± 0,237	73,1	-
AP MAC	4,853 ± 0,006	5,280 ± 0,346	8,8	-
MG KLS	1,693 ± 0,081	2,577 ± 0,003	52,2	-
Average	3,268	4,47		
		TRIGONA		
TR SLT	3,330 ± 0,121	2,638 ± 0,118	-	20,8
TR BIR	3,880 ± 0,118	6,160 ± 0,301	58,8	-
TRG BGR	2,224 ± 0,120	2,022 ± 0,475	-	9,1
TR LMB	4,917 ± 0,108	7,097 ± 0,237	44,3	-
TR SLS	7,863 ± 0,531	5,897 ± 0,006	-	25
Average	4,443	4,760		

Note : GAE = *Gallic Acid Equivalent*

AP LK (apis Klengkeng); AP LMB (apis North Lombok); AP MG (apis mangga); AP MAC (honey from Aceh, Buloh Seuma); MG KLS (apis mangrove South Kalimantan), TR SLT (trigona South East Sulawesi); TR BIR (trigona tetroginola biroi South Sulawesi selatan); TRG BGR (trigona Bogor); TR LMB (trigona North Lombok utara); TR SLS (trigona Genotrigona Insica South Sulawesi).

The decrease in total phenolic after heating occurred in three samples of trigona honey, namely TR SLT (Trigona Southeast Sulawesi), TRG BGR (Trigona Bogor), and TR SLS (Trigona Sulawesi). This is possible because trigona honey is rich in phenolic compounds, which are more straightforward than polyphenols. Moreover, it is more easily degraded than apis honey (Figure 6). According to reports, trigona honey has more aglycone compounds (flavonoids or phenolics that are not bound to sugar) than glycoside compounds (phenolic or flavonoids bound to sugar). Because they contain more water, bacteria are easier to live and deglycosylated phenolic or phenolic compounds. Flavonoids in simpler forms or their aglycone forms (Adriane Costa dos et al., 2021) are more easily degraded by heat.

Heating for a long time will damage the structure of phenol compounds, in this case, gallic acid into more superficial structures, namely pyrogallol and tetrahydroxybenzene. Further, it degraded into oxalic acid and maleic acid to become CO_2, and H_2O (Figure 6), resulting in total phenolic content decreased (Bensalah et al., 2018) and could no longer react with Folin-Ciocalteu.

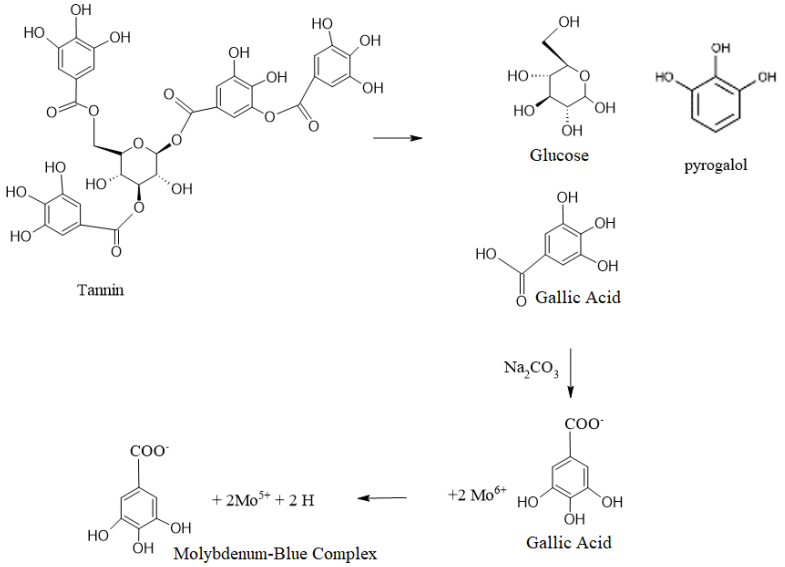


Figure 5. Hydrolysis of Tannins to Glucose, Gallic Acid and Pyrogallol

Flavonoids

This test is carried out with the principle of colorimetry. The sample reacted with AlCl3 and CH3COONa will produce a yellow color. Then, the wavelength will shift towards the visible, complex compounds that can be measured using a UV-Vis spectrophotometer at 430 nm (Fadillah et al., 2017). The addition of AlCl3 serves to chelate flavonoids (Figure 7). The addition of CH3COONa is intended to make the atmosphere alkaline because Al chelates with ortho hydroxy in the C3' and C4' rings are unstable in acidic conditions.

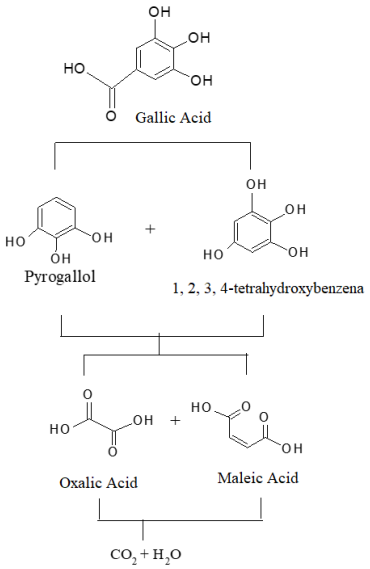


Figure 6. A simple scheme of phenolic degradation (Bensalah et al., 2018)

After the total flavonoid test results in each sample of apis honey and trigona honey after being heated, several samples increased and some samples decreased. However, the average total flavonoid in trigona honey was greater than that of apis honey (Table 4). The average flavonoid in apis honey was 0.194 mgQE/g sample after heating, while in trigona honey, it was 0.343 mgQE/g sample after heating (Table 4). This follows the research of Ismail et al. (2021), which stated that the total flavonoids in trigona honey were higher than apis honey.

The analysis showed that apis honey decreased in samples of AP LK by 47% and MG KLS by 11.8%, trigona honey decreased in samples of TRG BGR by 57.6% and TR SLS by 13.9%, while the sample others increased (Table 4). Increased levels of flavonoids in heated honey can be caused by the release of flavonoid compounds that are still bound to other compounds in food (Jeong et al., 2004). In this case, flavonoid compounds bound to sugar can become more free simple compounds or flavonoid aglycones.

Table 4. Total flavonoids before and after heating

Name	Before heating (mg QE/ g sample)	After heating (mg QE/ g sample)	Increase (%)	Decrease (%)
		APIS		
AP LK	0,117 ± 0,021	0,062 ± 0,004	-	47
AP LMB	0,198 ± 0,013	0,374 ± 0,008	88,8	-
AP MG	0,045 ± 0,003	0,096 ± 0,023	114	-
AP MAC	0,128 ± 0,014	0,383 ± 0,008	199	-
MG KLS	0,062 ± 0,004	0,055 ± 0,003	-	11,8
Average	0,110	0,194		
		TRIGONA		
TR SLT	0,371 ± 0,014	0,504 ± 0,039	35,8	-
TR BIR	0,289 ± 0,010	0,712 ± 0,010	146	-
TRG BGR	0,191 ± 0,032	0,081 ± 0,005	-	57,6
TR LMB	0,292 ± 0,012	0,540 ± 0,070	84,9	-
TR SLS	0,423 ± 0,015	0,364 ± 0,041	-	13,9
Average	0,313	0,343		

Note : QE = quercetin equivalen

AP LK (apis Klengkeng); AP LMB (apis lombok utara); AP MG (apis mangga); AP MAC (Honey from Aceh, Buloh Seuma); MG KLS (apis mangrove South Kalimantan), TR SLT (trigona South East Sulawesi); TR BIR (trigona tetroginola biroi South Sulawesi); TRG BGR (trigona Bogor); TR LMB (trigona North Lombok); TR SLS (trigona Genotrigona Insica South Sulawesi).

One example of a compound widely contained in honey is Isorhamnetin 3-O-neohesperidoside, which reaches 407 g/100g sample (Pilar et al., 2009). This compound is a flavonoid compound with a glycoside bond or a neohesperidoside

bond in the C3 ring, neohesperidoside is a combination of glucoside and rhamnose (Figure 7).

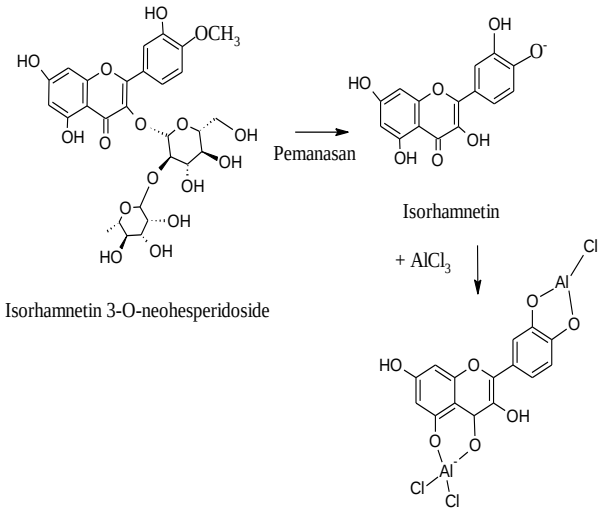


Figure 7. Glucoside Flavonoid in Heating Reaction

Heating can break the bonds between sugars and flavonoids. When heated, the levels of flavonoids increase along with the increase in flavonoid aglycones. This indicates the change of flavonoid glycosides into flavonoid aglycones (Sung et al., 2019). The release of neohesperidoside in the C3 ring of flavonoids makes it easier for $AlCl_3$ to react with flavonoids to form a yellow color (Figure 4) and can be absorbed at a wavelength of 430 nm on a UV-Vis spectrophotometer (Indrayani, 2008). The type of structure influences the ease of reaction of $AlCl_3$ with flavonoids; flavonoid aglycone compounds react more quickly with $AlCl_3$ than flavonoid glycosides to produce greater absorbance (Struchkov et al., 2018).

The decrease in total flavonoids in the AP LK, MG KLS, TRG BGR, and TR SLS samples could be possible in the four samples containing more simple flavonoids so that they were easier to degrade than in other samples. Therefore, the total flavonoids in the four kinds of honey decreased.

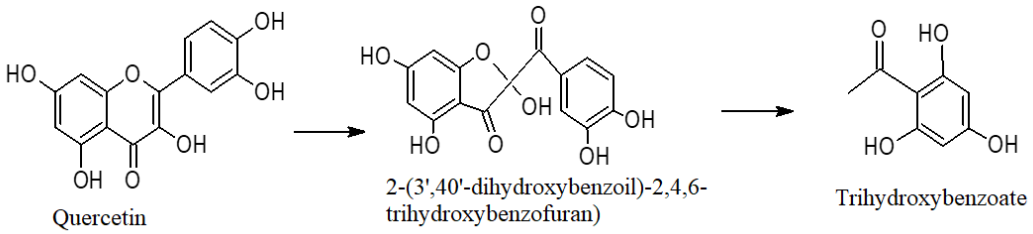


Figure 8. Denaturation Reaction of Flavonoid Structure (Chaaban et al., 2017)

Quercetin will be degraded so that it changes structure when heated to trihydroxylbenzoate (Figure 8). This structural change reduces and eliminates the

amount of ortho-hydroxy in flavonoids so that flavonoid complexes cannot form with AlCl3, which causes the absorbance to decrease. The structure influences the resistance of flavonoids to heat. The more double bonds, the more stable it is

This study shows the possible structural changes of phenolics and flavonoids due to heating. Heating can cause some samples to decrease due to degradation, while samples increase due to changes in phenolic or flavonoid glycosides. The changes are to their aglycone form. These are caused by breaking glycoside bonds and esterification bonds in flavonoids or flavonoids as a result of heating (Jeong et al., 2004; Sung et al., 2004; Sung et al. ., 2019).

Brown Pigment

The brown pigment is a compound resulting from the Maillard reaction, a non-enzymatic reaction between reducing sugars and free amino acids (Noda et al., 2016). Brown pigment measurements were carried out using a UV-Vis spectrophotometer at 420 nm (De la Cueva et al., 2017).

The brown pigment was measured on honey that had previously been prepared with a concentration of 1 Brix. Brix is a unit used to express the amount of solute content in a solution in a food (Putrianti 2013). Pyrolthiazolate (Figure 9) is an example of a brown dye (Noda et al., 2016). This compound is formed from cysteine with glucose, while acetylpyrrole is formed from glucose and proline (Yanagimoto et al., 2002). Trigona honey has more amino acid cysteine than proline, the cysteine content of trigona honey is around 34.67 ± 2.09 mg/Kg sample, and proline is around 16.23 ± 5.68 mg/Kg sample (Shamsudin et al., 2019).

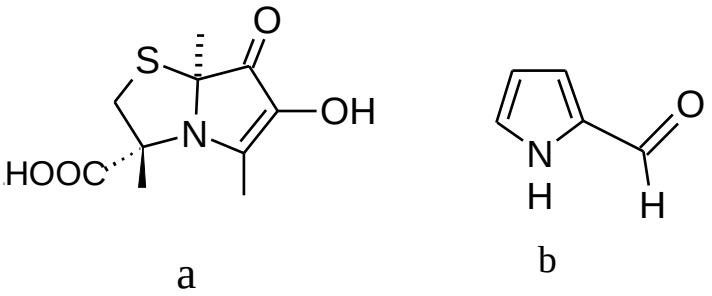


Figure 9. Structure of (a) Piroltiazolat (b) Asetilpirol

The TR SLS sample increased the absorbance of the brown pigment. That happened after heating from 0.108 ± 0.002 to 0.603 ± 0.002. The TRG BGR sample increased in the absorbance of the brown pigment from 0.594 ± 0.006 to 1.369 ± 0.048 (Table 5). Changes in brown pigment are characterized by an increase in absorbance at 420 nm and temperatures of 50, 70, and 80 °C within 1 to 10 days. This phenomenon was also shown in Turkish commercial honey, which experienced an increase in brown pigment after heating (Turkmen et al.,

2006). In practical applications, brown pigment formation is an internal parameter used to limit thermal treatment and during honey storage (Bult & Kilic, 2009).

In addition, based on research by Starowicz et al. (2021) stated that the formation of brown pigment is strongly correlated with the appearance of honey, the content of bioactive compounds, and antioxidant activity. It was proven that honey with high browning index had high antioxidant activity.

Table 5. The absorbance of brown pigment before and after heating

Sample	Before heating	After heating	Increase (%)
APIS			
AP LK	0,057 ± 0,001	0,872 ± 0,001	1.428
AP LMB	0,297 ± 0,090	1,215 ± 0,041	309
AP MG	0,069 ± 0,002	0,597 ± 0,019	765
AP MAC	0,169 ± 0,005	0,969 ± 0,009	473
MG KLS	0,128 ± 0,001	0,817 ± 0,006	538
TRIGONA			
TR SLT	0,220 ± 0,010	0,568 ± 0,027	158
TR BIR	0,210 ± 0,031	1,175 ± 0,014	434
TRG BGR	0,594 ± 0,006	1,369 ± 0,048	130
TR LMB	0,349 ± 0,006	0,461± 0,001	32
TR SLS	0,108 ± 0,002	0,603 ± 0,002	458

Note : AP LK (apis Klengkeng); AP LMB (apis North Lombok); AP MG (apis mangga); AP MAC (Honey from Aceh, Buloh Seuma); MG KLS (apis mangrove South Kalimantan;TR SLT(trigona South East Sulawesi); TR BIR (trigona tetroginola biroi South Sulawesi); TRG BGR (trigona bogor); TR LMB (trigona North Lombok); TR SLS (trigona Genotrigona Insica South Sulawesi.

Conclusion

The results showed that all honey samples showed the presence of phenolics and flavonoids. Tests of total phenolic and flavonoid levels showed a decrease in several samples, including TR SLT (Trigona Southeast Sulawesi, TRG BGR (Trigona Bogor), TR SLS (Trigona Genotrigona Insica South Sulawesi) in the range of 11.8 – 57.6%. Meanwhile, some of the total phenolic and flavonoid content increased after heating. It was found in the samples of AP LMB (Apis Lombok Utara) (25.3% and 88.8%), AP MG (Apis Mango) (73.1% and 114%), AP MAC (honey from Aceh, Buloh Seuma) (8.8% and 199%), TR BIR (Trigona Tetroginola Biroi south Sulawesi) (58.8% and 146%), TR LMB (Trigona Lombok north) (44.3% and 84.9). While the formation of brown pigment, there was an increase in all honey samples between 32 - 1.428%. The presence of brown pigment at the end of the heating process of honey samples is thought to have the same role as phenolic compounds and flavonoids, which change the heating

process, especially on antioxidant activity and other bioactivities. However, further analysis is needed to prove this hypothesis.

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References

Adriane Costa dos, S., Carina, B. F., Francieli, B., Gonzaga, L. V., Costa, A. C. O., & Roseane, F. (2021). Phenolic Composition and Biological Activities of Stingless Bee Honey, An Overview Based on its Aglycone and Glycoside Compounds. *Food Research International*, *147*(2), 110553.

Alqarni, A.S.; Owayss, A.A., Mahmoud, A.A, & Hannan, M. A. 2014. Mineral content and physical properties of local and imported honeys in Saudi Arabia. Journal of Saudi Chemical Societyxic. **2012**, 18(5), 618-625, 1508–1516. https://doi.org/10.1016/j.jscs.2012.11.009

Agustina, W., Nurhamidah, & Handayani, D. (2017). Skrining Fitokimia Dan Aktivitas Antioksidan Beberapa Fraksi Dari Kulit Bantang Jarak (*Ricinus communis* L.). *Jurnal Pendidikan dan Ilmu Kimia, 1*(2),117-122. DOI: https://doi.org/10.33369/atp.v1i2.3529.

Almey, A.A., Khan, A. J., Zahir, S., Suleiman, M., Aisyah, M. R., & Kamarul Rahim, K. (2010). Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. *International Food Research Journal*, *17*,1077–1084

Bensalah, N., Chaira, K., & Bedoui, A. (2018). Efficient degradation of tannic acid in water by UV/H2O2 process. *Sustainable Environment Research*, *28*(1),1-11. https://doi.org/10.1016/j.serj.2017.04.004.

Blainski, A., Lopes, G. C., & De Mello, J. C. P. (2013). Application and analysis of the Folin-Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. *Molecules*, *18*(6), 6852-6865. DOI: 10.3390/molecules18066852.

Brudzynski, K., & Miotto, D. (2011). Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. *Food Chemistry*, *129*(7), 1023–1030. https://doi.org/10.1016/j.foodchem.2011.01.075

Bulut, L., & Kilic, M. (2009). Kinetics of hydroxymethylfurfural accumulation and color changes in honey during storage in relation to moisture content. *Journal of Food Processing and Preservation*, *33*(1), 22–32. doi 10.1111/j.1745-4549.2008.00233.x

Carciochi, R. A., Dimitrov, K., & Galván D´Alessandro, L. (2016). Effect of malting conditions on phenolic content, Maillard reaction products formation, and antioxidant activity of quinoa seeds. *Journal of Food Science and Technology*, *53*(11), 3978-3985. DOI: 10.1007/s13197-016-2393-7.

Chaaban, H., Ioannou, I., Chebil, L., Slimane, M., Gérardin, C., Paris, C., Charbonnel, C., Chekir, L., & Ghoul, M. (2017). Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *Journal of Food Processing and Preservation*, *41*(5), 1-12. https://doi.org/10.1111/jfpp.13203.

De la Cueva, S.P., Seiquer, I., Mesías, M., Rufián-Henares, J., & Delgado-Andrade, C. (2017). Evaluation of the Availability and Antioxidant Capacity of Maillard Compounds Present in Bread Crust: Studies in Caco-2 Cells. *Foods*, 6(1), 5. doi:10.3390/foods60100.

Ergina, Nuryanti, S., & Pursitasari, I. D. (2014). Uji Kualitatif Senyawa Metabolit Sekunder pada Daun Palado (*Agave angustifolia*) yang Diekstraksi dengan Pelarut Air dan Etanol. *Jurnal Akademika Kimia*, 3(3), 165-172.

Fadillah, A., Rahmadani, A., & Rijai, L. (2017). Analisis Kadar Total Flavonoid dan Uji Aktivitas Ekstrak Daun Kelubut (*Passiflora foetida* L.). Proceeding of the 5th Mulawarman Pharmaceuticals Conferences, Samarinda, 5, DOI: https://doi.org/10.25026/mpc.v5i1.217

Huvaere, K., & Skibsted, L.H. (2014). Flavonoids protecting food and beverages against light. *Journal of the Science of Food and Agriculture*, *95*(1), 20-35. DOI 10.1002/jsfa.6796.

Idris, N. A. (2016). Uji Aktivitas Antioksidan Ekstrak Sarang Lebah dan Madu Hutan dari Luwu Utara dengan Metode DPPH (1,1-Difenil-2-Pikrilhodrazil), [Skripsi], 2016, Makasar

Imtara, H., Kmail, A., Touzani, S., Khader, M., Hamarshi, H., Saad, B., Lyoussi, B. (2019). Chemical Analysis and Cytotoxic and Cytostatic Effects of Twelve Honey Samples Collected from Different Regions in Morocco and Palestine. *Evidence-based Complementary and Alternative Medicine*, 19,1-11, https://doi.org/10.1155/2019/8768210.

Indrayani, S. (2008). Validasi penetapan Kadar Kuersetin dalam Sediaan Krim SecaraKolorimetri dengan Pereaksi $AlCl_3$, Fakultas Farmasi Universitas Yogyakarta.

Ismail, N. I., Kadir, M. R. A., Zulkifli, R. M., & Mohamed, M. (2021). Comparison of Physicochemical, Total Protein and Antioxidant Profiles Between Malaysian Apis and Trigona Honeys. *Malaysian Journal of Analytical Sciences*, *25*(2), 243–256.

Jeong, S. M., Kim, S. Y., Kim, D. R., Jo, S. C., Nam, K. C., Ahn, D. U., & Lee, S. C. (2004). Effect of heat treatment on the antioxidant activity of extracts

from citrus peels. *Journal of Agricultural and Food Chemistry*, 52(11), 3389-3393. https://doi.org/10.1021/jf049899k.

Kambourakis, S., Draths, K. M., & Frost, J. W. (2000). Synthesis of Gallic Acid and Pyrogallol from Glucose: Replacing Natural Product Isolation with Microbial Catalysis. *Journal of the American Chemical Society*, 122(37), 9042–9043. DOI: 10.1021/ja000853r.

Kim, T. J., Silva, J. L., & Jung, Y. S. (2011). Enhanced Functional Properties of Tannic Acid after Thermal Hydrolysis. *Food Chemistry*, *126*(1),116–120. https://doi.org/10.1016/j.foodchem.2010.10.086.

Krishnan, S. (2020). Penetapan Kadar Total Fenol dan Total Flavonoid dari Ekstrak Etanol daun Sibo (*Leea indica* F.). [Skripsi]. Program Studi Sarjana Farmasi Fakultas Farmasi. Universitas Sumatera Utara.

Martono, Y., Yanuarsih, F., Aminu, N. R., & Muninggar, J. (2019). Fractionation and Determination of Phenolic and Flavonoid Compound from *Moringa Oleifera* Leaves. *Journal of Physics: Conference Series*, *1307*(1),1-8. https://doi.org/10.1088/1742-6596/1307/1/012014

Molaveisi, M., Beigbabaei, A., Akbari, E., Noghabi, M. S., & Mohamadi, M. S. (2019). Kinetics of temperature effect on antioxidant activity, phenolic compounds and color of Iranian jujube honey. *Heliyon*, *5*(1), 01129. https://doi.org/10.1016/j.heliyon.2019.e01129.

Mukhriani, Rusdi, M., Arsul, M. I., Sugiarna, R., & Farhan, N. (2019). Kadar Fenolik dan Flavonoid Total Ekstrak Etanol Daun Anggur (*Vitis vinifera* L). *Ad-Dawaa'. Journal of Pharmaceutical Sciences*, *2*(2), https://doi.org/10.24252/djps.v2i2.11503.

Noda, K., Terasawa, N., & Murata, M. (2016). Formation scheme and antioxidant activity of a novel Maillard pigment, pyrrolothiazolate, formed from cysteine and glucose, *Food and Function*, 7(6), 2551-2556.

Parbuntari, H., Prestica, Y., Gunawan, R., Nurman, M. N., & Adella, F. (2018). Preliminary Phytochemical Screening (Qualitative Analysis) of Cacao Leaves (*Theobroma cacao* L.). *EKSAKTA: Berkala Ilmiah Bidang MIPA, 19*(2),40-45.

Pilar, T., Federico, F., & Tomas-Barberan, F. A. (2009). Liquid Chromatography-Tandem Mass Spectrometry Reveals the Widespread Occurrence of Flavonoid Glycosides in Honey, and Their Potential as Floral Origin Markers. *Journal of Chromatography A, 1216*(43), 7241-7248.

Putri, H. D, Sumpono, & Nurhamidah. (2018). Uji Aktivitas Asap Cair Cangkang Buah Karet (*Hevea brassiliensis*) dan Aplikasinya dalam Penghambatan Ketengikan Daging Sapi. *Jurnal Pendidikan dan Ilmu Kimia, 2*(2), 97-105. doi: https://doi.org/10.33369/atp.v2i2.7474.

Putrianti, R. D. (2013). Pengaruh Lama Penyimpanan Batang Sorgum Manis Terhadap Rendemen dan Brix Nira yang Dihasilkan, Makasar: Universitas Hasanuddin, 2013.

Putu, N., Savitri, T., Hastuti, E. D., Widodo, S., & Suedy, A. (2017). Kualitas Madu Lokal dari Beberapa Wilayah di Kabupaten Temanggung The Local Honey Quality of Some Areas in Temanggung. *Buletin Anatomi dan Fisiologi*, 2(1), 58-66

Rakic, S., Maletic, R., Perunovic, M., & Svrzic, G. (2004). Influence of Thermal Treatment on Tannin Content and Antioxidation Effect of Oak Acorn Quercus Cerris Extract. *Journal of Agricultural Sciences, Belgrade*, *49*(1), 97-107

Shamsudin, S., Selamat, J., Sanny, M., Shamsul, B. A. R., Jambari, N. N., & A. Khatib, (2019). A comparative characterization of physicochemical and antioxidants properties of processed Heterotrigona itama honey from different origins and classification by chemometrics analysis. *Molecules, 24*(21), 1-20. DOI: 10.3390/molecules24213898.

Starowicz, M., Ostaszyk, A., & Zieli´nski, H. (2021). The Relationship between the Browning Index, Total Phenolics, Color, and Antioxidant Activity of Polish-Originated Honey Samples. *Foods*, 10, 967. https://doi.org/10.3390/foods10050967

Struchkov, P., Beloborodov, V., Kolkhir, V., Voskoboynikova, I., & Savvateev, A. (2018). Comparison of Spectrophotometric Methods of Total Flavonoid Assay Based on Complex Formation with Aluminum Chloride as Applied to Multicomponent Herbal Drug Angionorm. *Journal of Pharmaceutical, 9*(1), 39-43. DOI:10.4103/jpnr.JPNR_22_17.

Sung, J., Suh, J. H., & Wang, Y. (2019). Effects of heat treatment of mandarin peel on flavonoid profiles and lipid accumulation in 3T3-L1 adipocytes. *Journal of Food and Drug Analysis, 27*(3), 729-735. https://doi.org/10.1016/j.jfda.2019.05.002.

Sumarlin, L., Muawanah, A., Afandi, F.R., & Adawiah. (2019). Inhibitory, Activity of HEp-2 Cells by Honey from Indonesia. *Jurnal Kimia Sains dan Aplikasi*, *22*(6), 317-325 https://doi.0rg/10.14710/jksa.22.6.317-325.

Tandi, J., Melinda, B., Purwantari, A., & Widodo, A. (2020). Analisis Kualitatif dan Kuantitatif Metabolit Sekunder Ekstrak Etanol Buah Okra (*Abelmoschus esculentus* L. *Moench*) dengan Metode Spektrofotometri UV-Vis. *Kovalen: Jurnal Riset Kimia*, *6(*1),74–80. https://doi.org/10.22487/kovalen.2020.v6.i1.15044

Turkmen, N., Sari, F., Ender, P., & Sedat, V. (2006). Effects of Prolonged Heating on Antioxidant Activity and Colour of Honey. *Food Chemistry, 95*(4), 653-657. https://doi.org/10.1016/j.foodchem.2005.02.004

Yalçin, G. (2021). Effects of Thermal Treatment, Ultrasonication, and Sunlight Exposure on Antioxidant Properties of Honey. *Turkish Journal of*

Pharmaceutical Sciences, *18*(6), 776-780. https:10.4274/tjps.galenos.2021.53810i.

Yanagimoto, K., Lee, K. G., Ochi, H., & Shibamoto, T. (2002). Antioxidative activity of heterocyclic compounds formed in Maillard reaction products. *International Congress Series,* 1245, no. C, (2002) 335-340