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Quality Analysis Based on Organoleptic Properties, Water Content, and Total Reducing Sugars Content in the Raw Honey (Apis dorsata) and Processed Honey

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A B S T R A C T

Measuring honey quality standards aims to protect consumers from products not meeting the criteria and counterfeit honey. This paper compares differences in quality analysis based on organoleptic properties, water content, and total reducing sugars content between raw and processed honey. This study used a true experimental research design. The independent variables were raw and processed honey, while the dependent variables were organoleptic properties, water content, and total reducing sugars content. Instruments to determine water contents used a refractometer. In addition, we calculated the total reducing sugars in honey through the iodometry titration method. Data analysis in the water content and total reducing sugar used the independent T-Test with a 95% confidence level (p=0.05). In addition, we descriptively analyzed the colour, aroma, taste, and viscosity of raw and processed honey. Organoleptic analysis showed differences between the colour, taste, and viscosity of both kinds of honey. The mean water content was 19.01% in raw honey and 20.83% in processed honey, indicating that much water was used as a mixture when processing honey. The mean total reducing sugars in raw honey were 15.92% and 25.38% in processed honey, indicating that the processed honey contained a lot of sugar or additional sweeteners such as granulated sugar. The Independent Sample T-Test resulted in p=0.00 (p<0.05). Thus, there was a significant difference between water content and total reducing sugars in raw and mixed honey. Overall, the quality of raw honey is better than processed honey.

INTRODUCTION

Honey is natural product bees produce for consumption because it contains essential nutrients. It is not only a sweetener or food flavoring but is often used to relieve fatigue, smooth skin, and grow hair (Cianciosi *et al.*, 2018). Measuring honey quality standards aims to protect consumers from products not meeting the criteria and counterfeit honey. Each country has different honey quality standards. The National Standardization Agency of Indonesia regulates honey quality standards in Indonesian National Standards (INSs) (Sahlan *et al.*, 2019). Quality is crucial for honey consumers, both in industry and importers. Therefore, quality control in honey-producing countries is essential, especially for exporters (Rupilu, Pattiselanno and Papilaya, 2022).

Honey has a unique chemical composition. However, the elements of carbohydrates (sugars) are the most dominant. Honey contains 70-80% sugar. The honey contains sucrose, maltose, and dextrin. In addition, there are vitamin C, vitamin B1, vitamin B2, vitamin B6, pantothenic acid, folic acid, and minerals (Na, K, Ca, Fe, Cu, P, S). Furthermore, honey has hormones, enzymes, bactericidal substances, and fungicides. Honey also contains aromatic substances, waxes, proteins, essential oils, formic acid, and flower pollen. Researchers at the Department of Biological Sciences, University of Waikato, also proved that honey had

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active antibiotic substances against various pathogenic bacteria that cause disease. In addition, it can potentially cure and prevent several infectious diseases, such as cough, fever, heart, liver, and lung disease. Honey is also effective for illnesses that can interfere with the function of the eyes, nerves, ears, and respiratory tract infections.

Total water content is one of the quality standards of honey, which is a maximum of 22%. The honey production process by the bees is complex, so there will likely be differences in water content and composition between types of honey (Chen, 2019) (Otmani *et al.*, 2019). In addition, the water content in honey may affect its efficacy, especially in the treatment process (Nuraini, Hastuti and Husnaeni, 2021).

Based on the organoleptic analysis, pure honey has characteristics. The organoleptic analysis includes honey's taste, colour, aroma, and viscosity. The taste is something that the sense of taste can feel, and the colour is visible and can be observed in honey. Aroma is the result of sensing through smell. The smell is also an indicator of damage to the product. For example, a foul odor indicates the product has been damaged (Prabowo *et al.*, 2019). In honey products, the quality standard for the aroma and colour of honey depends on the origin and type of flower. In addition, the colour varies, white, yellow, brown, red, and black. The colour variation depends on the nectar of the flower. The plant pigments are xanthophyll, carotin, and chlorophyll. The colour of honey can be divided into white, light, and dark honey (Prabowo, Prayitno and Yuliani, 2020). Red honey is usually dark honey, which was previously yellow, then brownish yellow, and then becomes slightly reddish. White honey is not much different from red honey. The only difference is the flower nectar that is sucked by the worker bees. White honey is mainly obtained from citrus, kapok, and durian trees, while dark honey is from daisies and insect fluids.

Honey is hygroscopic and easy to absorb water from the surrounding environment. It will undergo fermentation when the water content increases. Furthermore, fermentation will decrease its qualities and damage its properties. Many types of honey come from various regions. The different types of honey are based on the food source and the type of bee. There are differences between raw and processed honey. Raw honey is pure honey that has not been processed and mixed with additives. Meanwhile, regular honey is processed honey and has been mixed with certain additives.

A preliminary study indicated that processed honey in the community often found several problems related to its authenticity. There were honey deposits at the bottom of the bottle. In addition, processed honey freezes when stored in the refrigerator due to the increased water content. At the same time, previous research revealed that high water content in honey could reduce its durability and affect its effectiveness in the treatment. Unfortunately, reducing the water content in honey is challenging and requires relatively expensive costs. This paper compares differences in quality analysis based on

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organoleptic properties, water content, and total reducing sugars content between raw and processed honey.

METHOD

This study used a true experimental research design conducted in the laboratory to discover specific treatments' symptoms or effects. The researchers examined the possible causes and effects between processed honey (experimental group) and raw honey (control group) and then compared both. The research was conducted at the Chemical Laboratory of the Health Analyst Study Program, Mataram Health Polytechnic of the Health Ministry, from January to May 2022. On the first day, reagents were prepared and manufactured and then continued with the organoleptic analysis (colour, smell, taste, and viscosity) in raw and processed honey. Then, we analyzed water content and total reducing sugars on the second day.

This study used several treatments as follows:

t_1 = water content of raw honey

t2 = water content of processed honey

t3 = total reducing sugars content of raw honey

t_4 = total reducing sugars content of processed honey

The steps in determining the layout of the experimental units were:

1. Determine the number of replications.

   $(t-1).(r-1) \geq 15$

   $(4-1).(r-1) \geq 15$

   $(3).(r-1) \geq 15$

   $3r - \geq 15$

   $3r \geq 15 + 3$

   $3r \geq 18$

   $r \geq \frac{18}{3} = 6$

   $r \geq 6$

2. Determine the number of experimental units.

   N= t x r

   4 x 6

   24 trial units

   Note: n = number of experimental units

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t = Treatment

r = Replication/replication

We weighed 0.1 grams of a sample obtained from 24 experimental units from each of 12 raw and 12 processed honey with twice the replication for each. This research used purposive sampling or based on considerations according to the population characteristics. The samples were raw honey collected from giant honeybees (*Apis dorsata*) and processed honey sold in the market. Both samples were taken in Lantung Village, Lantung District, Sumbawa Besar Regency, West Nusa Tenggara. The independent variables were raw and processed honey, while the dependent variables were organoleptic properties, water content, and total reducing sugars content. The operational definitions of the variables were:

1. Raw honey was pure honey without additives produced by bees (*Apis dorsata*) obtained from forest bee honey collectors assisted by the Forestry Service of West Nusa Tenggara Province in Lantung Village, Lantung District, Sumbawa Besar Regency.
2. Processed honey was natural honey with certain additives, such as traditional medicinal concoctions or concentrated fruit juice.
3. The organoleptic properties of honey were the standard for purity of honey quality, namely taste, colour, smell, and texture.
4. Total reducing sugars content was the properties of honey as a standard for the purity of honey quality, namely glucose in honey.
5. The water content was the amount of water contained in honey.

Instruments to determine water contents used a refractometer through the refractive index. In addition, we calculated the total reducing sugars in honey through the iodometry titration method. Some tools and materials used in the data collection technique were a glass beaker, dropper pipette, petri dish, drying oven, cup clamp, desiccator, electrical analytical balance, burette, Erlenmeyer pumpkin, measuring flask, and blank. At the same time, the materials used are pure honey and mixed honey, aqua dest, KIO_3 0.1N, KI 10%, Sulfuric acid 4N, Sodium thiosulfate 0.1N, Pb Acetate 10%, K_4FeCN_6 10%, Luff Schrool, and Amilum.

Work procedures

Sample Preparation

We poured both samples into a 20 ml glass beaker with a volume of 5 ml from the packaging container. The sample used must be in ready-to-use or liquid form. To prevent damage, the temperature of the honey should not exceed 40°C. If the honey sample did not contain lumps, then the sample was shaken or stirred well. The measurement of water content in honey did not undergo special treatment.

Determination of Water Content

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1. The empty cup was placed in an oven at 105°C for 1 hour, cooled in a desiccator, and then weighed, repeated until there was a constant weight.
2. We carefully weighed the 2-5 g sample into the cup, and the sample's surface was made evenly in the cup and heated in the oven at 100°C for 1 hour.
3. We cooled it in a desiccator for ± 15 minutes, then weighed it.
4. This treatment was repeated (30 min heating briefly) until there was a constant weight. Then, we calculated the water content percentage.

Formula : $\%H_2O = \frac{W1 - W2}{W1 - Wo}$

Note: Wo = Empty Container

W_1 = Empty container + sample before heating

W_2 = Empty container + sample after heating

Determination of total reducing sugar content through iodometry titration method

1. 0.1N. KIO_3
   a. We pipetted 10.0 ml KIO_3 0.1N and added 15 -25 ml of distilled water
   b. We added 7.5 ml of 10% KI and 10 ml of 4N. sulfuric acid
   c. We titrated with 0.1N sodium thiosulfate until pale yellow
   d. We added a starch indicator
   e. We titrated with 0.1N Sodium thiosulfate to an endpoint

   Formula: $N_1 . V_1 = N_2 . V_2$

   Note: N_1 = KIO Normality $_3$

   V1 = KIO $_{\text{Volume } 3}$

   N2 = Normality of Natrium thiosulfate

   V2 = Average titration volume
2. Determination of total reducing sugars content.
   a. We weighed 0.1 grams of the sample and put it into a 250 ml volumetric flask.
   b. We added 5 ml of Pb Acetate 10% + 5 ml K_4FeCN_6 10%
   c. We added aqua dest up to the marked line
   d. We filtered with filter paper (as much as ± 30 ml)
   e. We pipetted 25.0 ml of this solution and added 25.0 ml of the Luff-Schoorl solution
   f. We heated 10 minutes after boiling and let it cool
   g. We added 15 ml of 10% KI + 25 ml of 6 N sulfuric acid (acid)
   h. We titrated with 0.1 N sodium thiosulfate until pale yellow
   i. We added an amylum indicator

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j. We titrated with 0.1 N sodium thiosulfate to an endpoint

k. We calculated the total reducing sugars in honey.

l. A blank titration was carried out simultaneously with aqua dest as a substitute for the sample.

Formula : F= $\frac{Ns}{Nf} x\ (VtBlkxVtspl)$ x ~

Note: N_s = True Normality Sodium Thiosulfate

N_f = Normality factor = 0.1N

Formula to find the value of F

$$\%\ Gula\ Reduksi = \frac{F\ X\ P}{W\ (mg)}\ x\ 100\ \%$$

Note: F = sugar equivalence factor

P = Dilution

W = Mass of sample

Data analysis in the water content and total reducing sugar used the independent T-Test with a 95% confidence level (p=0.05) using the IBM Statistical Product and Service Solutions (SPSS) 20.0 analysis software program. When p<0.05, it means that there was a difference in water content and total reducing sugar in raw and processed honey. Meanwhile, p≥0.05 means there was no difference in water content and total reducing sugar content in both independent variables. In addition, we descriptively analysed the colour, aroma, taste, and viscosity of raw and processed honey.

RESULT

Raw honey had red brown, a caramel-like aroma, tasted sweet, and had high viscosity. Meanwhile, processed honey had a light yellow colour, caramel aroma, sour taste, and watery consistency. Thus, raw and processed honey differed in colour, taste, and viscosity (table 1).

Table 1 Organoleptic Test on Raw and Processed Honey

Variable	Parameters			
	Colour	Smell	Flavour	Viscosity
Raw Honey	RB	C	Sw	T
Processed Honey	LY	C	S	W

Note: RB: Red Brown; S: Sour; LY: Light Yellow; T: Thick; C: Caramel; W: Watery and Sw: Sweet

In addition, the mean water content was 19.01% in raw honey and 20.83% in processed honey, indicating that much water was used as a mixture when processing honey (table 2).

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Table 2 Water Content Test on Raw and Processed Honey

Sample number	Raw honey (%)	Processed honey (%)
1	18.46	20.53
2	19.76	21.53
3	19.12	20.56
4	18.80	20.87
5	18.64	21.78
6	19.10	20.39
7	18,50	20.45
8	18.78	21.06
9	18.64	20.27
10	18,80	20.68
11	19.02	20.87
12	18.75	21.04
Total	228.17	250.03
Mean	19.01	20.83

Furthermore, the mean total reducing sugars in raw honey was 15.92% and 25.38% in processed honey, indicating that the processed honey contained a lot of sugar or additional sweeteners such as granulated sugar (table 3).

Table 3 Total reducing Sugars Test in Raw and Processed Honey

Sample number	Raw honey (%)	Processed honey (%)
1	15.83	26.54
2	15.90	24.33
3	16.05	25.45
4	15.85	24.60
5	15.90	25.54
6	16.00	26.25
7	15.83	24.75
8	15.95	25.25
9	16.02	25,40
10	15.85	25.56
11	15.95	26.05
12	16.02	24.86
Total	191.15	304.51
Mean	15.92	25.38

The normality and homogeneity tests of water content and total reducing sugars obtained $p > 0.05$. Thus, the data was normally distributed. Furthermore, both dependent variables were analysed using the independent sample T-test (table 4).

Table 4 Normality and homogeneity tests

Normality test		Kolmogorov-Smirnov [a]	
		Statistics	df
Water content (%)	Raw Honey	0.239	12
	Processed Honey	0.146	12
Reducing Sugar Content (%)	Raw Honey	0.171	12
	Processed Honey	0.146	12

Homogeneity test	Mean Median	Levene statistics	df_1	df_2	Sig
Water content (%)	Mean	0.924	1	22	0.347
	Median	1.169	1	22	0.291
Reducing Sugar Content (%)	Median and with adjusted df	1.169	1	21,943	0.291
	Trimmed mean	1.050	1	22	0.317

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The Independent Sample T-Test resulted in p=0.00 (p<0.05). Thus, there was a significant difference between water content and total reducing sugars in raw and mixed honey (Table 5).

Table 5 Results of Statistical Analysis Using the Sample T-Test

Variable	Assumption	t-test for Equality of Means		
		df	Sig. (2-tailed)	Mean Difference
Water content (%)	Equal variances assumed	22	0.000	-1.97167
	Equal variances not assumed	20,623	0.000	-1.97167
Reducing Sugars Content (%)	Equal variances assumed	22	0.000	-9.45250
	Equal variances not assumed	11,311	0.000	-9.45250

DISCUSSION

The organoleptic properties of honey are the properties of honey as a standard for purity of honey quality. This research found that raw honey had red brown, a caramel-like aroma, tasted sweet, and had a high viscosity. Meanwhile, processed honey had a light yellow colour, caramel aroma, sour taste, and watery consistency. So, the quality of raw and processed honey was dissimilar. The honey harvesting time, when the honey is ripe and the honey cells begin to be closed by the bees, influences the quality of honey.

Based on the Indonesian National Standard, high-quality honey has a high viscosity. Processed honey in this research had a runny consistency, so there was fermentation in the honey. Good quality honey has a high viscosity and low water content. So, the honey will not penetrate the newsprint. The viscosity of honey depends on its water content. The maximum water content in good honey is 22%. Foamy honey could be because it has been left in an open container for a long time and has undergone fermentation (Palilati et al., 2021).

In addition, our study indicated that the taste of both kinds of honey was different. Raw honey had a sweet taste, while processed honey had a sour taste. A previous study also showed that raw honey had a sweet taste compared to processed honey, which has a slightly sour taste (Kornienko et al., 2020). The type of flower the bees visit to collect the nectar as a raw material for honey can influence the taste of honey. Currently, there are various types of honey, such as Randu honey, longan honey, sour honey, mango honey, apple honey, cherry honey, orange honey, peer honey, etc. Honey has a sweet and slightly sour taste due to its high acid content. The sour taste is because the pH in honey is acid (pH: 3.4-6.1). Several organic acids, namely gluconic, acetic, butyric, citric, formic, lactic, malic, pyroglutamic, and succinic acids cause low pH in the honey. The honey's pH analysis can use pH indicator paper. The procedure is dipping the pH paper and comparing the results with the existing colour standard (Chen, 2019).

Furthermore, the colour of raw honey differed from processed honey. Raw honey had red-brown, while processed honey had a light yellow colour. Colour is one of the criteria for the quality of honey. Usually,

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the honey colour tends to follow the nectar-producing plants. For example, honey from radish plants will be white like water, honey from acacia and apple plants will be bright yellow, and honey from lime plants will be bright green. In addition, honey that has been stored for a relatively long period will tend to experience a darker colour change (Groposila-Constantinescu et al., 2020). Based on the colour, honey can be divided into white or light honey and dark honey. Red honey is usually dark honey, which was previously yellow, then brownish yellow became slightly reddish. White honey is not much different from red honey. What distinguishes it is the flower nectar sucked by the bees as workers. White honey is mainly obtained from citrus, kapok, and durian plants, while dark honey is from daisies and insect fluids.

Meanwhile, both kinds of honey in this research had a similar smell. The honey smell correlates with its colour. The darker the colour, the stronger or sharper the aroma. But its scent evaporates quickly. Therefore, honey must be stored and closed tightly. Heating can remove smells, and the extraction process decreases the aroma (Nicewicz et al., 2021).

This study showed that processed honey's water content was significantly higher than raw honeys. The mixing process in the processed honey used too much water. The second largest composition after carbohydrates in honey is water, with a percentage of 15%-25%. Air humidity, type of nectar, and production and storage processes cause variations in the water content. The water content in honey determines its durability. Honey with a high-water content will quickly ferment. Fermentation occurs because the fungi contained in honey grow actively because of the high-water content. In addition, the water content in honey will affect its shelf life because it is closely correlated with the metabolic activity that occurs while the honey is stored. Water content in foodstuffs is also critical because water can affect appearance, texture, and taste. In addition, it determines the freshness and durability of the food. The high-water content will cause bacteria, moulds, and yeasts to breed, so there will be changes in food ingredients. The role of water in food is one factor that affects metabolic activities such as enzyme activity, microbial activity, and enzymatic reactions, causing changes in organoleptic properties and quality values (Majid et al., 2019).

Honey results from bee secretion but does not its poop. Bees have two stomachs - one for eating and the other for storing nectar. The nectar contains 60% water, so bees must reduce it to 20% or lower to make honey. The decrease in water content is through physical and chemical processes. Decreasing water content begins when the bee sticks out its tongue (proboscis) to move honey from the honey stomach to the beehive. In the hive, the water content continues to be lowered through the rotation of the bee's wings, which circulates warm air into the beehive. Meanwhile, the chemical process occurs in the bee's stomach. The invertase enzyme in its stomach converts sucrose (disaccharide) into glucose and fructose (monosaccharides) (Chen, 2019).

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Our findings found that the total reducing sugars in processed honey were significantly higher than in raw honey. Total reducing sugars is one of the quality standards of honey, at least 60%. The total reducing sugars in honey contain glucose, fructose, maltose, and dextrin. The honey production process by bees is complex, so there will likely be differences in the levels and composition of reducing sugars between various types of honey (Dzugan *et al.*, 2018).

Honey is nectar or sugar exudate from plants collected by honeybees, processed, and stored in a beehive. The main components of honey are fructose, glucose, a little saccharose, minerals, vitamins, and enzymes. It Contents high nutrition, so honey consumption by adults, children, and babies is beneficial. The main sugar of nectar is sucrose. During the honey production process, sucrose was broken down by the enzyme invertase into simple sugars (glucose and fructose). The higher demand and limited availability of honey led to different forms of honey adulteration. Honey adulteration is by adding various syrups to natural honey or feeding honeybees with sugar syrups.

Honey also contains antibiotic substances against various disease-causing pathogenic bacteria. A study revealed that high honey sugar content, hydrogen peroxide radical compounds, a high acidity of honey, and organic antimicrobial agents were responsible for antibacterial activity in honey (Johnston *et al.*, 2018). High sugar levels inhibited the growth of bacteria so that bacteria could not live and reproduce. Hydrogen peroxide radicals were responsible for killing pathogenic microorganisms. The high acidity of honey reduced the growth and viability of bacteria. In addition, organic antimicrobial agents in honey were polyphenols, flavonoids, and glycosides (Johnston *et al.*, 2018).

CONCLUSION

In conclusion, raw honey has different organoleptic properties (colour, flavour, and viscosity) than processed honey. In addition, water content and total reducing sugars in processed honey were significantly higher than in raw honey. Overall, the quality of raw honey is better than processed honey. In addition, raw honey has met the quality standard for consumption.

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