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Water Content of Stingless Bee Honey Varies by Season

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Abstract: The relative humidity of the air in the region where honey is produced has a significant influence on the moisture content of the honey. The production season, feed source, nectar type and concentration, colony strength, and physical environmental conditions are all factors that influence honey's moisture content. The goal of this study was to determine the moisture content of honey collected over the course of a year to learn more about the quality and safety of honey. One hundred fifty samples of honey gathered from Bone, Indonesia, during both the wet and dry seasons were analyzed to assess the percentage of moisture present in the honey. Honey's physical properties, microbiological value, sensory qualities, and economic worth are all affected by its moisture content. At a temperature of 25 degrees Celsius, an Abbetype standard model refractometer was used to measure the refractive index (RI) in accordance with the method recommended by the International Honey Commission. Comparing honeys produced during the wet season with those produced during the dry season revealed that there is a statistically significant variation in the quantity of moisture that is present ($p = 0.0029$). This demonstrated that the moisture content of honey during the dry season had a substantially different value compared to the wet season at the 0.01 level ($p = 0.00024$). Using the F test, it was determined that there was not a significant difference in the amount of moisture contained in specific varieties of honey that were produced during the wet seasons and those that were produced during the dry seasons.

Keywords: beekeepers, feed resources, moisture content, nutrition, raw honey.

Introduction

Raw honey is a natural product that is defined by its complex composition, which varies according to the kind of bee, geographic region, accessible food sources, and storage conditions. Honey is often taken in its purest form (Karabagias *et al*., 2014). Honey is a sticky, viscous solution that consists of approximately 88.5 percent carbohydrates (mostly glucose and fructose), 151.7 percent water, 0.10.4 percent protein, 0.2% ash, and small amounts of amino acids, enzymes, vitamins, and other substances such as phenolic antioxidants. Honey is extracted from the nectar of the honeybee (Buba *et al*., 2013; Kek *et al.*, 2017).

The relative humidity of the air in the region where honey is produced has a significant impact on the honey's moisture content. Other factors that influence honey's moisture content include the production season, feed source, nectar type and concentration, colony strength, and physical environmental conditions. Escuerdo *et al*., 2014; Lazarević *et al*., 2017; Sousa *et al*., 2016). In addition, the level of maturity of the honey as well as the time of harvest are crucial elements that can affect its level of wetness. Honeybees should be harvested according to the recommendations of those who practice husbandry when two-thirds of the wax comb has been covered with wax.

The presence of water in honey is necessary for maintaining its resistance to fermentation and granulation. Honey that has a low moisture content is resistant to the growth of microorganisms and can be preserved for a longer amount of time as a result (Akhtar *et al*., 2014). Honey's physical qualities are affected in

a variety of ways by the presence of moisture. The presence of high moisture levels in honey is another sign of honey that has been adulterated (Nyau *et al*., 2010; Obiegbuna *et al*., 2017). The higher the honey's moisture level, the greater the possibility that osmotolerant yeasts will ferment the honey while it is being stored (Viuda-Martos *et al*., 2010).

When there is less than 17.1% moisture in the honey, the fermentation process cannot take place. In addition, the microbiological load influences honey's stability when the moisture content is between 17.1% and 20%, whereas the presence of osmophilic yeasts can occur when the moisture content is greater than 20%. In addition to this, the ratio of glucose to water (G/W) can be utilized to forecast the crystallization of honey (Manikis and Thrasivoulou, 2001).

Many research have been conducted on the water content of honey obtained from honeybees. However, no research has been conducted to examine the water content of honey produced by stingless bees. The purpose of this study was to assess the moisture content of honey that had been gathered over the course of the year to acquire information regarding the quality and safety of honey. In addition, the F-test and analysis of variance were utilized to establish the significance of statistical differences in the amount of moisture present in the various varieties of honey as well as the seasons during which they were produced.

Material and methods

Research sample

Honey as many as 150 samples were produced by stingless bees obtained from local beekeepers in Bone District, South Sulawesi. A total of 75 samples were taken in 2020, and the same number of samples will be taken again in 2021. During the honey pot inspection, a sample of the honey is taken and placed in a container for analysis in the laboratory.

Water content analysis

To determining the amount of water present, refractometry was utilized. This technique involved measuring the refractive index (RI) at 25 degrees Celsius utilizing a standard model Abbetype refractometer in line with the Methods of the International Honey Commission (2009). After then, the percentage of water was determined with the use of the Chataway table.

Statistical analysis

The statistical analysis was performed with the help of the PAST software package, version 2.12, which was developed in Oslo, Norway. The information was arranged according to the various types of honey and displayed with a mean, standard error, minimum, and maximum for each value. In order to examine the data, we employed both a one-way analysis of variance (ANOVA) and Tukey's method for performing pairwise comparisons. The F test was carried out in order to ascertain whether or not there was a discernible change in the amount of moisture contained within particular varieties of honey between the two years.

Results and discussion

Variations in water content in stingless bee honey of several colonies

The amount of water that is present in a food product, as well as the materials that go into making that product, is a very essential quality criterion. It has a very major, if not crucial, influence on the quality, and more specifically on the shelf life, of nearly any item that is biotic in origin. When referring to quality control, it is common practice to make remarks and have conversations about the water content of the product. However, it is essential to be aware that it is considerably superior and far more significant to speak of water activity rather than simply of water content. This rule applies equally to honey as it does to everything else.

Water content may appear to be a more straightforward and clearly defined quantity than water activity at first look; yet we should be aware that water activity is more strongly related with concerns of product quality (stability, viscosity, and crystallization of honey) (Abramovic *et al*., 2008). There is no single test that can guarantee that a product is pure honey, but a variety of analyses can confirm that nothing has been added to or substituted for the real thing. Furthermore, we must consider the fact that the standard method for determining water content is based on refractometric data. As a result of the

nature of these measures, this procedure is not always immediately applicable to crystalline honey. When opposed to water activity, moisture content has the advantage of being an excellent and extremely comprehensible definition, making it the preferable criterion.

Several studies examining the water content of honey have also been published using various approaches or methods. Using the dilectric property sensing approach, Guo *et al.*, (2010) discovered that the amount of water content in honey ranged from 18 to 42.6%. The research reported that pure honey and water-added honey dielectric constants decreased monotonically with increasing frequency and increased monotonically with increasing water content. The dielectric loss factors revealed dielectric relaxation. With increasing water content, the critical frequency and maximum loss factor increased. The dielectric constant had strong linear correlations with the total soluble solids and water contents. Aside from dielectric property sensing, another method for determining the water content of honey is Karl Fischer titration (Scholz 1984; Isengard *et al*., 2001).

Based on the research results of Sanchez, et al., (2010) using this Karl Fischer titration method, the results showed that the RI and KF methods produced comparable results for determining water content in honeys. The solvent mixture also allowed for a shorter titration time, which may be advantageous when measuring water content in honey. In this current study, water content in honey samples tested during the wet season exceeded 20% in eight samples (28.6%). During the dry season, the moisture content of the honey samples tested was less than 18%, or ranged between 14.6 and 18.2%, with an average of 17.5±1.31% (Table 1).

Table 1. Indonesian stingless bee honey water content in Bone Regency, South Sulawesi

	Water content (%)			
	Production season			
Number of bee colony	**Wet season**		**Dry season**	
	Number of samples	**Average ± SD range**	**Number of samples**	**Average ± SD range**
Colony 1	1	20.3±1.7	1	16.3±1.5
Colony 2	2	22.4±1.5	2	17.4 ± 2.2^{bxy}
Colony 3	3	21.5±1.8	3	16.4 ± 1.4^{bxy}
Colony 4	4	23.1 ± 1.3^{ax}	4	18.4 ± 1.1^{ax}
Colony 5	5	22.5±1.1	5	17.3±1.4
Colony 6	6	21.6±1.2	6	16.5±1.5
Colony 7	7	23.3±1.3	7	14.6±2.4
Colony 8	8	22.4 ± 1.4^{by}	8	16.5 ± 1.2^{by}

^{a,b} $p<0.05$. ^{x,y} $p<0.01$

When honey from the rainy season and the dry season were compared, the results showed a statistically significant difference in the amount of moisture present ($p = 0.0029$). This suggests that there is a significantly different moisture content of honey during the dry seasons compared to the wet seasons at the 0.01 level ($p = 0.00024$). Previous literature reports indicated that Karl Fischer water content values differed slightly from Refractometric water content values. These variations could be attributed to the botanical origin and the composition of honey's dry matter (Isengard & Schultheiß, 2003; Isengard *et al*., 2001). In recent years, water activity has been studied as an alternative to water content as a characteristic of microbes' stability of honeys (Chirife *et al.*, 2006), and efforts have been made to correlate water activity with methods can be broadly classified water content.

The percentage variation in the water content of stingless bee honey between rainy and dry seasons

The results of the F-test indicated that there was not a statistically significant variation in the amount of water content present in the various varieties of honey that were produced

during the two different seasons. In terms of the average meteorological conditions, the flowering duration of a plant during the dry season is longer and more varied, whereas the flowering period of a plant during the wet season is shorter. Even though each season has its own unique weather patterns, we could not find any statistically significant changes in the amount of water that was contained in the honey that was produced during each season. The water content of all honey groups during both summer and fall may be found summarized in Figure 1.

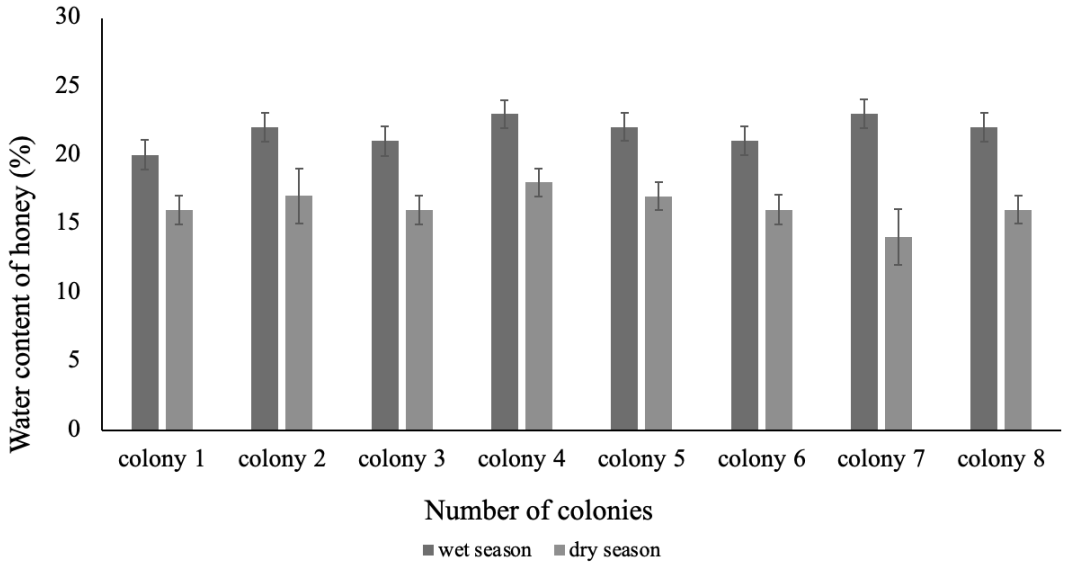

Figure 1. Water content of stingless bee honey summarized for wet and dry seasons

The proportion of stingless bee honeys appears to differ significantly from that of Apis honey. These honeys are frequently bitter. Some stingless bee honeys were reported to taste acidic by Cortopassi & Gelli (1991). Several researchers have discovered a high-water content in stingless bee honey (Cortopassi & Gelli, 1991; Vit *et al*., 2004; De Bruijn and Sommeijer, 1997; Torres *et al*., 2004). In a survey of 27 species, Roubik (1983) discovered an average water content of 31%. The volume of honey produced by stingless bees reflects their diversity in body size and colony population.

The presence of watery honey in stingless bees may be related to the humid tropical environment, where extracting water to low concentrations from nectar is difficult. When it is difficult to produce highly dehydrated honey, there is a risk of spoilage (Bijlsma *et al.,* 2006). Most honey is composed of sugars, with the monosaccharides fructose and glucose dominating and just trace amounts of the disaccharide's maltose and sucrose present. Other disaccharides and higher sugars (trisaccharides and oligosaccharides) can be detected in trace amounts.

Honey's water activity is typically, but not always, less than 0.60 because it includes a considerable number of monosaccharides (fructose and glucose) but only a little amount of water. Because honey has relatively little moisture, this is the case. This level of water activity is adequate to prohibit the growth of osmotic-tolerant yeast (Chrife *et al*., 2006). Fructose and glucose are the two primary sugars that are found in honey, along with trace amounts of many additional complex sugars. Honey is made up of concentrated water solutions of these sugars. International food standards state that honey is a pure product that cannot be contaminated with any other ingredient, including but not limited to water and other sweeteners (Diacu and Tantaveanu, 2007).

According to Valdés-Silverio *et al.*, (2018), the amount of water present in honey influences its physiology, microbiology, flavor, and commercial value. Most honeys have glucose as their primary sugar component, and this glucose can precipitate as glucose

monohydrate. The carbohydrate content of honey is mostly responsible for its crystalline structure, which is an important property of this substance. Honey has a propensity to crystallize with age. Honey that has a low glucose-to-water (G/W) ratio does not crystallize as easily as honey with a higher G/W ratio. The ability of honey to crystallize can be determined, in part, by a metric known as the G/W ratio (Zamora and Chirife, 2006; Escuredo *et al*., 2014).

The number of different kinds of microorganisms that can be found in honey is mostly determined by its type of honey and the amount of moisture it contains (Namini *et al*., 2018). There is a possibility that the growth of microbes is inhibited by the inherent qualities of honey, such as the pH level, the amount of water present, the oxidation-reduction potential, the nutrient content, and so on (Iurlina and Fritz, 2005). These findings make it abundantly clear that it is difficult to produce general quality standards for all types of honey produced by stingless bees. According to the findings of our research, which was conducted with the sole intention of determining the amount of water present in these honeys, the amount of water present in natural colonies ranges anywhere from 14 to 20%.

The discovery that the water content of honey produced by the same species of stingless bees appears to vary depending on the region in which it is produced further complicates the process of establishing quality standards. According to the suggestions made by Vit *et al*., (2004), the maximum amount of water that should be present in honey made from Melipona, Scaptotrigona, or Trigona honey should be 30 percent. It is remarkable that almost none of the honeys that were collected for this research reached levels that were so low.

Conclusion

According to the results of this study, the amount of water that was found in the different varieties of honey that were tested varies, which may be due to the different kinds of feed that are available. There was not a discernible change in the amount of water present in particular varieties of honey that were produced during either the wet or dry seasons. The findings of this research might be helpful for comparing our study to others like it that have been conducted in other parts of the world. Additionally, they might contribute information that is pertinent to predicting crystallization, viscosity, behavior, and microbiological quality in various kinds of honey.

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