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Original Article

CORRELATION OF ALKALOID CONTENT AND TASTE OF HONEY FROM VARIOUS PROVINCES IN INDONESIA

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ABSTRACT

Objective: This study analyzed the correlation between alkaloid contents and taste (bitter and rough taste).

Methods: Qualitative analysis was analyzed using Mayer and Draggendorff methods, while quantitative analysis was analyzed using spectrophotometric methods. Taste evaluation in each sample was carried out by descriptive method with parameters including bitter and rough taste. The correlation between alkaloid content and taste in honey was statistically analyzed using a correlation test.

Results: The alkaloids testing showed that the sample with the highest alkaloid content was *Tetragonula fuscobalteata* honey from Sumbawa province, with an alkaloid content of 119.71 mg/g. In contrast, the sample with the lowest alkaloid content was *Geniotrigona thoracica* from West Sumatra at 21.24 mg/g. Taste evaluation results showed that the sample with the highest alkaloid content had the most bitter and rough taste.

Conclusion: Honey that has the highest alkaloid content is *T. fuscobalteata* Sumbawa, with an alkaloid content of 119.71 mg/g, because the availability of adequate alkaloid feed supports this species. In contrast, the honey with the lowest alkaloid content was *G. thorasica,* with an alkaloid content of 21.24 mg/g, because the availability of adequate alkaloid feed did not support this species. Based on the data, the alkaloid content of honey has a linear correlation with the bitter and rough taste. The higher alkaloid content in honey causes the honey taste to become more bitter and rough.

Keywords: Alkaloids, Honey, Taste

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INTRODUCTION

Honey is a superior product in the forestry sector with many health benefits [1]. Honey can be obtained from bees, which are based on biological characters divided into two groups: stinging bees and stingless bees. In general, honey has a very high nutritional content, including sugar, various minerals, as well as various phytochemical compounds, including phenols, flavonoids, and alkaloids [2] which can be beneficial for the body including as an anti-inflammatory, antibacterial, antiviral, antidiabetic, and antioxidant [3].

Alkaloids are a class of alkaline organic compounds with toxic characteristics that can be used as detoxifiers to neutralize toxins in the body [4]. These characteristics cause alkaloids to become one of the components that cause a bitter taste in honey [5]. In addition, alkaloids specifically act as antimicrobials that can prevent various diseases caused by microbial infections [7].

Taste is one of the most important aspects in determining consumer acceptance of food. In addition, honey is a food commodity known for its sweet taste [1]. Thus, it is important to analyze the levels of alkaloids as the potential compound that cause the bitter and rough taste of honey and how much influence the levels of alkaloids have on honey's bitter and rough taste. Furthermore, since alkaloids are good for health, an analysis of the alkaloid content in honey is also needed to determine potential honey sources to produce optimal alkaloid levels of honey.

This study aims to analyze the correlation between alkaloid contents and honey's bitter and rough taste.

MATERIALS AND METHODS

Materials

The materials used in this study included honey samples from the bees *Tetragonula laevicep, Tetragonula biroi, Heterotrigona itama, Geniotrigona thorasica, Wallacetrigona incisa, Tetragonula fuscobalteata, Apis. cerana, Apis melifera* and *Apis dorsata* collected by CV. Nutrima, Bogor, 1, 10-phenanthroline, aquadest, hydrochloric acid (HCl), bismuth nitrate ($Bi(NO_3)_3$), ethanol (C_2H_5OH), Ferrum chloride ($FeCl_3$), potassium iodide (KI), colchicine, and mercury chloride ($HgCl_2$).

Qualitative analysis of alkaloids

Qualitative analysis of alkaloids was carried out using the Mayer (M) and Draggendoff methods (D) [10]. 3 ml of honey sample in a test tube. Add 0.3 ml of 2N HCL. Heat the test tube for 30 min, then drain the test tube. Add three drops of Mayer/Draggendorff reagent and observe the formation of a precipitate.

Quantitative analysis of alkaloids

Quantitative analysis of alkaloids was carried out using the spectrophotometry method [11]. First, prepare a honey sample, add 1 ml of 0.025 $FeCl_3$ in 0.5M HCL, and then add 1 ml of phenanthroline. Next, place in a 25 ml volumetric flask using distilled water. Incubate at 70 °C for 30 min. Then, measure the absorbance of the sample using UV-vis spectrophotometry with a wavelength of 510 nm.

Taste evaluation

Sensory evaluation begins with a threshold test to test the sensitivity of the panels. The threshold test was carried out using the limit method [12] with coffee samples with a concentration of 0.01%-0.05% to obtain 15 semi-trained panellists, and taste evaluation was carried out using a descriptive way with parameters such as bitter and rough taste.

Statistical analysis

The relationship between alkaloid content with the bitter and rough taste was tested statistically using a correlation test.

RESULTS AND DISCUSSION

Qualitative analysis of alkaloids

Qualitative alkaloids were analyzed using Mayer and Draggendorff reagents [10]. The data result is shown in fig. 1.

Fig. 1: Qualitative analysis result of alkaloid

S. No.	Sample	Province	M	D
1	*A. melifera*	Central Java	-	+
2	*A. melifera*	West Java	-	+
3	*A. dorsata*	East Nusa Tenggara	+	+
4	*A. dorsata*	Bangka Belitung	+	+
5	*A. cerana*	North Sumatra	+	+
6	*A. cerana*	West Java	+	+
7	*T. laevicep*	Central Java	+	+
8	*T. laevicep*	Banten	+	+
9	*T. biroi*	Sulawesi Tenggara	+	-
10	*T. biroi*	South Sulawesi	+	+
11	*H. itama*	West Kalimantan	+	+
12	*H. itama*	South Sumatra	-	+
13	*G. thorasica*	North Sumatra	-	+
14	*G. thorasica*	West Sumatra	+	-
15	*T. fuscobalteata*	Sumbawa	+	+
16	*T. fuscobalteata*	Lombok	+	+
17	*W. Incisa*	Sulawesi Tenggara	+	+
18	*W. Incisa*		+	+

Mayer's reagent is made of mercury chloride (II), which is reacted with potassium iodide to produce mercury (II) iodide, which in excess will have potassium tetraiodomercurate (II) [13]. The reaction between Mayer's reagent and the alkaloid component causes the nitrogen atom in the alkaloid to form a covalent bond with the metal ion K^+ from the potassium tetraiodomercurate (II) complex. This reaction can occur because the nitrogen atom in the alkaloid has a lone pair of electrons so that it can form coordinate covalent bonds with metal ions. The reaction between nitrogen atoms and metal ions K^+ produces a white precipitate of potassium-alkaloid, which indicates the results of qualitative analysis of alkaloids can be declared positive [14].

Draggendorff is made by dissolving bismuth nitrate with potassium iodide to prevent hydrolysis. Hydrolysis reactions occur because bismuth salts quickly form bismuthyl ions (BiO^+). Adding HCl to bismuth nitrate aims to make the equilibrium move to the left so that Bi^{3+}ions can be maintained. After being dissolved in HCl, Bi^{3+} from bismuth nitrate reacts with excess potassium iodide to form a potassium-tetraiodobismuthate complex [13].

The results in fig. 1 show that *A. melifera* West Java, *A. melifera* Central Java, *H. itama* South Sumatra, and *G. thorasica* North Sumatra showed negative results on Mayer's reagent, and *T. biroi* Sulawesi Tenggara and *G. thorasica* West Sumatra showed negative results on Draggendorff's reagent. In conclusion, it can be stated that all samples showed positive results because the results of the analysis can be declared positive if at least one of the two reagents used shows a positive result [10]. Most of the previous study also states that qualitative analysis of alkaloids in honey showed positive results in *Apis dorsata* species from North Sulawesi [15], *Apis melifera* from Nigeria [16], and *Apis cerana* from India [17] as part of the stinging bee group. In addition, various works of literature also state that honey from the stingless bee group also showed positive results in qualitative analysis of alkaloids, including *Tetragonula biroi* from South Sulawesi [18], *Heterotrigona itama* from West Kalimantan [19], *Trigona incisa* from West Kalimantan [20], and *Tetragonula fuscobalteata* from Lombok [21].

Quantitative analysis of alkaloids

Quantitative analysis of alkaloid content was carried out using a set of UV-vis spectrophotometry tools. The alkaloid content was analyzed by adding 1 ml of $FeCl_3$ and 1 ml of 1,10-phenanthroline. The reaction occurs when $FeCl_3$ oxidizes the alkaloids contained in the sample to produce 3,4,5,6 tetrahydroreserpine and Fe^{2+}. The Fe^{2+}ion from the response reacts with 1,10-Phenanthroline and produces tris-1,10-phenanthroline, which has a reddish-orange colour [11]. The data obtained are shown in fig. 2.

The data from fig. 2 show that honey from different species and provinces may exhibit different levels of alkaloids. The differences of alkaloids content was caused by differences in the availability of feed sources [23].

The quantitative analysis of alkaloid content showed that the honey with the highest alkaloid content was *T. fuscobalteata* honey from Sumbawa province. *T. fuscobalteata* from Sumbawa has an alkaloid content of 119.71±1.66 mg/g. According to previous research, honey has an alkaloid content of 2,580 mg/100g or 25.8 mg/g [23]. The high content of alkaloids in honey obtained from Indonesia is influenced by the availability of feed sources which are the primary source of nutrition for bees. Therefore, Indonesia has the potential to show higher yields because Indonesia has a very fertile and diverse diversity of feed plants. In addition, according to [25], Indonesia has the 3rd largest tropical forest area after Brazil and Congo, with a high abundance of flora and fauna and the potential for food sources for bees.

T. fuscobalteata honey obtained from different regions has different levels of alkaloids. For example, in contrast to Sumbawa *T. fuscobalteata* honey which has an alkaloid content of 119.71±1.66 mg/g, *T. fuscobalteata* honey obtained from Lombok has an alkaloid content of 68.33±0.99 mg/g. These differences are caused by differences in the availability of feed sources in each province.

T. fuscobalteata from Sumbawa has a higher alkaloid content because potential food source species surround it. The Sumbawa *T. fuscobalteata* bee has a source of propolis in the form of resin obtained from mango trees and guava trees. The mango tree *(Mangifera indica)* is one species with potential feed sources in producing resins with high alkaloid content. This statement is supported by research which states that mango leaf extract *(Mangifera indica)* showed positive results in qualitative analysis of alkaloids [25]. In addition, other studies also say that mango leaf extract *(Mangifera indica)* is rich in alkaloid compounds that can act as antimicrobials by interfering with the formation of cell walls in bacteria that causing bacteria to become efficiently lysed and cause cell death [26]. In addition to the mango tree *(Mangifera indica),* the cashew tree *(Anacardium occidentale)* is also a potential food source species in producing resins rich in alkaloids because it shows positive results in qualitative analysis of alkaloids [27]. According to [31], cashew leaf extract *(Anacardium occidentale)* is rich in alkaloid compounds that work effectively as antimicrobials, especially against *Salmonella thypi.*

T. fuscobalteata Sumbawa obtains feed sources not only to obtain resin as a source of propolis but also as a source of nectar which will be processed into honey. Data from interviews with the farmers also stated that *T. fuscobalteata* Sumbawa honey has a variety of primary feed sources and contains alkaloids. There are mangrove trees *(Avicenna marina)* [29], acacia *trees (Acacia auriculiformis)* [30] and also teak *(Tectona grandis)* [34], which showed positive results in qualitative analysis of alkaloids. In contrast to *T. fuscobalteata* honey from Sumbawa, *T. fuscobalteata* honey from Lombok has more limited availability of alkaloid feed sources, including those obtained from teak trees *(Tectona grandis)* [31], avocado trees *(Persea americana).* [32] and coconut trees *(Cocos nucifera)* [33] showed positive results on the qualitative test of alkaloids. Meanwhile, extracts obtained from banana trees *(Musa paradisiaca)* [34], and longan *(Dimocarpus longan)* [35] showed negative results in the qualitative alkaloid test.

Honey with the lowest alkaloid content, namely *G. thorasica* from West Sumatra has an alkaloid content of 21.24±0.49 mg/g, and honey of *G. thorasica* from North Sumatra has an alkaloid content of 34.77±3, 87 mg/g. The low level of alkaloids in honey is caused by the characteristics of feed sources that do not provide optimal alkaloids. Based on several previous studies. *G. thorasica* honey only obtained feed sources containing alkaloids from the ambacang tree *(Mangifera foetida)* [36] and johar tree *(Cassia siamea)* [41], which showed positive results in the qualitative analysis of alkaloids.

In contrast, matoa *(Pommetia pinata)* leaf extract [38], bridal tear tree *(Antigonon leptopus)* [39] and rubber tree leaf extract *(Ficus elastica)* [40] showed negative results in qualitative analysis of alkaloid content. Meanwhile, *G. thorasica* honey from North Sumatra has an alkaloid source from the sidaguri tree *(Sida rhombifolia)* [41], coconut tree *(Cocos nucifera)* [33] and water guava tree *(Syzygium aqueum)* [42] which were positive for alkaloids. In contrast, the tarap tree *(Artocarpus odoratissimus)* [43] showed negative results in the qualitative analysis of alkaloids.

Fig. 2: Alkaloids content and their bee species and plant origin of honey from various provinces in Indonesia

S. No.	Bee species of honey	Alkaloid content of honey (mg/g)	Plant origin
1	*T. fuscobalteata* (Sumbawa)	119,71±1,66	*Mangifera indica* *Anacardium occidentale* *Avicenna marina* *Tectona grandis* *Acacia Aauriculiformis*
2	*T. fuscobalteata* (Lombok)	68,33±0,99	*Persea americana* *Musa paradisiaca* *Tectona grandis* *Dimocarpus longan* *Cocos nucifera*
3	*H. itama* (South Sumatra)	119,13±0,83	*Hevea brasiliensis* *Melastoma malabathricum* *Macarang apruinosa*
4.	*H. itama* (west Kalimantan)	35,90±1,66	*Acacia auriculiformis* *Mangifera orodata* *Rhizophora mucronatta* *Xylocarpus moluccensi* *Vitex pinnata L.*
5.	*A. dorsata* (Bangka Belitung)	104,79±2,21	*Elaecarpus sp.*
6.	*A. dorsata* (East Nusa Tenggara)	53,92±4,98	*Schleichera oleosa*
7	*A. cerana* (North Sumatra)	88,58±4,15	*Calliandra spp* *Cocos nucifera* *Arenga pinnata* *Syzygium aqueum* *Theobroma cacao*
8	*A. cerana* (West Java)	43,31±2,49	*Citrus limon* *Cocos nucifera* *Muntingia calabura* *Dombeya ratudifolia* *Calophyllum soulattri*
9	*T. laevicep* (Banten)	88,42±1,11	*Swietania mahagony* *Acacia auriculiformis* *Cocos nucifera* *Garcinia mangostana*
10	*T. laevicep* (Central Java)	87,17±1,25	*Swietania mahagony* *Mangifera indica* *Theobroma cacao* *Syzygium aqueum*
11	*T. biroi* (South Sulawesi)	83,65±1,11	*Theobroma cacao* *Mangifera indica* *Calliandra spp* *Michelia campaca* *Syzygium aqueum*
12	*T. biroi* (Sulawesi Tenggara)	62,88±1,33	*Dimocarpus longan* *Cocos nucifera* *Mangifera indica* *Citrus aurantiifolia*
13.	*A. melifera* (Central Java)	75,50±0,55	*Ceiba pentandra*
14	*A. melifera* (West Java)	53,57±2,77	*Nephelium lappaceum*
15.	*W. incisa* (Sulawesi Tenggara)	52,79±0,55	Pohon *Dillenia serrata* *Macadamia integrifolia*
16	*W. incisa* (South Sulawesi)	31,25±2,21	*Anacardium occidentale*
17	*G. thorasica* (West Sumatra)	21,24±0,49	*Cassia siamea* *Antigonon leptopus* *Pommetia pinata* *Mangifera foetida* *Pommetia pinata*
18	*G. thorasica* (North Sumatra)	34,77±3,87	*Artocarpus odoratissimus* *Sida rhombifolia* *Syzygium aqueum* *Cocos nucifera*

Values are expressed as mean±standard deviation (SD) each data is obtained based on the results of duplicate analysis

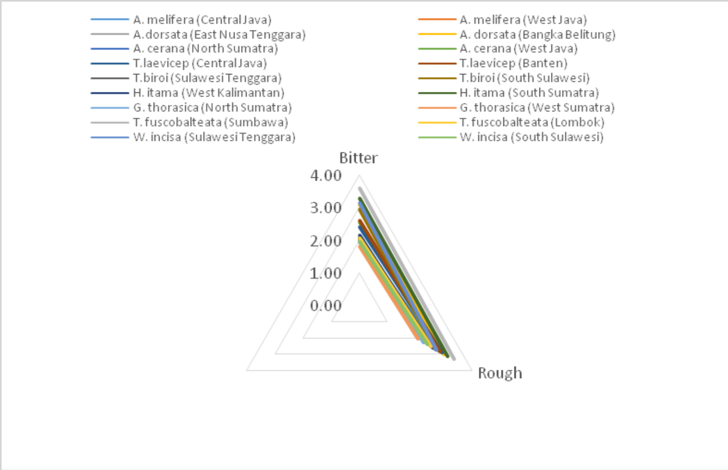

Fig. 3: The taste evaluation results

Taste evaluation

Taste evaluation of 18 types of honey was carried out on 15 semi-trained based on the sensitivity test stage using the threshold test method. Taste evaluation was done on two parameters: bitter taste and rough taste. Taste evaluation results are shown in fig. 3.

According to [44], honey has a taste that tends to be sweet, a colour that ranges from yellowish brown to blackish brown, and a variety of viscosities. But in addition to the sweet taste that is identical to the high sugar content of honey, some honey also has a bitter taste caused by the presence of alkaloid compounds [45].

The results of the taste evaluation showed that *T. fuscobalteata* honey from Sumbawa had the most bitter and rough characteristics. Meanwhile, *G. thorasica* honey from West Sumatra has the sensory attributes of being the least bitter and rough.

The bitter and rough taste of *T. fuscobalteata* honey is caused by the high content of alkaloids in this type of honey. According to a previous study [50], Alkaloid compounds cause honey's bitter and rough taste. The toxic characteristics of the alkaloids cause the bitter taste, and the rough taste is caused by the alkaline elements of the alkaloids [4]. The level of bitter taste contained in honey is also influenced by the concentration of alkaloids in it, following the previous study [51], which states that the higher the concentration of alkaloids, the stronger the bitter taste. In contrast, *G. thorasica* honey has sensory characteristics that are the least bitter and not abrasive, which correlates with its very low alkaloid content compared to *T. fuscobalteata* honey.

Correlation between alkaloids content and taste characteristics

Alkaloids naturally have a characteristic bitter and rough taste [4]. The correlation between alkaloid levels and bitter taste in honey is shown in fig. 4.

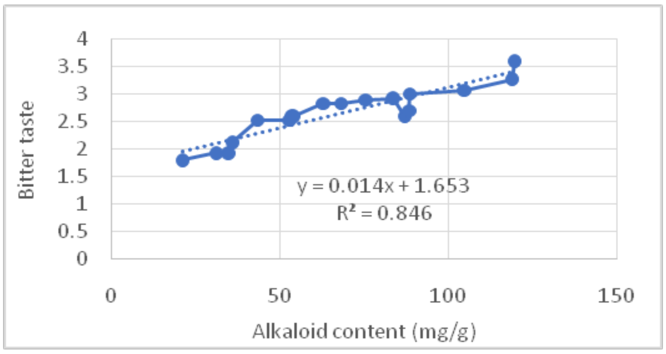

Fig. 4: Correlation between alkaloids content and bitter taste

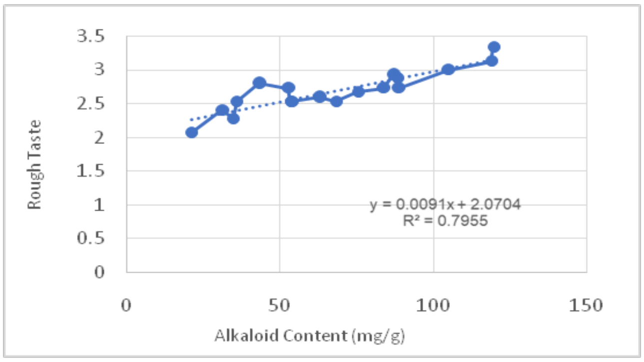

Fig. 5: Correlation between alkaloids content and rough taste

The results indicate that the linear equation between alkaloids and rough taste levels has an R square value of 0.7955, indicating a relatively strong correlation. Furthermore, this equation shows that the higher the alkaloid content in honey, the stronger the intensity of the rough taste.

CONCLUSION

Honey that has the highest alkaloid content is *T. fuscobalteata* Sumbawa, with an alkaloid content of 119.71 mg/g because the availability of adequate alkaloid feed supports this species. At the same time, the honey with the lowest alkaloid content was *G. thorasica,* with an alkaloid content of 21.24 mg/g, because the availability of adequate alkaloid feed did not support this species. Based on the data, the alkaloid content of honey has a linear correlation with the bitter and rough taste. The higher alkaloid content in honey causes the honey taste to become more bitter and rough.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author declares no conflict of interest associated with this study

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