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ADULTERATION IDENTIFICATION FOR BITTER TASTE HONEY SAMPLES FROM MARKET

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Abstract

Honey with a bitter flavour available on the market is expected to meet quality standards outlined by the Indonesian National Standard (SNI). This study aimed to determine adulteration in such honey products using SNI as the benchmark. Tests conducted included analyzing moisture content through Karl Fischer titration, diastase activity, hydroxymethylfurfural (HMF) levels, sucrose concentrations via the Anthrone method by UV-visible spectrophotometry, and reducing sugar content using HPLC-RI. Eight honey samples were assessed—seven bitter and one sweet (as a reference). Findings revealed several bitter honey samples failed to comply with SNI criteria, signalling possible adulteration. These results underscore the importance of adhering to SNI to ensure honey quality.

Keywords: adulteration, bitter taste honey, honey quality, SNI

Introduction

Honey has historically been acknowledged for its nutritional and medicinal benefits. In Indonesia, honey production mainly utilizes the Apis indica and Apis mellifera bee species. Notwithstanding its advantages, adulteration—particularly in bitter honey—represents an escalating concern. This study assesses bitter honey products concerning SNI criteria, emphasizing the impact of adulteration on consumer safety and product integrity.

Honey is inherently sweet and has historically been used as a health cure. Domestic and industrial honey production in Indonesia predominantly employs Apis indica and Apis mellifera.[1] The demand for honey encompasses the pharmaceutical, food, and cosmetics sectors, propelled by its antioxidant properties and many health advantages.[2]

The quality of honey is assessed according to characteristics specified in SNI, which also facilitates the identification of adulteration.[3,4] Numerous improvements have resulted in the amalgamation of honey with herbal constituents, accompanied by assertions of particular health advantages. Bitter honey has acquired appeal for these

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reasons but is accused of adulteration due to its distinctive flavour profile. These products may fail to comply with SNI's standards and be deemed counterfeit or inferior.[5]

The SNI delineates quality standards for honey, including a maximum water content of 22% w/w, a minimum reducing sugar content of 65% w/w, a maximum sucrose content of 5% w/w, a maximum HMF level of 50 mg/kg, and a minimum diastase activity of DN 3. Verifying that bitter honey complies with these standards is crucial for evaluating possible adulteration.[4]

Method

Apparatus

The instruments used included a Microscope (Olympus CKX41, Olympus, Japan), Karl Fischer titrator (Mettler Toledo, USA), visible spectrophotometer (Shimadzu, Japan), High-Performance Liquid Chromatography (HPLC) system (BIORAD, CA, USA), Aminex HPX87H column (BIORAD, CA, USA).

Material

The samples comprised seven varieties of bitter honey, designated A-G, sourced from various markets in Java and Sumatra, and a sweet honey sample labelled X for reference.

Procedure

Organoleptic and Microscopic Characterization

This investigation utilized methanol (Merck, Germany) and water as solvents. Iodine solution, Anthrone reagent (Merck, Germany), and saturated lead acetate (Merck, Germany) were utilized as reagents. Supplementary reagents comprise Carrez I and II solutions (Merck, Germany) and sodium oxalate (Merck, Germany).

Organoleptic evaluations measured colour, flavour, and fragrance, performed subjectively by researchers. The microscopic study assessed the presence of pollen grains and plant cells indicative of honey authenticity.

Determination of Moisture Content

The moisture content was determined via the Karl Fischer titration method with a Karl Fischer titrator (Mettler Toledo, USA). Approximately 200 mg of the material was combined in a titration flask with an iodosulfur solution (Merck, Germany).[6] The mass of water in the sample was calculated using the formula:

$$B=VxF$$

Where:
B = weight of water in the sample (mg)
V = volume of iodosulfur solution used in the titration
F = water equivalence of the iodosulfur solution

Diastase Activity and HMF Analysis (SNI Methods)

The activity of the diastase enzyme was assessed utilizing a standard starch solution (Merck, Germany) at 40˚C, with absorbance recorded every 5 minutes at λ 660 nm via a visible spectrophotometer (Shimadzu, Japan). The activity was determined using reaction time data, and a graph was constructed mapping absorbance against reaction time to ascertain enzyme activity at an absorbance of 0.235. Subsequently, the diastase enzyme activity was computed using the following formula:

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$$\text{Diastase Number} = \frac{300}{t_{A_{660}}0{,}235}$$

Where: t_{A660} = time when the absorption is at 660.

HMF concentrations were evaluated by extracting 20 mg of the sample in 60 mL of methanol (Merck, Germany). The obtained solution was subjected to treatment with Carrez I and II reagents (Merck, Germany) and examined via UV spectrophotometry (Shimadzu, Japan) at wavelengths of λ284 nm and λ366 nm, using a comparative solution of 0.2% $NaHCO_3$ (Merck, Germany). The HMF concentration is determined utilizing the subsequent equation:

$$\text{HMF}\left(\frac{\text{mg}}{100\text{g}}\text{sample}\right) = \frac{(A_{284}\text{-}A_{336})\times\text{Correction Factor}}{\text{Sample Masses (g)}}$$

Where:
HMF: Hydroxymethylfurfural
A284: Absorbance at λ 284
A336: Absorbance at λ 336
Correction Factor: based on the dilution factor used.

Determination of Sucrose Content Utilizing the Anthrone Method

The Anthrone reagent, a reactive chemical for sucrose detection, was employed in this approach. A calibration curve was established by creating standard sucrose solutions at 40, 80, 120, and 160 g/L concentrations. To make the standard solution, 1 gram of $CaCO_3$ (Merck, Germany) was dissolved in 50 mL of distilled water. The mixture was heated for 30 minutes and thereafter permitted to cool. After cooling, 2 mL of saturated lead acetate (Merck, Germany) was added, followed by distilled water, to achieve 100 mL. The resultant solution was filtered, and sodium oxalate (Merck, Germany) was subsequently included. Subsequently, 10 mL of the filtrate was centrifugated until a precipitate was generated. Subsequently, 100 µL of the clear supernatant was transferred to a new tube, combined with 900 µL of distilled water, and reacted with 5 mL of 0.1% Anthrone reagent (Merck, Germany). The tube was sealed, homogenized, and subjected to boiling water for 12 minutes. After removal, the tube was swiftly cooled with running water, homogenized once again, and the absorbance was assessed at λ 630 nm using a visible spectrophotometer (Shimadzu, Japan).[7]

For sample analysis, 5 grams of honey were dissolved in 50 millilitres of distilled water.The prepared sample solution was subjected to the previously described procedure to quantify sucrose concentration.

Determination of Glucose and Fructose Content by HPLC Refractive Index Method

As sugars were reduced in the honey sample, the glucose and fructose constituents were quantified using high-performance liquid chromatography (HPLC).[8] The ideal formulation for the HPLC mobile phase comprised 0.005 M H_2SO_4, employing an Aminex HPX87H column (BIORAD, CA, USA) with a refractive index detector. A calibration curve was created using standard glucose and fructose solutions at concentrations of 60, 80, 100, 120, 140, and 160 g/L. Standard solutions were injected into the system in 20 µL increments, and their peak areas were documented to formulate equations linking peak area to concentration.

To evaluate glucose and fructose concentrations in the honey samples, roughly 0.1 g of each sample was precisely measured and transferred into a 10 mL volumetric flask. Distilled water was included, and the mixture underwent ultrasonic agitation for 30 seconds to guarantee thorough dissolving and homogeneity. The solution was diluted to

the specified mark, filtered, and 20 µL was fed into the HPLC apparatus. The resultant peak areas were compared to the calibration curves to determine the amounts of glucose and fructose in each sample.[9]

Result

The organoleptic characteristics of bitter honey are summarized in Table 1:

Table 1. Organoleptic Characteristics of Honey Samples

Sample	Code	Color	Taste	Aroma
Honey "PMA"	A	Black	Bitter	Jamu Aroma
Honey "PMB"	B	Black	Bitter	Jamu Aroma
Honey "PMC"	C	Black	Bitter	Jamu Aroma
Honey "PKG"	D	Bright Yellow	Bitter	Honey Aroma
Honey "PHJ"	E	Dark Brown	Bitter	Honey Aroma
Honey "PAB"	F	Cloudy Brown	Bitter-Sour	Honey Aroma
Honey "PBB"	G	Cloudy Brown	Bitter-Sour	Honey Aroma
Honey "MRM"	X	Golden Yellow	Sweet	Honey Aroma

Explanation:
PMA: Bitter label A
PMB: Bitter label B
PMC: Bitter label C
PKG: Bitter Yellow Garut
PHJ: Bitter Black Jogja
PAB: Pelawan A Bangka
PBB: Pelawan B Bangka
MRM: Sweet Randu Malang

Organoleptic characteristics revealed variations in colour, taste, and aroma among samples. Darker colors and bitter tastes were predominant in sample A-C, while sample X exhibited characteristics of pure honey.

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The Microscopic Characteristics of Bitter Honey are summarized in Figure 1:

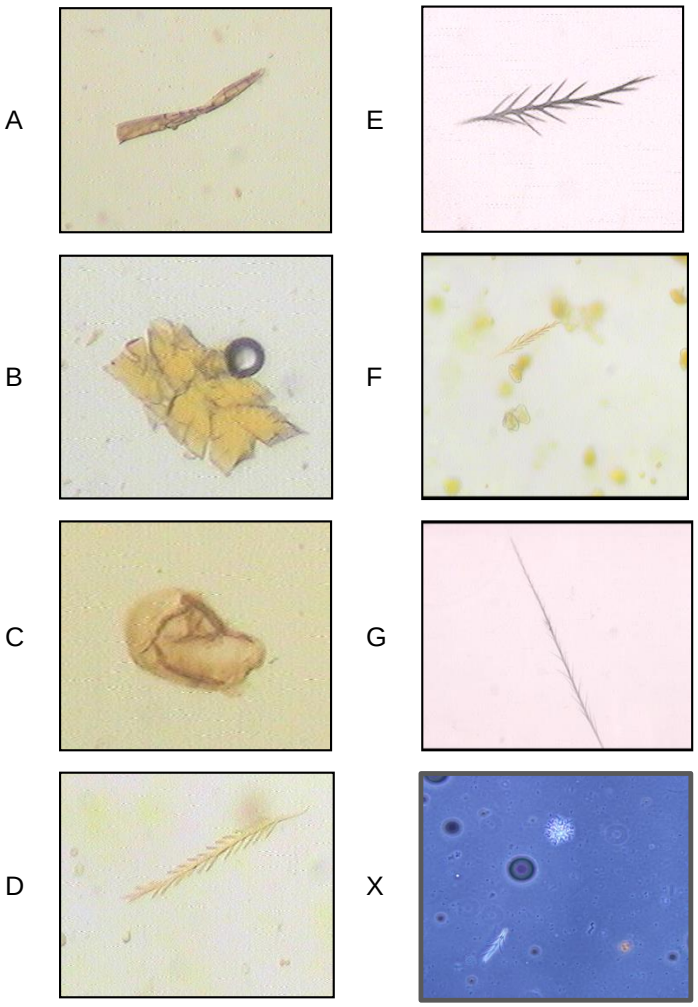

Figure 1. Microscopic characteristics of honey; samples A-G: bitter honey (Magnification 5/0.10) and X: sweet honey (Magnification 10/0.25

Microscopic analysis identified pollen grains in some bitter honey samples, but others displayed foreign plant fragments, indicating potential adulteration.

Data on the Adulteration Examination of Bitter Honey Circulating in the Market is summarized in Table 2:

Table 2. Data on the Adulteration Examination of Bitter Honey Circulating in the Market

No.	Samples	Water Content (% b/b)	Diastase Number	HMF Content (mg/kg)	Reducing Sugar (% b/b)	Sucrose Content (% b/b)
1	X	20,15±0,02	4,35±0,02	0,01±0,01	104,34	4,35±0,02
2	A	22,03±0,02*	3,81±0,03	68,99±0,01*	76,86	83,87±0,02*
3	B	15,75±0,05	4,20±0,03	60,70±0,02*	64,91*	64,20±0,02*
4	C	24,54±0,04*	2,78±0,01*	36,57±0,02	78,78	62,78±0,03*

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Table 2. (Extension)

No.	Samples	Water Content (% b/b)	Diastase Number	HMF Content (mg/kg)	Reducing Sugar (% b/b)	Sucrose Content (% b/b)
5	D	17,86±0,02	4,17±0,04	36,88±0,02	78,95	84,17±0,01*
6	E	16.,44±0,04	2,65±0,02*	68,47±0,03*	59,30	82,64±0,02*
7	F	19,57±0,03	3,02±0,02	40,56±0,01	61,07*	63,02±0,02*
8	G	18,74±0,03	3,57±0,03	56,72±0,02*	68,44	63,56±0,02*
	SNI	Maks. 22	Min. 3	Maks.50	Min. 65	Maks. 5

Explanation: Mean value ± standard deviation, with the analysis repeated three times for each procedure.
*) Does not meet the SNI criteria

Quality testing showed that samples A, B, C, E, F, and G failed to meet SNI criteria for water content, HMF, and sucrose levels. In particular, high sucrose content in all bitter honey samples suggested adulteration.

Discussion

Organoleptic Characteristics of Bitter Honey

The organoleptic assessment of colour in the bitter honey samples indicated that the majority possessed a rich, dark shade, and all displayed a distinct bitter taste. Furthermore, samples A, B, and C exhibited an aroma akin to herbal medication or jamu. The dark hue indicates that caramelization and the Maillard reaction have transpired in the honey due to further processing.[10]

Microscopic Characteristics of Bitter Honey

All honey samples were examined for macroscopic organoleptic characteristics and microscopic attributes at 5/0.10 and 10/0.25 magnifications. Microscopic examination of the honey disclosed many bits of pollen, plant cells from the nectar source, and a few sugar crystals. Honey contaminated with sugar syrup had no discernible pieces of pollen or plant cells. Honey infused with herbal extracts exhibited the presence of supplementary plant cell fragments.

Figure 1 illustrates that bitter honey samples D-G and sweet honey sample X exhibited unique bits of pollen and plant cells originating from their respective honey sources. Conversely, samples A-C exhibited no pollen or plant fragments; however, pieces from other plants—presumably from incorporated herbal extracts—were evident. Furthermore, sucrose sugar crystals were detected, which should not be seen in pure honey unless there was a substantial addition of sucrose.[11]

Parameters of Honey Quality As per SNI

The Indonesian National Standard (SNI) specifies the quality criteria for premium honey, which encompass a maximum moisture content of 22% (w/w), a minimum reducing sugar content of 65% (w/w), a maximum sucrose content of 5% (w/w), a maximum ash content of 0.5% (w/w), a maximum hydroxymethylfurfural (HMF) content of 50 mg/kg, and a minimum diastase enzyme activity of 3 DN.

The moisture content in bitter honey samples A and C surpassed the allowable limit set by the SNI. Increased moisture levels in honey can compromise its quality, potentially impacting enzymatic processes like hydrolysis and heightening the likelihood of microbial proliferation and deterioration. The adulteration of honey with syrups, such as corn syrup, can result in elevated moisture levels.[12]

In samples C and E, the diastase number (DN) fell short of the criterion established by SNI. The diastase number signifies the enzyme activity characteristic of authentic

honey from bees. A high diastase number indicates active enzymatic activity, signifying honey freshness and confirming its safety for eating.[2]

Four of the seven bitter honey samples (A, B, E, and G) surpassed the maximum permissible HMF concentration stipulated by SNI. HMF is an essential quality metric to evaluate the freshness of sugar-laden products such as syrup, dairy, and honey.[13] It is also a marker for honey adulteration with fructose or sucrose syrups. HMF may present health hazards as it is possibly poisonous, mutagenic, and carcinogenic.[1] Although usually found in honey, elevated levels of HMF indicate sugar breakdown in honey products. HMF is produced during the Maillard reaction, a non-enzymatic, acid-catalyzed browning process that transpires when sugars dehydrate.[14,15]

The Anthrone reagent, which interacts with sucrose, facilitates the measurement of sucrose concentrations by spectrophotometry.[7] This method's regression equation for sucrose content is $y = 0.004x + 0.003$, with an R^2 value of 0.996. All bitter honey samples surpassed the sucrose content threshold established by SNI, suggesting the probable addition of sucrose syrup to these samples.

A chromatographic system operating at a flow rate of 0.6 mL/min and a column temperature of 60°C yielded the following regression equations: for glucose, $y = 82.64x - 994.39$ ($R^2 = 0.9898$), and for fructose, $y = 86.32x - 1695.88$ ($R^2 = 0.9854$). The resulting peak regions were used in the conventional regression models for glucose and fructose to ascertain their percentage content, subsequently aggregated as reducing sugars.

All bitter honey samples exhibited a greater glucose content compared to fructose. Sweet honey sample X exhibited the most significant glucose and fructose levels, validating its classification as pure honey with a reducing sugar content of over 65%. Nevertheless, samples B and F failed to satisfy the SNI criteria.

Prevalent techniques for honey adulteration encompass dilution with sugar syrup, fructose syrup, or corn syrup, and incorporating artificial sweeteners, granulated sugar, and food colouring.[1]

Conclusion

This study demonstrated that multiple bitter honey samples failed to comply with the quality standards established by SNI, including moisture content, diastase activity, HMF levels, reducing sugars, and sucrose concentration. The variations indicate potential adulteration, highlighting the necessity of enforcing rigorous quality control measures in honey production. Macroscopic analysis revealed significant changes in taste and colour between the bitter honey samples and the sweet honey sample. The bulk of the bitter honey samples had a black hue and a pronounced bitter taste, with several (A, B, and C) furthermore possessing a herbal or jamu-like aroma.

The microscopic analysis verified that both the bitter and sweet honey samples exhibited standard honey attributes and supplementary plant fragments in samples A, B, and C. The sweet honey sample satisfied all SNI quality characteristics. However, many bitter honey samples (A, B, C, E, F, and G) did not comply with these standards. All bitter honey samples (A-G) specifically surpassed the sugar concentration threshold. The inability of specific bitter honey samples to comply with SNI quality criteria is believed to stem from adulteration, including the incorporation of non-honey compounds or additional processing of fresh honey before packaging and distribution by the manufacturers.

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