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Impact of Storage Duration and Container Materials on Hydroxy Methyl Furfural Levels in Indonesian Trigona Honey

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ABSTRACT

Hydroxymethyl Furfural (HMF) is a six-carbon heterocyclic organic compound containing aldehyde and alcohol functional groups. It is formed from reducing sugars when heated through the Maillard reaction. HMF is widely recognized as an indicator of honey quality, reflecting the time and type of storage container used. In this study, we analyzed HMF content to investigate the effects of different storage container types and durations on HMF levels in honey. The analysis was conducted using High-Performance Liquid Chromatography (HPLC) with the following parameters: a mobile phase of acetonitrile:water in a 10:90 ratio, a stationary phase of octadecylsilane (C18), a flow rate of 1.0 mL/min, and a UV detector set to 280 nm. The results showed an increase in HMF content during the storage process, with variations depending on the container type and the storage duration. The highest HMF level recorded was 47.7931 µg/g in honey stored in transparent glass bottles for 8 months. These findings indicate that both the container type and the storage duration significantly influence HMF accumulation in honey, making it an important parameter for evaluating honey quality.

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1 Introduction

In recent years, there has been increased public concern regarding health issues related to food storage materials. Among dietary choices, unprocessed options like honey, a natural sweetener with a long history, have gained popularity. Honey is preferred because it undergoes minimal processing, helping to preserve its natural properties and qualities (Obiedzińska et al. 2018). Composed primarily of fructose and glucose, honey also contains various proteins, minerals, enzymes, and other substances. Its composition can vary based on the flowers, geographical location, and the insects responsible for its production. Environmental factors that change with seasons, processing techniques, and storage conditions significantly influence honey's composition (Shapla et al. 2018). The main component of honey is sugar, which plays a crucial role in its crystallization. Importantly, crystallization does not detract from honey's quality. However, many consumers prefer honey with a relatively liquid texture and may perceive crystallized honey as inferior quality. This consumer preference influences beekeepers and manufacturers to adopt technological processes, such as carefully timed heating and storage for decrystallization. If not executed properly, these decrystallization attempts can lead to changes in honey's composition (Obiedzińska et al. 2018). One commonly used quality criterion for assessing honey's ripening time and location is the hydroxymethylfurfural (HMF) content. HMF is a byproduct of sugar decomposition, formed during heating and storage (Suhaela et al. 2016). Both temperature and storage duration significantly impact the increase in HMF levels. Quantitative analyses of HMF in honey using spectrophotometry indicate that honey stored at high temperatures tends to have higher HMF levels, influenced by the duration of heating and storage (Shapla et al. 2018). Furthermore, prolonged storage of honey can lead to elevated HMF concentrations. High levels of HMF in honey are associated with various chemical properties, such as water content, pH, free acid concentrations, reduced sugar levels, and enzymatic activity (Suhaela et al. 2016).

The Indonesian National Standards (SNI) regulate honey quality by establishing a maximum limit of 40 mg/kg for HMF content. This regulation aims to help ensure that honey retains its natural properties and is safe for consumption (Badan Standarisasi Nasional 2018). Many researchers are exploring the impact of HMF content on human health regarding honey quality, as this component is linked to honey's quality and chemcial composition. (Fallico et al. 2004). Additionally, previous studies suggest that HMF and its compounds may offer positive health effects, although the concentration of HMF can lead to various consequences, its effects depend on exposure levels. Alongside potential benefits, HMF and its derivatives can have detrimental effects on human health, including genotoxic, mutagenic, carcinogenic, organotoxic, enzym-inhibitory, and DNA-damaging effects. (Suri and Chhabra 2020).

The primary metabolic pathway for HMF involves its oxidation to form 5-hydroxymethyl-2-furanoic acid (HMFA), followed by glycine conjugation to create N-(5-hydroxymethyl-2-furoyl) glycine (HMFG), the main metabolite which is excreted through urine. Additionally, under in vivo conditions, HMF can be converted into 5-sulfoxymethylfurfural (SMF) through the sulfonation of its allylic hydroxyl group, facilitated by sulfotransferases (SULTs) and the sulfate donor, known as 3-phosphoadenosine-5-phosphosulfate (PAPS) (Farag et al. 2020). This highlights the need for further laboratory research to examine the HMF content of honey stored in various containers and over different time intervals and to explore the synthesis and metabolism of HMF concerning human health. One recommended method for measuring HMF levels, suggested by the International Honey Commission (IHC), is High-Performance Liquid Chromatography (HPLC) (Suri and Chhabra 2020). System suitability tests on HMF solutions confirm that the HPLC method is effective for analyzing HMF, offering the advantages of short analysis times and strong separation capabilities, which support its selection among various analytical methods (Dimyati and Marzuki 2023). The current study has evaluated the effect of various storage conditions on the quality of Hydroxy Methyl Furfural content in Indonesian Trigona honey.

2 Materials and Methods

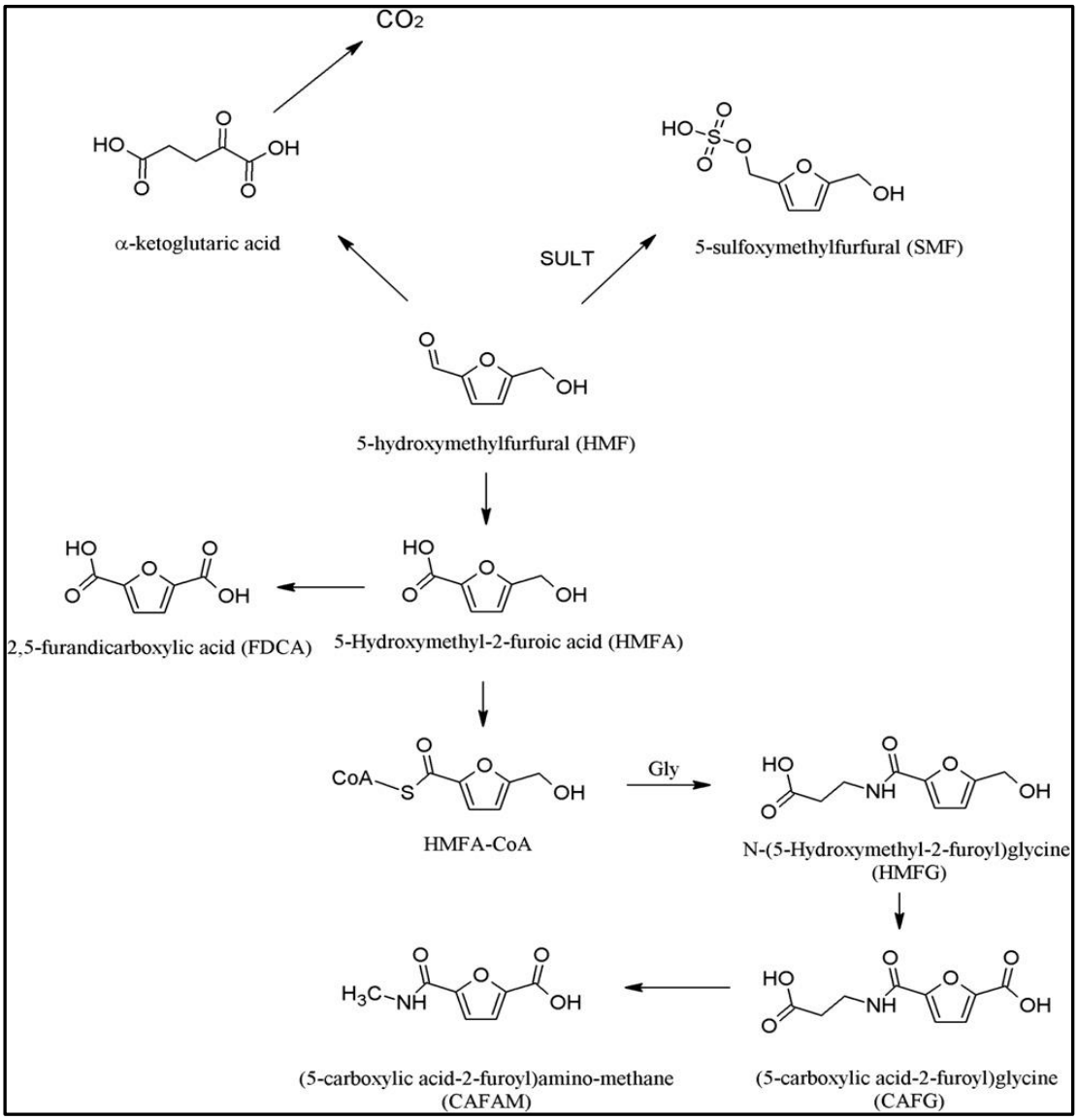
2.1 Protein Identification

Protein identification was conducted by adding biuret reagent to a test tube containing Trigona honey as the sample, chicken egg yolk as the positive control, and distilled water as the negative control. The color changes were then evaluated.

2.2 HMF Content Determination

2.2.1 Honey Sample Preparation

The water content of Trigona honey was measured monthly at room temperature throughout the storage period using a honey refractometer. At the same time, the concentration of hydroxymethylfurfural (HMF) was analyzed using High-Performance Liquid Chromatography (HPLC). For the HMF analysis, 5 grams of honey were dissolved in 25 mL of demineralized water, and then 0.5 mL each of Carrez I and II solutions were added to precipitate proteins. The solution was diluted with demineralized water to reach the required volume, filtered through a 0.45 µm nylon filter, and injected into the HPLC system. The chromatographic conditions were set to use a mobile phase consisting of demineralized water and acetonitrile in a 90:10 ratio, with a C18 column, a flow rate of 1.0 mL/min, and a UV detector set to 280 nm. This method allowed for precise and periodic monitoring of both the water content and HMF levels in Trigona honey, providing insights into the effects of storage duration and container material on honey quality.



2.2.2 Chromatographic Condition

Mobile phase: Deionized water:acetonitrile (90:10); Column: C18 (4.6 mm x 150 mm, particle size 5 µm); flow rate: 1.0 ml/min; injection volume: 20 µl; Detector: UV 280 nm.

2.2.3 Method Validation

Method validation was conducted by evaluating different parameters: linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Analyte standards were spiked into seven sample replications to assess accuracy until a final concentration of 10 ppm was achieved. These samples were then injected into an HPLC system. The accuracy was determined by calculating the percentage recovery (%recovery). Precision testing involved seven honey samples, each spiked with standard analytes at a concentration of 10 ppm. A volume of 20 µL from each sample was analyzed using HPLC. The peak area obtained from the chromatogram was used to calculate the standard deviation (SD) and the relative standard deviation (RSD). The linearity test was performed by preparing a standard series of six concentrations ranging from 5 to 50 ppm at a wavelength of 280 nm. Linearity was assessed by measuring the slope, intercept values, and correlation coefficient. Determination of detection limits was performed using statistical methods based on a linear regression line derived from the calibration curve. The measurement value corresponds to the intercept (b) in the linear regression equation $y = a + bx$, while the blank standard deviation equates to the residual standard deviation (Sy/x).

3 Results and Discussion

3.1 Protein Identification

The Carrez solutions I and II are used to precipitate proteins found in honey analyzing hydroxymethylfurfural (HMF) content. Proteins are complex organic compounds with high molecular weights, so the Carrez solutions are essential for extraction. This facilitates HMF analysis using High-Performance Liquid Chromatography (HPLC). We conducted the Biuret test to determine the presence of proteins in Trigona honey samples. These findings confirmed the need for Carrez solution in the HMF analysis (Wahdania et al. 2022). We performed tests by adding Biuret reagent was added to three test tubes containing chicken egg yolk (positive control), Trigona honey (sample), and distilled water (negative control). The results of the Biuret test indicated that the Trigona honey sample contained proteins, as the color shifted from yellow to dark yellow or yellowish-purple. While the color change observed did not align precisely with the Biuret test guidelines, where a purple or blue color indicates a positive protein result (Jain et al., 2021), the change in the honey samples still occurred, suggesting the presence of a small percentage of protein. The positive protein result in the Trigona honey samples allows us to use the Carrez solution to analyze HMF content. This is important for precipitating proteins to ensure the analysis yields accurate results for HMF compounds, free from interference by impurities such as proteins (Kurtagić 2021). The Carrez solutions are necessary due to the high molecular weight of proteins, enhancing the effectiveness of HMF analysis using HPLC.

3.2 Suitability System Testing

To achieve reliable results, instrument parameters must be adjusted when analyzing with HPLC instruments. Key factors that can be modified include the flow rate, mobile phase composition, and column type. The chosen system must be appropriate and tailored to optimum conditions to ensure that the results are accurate and valid (Kumar et al. 2023).

The retention time refers to the duration it takes for compounds in a sample to reach the detector. Ideally, the desired retention time (Rt) should not be too short, as this may hinder effective separation, nor should it be too long, as that could impact processing efficiency. For the compound HMF, a retention time of approximately 4 minutes is considered adequate, indicating that the detection time for this compound is efficient. This finding aligns with previous research (Lamerkabel 2011). The results demonstrate that both the Rt and Area Under the Curve (AUC) data for evaluating the suitability of the HMF system using HPLC meet the necessary criteria, with a %RSD value of ≤2.0%.

3.3 Method Validation

3.3.1 Accuracy and Precision

The accuracy test was conducted using an analytical standard (Hydroxymethyl Furfural) spiked method on seven samples, with a reference material concentration spiked at 10 ppm. The results showed a recovery percentage of 106.7% for HMF in honey samples. This recovery value meets and aligns with the established requirement within the 90-107% range. This alignment instills confidence in the accuracy of our method. Since the accuracy test

Table 1 Biuret Test for Protein Identification

Sample	Colour	Results
Trigona honey solution	Yellow-dish purple	Positive
Aquadest as a negative control	No colour	Negative
Egg yolk as positive control	Purple	Positive

Tabel 2 Results of Suitability System

Replication	Rt	AUC
1	4.313	1065.397
2	4.295	1061.774
3	4.268	1065.407
4	4.275	1061.014
5	4.335	1069.757
Average	4.297	1064.669
SD	0.0275	3.4893
%RSD	0.6403	0.3227

Table 3 Results of Accuracy and Precision Test

No.	Replication	Experimental concentration (ppm)	AUC (mAU)	% Recovery
1.	Sample 1	11.15	1065.40	106.50
2.	Sample 2	11.12	1061.77	106.20
3.	Sample 3	11.15	1065.41	106.50
4.	Sample 4	11.11	1061.01	106.10
5.	Sample 5	11.20	1069.76	107.00
6.	Sample 6	11.28	1077.88	107.80
7.	Sample 7	11.16	1066.27	106.60
		Σ		78.17
		Average		11.1671
		Standard Deviation		0.0576
		%Relative Standard Deviation		0.5162
		% Recovery		106.6714
		CV Horwitz		1.3909
		2/3 CV Horwitz		0.9319

yielded recoveries that align with these standards, the tested HPLC method can be considered accurate and capable of providing reliable analysis results.

The precision tests, a key aspect of our evaluation, were performed on the seven honey samples, each representing a separate replication spiked with standard analytes at a concentration of 10 ppm. A volume of 20 µL from each sample was analyzed using HPLC. After measuring the peak area on the chromatogram, we determined the standard deviation (SD) and relative standard deviation (RSD). The % RSD values, less than 2/3 of the CV Horwitz, demonstrate high precision in the testing system, equipment, and analysis method. This HPLC method's precision ensures the validity of the measurement results, which meets the necessary precision criteria.

3.3.2 Linearity, Limit of Detection (LOD), and Limit of Quantification (LOQ)

A linearity graph was constructed using external standardization with six HMF standard solutions at 5 to 50 ppm concentrations. The linear equation obtained from HMF testing on honey is expressed as y = 45.054x + 1.1434, with a correlation coefficient 1. This high correlation coefficient value, which meets the specified requirement of being greater than 0.9997, is a strong indicator of

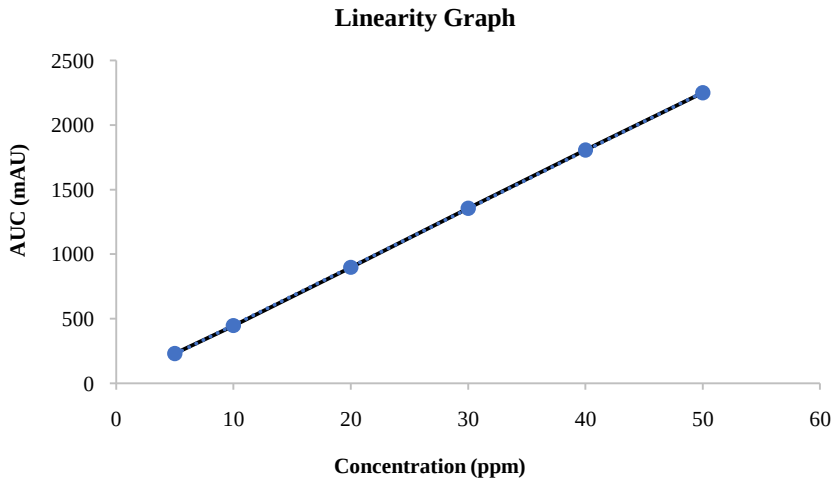


Figure 2 Linearity Graph for HMF standardization

the reliability of the results. This indicates that the method for determining HMF in honey using HPLC exhibits good linearity, as it fulfills the criteria for an acceptable correlation coefficient (r). The limit of detection (LOD) value obtained for HMF was 0.0590, and the limit of quantification (LOQ) was 0.1968. These values demonstrate that the HPLC method for measuring HMF in honey samples is sensitive and can accurately detect HMF concentration levels.

3.4 Evaluation of Water Content and HMF Concentration in Several Period Storage

The water content in Trigona honey samples was determined by measuring the refractive index of honey at room temperature, a crucial step conducted periodically once a month during storage. The results of the analysis showing the relationship between water content and HMF (hydroxy methyl furfural) compound content in Trigona honey, based on variations in container material and storage time, are presented in Table 4. These findings are significant as they shed light on the impact of storage conditions on the quality of Trigona honey. On the day of harvest, the water content of Trigona honey stored in a white plastic container was recorded at 26.0%, with an HMF content of 6.8936 µg/g.

Based on Table 4, it can be observed that there is a decrease in water content accompanied by an increase in HMF (hydroxyl methyl furfural) levels with each increase in honey storage time (Suhaela et al. 2016). However, the decrease in water content across different container types did not significantly correlate with the rise in HMF content. The analysis of container materials and storage duration revealed that the highest water content was found in dark glass containers after 8 months of storage, with a moisture level of 25.5%, while the lowest water content was recorded in dark plastic containers at 24.0%. Conversely, the highest HMF levels were detected in Trigona honey samples stored in transparent glass containers for the same duration, with a concentration of 47.7931 µg/g. In comparison, the lowest HMF levels were found in samples stored in white plastic containers, which exhibited a concentration of 6.8936 µg/g.

The changes in water content during the Trigona honey samples' storage period were insignificant; therefore, no meaningful conclusion can be drawn regarding the relationship between water content and HMF levels. However, it is important to note that the Maillard reaction, a complex chemical process that occurs during prolonged honey storage, alongside the degradation of reducing sugars, coincides with the decrease in water content (Capuano and Fogliano 2011; Hustiany 2016; Shapla et al. 2018; Farag et al. 2020). The increased HMF levels observed were insignificant because no heat treatment was applied to the Trigona honey samples. This underscores the role of the Maillard reaction in HMF formation, a fascinating aspect of honey production that professionals in the field will find intriguing.

3.5 Evaluation of HMF Content in Storage Variation

Hydroxymethyl Furfural (HMF) levels in honey were determined using validated High-Performance Liquid Chromatography (HPLC). The study analyzed HMF compound levels in Trigona honey samples that were treated and stored in four containers for eight months.

The hydroxy methyl furfural (HMF) levels in honey samples were measured in triplicate on the first day of storage, yielding 6.8936,

Table 4 Water Content and HMF Concentration in Several Months of Storage

Storage Container	Monthly storage	Water content (%b/b)	HMF concentration (µg/g)
Transparent Plastic	6	25.75	9.4841
Dark Plastic		24.5	11.5254
Transparent glass		25.5	11.0133
Dark glass		26.0	12.0204
Transparent Plastic	7	25.5	11.3612
Dark Plastic		24.0	14.7059
Transparent glass		25.5	11.9216
Dark glass		26.0	13.9661
Transparent Plastic	8	25.0	31.9106
Dark Plastic		24.0	41.3719
Transparent glass		25.0	47.7931
Dark glass		25.5	42.8390

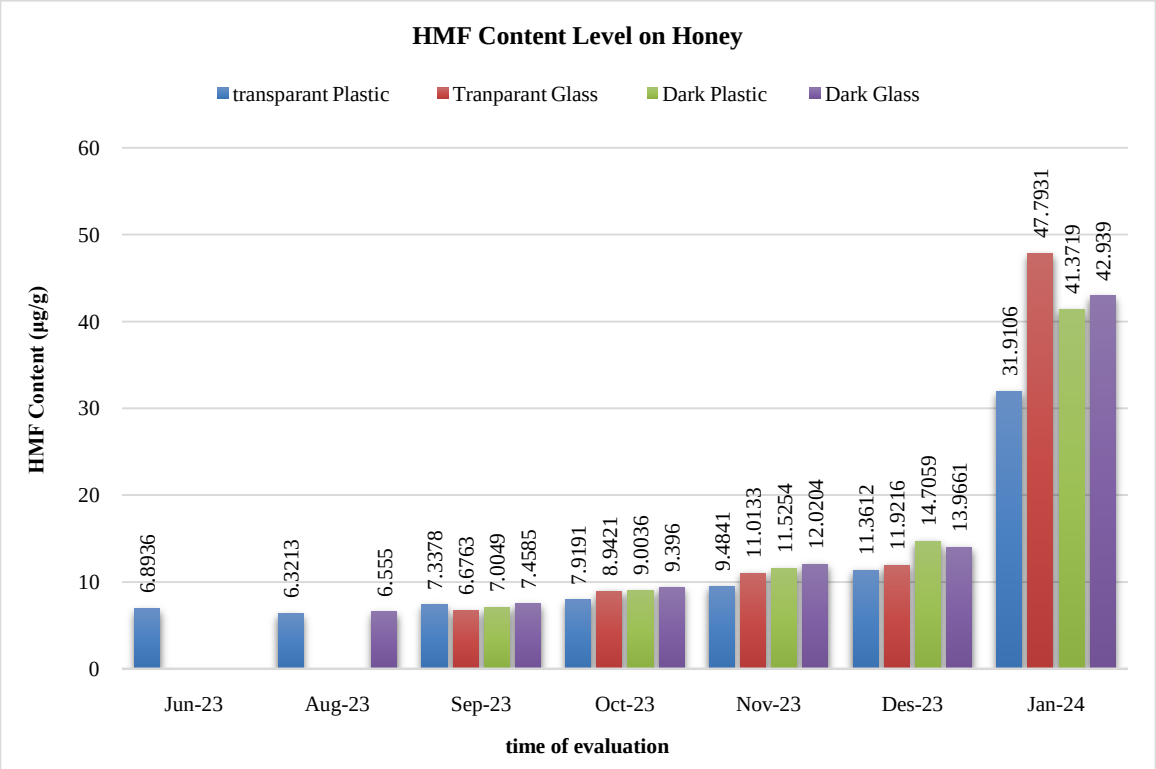


Figure 3 Graph of HMF Content Determination in Honey

6.2884, and 6.5638 ppm, respectively. These measurements establish an initial baseline for evaluating HMF content. Monitoring these levels over time will help assess the impact of storage conditions on honey quality. The resulting HMF levels indicate that fresh honey samples contain only small amounts of HMF. In an experiment to determine HMF levels in honey stored in containers made of four different materials (dark glass, dark plastic, white plastic, and white glass), the highest HMF result was observed in the transparent glass bottle, with levels reaching 47.7931 µg/g after 8 months. This finding is consistent with research indicating that HMF is sensitive to light (Kukurová et al. 2006; Capuano and Fogliano 2011; Suhaela et al. 2016; Ariandi and Khaerati 2017; Obiedzińska et al. 2018; Shapla et al. 2018; Suri and Chhabra 2020; Kurtagić 2021; Petrarca et al. 2020; Pujiarti et al. 2021; Dimyati and Marzuki 2023). The formation of HMF in the transparent glass bottle is more pronounced than in the other three bottles because the transparent nature of the glass allows more light to enter.

Conversely, the transparent plastic bottle had the lowest HMF levels, measuring 31.9106 µg/g. The results indicate lower HMF levels in plastic containers than in glass ones, likely due to the superior insulating properties of plastic. Studies suggest that the insulating ability of a material largely depends on its thickness and thermal conductivity. For optimal insulation, a greater thickness and lower thermal conductivity are necessary. Plastic insulates 5 to 10 times better than glass, with a significantly lower thermal conductivity (Alhamidi et al. 2022). This allows heat to transfer more rapidly in glass containers than in plastic.

Regarding storage duration, the highest HMF levels after 8 months were found as follows: 31.9106 µg/g in transparent plastic bottles, 41.3719 µg/g in dark glass bottles, 47.7931 µg/g in transparent glass bottles, and 42.839 µg/g in dark glass bottles. HMF levels during this 8-month storage period were significantly higher than those measured in fresh honey immediately after harvest, which were 6.8936, 6.2884, and 6.5638 µg/g when stored in a transparent plastic bottle. This increase can be attributed to the absence of significant driving factors influencing large-scale HMF formation during the initial storage period. However, the HMF levels in fresh honey can result from various environmental factors, including the conditions of the bees, the environment where the honey is produced, and the bees' diet during honey production. This complexity in HMF formation underscores the intricate nature of honey production. Additionally, the increase in HMF levels in honey generally coincides with a reduction in water content, although this decrease is relatively stable and not substantial. Water content plays a role in HMF formation through the Maillard reaction, which involves the degradation of reducing sugars.

According to the study's findings, the HMF levels in honey stored in dark plastic bottles, white glass bottles, and dark glass bottles

after 8 months exceeded the maximum allowable limit set by the Indonesian National Standard (SNI) of 40 mg/kg (or µg/g) for HMF content in honey (Badan Standarisasi Nasional 2018; Hidayatullah et al. 2022). This indicates that both the type of storage container and the duration of storage significantly influence the HMF content in honey. This information underscores the responsibility of both producers and consumers to consider storage times for optimal honey quality and safety and to take proactive measures to ensure these standards are met.

Conclusion

Storage time and the type of container significantly impact the levels of Hydroxymethyl Furfural (HMF) in Trigona honey. After eight months, the highest concentration of HMF, measuring 47.7931 µg/g, was found in transparent glass bottles, likely due to exposure to light. In contrast, lower HMF levels were observed in plastic containers, which were better insulated against heat transfer. As storage duration increased, HMF levels rose and were positively correlated with a reduction in water content, which was linked to the Maillard reaction. Notably, the choice of container played a crucial role in reducing this increase in HMF. These findings underscore the potential impact of our research on your work, as they highlight the importance of selecting appropriate storage materials and durations. This is crucial to maintaining honey quality in compliance with regulatory standards, ensuring safety, and satisfying consumers.

Acknowledgment

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Conflict of Interest

The author declares that there are no conflicts of interest.

Ethical Clearance

No animal model was used in this study; therefore, ethical clearance is not required.

References

Alhamidi, A., Anis, A., Al-Zahrani, S. M., Bashir, Z., & Alrashed, M. M. (2022). Conductive Plastics from Al Platelets in a PBT-PET Polyester Blend Having Co-Continuous Morphology. *Polymers*, *14*(6). https://doi.org/10.3390/polym14061092.

Ariandi, & Khaerati (2017). Uji Aktivitas Enzim Diastase, Hidroksmetilfurfural (HMF), Kadar Gula pereduksi, dan Kadar Air Pada Madu Hutan Battan. In *Prosiding Seminar Hasil Penelitian (SNP2M), 1-4*.

Badan Standarisasi Nasional. (2018). *SNI 8664:2018 Madu*. Retrived from www.bsn.go.id.

Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A Review on Metabolism, Toxicity, Occurrence In food and Mitigation Strategies. *Food Science and Technology Journal*, *44*(4), 793–810. https://doi.org/10.1016/j.lwt.2010.11.002.

Dimyati, E., & Marzuki, H. (2023). Penetapan Kadar 5-Hydroxymethyl Furfural Dalam Menggunakan Kromatografi Cair Kinerja Tinggi. *Jurnal Multidisipliner Bharasumba*, *2*(1), 672–684.

Fallico, B., Zappalà, M., Arena, E., & Verzera, A. (2004). Effects of conditioning on HMF content in unifloral honeys. *Food Chemistry*, *85*(2), 305–313. https://doi.org/10.1016/j.foodchem.2003.07.010.

Farag, M. R., Alagawany, M., Bin-Jumah, M., Othman, S. I., Khafaga, A. F., et al. (2020). The Toxicological Aspects of the Heat-Borne Toxicant 5-Hydroxymethylfurfural in animals: A Review. *Molecules*, 25 (8). https://doi.org/10.3390/molecules25081941.

Hidayatullah, M., Handoko, C., & Maring, A. (2022). SNI Madu dan Manfaat Madu Untuk Kesehatan. *Standarisasi LHK*, *1*(6), 23–26.

Hustiany, R. (2016). *Reaksi Maillard Pembentuk Citarasa dan Warna Pada Produk Pangan*. PhD Thesis, Lambung Mangkurat University, Indonesia. https://repo-dosen.ulm.ac.id//handle/123456789/17832.

Jain, B. P., Goswami, S. K., & Pandey, S. (2021). *Protein*. In B. P. Jain, S. K. Goswami, & S. Pandey (Eds.), Protocols in Biochemistry and Clinical Biochemistry (pp. 31-48). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-822007-8.00008-8.

Kukurová, K., Karovičová, J., Greif, G., Kohajdová, Z., & Lehkoživová, J. (2006). Determination of 5-Hydroxymethylfurfural After Winkler and By The HPLC Method For Authentication of Honey. *Chemical Papers*, *60*(3), 186–191. https://doi.org/10.2478/s11696-006-0034-8.

Kumar, G. T. J., Andrews, B. S. A., & Abbaraju, V. D. N. K. (2023). The Significance of System Suitability in High-Performance Liquid Chromatography (HPLC) Analysis: Ensuring Accurate and Reliable Results. *International Journal for Research in Applied Science and Engineering Technology*, *11*(10), 29–35. https://doi.org/10.22214/ijraset.2023.56435.

Kurtagić, H. (2021). Determination of Hydroxymethylfurfural Content (HMF) in Fresh Bee Honey Produced in Bosnia and Herzegovina (B&H) by HPLC DAD Method. *International*

Journal of Environmental Sciences & Natural Resources, *26*(5), 556199. https://doi.org/10.19080/ijesnr.2021.26.556199.

Lamerkabel, J. (2011). Mengenal Jenis-Jenis Lebah Madu, Produk-Produk Dan Cara Budidayanya. *Jurnal Ilmu Pengetahuan Dan Teknologi* , *9*(1), 70-78.

Obiedzińska, A., Biller, E., Obiedziński, M. W., & Tyszka, M. (2018). Determination of Hydroxymethylfurfural Content In Natural Honeys In Poland. *Polish Journal of Applied Sciences*, *4*, 60–64. https://doi.org/10.34668/PJAS.2018.4.2.04.

Petrarca, M. H., Meinhart, A. D., & Godoy, H. T. (2020). Dilute-and-Shoot Liquid Chromatography Approach for Simple and High-throughput Analysis of 5-Hydroxymethylfurfural in Fruit-based Baby Foods. *Food Analytical Methods*, *13*(4), 942–951. https://doi.org/10.1007/s12161-020-01713-7.

Pujiarti, R., Amin, A., Ngadianto, A., Septiana, R. M., Purba, B. A. V., & Permadi, D. B. (2021). Quality of Three Forest Honey Types from Baduy Tribe, Lebak District, Banten Province. *Jurnal Ilmu Kehutanan*, *15*(2), 123–136. https://doi.org/10.22146/jik.v15i2.1529.

Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I., & Gan, S. H. (2018). 5-Hydroxymethylfurfural (HMF) Levels In Honey and Other Food Products: Effects On Bees and Human Health. *Chemistry Central Journal*, *12*, 35. https://doi.org/10.1186/s13065-018-0408-3.

Suhaela, Noor, A., & Ahmad, A. (2016). Effect of Heating and Storage Time Levels 5-(Hydroxy Methyl) furan-2-Karbaldehida (HMF) In Honey Origin Mallawa. *International Journal*, *17*(2), 24–31.

Suri, P.S., & Chhabra, P. (2020). A Review Presence of 5-Hydroxymethylfurfural (HMF) in Food Products: Positive and Negative Impacts on Human Health. *International Journal of Forensic Sciences*, *5*(2), 1–10. https://doi.org/10.23880/ijfsc-16000194.

Wahdania, Porodjia, F., & Nurkamiden, S. (2022). Protein Test With Chemical Solution in Identifying Nutrient Content in Food Material. *Journal of Health, Technology and Science*, *2*(4), 41–49. https://doi.org/10.47918/jhts.v2i4.252.