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The effect of processing and storage on Klanceng honey (*Tetragonula laeviceps*)[1]

El efecto del procesamiento y almacenamiento en la miel Klanceng (*Tetragonula laeviceps*)

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Abstract

Introduction. Klanceng honey (*Tetragonula laeviceps*) is in great demand because of its benefits. There is no information on the expiration date of the Klanceng honey. **Objective.** To predict the shelf life of Klanceng honey by evaluating the effect of heating (40 ºC / 48 h and 70 ºC / 7 h) and storage for two years. **Materials and methods.** The analysis was carried out three times (2020, 2021, and 2022) at the National Innovation Research Agency Laboratory, Jakarta, Indonesia. Samples of Klanceng honey (*Tetragonula laeviceps*) were taken from a bee farm in Magetan, East Java, Indonesia. The sample (5 kg) was divided into 3: without heating process (UT), heating at 40 ºC / 48 h (T1), and heating at 70 ºC / 7 h (T2). Then analyzed the activity of HMF, diastase, invertase, acid phosphatase (AP), glucose oxidase (GO), DPPH, honey color, and phenolic at 0, 12 and 24-months. **Results.** The results showed that short heating at high temperature (70 ºC / 7 h) had a greater impact on decreasing enzyme activity compared to prolonged heating at low temperature (40 ºC / 48 h). Storage had a major impact on the increase of 5-hydroxymethyl (furan)-2-carbaldehyde (HMF) compared to the heating process. During storage (24-months) the HMF value exceeded the maximum limit. **Conclusions.** The HMF value (55.33 ± 0.57 mg/kg) exceeded the maximum allowable limit (max 40 ppm), this was due to the significant effect of heating on T2 and the storage process. The shelf life of Klanceng honey was two years, as long as it was not heated to high temperatures (70 ºC).

Keywords: honey quality, heating, invertase, diastase.

Resumen

Introducción. La miel Klanceng (*Tetragonula laeviceps*) es muy demandada por sus beneficios. No hay información sobre la fecha de caducidad de la miel Klanceng. **Objetivo.** Predecir la vida útil de la miel Klanceng mediante la evaluación del efecto del calentamiento (40 ºC / 48 h y 70 ºC / 7 h) y el almacenamiento durante dos años.

Materiales y métodos. El análisis se llevó a cabo tres veces (2020, 2021 y 2022) en el Laboratorio de la Agencia Nacional de Investigación de Innovación, Yakarta, Indonesia. Se tomaron muestras de miel Klanceng (*Tetragonula laeviceps*) de una granja de abejas en Magetan, Java Oriental, Indonesia. La muestra (5 kg) se dividió en 3: sin proceso de calentamiento (UT), calentamiento a 40 °C / 48 h (T1) y calentamiento a 70 °C / 7 h (T2). Luego se analizaron HMF, diastasa, invertasa, fosfatasa ácida (AP), glucosa oxidasa (GO), DPPH, absorbancia y actividad fenólica a los 0, 12 y 24 meses. **Resultados.** Los resultados mostraron que el calentamiento corto a alta temperatura (70 °C / 7 h) tuvo un mayor impacto en la disminución de la actividad enzimática en comparación con el calentamiento prolongado a baja temperatura (40 °C / 48 h). El almacenamiento tuvo un impacto significativo en el aumento de 5-hidroximetil (furano)-2-carbaldehído (HMF) en comparación con el calentamiento. Durante el almacenamiento (24 meses) el valor de HMF superó el límite máximo. **Conclusiones.** El valor de HMF (55,33 ± 0,57 mg/kg) superó el límite máximo permitido (máximo 40 ppm), esto se debió al efecto significativo del calentamiento en T2 y el proceso de almacenamiento. La vida útil de la miel Klanceng fue de dos años, siempre que no se calentara a altas temperaturas (70 °C).

Palabra clave: calidad de la miel, calentamiento, invertasa, diastasa.

Introduction

Many parameters of honey quality test make honey breeders and traders pessimistic. It is necessary to select from many parameters in order to obtain only two or three that represent the overall quality. The honey quality test parameters that are sensitive to heating in the history of honey are diastase and 5-hydroxymethyl (furan)-2-carbaldehyde (HMF) (Cozmuta et al., 2011; Tosi et al., 2004). The results of other research stated that invertase activity and HMF are the most sensitive (Karabournioti & Zervalaki, 2001; Vorlova & Pridal, 2002).

Many nutrients in honey are important for digestive processes, such as diastase (digests starch), invertase (produces glucose), AP (removes phosphate from other molecules by hydrolyzing monoester phosphoric acid), and DPPH (antioxidant). So, efforts are needed to maintain the quality of honey from biochemical damage. For other biochemical compounds, which are the result of the Maillard reaction due to heating and storage (HMF, honey color, and phenolic) it is also effective if used as an indicator of damage to the quality of honey (Haouam et al., 2019).

Klanceng honey (*Tetragonula laeviceps*) is a type of honey that is in great demand by Indonesians for its benefits. The quality of honey is strongly influenced by its botanical origin, processing and storage (Machado De-Melo et al., 2018). The composition and nutritional value of honey is often used as an indicator of its quality, in which its composition and biological properties are very sensitive to heat and unstable in storage and processing (Nguyen et al., 2012; Önür et al., 2018). So far, there is no information on the expiration date of the Klanceng honey product.

Before honey is sold, usually there is processing to prevent caramel/crystallizing decrease in viscosity, engineered enzyme inhibition to make it stable in storage. Farmers and traders in Indonesia still use heating to get the desired conditions. In fact, there are many innovative treatments that prevent the damage of honey (Akhmazillah et al., 2013; Amariei et al., 2020). The high temperature heating process is often used to inhibit the fermentation process due to bacteria and fungi (Tosi et al., 2004).

In addition, packaging has an important role in maintaining sensory quality (aroma, color, and taste), decreased enzyme activity, antibacterial, and other biological functions (Missio da Silva et al., 2020; Samborska et al., 2015). The critical factors in the honey storage process are temperature, light, and relative humidity (Abou-Shaara et al., 2017). Materials that are safe for honey are usually made of bottles or dark plastic to protect the honey from damage caused by light (Pohl et al., 2009).

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Tetragonula laeviceps honey is very important in Indonesia beekeepers meliponiculture this bee a lot, so there is plenty of research related to this honey. The research includes: (i) bee feed sources (Nugroho & Soesilohadi, 2014; Zulkan Jayadi & Susandarini, 2020), (ii) honey function as antibacterial (Al-kafaween et al., 2020; Al Kafaween et al., 2019), (iii) honey function as an antioxidant (Ng et al., 2017; Oddo et al., 2008), (iv) honey nutritional content (Fatima et al., 2018; Syam et al., 2016), (v) effect processing (Agussalim et al., 2017). (vi) the physisochemical quality for Klanceng honey (Agus et al., 2019; 2021; Sabir et al., 2021). There is no information regarding the estimated age of Klanceng honey based on the treatment or processing so far.

This research to look at the sensitivity of HMF, diastase, invertase, acid phosphatase (AP), glucose oxidase (GO), antioxidant with DPPH, honey color, and phenolic. In order to recommend the right parameters for the honey quality test due to heating and storage.

This study aimed to predict the shelf life of Klanceng honey by evaluating the effect of heating (40 ºC / 48 h and 70 ºC / 7 h) and storage for two years.

Material and methods

Sample

The sample was taken from a bee farm in Jambangan village, Magetan district, East Java, Indonesia on March 28, 2020. The sampling area was on site S7º40'39,47232 and E111º24'15,2247. The sample (5 kg) was placed in a large container for a homogeneous process, then the sample was divided into three (UT, T1, and T2). The samples (UT, T1 and T2) were placed in small glass containers, identified and sealed (not opened until the analysis time limit). Each sample (UT, T1, and T2) was divided into three for analysis at 0, 12, and 24 months' storage.

UT samples were analyzed without heating, while T1 samples were heated at 40 ºC for 48 h and T2 was heated at 70 ºC for 7 h. Heating was carried out in a heating room. Cooling was carried out at room temperature (25 - 27 ºC), then analyzed 5-hydroxymethyl (furan)-2-carbaldehyde (HMF), diastase, invertase, acid phosphatase (AP), glucose oxidase (GO), antioxidant with DPPH, honey color, and phenolic. Analysis results (triple) were recorded at T=0 months (T0).

The three samples (UT, T1, and T2) were stored in the dark at room temperature. Determination of HMF, diastase, invertase, acid phosphatase (AP), glucose oxidase (GO), antioxidant with DPPH, honey color, and phenolic was performed after 12 and 24 months. The analysis was carried out three times in different years (2020, 2021, and 2022) at the National Innovation Research Agency Laboratory, Jakarta, Indonesia.

Methods

Pollen observations were carried out using the acetolysis method (Erdtman, 1954), adding 30 % glycerin. Then, the liquid was stirred and the solution containing the pollen was dropped onto an object glass on an optilab microscope (CX-23). Melissopalynological analysis refers to Von Der Ohe et al. (2004). Identification was carried out based on melissopalynological and physicochemical analysis to determine the origin of the food for the Klanceng bee (*Tetragonula laeviceps*).

Geographic origin based on Maurizio (1975). The identification and counting of pollen grains and other particles in honey. From 200-300 pollen grains. Frequency calculation was as follows: Predominant (more than 45 %), secondary (16-45 %), important minor pollen (3-15 %), minor pollen (less than 3 %). Botanical origin was deduced from the frequency of pollen and honeydew.

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In radius 500 m, % plant spread = (number of plants found: number of plants) x 100. Nectar producing plants, take one sample or two flowers then check availability of nectar by opening the flower crown, then check the liquid nectar on the base of the flower and the volume is not be measured. For extraflora nectar was done by checking the liquid nectar that comes out from the leaves and stems of plants. Nectar extraflora secreted by glands nectarines that can develop on stems, leaves or other parts plant.

Pollen producing plants, take one sample or two flowers, then check pollen on the anther, The pollen was in the form of powder or flour and generally mostly yellow and the weight of each flower was not weighed.

The type of honey was determined based on the provisions of Wingenroth (2001), namely monofloral (one species of pollen that has a pollen frequency >45%), bifloral (two types of pollen that has a pollen frequency of >22.25% in one honey), and multifloral (three pollen or more that has a frequency of <16% in one honey).

The physicochemical test refers to the International Honey Commission (2009) and Association of Official Analytical Chemists (AOAC, 2016). All physicochemical analyzes were repeated three times. Determination of total sugar content and water content used a honey refractometer, determination of pH using a pH meter, electrical conductivity in 20 g of dry honey in distilled water with no conductivity, and determination of acid and lactone levels using titrimetric with the official AOAC method (AOAC, 2016).

Determination of HMF, diastase and invertase was made used a spectrophotometer with reference to the standard analysis of the International Honey Commission (2009).

Invertase activity was tested photometrically, based on decomposition of p-nitrophenyl α-D glucopyranoside substrate to p-nitrophenol (maximum absorbance at 400 nm). 1IN is the amount of sucrose (g) which is hydrolyzed by enzymes in 100 g of honey for 1 h of incubation.

Diastatic activity was expressed in DN. One unit was the enzyme activity in I g honey which can hydrolyze 0.01 g starch for 1 h incubation at 40 ºC.

Determination of HMF using 0.5 mL of Carrez I solution and 0.5 mL of Carrez II with 0.2 % sodium bisulphite standard solution. Then measured with wavelengths at 284 nm and 336 nm.

Phenolic analysis (mg gallic acid/kg honey) refers to the modified Folin-Ciocalteu method (Cao et al., 2020). The analysis was made with 200 mL of honey plus 1 mL of Folin and Ciocalteu phenol reagent. Wait for 4 min, then add 1 mL of 10 % Na_2CO_3 solution and 10 mL of distilled water. Solution stored in the dark for 120 min, the absorbance is read at 725 nm by UV/VIS spectrophotometer.

The analysis of acid phosphatase (AP) refers to the analysis of previous researchers (Tomazic, 2001), the activity of the phosphatase enzyme is defined as Ca_3PO_4 which undergoes hydrolysis to soluble PO_4^{-3} (phosphate) during 24 h of incubation. AP activity is expressed in mg phosphate/100 g honey / 24 h.

Determination of glucose oxidase activity was obtained according to Trinder (1969), the activity was expressed in μg H_2O_2/g honey. Test was done through colorimetry with λ 505 nm for 5 min.

Antioxidant analysis using spectrophotometry (λ 517), the amount of 1,1-diphenyl -2-picrilhydrazine (DPPH) is marked with a pink to yellow color. The antioxidant in the sample is expressed in IC_{50}. This means that there is a decrease in the levels of DPPH radicals (50 %) at the beginning of the sample concentration (mg/mL). The test refers to Molyneux (2004), with standard solution of $FeSO_4.7H_2O$ from 10 % honey sample.

Honey color was performed spectrophotometrically, with wavelengths of 450 nm and 720 nm, the results were expressed in mAU (Beretta et al., 2005).

Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics version 26 (SPSS Inc.). ANOVA test was used to determine the mean value, range of variation, standard deviation and post hoc Tukey HSD test to see differences in treatment and storage conditions. The research conducted an analysis of honey on heating and storage effects. The significant difference test for heating uses subscription letters (a, b, and c) while the storage test uses x, y, and z. Results were presented as mean ± standard deviation. Statistical significance was considered at $p<0.05$.

Results

Plant origin

Melissopalynology test was used to detect the source of pollen in honey samples, as well as to provide information of types of plants frequented by Klanceng bee (*Tetragonula laeviceps*). The results of the melissopalynological analysis are shown in Table 1.

Table 1. Melissopalynological test, botanical origin of the food of the Klanceng bee (*Tetragonula laeviceps*) in the Magetan area of East Java, Indonesia. 2020.

Cuadro 1. Prueba melisopalinológica, origen botánico del alimento de la abeja Klanceng (*Tetragonula laeviceps*) en el área de Magetan en Java Oriental, Indonesia. 2020.

Local name	Scientific name	Family	% Pollen frequency	% Plant spread	Source	
					Pollen	Nectar
Corn	*Zea mays spp. Mays* L	Poaceae	40.58	29.7	√	
Papaya	*Carica papaya* L	Caricaceae	21.5	12.6	√	√
Noodle bean	*Vigna unguiculate sesquipedalis (L)*	Fabaceae	14.7	25	√	
Egg plant	*Solanum melongena* L	Lauraceae	11.5	11	√	√
Mango	*Mangifera indica*	Anacardiaceae	3.1	3	√	√
Water apple	*Syzygium aqueum*	Myrtaceae	2.05	2.6	√	√
Rambutan	*Nephelium lappaceum* L	Sapindaceae	2.1	0.7	√	√
Coconut	*Cocos nucifera* L	Araceae	1.8	not be found	√	√
Guava	*Psidium guajava* L	Myrtaceae	2.67	0.6	√	√
cottonwood	*Ceiba pentadra*	Malvaceae		1.2		√
Mahogany	*Swietenia macrophylla*	Maliaceae		0.4		√
Avocado	*Persea Americana*	Lauraceae		1.8		√
Melinjo	*Gnetum gnemon*	Gnetaceae		1.4		√
Sapodilla	*Manikara zapota*	Sapotaceae		0.8		√
Sengon	*Albizzia falcata*	Mimosoidae		0.2		√
Breadfruit	*Artocarpus altilis*	Moraceae		1.2	√	√
Red chili	*Capsicum annum*	Solaneceae		5	√	√
Sonokeling	*Dalbergia latifolia*	Leguminoseae		0.8		√
Lamboro	*Leucaena leucocephala*	Fabaceae		2	√	√

Based on the number of pollen frequencies, secondary pollen was found from two types of plants (*Zea mays* spp. *Mays* L. and *Carica papaya* L.). Important minor pollen for three types of plants and the rest was small (less

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than 3 % pollen). Observing the spreading plant at a radius of 500 m, no *Cocos nucifera* L. species were found, but 1.8 % (pollen frequency) was detected.

The results in Table 2 showed a physicochemical analysis to determine the general characteristics of Klanceng honey (*Tetragonula laeviceps*).

Table 2. Physicochemical test results of fresh Klanceng honey (*Tetragonula laeviceps*) from beekeepers in Magetan, East Java, Indonesia. 2020.

Cuadro 2. Resultados de las pruebas fisicoquímicas de miel Klanceng (*Tetragonula laeviceps*) fresca de apicultores en Magetan, Java Oriental, Indonesia. 2020.

Parameter	Average	SD	Minimum	Maximum
Moisture (%)	14.80	0.5000	14.30	15.30
Total sugar (Brix)	77.23	0.3785	76.80	77.50
Total acidity (mmol/kg)	41.76	0.3055	41.50	42.10
Electrical conductivity (mS/cm)	0.26	0.0435	0.22	0.31
pH	5.06	0.4041	4.70	5.50
Lactones (mmol/kg)	5.66	0.8504	4.80	6.50

Note: The Klanceng honey (*Tetragonula laeviceps*) quality as a whole still meets the requirements of honey quality in Indonesia (Indonesian National Standard, 2021). Moisture standard: max 22 %; total sugar: 76-83 ºBrix; total acidity: max 50 mmol/kg. / Nota: la calidad de la miel de Klanceng (*Tetragonula laeviceps*) en su conjunto todavía cumple con los requisitos de calidad de la miel en Indonesia (Indonesian National Standard, 2021). Estándar de humedad: máx. 22 %; azúcar total: 76-83 ºBrix; acidez total: máx. 50 mmol/kg.

The effect of heating and storage on enzyme activity and chemical composition

Heating in this study refers to the activities of farmers and traders in the processing process. Heating is often done at a temperature of 40 ºC for 48 h and a temperature of 70 ºC for 7 h. These conditions will result in changes in the activity of HMF and invertase. The relationship between the two activities can be seen in Figure 1.

Invertase activity in the heating process had a significant effect ($p<0.05$) on T1 and T2. Storage factors showed significant differences ($p<0.05$) at 0, 12, and 24 months. The increase in HMF showed a significant difference on T2, whereas on T1 it did not show a significant difference. The storage factor has a significant effect on the increase in HMF.

From the table above, a drastic decrease in invertase is seen in the heating process. In the T1 and T2 processes without storage (T1-0 and T2-0) there was a decrease from the control sample (UT0) an average of 98.52 ± 0.16 to 68.83 ± 2.88 (UT12) and continued to decrease until 7.80 ± 0.72 (UT24). The same pattern occurred for T1 and T2 when stored for 12 months and 24 months.

The effect on invertase of storage without heating (UT) decreased from an average of 98.52 ± 0.16 (UT0) to 62.45 ± 0.10 (UT12) and at 24 months of storage it became 51.71 ± 0.11. These conditions indicate that invertase is very sensitive to heating compared to storage effects.

The storage process has more effect on the increase in HMF than heating. It can be seen from UT. HMF at UT0 was 3.50 ± 0.50, there was an increase in UT12 by 21.31 ± 0.17 and continued to increase at T24 (34.83 ± 0.06). Heating at 40 ºC (UT1-0 = 5.20 ± 0.72) increased after 12 months of storage (24.31 ± 0.89) and continued to increase at 24 months (37.23 ± 0.49). Process T2 experienced a greater increase than T1 (at T2-0 = 6.33 ± 0.57;

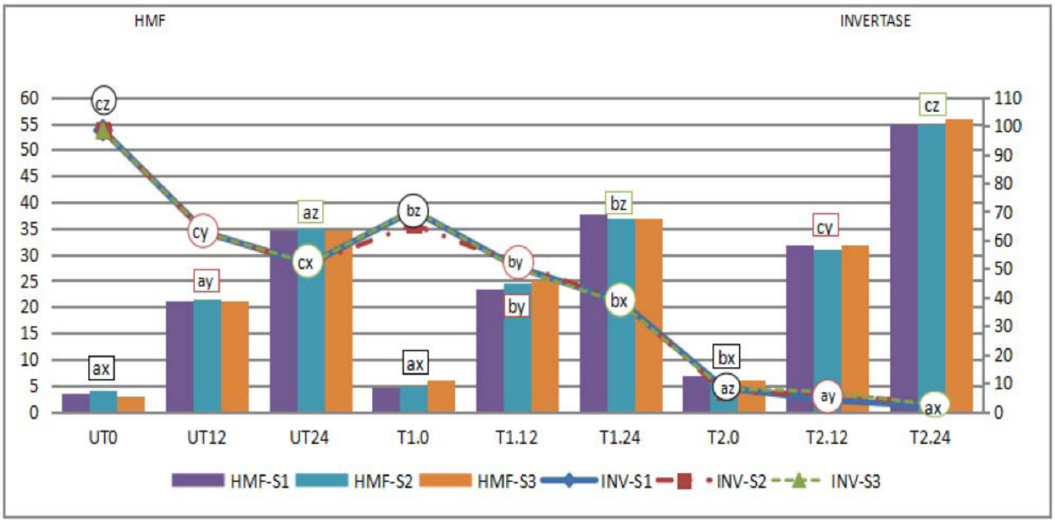

Figure 1. Effect of heating and storage on the activity of invertase and HMF in Klanceng honey. The samples of Klanceng honey (S1, S2, S3) were analyzed for their invertase (INV-S1, INV-S2, INV-S3) and HMF (HMF-S1, HMF-S2, HMF-S3) activity. The same superscript used (two letters) indicates no significant difference. Superscripts for UT, T1, and T2 were a, b, c. Superscripts used for storage times (0, 12, and 24) were x, y, z. National Innovation Research Agency Laboratory, Jakarta, Indonesia. 2022.

Figura 1. Efecto del calentamiento y almacenamiento sobre la actividad de invertasa y HMF en miel Klanceng. Las muestras de miel Klanceng (S1, S2, S3) fueron analizadas por su actividad invertasa (INV-S1, INV-S2, INV-S3) y HMF (HMF-S1, HMF-S2, HMF-S3). El superíndice (dos letras) igual indica que no hubo diferencias significativas. Los superíndices utilizados para UT, T1 y T2 fueron a, b, c. Superíndices utilizados para tiempos de almacenamiento (0, 12 y 24) fueron x, y, z. Laboratorio de la Agencia Nacional de Investigación de Innovación, Jakarta, Indonesia. 2022.

T2-12 = 31.66 ± 0.57 and continued to increase to 55.33 ± 0.57). This condition indicates that HMF is sensitive to storage and heating at high temperature (only T2). The effect of heating and storage on other activities can be seen in Table 3.

The heating effect had a significant effect ($p<0.05$) for diastase, invertase, GO, DPPH, and honey color. The storage process had a significant effect ($p<0.05$) on HMF, diastase, invertase, AP, honey color, and phenolic. Parameters that have sensitivity to both processes were: diastase, invertase, and honey color.

Parameters are the most sensitive due to the heating process (T1, T2) and storage (Table 3). The heating process at a temperature of 40 ºC for 48 h (T1) has a statisticals effects on the parameters (in order of greatest effect): (a) invertase, which refers to the initial standard (UT0=98.52 ± .16) decreased by 29.69 U/kg, (b) honey color, decreased by 18.5 mAU, (c) GO decreased by 14.07 μg/g H_2O_2, (d) other parameters (HMF, diastase, DPPH, phenolic) have shown a small change, but there was no significant difference for AP ($p>0.05$).

Parameters which are sensitive to heating at 70 ºC for 7 h (T2) were: (a) glucose oxidase (difference 193.33 μg/g H_2O_2 from UT0), (b) invertase (difference 90.72 U/kg from UT0), (c) honey color (44.17 mAU), (d) other. All parameters showed significant differences ($p<0.05$).

Parameters that were sensitive and significantly different ($p<0.05$) on storage for 12 months (UT0 control standard) were: honey color (109.9 mAU), invertase (36.07 U/kg), HMF (17.81 mg/kg), phenolic (17 Mg/kg gallic acid) and followed by other components except AP ($p>0.05$).

Parameters that were sensitive and significantly different ($p<0.05$) to 24 month storage were: honey color (180 mAU), invertase (46.81 U/kg), HMF (31.33 mg/kg), and other components (AP and GO). Parameters that were not significantly different ($p>0.05$) are phenolic, diastase and DPPH.

Table 3. Effect of heating and storage processes on physicochemical parameters, tested three times at the National Innovation Research Agency Laboratory, Jakarta, Indonesia. Testing in 2020, 2021 and 2022.

Cuadro 3. Efecto de los procesos de calentamiento y almacenamiento en los parámetros fisicoquímicos, probado tres veces en Laboratorio de la Agencia Nacional de Investigación de Innovación, Jakarta, Indonesia. Pruebas en 2020, 2021 y 2022.

Parameter	Storage (month)	Untreatment (UT)	T1 (40 ºC; 48 h)	T2 (70 ºC; 7 h)
5-hydroxymetyl (furan)-2-carbaldehyde (HMF) mg/kg	0	3.50 ± 0.50^{ax}	4.20 ± 0.72^{ax}	6.33 ± 0.57^{bx}
	12	21.31 ± 0.17^{ay}	24.31 ± 0.89^{by}	31.66 ± 0.57^{cy}
	24	34.83 ± 0.06^{az}	37.23 ± 0.49^{bz}	55.33 ± 0.57^{cz}
Diastase DN	0	19.13 ± 0.15^{cz}	17.40 ± 0.36^{bz}	12.60 ± 0.43^{az}
	12	14.47 ± 0.14^{cy}	13.30 ± 0.43^{by}	10.80 ± 0.10^{ay}
	24	12.85 ± 0.10^{cx}	12.15 ± 0.44^{bx}	9.50 ± 0.10^{ax}
Invertase U/kg	0	98.52 ± 0.16^{cz}	68.83 ± 2.88^{bz}	7.80 ± 0.72^{az}
	12	62.45 ± 0.10^{cy}	50.80 ± 0.81^{by}	5.66 ± 1.52^{ay}
	24	51.71 ± 0.11^{cx}	38.63 ± 0.47^{bx}	2.50 ± 0.50^{ax}
Acid phospatase (AP) Mg P/100g/24h	0	45.73 ± 0.05^{bz}	45.50 ± 0.43^{bz}	29.33 ± 0.57^{az}
	12	44.76 ± 0.12^{by}	44.36 ± 0.10^{by}	24.66 ± 0.58^{ay}
	24	34.83 ± 0.15^{bx}	34.836 ± 0.05^{bx}	23.33 ± 0.57^{ax}
Glucose oxidase (GO) μg H_2O_2/g honey	0	226.63 ± 0.20^{cy}	212.86 ± 0.83^{bx}	33.33 ± 0.57^{ay}
	12	226.02 ± 0.45^{cy}	212.66 ± 0.53^{bx}	17.66 ± 0.58^{ax}
	24	224.50 ± 0.85^{cx}	211.00 ± 0.93^{bx}	17.00 ± 0.95^{ax}
DPPH-IC_{50} Mg/mL	0	25.40 ± 0.10^{cz}	22.73 ± 0.58^{by}	18.66 ± 0.47^{az}
	12	17.05 ± 0.01^{by}	18.71 ± 0.57^{cx}	13.66 ± 0.38^{ay}
	24	14.36 ± 0.01^{bx}	17.69 ± 0.53^{cx}	11.33 ± 0.75^{ax}
Honey color mAU	0	230.50 ± 0.10^{ax}	249.00 ± 0.15^{bx}	275.33 ± 0.35^{cx}
	12	340.40 ± 0.45^{ay}	360.66 ± 0.35^{by}	409.66 ± 0.55^{cy}
	24	410.50 ± 0.01^{az}	440.83 ± 0.25^{bz}	560.33 ± 0.15^{cz}
Phenolic mg/kg gallic acid	0	102.50 ± 0.10^{ax}	112.50 ± 0.65^{bx}	116.33 ± 0.45^{cx}
	12	119.50 ± 0.45^{ay}	129.50 ± 0.15^{by}	142.33 ± 0.75^{cy}
	24	132.50 ± 0.35^{az}	132.40 ± 0.15^{az}	160.66 ± 0.35^{bz}

Note: ANOVA test_Tukey HSD post-hoc using $p<0.05$. The same superscript (two letters) indicates no significant difference. Superscripts used for UT, T1, and T2 were a, b, c. Superscripts used for storage times (0, 12, 24) were x, y, z. Using standard control on UT0 (without processing and storage). / Nota: ANOVA test_Tukey HSD post-hoc usando $p<0.05$. El mismo superíndice (dos letras) indica que no hubo diferencias significativas. Los superíndices utilizados para UT, T1 y T2 fueron a, b, c. Superíndices utilizados para tiempos de almacenamiento (0, 12, 24) fueron x, y, z. Usando control estándar en UT0 (sin procesamiento y almacenamiento).

The effect of honey treatment on changes in activity on diastase, HMF, invertase, GO, DPPH, honey color, phenolic and AP with standard control UT.0 can be seen in Figure 2.

There was a striking change for glucose oxidase and honey color in GO, there was a change in T2 (T2.24 and T2.0), while the honey color was only at T1 (T1.24) and T2 (T2.24). Uniformity of changes and sensitivity occurred in the activity of invertase, HMF, phenolics. Uniformity of changes occurred also for the parameters: diastase, DPPH and AP.

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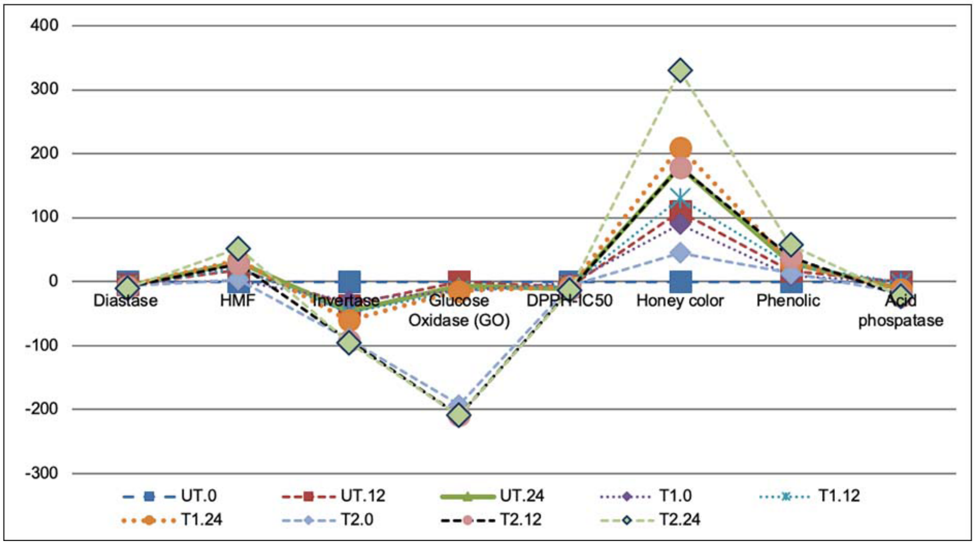

Figure 2. Physicochemical changes selected for the treatment and storage process. Standard control UT0 (unheated and not stored), UT1 (heated at 40 ºC for 48 h) and UT2 (heated at 70 ºC for 7 h). UT was stored for 12 months (UT12) and for 24 months (UT24). T1 was not stored (T1.0), stored for 12 months (T1.12) and for 24 months (T1.24). T2 without storage (T2.0), stored for 12 months (T2.12), and for 24 months (T2.24). Chemical engineering Department. Institute Sains and Technology Al Kamal-Jakarta, Indonesia. 2022.

Figura 2. Cambios fisicoquímicos seleccionados para el proceso de tratamiento y almacenamiento. Control estándar UT0 (sin calentar y sin almacenar) UT1 (calentado a 40 ºC por 48 h) y UT2 (calentado a 70 ºC por 7 h). UT almacenado por 12 meses (UT12) y por 24 meses (UT.24). T1 sin almacenamiento (T1.0), almacenado durante 12 meses (T1.12) y por 24 meses (T1.24). T2 sin almacenamiento (T2.0), almacenado durante 12 meses (T2.12) y por 24 meses (T2.24). Departamento de Ingeniería Química Instituto Sains y Tecnología Al Kamal-Jakarta, Indonesia. 2022.

Discussion

Honey samples in the study area were multifloral honey (three pollen or more that had a frequency of <16 % in one honey). The pollen frequency was identified as 1.8 % for *Cocos nucifera* L., but the spread of this plant was not found in 500 m radius. This indicates that the bee had a flight range of more than 500 m radius in search of food. The results of the identification and counting of pollen based on frequency did not find a predominant pollen (more than 45 %). Pollen *Zea mays* spp. *Mays* L. and *Carica papaya* L. were secondary (16-45 %). *Vigna unguiculate sesquipedalis* (L.), *Solanum melongena* L., and *Mangifera indica* were important minor pollen (3-15 %) and the rest were minor pollen (less than 3 %).

Estimating the shelf life of Klanceng honey (*Tetragonula laeviceps*) was done by making comparisons of processing (T1, T2) and different storage times. The heating process at high temperature (T2) within 7 h has a major influence on the selected physicochemical parameters. Heating at low temperatures (T1) will be a dilemma for countries with high temperatures such as Indonesia. Without doing treatment (T1) there will be small damage that accumulates so that it becomes significant damage (Castro-Vázquez et al., 2012).

All physicochemical parameters showed sensitivity to high heating. In this study, the effect was clearly seen on GO, invertase, honey color, and other components, at low-temperature heating (T1), only some sensitive physicochemical parameters such as invertase. Referring to the standard control (UT0), the heating effect on the selected physicochemical was:

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At T2, GO has the largest difference (193.33 μg g^{-1} H_2O_2). High heating effect (T2) affects the decrease in the value of GO. This causes the function of the glucose oxidase catalyst to decrease in the β-D- glucose oxidation reaction in Klanceng honey. Low temperature has not shown sensitivity (14 μg g^{-1} H_2O_2). Storage showed only small changes in UT (1.52 μg g^{-1} H_2O_2), as well as in T1 and T2.

Invertase continued to decrease during high heating. This results in a decrease in the hydrolysis process of sucrose (disaccharide) into glucose and fructose (monosaccharide). Given the importance of this role, invertase activity is usually used to test the quality of honey (Karabournioti & Zervalaki, 2001). At low heating, invertase is very sensitive to T1. Storage has a great influence on this enzyme. The decline reached 47.5 % (UT), 43.8 % (T1) and 67.9 % (T2). The sensitivity of invertase to processing (T1, T2) and storage is feasible to be used as a parameter of fresh honey quality. The biggest damage to honey in Indonesia is this invertase enzyme, because without the process (UT) it also decreases due to climate.

Honey color increased in processing (T1, T2) and storage. The increase in honey color indicates the addition of melanoidin formed at the end of the MRPs (Nagai et al., 2018), which have honey colors in the range of 420 nm and 450 nm. The increase in honey color in the range of 450 nm indicates the addition of melanoidin in processing (T1, T2) and storage (12, 24 months).

There was a significant difference ($p<0.05$) in the decrease in the diastase enzyme, a large change at T2 (6.53 DN), while at T1 it showed a small change (1.73 DN). The same conditions occur for storage in processes T0 and T1 (small change) and T2 (large change). The decrease in the diastase enzyme causes disruption of the maltose digestion process and the conversion of other sugars in honey. This enzyme comes from bee saliva, so the higher diastase indicates the purity of the honey. This is a benchmark so that the diastase parameter is used as the main parameter of honey freshness (Pasias et al., 2018).

There was a significant difference ($p<0.05$) in the increase in HMF at T2, but not significant at T1. The highest increase occurred at T2 (2.83 mg/kg) while at T1 (1.7 mg/kg). The effect of storage has a large enough effect, the difference of 17.91 mg/kg in UT for 12 months, 19.11 mg/kg at UT1 for 12 months and 25.3 mg/kg at UT2 for 12 months. The increase in HMF indicates the amount of cyclic aldehyde compounds obtained from the degradation of sugar compounds (Maillard reaction / MRPs). HMF is highly soluble in water as well as other organic solvents. The molecular ring is a Furan containing an alcohol and an aldehyde group. In this study, the HMF was still within the standard limits (< 40 mg/kg) for T1 and T2 (T2.0 and T2.12) but for T2.24 it was outside the standard limits.

Acid phosphatase (AP) decreased drastically at T2 (16.4 Mg P/100 g/24 h), while T1 (0.3 Mg P/100 g/ 24 h). Storage had the greatest effect at 24 months (UT: 10.9; T1: 10.66; T2: 22.4). The decrease in AP will make the pH of the honey tend to be low due to the hydrolysis effect of various phosphate esters in the liquid honey.

Phenolic increased in the T2 process (13.83 mg/kg gallic acid), while at T1 (10 mg/kg gallic acid). Storage has a greater effect than heating. There was a difference in storage for 24 h (std=UT0) at UT= 29.5, T1= 29.6 and T2= 58.16. The increase in phenolic will make the pH of honey become acidic because it is easy to remove H^+ ions from the hydroxyl group. These conditions actually stabilize free radicals when releasing hydrogen.

Antiradical/antioxidant through the DPPH test after the T1 process decreased, and the decrease in the DPPH value continued after the T2 process. The effect of storage can reduce DPPH greater than the heating process. This can be seen from UT24 to UT0 where there is a difference of 11.04 mg/mL. The value of DPPH-IC_{50} in the sample which shows a strong antioxidant was <50 mg/mL (Molyneux, 2004). However, this antioxidant value cannot be separated from the formation of MRPs which have antiradical activity (Akhmazillah Fauzi & Mehdi Farid, 2017).

From the description above, the biochemical compounds that were sensitive to heating and storage were invertase, GO, and diastase. The heating effect caused a decrease in the concentration (UT0 control) of invertase (92 %), GO (85 %), and diastase (36 %). While the effect of storage on invertase (43-68 %), diastase (36-52 %), and GO (0.85-58 %). This study showed that invertase was most sensitive than diastase and GO due to heating and storage.This study supports the statement of Al-Rubaie and Al-Fekaiki (2022), Kekeçoğlu et al. (2022), and

Makhloufi et al. (2020); which states that invertase is the most sensitive to heating and storage, although many previous researchers think that diastase is the most sensitive (Akalın et al., 2017; Singh & Singh, 2018).

Referring to the results of the analysis and discussion, while still taking into account the standards (International Honey Commission, 2009), heating of Klanceng honey at low temperatures (T1) with 12 and 24 months storage still meets the honey eligibility standards. However, on high heating (T2) at 24 months storage did not enter the standard (HMF=55.33 ± .57 mg/kg) because the standard HMF = max 40 mg/kg.

The shelf life of Klanceng honey can reach two years with a record that there is no high heating (70 ºC). Heating is tolerated only low temperature (40 ºC). This finding will certainly be different from other studies because the Klanceng honey sample is closely related to the type of feed (flower pollen) and the location of the bee. This research can at least provide initial information regarding the shelf life of Klanceng honey.

Conclusion

Heating at high temperatures for a short time had a greater effect on damage to honey than heating at low temperatures for a long time. Parameters that were sensitive to heating and storage were diastase, invertase, and honey color. The test results for these three parameters were still within tolerance limits, as well as for DPPH, GO, phenolic, and AP.

The HMF value (55.33 ± 0.57 mg/kg) exceeds the maximum allowable limit (max 40 ppm), this was due to the significant effect of heating on T2 and the storage process. The shelf life of Klanceng Honey was two years, as long as it was not heated to high temperatures (70 ºC). The recommendation to obtain a long honey shelf life was to process honey at the lowest possible temperature or avoid the heating process.

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