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EFFECT OF DIFFERENT PACKAGING MATERIALS ON THE QUALITY OF STINGLESS BEE HONEY (*Heterotrigona itama*) DURING STORAGE

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Abstract: Bee can be categorised as either honeybee or a stingless bee. Both produce honey, which is beneficial for human consumption because it is rich in nutrients. However, there is no study done on its quality affected by packaging materials during storage. This study evaluated the physicochemical properties of stingless bee honey (*Heterotrigona itama*) when stored in different packaging materials, clear and amber glass bottles, and clear and amber plastic bottles, stored at room temperature for three months. Honey was harvested from the pots to test its quality after some time. The physicochemical analyses were colour L*, a*, and b*, pH value, total titratable acidity, antioxidant activity, ashes, moisture content, and water activity. During three months of storage, none of the storage materials showed a significant difference from each other. If the honey storage is extended, honey stored in amber glass will give better quality at the end of the storage day due to the consistent parameter such as antioxidant activity, which is considered one of the valuable components in stingless bee honey.

Keywords: Stingless bee, physico-chemicals, packaging, quality, storage, antioxidant.

Introduction

Stingless bee honey (*Heterotrigona itama*) is also known as *Kelulut* honey or Meliponines honey as it comes from a subfamily of Meliponinae. Stingless bees are easily distinguished from other bees by three characteristics: Reduction and weakness of the wing venation, presence of penicillium and reduction of the sting (Wille, 1983). The stingless bees produced such valuable honey and propolis as it is rich in antioxidants. The growing interest in honey produced by a stingless bee is possibly due to its beneficial properties such as antiseptic, antimicrobial, anticancer, anti-inflammatory, wound-healing and promote cell functions in erythrocytes (Vit *et al.,* 1998; Alvarez-Suarez *et al.,* 2012; Silva *et al.,* 2006; 2013). Stingless bee honey exhibits higher antioxidants than honey, providing plenty of benefits for human health (Mohammed, 2010). Natural antioxidants exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, anti-allergic, anti-thrombotic, and vasodilatory actions (Al-Mamary, 2002). Honey contains cinnamic acid, an antioxidant agent and some flavonoids approved for antibacterial applications (Rahman *et al*., 2010). Many studies have demonstrated that honey is a source of natural antioxidants, effectively reducing the risk of heart disease, cancer, immune system deficiency, cataracts, different inflammatory processes, and so on (National Honey Board, 2002). The nutrient is mainly contributed by antioxidants such as flavonoids, phenolic beta-carotene, vitamins, and many more. Besides sugars, honey also contains several vitamins, especially B complex and vitamin C, and many minerals (Vallianou *et al*., 2014). However, one of the largest obstacles to meliponiculture lies in the market chain (Drummond, 2013). Naidu (2003) reported that L-ascorbic acid ($C_6H_8O_6$); vitamin C is sensitive to air, light, and heat and easily destroyed by prolonged storage and over-processing of food. Since vitamin C is also considered one of the antioxidants in stingless bee honey (Muruke, 2014), exposure of the honey to light may degrade the vitamin C content in the honey. Packaging will play a very important role in avoiding any degradation.

Most of the honey is packaged with clear glass or plastic packaging. Unfortunately, no studies are being focused on the effect of different packaging materials on stingless bee honey quality. The excess light transmission on the honey may degrade the antioxidant (Ioannou *et al*., 2020) and subsequently reduce the nutrient if not properly packed. Light exposure can be natural or artificially exposed during processing, packaging, storage, shipping, and marketing. The manufacturer needs to be concerned about reducing it by packing it in suitable packaging materials as some other chemical reactions also accelerate under light exposure. Those reactions might deteriorate the food itself and reduce its quality. According to Garedew *et al.* (2003), Trigona honey produced by a stingless bee is less viscous, darker in colour and has a strong acid flavour. Unsuitable packaging may corrode due to high acidity in the packaging and negatively affect human health. Based on previous studies, some reports showed changes happen to honey during storage due to storage temperature and affected the antioxidant pigments such as carotenoids and flavonoids (Terrab *et al.,* 2004; Baltrusaityte *et al.,* 2007). The packaging materials may also affect the nutrient inside the honey due to the plastic material's permeability properties, which allow oxygen transmission (Siracusa, 2012). Thus, to preserve the nutrient content and freshness during commercialisation, proper and suitable packaging should be used to maintain the quality of the honey. Therefore, the packaging material is one of the important factors during processing before commercialisation to the market. It is one of the issues among local industries, especially when they want to transport and market honey globally. Thus, the objective of the study is to determine the physico-chemical properties of stingless bee honey in different packaging materials during storage.

Materials and Methods

Samples Collection and Packaging Materials

The experiment was conducted at the Postharvest Laboratory of Universiti Malaysia Terengganu. Stingless bee honey was bought at Kg. Gong Beris, Jalan Teja 3, Marang, Terengganu. Transparent and plastic bottles, amber glass and plastic bottles were purchased at Dungun, Terengganu and sterilised before the experiment.

Samples Preparation

Harvested honey was separated into four different types of bottles: Transparent glass and plastic bottles, amber glass and plastic bottles with two replicates for each. Each packaging was filled with 30 ml of stingless bee honey and stored at room temperature for three months and analysed every 18 days interval.

Parameters Evaluation

Samples were analysed with parameters such as colour, pH value, total acidity, moisture content, water activity, ash content, and antioxidant activity. Konica-Minolta colourimeter (Japan) was used for colour analysis and a pH meter was used for pH value determination. Total acidity was evaluated using the titration method with 0.1 N sodium hydroxide (NaOH) solution. The samples' moisture content was analysed using a moisture analyser (Radwag, Poland) and water activity was measured using AquaLab Dew Point Water Activity Meter 4TE (Aqualab, USA). The ash content in the samples was determined by leaving them in a furnace and incinerating them at 550^{o}C for 3 hours. The antioxidant activity of the samples was measured by using the 2-2-diphenyl-1-picrylhydrazil (DPPH) assay method (Alzahrani *et al*., 2012). The radical inhibition activity of the sample against the reagent was recorded at 517 nm using a UV-Vis spectrophotometer. The experiment was laid out Completely Randomised Design (CRD). The results were subjected to Kruskal-Wallis using the SPSS program.

Results and Discussion

*Colour (L*a*b* value)*

Results showed no significant difference for the L*, a*, and b* values for all samples during storage (Figures 1-3). However, from the graph's trend, if the storage day was extended up to 12 months, it may show a significant difference since the composition of honey, which is related to antioxidants and colour showed changes during the storage time due to the penetration of light and oxidation (Chou *et al*., 2020).

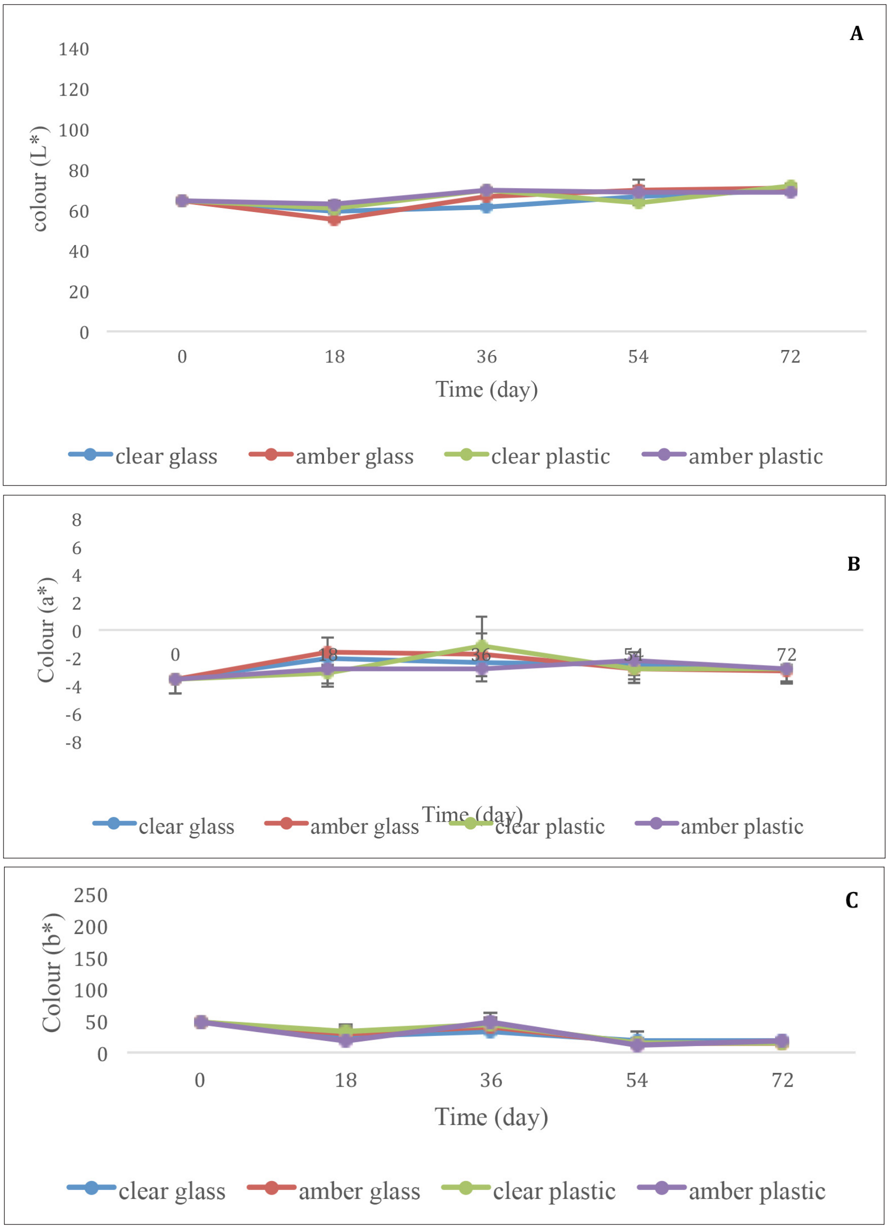

Figure 1: (A-C) Colour value of stingless bee honey in different packaging materials during 72 days of storage

pH Measurement

Based on the results obtained, the pH value for all treatments was still in a given range. All treatments showed no significant difference ($p > 0.05$) in pH value for all packaging during storage (Figure 2). According to Souza *et al.* (2006), the pH value for stingless bee honey ranged between pH 3.15-4.66. The acidity of the honey may be due to the survival of bacteria under high acidic condition (less than pH 3) called acidophilic bacteria (Baker-Austin & Dopson, 2007). Although the bottles were kept closed, this bacteria might exist and still live while respiring anaerobically or without the presence of oxygen. Since bacteria were living in the packaging, fermentation could occur due to the anaerobic respiration and lactic acid produced as a by-product (BBC, 2014) which increased the acidity of the honey itself.

Hence, the results suggested that different types of packaging material do not significantly influence the pH value during storage. Although the results of the pH value throughout the storage were not significant, it is suggested to the manufacturer use packaging material that may resist high acidity levels such as glass, to store stingless bee honey. It is recommended as if the acidity level keeps increasing for some time, the inner surface of the packaging will become corrosive and affect the honey, especially plastic material. The phthalate content related to acidic products packed in plastic packaging is a concerning issue found in a study by Rastkari *et al*. (2017).

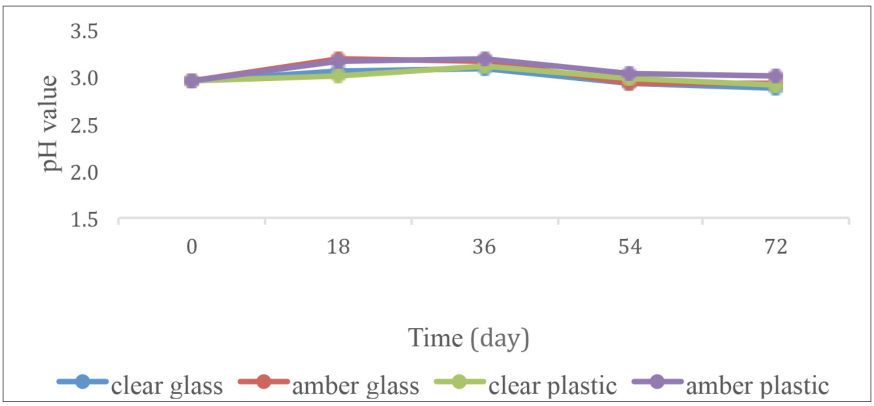

Figure 2: pH value of stingless bee honey in different packaging materials during storage

Total Acidity

In this study, the total acidity of honey is still in the range of 30.0 to 90.0 meq/kg (Cortopassi-Laurino & Gelli, 1991). The result (Figure 3) is reliable according to Nascimento *et al*. (2015), stingless bee honey usually features high acidity compared to honey from *Apis mellifera.* However, the total acidity during the experiment was insignificant ($p>0.05$). The trend of the acidity from the initial to end storage day tends to increase as this may be due to the maturation state of honey differing from the state at the beginning of the storage with fermentation occurring or by the activity of lactic acid bacteria during the storage.

The high value of acidity will affect the quality control of stingless bee honey if stored for longer periods and not safe to be consumed by humans as too acidic may erode the packaging, thus affecting the quality of the honey itself. The main contributor to the phthalate syndrome in humans, which is dibutyl phthalate (Henkel, 2018), may come from the packaging material and is not strongly bound to the polymer structure (Koo *et al.* 2017). This harmful substance can potentially migrate into honey and affect human health.

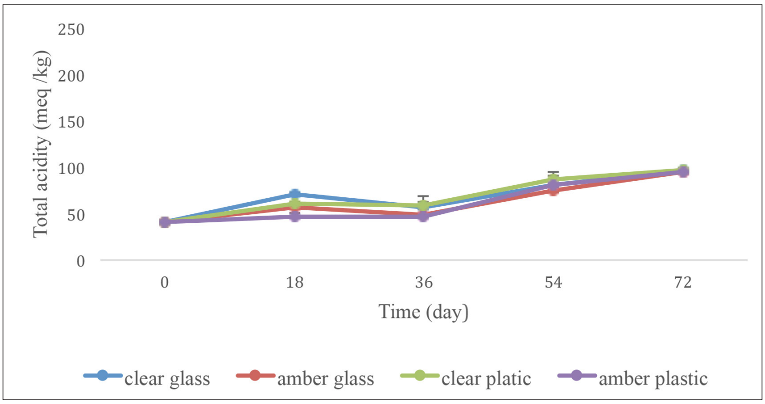

Figure 3: Total acidity of stingless bee honey in different packaging materials during storage

Moisture Content

Figure 4 shows the percentage of moisture content of stingless bee honey in different packaging materials during storage. The average moisture of all samples ranged from 24.14% to 28.53%. The threshold of moisture for stingless bee honey is 35% (Villas-Boas & Malaspina, 2005). Even though the moisture content was still in the range, there was no significant difference ($p>0.05$) between all the honey samples stored in different packaging materials. Based on the previous study, the moisture content in stingless bee honey can be influenced by the relative humidity and possibly by the packaging materials used after harvesting (Chaves *et al*., 2012). Stingless bee honey is high in hygroscopicity (Nascimento *et al*., 2015) which tends to absorb moisture from the environment. Therefore, proper packaging and suitable relative humidity should be properly organised during harvest until storage.

Plastic materials with no barrier toward air absorption should be avoided so that the honey's shelf life will not be reduced but can be maintained or extended. It is due to this fact that excess moisture content may have a detrimental effect on the sensory properties of honey if it exceeds the maximum permitted level (Prica *et al*., 2014).

The moisture content in all packaging is still satisfactory as the higher moisture content in honey can be beneficial or harmful to the honey and consumers, where the honey will facilitate the proliferation of yeasts that cause a fermentation process to occur (Ribeiro *et al*., 2009). Thus it made the honey unsafe to be consumed by humans and hindered its marketing (Ribeiro *et al*., 2009). However, with the moisture content recommended by Malaysian Standard for stingless honey (Malaysian Standard, 2017; Yap *et al.,* 2019), the shelf life of honey can be enhanced due to unfavourable conditions for microbial development (Bertoldi *et al*., 2007).

Hence, glass bottle treatment tends to have less moisture content at the end of the storage day. It is because the properties of glass do not allow any moisture to be absorbed, whereas plastic can still transmit water. It is also recommended that honey should be stored in refrigerated conditions to avoid any degradation or modification of physico-chemical properties to ensure the good quality of honey products with long periods of storage (do Nascimento *et al*., 2015).

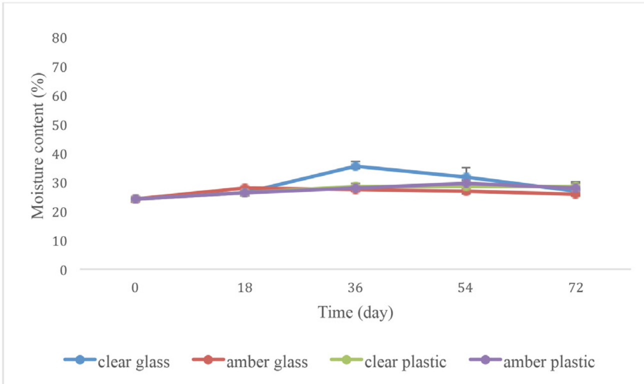

Figure 4: Moisture content (%) of stingless bee honey in different packaging materials during storage

Water Activity

The stability of honey eventually affects water availability, which is characterised by water activity (a_w) (Figure 5). During three months of the experiment, the water activity of all packaged honey was not significantly different ($p>0.05$). According to Wilczyńska and Ruszkowska (2014), the water activity of honey during storage may vary and can experience many physical and chemical changes. The property changes may be due to the packaging material's permeability, which allows the humidity from the surrounding into the stored food (Othman *et al*. 2017). Too high humidity is not a suitable atmosphere to store honey, especially raw or unprocessed honey, as it may cause water vapour adsorption from the environment, thus increasing its water activity (Wilczyńska & Ruszkowska, 2014).

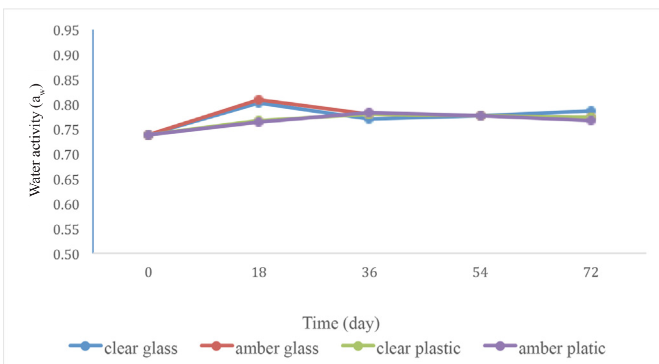

Figure 5: Water activity (a_w) of stingless bee honey in different packaging materials during storage

Total Ash Content

In this study, the percentage of ashes from all treatments at the storage end ranged from 0.02 to 0.03 % (Figure 6). Although the results obtained did not have any significant difference ($p>0.05$), similar results were detected by Souza *et al*. (2009) for Melipona honey from the Northeast area of Brazil. Thus, a similar mineral amount can still be obtained by consuming this honey in any packaging material for up to three months. However, amber glass bottle treatment showed that it might have the potential to be higher in ashes in longer storage as it experienced the least decreasing pattern compared to other treatments and retained most of the minerals inside the honey sample. Based on previous studies, the storage container may affect the mineral content in the honey, it generating or contaminating Since honey can be conserved without the need for refrigeration while the shelf life extended over two years (Drummond, 2013), three months of storage may not give any significant effect to the honey. A previous study documented that honey from bees undergoes the least change from the fresh one when store at room temperature with no preservative or heating process for eight months but showed a significant effect after the eighth month to the 16th month (Qamer *et al*., 2013). Since stingless bee honey contains higher moisture content (Bijlsma *et al*., 2006), which can be subjected to deterioration of honey, it may take less than eight months. However, according to a study by Chuttong *et al.* (2015), changes in the stingless bee honey quality parameters started during the sixth month of storage. Thus, it is proved that longer than three months of storage should be done to identify the effect of mineral content inside different packaging materials.

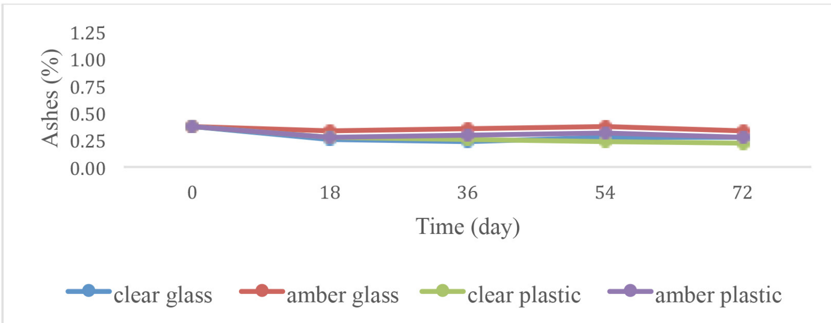

Figure 6: Ash content (%) of stingless bee honey in different packaging materials during storage

Antioxidant Activity

Results showed no significance different ($p>0.05$) for antioxidant activity during three months of storage between days and all treatments applied (Figure 7). Based on the results, the antioxidant activity still exists in the honey after some time. However, it tends to decrease due to oxygen, light, and heat during storage, as those factors may degrade and oxidise polyphenolic compounds (Fender, 2005). However, the results were not enough to prove the antioxidant's degradation process since it was insignificant. Extending the storage of this stingless bee honey up to 6 months may prove that penetration of light, adsorption of oxygen and presence of heat may destroy the antioxidant activity. Besides, as Rakkimuthu *et al.* (2016) stated, pH 1 to 3 may stabilise the antioxidant content. According to Cavia *et al.* (2007), acidity can improve the antioxidant activity against the action of microorganisms. The acidic property of stingless bee honey is high enough for the antioxidant activity to be detected (Figure 3). This is also supported by Nayik and Nanda (2016), where the antioxidant properties of saffron honey were significantly ($p<0.05$) decreased with the increase in pH from 3 to 6. Hence, it can be proved that antioxidant is stable in acidic condition making stingless bee honey beneficial for human consumption.

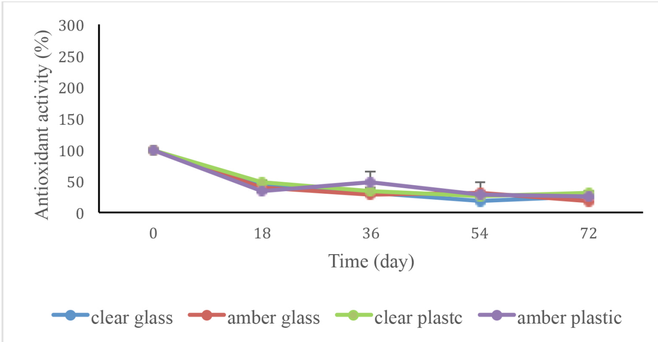

Figure 7: Antioxidant activity (%) of stingless bee honey in different packaging materials during storage

Conclusion

All quality parameters of stingless bee (*Heterotrigona itama)* honey in different packaging materials showed no significant difference ($p>0.05$) during three months of storage. As all of the physico-chemicals analyses tested on stingless bee honey were not affected by the packaging materials applied during storage, it is revealed that the nutrients content, mainly antioxidants and total ash, are stable and still beneficial for human consumption and can be retained by using any kind of packaging materials up to three months. The results obtained from this study can help guide the manufacturer in determining the storage packaging material to store stingless bee honey at room temperature for longer storage time to minimise the changes and maintain the shelf life. Overall, the trend found in this study suggested that stingless bee honey is better to be kept in amber glass packaging to retain the maximum beneficial properties during long storage. Worldwide and domestic meliponiculture has faced great difficulties concerning the conservation of this product due to high moisture content, resulting in fermentation and consequent deterioration.

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