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Effect of storage conditions on the crystallisation behaviour of selected Malaysian honeys

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Abstract

Honey is a sweet liquid food of high nutritional value and it provides immense health benefits. It is highly concentrated with sugar and contains mostly glucose and fructose, which will crystallize over a period of time. Crystallisation of honey will affect its quality, as well as consumers' acceptability. Storage condition is one of the factors that influence the crystallisation of honey. Different types of honey may need different storage conditions to retain the quality. This research was conducted with the aims to study the crystallisation behaviour of the selected Malaysian honeys and to determine the storage conditions that influence the formation of crystal. The crystallisation of Malaysian honeys (Hutan, Kelulut, Acacia, Gelam) stored at 25, 4 and -20°C for different storage times of 0, 5, 14, 30, 60 and 180 days was analyzed by a differential scanning calorimeter (DSC), and sugar composition was analyzed using a high performance liquid chromatography (HPLC). The results showed that Hutan honey had the greatest crystal formation at the storage temperature of 4°C even after 14 days of storage. Glucose compositions in Hutan and Gelam honeys were also high which were 33.49 ± 0.53 % and 33.93 ± 0.15 %, respectively. The enthalpy value for the storage temperature of 25°C, which represents the amount of heat needed to melt crystals present in honey, was the lowest (0.37 ± 0.1 – 2.56 ± 0.5 J/g) compared to other storage temperatures, which showed only a small amount of crystals was formed at this temperature. Thus, this study suggested that the crystallisation behaviour of Malaysian honeys is influenced by the storage condition and will be different for each type of honey.

Introduction

Honey mainly consists of sugar and water (Natalia *et al*., 2014). It is highly concentrated with glucose and fructose, as well as other complex sugars (Laos *et al.*, 2011). There are more than 25 types of sugar in honey,which make up about 95% of total dry weight of honey (Weston, 1999). Honey also contains minor components such as vitamins, especially B complex and vitamin C, minerals, amino acids, and various phytochemicals (Kaskoniene *et al*., 2010).The presence of enzymes such as glucose oxidase, diastase, invertase, phosphatase, catalase and peroxidase has also been documented in honey (Crane, 1975). According to Tosi *et al.* (2004), biochemical composition of honeys varies greatly, depending on the floral, regional, and climatic conditions. In Malaysia, there are varieties of honey produced from honeybee *(Apismellifera)*, which are Tualang, Gelam, Acacia, Hutan and Nenas while Kelulut honey produced from stingless bees.

Crystallisation is a mass transfer phenomenon that leads to the creation of a solid-liquid interface and results in a positive contribution to the free energy of nucleation (Marangoni and Wesdorp, 2013). Supersaturation is the driving force of crystallisation (Chen and Chou, 1993). Honey is a glucose supersaturated solution and can granulate or crystallize spontaneously at room temperature (Zamora and Chirife, 2006) during storage (Lupano, 1997). In food products, crystallisation somehow may be important or cause defects to the quality, texture, and shelf life (Hartel, 2001). However, crystallisation is an undesirable process in liquid honey (Assil *et al*., 1991) and has been misunderstood by most of the customers as they claim it as an adulterated honey. As crystallisation occurs naturally in honey, it can cause major problems during handling and processing (Assil *et al.*, 1991; Venir *et al*., 2010) and also makes fractionating and pouring difficult (Tosi *et al*., 2004).

Honey tends to crystallize over a period of time due to the loss of water in glucose and become precipitate. These precipitate is in the form of glucose monohydrate. It will then revert to a more stable saturated state (Zamora and Chirife, 2006; Venir *et al*., 2010). Crystallisation in honey causes

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several physical, chemical and biological changes in the honey,which leads to quality degradation. On the other hand, uncontrolled crystallisation may cause phase separation that results in a crystalline phase at the bottom and a dark-coloured supernatant liquid phase that makes honey less attractive to consumers (Zamora and Chirife, 2006). Crystallize honey will become opaque and show a waxy appearance (Tosi *et al*., 2004). Nearly all types of honey crystallize but the time period varies for different types (Shafiq *et al.*, 2014).

Many factors influence the crystallisation of honey. According to Jamieson (1954), honey that crystallizes quickly has high dextrose content,whereas honey with high levulose content will not form crystals. The tendency of honey to crystallize depends primarily on its glucose content and moisture level (Doner, 1977). Hartel (2001) stressed that glucose content in honey is probably the main determinant of whether a particular honey crystallizes or not. Storage temperature also affects honey crystallisation. Zamora and Chirife (2006) reported that the temperature between 10 and 15oC are the optimum temperature to increase crystallisation in honey. Moreover, crystallisation rate at any temperature depends on 2 factors, which are glucose diffusivity that depends on honey viscosity,as well as saturation solubility. As fructose is more soluble in water, it can delay the onset of crystallisation of honey (Hartel, 2001),whereas the presence of glucose that is lower in solubility can enhance the crystallisation process. White (1975) found that glucose/fructose (G/F) ratio is another parameter that has the tendency to crystallize honey.

Dynamic thermal analysis methods such as using the differential scanning calorimetry have been proven as powerful tools to probe the extent, rate, and sequence of thermal events in both pure and complex carbohydrate systems (Biliaderis, 1990). DSC can measure the heat flow into or from a sample as it is heated, cooled or under isothermal condition. Hohne *et al.* (2003) reported that DSC is most commonly used for thermal analysis as it can detect most of the physical and chemical changes involving heat absorption or liberation. DSC has been used by Lupano (1997) to study the crystallisation kinetics in honey stored at various temperatures by identifying the melting enthalpy (ΔH) as an expression of honey granulation. The aims of this present work are to study the crystallisation behaviour of the selected Malaysian honeys subjected to different storage times and temperatures up to 180 days at -20, 4 and 25^{o}C and to determine the storage conditions that influence the formation of crystal.

Materials and methods

Materials

Four different types of honey sample (Hutan, Kelulut, Gelam, Acacia) were purchased from a local honey manufacturer as fresh, non-crystallized honeys. Samples of 40 mL of each type of honey were kept in a clear glass container and stored at different temperatures (-20, 4, 25^{o}C) for different storage times ranging from 0, 5, 14, 30, 60 and 180 days. In all cases, the measurement was performed after gently stirring the honey in order to obtain samples that are as homogeneous as possible.

Determination of sugar composition

Analysis of sugars (glucose and fructose) was performed by high pressure liquid chromatography (HPLC Prominence System from Shimadzu) with a refractive index detector (RID-10A Shimadzu). For the column Shim-Pack CLC-NH_2 (6.0 9150 mm), 5 µm was eluted by the use of an isocratic system with acetonitrile (pump A – LC 20AT Shimadzu) and water (pump B – LC 20AT Shimadzu) (80:20, v/v) previously filtered through a 0.45 µm filter. The separation was performed at a flow rate of 1.3 mL min^{-1}, with the column and detector temperature set at 30°C using an autosampler (SIL20A-Shimadzu). Quantification was achieved by the external calibration method, and the calibration curves ranged from 50 to 500 µg mL^{-1} for glucose and fructose. Sugar contents were further expressed in gram; per 100 g of dry weight.

Determination of crystallisation behaviour of honey

Crystallisation behaviour of honey was determined using DSC (TA 4000 system equipped with TC15 TA Processor, DSC 30 measuring cell and Star software V8.10, Mettler, Greinfensee, Switzerland). Heat flow calibration was performed with indium. Honey samples (10-15 mg) were placed in a 100 µl aluminium DSC crucible pan and an empty crucible with cover was used as reference. The samples and the reference were heated to 80^{o}C at a heating rate of 5^{o}C min^{-1}. The thermograms were obtained in triplicate. The average value of enthalpies of melting was computed from the endothermic peaks.

Statistical analysis

Data were analysed statistically by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test. Statistical processing was carried out using SPSS 20. A difference was considered significant at $p < 0.05$ levels. All the data

Table 1. Composition of glucose and fructose in honey samples

Honey type	Glucose (%)	Fructose (%)	G/F
Hutan	33.49 ± 0.53^a	35.15 ± 0.49^a	0.95 ± 0.00^a
Kelulut	9.19 ± 0.06^c	9.69 ± 0.07^c	0.95 ± 0.00^a
Acacia	23.38 ± 0.22^b	24.64 ± 1.31^b	0.95 ± 0.04^a
Gelam	33.93 ± 0.15^a	38.08 ± 0.09^a	0.89 ± 0.01^a

Each value is expressed as mean ± S.D (n=3). Different superscript letters in the same column indicate significant differences at $p<0.05$.

shown in figures and tables are the means of triplicate determinations.

Results and discussion

Based on the results shown in Table 1, Hutan and Gelam honey have higher glucose levels, which are 33.4 ±0.53% and 33.93±0.15%, respectively. Higher glucose concentration can initiate crystallisation in honey. This fact is consistent with this study as the formation of crystals in Hutan and Gelam honeys is higher compared to Kelulut and Acacia honeys. Moreover, Bogdonov (1993) also reported that honey will crystallize faster if the glucose content is greater than 28% to 30%. As can be seen in Table 1, Hutan and Gelam honey have a glucose concentration of more than 30%, which strongly shows that both honeys have tendency to initiate crystallisation.

Generally, the composition of fructose and glucose in honey ranges from 38 – 40% and 33 – 35% (Doner, 1977). As composition of sugar in honey is higher, thus it leads to crystallisation. Different honeys have different percentage of fructose and glucose that will determine the crystallisation rate. According to Assil *et al.* (1991) and Bogdanov (1993), crystallisation rate increases when glucose concentration in honey is increased. This is because glucose has lower solubility in water compared to fructose, which is more soluble. Thus, honey with a higher fructose concentration will remain in solution for a longer period of time (Gleiter *et al*., 2006).

According to White (1975), honey will crystallize rapidly when the G/F ratio is less than 1.14. In this research, all types of honey have ratio less than 1. Theoretically, based on the results obtained, all honeys will form crystal rapidly. The G/F ratio cannot determine the crystallisation rate well. Manikis and Thrasivoulou (2001) found that G/F ratio cannot be used as predictor indicator for crystallisation. However, they believed that the higher percentage of glucose contained in honey will distinguish the rate of crystallisation.

Figure 1 shows the thermograms of honeys stored

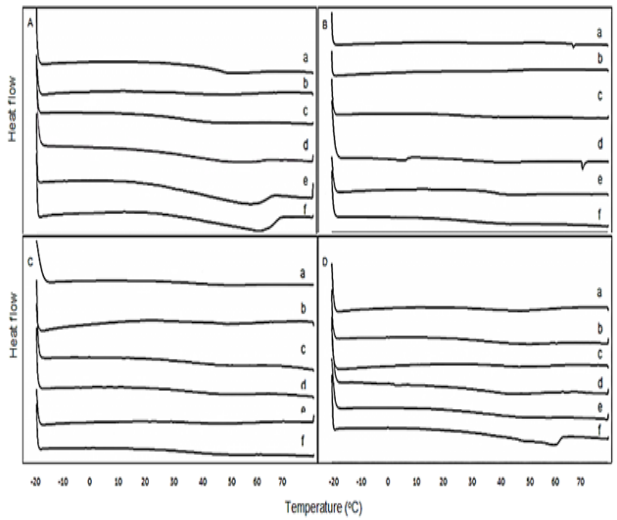

Figure 1. Differential scanning calorimetry thermograms of different honey at -20oC from 0 days to 180 days: (A) Hutan; (B) Kelulut; (C) Acacia; (D) Gelam; Storage time: (a) 0 days; (b) 5 days; (c) 14 days; (d) 30 days; (e) 60 days; (f) 180 days

at -20°C. The existence of peak in thermograms corresponds to the melting of crystals in honey. If more than one peak present in the thermogram, it shows that there are more than one type of crystals with different melting points present in the sample. In all graphs, a single peak can be observed starting from 60 days storage time. The peaks are visible at 60 days for Hutan (Figure 1A) and Gelam (Figure 1D) honeys, but thermograms for Kelulut (Figure 1B) and Acacia (Figure 1C) honeys do not show any peak at 60 days of storage time. This explains that more crystallized honey was formed in Hutan and Gelam honeys as both honeys contain higher glucose level. The crystallisation process in honey slowed down and delayed at the freezing temperature (White, 1974) as water cannot precipitate out of the glucose if it was kept frozen. Consequently, nucleation cannot occur as rapid cooling of a sugar solution below the glass transition temperature will yield a stable amorphous solid (Hartel and Shastry, 1991). According to Johnson *et al.* (1975) and Gómez-Díaz *et al.* (2009), decreasing temperature

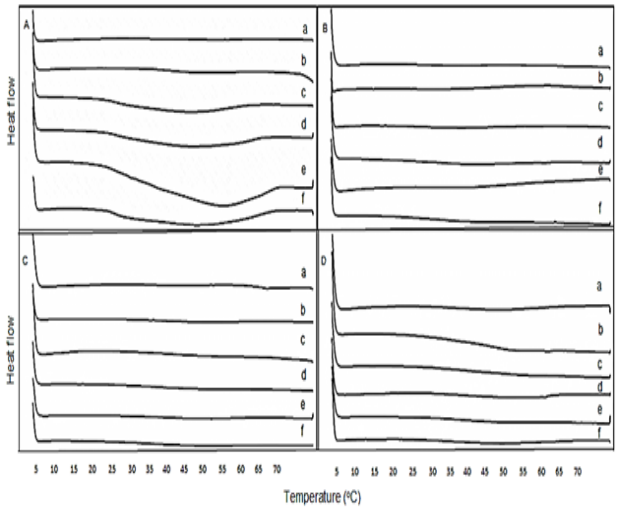

Figure 2. Differential scanning calorimetry thermograms of different honey at 4°C from 0 days to 180 days: (A) Hutan; (B) Kelulut; (C) Acacia; (D) Gelam; Storage time: (a) 0 days; (b) 5 days; (c) 14 days; (d) 30 days; (e) 60 days; (f) 180 days

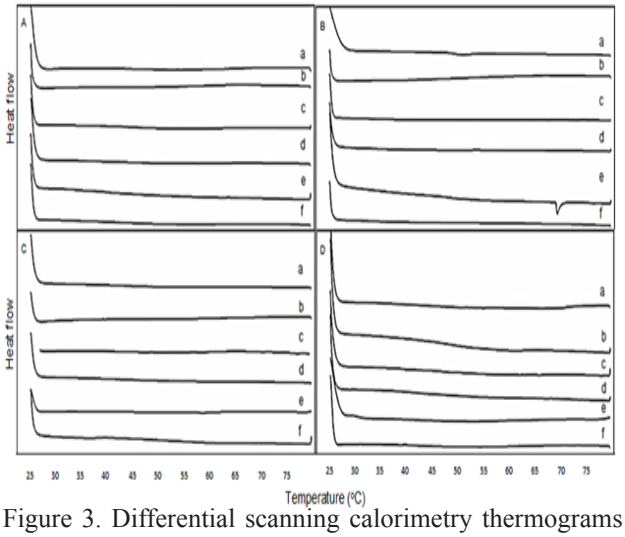

Figure 3. Differential scanning calorimetry thermograms of different honey at 25oC from 0 days to 180 days: (A) Hutan; (B) Kelulut; (C) Acacia; (D) Gelam; Storage time: (a) 0 days; (b) 5 days; (c) 14 days; (d) 30 days; (e) 60 days; (f) 180 days

will increase the viscosity of honey, thus slowing down the mobility of the molecules and lower the rate of crystal growth. Honey is known as Newtonian liquid and it is expected to increase in viscosity as it is highly sensitive to temperature (Sapode *et al.*, 2002). Zamora and Chirife (2006) stated that when the temperature is lowered, the diffusion coefficient of glucose will drop and delay the crystallisation.

Figure 2 shows thermograms of honeys stored at 4°C. It can be seen that the peak area for Hutan honey is wider in Figure 2(A) when compared to the peak area for Kelulut, Acacia and Gelam honeys presented in Figure 2(B-D). According to Lupano (1997), the greater the peak area, more granulated honey crystals are present. In addition, the greater peak area shows that more activation energy is needed to melt the crystals. Honey stored in chiller at 4°C shows the greatest crystals formation among the storage temperatures used in this study. This reveals that slower reduction in temperature may initiate the nucleation process as sugar molecules have sufficient mobility to form crystal lattice (Hartel and Shastry, 1991). The peak can be observed starting from 14 days storage and the peak area increased as it is stored for longer storage time. An increase in storage time could make the crystallisation process longer, thus producing greater peak area, which is related to crystal growth (Al-Habsi *et al*., 2013).

The thermograms for all types of honey stored at 25°C are shown in Figure 3. It can be seen that most of the peaks are not well defined, suggesting that the formation of crystals is minimum for this storage temperature. According to Sopade *et al*. (2002), high storage temperature may slow down the growth of crystal as it can reduce the viscosity in honey. Zamora and Chirife (2006) and Costa *et al.* (2015) noted that when temperature increases, the solubility of glucose increases and the tendency to crystallize is low, which explains the slow crystal growth in all honeys observed at 25°C.

Enthalpy is a function related to the heat absorbed by a chemical system (Blackman *et al*., 2008). Based on Figure 4, enthalpy increases rapidly with storage time for honeys stored at 4 and -20°C as more activation energy is needed to melt the crystals. The enthalpy value for 25°C also increases but it is the lowest compared to other storage temperatures. This explains that only a small amount of crystals are formed at this temperature. As mentioned earlier, glucose is soluble as temperature increases, thus it can be observed that the crystallisation rate is the slowest at 25°C. All honeys showed significant difference ($p<0.05$) in enthalpy value for every storage temperature throughout 180 days of storage. This indicates that honey is sensitive to surrounding temperature as it may affect the crystallisation rate. According to Figure 4(A), it can be seen that enthalpy for 4°C is more noticeable as it increased rapidly among other storage temperatures. The values increased from 0.96 J/g to 21.79 J/g, respectively. It shows that unexpected crystallisation process occurred, thus more energy is needed to melt the crystal. It is correlated with the thermogram presented, which shows a wider peak area for the storage temperature of 4°C for Hutan honey (Figure 2A). Hutan honey also shows a significance difference ($p< 0.05$) with storage temperature between 25 and 4°C, with the enthalpy value of 1.54 J/g and 8.32 J/g starting from

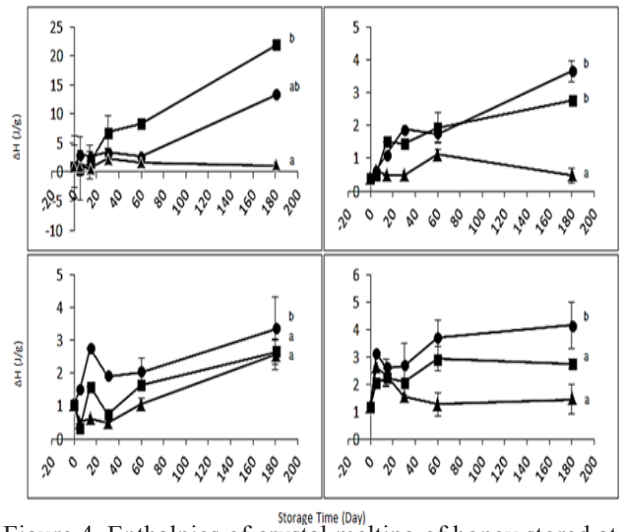

Figure 4. Enthalpies of crystal melting of honey stored at different temperatures and storage time: (A) Hutan; (B) Kelulut; (C) Acacia; (D) Gelam; (—•—) -20°C; (—▪—) 4°C; (—▲—) 25°C

60 days. Compared to Gelam honey, it shows a significance difference ($p < 0.05$) in enthalpy, starting from 60 until 180 days at 25 and -20°C.

Conclusion

According to the present findings, variation in the crystallisation behaviour of the selected honeys is influenced by the sugar composition and storage conditions. Considering the higher glucose level and rapid increment in enthalpy for Hutan honey, it can be concluded that Hutan honey is very sensitive to cold temperature compared to other types of honeys, and the greatest crystal formation was found to occur at the storage temperature of 4°C, even after only 14 days of storage. Kelulut and Acacia honey can be stored at lower temperature (4 and -20°C) or room temperature as both honey did not show any clearly peak existence in thermograms. Meanwhile, Gelam honey should be avoided from being stored at -20°C to prevent crystallisation. Storing honey at room temperature (25°C) may delay the formation of crystallisation.

Conflict of Interest

The composition of Hutan honey should be further studied in order to determine the compound that promotes the occurrence of crystallisation.

Acknowledgments

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