



Physical properties of kelulut honey emulsions with different oil types and emulsifier concentrations

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ABSTRACT

Kelulut honey (KH) is a honey produced by stingless bee species, known for its high nutritional content and health benefits. Even though it is very healthy, some people cannot consume it directly. Therefore, incorporating this type of honey into an emulsion system is a good strategy to increase its consumption. Emulsion technology is one of the alternative technologies nowadays which combines two or more immiscible liquids to form a solution. In this study, KH emulsions were emulsified with glyceryl monostearate (GMS) and stabilised by xanthan gum. There were two stages involved to determine the effects of variations in oil types and emulsifier concentrations on the physical properties of KH emulsions. In the first stage, three types of oil were used as the dispersed phase, which were palm oil, canola oil, and sunflower oil. Then in the second stage, different concentrations of GMS (0.25, 0.5, 0.75 and 1%) were applied. From the droplet size analysis, the emulsion with sunflower oil and the highest concentration of GMS gave the smallest diameter of $0.78 \pm 0.01 \mu\text{m}$ and the lowest polydispersity index (pDI) of $16.2 \pm 2.03 \%$. Meanwhile, the texture analysis shows that emulsion with palm oil has the highest firmness ($45.16 \pm 2.83 \text{ g}$) and consistency ($305.44 \pm 14.91 \text{ g}\cdot\text{sec}$) values. The foaming index on the other hand depicts that emulsion with palm oil and that with the lowest concentration of GMS gave the highest foam stability. From this study, a physically stable oil-in-water KH emulsion was produced, which should undergo further chemical, sensory and storage studies for commercial production in the future.

Keywords: kelulut honey, glyceryl monostearate, emulsion, palm oil, sunflower oil

INTRODUCTION

Nowadays, honey is known for its sweet tasting and benefits for health. People consume honey not only in the form of food, but apply it in the form of skincare and cosmetics. Among many types of honey available in the market, kelulut honey (KH) is popular due to its distinct flavour and unique taste (Keng et al., 2017). In Malaysia, KH is mainly produced by the stingless bee of *Trigona* spp. (Keng et al., 2017) and is also known for

its nutritional properties. KH has a higher content of polyphenol than any other kind of honey (Ranneh et al., 2018). In a comparative study between KH and Tualang honey, it was revealed that KH has higher amount of protein and ash compared to Tualang honey (Ranneh et al., 2018). However, KH is more diluted and has a special sour-like taste and smell, which some people do not prefer to consume it regularly (Rashid et al., 2019). Therefore in this study, KH was incorporated in an emulsion system to increase its consumption, while stabilising the easily oxidised food thus extending its shelflife. This emulsion can be classified as an oil-in-water (O/W) emulsion as the oil was dispersed in kelulut honey (KH) dissolved in water (aqueous phase).

Emulsion technology is one of the stabilization strategies applied widely in many fields especially in food. Emulsion is a dispersion of one liquid into the second immiscible liquid in the form of fine droplets (Narsimhan & Wang, 2008). Some of its applications in the food products are cream, mayonnaise, butter, and even chocolate spread (Said et al., 2019). The formation of emulsions depends on various factors such as the composition, processing and storage conditions. In an oil-in-water emulsion, where the oil phase is dispersed in the aqueous continuous phase, the type of oil and the emulsifier concentration used are among the important factors to consider. Emulsifiers act as stabilizers that have both a hydrophobic tail (non-polar) and a hydrophilic head (polar), which will attach the immiscible liquids e.g. oil and water, and form stable emulsions (Wan Mohamad et al., 2017).

Producing a physically stable emulsion system is the first step to producing a commercial product, therefore in this study, the effects of different types of oil and concentrations of GMS on the physical properties of KH emulsions were determined, such as the size of particles, polydispersity index, stability of emulsions, and texture. There were two stages involved to determine the most stable emulsions. In the first stage, three types of oil (palm oil, canola oil, and sunflower oil) were used as the oil phase. The oil that produced the most physically stable emulsion was then chosen for the next stage, where the concentration of glyceryl monostearate (GMS) as an emulsifier was varied.

MATERIALS AND METHODS

Material

In this study, three types of oil (palm oil, sunflower oil and canola oil), kelulut honey (KH), distilled water, glyceryl monostearate (GMS) and xanthan gum (XG) were used. Palm oil was purchased from a local supermarket in Besut while canola and sunflower oil were purchased from a food ingredient store in Jerteh. KH were bought from a local kelulut farm in Besut. GMS and XG were readily available in UniSZA laboratory.

Preparation of Kelulut Honey Emulsions

A stand homogenizer (D-500 Homogenizer, Wigger Hauser, Berlin, Germany) was used to prepare the emulsions at speed 10000-29000 min^{-1} . Formulations used in Stage 1 and 2 are summarised in Table 1 and 2 respectively. Firstly, the solution of XG and GMS were prepared by diluting with distilled water. The solution of XG and GMS, remaining water and KH were mixed together as aqueous phase using homogenizer about 2 minutes until well mixed. The oil was slowly mixed into the aqueous phase. The solution was then homogenised together for about 2 minutes per 100ml emulsion. The final emulsion samples were immediately analysed for their physical properties.

Table 1. List of ingredients and formulations of kelulut honey emulsions in Stage 1.

Ingredients	Sample A	Sample B	Sample C
Palm oil	20%	-	-
Sunflower oil	-	20%	-
Canola oil	-	-	20%
Distilled water	73.75%	73.75%	73.75%
GMS	0.75%	0.75%	0.75%
XG	0.5%	0.5%	0.5%
Kelulut Honey	5%	5%	5%

Table 2.List of ingredients and formulations of kelulut honey emulsions in Stage 2.

Ingredients	Sample D	Sample E	Sample F
Sunflower oil	20%	20%	20%
Water	74.25%	74%	73.5%
GMS	0.25%	0.5%	1%
XG	0.5%	0.5%	0.5%
Kelulut Honey	5%	5%	5%

Droplet Size Analysis

The diameter, distribution and polydispersity index (pDI) of oil droplets in KH emulsions in this study were analysed using a zeta sizer (Litesizer 500 Anton Paar, Austria) at a fixed detector angle of 90° (Zhang et al., 2016). Emulsions were diluted in 200 ml of distilled water. The refractive index used for this analysis was based on the type of oil used and the material absorption coefficient used was 1.3300. All measurements were made in triplicate and conducted at 25°C.

Texture Analysis

Texture analysis of KH emulsions were performed using a Texture Analyzer TA.XT Plus (Stable Micro Systems, Cardiff, UK) in compression test mode. The test was carried out using a cylindrical probe P/35, 35mm diameter cylinder aluminium probe. The characteristics of texture (firmness and consistency) were analysed (Gilbert et al., 2013).

Foaming Index

The freeze-thaw method for creaming index analysis by (Hong et al., 2018) was modified into foaming stability analysis in this study. Each emulsion sample was poured into a test tube to the height of 10 cm, then sealed with aluminium foil and stored. All samples were assessed using a freeze-thaw method (-10°C for 15 h / 90°C for 10 min). After storing the samples according to the set temperature and time, the changes in the height of the samples in the test tube were observed.

Statistical Analysis

Statistical analysis was performed by using the software of Statistic Package for Social Science (SPSS) to analyse data. All measurements were performed in triplicate and results were expressed as mean \pm standard deviation (SD). The analysis used was the Analysis of Variance (ANOVA) to compare different groups and a value of $p < 0.05$ is considered statistically significant (Li & Xiang, 2019).

RESULTS AND DISCUSSION

Stage 1: Effects of varying the oils types on the physical properties of kelulut honey emulsions

In Stage 1, three types of oils were used (palm oil, canola oil and sunflower oil) as the dispersed phase of emulsions to differentiate the physical properties of the KH emulsions. Table 3 shows the results of the droplet size (μm), polydispersity index, pDI (%), firmness (g), consistency (g.sec) and foaming index (cm) of the KH emulsions varied in oil types.

Table 3. Physical properties of kelulut honey emulsions with different oil types.

Oil type	Palm oil	Canola oil	Sunflower oil
Droplet size (μm)	2.84 \pm 0.4 ^a	1.71 \pm 0.42 ^b	0.97 \pm 0.01 ^c
pDI (%)	29.97 \pm 1.46 ^a	28.55 \pm 0.21 ^a	28.8 \pm 6.36 ^a
Firmness (g)	45.16 \pm 2.83 ^a	27.68 \pm 0.81 ^b	26.81 \pm 0.76 ^b
Consistency (g.sec)	305.44 \pm 14.91 ^a	179.83 \pm 5.08 ^b	174.75 \pm 4.33 ^b
Foaming index (cm)	7.87 \pm 0.38 ^b	9.47 \pm 0.15 ^a	9.13 \pm 0.12 ^a

Each value is expressed as mean \pm standard deviation (n=3).

^{a-c}means with the same superscript letter in a row do not differ significantly ($p < 0.05$).

Effects on droplet size and polydispersity index (pDI)

The average means of droplet size in Table 3 shows that KH emulsion with the sunflower oil had the smallest droplet size of 0.97 \pm 0.01 μm while the emulsion with palm oil had the biggest droplet size of 2.84 \pm 0.4 μm . For this droplet size analysis, there were significant differences ($p < 0.05$) between the three samples with different oil types. Smaller droplet size of the emulsions indicates more stable emulsions. Based on the results, the smaller droplet of KH emulsions with sunflower oil means that sunflower oil can easily disperse in the aqueous phase. Referring to Table 4, this might be due to the sunflower oil having the lowest viscosity compared to the other two oils (Siddique et al., 2010). This is consistent with study by Wooster, Golding and Sanguansri (2008) who compared the droplet breakup in emulsions with peanut oil and hexadecane. Oil viscosity is influenced by its molecular structure and decreases with the unsaturation of fatty acids (polyunsaturated, PUFA and monounsaturated, MUFA) (Yalcin, Toker and Dogan, 2012). The palm oil on the other hand has the highest viscosity with the lowest content of PUFA and highest saturated fatty acid (SFA).

Table 4. Melting point and fatty acid compositions of oils (Siddique et al., 2010)

Oil type		Palm oil	Canola oil	Sunflower oil
Viscosity (mPa.s) at	23°C	79.7	67.1	62.0
	33°C	63.2	53.1	44.0
	43°C	36.7	32.4	30.3
SFA (%)		52	7	11
MUFA (%)		38	58	20
PUFA (%)		10	35	69

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid

Polydispersity index (pDI) is a parameter used to define the size range of the lipidic nanocarriers systems with respect to particle size distribution characterization. The term “polydispersity” (or “dispersity” as recommended by IUPAC) is used to denote the non-uniformity degree of particle size distributions, therefore it basically represents the distribution of size populations within a sample. The value of pDI ranges from 0% for a perfectly uniform particle size, to 100% for multiple particle size populations within a highly polydispersed sample (Kowalska et al., 2020).

Figure 1 depicts the plots of particle size distribution of KH emulsions varied in oil types. It is obvious that the particle size of the sample with palm oil was more widely distributed, compared to that with canola and sunflower oils. Based on Table 3 however, KH emulsion with palm oil recorded the highest pDI value of $29.97 \pm 1.46\%$, while the one with canola oil recorded the lowest pDI of $28.55 \pm 0.21\%$. However, there were no significant differences at ($p < 0.05$) between the pDI of the three samples. In pharmaceutical applications using lipid-based carriers, a pDI of 30% and below is acceptable and considered as a homogenous population of oil droplets (Danaei et al., 2018).

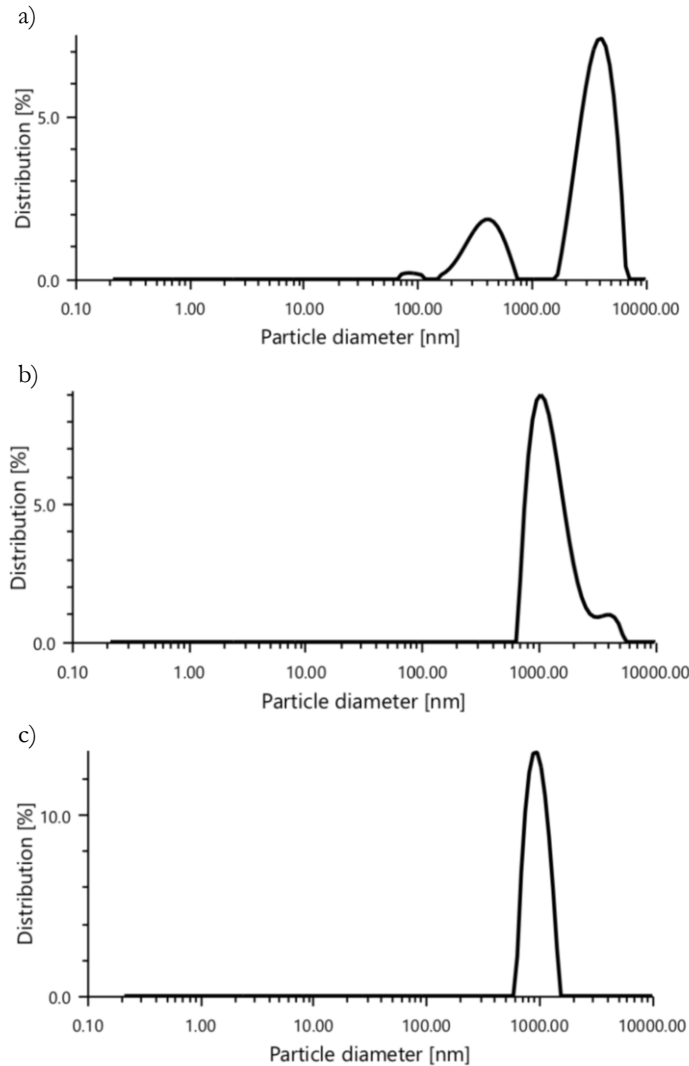


Figure 1. Particle size distribution of kelulut honey emulsions varied in oil types: a) palm oil, b) canola oil and c) sunflower oil.

Effects on texture

Compression test was run on the three emulsion samples with different oil types to measure the force needed to shear the emulsions texture. The firmness (g) and consistency (g.sec) reading were recorded. Table 3 shows that palm oil has recorded the significantly highest value of firmness (45.16 ± 2.83 g), indicating that the sample needs the highest force to shear its texture, compared to the other two samples. On the other hand, the KH emulsion with palm oil depicted the highest consistency (305.44 ± 14.91 g.sec), which were significantly different ($p < 0.05$) compared to the other two samples. The highest in consistency gives the most viscous and

thickest texture of emulsion sample. Palm oil is known for its high content of palmitic acid which is saturated fats. The high amount of saturated fats in palm oil causes its emulsion to be firm, compact and dense in texture.

Effects on foaming index

Table 3 also depicts the average height of every sample after undergoing intense cooling and heating to destabilise its emulsion structure.

The sample with palm oil has recorded the significantly lowest height of 7.87 ± 0.38 cm compared to the other two samples. This shows that the emulsion with palm oil has the largest decrease in height from the original 10 cm height. The decrease in volume of emulsions may be caused by the foam formed inside the test tube of each sample evaporates during heating at 90°C . Since the palm oil is more hydrophobic than the sunflower and canola oils, the emulsion particles are large enough to touch both surfaces of the thin liquid film between a pair of bubbles, causing the Laplace pressure in the film adjacent to the extraneous particle to become positive. As a result, the liquid flows away from the particle and breaks contact with the particle, thus leading to film rupture (Dickinson, 2010).

Stage 2: Effects of varying emulsifier concentrations on physical properties of kelulut honey emulsions with sunflower oil

In Stage 2 of this study, the sunflower oil was applied as the dispersed phase of KH emulsions varied in emulsifier (GMS) concentrations. The sunflower oil was chosen due to its KH emulsion depicting the smallest droplet size in Stage 1. Four different concentrations of GMS emulsifier (0.25, 0.5, 0.75 and 1%) were applied in order to determine the most stable KH emulsion.

Table 5 shows the results of the droplet size (μm), polydispersity index, pDI (%), firmness (g), consistency (g.sec) and foaming index (cm) of the KH emulsions varied in GMS concentrations.

Table 5. Physical properties of kelulut honey emulsions with sunflower oil at different emulsifier (GMS) concentrations.

GMS concentration (w/w%)	0.25	0.50	0.75	1.00
Droplet size (μm)	1.20 ± 0.05^a	1.00 ± 0.02^b	0.97 ± 0.01^b	0.78 ± 0.09^c
pDI (%)	24.6 ± 2.55^a	24.9 ± 0.71^a	28.8 ± 6.36^a	16.2 ± 2.03^b
Firmness (g)	25.22 ± 0.46^c	27.75 ± 0.94^{ab}	26.81 ± 0.76^{bc}	28.97 ± 1.44^a
Consistency (g.sec)	164.38 ± 3.43^b	179.02 ± 5.44^a	174.75 ± 4.33^{ab}	185.83 ± 9.15^a
Height (cm)	9.97 ± 0.06^a	9.77 ± 0.12^b	9.13 ± 0.12^c	9.1 ± 0.10^c

Each value is expressed as mean \pm standard deviation (n=3).

^{a-c} means with the same superscript letter in a row do not differ significantly ($p < 0.05$).

Effects on droplet size and polydispersity (pDI) index

Based on Table 5, it shows that the KH emulsion with 1% (w/w) GMS had the smallest droplet size of $0.78 \pm 0.09 \mu\text{m}$, while that with 0.25% (w/w) GMS had the biggest droplet size of $1.20 \pm 0.05 \mu\text{m}$. Therefore the higher the emulsifier concentration, the smaller the droplet size of the emulsion.

It has been reported that different ratios or concentrations of emulsifier used to stabilise an emulsion could alter the viscosity, droplet size and stability of the emulsion (Ilia Anisa & Nour, 2010). The concentration of emulsifier plays an important role in determining how small the average size of particles is. Since emulsifier molecules form a monolayer at the internal surface of the two phases, it is important that the

complete surface of each oil droplet is “covered” by the emulsifier molecules so the emulsion will be more stable (Masucci, 2017). Insufficient of emulsifier concentration can cause the instability phenomenon like coalescence to occur. The result shows that the oil had the best ability to disperse with aqueous phase in the presence of 1% (w/w) GMS. There are significant differences ($p < 0.05$) of droplet size between all the KH emulsion samples, except for that with 0.5 and 0.75% (w/w) GMS, which show no significant difference in their droplet sizes.

Based on the values of pDI in Table 5, there were no significant differences between the samples with 0.25, 0.5 and 0.75% (w/w) GMS, but the emulsion with the highest GMS concentration of 1% (w/w) gave the significantly lowest pDI value ($p < 0.05$), indicating better homogeneity and uniformity of its oil droplets. The plots of particle size distribution of all samples varied in GMS concentration were similar, as depicted by Figure 1 (c).

Effects on texture

The results of the sample firmness in Table 5 show that 1% (w/w) GMS gave the highest value of 28.97 ± 1.44 g, denoting that more force is needed to shear the emulsion. The same sample depicts the highest consistency value of 185.83 g.sec, in comparison with the other samples.

The highest firmness and consistency values resulted in the sample having the thickest and most viscous texture. This is due to the high concentration of GMS molecules that surround the oil droplets and protect the structure of the emulsion. At $p < 0.05$ of firmness and consistency, the KH emulsion with 0.25% (w/w) GMS gave the lowest values, giving the least thick and viscous texture of sample.

Effects on foaming index

Based on the foaming index in Table 5, the results show that emulsion with 1% (w/w) GMS concentration recorded the lowest value of 9.1 ± 0.10 cm. This shows that the sample had the highest reduction in height from its initial height.

Among the four samples, the KH emulsion with 1 and 0.75% (w/w) shows no significant difference ($p < 0.05$) in the foaming index, while there were significant difference between emulsions with 0.25 and 0.5% (w/w) GMS to the other two concentrations. GMS is considered as a low molecular weight surfactant and foaming agent. Surfactants usually form a compact adsorbed layer with the low interfacial tension (Wilde, 2000). The lowest concentration of GMS at 0.25% (w/w) was actually enough to reduce the surface tension between the liquids, thus increasing the stability of the large interfacial area related with emulsion. Dickinson (2010) has stated that the high free energy of the gas–liquid interface leads to the inherent instability of foam. The interface is stabilised through the Gibbs-Marangoni mechanism, which depends on rapid diffusion of molecules along the interface when surface tension gradients occur due to deformations. During diffusion, the low amount of GMS can drag along some of the continuous phase, causing the drainage of liquid from the films to slow down, hence increasing the foam stability (Van Kempen et al., 2014).

CONCLUSION

In conclusion, the kelulut honey emulsions varied in oil types and glyceryl monostearate (GMS) concentrations in this study showed different physical properties. Emulsions that have the smallest droplet size were the emulsions with sunflower oil and that with the highest (1% w/w) concentration of GMS. The smallest droplet size means the oil droplets can easily disperse in the continuous phase. For polydispersity index, there was no significant different ($p < 0.05$) between the samples with different oil types while polydispersity index of emulsion with varying GMS concentration shows it fluctuates alternately with increasing concentrations. For texture properties, emulsions with palm oil and that with the highest (1% w/w) concentration of GMS were significantly high in firmness and viscosity compared to other samples. Besides

that, the emulsion with palm oil was significantly higher in the foaming index ($p < 0.05$) in comparison with the emulsions containing the other two oil types. Meanwhile, the emulsion with the lowest GMS concentration at 0.25% (w/w) gave the highest foaming index ($p < 0.05$) indicating good forming stability. Production of a physically stable kelulut honey emulsion will hopefully preserve the nutritional content of kelulut honey while giving an alternative way to consume it. The emulsion samples nevertheless need to undergo sensory evaluation, chemical analyses and storage studies before being commercialised at a larger scale.

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