Stability of Mixture Honey, Black Seed Oil and Olive Oil With Tween 80 as Emulsifier

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Abstract. The purpose of this study is to view stability a mixture of honey, black seed oil, and olive oil. There have many benefits and widely distributed in the market. However, this mixture has instability problem because it has different properties of polarity or phase, to solve this problem required emulsifier Tween 80, an emulsifier food grade to stabilize the mixture. This research is expected to be aim for mixture with the addition of the emulsifier. In the addition of 1.25 grams, Tween 80 could mix up to 7.5% of the total oil in the mixture. These are showing the process of the distribution particle, viscosity measurement, phase separation by centrifugation and storage for eight weeks in room temperature.

1. Introduction

Currently, back to nature tends to use of drug, food, cosmetic were made of natural or called its herbal more increasingly in the world. The background of this matter was environmental change, human lifestyle, and disease progression. Use of natural resources to develop the medicinal plants can also provide benefits in aspect economic and the public of welfare Indonesia also. Herbal medicine is the nation's cultural heritage that is the type of traditional Indonesia.

Characterization of phenolics and other components in honey that might be responsible for its antioxidant and antimicrobial effects is essential to improve our knowledge about the potential honey effects in the human health (1). However, the use of honey as a wound treatment has become a developing area of research, with a positive clinical result. Honey has several properties that contribute to its wound-healing effects, including antibacterial, debriding and deodorizing activity, as well as anti-inflammatory and tissue growth-promoting properties (2). Therefore, the use of honey is often very diverse causes its use varies widely.

Some use the manufacture of the increase the value of honey by adding other herbs that have specific benefits for health. Herbs are often used as a mixture of honey, or black cumin oil is Black Seed (Nigella sativa), essential oils, olive oil, ginger, turmeric, commonly a traditional honey-herbal (3).

A mixture of honey and black seed oil of Nigella sativa is proven to accelerate the process of cell death in liver cancer (4). So that the mixture to be plenty in the market. Also, there are also other herbal plant extracts that are beneficial to health such as olive oil, which is mixed into a mixture of honey and the Black Seed Oil as well as available in the market. The related problem with mixing Honey, Black Seed oil and olive oil has instability because they have the different properties of polarity or phase, so that the required emulsifier. The emulsifier will be used must be food grade emulsifier such as Tween.
80 (dissolved in water). Tween 80 is an oil in water emulsion type O/W. This emulsion type most widely used in oral dosage formulations, because it can eliminate unpleasant odors in the oil mixture.

2. Material and Methods

2.1. Preparation
Honey was purchased from Herbal Spirit Company, Indonesia. For the first time before blended all of the materials must be through the preparation, including measurement of viscosity, surface tension, water content and density at each raw material. And then mixed all of the raw material and determined of solution simultaneously. The determination of the solution is used by adding a surfactant of 1.25 g (5). Appropriate discussions with the company Herbal Spirit, honey and an oil volume ratio (black seed oil and olive oil) will be blended with a ratio of 7:3. For comparison of olive oil and black seed oil 2:1. In the next experiment performed by measuring the surface tension and conductivity the mixture of honey both of randu honey and rubber honey to determine the critical micelle concentration (CMC).

The last analysis stability emulsion is used to observe the separation phase (bottle test) with earth gravity (storage at room temperature for eight weeks). It has given the centrifugal force at a speed 3500 rpm for 5 hours and distance of diameter at 10 cm to precipitate the emulsion. Measure the viscosity of the emulsion samples during eight weeks, and the last measured the diameter of the particle using the Particle Size Analyzer (PAS) and viewed the distribution of particles under the microscope (6,7).

The preparation includes measurement of viscosity, surface tension and density of each material. Oil viscosity is measured using Ostwald because it has a low viscosity or below 1.0 cP, whereas the viscosity of honey is measured using a Brookfield viscometer. Olive oil viscosity and density of 0.985 cP and 0.9438 g / mL and Black Seed Oil 0.88 cP and 0.928 g / mL. The viscosity of randu honey is 30 cP and surface tension 79.5 dyne/cm and for rubber honey 17 cP and surface tension 78.9 dyne/cm. The water content of honey is measured using a refractometer. The moisture content of randu honey is 17.9%, and rubber honey is 18.9%. And the last density of randu honey is 1.4465 g/ml and the density of rubber honey 1.4116 g / ml.

2.2. The Formulation Ratio Phase Internal
The first for determination of the composition of honey (MR and MK) and oil (MH and MZ) by adding a surfactant of 1.25 g (5). Appropriate discussions with the company Herbal Insani, honey and oil volume ratio (MH and MZ) will be conducted with a ratio of 7:3. For comparison of olive oil and black seed oil is 2:1, then performed the experiment with its composition, so obtained the conclusion that a mixture of honey and oil in the ratio 7:3 and added of 1.25 grams of Tween 80 cannot be showed good quality product evidently was occurred breaking of emulsion (breaking) which is one of the instability situations of emulsions. The ratio volume phase is one of the determinants of emulsion stability properties. Based on the experiment was conducted, so the determination of solutions by changing the composition of the mixture for each honey (MR and MK) and total volume oil until the mixture obtained is stable with the details as Table 1 below:

| Exp | Randu Honey (ml) | Oil (ml)   | % of Total Oil | Result of Randu Honey | Result of Rubber Honey |
|-----|-----------------|------------|----------------|-----------------------|-----------------------|
|     |                 | Olive oil  | Nigella seed   |                       |                       |
| 1   | 70              | 20         | 10             | 30%                   | Insoluble             | Insoluble             |
| 2   | 73              | 18         | 9              | 27%                   | Insoluble             | Insoluble             |
| 3   | 76              | 16         | 8              | 24%                   | Insoluble             | Soluble               |
| 4   | 79              | 14         | 7              | 21%                   | Soluble               | Soluble               |
| 5   | 85              | 10         | 5              | 15%                   | Soluble               | Soluble               |
| 6   | 92.5            | 5          | 2.5            | 7.5%                  | Soluble               | Soluble               |

**Table 1.** The composition of oil and honey.
As the results of the experiment, as the table above, the maximum volume of oil in honey can be used and mixed well with no visible show two separate phases. On the rubber, honey is 24% oil in the total mix (for surfactants of 1.25 gram) can be mixed well after homogenized using an agitator. Meanwhile, the mixture using rubber honey is 21% — the results of this experiment as shown in Figure 1.

![Figure 1. The separation phase of a mixture of honey and oil](image)

2.3. The determination of CMC
In this experiment carried out by measuring the surface tension and conductivity of the mixture. Determination of CMC aimed to determine the appropriate concentration and the maximum use of Tween 80 as an emulsifier. Determination of the CMC conducted on a comparison of Black Seed oil, olive oil and honey 1:2:100. Surface tension and conductivity measurements performed every 0.1 gram increase in the concentration of Tween 80 on the oil percentage of 2.91% of the total mixture. Critical Micelle Concentration curve can be determined by looking at the relationship between the increase of emulsifier with surface tension and conductivity of the mixture. The relationship graph of surfactant concentration and surface tension of the mixture of randu honey and rubber honey can be viewed in Figure 2 and Figure 3.

In Figure 2 can be viewed both of randu honey and rubber honey were occurs the decreased surface tension or the surface free energy so that the micelles are easily formed. The surface tension decreased to 0.5 grams of surfactant concentration; this condition is called perfectly micelles (critical micelle Concentration). After passed the through its condition, the surface tension will be increased and tend to form new micelles and reverse emulsion condition (emulsification) (8).

Meanwhile in Figure 3 measurement of electrical conductivity (conductive) both of randu honey and rubber honey relatively did not change significantly. That's because the type of fluid is too thick (viscous) which causes the movement of the conducting ion is weak so it can not be measured properly.

![Figure 2. Effect of amount of Tween 80 to the surface tension](image)

![Figure 3. Effect of amount of Tween 80 to the conductivity](image)
3. Results and Discussion

Analysis stability emulsion is used to observe phase separate with earth gravity, giving the centrifugal force to precipitate the emulsion, measure the particle size diameter of emulsion particles as well as see the distribution is dispersed and measure the viscosity of the emulsion samples during eight weeks.

3.1. Bottle test (Storage)

Bottle test is to see directly phase separate, such as coalescence, flotation and breaking/demulsification was formed during storage. In this experiment, almost all the honey mixture mainly of rubber was creaming during storage for eight weeks. This mixture instability process can be viewed in Figure 4 and 5.

![Figure 4. After eight week storage in room temperature (a) Randu honey (b) Rubber honey](image)

The results obtained for randu honey in Figure 4 is not the same as rubber honey. For a total of 15% and 21% oil is in a mixture of honey was occurred instability during storage, but at concentrations of 7.5% of total oil in the mixture proved to be stable without phase separation (creaming) when was stored during eight weeks can be proved in Figure 4.

The water content of rubber honey causes this phenomenon is higher than the water content of randu honey so that the viscosity of the rubber honey, is called the continuous phase is lower than randu, so the creaming was formed easily in the mixture of rubber honey during storage.

Creaming is reversible; it means when the mixture was shuffled will be dispersed again. During the creaming was no breaking of the emulsion, but if continued will be the incorporation of particles into larger that will initiate the demulsification.

Creaming can be prevented by the reduced density difference between internal and continuous phases, increased the viscosity of continuous phase and the reduced of the particle size diameter (9).

3.2. Viscosity

Viscosity was measured every week for eight weeks at room temperature. The influence of storage time on the viscosity of honey cottonwoods can be seen in Figure 5.a.

In the measurement of viscosity the mixture of rubber honey, the solution decreased until at third weeks measurement, it signed the decrease of particle size. But viscosity decreased gradually and became almost constant till eight weeks. And that may be caused by mixed surfactant (Tween 80) only works effectively until third weeks, and then after third weeks of emulsifier are not able to hold the particles of oil, or there is some micelles is not strong enough to hold the emulsion. It makes larger formation droplets from the incorporation of small droplets so that the influenced measurement of viscosity being lower.

Furthermore, the rate of creaming is high, which can bother while the measurement using a spindle. At zero week until the third weeks of the measured emulsion still homogeneous, but because there is movement droplet in the next of measurement can be messed. On the measured viscosity of the rubber, honey decreased in the second weeks. Because the rubber honey has a higher water content which its viscosity is lower than randu honey.

Ultimately the rate of creaming is higher than randu honey. The increased the rate of creaming is caused by three factors including higher the difference density internal and continuous phase, lower
viscosity of phase continues and larger particle diameter. The results of viscosity measurements of rubber honey can be seen in Figure 5.b

**Figure 5.a.** Effect of time on the viscosity of randu honey

**Figure 5.b.** Effect of time on viscosity rubber honey

Viscosity measurements, in this case, did not become an accuracy to determine the stability of solutions, so to see the stability we have to give centrifugal force by centrifuged, and view the distributed particles under a microscope. Moreover, the significantly increased the viscosity of each week (too thick) will be difficult to make the mixture was poured or consumed.

3.3. Gravitational stress

Evaluated the stability gave the centrifugal force at a speed 3500 rpm for 5 hours and distance of diameter at 10 cm which is equivalent to one year of storage (7). The form that occurs after the centrifuge for randu honey can be viewed in Figure 6. The percentage of the oil phase separation results are presented in Table 2.

**Figure 6.** The Centrifugation of Result of Randu Honey
Table 2. The Centrifugation of Result of Randu Honey

| No | Sample                | Percentages phase separation |
|----|-----------------------|-----------------------------|
| 1  | Total oil 7.5%        | -                           |
| 2  | Total oil 15%         | 15.25%                      |
| 3  | Total oil 21%         | 27.27%                      |

At 2.91% of total oil in a mixture with the addition of an emulsifier or before and after CMC concentration, 0.4 grams and 0.6 grams of Tween 80 is mixed with no phase separation occurs after centrifuging. It signed that the third mixture is stable when is given gravitational stress. Besides, that signed no creaming occurred at room temperature when is stored for eight weeks and the centrifugal force by centrifugation. The results of the mixture centrifuged at 2.91% of total oil can be seen in Figure 7.

![Figure 7](image)

Figure 7. The results of centrifugation of randu with concentration variation emulsifier

3.4. Diameter of Particle
The stability of emulsion depends on droplet size at dispersed phase. Emulsion droplet sized was measured by using a Coulter Particle Size Analyzer. The size of mixture randu honey without used emulsifier had a particle diameter of approximately 14 μm and 7μm — meanwhile mixture of randu honey with emulsifier about 5μ and 2μ. This signed particle size is smaller and more stable due to the small droplet size, the collision rate and the rate of incorporation will be smaller. The smaller of the rate the incorporation is not easily occurred creaming, breaking, coalescence, flocculation, and so forth.

Also, with smaller particle size can be kept much longer, and not easily damaged, do not change the flavor, easily absorbed by the body and is safe for consumption especially for medicine and food (9). Then the next thing to ensure a stable form of the mixture is done by looking at the picture of the particles are distributed under the light microscope. Used ten times magnification and then photographed to show. The image can be seen in Figure 8.

![Figure 8](image)

Figure 8. Mixed Particle A) Total Oil 7.5% With Tween 80 B) Total Oil Without Tween 80

In Figure 8 the size of particle diameter used Tween 80 tends to have the same shape as compared without Tween 80. Particle size is a very important factor to determine the stability of an emulsion (10). While in Figure 8 mixture did not form droplets that are uniform so it would be more susceptible to processes instability emulsion such as creaming in that case.
4. Conclusion
Study of the stability a mixture of honey, Black Seed oil and olive oil using Tween 80 emulsifier can be obtained that either mixture of rubber honey or randu honey with black seed oil and olive oil successfully made with the addition of 1.25 gram Tween 80 in 100 mL of a mixture. A mixture of Black Seed oil, olive oil, and honey are the most stable has a composition ratio of 1: 2: 37 or 7.5% total oil with the addition of 1.25 grams Tween 80.

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