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The Characteristics of Coconut Phospholipids as Biosurfactants

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ABSTRACT

All living cells consist of phospholipids. Phospholipids are known as biosurfactants. Here we report our study of the physico-chemical characteristics of phospholipids derived from coconut endosperm as biosurfactants. We studied the aggregation and emulsification characters. The aggregation proccess was examined by optical microscope. The stability of emulsi formed was montitored at different temperature and pH. Where as emulsification capability was investigated by constructing a ternary diagram. Depending on the amount of phospholipids and additive substances the emulsification capability changes. The coconut phospholipids will form vesicles in various size (polydisperse). We noticed that coconut phospholipids emulsification stability was affected by temperature, pH, and phospholipids composition. It can be concluded that the prospect of phospholipids from coconut endosperm as biosurfactants is promising.

Keywords: coconut phospholipid, emulsification capability, aggregation, ternary diagram, vesicle.

INTRODUCTION

Phospholipids are essential constituents of all living tissues. They also are becoming important, and often crucial, components in numerous medicinal, biotechnological, cosmetic, and industrial applications Today the source of phospholipids is mainly soybean and egg. But the demand for and interest in various types of industrial phospholipids are increasing, because of the number of applications for human use also increasing. There is also tendency to replace materials of synthetic origin, such as tensides, with natural products for biocompatibility reasons. This growing demand for natural phospholipids has encouraged a search of phospholipids source from natural origin.

In many industrial applications the ability of phospholipids to interact with water and other compounds is part of the necessary performance criteria. The combination of chemical composition and physicochemical tests are therefore important as part of the quality concept [1]. One of the test would be the dispersion and aggregation behavior which are required to be reproducible to maintain the quality.

It is for the above reasons, our research was done. we report herein the results of the study of the physico-chemical characteristics of phospholipids derived from coconut endosperm as an emulsifier. The experiments were cmc determination, emulsion stability, emulsification capability [2, 3, 4, 5]. Hopefully the result would bring to light the prospect of phospholipids from coconut endosperm as an emulsifier and other functionalities in practice.

MATERIALS AND METHODS

Phospholipids from coconut endosperm were prepared in-house [6]. Albumin was obtained from Sigma Aldrich (A5253), Casein was obtained from Sigma Aldrich (C3400). Vegetable oil ("B" brand) was purchased from supermarket. All solvents used were of analytical grade.

Methods

Cmc determination was done by measuring turbidity of a series of phospholipids dispersion with various concentration. The phospholipids were dispersed in distilled water. The concentration of the phospholipids were varied from 5.05 to 5.5 mg/mL. The turbidity of each dispersion then was measured using Orbeco-Helige Turbidimeter. Cmc value was obtained by plotting the turbidity data with the concentration.

Emulsion stability was determined by measuring the breakdown duration for emulsion with various composition in various temperature, and pH. The phospholipids were first dispersed in the aqueous phase. After addition of the oil, the emulsion was mixed in a shaker for about 30 minutes. The breakdown duration was determined after the emulsions were left to stand for about 5 minutes.

Emulsification capability was determined by observing the number of phases of the emulsion 5 minutes after the mixing performed. All emulsions were mixtures of vegetable oil in distilled water, stabilized by coconut phospholipids in various concentration. The composition of emulsion components then were plotted in a ternary diagram. The procedure was repeated for emulsion added with protein albumin and casein in different concentration.

Phospholipids aggregation behavior in water was investigated by visual and microscopic observation of phospholipids solution in water. The dispersion of phospholipids was performed by two different procedures: (a) certain amount of phospholipids were added into distilled water and then dispersion was done in a shaker until all the phospholipids were dispersed. After the dispersion were left to stand for about 10 minutes the observation was performed visually and microscopically. (b) The dispersion was made through formation of thin film first. Certain amount of phospholipids were dissolved in a chloroform-methanol (2/1) mixture. The solution then evaporated in a rotary evaporator until all the solvent was gone and thin film of phospholipids was formed on the glass container. Distilled water was added to the container and the mixture was rotated in a rotary evaporator at 50 ^{o}C followed by ultrasonication until the film was dissolved. After the dispersion were left to stand for about 10 minutes the observation was performed visually and microscopically .

RESULTS AND DISCUSSION

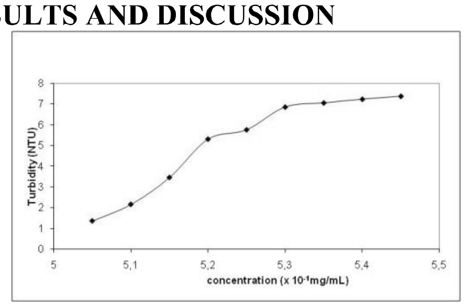

Figure 1. Cmc Determination of Coconut Phospholipids

The curve in Fig. (1) was obtained when coconut phospholipids were dispersed in water at 25 ^{o}C. It showed that the turbidity was increased as the concentration increased. Despite the steep increase of turbidity at lower concentrations, the turbidity increases only gradually after 5.3 mg/mL. it seemed that at lower concentration coconut phospholipids molecules were dispersed individually but when the concentration reach 5.3 mg/mL it began to form micelles or other aggregates. The cmc was found around 5.3 mg/mL.

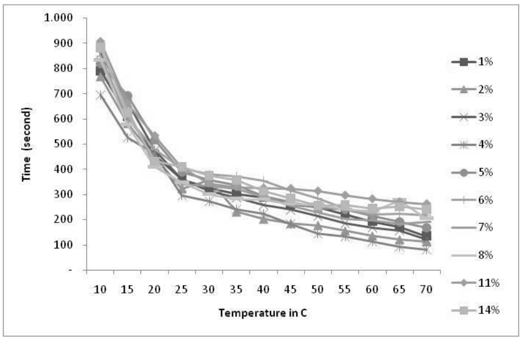

Figure 2. Effect of Temperature on Emulsion Stability for Different Composition of Coconut Phospholipids

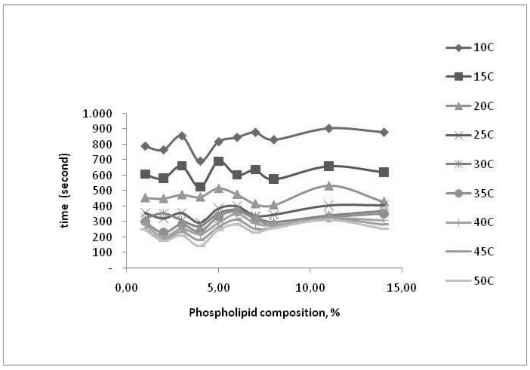

Figure 3. Effect of Composition of Coconut Phospholipids on Emulsion Stability at Different Temperature

Emulsion stability observation were performed by preparing series of mixture of vegetable oil, water, and phospholipids with composition varied. The weight ratio of each component were adjusted so that the total final weight was 100 g. The breakdown duration of each emulsion was observed at different temperature, range from 10 ^{o}C to 50 ^{o}C. Curves in Fig. (2) and (3) showed that temperature would give the same effect to all phospholipids composition. Increasing temperature would decrease the duration of emulsion breakdown, i.e. destabilized the emulsion. The figure also showed that the duration of breakdown were not change significantly at temperature above 30 °C. From Fig. (3) it was cleared that emulsion would be more stabile when kept in 10 °C compared to other temperature. After 25 °C the stability was not changed significantly by changing the temperature.

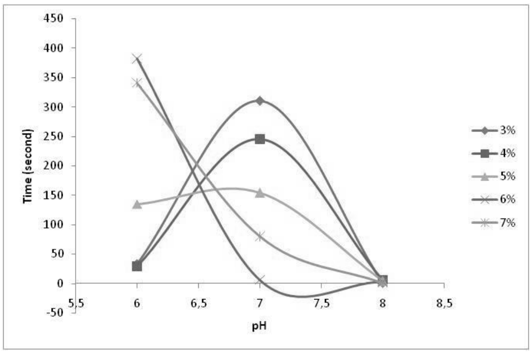

Figure 4. Effect of pH on Emulsion Stability for Different Composition of Coconut Phospholipids

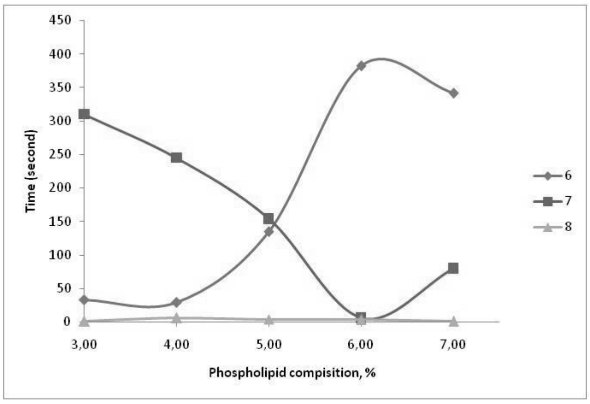

Figure 5. Effect of Coconut Phospholipids Composition on Emulsion Stability in Different pH

Curves in Fig. (4) and (5) showed that emulsion stability were affected by pH but the effect was not similar for each composition and pH. At composition of coconut phospholipids 5% and below the emulsions were most stabile at pH 7. At composition of coconut phospholipids 6% and up the stability was decreasing as the pH increasing (see fig. 4). Fig. (5) showed that at pH 6 the stability increased to a maximum at composition around 6% and then decreasing. At pH 7 the emulsion stability decreased to a minimum at composition around 6% and then increasing. At pH 8 the emulsions were not stabile at all composition. This results were still inconclusive, however, we could see that changing the pH would affect the emulsion stability. We needs further research to explained this phenomena.

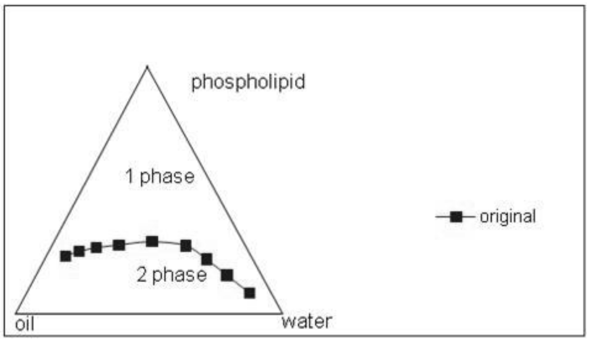

Figure 6. Emulsification Capablility of Coconut Phospholipids

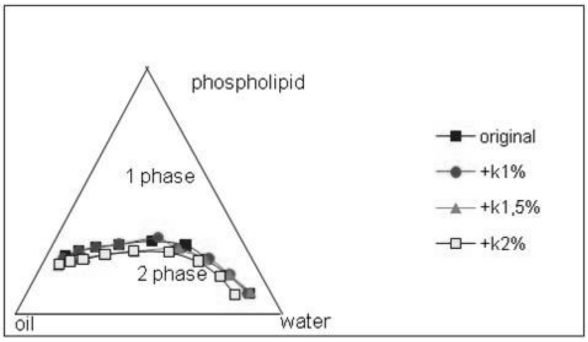

Figure 7. Effect of Different Concentration of Casein on Emulsification Capability of Coconut Phospholipids

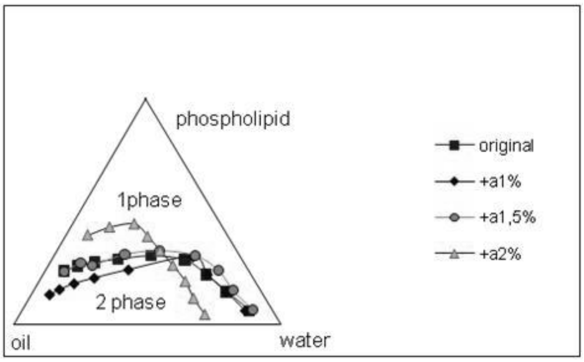

Figure 8. Effect of Different Concentration of Albumin on Emulsification Capability of Coconut Phospholipids

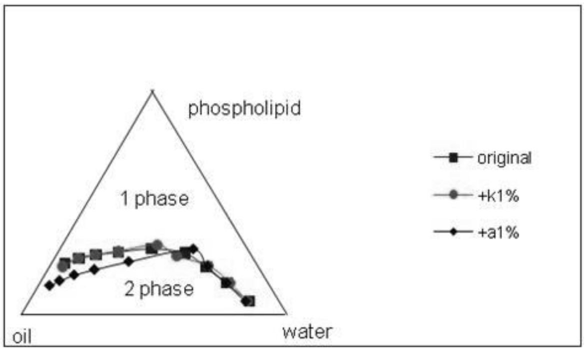

Figure 9. Effect of 1 % Albumin and 1 % Casein on Emulsification Capability of Coconut Phospholipids

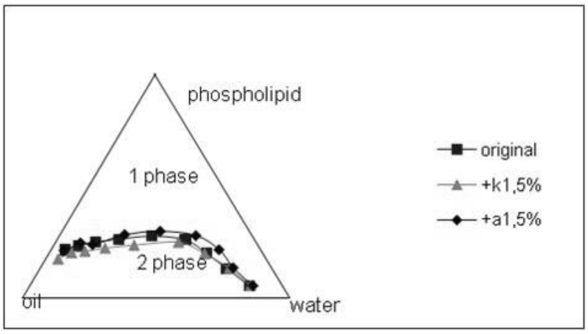

Figure 10. Effect of 1.5 % Albumin and 1.5 % Casein on Emulsification Capability of Coconut Phospholipids

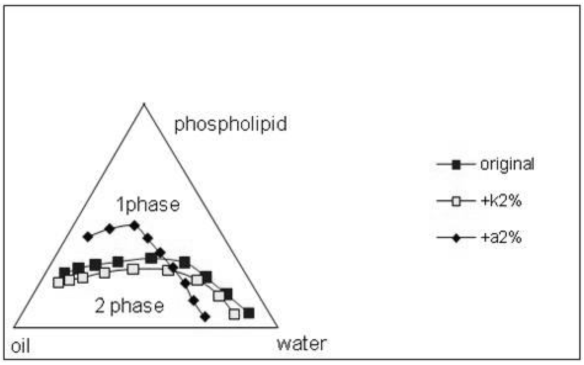

Figure 11. Effect of 2 % Albumin and 2 % Casein on Emulsification Capability of Coconut Phospholipids

Emulsification capability observation were showed in Fig. (6) to (11). The emulsification capability of coconut phospholipids were presented by the 1 phase region (see fig. 6). It was considerably good. In the present of casein the emulsification capability were increased and the capability was increased as the casein concentration increased (fig. 7). For albumin the capability was affected by the albumin concentration but the effect was not similar as the concentration increased (fig. 8). For low concentration, 1%, the effect of albumin and casein on emulsification capability was nearly the same (see fig. 9). For higher concentration, 1.5% and 2%, the effect of albumin and casein on emulsification capability were different (see fig. 10 and 11). Casein tend to increase the capability while albumin on the contrary tend to decrease it. Probably it was related to the structural characteristic of the protein. Casein is a phosphorous protein while albumin is not.

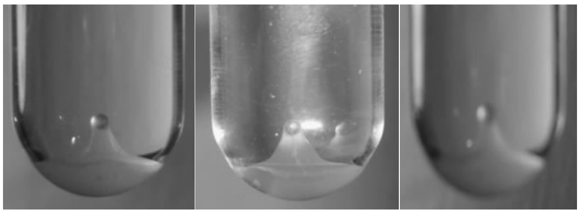

Figure 12. Observation of Vesicle Formation Mechanism of Coconut Phospholipids

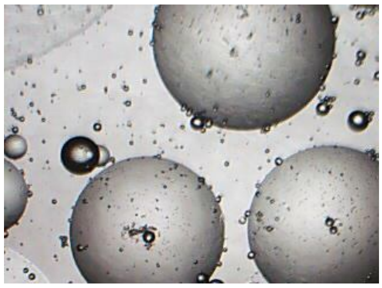

Figure 13. Microscopic Observation of Vesicles Formation by Coconut Phospholipids

Figure 14. Vesicle Mixture in Distilled Water

Observation of aggregation phenomena in fig. 12 showed that coconut phospholipids might form vesicles. Fig. (12) suggested the mechanism of vesicle formation was occurred when coconut phospholipids were dispersed in water. This phenomena is in agreement with the mechanism of vesicle formation suggested by Lasic (1988). This vesicle was in various size (polydisperse) with the biggest size around 10 μm (see fig. (13). This dispersion which was formed by the second method were stabile for more than 2 weeks. Visual observation also showed that the dispersion has bluish appearance which is consistent with the visual appearance of vesicle dispersion, fig.14. This result is not final yet since the experiment is still in progress at the moment.

CONCLUSION

It can be concluded that the prospect of phospholipids from coconut endosperm as an emulsifier is promising, but still it should be noted that more thorough research should be done to reach to its utilization as an emulsifier and other functionalities in practice.

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