



## **Performance of Lecithin Isolate from Vegetable Oil as an Emulsifier on the Beeswax Coating Characteristics**

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**Abstract :** Beeswax is a naturally occurring wax that generally consists of fatty acid esters and various long-chain alcohol compounds. One of the main components in wax coating is an emulsifier that functions to form products. Vegetable oils have the potential to be the source of materials for production of lecithin. Rice bran oil contains 1.0 – 2.0% phosphatidate gum, which is used in crude lecithin production. This study aimed to isolate lecithin from crude rice bran oil, characterize the isolate, and determine the effects of lecithin concentrations as an emulsifier on the characteristics of beeswax coating. The variables consisted of the lecithin concentrations (%) of 0, 0.25, 0.5, 0.75, and 1 in the beeswax coating formulation. The procedures are extracting crude rice bran oil, isolating lecithin from the oil, characterizing the lecithin isolate, mixing rice bran and soy lecithin, and incorporating the lecithin mixture in beeswax coating formulation at different concentrations mentioned above. The rice bran and soy lecithin mixture, at the ratio of 1:6 and to be used as an emulsifier, was analyzed for Creamy Index (%) and HLB. The beeswax coating preparations, containing different concentrations of emulsifier, were analyzed for visual appearance, pH, density, and viscosity. The yield of crude rice bran oil 9.989%. The lecithin isolate 1.1% and contained phospholipid based on the FT-IR spectroscopy. The rice bran-soy lecithin mixture exhibited 19.4% Creamy Index and 8.29 HLB. The analyses of beeswax formulations showed increasing trends on all parameters tested as the result of increasing concentrations of emulsifier.

**Keywords :** beeswax, coating, emulsifier, rice bran lecithin.

## Introduction

Indonesia is an agricultural nation because most of its citizens are farmers and/or make a living in the agricultural sector. Fruits are some of the important commodities in the agroindustry. Efforts have been taken to increase the shelf life of these farm products by lowering the storage temperature and applying layers of food-grade wax.

One of the main components in wax coating formulations is the emulsifier, which functions to form the products. The types of emulsifiers that are used in edible films and coatings include emulsifiers (lecithin, Tweens, Spans) and lipid emulsions (edible waxes, fatty acids [1]. Lecithin is present in the cytoplasm and obtained through extractions of compounds containing fatty acids from animals and plants. Some of the methods in lecithin extractions include water degumming, acid degumming, and enzymatic degumming.

One of the vegetable oils that can be used as a source of lecithin is rice bran oil because it contains 1.0 – 2.0% phosphatidate gum [2]. The utilization of lecithin from rice bran oil can increase the added value to and be a part of product diversifications of rice bran. The aims of this study were to isolate lecithin from crude rice bran oil, characterize the isolate, and determine its performance as an emulsifier in a beeswax coating formulation.

## Experimental

### Equipment and Materials

The equipment consisted of magnetic stirrer, centrifuge, oven, filter paper, glass beaker, hotplate, digital scale, glass bottles, graduated cylinders, thermometer, pH meter, rheometer (Brookfield<sup>R</sup>), pycnometer, and Erlenmeyer flasks. The materials included crude rice bran oil, soy lecithin, distilled water, beeswax, stearic acid, triethanolamine (TEA), 0.5N HCl, 0.5N alcohol-KOH, PP indicator, 0.1N oxalic acid, ethanol p.a., n-hexane, and rice bran.

### Methods

The procedures comprised of extracting crude rice bran oil by maceration, isolating lecithin by water degumming, and characterizing the lecithin by Fourier Transform Infrared (FT-IR) spectroscopy. The rice bran oil lecithin was mixed with soy lecithin at the ratio of 1:6, and the resulting mixture was analyzed for its characteristics as an emulsifier by determining the values of the creamy index and hydrophilic-lipophilic balance (HLB). These processes are presented in flow charts in Figure 1 and Figure 2.

The above mixture was used in the beeswax coating formulation at different concentrations of 0, 0.25, 0.5, 0.75, and 1 % (v/v). The resulting preparations were analyzed for visual appearance, pH, density, and viscosity. The steps of this part are presented in Figure 3. The **Creamy Index** of the preparations was determined by mixing 10% (v/v) plant-based oil with water then adding 1% emulsifier containing plant-based lecithin. This mixture was stirred for three hours at 3000 rpm. The resulting emulsion was incubated for 24 hours, after which cream and serum were separated. The Creamy Index was determined by dividing the height of serum (HS) by the height of cream (HC) and then multiplied by 100 per cent. The following is the formula for the Creamy Index.

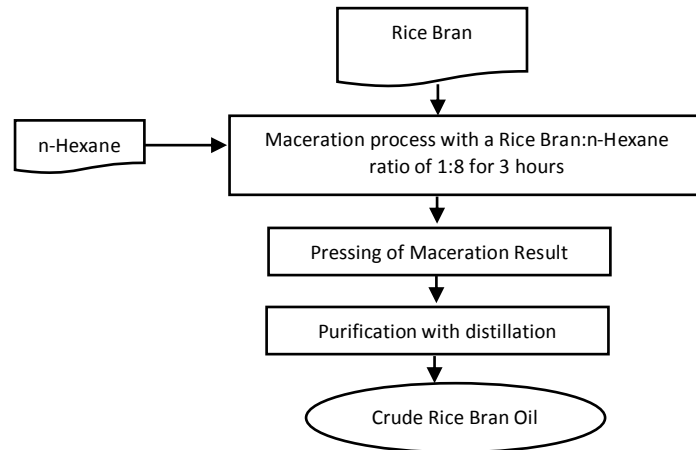
$$\text{Creamy Index} = 100\% * (\text{HS} / \text{HC}) \dots \dots \dots [3]$$

The following steps were taken to obtain **HLB**. First, the acid value (**A**) was determined by dissolving one gram of sample in 10 mL ethanol and adding three drops of PP indicator. The solution was titrated with alcohol-KOH standard until it turned to purple red. Second, the saponification value (**S**) was generated from the blank solution, in which 25 mL of 0.5N alcohol-KOH was refluxed in a water bath for one hour to boil. The solution was cooled to room temperature, in which 1 mL PP indicator was added and the mixture was titrated with 0.5N HCl. Testing of samples were conducted by obtaining 2 grams of sample and dissolving it in 25 mL of 0.5N alcohol-KOH. The mixtures were treated in the same procedure for obtaining the saponification value.

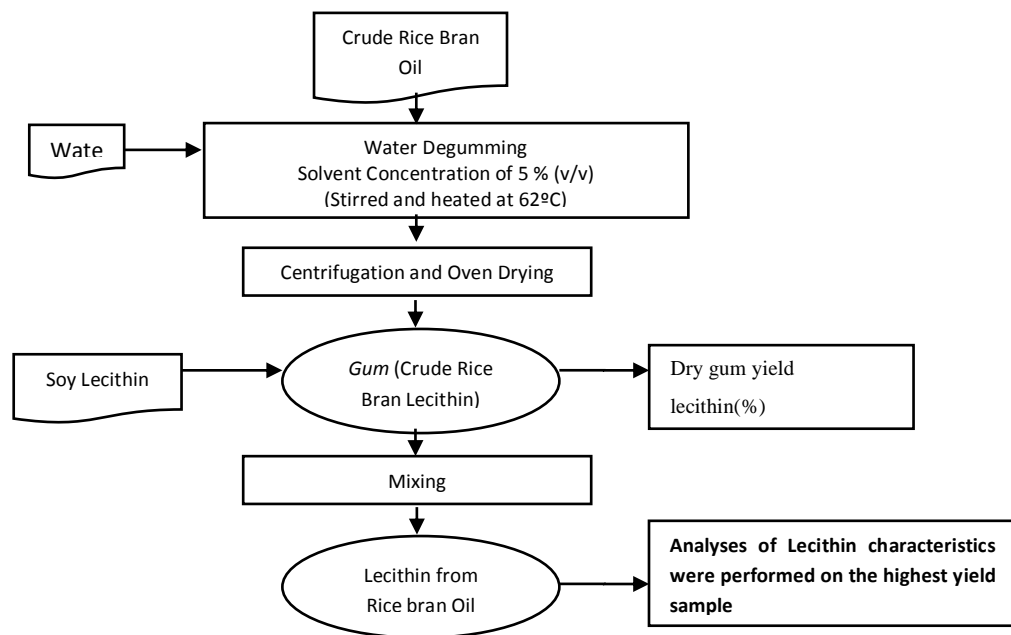
The HLB was calculated using acid and saponification values in the following formula:

$HLB = 20 \times (1 - S/A) \dots\dots[4]$

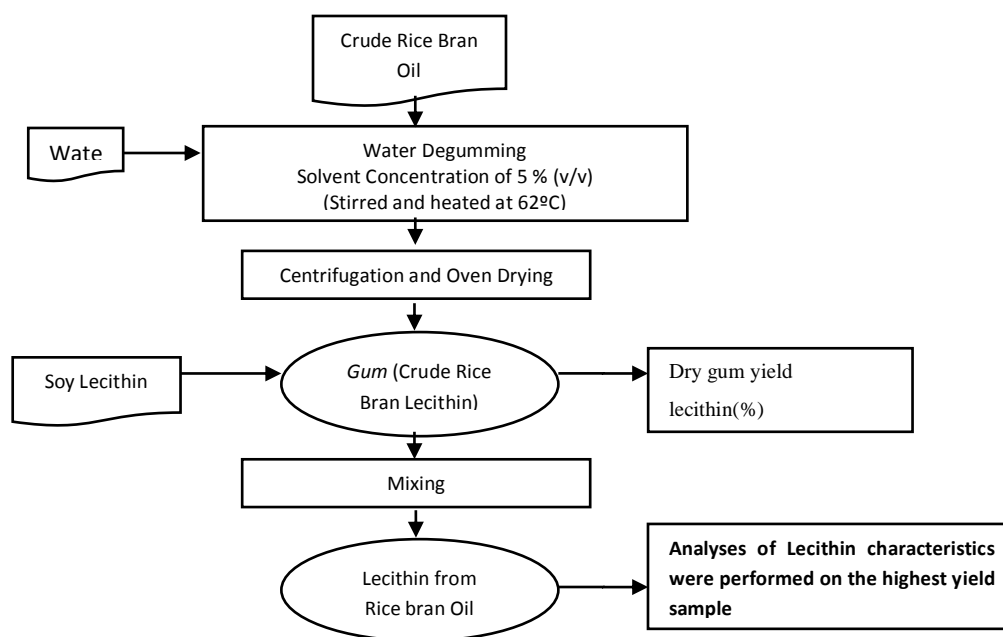
The analyses of beeswax coating characteristics include visible appearance (homogeneity, coagulation, and odor), pH (to be edible, this value should be close to 8 – 10), viscosity using rheometer [5], and density using pycnometer [6].



**Figure 1. The flow chart for the production of crude rice bran oil**



**Figure 2. Flow chart for Lecithin Production from Vegetable Oil**



**Figure 3. Flow chart for Beeswax Coating Production**

## Results and Discussion

### Crude Rice Bran Oil Yield

This trial employed extraction with n-hexane at the ratio of 1:8, in a maceration process that took place for three hours. The yield of crude rice bran oil 9.989%. [7] conducted a research using ethanol at the ratio of 1:6 and obtained a yield of 7.51%. They further concluded that the amount of solvent determined the amount of yield, in which an increased solvent produced a larger yield. This phenomenon might be the result of increased distribution of solutes that leads to increased contacts between solutes and solvent. And therefore, the difference in oil and solids becomes greater.

### Lecithin Yield from Crude Rice Bran Oil

Previous trials on isolation of lecithin from rice bran using water degumming produced different results. [8] set their parameters at  $T = 62\text{ }^{\circ}\text{C}$ ,  $t = 30$  minutes,  $k = 5\%$  and obtained a yield of 1.1%. Meanwhile, [3] performed their research at  $T = 80\text{ }^{\circ}\text{C}$ ,  $t = 2$  hours,  $k = 6\%$  and produced a yield of 0.683%. Additionally, [8] produced 1.55% of lecithin from soy at the optimal condition. This might be explained by the higher phospholipid content of soy (2-4%) than that of rice bran (1-2%) [9].

### Result of FT-IR Analysis of Lecithin from Crude Rice Bran Oil

FT-IR testing was conducted to determine the characteristics of phospholipids based on the functional groups and polar bonds of a compound. The results of this analysis on lecithin obtained from crude rice bran oil are presented in Figure 4. The following groups were detected on the sample: ester (peaks of  $1750\text{--}1725\text{ cm}^{-1}$ ),  $\text{PO}_2$  of phospholipids (peaks of  $1350\text{--}1250/1200\text{--}1145\text{ cm}^{-1}$ ), P-O-C (aromatic phosphates – peaks of  $995\text{--}850\text{ cm}^{-1}$ ), P-O-C (aliphatic phosphates – peaks of  $1145\text{--}970\text{ cm}^{-1}$ ) and amine compounds (peaks of  $1000\text{--}1080/1050\text{--}1200\text{ cm}^{-1}$ ) [10]. This result is in accordance with that of [11], in which the infrared spectrum for lecithin shows the presence of a peak at  $1720\text{ cm}^{-1}$  corresponding to glycerophosphatide carbonyl ester. Whereas the peak at  $970\text{ cm}^{-1}$  is an indication of the presence of phospholipid-containing choline [11].

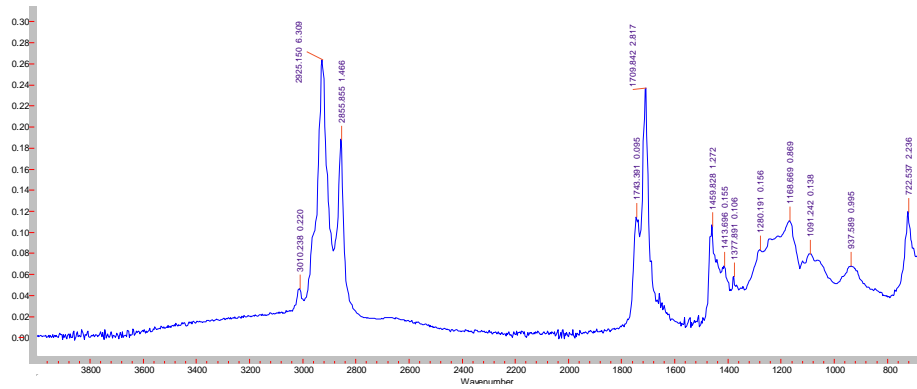


Figure 4. Fourier Transform Infrared (FT-IR) Spectrum for Rice Bran Lecithin



Figure 5. Lecithin Emulsion in a Graduated Cylinder for Creamy Index Analysis

### Results of Analyses on Lecithin from Vegetable oil

The lecithin isolates from soybean oil and rice bran oil at the ratio of 1:6 was analyzed for Creamy Index to determine the emulsion stability and HLB.

#### Creamy Index Analyses

Index Creamy is an indicator for emulsion stability and formulated as:

$$\text{Creamy Index} = 100\% * (\text{HS} / \text{HC}) \dots \dots \dots [3]$$

Figure 5 describes the data collection for HS and HC.

$$\text{HS (Height of Water Layer)} = 8$$

$$\text{HC (Total Height of Emulsion)} = 42$$

$$\text{Creamy Index} = 100\% \times (8/42) = 19,04\%$$

Creamy Index describes the percentage of cream formed in 24 hours after the emulsion is generated. The smaller percentage of cream produced indicates that the emulsion is more stable [3].

#### HLB Analyses

The HLB graph shows the values for predicting the hydrophilic-lipophilic balance and can be used to determine whether an emulsifier would prefer water or fat/oil. The obtained value determines whether lecithin as an emulsifier should be added to the oil or water phase during the beeswax coating production. Liquid lecithin tends to spread more easily in oil, while the powder form does in water. Heating lecithin up to 120 °F assists in dispersion and increasing the handling and mixing characteristics [12].

$$\text{HLB} = 20 \times (1 - \text{S/A}) \dots \dots \dots [4]$$

$$\text{A} = \text{Acid Value} = \frac{nmolKOHxNKOHx 56,1}{\text{Sample Mass}} = \frac{0,5 \times 0,5102 \times 56,1}{1 \text{ gr}} = 14,03$$

S = Saponification Value

$$\frac{28.05 \times (\text{volblanko-sample})}{\text{Sample Mass}} = \frac{28.05 \times (19.4-18.8)}{2 \text{ gr}} = 8.21$$

$$\text{HLB} = 20 \times \left(1 - \frac{S}{A}\right) = 20 \times \left(1 - \frac{8.21}{14.03}\right) = 8.29$$

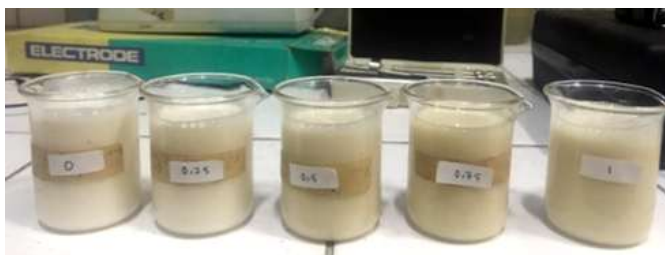
The HLB value of lecithin was 8.29. This value is close to that of soy lecithin based on the finding of [13], which is 8. This result indicates that the lecithin can be added to the formulation to either oil or water phase.

## The Effects of Lecithin Concentrations on Beeswax Coating Characteristics

### Visual Appearance

One of the key components in was emulsion formulation is an emulsifier to emulsify the product. The visual appearance of the edible coating formulation containing, beeswax, stearic acid, TEA, and water produced a satisfactory product. Homogeneity was accomplished by high speed stirring and exhibited by all five formulations prepared at 80-90°C.

The observation for visual appearance is presented in Figure 6. The color gradation can be observed, in which the absence of lecithin exhibits white coloration, while increased lecithin concentrations causes deeper yellow shades. This phenomenon is a result of lecithin initial color, which is brownish yellow. Therefore, the higher the lecithin concentrations, the darker the formulations.



**Figure 6. Visual Appearance of Five Beeswax Coating Formulations at Lecithin concentrations (%) of (left to right) 0, 0.25, 0.5, 0.75, and 1**

### pH

The result of pH analyses is presented in Figure 7 and shows that the effect of lecithin concentrations on preparation pH follows a linear regression with the formula of  $y = 0.22x + 8.032$ , with y representing pH and x representing lecithin concentration. The  $R^2$  value is 0.811, which shows that there is a significant positive correlation between the variables.

The pH values obtained in this trial range from 7.98-8.22. These values fall within the range of previous studies by [14], which was 8-10, and 8.5-10 [15]. Coating formulation is optimized to alkaline condition of 8-10 pH. Moreover, acidic condition is vulnerable to bacterial and fungal growths, which lead to faster spoilage of beeswax coating formulation.

### Density

Analyses on density is presented in Figure 8 and resulted in a linear regression of  $y = 0.033x + 0.992$ , with y representing density and x representing lecithin concentration. The  $R^2$  is 0.910, which shows a positive correlation between lecithin concentrations and density. The density values in this study fall in the range of 1.005-1.022 g/ml and are in accordance with the finding of which was 0.98-1.02 g/ml [15].

## Viscosity

Figure 9 represents the results from viscosity analyses. Lecithin concentrations significantly affect the viscosity of beeswax coating formulation ( $R^2 = 0.925$ ). The correlation produced a linear regression with the formula of  $y = 20x + 11$ , with  $y$  representing viscosity and  $x$  representing lecithin concentration. The viscosity values were in the range of 10-30 cp. This result is close to that of [15] which was 9-15 cp. The lower value of the previous study was a result of 10% wax used by [15], while this trial employed beeswax concentration of 12%. Coating can be applied optimally when it has the appropriate viscosity.

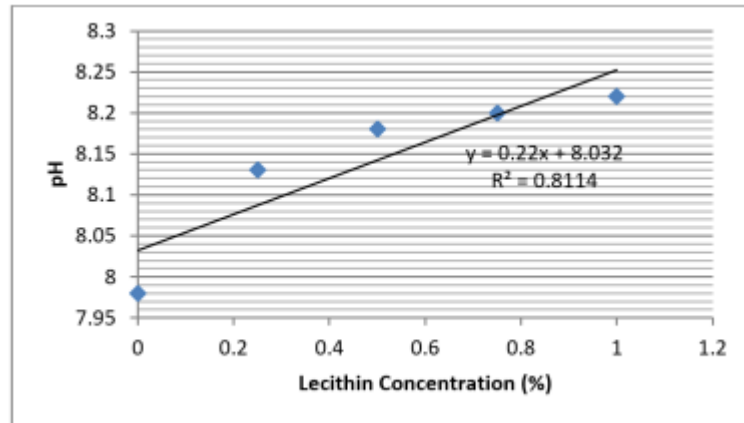


Figure 7. The effects of Lecithin Concentrations on pH.

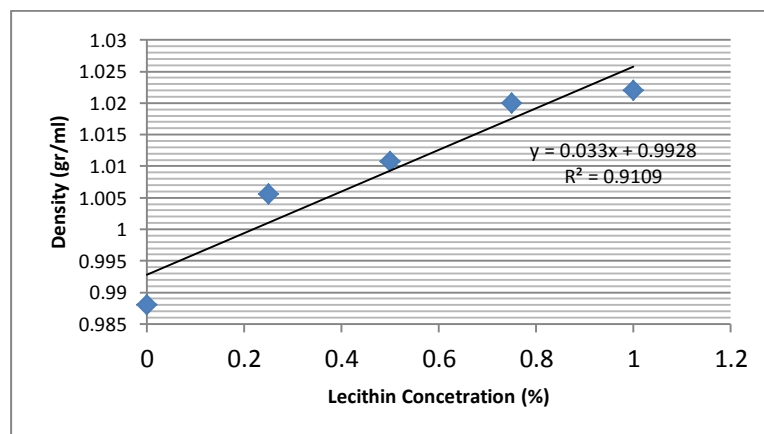


Figure 8. The effects of Lecithin Concentrations on Density.

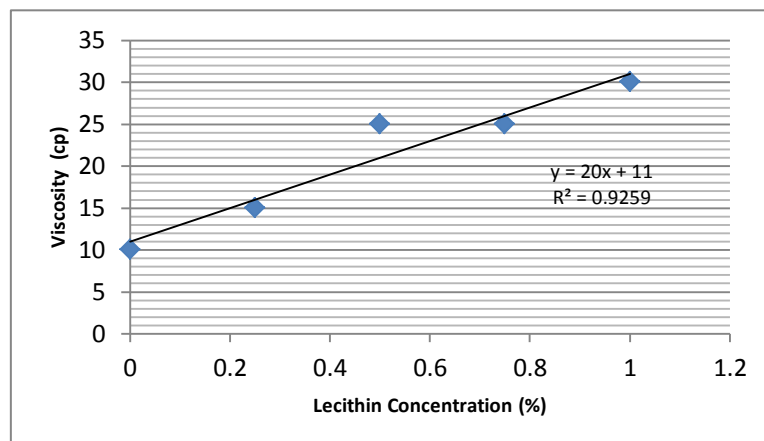


Figure 9. The effects of Lecithin Concentrations on Viscosity

## Conclusions

The results of this study can be concluded as follow:

1. Maceration process using n-hexane of rice bran produced 9.989% crude oil.
2. Water degumming on crude rice bran oil yielded 1.1% lecithin, which according to FT-IR analysis containing phospholipids. Mixture of rice bran and soy lecithin (1:6) lecithin with Creamy Index of 19.04% and HLB of 8.29.
3. Different concentrations (%) of plant-based lecithin of 0, 0.25, 0.5, 0.75, and 1 had significant effects on beeswax coating characteristics:
  - pH values increase as lecithin concentration increase, and those were 7.98, 8.13, 8.18, 8.2, and 8.22.
  - Densities (gr/ml) increase as lecithin concentration increase, and those were 0.98, 1.005, 1.01, 1.02, and 1.022.
  - Viscosities (cp) increase as lecithin concentration increase, and those were 10, 15, 25, 25, and 30.

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