Optimizing Biosurfactant Sorbitol Ester Synthesis Enzymetically From Fatty Acid Methyl Ester Using Coarse Papaya Resin

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Abstract. FASE is sorbitol ester from fatty acid with esterification degree point ranges from one to six. FASE with esterification degree point above three is polyester, which can be used as fat replacer. It has low calories. While FASE with esterification degree point below three can be used as surfactant. Food and beverages industries use surfactant as stabilizer in food and beverages composition. The making of FASE as biosurfactant brings advantages, i.e. it is save to be consumed, relatively cheap because the material needed in the making is easy to obtain, and it does not generate negative side effects. This research consists of three phases, there are preparation phase of papaya resin and making of Fatty Acid Methyl Ester (FAME). The next phase is optimum conditions (temperature, time, and enzyme concentration) defined using Response Surface Methodology and FASE synthesis. The last phase is FASE characterization. In this research, FASE is synthesizes, through sorbitol esterification with palmitat-stearat FAME by using papaya’s resin as the catalyst. The result of the research shows that the optimum condition of FASE synthesis at 41.2°C, in 52.35 minutes, with concentration of lipase enzyme from papaya’s resin 10.30 %, and at this condition, sorbitol conversion into FASE reaches 31.43 %.

1. Introduction

Surfactants are used in food processing to improve product quality and reduce the difficulty of handling perishable materials. The function of surfactants is to stabilize emulsions, for example in making bread, ice cream, and detergents. During product storage, surfactants will maintain viscosity, texture, mouthfeel, and extend shelf life. A surfactant is a bipolar molecule consisting of a polar group (interacting with the water phase) and a non-polar group which can interact with the oil phase. Energy supply into the system causes an increase in the contact area between water and oil molecules. The amount of energy given to the system must be proportional for increasing contact area between water and oil molecules. In this situation, thermodynamic free energy in the system is at a minimum level. Free energy reduction cause surface tension decreasing between the oil and water phases so that both fluids can be mixed homogeneously [1].

Biosurfactants are surfactants that are synthesized from biological compounds without using Synthetic chemicals and safe for consumption because they do not contain dangerous chemicals. Sorbitol and fatty acids are used for making biosurfactants. Sorbitol is non-sugar sweeter with 6 carbon atoms. Sorbitol also used in making ascorbic acid, propylene glycol, plate lubricants, humectants (humidity regulators), making printing inks used to give evenly distributed properties on the surface of
the paper and keep it from drying quickly [2]. Sorbitol used in candy, bread and chocolate products as stabilizer so that the product maintained from dryness and hardening. Sorbitol can be esterified with fatty acids because it has hydroxyl group which bind to FAME to produce FASE. FASE can be used as surfactant because it has head polar group, namely sorbitol and tail nonpolar groups, namely FAME.

FAME from palm oil used as fatty acids source in the manufacture of biosurfactants. FAME has several advantages than fatty acids, including the easier fractionation because the boiling point is lower than the fatty acid. FAME has stability in colour formation and oxidative degradation especially when heated, and also more stable against corrosivity. The use of FAME as a base material increases yields by 25-30% greater than using fatty acid.

In this study, synthesis was carried out enzymatically to prevent problems encountered in chemical processes which produce brown colour product due to high temperature and solvent toxicity. FASE synthesis used lipase enzyme from papaya resin as catalyst. The papaya lipase enzyme was chosen because it has several advantages including low prices, easy to obtain, and simple separation. Papaya production in Indonesia in 2017 reached 875,112 tons [3]. The purpose of his study are 1) Determine optimum conditions which include temperature, time, and concentration of papaya latex lipase enzyme in the synthesis of FASE as biosurfactant. 2) Evaluation the characteristics of FASE, including hydroxyl numbers, HLB, fatty acid composition, melting point, smoke point, and refraction index to find out potential uses in the food industry.

2. Methodology

2.1. Tools

The FASE reactor is Erlenmeyer with magnetic stirrer. The reactor is equipped with thermometer, vacuum pump, and inlet material. Heat reaction conditions can be achieved using heater equipped with thermostat. Stirring is carried out around 200 rpm. FASE purification and characterization using separating funnels, abbe refractometers, and spectrophotometers. Kotiermann Shaker water bath set on of 100°C, 120 rpm, Memmert 200°C oven, UD Rekayasa water bath ype fryer, Analytical scale Sartorius 160 gr, UV Visible Spectrophotometer Shimadzu, Sartorius moisture meter, freeze dryer Christ, IKA vacuum controller, pH Schott meter, micropipette, Erlenmeyer 100 ml Pyrex, pro pipette, drop pipette, measuring pipette, syringe, gas chromatogram, 50 mesh sieve. This research was carried out at Laboratory of Food Chemistry and Biochemistry, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta.

2.2. Research Procedure

2.2.1. Papaya latex lipase enzymes preparation

Papaya is between 2-4 months old. Sampling is carried out at 05.00-08.00 pm, using stainless steel knife with 2 mm scratch depth. Papaya resin that comes out then added by CaCl2, phosphate buffer pH 6, and store at freeze dryer. Papaya resin dried with freeze dryer at -40°C, vacuum pressure 0.5-0.1 bar (1 bar 1.0197 atm) for 48 hours. Dried resin powdered and filtered at 50 mesh. Papaya resin can be collected again in an interval of 1 week.
Determination of the optimum point is obtained based on the results of FASE synthesis preliminary with variations in temperature (at fixed enzyme time and concentration), time (at fixed temperature and concentration), and enzyme concentration (at fixed temperature and time). The synthesis was carried out at 45 °C, 90 minutes, and 15% enzyme concentration. In this study, acetate was used for acetylation, namely the formation of acetate esters. Reflux is carried out to prevent acetic acid loss from evaporating and help acetylation. Addition of butanol is carried out to prevent acetic acid loss. Isooktan and pyridine are used as solvents.

Incubation is carried out not in optimal conditions according to RSM calculations because it is difficult to place at temperature of 41.02 °C, so the incubation time is added ± 60 minutes to give longer alcohoholysis reaction so that more FASE are obtained. Shakers are carried out to provide greater opportunities for contact between sorbitol, FAME, and lipase. FAME added based on the mole ratio with sorbitol which has 6 hydroxyl groups which are assumed require 6 moles of FAME and given excess with a ratio of 1: 2 to sorbitol: FAME to shift the reaction equilibrium to the right so that more FASE are obtained.

Figure 1. Sorbitol Esther Biosurfactant Synthesis

100 mL FAME + 3.35 g sorbitol + 15 g papaya resin enzyme

Incubation at 45°C selama 90 menit

Crude FASE

Water at 80°C Fractionation Sorbitol Bottom Faction

Upper Faction

Ethanol at 70°C Fractionation Bottom Faction

Upper Faction TLC
2.2.2. FAME Synthetic

![Diagram of FAME Synthetic Path]

**Figure 2. FAME Synthetic Path**

2.2.3. Analysis of FASE properties

2.2.3.1. Hydroxyl Number Analysis (AOAC, 1995)

The hydroxyl number shows the milligrams of KOH which is equivalent to the hydroxyl content in 1 gram of sample. Sorbitol has 6 hydroxyl groups, if sorbitol is stabilized with fatty acids, the hydroxyl group content decreases and the hydroxyl number decreases.

The hydroxyl number analysis procedure is as follows: 0.067 g sorbitol, coarse papaya latex (5%, 10%, 15%), weighed into Erlenmeyer 100 mL (A) for acetylation plus 2 mL FAME and isooctane. Into another Erlenmeyer (B) weighed 0.5 g of a sample for determination of acidity. 1 mL of reagent C (the mixture of 3 volumes of pyridine and 1 volume of anhydrous acetate) pipetted into Erlenmeyer. Erlenmeyer is placed on a steam bath with a banded reflux condenser and heated for 1 hour. 1 mL and so on aquadest is added through the condenser and reheated for 10 minutes. Over 1.25 ml of butanol and Erlenmeyer were left to cool with a condenser, then released and condenser. Added 1.25 ml butanol again and 0.1 ml pp then titrated to a pink endpoint with 0.5 N KOH alcoholic standardized with 0.1 N HC1. The previously used butanol was neutralized with 0.5 N alcoholic KOH until the colour turn to it was pink. In the sample for determination of acidity (B) 1 ml of pyridine was added and mixed, then 0.1 ml pp was added, and titrated with 0.5 N KOH alcoholic until the pink endpoint.
If FASE is perfectly formed, the hydroxyl number is close to 0, because it is measured and titration is only acids and impurities and the B and S values are the same because there is no hydroxyl residue which binds to acetic acid so that the acetic acid remains intact.

0.067 g sorbitol + 2 mL FAME + 0.2 g papaya resin enzyme

Figure 3. Calculate Acidity
2.2.3.2 Analysis of fatty acid composition by GC (AOAC 963.22)
The column temperature is set at 195 °C, injection temperature is 240 °C. The carrier gas uses N2 at a pressure of 200 kPa (20 ml/minute, 1121 kg/cm², 02 1.5 kg/cm²). The column used has a polar / non-polar 10% DEGS phase with a column length of 2 m and a sample injection of 1 mL and so on. The injected solution was methylated in the following way: a 5% fat (FASE) solution was made in hexane, 6 ml of the solution was taken, then put in a closed test tube (12 ml capacity). Added 150 mL of methanolic 2 N KOH, vortexed for 5 minutes. Then centrifuged for 5 minutes at a speed of 2000 rpm, the supernatant obtained is ready to be injected into the GC. For comparison, standard fatty acids are used (lauric, myristic, palmitic, stearic, oleic, linoleic, and linolenic).

According to Hui (1996a) fatty acids in FAME and palm oil are composed of lauric (C120) 0.1-0.3%, myristate (C140) 0.9-1.5%, palmitate (C160) 42-51%, stearic (C180) 0.2-0.6%, oleic (C181) 32.8-39.8%, linoleic (C182) 8.6-11.3%, linolenic (C183) 0.2 -0.6%.

![Figure 4. Chromatogram of Origin of Fat Methyl Esters](image)

2.2.3.3 Confirmation of Fatty Acid Sorbitol Ester with TLC
TLC measuring 20 x 20 x 0.25 cm with silica gel binder 60. The car phase consists of petroleum ether: diethyl ether: glacial acetic acid 85: 35: 1 (v / v / v) Samples made by dissolving 0.5 ml FASE in 1 ml of hexane, 10-15 µl of aliquots are used to form spots on the TLC. The development lasts 20-30 minutes. Visualization was carried out by spraying 50% sulfuric acid solution, dried and heated at 100-110 °C for 30 minutes. Determination of sample and standard retention times was carried out with the help of UV lamp 254 um.

2.2.3.4 Determination of Hydrophilic-Lipophilic (HLB) balance
The HLB value is determined by the titration method. (Amount of water) according to Gupta et. al. (1983). One g of the sample was dissolved with dimethylformamide (DMF): benzene 100: 5, then titrated with distilled water until it was permanently cloudy (turbidity did not disappear with shaking out for 1 minute). Each addition of water needs to be followed by cooling to a temperature of 20 ± 1 °C because the addition of water increases the temperature of the mixture. The HLB value is determined by entering the volume of water needed for titration on the standard curve of water titration vs. HLB.

2.2.3.5 Determination of melting point (AOAC 920.157)
FASE is included in a capillary tube that is 1 mm in diameter, then closed using a flame without burning oil. After being incubated overnight in a cooler (4-10 °C), dip it in a glass cup containing 600 ml of water and equipped with a thermometer. Starting from 8-10 °C below the melting point of the product, heating is done to increase the temperature (0.5 °C/minute). The water in the container is stirred slowly. Melting points are indicated when the product becomes transparent.
2.2.3.6. Determination of smoke points (smoke points)
The smoke point is the temperature at which oil produces thin smoke which is bluish on heating (Ketaren, 1986). The principle of determining smoke points is the measurement of temperature when thin smoke arises from heated FASE. FASE is inserted in a beaker and other heat is measured at the highest temperature reached by a thermometer.

2.2.3.7. Determination of the refractive index (AOAC 921.08)
The refraction index is measured by the Abbe refractometer which is equipped with a temperature regulator. The method of determination is that the refractometer is set to 25 °C (4), then the prism is cleaned to dry and the number of samples is determined by reading the average price obtained from the refractometer. The refractive index is determined using the Abbe refractometer at a temperature of 20-25 °C. A double prism is opened, the sample or water is dropped between them and then closed by tightening the thread. Left for a while so that the temperature of the sample and the tool are the same, then the reading is done. The tool is cleaned using a soft cloth after that with cotton soaked in ethanol then dried. Observations based on position on boundary lines and total reflection.

3. Results and Discussion

3.1. Determination of Optimal FASE Synthesis Conditions
The results of the optimization analysis of FASE synthesis are presented in Table 1.

| Temperature (°C) | Time (Minutes) | Enzyme (%) | Temperature (°C) | Time | Enzyme (%) | Conversion (%) |
|-----------------|----------------|------------|-----------------|------|------------|----------------|
| 40              | 90             | 10         | 0               | 1    | 0          | 0.0000         |
| 45              | 30             | 10         | 1               | -1   | 0          | 0.0000         |
| 45              | 60             | 10         | 1               | 0    | 0          | 0.0000         |
| 35              | 90             | 10         | -1              | 1    | 0          | 0.0000         |
| 40              | 30             | 5          | 0               | -1   | 0          | 0.0000         |
| 35              | 60             | 5          | -1              | 1    | -1         | 0.9130         |
| 35              | 90             | 5          | -1              | 1    | -1         | 5.4588         |
| 45              | 30             | 15         | 1               | -1   | 1          | 6.6038         |
| 45              | 90             | 15         | 1               | 1    | 0          | 7.5472         |
| 40              | 60             | 10         | 0               | 0    | 0          | 11.5217        |
| 35              | 60             | 15         | -1              | 0    | 1          | 11.8835        |
| 45              | 90             | 10         | 1               | 1    | 0          | 11.9565        |
| 45              | 90             | 5          | 1               | 1    | -1         | 11.9679        |
| 35              | 30             | 10         | -1              | -1   | 0          | 12.1304        |
| 40              | 60             | 5          | 0               | 0    | -1         | 12.8662        |
| 35              | 90             | 15         | -1              | 1    | 1          | 13.2241        |
| 40              | 90             | 5          | 0               | 1    | -1         | 13.2532        |
| 45              | 30             | 5          | 1               | -1   | -1         | 13.9718        |
| 35              | 60             | 5          | -1              | 0    | -1         | 14.9254        |
| 40              | 90             | 15         | 0               | 1    | 1          | 14.9785        |
| 45              | 60             | 5          | 1               | 0    | -1         | 21.9735        |
| 35              | 30             | 15         | -1              | -1   | 1          | 22.4760        |
The results of the study as in Table 1 are contour graphs (not shown) which show the optimal area to be used for FASE synthesis.

The orientation results indicate the optimal area of FASE synthesis, the optimum condition is the optimum temperature of 40 °C, the optimum time of 60 minutes, and the optimum enzyme concentration at 15%. In all three regions, the graph results show the maximum optimal form of contour. Through processing statistical data, the conversion equation (KV) is obtained in the variables x1, x2, and x3, each of which states the code and temperature, time, and concentration of the enzyme.

Table 2. RSM Calculation Results

| Model                | Dep.var: KV Loss: (OBS-PRED)**2 | Final loss: 1069.8477842 R=,81744 Variance explained: 66.820% |
|----------------------|---------------------------------|---------------------------------------------------------------|
| A0                   | Estimate                        | Std.Err                                                      | t(5)  | p-level |
| A1                   | 30.65275                        | 7.602921                                                     | 4.031683 | 1.010005 |
| A2                   | -4.97786                        | 24.20198                                                     | -0.20568 | 0.845153 |
| A3                   | -9.27870                        | 19.37395                                                     | -0.47892 | 0.652202 |
| A4                   | 3.489145                        | 8.750288                                                     | 0.389746 | 0.706539 |
| A5                   | 9.28438                         | 31.88033                                                     | 0.290599 | 0.783026 |
| A6                   | -18.2994压                        | 13.55567                                                     | -1.34994 | 0.234924 |
| A7                   | -23.062压                        | 10.0866                                                     | -2.28646 | 0.070962 |
| A8                   | -2.86348压                        | 19.53942                                                     | -0.14654 | 0.889215 |
| A9                   | 7.479376压                        | 10.83852                                                     | 0.690074 | 0.520864 |
| A10                  | 8.759701压                        | 9.48524                                                      | 0.923509 | 0.39812 |

The variance and equation are 66.82% and R is 0.82 indicating that the conversion equation can be accepted as an equation model.

Table 3. Level 2 Polynomials

| Optimum Condition | Conversion at Optimal Condition | Nilai Eigen |
|-------------------|---------------------------------|-------------|
| X1                | 0.2048                           | KV = 31.34  |
| X2                | -0.2551                          | E           |
| X3                | 0.0604                           | -15.7130    |
|                   | 0                                | 0           |
|                   | 0                                | -26.1074    |

With these three optimum variables produced the optimum response (maximum FASE produced) of 31.643. The Eigen value is positive and negative, indicating that the response obtained forms a saddle curve (not maximum or minimum). Based on the results of this orientation, further optimization of FASE synthesis is carried out. The canonical analysis for the second level polynomial equation is:

\[ KV = 31.43 + 9.7226 x1^2 -15.7130x2^2 -26.1074x3^2 \]

The polynomial equation shows the existence of positive and negative numbers which means the form of this equation is the saddle, the optimal peak point is more than one so that if there is an addition or subtraction on one of the variables it will find another optimal peak condition. Based on the conversion, the FASE produced is 31.43% from 6 sorbitol hydroxyl groups, so the hydroxy groups are as many as 1-2 groups with the proportion of diesters more than the monoester which means FASE is diester.

FASE products have 1-2 substitution degrees as indicated by% conversion of 31.43%. In figure 10 displayed at each temperature (35, 40, 45 °C), time (30, 60, and 90 minutes), and enzyme concentration (5, 10, and 15%) which shows the optimal area that will be used for the synthesis of FASE.
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a. Temperature at 41.07°C

b. Time at 52.35 minutes

c. Enzyme Concentration at 10.30%

Figure 5. Contour 3 Dimensions of Surface Response of FASE Synthesis in Optimum Conditions

3.2. Determination of FASE properties

3.2.1. Hydroxyl number

The success of the reaction is expressed by % conversion which states the ratio of the hydroxyl number of the product to the hydroxyl sorbitol number. Therefore the hydroxyl number is used as a parameter of the success of the esterification process, ie the smaller the hydroxyl number indicates that the esterification is getting better (the degree of esterification obtained is high). The remaining acetic acid which is not used to bind the remaining hydroxyl will bind to KOH. When titrating the amount of acetic acid in the reagent, it is indicated by the result of titration of blank (B) and the remaining acidity titration shows the number of acidic impurities in the sample originating and breaking ester (V) the calculation functions as a correction for sample titration. The result of the titration of the sample (S) is the result of titration of KOH with acetic acid and acid derived from impurities. The more KOH that is used for titration, the less hydroxy groups that react with acetic acid. Sorbitol validates 6 hydroxyl groups. The smaller number of hydroxyl esters of fatty acid sorbitol shows that the esterification process is getting better (the degree of esterification obtained is high. The hydroxyl number can be seen in appendix 1.

3.2.2. Fatty acid composition

Fatty acids in FASE consist of palmitic acid (C16: 0) 59.21% and stearic acid (C18: 0) 38.98%.

\[
C_{16:0} = \left(\frac{23372636}{52496496 - 13027315}\right) \times 100\% = 59.21\%
\]

\[
C_{18:0} = \left(\frac{15383094}{52496496 - 13027315}\right) \times 100\% = 38.98\%
\]
The percentage of palmitic fatty acids in FASE tends to be greater compared to stearic fatty acids. This is due to the palmitic-stearic ratio in FAME is 53.75% and 46.25%. In addition, the palmitic molecular weight is smaller so that it is easier to test because it is more reactive than stearic. Papaya latex lipase is more effective or shows maximum activity for short chain fatty acids, so lipase activity is more likely to palmitic acid than stearic. According to (5), the suitability of oil for frying is directly related to the content of unsaturated fatty acids, especially linolenic acid. This is described as inherent stability calculated from the level of each unsaturated fatty acid (oleic, linoleic, linolenic) and the relative reaction speed with oxygen. Higher inherent stability causes oil to be less suitable for frying.

3.2.3. Thin layer chromatography
Thin layer chromatography (TLC) analysis based on the principle of like dissolves like, meaning that to separate non-polar samples, a solvent system that is non-polar is used (6). The stationary phase used is silica gel G 60 which is polar and as the mobile phase (solvent system or developer solution) PE: DE: glacial acetate (85: 35: 1) is used which is non-polar. Therefore the more polar sample will be below (the Rf value is smaller), while the non-polar sample will be above (the Rf value is greater).

The results of the confirmation structure show that the FASE sample has an Rf of 0.32. Rf values for the sorbitan monopalmitate, sorbitan monooleate and sorbitan trioleate standards were 0.25, 0.46 and 0.65. If the sample of the fatty acid sorbitol ester is compared to the standard, the formed band can be interpreted to have one esterification degree. TLC results show that FASE is formed as monoester. According to (1), the structure of the sorbitol ester must have more than four fatty acid esters to provide
steric hindrance needed to inhibit digestion. Esters that have less than four degrees of esterification do not function as oil replacer but as emulsifiers.

3.2.4. Hydrophilic-Lipophilic Balance

HLB value shows the function of the product as an emulsifier. Determination of HLB values using the amount of water method (7) shows the volume of water (mL) that must be added to sorbitol ester solution in DMF: benzene with a ratio of 100: 5 to form stable emulsions characterized by permanent turbidity. According to (7), HLB values are determined by type, a chain length of fatty acids and the number of fatty acids bound to sorbitol. The lipophilic nature of the sorbitol ester with a wide range can be made with variations in the length of the FAME chain, the unsaturation level of the ester group, and the number of ester groups of each sorbitol ester molecule.

| Type                      | Number of Water Titrations (mL) | HLB   |
|---------------------------|---------------------------------|-------|
| Sorbitan monopalmitate    | 1,5                             | 6.7*  |
| Sorbitan monoleate        | 0.75                            | 4.3*  |
| Sorbitan trioleate        | 0.3                             | 1.8*  |
| Oleat Acid                | 2.7                             | 17*   |
| FASE (product)            | 1,7                             | 7.5   |

* [1]

Determination of HLB values uses linear equations between the amount of water for titration (mL) and standard HLB values as in the following graph:

The HLB value of 7.5 indicates that FASE is closer to the sorbitan monopalmitate standard than the sorbitan monoleate and Sorbitan trioleate standards. Sorbitan esters that are used as a standard have nonionic emulsifier characteristics.

Stability of dispersion can be considered that attractive forces between particles (van der Waals strength) are balanced by electrostatic forces or resistive repulsive forces (1). The characteristic of surfactant is that it can reduce surface tension, from the results of FASE surface tension measurements having a surface tension of 28.75 mN / m (1 mN / m = 1 dyne/cm). The standard of sorbitan monoleate and trioleate has a surface tension of 17.73 and 20.37 mN / m. Water itself has a surface tension of 52.63 mN / m. 1% FASE can hold oil emulsion in water (o / w) for 22 seconds, while in water-in-oil emulsion (w / o) for 92 minutes. Sorbitan monopalmitate 1% can emulsify water in oil for 105 minutes. While the Sorbitan monoleate and Sorbitan 1% trioleate standard can emulsify for 115 minutes and 120 minutes. Observations are based on the level of turbidity due to the dispersion of oil in water. time is calculated after the oil and water mix until oil and water separate.

3.2.5. Smoke point

Another characteristic that needs to be analyzed is the smoke point because it is useful to find out whether the product can be used for frying or not. FASE has a smoke point 140 ° C. While the oil smoke point used to fry around >160 ° C (8). FASE smoke points tend to decrease during heating. Decreasing smoke points is closely related to oxidation and hydrolysis reactions during heating which causes accumulation of free fatty acids. According to (4), the smoke point depends on the level of free fatty acids, the freer fatty acids the lower the smoke point.

3.2.7. Refractive index

The oil refractive index is the ratio of the sine of the falling beam angle and the reflected angle of light through oil. This refraction is due to the interaction between electrostatic forces and electromagnetic atoms in oil molecules (9). Refractive index is influenced by the degree of oil unsaturation, long chain fatty acids, thermal polymerization. Changes in the refractive index are caused by heating which causes
thermal polymerization so that the C chain is longer. Measuring the index to test the purity of oil, increases with the length of the C chain, the higher the degree of unsaturation, and the higher the temperature. Index of refraction of palm oil 1.435 (10). FASE refraction index 1.441 (26 °C). The refractive index of carbohydrate polyester is generally greater than the index of oil refraction because it has a larger molecule, which is caused by many fatty acids that are bound to the core of carbohydrates.

4. Conclusion and Suggestion

4.1. Conclusion
Papaya resin has activity as a lipase enzyme in catalyzing FAME and sorbitol into fatty acid sorbitol esters (FASE). FASE synthesis can be obtained maximally in the optimal treatment, namely the temperature of 41.02 °C, time of 52.35 minutes. Coarse papaya lipase enzyme concentration of 10.30%, and resulted conversion of 31.43% (FASE formed in the form of diester).

Based on the TLC results that have been carried out, show that FASE Rf is 0.32. Based on the determination of FASE characters to find out the potential use in the food industry, biosurfactant sorbitol ester has a melting point of 27-30 ° C, smoke point 140 °C, refraction index 1.44, and HLB value 7.5. 1% FASE can hold emulsion of water in oil for 92 minutes.

4.2. Suggestion
It is necessary to purify raw materials both FAME and papaya latex lipases and review process conditions (temperature, time, and enzyme concentration) and vacuum to produce optimal FASE. The potential use of biosurfactant Sorbitol Esters as an emulsifier for water in oil (w/o) needs to be investigated further.

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