Enzymatic Synthesis of Sucrose Polyester as Food Emulsifier Compound

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Abstract

Sucrose polyester (SPE) is a carbohydrate ester compound that has diverse functions, from surfactant to low-calorie food products. Sucrose fatty acid ester with the degree of substitution 1-3 can be used as emulsifier in foods and cosmetics. The enzymatic synthesis of sucrose polyesters can be carried out using lipase in organic solvent and contain small amount of water. In this study sucrose esters were synthesized by esterification reaction between sucrose with fatty acids from coconut and palm oil using *Candida rugosa* lipase in n-hexane. Optimization esterification reaction was carried out for parameters of incubation time, temperature, and the ratio of the substrate. The optimum incubation time was at 18 hours for coconut oil and 12 hours palm oil, the optimum temperature was 30 ^{o}C for coconut and palm oil, and the mole ratio of fatty acid to sucrose was 40:1 for coconut oil and 64:1 for palm oil. Esterification products were characterized by FT-IR. The FT-IR spectrum showed the ester bond was formed as indicated by the wave number 1739.79/cm. Esterification products have 2 substitution degrees.

Abstrak

Sintesis Poliester Sukrosa secara Enzimatik sebagai Emulsifier pada Makanan. Poliester sukrosa merupakan senyawa ester karbohidrat yang memiliki berbagai fungsi, dari surfaktan hingga produk makanan rendah kalori. Ester asam lemak sukrosa dengan derajat substitusi 1-3 dapat digunakan sebagai emulsifier pada makanan dan kosmetik. Sintesis poliester sukrosa secara enzimatik dilakukan dalam pelarut organik dan dalam keadaan terdapat sedikit air. Pada penelitian ini, ester sukrosa disintesis melalui reaksi esterifikasi antara sukrosa dengan asam lemak dari minyak kelapa dan kelapa sawit menggunakan lipase *Candida rugosa* dalam n-heksana. Optimasi reaksi esterifikasi dilakukan terhadap waktu inkubasi, temperatur, dan rasio substrat. Hasil optimasi menunjukkan bahwa waktu inkubasi optimum adalah 18 jam untuk minyak kelapa dan 12 jam untuk minyak kepala sawit, temperatur optimum pada 30 ^{o}C untuk minyak kelapa dan kelapa sawit, dan rasio mol asam lemak terhadap sukrosa optimum adalah 40:1 untuk minyak kelapa dan 64:1 untuk minyak kelapa sawit. Produk hasil esterifikasi dikarakterisasi menggunakan FT-IR. Spektrum FT-IR menunjukkan telah terbentuknya ikatan ester, yang ditunjukkan dengan adanya serapan pada bilangan gelombang 1739,79/cm. Produk esterifikasi memiliki derajat esterifikasi 2.

Keywords: coconut oil, lipase, palm oil, substitution degree, sucrose polyester

1. Introduction

Indonesia is the largest producer of palm oil and second largest producer of coconut oil in the world [1]. Primary utilization of the products of these plants is for food preparation particularly as food modifier to improve the taste properties (palatability) such as smoothness and flavor [2]. Therefore, the need for oils and fats in the dietry are quite significant.

However, excessive fat intake can lead to specific-diseases, such as coronary heart disease, cancer, and obesity [2]. To avoid this negative impact, materials which have lower caloriy for fat substitute without sacrificing the taste and the texture of food is needed.

Sucrose polyester (SPE) has similar structure to natural fats. This compound is not well-digested and can not be absorbed. So that SPE potentially can be used for fat substitution. Mainly, SPE is prepared chemically and very little is known for the enzymatic preparation. Enzymatic preparation of SPE was conducted by free and immobilized enzyme. Enzymatic preparation offers some advantages. One of them is that it allows us to control the degree of esterification. Depending upon the degree of esterification, SPE may function as an

emulsifier for food or as fat substitute. Sucrose fatty acid ester with the degree of substitution up to three can be used as an emulsifier in foods and cosmetics [3-5].

In this work, SPE was synthesized by enzymatic esterification reaction between sucrose and fatty acids from coconut and palm oil using *Candida rugosa* lipase in n-hexane. The objectives of this research were to ensure the possible use of coconut and palm oil as raw materials for the manufacture of SPE and to determine the optimum conditions for the production of SPE with high degree of esterification.

The aim of this study was to obtain optimal conditions for the manufacture of sucrose polyester, as well as studying the physical properties of chemical compounds for use as an oil substitute material that has characteristics of low-calorie. The results of this study is expected can contribute in the field of food science, especially concerning the development and empowerment of sucrose modified vegetable oils as one of the natural wealth of Indonesian origin.

2. Experiment

Hydrolysis of Triglycerides. Hydrolysis of triglycerides were carried out by mixing 20 g of coconut and palm oil each to 100 ml of 1.0 M KOH in 95% alcohol. This mixture is then refluxed for 1 h at 62 ± 2 °C. After heating, the mixture was added into 50 ml water and acidified with 30 ml 3N of HCl. The mixture was extracted with 50 ml n-hexane. The top layer was separated and added by 1.0 g anhydrous Na_2SO_4. The solution was separated from Na_2SO_4 by decantation and the solvent was evaporated by rotary evaporator at 40 °C.

Synthesis of SucroseEsters (Esterification reaction). The enzyme used in this study was *C. rugosa* lipase obtained from Sigma Aldrich. Sucrose, fatty acids, and n-hexane were mixed in Erlenmeyer then incubated for ±10 minutes in a horizontal shaker incubator. The *C. rugosa* lipase was added to the mixture. The enzyme was dissolved in phosphate buffer pH 8. The mixture was incubated in a horizontal shaker incubator at 200 rpm at 40 °C for 18 h. Esterification reaction was terminated by heating the mixture at temperature of 80 °C for enzyme denaturization. The product of esterification was centrifuged for 15 min at 3400 rpm. The layers formed were separated. Then - hexane phase was diluted into10 ml volumetric flask and then titrated with 0.5 M NaOH to determine the yield percentage. The layer between n-hexane and water phase was separated. The formation of the ester group was characterized by FT-IR method for sucrose, fatty acid from coconut and palm oil, and esterification products. The esterification product should be dried at 60 °C to remove any residual water or solvent, before it was

characterized. Standard sucrose ester used in this study is ryoto sugar ester S-1170 from Mitsubishi-Kagaku Foods Corporation, with stearic acid as fatty acid esterified at the sucrose group and consist of a mixture of mono, di, and tri ester sucrose.

Optimization of reaction conditions. Optimization of the reaction conditions was done by vary the reaction of time, temperature, and the ratio of the sucrose concentration to fatty acid obtained from hydrolisis. The reaction time variations used were at 6, 12, 18, 24, 30, and 36 h; the reaction temperature variations were at 25, 30, 35, 40, and 45 °C, and the variation ratio of the concentration of sucrose to fatty acids from hydrolyzed coconut oil were 1:16, 1:40, 1:80, 1:120, whereas for sucrose with fatty acids from hydrolyzed palm oil were 1:16, 1:40, 1:64, 1:90. The sucrose ester obtained was analyzed by high performance liquid chromatography (HPLC), using C18 column, maintained at 40 °C, and refractive index detector. The separation of sucrose esters was achieved with methanol/water 7:1 (v/vr) as the mobile phase at a flow rate of 1.1 ml/min. This method was adopted from Cruses [6].

Determination of emulsion types using microscopes. A test for the emulsion was carried out by mixing a drop of emulsion and eosin on an object glass. The mixture was then observed under a microscope to determine whether the emulsion formed is oil in water (o/w) or water in oil (w/o) emulsion.

3. Results and Discussion

Fatty acid composition. The experiment to determine the composition of the fatty acid was caried out at *Laboratorium Analisis dan Kalibrasi Balai Besar Industri Agro*. The fatty acids composition are listed in Table 1.

In Table 1, it can be seen that most fatty acid in coconut oil is lauric acid (54.1%), which is a medium-chain fatty

Table 1. Fatty Acids Composition

Fatty cids	Persentase (%)	
	Coconut oil	Palm oil
Capyilic (C8)	7.2	0.09
Capric (C10)	8.02	0.13
Lauric (C12)	54.1	0.51
Myristic (C14)	17.4	1.24
Palmitic (C16:0)	6.64	35.5
Stearic (C18:0)	1.86	2.82
Oleic (C18:1)	3.99	41.1
Linoleic (C18:2)	0.81	17.8
Linolenic (C18:3)	0.02	0.78

acids. Meanwhile, most fatty acids in palm oil are oleic acid (41.1%) and palmitic acid (35.5%) which are long chain fatty acids.

Hydrolysis of Triglycerides. The hydrolysis of the triglycerides content in coconut and palm oil to form fatty acids was performed using KOH in ethanol as catalyst at 60 ^{o}C. KOH in ethanol was used to avoid the formation of soap. The function of ethanol is to lower the polarity difference between KOH and oil which in the end permits the reaction. The potassium salt of the fatty acid was then acidified with HCl to form fatty acid. These fatty acids obtained from this process were used for SPE formation.

Esterification. Lipase is a hydrolase class of enzyme that catalyzes the triglyceride hydrolysis reaction produces fatty acids and glycerol. However, on certain conditions, lipases can also catalyze the reverse reaction of hydrolysis, namely the esterification reaction. Esterification reactions catalyzed by lipase occur in the system with small amount of water. The organic solvent is commonly used for esterification reaction. In this study, n-hexane was used as solvent in the enzymatic esterification reaction between sucrose and fatty acids from coconut and palm oil.

In performing its function as a catalyst, an enzyme must be in a good condition to support its catalytic activity. A study on catalytic activity of enzymes in organic solvents showed that solvents with log P values between 2 and 4 can maintain enzyme activity and stability [7]. Moreover solvent hydrophobicity effects the amount of essential water needed by an enzyme for its catalytic activity. This is understandable since water contributes directly or indirectly in all non-covalent interactions that maintain the natural catalytic conformation of the enzyme. Water also plays an important role in the dynamics of the enzyme. Small amount of water is required to maintain the conformation of the enzyme. If the essential water molecules still coat the enzyme molecules, then the replacement of the remnants water with organic solvents will not interfere the enzyme catalytic activity [8]. The solvent used in this study was n-hexane which has a log p value of 3.5. N-hexane is known as good solvent that can increase the stability of the enzyme lipase in the esterification reaction [9].

The product obtained from esterification reaction formed an emulsion system. To break the emulsion system the mixture was centrifugated. The three layers formed were separated.

The top layer, the fatty acids layer, was dissolved in n-hexane and then titrated with 0.5 N NaOH to determine the amount of residual fatty acids to calculate the value of esterification reaction yield.

Preliminary test the product of sucrose esters synthesis as emulsifier. The middle layer, which is estimated as the product of esterification, was used for emulsifier test. Water and oil that was initially separated could be mixed well and form stable emulsion system for more than 24 hours.

Characterization of esterification product. Next, the esterification product was characterized by FT-IR. IR spectrum of sucrose, ryoto sugar ester S-1170, fatty acids from coconut and palm oil, and esterification products were shown in Figure 2-4.

It is clearly seen that in a new peak at wave number 1739.79/cm corresponds to C=O ester bond by comparing IR spectra of sucrose, fatty acids, and esterification product, a new peak observed in IR spectrum of esterification product from either coconut or palm oil. This new peak was observed at wave number 1739.79/cm and typical for C=O ester bond (Figure 3b and 4b). It was known that the spesific wave number for ester group is between 1720-1750/cm.

In addition, in IR spectrum of esterification product a wide absorption peak was observed at wave number 3325.28/cm for O-H group.This peak indicated that the esterification product still had several O-H groups that had not been esterified. When the IR spectrum of sucrose was compared to IR spectrum of esterification product, the esterification product had lower O-H absorption peak, indicating that the product had low estrification degree. Based on IR spectrum analysis, it can be concluded that the product obtained from eserification using lipase was ester.

Ryoto sugar S-1170 consist of sucrose mono-, di-, and tristearate with the composition of sucrose monostearate is 50%, while sucrose di- and tri-stearate are 30%. When IR spectrum of ryoto sugar compared with IR spectrum of esterification product, it was observed that there is a peak located at nearly the same wave number, namely 1739/cm for esterification product and 1737/cm for the Ryoto sugar (Figure 2b). The two peaks are a typical absorption for the ester functional group.

The other absorption peak observed in IR spectrum of Ryoto sugar ester S-1170 that is at the wave number 3286/cm. This peak indicated that Ryoto sugar still had
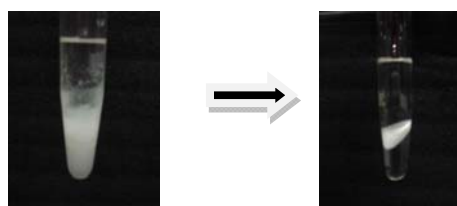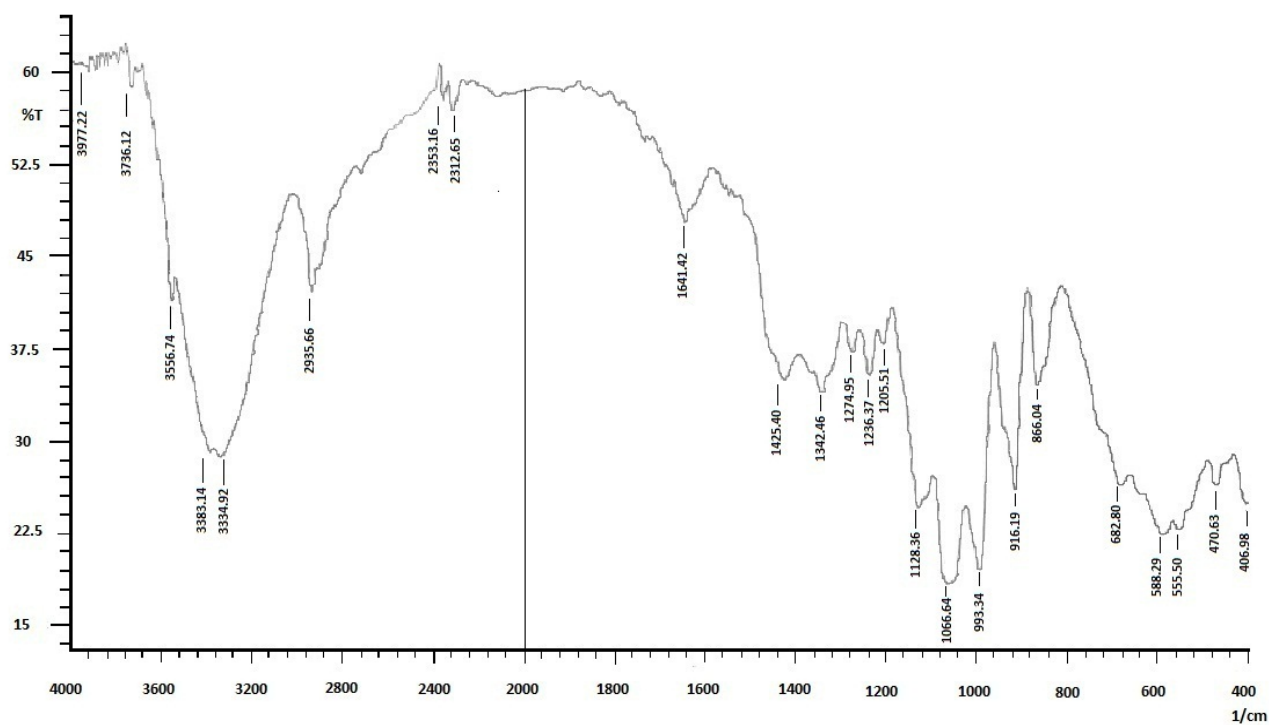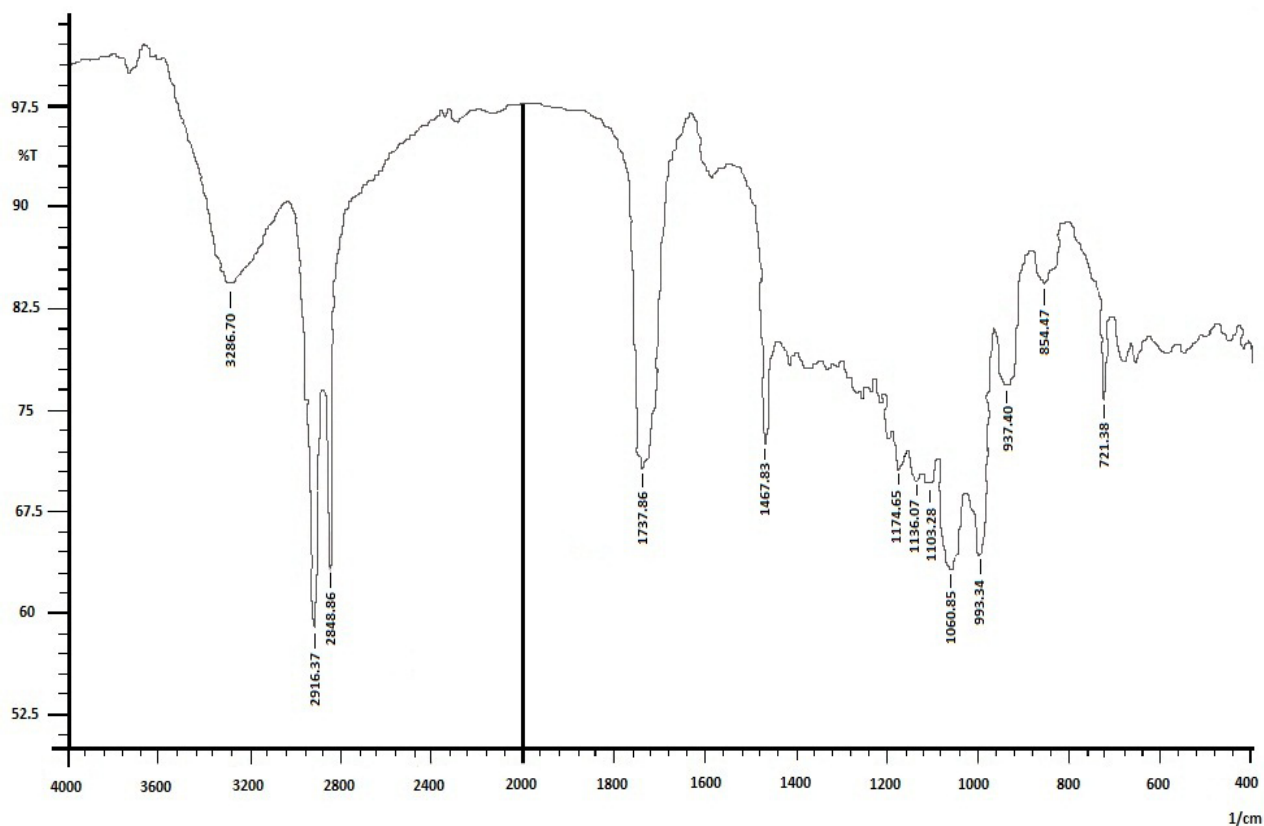

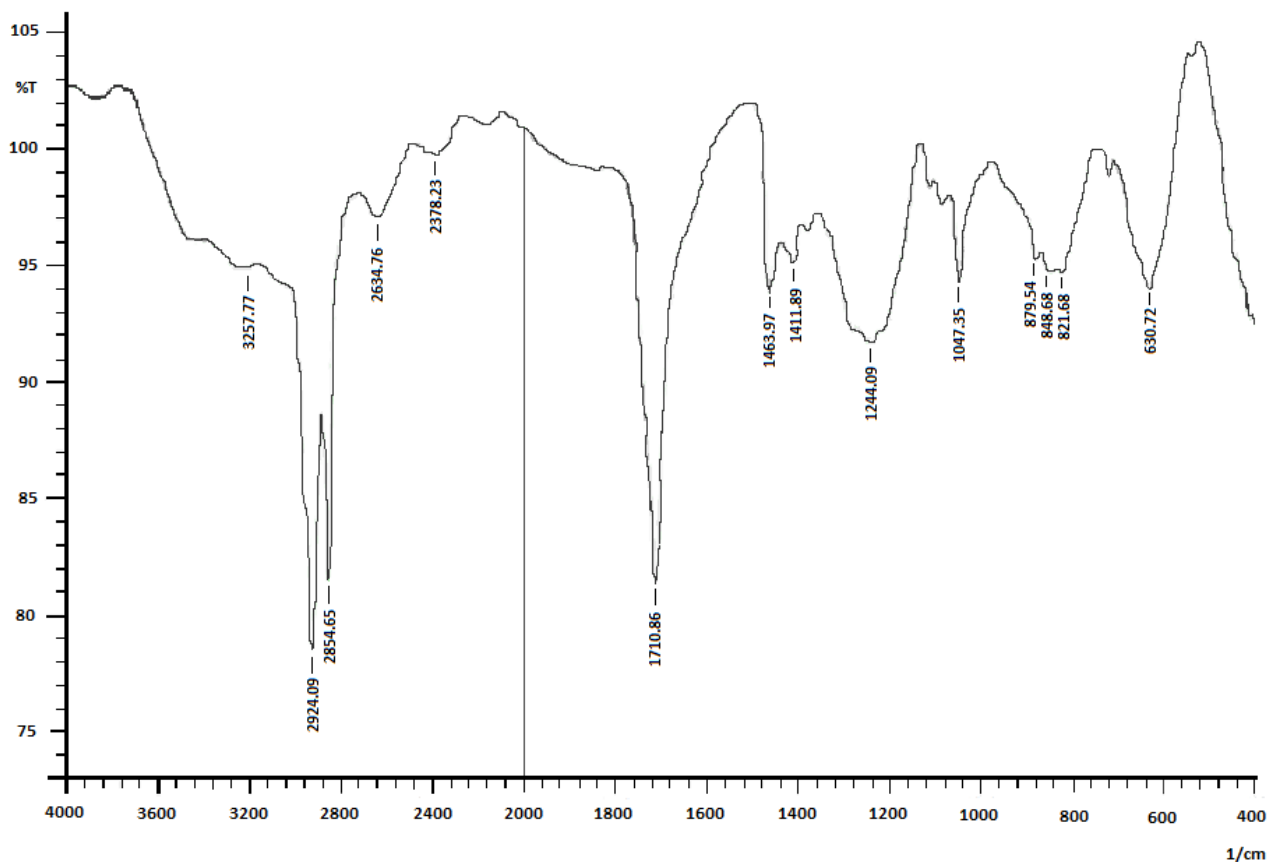**Figure 1. Esterification Product Before and After Centrifugation**
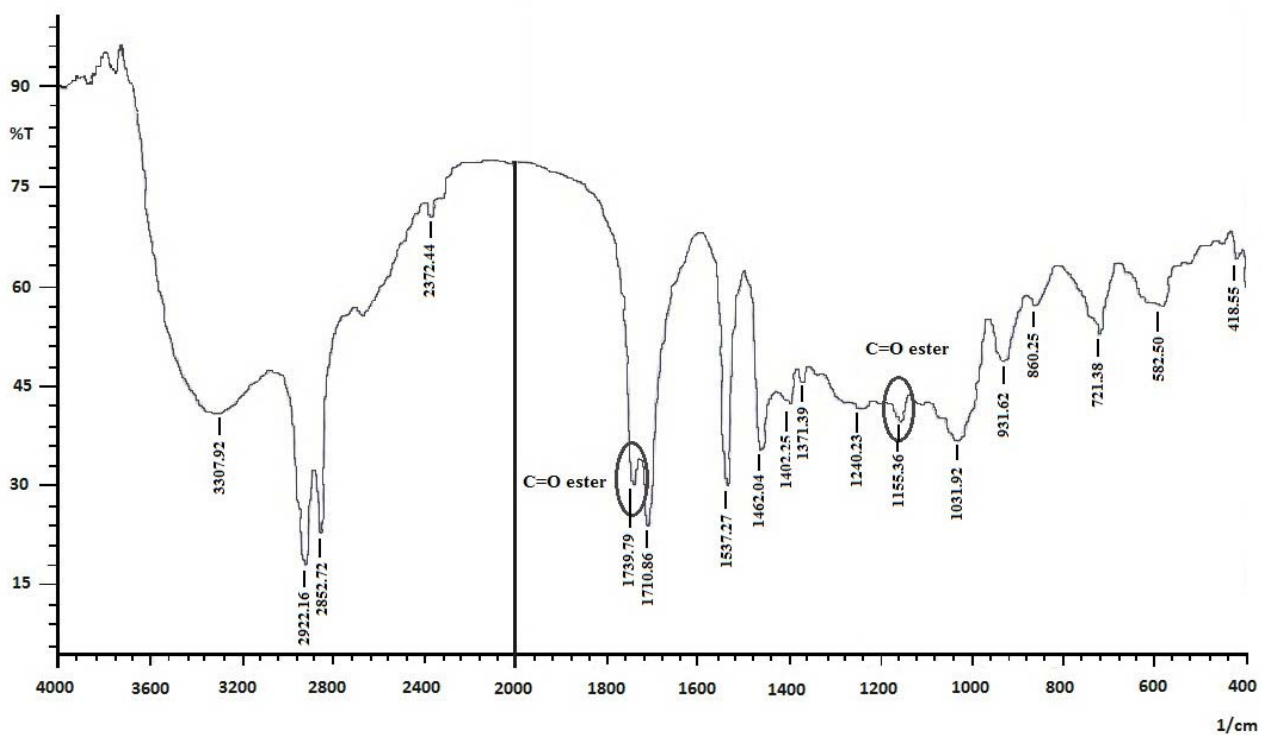
(a)

(b)

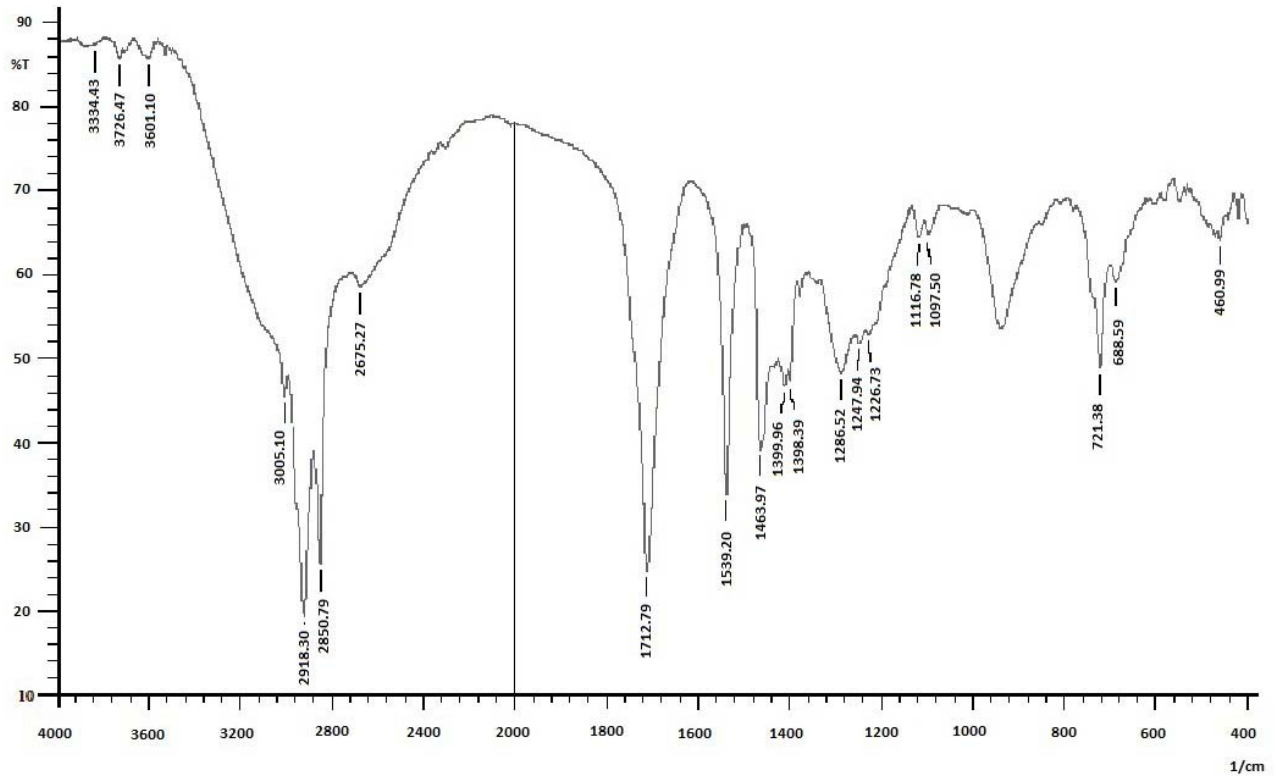**Figure 2. IR Spectrum of: (a) Sucrose, (b) Ryoto Sugar Ester S-1170**
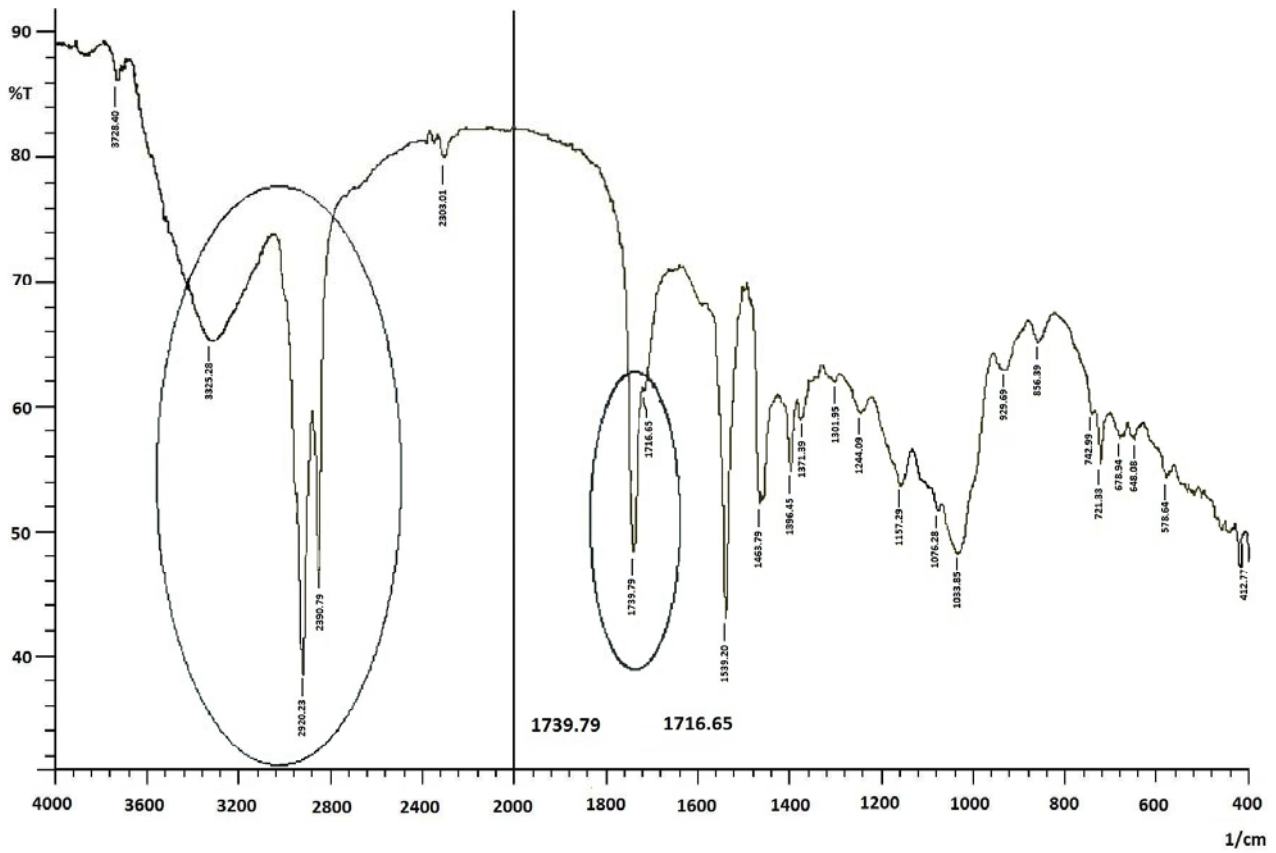
(a)

(b)

Figure 3. IR Spectrum of: (a) Coconut Oil Fatty Acid, (b) Esterification Product from Coconut Oil

(a)

(b)

Figure 4. IR Spectrum of: (a) Fatty Acid from Palm Oil, (b) Esterification Product from Palm Oil

several O-H groups that had not esterified. These data correspond to data specification of Ryoto sugar ester S-1170 that the standard was a mixture of mono, di, and sucrose trimester [10].

Optimization Esterification Condition: Optimization incubation time. The results of optimization of incubation time are shown in Figure 5. The time required to produce sucrose esters with the highest percentage was 18 h for the samples from coconut oil and 12 h for palm oil. At first there was an increase of % yield until the optimum incubation time was reached and then the % yield decreased.

The decrease in % yield can becaused by rehydrolyzed sucrose fatty acid ester produced into fatty acids and sucrose with increasing the water from the esterification reaction. Increasing water in the reaction medium can cause the enzyme to catalyze the reaction towards hydrolysis reaction compared to the esterification reaction.

Optimization Esterification temperature. The optimum temperature for esterification was investigated by observing % yield in various temperature. Changes in temperature could cause changes in the activity and stability of an enzyme and also reaction rate. In addition, temperature also affects the solubility of the substrate, ie fatty acids and sucrose.

Between temperatures of 25-30 ^{o}C enzymatic esterification rates increased with increasing temperature. This is due to the increased kinetic energy of the molecule so that the frequency of collisions between the molecules of the substrate with enzyme increased. Above 30 ^{o}C the reaction rate decreased because of disruption of the enzyme conformation, so the enzyme became inactive (Figure 6). The data was consistent with previous research results which suggest that the *Candida rugosa* lipase enzymes have a thermal stability and optimum temperature between 30-35 ^{o}C. The optimum temperature for *Candida rugosa* lipase in the esterification was at 30 ^{o}C, for both samples of coconut and palm oil.
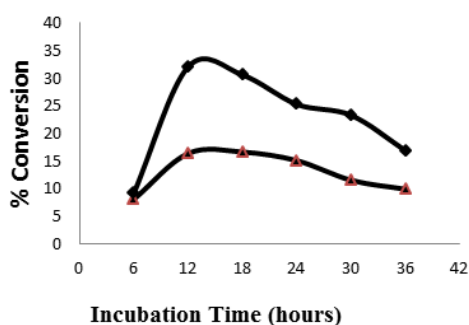

Figure 5. The Curve of Incubation Time vs % Yield, Coconut (▲), Palm Oil (♦)

Optimization subtrate ratio. One of the factors that influence the enzyme activity is substrate concentration. In this study the mole substrate ratio between sucrose to fatty acid was varied. The variations selection was based on the previous study who conduct the esterification using fructose. The optimum condition was shown at a ratio of 1:10 [11]. Another investigation that used glycerol for esterification showed optimum conditions at a ratio of 1:6 for glycerol to fatty acids [12]. This indicates that the ratio of the substrate associated with the number of hydroxyl groups on alcohol or sugar. Therefore the determination of substrate ratio variation in this study was done by considering the number of hydroxyl groups of sucrose and began at a ratio of 16:1. The influence of substrate ratio variations were shown in Figure 7.

The optimum conditions of esterification by *C. rugosa* lipase for palm oil sample was obtained at ratio 40:1, while for palm oil at ratio of 64:1. This indicated that the addition of fatty acids was no longer able to increase the production of sucrose esters because the enzyme used had been saturated.
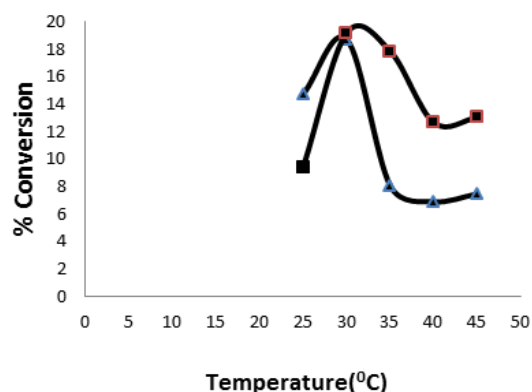

Figure 6. The Curve of Temperature Variations vs % Yield, Coconut (▲), Palm Oil (♦)
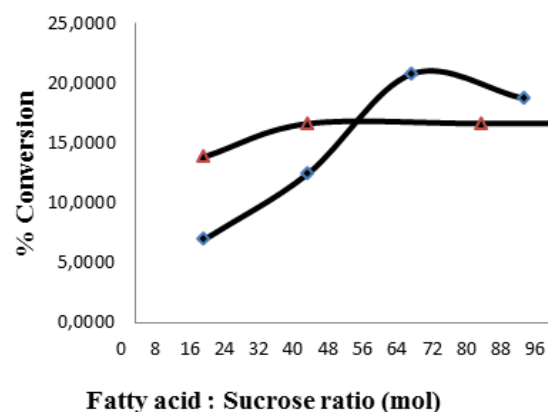

Figure 7. The Curve of Mole Ratio Variations Fatty Acid: Sucrose vs % Yield, Coconut (▲), Palm Oil (♦)

By calculating the ratio of moles of fatty acids that react with sucrose, sucrose esters produced in this study was estimated to have the degree of esterification is equal to two. This assumed that the ester products are mixture of mono- or diester sucrose. This calculation was based on the percentage yield of sucrose esters which only reached 16.62 % for coconut oil and 20.07% for palm oil at the optimum conditions.

Sucrose ester analysis using HPLC. Quantitative analysis using HPLC could be used to compare the composition of mono, di, tri, and tetraester of sucrose in the product. The comparison of the substitution degree of sucrose ester in the product shown in Table 2 and Figure 8.

Determination of emulsion types using microscopes. Figure 9 shows the microscopic photograph of emulsion. Emulsion obtained was added with eosin, a red dye which is soluble in water, so the water phase willbe red in color and easy to be observed anddistinguished from the oil phase.

Table 2. Composition of Substitution Degree of Sucrose Ester

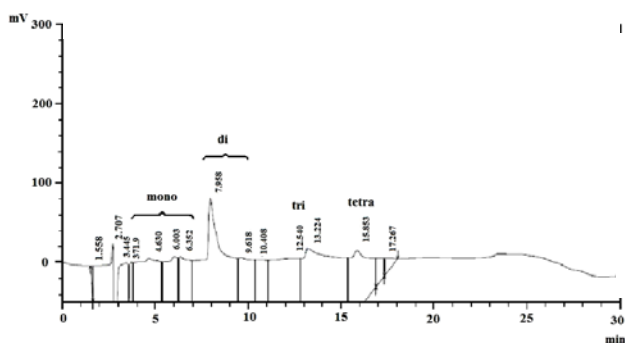| Substitution Degree	Sucrose Ester from Coconut Oil (%)	Sucrose Ester from Palm Oil (%)
Mono-	40.28	16.84
Di-	42.04	77.22
Tri-	13.65	3.52
Tetra-	4.03	2.42

Figure 8. Chromatographic Analysis of Synthesized Sucrose Ester

Figure 9. The Observed Emulsion Types with Microscopes

4. Conclusions

C. rugosa lipase can be used as catalyst in esterification. The optimum conditions of esterification using *C. rugosa* lipase obtained at the time of incubation for 18 hours for coconut oil and 12 hours for palm oil, temperature 30 $^{\circ}$C for coconut and palm oils, and the mole ratio fatty acids to sucrose 40:1 for coconut oil sample and 64:1 for palm oil sample. Ester bond formation evidenced by the absorption peaks in IR spectrum with the wave number 1739/cm. The esterification products obtained can be used as emulsifier and have esterification degree equal to 2.

Acknowledgment

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