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# HIGH-YIELD OPTIMISATION OF LIPASE-CATALYSED TRANSESTERIFICATION FOR SUCROSE ESTER PRODUCTION FROM PALM KERNEL OIL METHYL ESTER IN A SOLVENT-FREE SYSTEM

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## ABSTRACT

*The demand for sucrose esters (SEs) is increasing due to its important role as emulsifiers in the food, medicine and cosmetics industries. However, the conventional method of producing SEs commercially through chemical transesterification has several drawbacks, including a high-energy process, low product quality, waste production and the presence of toxic residues in the final product. To overcome these issues, a lipase-transesterification reaction was developed to produce SEs. This study aimed to optimise the lipase-transesterification reaction, catalysed by* Candida antarctica *Lipase B (CALB), using palm kernel oil methyl ester (PKOME) and sucrose to achieve the optimal SEs yield. The optimisation process was conducted following central composite design (CCD) and then analysed using response surface methodology (RSM). The independent variables used were sucrose concentration, CALB load and reaction temperature. The optimal SEs yield of 98.29% was obtained at a sucrose concentration of 400 mg mL$^{-1}$, a CALB load of 0.4 wt% and a reaction temperature of 40°C. The optimal SE characteristics were: acid value of 2.93 mg KOH g$^{-1}$, methanol content of 0.10%, hydrophilic-lipophilic balance (HLB) of 16.13 and soluble in oil, ethanol and $H_2O$. SEs have main functional groups of O–H, C=O, C–O and $CH_2$ (aliphatic chain).*

**Keywords:** emulsifier, lipase, palm kernel oil, sucrose esters, transesterification.

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## INTRODUCTION

Sucrose esters (SEs) are a class of non-ionic surfactants and belong to the larger group of sugar esters. They are synthesised through the esterification of sucrose with triglycerides and fatty acids or their derivatives. SEs occur naturally in certain plant species, including those in the families *Solanaceae* (e.g., potato, tomato, aubergine) and *Nicotiana* (e.g., tobacco) (Pyo et al., 2019). The commercial process involves the transesterification of sucrose with fatty acid methyl esters (FAMEs) or triglycerides, or direct esterification with fatty acids, employing alkaline catalysts at elevated temperatures (Reddy et al., 2015; Teng et al., 2021; Tongtummachat et al., 2023). An alternative approach that has been developed is enzymatic

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synthesis using the lipase enzyme. This method is considered more environmentally friendly (Kurniasih et al., 2023; Marathe et al., 2020; Shin et al., 2019). A significant advantage of SEs is that they are derived from renewable resources, such as sucrose and vegetable or animal-based triglycerides and fatty acids, which are abundant in nature (De Witte et al., 2024; Ren & Lamsal, 2017). Furthermore, SEs have good biocompatibility properties such as non-toxic, biodegradable (Pyo et al., 2019) and antibacterial properties (Zhao et al., 2015). These beneficial properties make SEs highly suitable for a wide range of applications, particularly in the food industry (Chen et al., 2023; Nagai et al., 2017; Zhang et al., 2019), medicines (El-Naggar et al., 2020; Vargas et al., 2020) and cosmetics (Pérez et al., 2017; Vassilev et al., 2021).

The SEs are surface-active compounds with excellent emulsifying and stabilising properties (Teng et al., 2021). Their structure consists of sucrose as the hydrophilic group (head) and fatty acids as the lipophilic group (tail) (Choi & Nidetzky, 2022; Delforce & Tcholakova, 2024; Huang et al., 2010; Zheng et al., 2015). Different types of fatty acids produce SEs with different hydrophilic-lipophilic balance (HLB) ranges (Ye & Hayes, 2014). The application of SEs mostly depends on their HLB value (Chansanroj & Betz, 2010; Pyo et al., 2019). The common fatty acids used in SEs production are lauric, myristic, palmitic, stearic, oleic and behenic acids (Marathe et al., 2020; Shin et al., 2019; Szuts & Szabó-Révész, 2012). Manufacturing of commercial SEs is generally based on the chemical esterification or transesterification of fatty acids, triglycerides, or their esters with sucrose in the presence of inorganic catalysts at elevated temperatures (Vargas et al., 2020). Nevertheless, technological progress has highlighted serious issues with chemical transesterification, such as its non-selectivity, high energy process, low productivity and darker colour products, toxic waste and chemical residues in final products (Vassilev et al., 2021). Therefore, developing lipase-catalysed transesterification for SEs production is a highly promising approach.

Lipase-transesterification is characterised by a high level of selectivity in the reaction (Li et al., 2015), a low-energy process (Pérez et al., 2017; Teng et al., 2021) and the waste that is biologically degraded, brighter colour products and non-chemical residues in the final product (Sasayama et al., 2021). The lipase-catalysed transesterification process is performed at moderate temperatures of 40°C–80°C, depending on the optimal temperature of the specific lipase enzyme used (Fernandes et al., 2012; Sindhu et al., 2021). In these temperature ranges, lipases such as *Candida antarctica* lipase B (CALB) exhibit high catalytic activity and remarkable operational stability. This allows them to maintain high cumulative yield over extended reaction period (≥ 12 hr) without significant denaturation**.** In contrast, temperatures below 40°C can slow the reaction rate due to insufficient molecular motion and reduced enzyme activity. Conversely, temperatures above 80°C can cause enzyme denaturation, which reduces catalytic performance and decreases yield. Furthermore, the utilisation of immobilised lipase enzyme such as CALB, offers the potential for reuse, which can help mitigate, though not fully offset, the high initial cost of the enzyme on industrial scale (Hidayat et al., 2016; Sindhu et al., 2021).

Currently, lipase-transesterification reaction to produce SEs can occur in solvent or solvent-free systems. Transesterification between sucrose and fatty acids is a slow process. A solvent is required to improve the homogeneity of the reaction and thus increase the reaction speed (Abdulmalek et al., 2016; Choi & Nidetzky, 2022). A previous study reported that a 6-O-glucose dodecanoic compound was synthesised from glucose and dodecanoic fatty acid in an ionic solvent comprising 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM]BF4), and 1-butyl-3-methylimidazolium trifluoromethane sulfonate ([BMIM]TfO) at 3:1 (v/v), using CALB as biocatalyst, and reaction time for 11 hr with an optimal yield of 82% (Lee et al., 2008). Another study has been conducted to produce fructose ester through direct esterification. Direct esterification of fructose with lauric acid in organic media using commercial immobilised CALB was investigated. Significant difference of direct esterification was observed between 2-methyl-2-butanol (2M2B) and methyl ethyl ketone (MEK), as mono-diester at a molar ratio of 1.05:1 in 2M2B and 2.79:1 in MEK. Based on Fourier transform-infrared spectra (FT-IR), showed that the secondary structure of the enzyme binding mono-ester presented distinct differences in 2M2B and MEK. Contents of β-turn and antiparallel β-sheet of CALB in 2M2B were 26.9% and 16.2%, respectively, but 19.1% and 13.2% in MEK (Li et al., 2015). Although these two studies were conducted in different solvent systems, the two solvents used did not increase the enzymatic reaction rate and achieving optimal SE yields required long reaction times.

On the other hand, transesterification between sucrose and FAMEs can be effectively performed in a solvent-free system (Pyo et al., 2019; Tongtummachat et al., 2023). Supporting this, (Gutiérrez et al., 2018) reported a 68.0% yield of SEs from sucrose and FAMEs using a $K_2CO_3$ as a catalyst at 100°C–140°C for just 5 hr in a solvent-free system. Similarly, another study has demonstrated the synthesis of xylitol esters with capric and caproic acid under solvent-free conditions. The utilisation of fatty acids as a substrate leads to $H_2O$ as a by-

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product. To overcome this, an amount of 0.30 g molecular sieve was added to the substrate mixture to adsorb $H_2O$. The study results showed that CALB catalysed the substrate with a yield of 74.1% xylitol caproate at a substrate ratio of 1:3 (molar), using 0.20 g of CALB and a reaction time of 29 hr. Meanwhile, 60.5% of xylitol caproate was obtained at a substrate ratio of 1:1 (molar), using 0.05 g of CALB and a reaction time of 18 hr (Adnani et al., 2011). Based on previous studies, the utilisation of methyl ester inhibits the formation of $H_2O$ during the synthesis of SEs (Kurniasih et al., 2023; Pyo et al., 2019). This significant advantage allows for the elimination of a high-temperature evaporation step (105°C–110°C), which is conventionally required to remove $H_2O$. Avoiding this step is crucial, as it prevents a Maillard reaction (browning reaction) that occurs when SEs are exposed to high temperatures, leading to undesirable darkening in colour and a burnt aroma (Vargas et al., 2020). Thermal degradation could damage the functional properties and sensory attributes of SEs, ultimately reducing their capabilities as emulsifiers (Gutiérrez et al., 2018). To produce SEs with optimal characteristics, it is necessary to utilise methyl esters with a high ester content as substrate. The higher the ester content, the greater the opportunity for ester bonds to form between sucrose and methyl esters (Pyo et al., 2019; Tongtummachat et al., 2023). Enzymatic synthesis of SEs is well-documented, achieving high yields often requires industrially challenging conditions, such as extended reaction times (≥ 48 hr) or high biocatalyst loadings. This study addresses these limitations by developing a highly efficient and optimised process. The novelty of this study lies in achieving high SE yields within a significantly reduced reaction time (≤ 12 hr) using a low loading of the biocatalyst CALB in a solvent-free system with palm kernel oil methyl ester (PKOME). This combination of high efficiency, mild conditions and a simplified process represents a significant advancement toward more economical enzymatic production (Guo et al., 2015; Hvidsten & Marchett, 2020).

In this study, PKOME was synthesised from crude palm kernel oil (CPKO) using esterification and transesterification methods. The resulting PKOME was characterised by a bright yellow colour and a high ester content of 96.59%, making it a suitable substrate for high-yield synthesis. The aim of this study was to determine the optimal transesterification conditions catalysed by CALB in order to increase the yield of SEs by investigating the influence of three independent variables that is sucrose concentration, CALB load and reaction temperature. Optimisation was conducted using a central composite design (CCD), and the interaction and influence of these variables on the response were analysed using response surface methodology (RSM). Finally, the characteristics of the optimal SEs synthesised from PKOME were analysed and compared against commercial SEs and Food and Agriculture Organization-Joint FAO/WHO Expert Committee on Food Additives (FAO-JECFA).

## MATERIALS AND METHODS

### Characteristics of Palm Kernel Oil Methyl Ester

The PKOME used in the SEs optimisation research was obtained from a previous study, which was synthesised from CPKO through a two-step reaction (esterification and transesterification reaction) (Kurniasih et al., 2025). Based on the analysis, PKOME has an ester content of 96.59%, an acid value of 0.20 mg KOH $g^{-1}$, a water content of 0.03% and a density of 0.86 g $mL^{-1}$. Other chemicals used for this study are of analytical grade, such as sucrose, KOH, methanol, ethanol, NaOH, HCl, $H_2SO_4$, n-hexane and phenolphthalein, which were purchased from Sigma-Aldrich (USA), and CALB was obtained from Novozyme Inc (Denmark). Commercial SEs were purchased from e-commerce sites in China.

### Lipase-Transesterification of Sucrose Esters

Substrates consisting of ± 87.5 mg of sucrose (0.25 mole) and 250 mL of PKOME (1 mole) were put into a 500 mL round-bottom glass reactor and homogenised at 400 rpm for 24 hr. All experiments were conducted with sucrose as the limiting reactant and a significant stoichiometric excess of PKOME. This approach drives the reversible reaction towards product formation and eliminates the need for a solvent to dissolve the sucrose. Subsequently, the substrates were heated to the specified temperature and CALB was slowly added to the substrates (following the CCD matrix design). The round-bottom glass reactor was equipped with a condenser for substrate to condense the substrate during the reaction. The heat source comes from a hot plate magnetic stirrer connected to an electric current. Based on the preliminary study results, the reaction time for SEs synthesis through lipase transesterification lasted 12 hr (Kurniasih et al., 2023), with a constant stirring speed of 250 rpm. After the reaction was completed, the crude SEs were separated from the reaction mixture by the vacuum filtration method. After that, SEs were gently washed with methanol to dissolve any PKOME that might remain in the crude product. The SEs were then rinsed with slowly dripped distilled water to remove any residual methanol. After rinsing, SEs were dried for 4 hr at 35°C–40°C and stored in a cool room at 20°C–25°C (Kurniasih et al., 2023).

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**Screening of Reaction Parameters**

This optimisation study involved three reaction parameters, which were selected through a preliminary study. The preliminary study examined four reaction parameters that affect the increase in SEs yield: (1) reaction time; (2) sucrose concentration; (3) CALB load; and (4) reaction temperature. It showed that the increase in SEs yield was influenced by sucrose concentration, CALB load and reaction temperature.

The following are the results of preliminary study: (1) at substrate concentration (400 mg $mL^{-1}$), the optimum SEs yield was 89.618%, so the substrate concentration at 400 mg $mL^{-1}$ was used as the center point in the CCD matrix design; (2) CALB load of 0.40 wt% produced the optimum SEs yield of 90.451%. This means that the CALB load of 0.40 wt% can be used as the center point in the CCD matrix design; (3) the reaction temperature of 40°C produced an optimal SEs yield of 93.953%. This result confirmed that the reaction temperature of 40°C can also be used as the centre point for the CCD matrix design; and 4) at reaction time 12 hr, the optimal SEs yield was 88.106%.

Among the four reaction parameters that were examined, reaction time exhibited a lower yield of SEs in comparison to the remaining three reaction parameters. The reaction time at the level of 12 hr was used for all the optimisation experiments.

**Determination of Sucrose Esters Yield**

The purified SEs were analysed using a UV-spectrophotometer to measure the initial and final sucrose concentration. The final SEs concentration was measured using the equation Y = 0.0011x – 0.0001 ($R^2$ = 0.9944) obtained from a standard curve of sucrose, which was made at a concentration of 50–1,000 mg $mL^{-1}$. SEs were dissolved in ethanol and stirred at 500 rpm until homogeneous. The SEs yield was determined from the difference between the initial SEs concentration and the final SEs concentration, following Equation (1).

$$\text{SEs yield (\%)} = \frac{S_o - S_1}{S_o} \times 100 \qquad (1)$$

where, $S_o$ is the initial SEs concentration (mg $mL^{-1}$) and $S_1$ is the final SEs concentration (mg $mL^{-1}$).

**Characteristics of Sucrose Esters**

The SEs were analysed using a Shimadzu FT-IR Prestige IR21 spectrophotometer (Shimadzu Corp., Japan) to identify the functional groups. Spectra were collected in the infrared region range of 400–4,000 $cm^{-1}$. The operation temperature range was between 0ºC–70ºC. A small amount of SEs ± 5-10 mg, which was obtained from lipase-transesterification, was bound with KBr and placed in a thin plate, then analysed. All spectra were measured against a background of air before analysing each sample (Rakmi et al., 1997). In this functional group analysis, SEs (commercial) and PKOME were also analysed. The functional groups of the SEs (synthesised) were compared with those of the SEs (commercial) and PKOME. Furthermore, physical, chemical and functional characterisation, including acid value (American Oil Chemist Society [AOCS], 1998), methanol content (FAO-JECFA, 2007), free sucrose (Indonesian Oil Palm Research Institute [IOPRI], 2004), HLB (Tadros, 2009), and solubility were conducted. The analytical results for SEs (enzymatic) were compared with those of SEs (commercial) and the specifications outlined in the FAO Compendium for Sucrose Esters of Fatty Acids (FAO-JECFA, 2007). The following is the procedure for analysing the characteristics of SEs, including acid value, saponification value, HLB, methanol content and solubility.

**Acid Value**

The acid value was determined according to the AOCS Official Method Ca 5a-40, 1998 (AOCS, 1998). About ± 2 g SEs was dissolved in neutralised ethanol and titrated with a standardised KOH solution using phenolphthalein as an indicator. The acid value was calculated using Equation (2).

$$\text{Acid value (mg KOH } g^{-1}) = \frac{V_s \times N \times 56.1}{W} \qquad (2)$$

where, $V_s$ is the volume of KOH, N is the normality of KOH (N); and W is the weight of the sample (g).

**Saponification Value**

The saponification value was determined according to the AOCS Official Method Cd 3-25, 1998 (AOCS, 1998). About ± 4 g SEs was melted in an erlenmeyer flask at 50°C. Then, added with 50 mL of 0.5 N KOH. A condenser was installed, and the mixture was heated for 60 min with continuous stirring to ensure a homogeneous reaction. The sample was then titrated with standardised HCl 0.1 N. A blank titration was performed, and the saponification value was calculated using Equation (3).

$$\text{Saponification value (mg KOH } g^{-1}) = \frac{(B - S) \times N \times 56.1}{W} \qquad (3)$$

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where, B is the volume of HCl for blank titration (mL); S is the volume of HCl for the sample titration (mL); N is the normality of HCl (N); and W is the weight of the sample (g).

### Hydrophilic Lipophilic Balance

The HLB is a value used in a scale to measure the balance of hydrophilic and lipophilic groups of a surfactant molecule. HLB value can be used to classify an emulsifier and calculate by using Equation (4). Acid numbers and saponification numbers have been obtained in previous analyses.

$$\text{HLB} = 20 \times \left(1 - \frac{\text{SV}}{\text{AV}}\right) \quad (4)$$

where, SV is the saponification value (mg KOH $g^{-1}$) and AV is the acid value (mg KOH $g^{-1}$).

### Methanol Content

***Preparation of a standard solution.*** Prepare a standard solution containing 4,000 mg $L^{-1}$ of methanol by accurately weighing ± 0.2 g of methanol into a 50 mL volumetric flask containing 20 mL of $H_2O$. Then add water to the volumetric flask until the volumetric flask volume reaches 50 mL (Standard Solution A). Using the same solution dilution method as Standard Solution A, prepare solutions containing 2,000 mg $L^{-1}$ (Standard Solution B) and 1,000 mg $L^{-1}$ (Standard Solution C).

***Analysed.*** Weigh accurately ± 1 g of SEs, and put it into each of the four sample bottles. To each sample bottle, add 5 µL of water. Next, add Standard Solutions A, B and C to each sample bottle, and seal it quickly. The concentrations of each sample were 20, 10 and 5 mg $kg^{-1}$, respectively. Place the sample vials in a headspace sampler and analyse using GC Shimadzu 2100 (Shimadzu Corp., Japan).

***Solubility.*** An amount of ± 25 g of SEs, and put it in a glass beaker. Add slowly ± 50 mL of $H_2O$ and stir it for 10 min. Observe whether the sample dissolves or not. Repeat the experiment using ethanol and oil as solvents.

### Optimisation Experiments

The optimisation study data were arranged following a CCD using three independent variables, namely: sucrose concentration; CALB load; and reaction temperature. The independent variables were determined based on the previous studies (Kurniasih et al., 2023). Each independent variable consists of five levels, which are indicated as actual and coded levels –1.682; –1.000; 0.000; +1.000; +1.682. For a coded level equal to zero (0.000), it refers to the centre point of the experimental data, which refers to the middle level of the data. Meanwhile coded level is equal to –1.682, –1.000, and coded level equal to +1.682, +1.000 as the lowest and highest level, respectively.

The CCD matrix was arranged with 4–8 replicates for each level of the independent variables, resulting in 20 experiments, and then the responses were analysed using RSM. This aims to obtain the lipase-transesterification reaction that experimentally produces the optimal SEs yield. RSM analysis would recommend lipase-transesterification reaction conditions that produce optimal yield using the mathematical Equation (5) (Dian et al., 2018). In Equation (5), Y refers to the yield of SEs, $\alpha_o$ is the constant, $\alpha_1$, $\alpha_2$, $\alpha_3$ are the linear coefficients, $\alpha_{11}$, $\alpha_{22}$ and $\alpha_{33}$ are the quadratic coefficients, $\alpha_{12}$, $\alpha_{13}$ and $\alpha_{23}$ are the variable interaction coefficients. The experimental data design according to the CCD can be seen in *Table 1*.

$$Y = \alpha_o + \alpha_1.X_1 + \alpha_2.X_2 + \alpha_3.X_3 + \alpha_{11}.X_1 + \alpha_{22}.X_2 + \alpha_{33}.X + \alpha_{12}.X_1.X_2 + \alpha_{13}.X_1.X_3 + \alpha_{23}.X_2.X_3 \quad (5)$$

The effect of the three independent variables on SEs yield was examined using analysis of variance (ANOVA) (Sukkathanyawat & Wichianwat, 2023). The primary benefit of RSM involves the

**TABLE 1. EXPERIMENTAL DATA RANGE IN CENTRAL COMPOSITE DESIGN (CCD)**

| Independent variables | | | |
|---|---|---|---|
| Coded levels | Actual levels | | |
| | Sucrose concentration ($X_1$) (mg $mL^{-1}$) | CALB load ($X_2$) (wt%) | Reaction temperatures ($X_3$) ($^o$C) |
| -1.682 | 316 | 0.23 | 31.6 |
| -1.000 | 350 | 0.30 | 35.0 |
| 0.000 | 400 | 0.40 | 40.0 |
| +1.000 | 450 | 0.50 | 45.0 |
| +1.682 | 484 | 0.57 | 48.4 |

Note: CALB - *Candida antarctica* lipase B.

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consolidation of data necessary for assessment, analysis and optimisation into a more compact set, thereby reducing the requirement for experimental treatments. RSM responds to the form of surface curves and contour areas for each independent variable to ensure efficiency and testing with equal precision. RSM also examines the interaction of the three independent variables and can provide predictions of reaction conditions that can be pursued to produce products with optimal yields through the use of mathematical equations. There are two forms of RSM data design: Box-Behnken design (BBD) and CCD. In this study, the CCD form is utilised due to its capacity to provide more precise response estimates and a wider experimental scope compared to the BBD (Carley et al., 2004; Krishnaiah et al., 2014; Shishir & Chen, 2017).

## RESULTS AND DISCUSSION

### Optimisation of Lipase-Transesterification

The lipase-transesterification between PKOME and sucrose was conducted with sucrose as the limiting substrate. Sucrose was maintained at a constant mass (± 87.5 mg) for each experiment, while the volume of PKOME was increased following the sucrose concentration design in the CCD matrix. The lipase-transesterification is a reversible reaction. Therefore, the use of an excess PKOME drives the reaction towards the product. It increases the possibility of ester bond formation. In this study, lipase-transesterification was conducted in a solvent-free system. The presence of organic or inorganic solvents is toxic to the enzyme. It can interfere with the biocatalytic activity of CALB and reduce the yield of SEs (Inprakhon et al., 2017).

The utilisation of PKOME as a substrate provides several advantages, including the elimination of $H_2O$ by-product formation, easier purification and no solvent residue. Although methanol is a by-product, which can have an inhibitory or deactivating effect on some lipases (Mangiagalli et al., 2022). The purification process involving washing and drying was designed to effectively remove it, resulting in a final product with low methanol content. Moreover, the solvent-free system is safer for SEs intended for use as food emulsifiers. The optimisation study was carried out on three independent variables: Sucrose concentration, CALB load and reaction temperature. Sucrose concentration is a significant factor in the formation of ester bonds, while the CALB load employed affects the biocatalytic activity, influencing the breakdown of the substrate into new compounds. The reaction temperature exerts an influence on the reaction speed, as it is related to the biological temperature of CALB. Lipase enzyme works optimally at an ambient temperature of 30°C–80°C (Kurniasih & Fachraniah, 2019; Manley & Mayer, 2012). Other variables that influence lipase-catalysed transesterification such as reaction time, $H_2O$ content, pH and stirring speed, were kept constant. The preliminary study reported that a reaction time of 10–12 hr has the potential to produce an optimal yield of SEs (Kurniasih et al., 2023). $H_2O$ content is an important variable for CALB catalytic activity. An excessive $H_2O$ content may lead to enzyme inactivation. PKOME has an $H_2O$ content of 0.03%, which is suitable for the biological $H_2O$ of CALB. Lipase-transesterification using PKOME as substrate inhibits $H_2O$ formation as a by-product. In this study, the $H_2O$ content did not increase during the lipase-transesterification reaction.

Meanwhile, CALB is a lipase immobilised in resin, so its characteristics against changes in pH and reaction temperature are more stable than crude enzyme (Brockman, 2013; Kumar et al., 2019). The stirring speed was maintained at a low range speed of 250 rpm to ensure sufficient contact between the CALB and the substrates. Although high stirring speeds can facilitate homogeneity and substrate collisions, the utilisation of stirring speeds above 250 rpm would damage the membrane of the immobilised enzyme. If the lipase membrane was damaged, it becomes more sensitive to environmental reaction changes, as indicated by a decrease in SEs yield. The enzymatic mechanism to synthesise SEs is shown in *Figure 1*.

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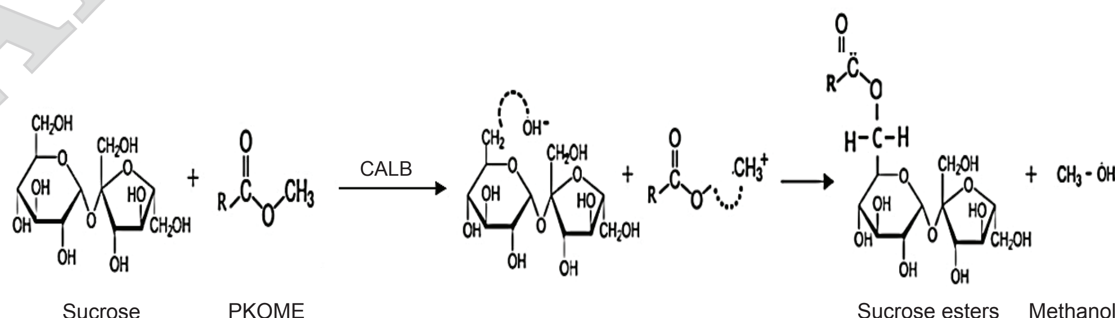


*Figure 1. Mechanism of lipase-transesterification between palm kernel oil methyl ester (PKOME) and sucrose to produce sucrose esters using* Candida antarctica *lipase B (CALB) as a biocatalyst.*

Based on the study results of the optimisation experiments, it is known that the interaction between the three independent variables produces SEs with optimal yield. The optimal SEs yield of 98.06%–98.29% was achieved in experiments 15–20. The optimal SEs results were obtained at the code level equal to 0.000 (center point) for the three independent variables, namely sucrose concentration of 400 mg mL$^{-1}$, CALB load of 0.4 wt%, and reaction temperature of 40°C. Furthermore, RSM analysis was conducted to determine the interaction of independent variables on responses and to provide reaction conditions that produce SEs yields ≥ 98.29%. The SEs yield (response results) with different treatments of sucrose concentration ($X_1$), CALB load ($X_2$) and reaction temperature ($X_3$) are shown in *Table 2*.

**Statistical Analysis Result**

The effect of the independent variables was observed based on the results of experimental data analysis. The statistical analysis results using ANOVA for the significance of the influence of the three independent variables, namely sucrose concentration ($X_1$), CALB load ($X_2$), reaction temperature ($X_3$), as well as their interactions and influence on SEs yield shown in *Table 3*.

The statistical analysis shows that increasing sucrose concentration yields a positive regression coefficient of 3.52, indicating that a 50 mg mL$^{-1}$ increase in sucrose concentration can raise SEs yield by 3.52% ($p$ = 0.078). Likewise, increasing the CALB load results in a positive effect of 3.51 on SEs yield, although this effect is not statistically significant ($p$ = 0.079). In contrast, increasing the reaction temperature produces a negative regression coefficient of -2.74. The $p$-value for each independent variable showed significance above 0.05, which means that the three independent variables have not shown a dominant influence on increasing SEs yield. Based on the $p$-value, the lipase-transesterification was influenced by $X_1$, $X_2$ and $X_3$, respectively. The same condition shown for the interaction between the independent variables is clear. The interaction between $X_1$ and $X_2$ has a negative effect on the SEs results, although this effect is not significant ($p$ = 0.885). The interactions between $X_1$ and $X_3$ ($p$ = 0.163) and $X_2$ and $X_3$ ($p$ = 0.300) had positive, but non-significant effects. This indicates that the interaction of the three independent variables did not have a dominant effect on lipase transesterification for SEs synthesis. The results of the ANOVA analysis for the determination of the regression model of experimental data are shown in *Table 4*.

**TABLE 2. EXPERIMENTAL CONDITION OF CENTRAL COMPOSITE DESIGN (CCD) AND CORRESPONDING RESPONSE (EXPERIMENTAL RESULTS)**

| Experiments | Sucrose concentration ($X_1$) (mg mL$^{-1}$) | | CALB load ($X_2$) (wt%) | | Temperature ($X_3$) (°C) | | SEs yield (%) |
|---|---|---|---|---|---|---|---|
| | Coded | Actual | Coded | Actual | Coded | Actual | |
| 1 | -1.000 | 350 | -1.000 | 0.30 | -1.000 | 35.0 | 89.09 |
| 2 | +1.000 | 450 | -1.000 | 0.30 | -1.000 | 35.0 | 81.26 |
| 3 | -1.000 | 350 | +1.000 | 0.50 | -1.000 | 35.0 | 87.66 |
| 4 | +1.000 | 450 | +1.000 | 0.50 | -1.000 | 35.0 | 84.13 |
| 5 | -1.000 | 350 | -1.000 | 0.30 | +1.000 | 45.0 | 57.02 |
| 6 | +1.000 | 450 | -1.000 | 0.30 | +1.000 | 45.0 | 69.01 |
| 7 | -1.000 | 350 | +1.000 | 0.50 | +1.000 | 45.0 | 84.96 |
| 8 | +1.000 | 450 | +1.000 | 0.50 | +1.000 | 45.0 | 89.87 |
| 9 | - 1.682 | 316 | 0.000 | 0.40 | 0.000 | 40.0 | 57.03 |
| 10 | +1.682 | 484 | 0.000 | 0.40 | 0.000 | 40.0 | 82.33 |
| 11 | 0.000 | 400 | -1.682 | 0.23 | 0.000 | 40.0 | 88.65 |
| 12 | 0.000 | 400 | +1.682 | 0.57 | 0.000 | 40.0 | 87.27 |
| 13 | 0.000 | 400 | 0.000 | 0.40 | -1.682 | 31.6 | 89.99 |
| 14 | 0.000 | 400 | 0.000 | 0.40 | +1.682 | 48.4 | 92.29 |
| 15 | 0.000 | 400 | 0.000 | 0.40 | 0.000 | 40.0 | 98.19 |
| 16 | 0.000 | 400 | 0.000 | 0.40 | 0.000 | 40.0 | 98.15 |
| 17 | 0.000 | 400 | 0.000 | 0.40 | 0.000 | 40.0 | 98.29 |
| 18 | 0.000 | 400 | 0.000 | 0.40 | 0.000 | 40.0 | 98.06 |
| 19 | 0.000 | 400 | 0.000 | 0.40 | 0.000 | 40.0 | 98.25 |
| 20 | 0.000 | 400 | 0.000 | 0.40 | 0.000 | 40.0 | 98.25 |

Note: SEs - sucrose esters; CALB - *Candida antarctica* lipase B.

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**TABLE 3. STATISTICAL ANALYSIS RESULT USING ANALYSIS OF VARIANCE (ANOVA)**

| Sources | Statistical analysis result | |
|---|---|---|
| | **Coefficient** | ***p*-value** |
| Constant | 98.25 | 0.000 |
| Sucrose concentration ($X_1$) | 3.52 | 0.078 |
| *Candida antarctica* Lipase B (CALB) load ($X_2$) | 3.51 | 0.079 |
| Reaction temperature ($X_3$) | -2.74 | 0.158 |
| $X_1X_1$ | -10.43 | 0.000 |
| $X_2X_2$ | -3.96 | 0.047 |
| $X_3X_3$ | -2.84 | 0.136 |
| $X_1X_2$ | -0.35 | 0.885 |
| $X_1X_3$ | 3.53 | 0.163 |
| $X_2X_3$ | 5.92 | 0.300 |

**TABLE 4. ANOVA MODEL REGRESSION EQUATION FOR OPTIMISATION OF LIPASE-TRANSESTERIFICATION OF SUCROSE ESTERS (SEs) PRODUCTION USING CALB AS BIOCATALYST**

| Source | DF | Sum square (SS) | Mean square (MS) | *f*-value | *p*-value |
|---|---|---|---|---|---|
| Model | 9 | 2,547.40 | 283.04 | 6.42 | 0.004 |
| Linear | 3 | 439.96 | 146.65 | 3.33 | 0.065 |
| $X_1$ | 1 | 169.40 | 169.40 | 3.84 | 0.078 |
| $X_2$ | 1 | 168.10 | 168.10 | 3.81 | 0.079 |
| $X_3$ | 1 | 102.47 | 102.47 | 2.33 | 0.158 |
| Quadratic | 3 | 1,726.23 | 575.41 | 13.06 | 0.001 |
| $X_1X_2$ | 1 | 1,566.77 | 1,566.77 | 35.56 | 0.000 |
| $X_2X_3$ | 1 | 226.39 | 226.39 | 5.14 | 0.047 |
| $X_1X_3$ | 1 | 115.97 | 115.97 | 2.63 | 0.136 |
| 2-Way interaction | 3 | 381.21 | 127.07 | 2.88 | 0.089 |
| $X_1X_2$ | 1 | 0.96 | 0.96 | 0.02 | 0.885 |
| $X_1X_3$ | 1 | 99.80 | 99.80 | 2.26 | 0.163 |
| $X_2X_3$ | 1 | 280.44 | 280.44 | 6.36 | 0.030 |
| Lack of Fit | 5 | 440.61 | 88.12 | 12,053.37 | 0.000 |
| Error | 10 | 440.65 | 44.07 | | |
| Pure Error | 5 | 0.04 | 0.01 | | |
| Total | 19 | 2,988.05 | | | |
| **$R^2$ = 85.25%, $R^2$ (adj) = 71.98%** | | | | | |

Based on ANOVA results, the quadratic equation gave a suitable model for the optimisation of lipase-transesterification ($p = 0.001$). Meanwhile, the linear regression and 2-way interaction resulted in an unsuitable equation model ($p \geq 0.05$). The following is the quadratic equation model for the optimisation of SEs production [Equation (6)].

$$\text{SEs yield (\%)} = 98.25 + 3.52\ X_1 + 3.51\ X_2 - 2.74\ X_3 - 10.43\ X_1X_1 - 3.96\ X_2X_2 - 2.84\ X_3X_3 - 0.35\ X_1X_2 + 3.53\ X_1X_3 + 5.92\ X_2X_3 \quad (6)$$

The accuracy of the equation model was measured by the $R^2$ determinant. Based on the analysis results, $R^2$ = 85.25% and R (adj) = 71.98% were obtained for the regression equation model. This indicates that the quadratic equation recommended by RSM using three independent variables can provide an accurate prediction of reaction conditions (Bouzaouit & Bidjou-Haiour, 2016).

**Effect of Sucrose Concentration ($X_1$) and CALB Load ($X_2$) on Sucrose Esters Yield**

The interaction of the three independent variables was analysed through response surfaces and contour areas, which were displayed in a three-dimensional graph. The effect of sucrose concentration and CALB load was plotted using $X_1$ as the X-axis, $X_2$ as the Y-axis, SEs yield as the Z-axis and $X_3$ as a fixed variable (coded level = 0, actual level = 40°C). The response surface illustrates that an increase in sucrose concentration and CALB load has a considerable effect on the enzymatic reaction. It has been demonstrated that the SEs yield increases in conjunction with an increase in sucrose concentration and CALB load, up to a

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specific threshold. Increasing $X_1 \geq 400$ mg $mL^{-1}$, and $X_2 = 0.4$ wt% decreased SEs yield by 40%–60%. This condition was observed using a response surface, which displayed a dome-like shape curve, indicating that the levels of $X_1$ and $X_2$ are located at the center point (coded level = 0). The curve surfaces for $X_1$ and $X_2$ exhibited comparable sharpness, indicating that $X_1$ and $X_2$ exerted comparable effects on the reaction. An increase in sucrose concentration and CALB load showed a positive influence on the increase in SEs yield. This finding was confirmed by ANOVA analysis, $X_1$ ($p = 0.078$) and $X_2$ ($p = 0.079$). From ANOVA, $X_1$ and $X_2$ showed a positive but non-significant effect as single variables, and the interaction between these two variables showed a negative effect. This condition indicates that increasing $X_1$ and $X_2$ can cause a decrease in SEs yield. The sucrose concentration and CALB load were at an accurate level to produce optimal SEs. The interaction between $X_1$ and $X_2$ with $X_3$ held at 40°C, is presented as a response curve in *Figure* 2.

The contour area (*Figure* 3) describes that SEs yields of $\geq 90\%$ are achieved when the $X_1$ is at the coded level of -0.5 to 1.0 (actual level = 375–450 mg $mL^{-1}$), and $X_2$ is at the coded level of -1.0 to 1.0 (actual level = 0.30–0.50 wt%). Further observation revealed that the utilisation of sucrose concentrations at coded level $\geq 1.0$ (actual value $\geq 450$ mg $mL^{-1}$), at both low and high CALB load levels, resulted in SEs yields $\leq 90\%$. This is because CALB only works selectively on the exact substrate and concentration level, as shown in *Figure* 3.

This condition was evidenced by the ANOVA results, which indicated a negative and non-significant interaction between $X_1$ and $X_2$ ($p = 0.885$). This shows that increasing the sucrose concentration and CALB load at a specific threshold resulted in a reduction in SEs yield. This condition is affected by two conditions. First, when the sucrose concentration is at coded level $\geq 0.5$ (actual level $\geq 425$ mg $mL^{-1}$) and CALB load is at coded level $\leq -0.5$ (actual level $\leq 0.35$ wt%). This condition is referred to as substrate inhibition. It occurs when the enzymatic activity

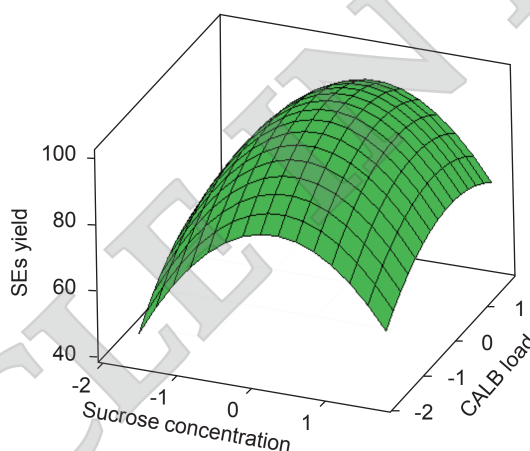


*Figure 2. Response surface of interaction between sucrose concentration ($X_1$) and* Candida antarctica *Lipase B (CALB) load ($X_2$), with reaction temperature held at 40°C (coded level = 0).*

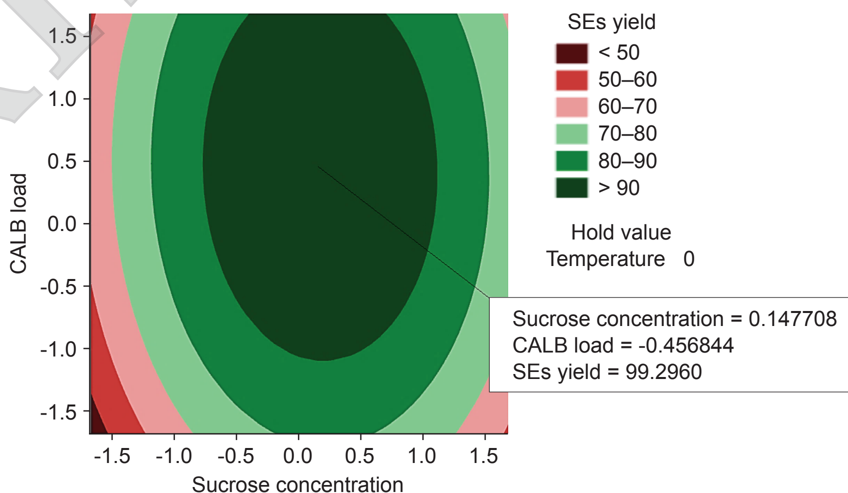


*Figure 3. Contour area of interaction between sucrose concentration ($X_1$) and* Candida antarctica *Lipase B (CALB) load ($X_2$), with reaction temperature ($X_3$) at actual level = 40°C (coded level = 0).*

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decreases because the substrate concentration has exceeded the quantity of lipase enzyme available. This is one of the most frequent phenomena in enzyme-catalysed reactions (Zhang et al., 2022). The opportunity for substrate inhibition is high, as the lipase enzyme has an active site, where the enzyme-substrate bond is formed and then converted into a new compound. Secondly, when $X_1$ is at coded level ≥ 0.5 (actual level ≥ 425 mg $mL^{-1}$), and $X_2$ is at coded level ≥ 1.5 (actual level ≥ 0.55 wt%). This condition is referred to as enzyme inhibition. It occurs when the availability of the lipase enzyme exceeds the substrate that is broken down into new compounds (Leow & Chan, 2019). Increasing $X_2$ can increase reaction speed, but it must be followed by an increase in $X_1$ to the exact level. This is needed to create a reaction equilibrium, to form enzyme-substrate bonds and to break them down into new compounds. The reactions catalysed by organic compounds, such as lipase enzymes, bacteria and fungi are more selective than reactions catalysed by inorganic compounds, because they only work with the proper type of substrate and concentration to produce an optimal product (Mardani et al., 2015).

**Effect of Sucrose Concentration ($X_1$) and Reaction Temperature ($X_3$) on Sucrose Esters Yield**

The interaction between $X_1$ and $X_3$ was observed on $X_2$ as a fixed variable (coded level = 0, actual level = 0.4 wt%). The response surface curve illustrates that SEs yield is linear with increasing $X_1$ and $X_3$, which indicates that the enzymatic reaction is affected by $X_1$ and $X_3$. However, $X_1$ affects the lipase-transesterification more than $X_3$, which is illustrated through the higher curvature of the response curve. At $X_2$ of 0.4 wt% (code level = 0), the optimal SEs yield is obtained if $X_1$ is at code level = 0 (actual level = 400 mg $mL^{-1}$), while $X_3$ is at code level = –2 to ≥ 1 (actual level = 30°C to ≥ 45°C). This was confirmed by ANOVA, which showed the effect of $X_1$ was greater ($p$ = 0.078) than $X_3$ ($p$ = 0.158). An increase in $X_3$ showed a negative and opposite effect to an increase in $X_1$. Thus, the interaction between $X_1$ and $X_3$ resulted in a non-significant effect ($p$ = 0.163) on the increase in SEs yield. Increasing temperatures can destabilise the substrate (sucrose) or alter the solubility of reaction components. This can lead to phase separation or reduced substrate availability at the enzyme active site. In reactions involving transesterification, high temperatures may cause thermal decomposition. This process can create new compounds, such as aldehydes and ketones. These compounds can divert the pathway from the desired SEs as the main product.

Based on the response surface analysis (*Figure 4*), the curves suggest that elevating the $X_1$ and $X_3$ at a wide level can increase the optimal SEs yield. This condition provides an opportunity to utilise $X_2$ at the centre point (actual level = 0.4 wt%), but still offers optimal SEs yield by controlling other variables, which is shown in *Figure 4*. The use of $X_2$ at code level = 0 provides a more efficient lipase-transesterification.

The contour areas predicted the reaction condition for lipase-transesterification that produce optimal SEs yields. It is estimated that the SEs yield reached ≥ 90% when the $X_1$ is at the coded level of ≤ -1 to 1 (actual coded = 350 to 450 mg $mL^{-1}$), while $X_3$ is at the coded level of –1.5 to ≤ 1.5 (actual coded = 32.5°C to ≤ 47.5°C). At these reaction temperatures, the CALB still performed as active biocatalyst. The interaction between $X_1$ and $X_3$ is presented in *Figure 5*.

The increase of $X_1$ affects the reaction even though $X_2$ is at code level = 0. In contrast, the utilisation of $X_1$ at coded levels ≥ 1 (± 450 mg $mL^{-1}$) at $X_3$ ≤ 40°C showed a decrease in SEs yield. Likewise, the use of $X_1$ at coded level ≤ –1.5 (actual level ≤ 325 mg $mL^{-1}$) at different levels of $X_3$ produces SEs yield ≤ 72%. The observed decrease in SEs yield

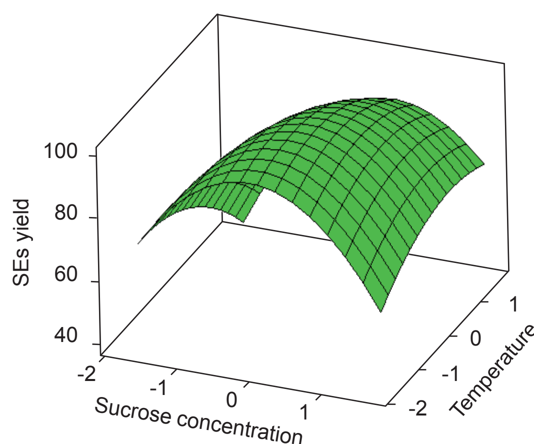


*Figure 4. Response surface of interaction between sucrose concentration ($X_1$) and reaction temperature ($X_3$), with* Candida antarctica *lipase B (CALB) load ($X_2$) at actual level = 0.4 wt% (coded level =0).*

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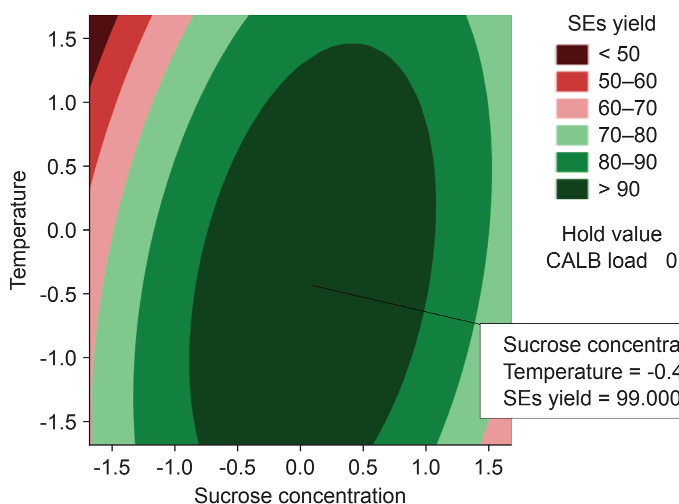


*Figure 5.* *Contour area of interaction between sucrose concentration ($X_1$) and reaction temperature ($X_3$), with* Candida antarctica *lipase B (CALB) load ($X_2$) at actual level =0.4 wt% (coded level = 0).*

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when increasing $X_1$ and $X_3$ at a fixed, optimal $X_2$ can be attributed to kinetic limitations rather than stoichiometric. At high substrate concentrations, the enzyme active sites become saturated, leading to a plateau in the reaction rate (substrate saturation). Furthermore, the accumulation of methanol, a by-product of the transesterification, could act as a non-competitive inhibitor. Methanol binds to the enzyme at a site other than the active site, inducing a conformational change that reduces catalytic efficiency. This inhibitory effect may be further enhanced at higher temperatures, which may also affect the stability of the enzyme during the 12 hr reaction period (Zhang et al., 2023). Non-competitive inhibition occurs when an inhibitor binds to the active site of the lipase enzyme to form enzyme-substrate-inhibitor bonding (Arsalan & Younus, 2018). The active site of the enzyme is deformed due to the presence of the inhibitor, which results in the substrate being unable to bind to the enzyme-substrate complex properly. In this condition, there is a decrease in reaction speed, or no reaction occurs. The inhibitor comes from the metabolic residue or $CH_3OH$. It is known that $CH_3OH$ has a hydrophobic group (O-H), so it can bind to the lipase enzyme and interfere with the activity of the CALB. Despite the reaction inhibition, there is a chance of producing optimal SEs using CALB load at coded level = 0. This lipase-transesterification condition provides an opportunity to optimise the other independent variables at a fixed level of CALB load.

### Effect of CALB Load ($X_2$) and Reaction Temperature ($X_3$) on Sucrose Esters Yield

The CALB activity depends entirely on the reaction temperature used during the synthesis. The reaction temperature used cannot be too high to optimise CALB activity, but should not be too low for the substrate to cause collisions between substrate particles. The ability of CALB to withstand temperature changes throughout the reaction is also influenced by the type of substrate used. For different substrate sources, CALB may exhibit optimal biocatalytic activity at different temperatures (Kumar et al., 2019; Neta et al., 2011). Further observations were at $X_1$ of 400 mg $mL^{-1}$ held as a fixed variable, with $X_2$ and $X_3$ as independent variables, presented in the response surface curve in *Figure 6*.

Based on the response surface analysis (*Figure* 6), it is known that the interaction between $X_2$ and $X_3$ affects the SEs yield. This was confirmed using ANOVA, which showed a significant effect for the interaction of the two independent variables ($p$ = 0.05). Based on the sharpness of the response surface curve, $X_2$ has a greater influence compared to $X_3$. This condition is reinforced by a regression coefficient of $X_2$ and $X_3$ ($p$ = 0.079 and 0.158), respectively. The single effect of $X_1$ and $X_3$ had an insignificant effect on increasing SEs yield, but their interaction had a significant effect. Thereby, the utilisation of $X_2$ at coded level = 0 (actual level = 0.4 wt%) showed an increase in SEs yield. The utilisation of $X_2$ at code level ≥ 0 (actual level ≥ 0.4 wt%), shows a decrease in SEs yield. In contrast, the $X_3$ showed linearity with an increase in SEs yield. Increasing the $X_3$ at code level = 0–0.5 (actual code = 40.0°C–42.4°C) produced a SEs yield of 80%–96%. Like most lipase enzymes, CALB has an optimal temperature range for catalytic efficiency. Meanwhile, moderate increases in temperature generally accelerate reaction rates by increasing molecular collisions and reducing viscosity. Temperatures that exceed the enzyme stability threshold can lead to partial denaturation or reduced catalytic performance. This results in a decline in enzyme activity and consequently, a lower SEs yield.

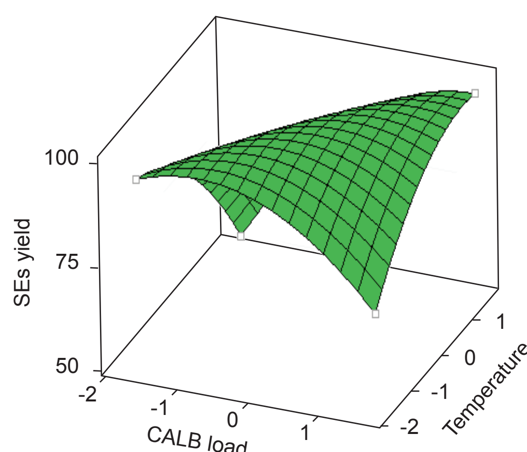


*Figure 6. Response surface of interaction between* Candida antarctica *lipase B (CALB) load ($X_2$) and reaction temperature ($X_3$), with sucrose concentration ($X_1$) at actual level = 400 mg mL$^{-1}$ (coded level = 0).*

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The contour area (*Figure 7*) reinforces the response surface analysis, which indicates that the SEs result reaches ≤ 90% when $X_2$ is at a coded level = -1.5 to 0.5 (actual level = 0.25–0.45 wt%), and $X_3$ is at a coded level = -1.5 to 1.5 (actual level = 32.5°C–47.5°C). This suggests that an increase in $X_2$ must be accompanied by an increase in temperature to induce lipase enzyme activity and facilitate substrate breakdown. The use of CALB as a biocatalyst can lower the activation energy so that enzymatic transesterification can take place at a lower temperature than chemical transesterification. However, increasing $X_2$ requires an increase in $X_3$ to trigger biocatalytic activity (Maghraby et al., 2023; Ortiz et al., 2019).

The effect of $X_3$ on lipase transesterification is apparent when interacting with other independent variables. This is based on the regression coefficient value, which shows that the interaction between $X_2$ and $X_3$ has a positive regression coefficient of 5.92, although the effect is not significant ($p$ = 0.30). This shows that increasing $X_2$ and $X_3$ can increase SEs yield by 5.92% for each treatment, although the effect is not significant. The most influential interaction of independent variables was given by the interaction between sucrose concentration and reaction temperature ($X_1X_3$), followed by the interaction of CALB load and reaction temperature ($X_2X_3$), and the interaction of sucrose concentration with CALB load ($X_1X_2$). The interaction between increasing $X_2X_3$ on SEs yield is presented in *Figure 7*.

The contour area (*Figure 7*) gave the comprehensive condition for the reaction temperature, at coded level = -1.5 to 0.5 (actual level = 32.5°C–42.5°C). This allows the utilisation of moderate temperatures to produce optimal SEs yields. The use of reaction temperatures ≥ 40°C is considered an ineffective treatment to increase the yield of SEs, considering that reaction speed is highly dependent on CALB function (Wardoyo & Hidayah, 2019). Although CALB is known for its robustness at higher temperatures (60°C–80°C), the observed negative effect of temperature in our system, particularly over the 12 hr reaction period, is likely due to a gradual loss of operational stability. The use of moderate temperature combined with stirring speed at 250 rpm may lead to changes in the structure of the immobilised enzyme or the release of substances that may reduce its catalytic performance. This is consistent with the findings of (Gumel et al., 2011; Yu et al., 2008), who also reported optimal yields at lower temperatures for long-duration reaction time (48–72 hr) for the synthesis of sugar esters using CALB as biocatalyst. The optimal product yield of 38%–63% was obtained at 35°C–45°C. The utilisation of reaction temperatures above 45°C caused a decrease in sugar ester yield.

**Recommendation of Lipase-Transesterification Reaction Conditions**

Based on ANOVA analysis, a quadratic regression equation was obtained, with response values influenced by the three independent variables. This quadratic regression equation was examined through a normality test model based on lack of fit (LOF), using the hypothesis; $H_o$: There is no LOF (the model fits the experimental data); and $H_1$: There is a LOF (the model that has been made does not represent the experimental data. Rejection area: The initial hypothesis ($H_o$) will be rejected if the $p$-value exceeds the $\alpha$-value (0.05), otherwise, the initial hypothesis will fail to be rejected if the $p \leq 0.05$.

Based on the ANOVA result in *Table 4*, it shows that LOF ≤ 0.05 ($p$ = 0.00), which indicates that the quadratic regression model is fit and represents the entire experiment data. When the examination results of the model equation show fit results, the lipase-

transesterification reaction conditions that produce optimal SEs yields can be predicted accurately (Kleijnen, 2014). Based on the RSM analysis in response surfaces and contour areas, it provides three recommendations for lipase-transesterification reaction conditions that would produce SEs yield ≥ 98.29%. Each recommended reaction condition involves three independent variables, with one of the independent variables held at coded level = 0 (centre point), shown in *Table 5*.

All the recommended reaction conditions of lipase-transesterification were predicted to produce SEs with a yield ≥ 98.29% (experimental). The first recommendation of lipase-transesterification reaction condition, that is the utilisation of a sucrose concentration ($X_1$) of 407.39 mg mL$^{-1}$, a CALB load ($X_2$) of 0.42 wt%, and a reaction temperature ($X_3$) of 40°C would result in a SEs yield of 99.29%.

## Characterisation of Optimal Sucrose Ester

Product characteristics are distinctive properties that are only possessed by a product and are not possessed by other products, even though they are processed through the same method. Therefore, analysis of the characteristics of a product can be used to identify the presence of an element or compound after going through several treatments. In this study, the functional groups of SEs (enzymatic) were compared with those of SEs (commercial) and the substrates consisting of sucrose and methyl ester were compared.

***Functional group.*** The identification of functional groups formed was determined through an FT-IR spectrophotometry analysis. The absorption of infrared radiation by a compound results in an increase in the vibration of its atoms. Molecular vibrations manifest in the form of stretching or bending. The quantity of infrared energy absorbed by a compound is contingent upon the bonding changes that occur within said compound. It has been established that distinct bonds exhibit varying degrees of susceptibility to infrared energy, with the capacity to absorb energy at disparate wavelengths. The infrared absorption region exhibits specific functional groups within the 4,000–400 cm$^{-1}$. In this study, the analysis was conducted on optimal SEs (enzymatic), commercial SEs, sucrose and methyl ester as a comparison, as shown in *Figure 8*.

Based on the functional group analysis, it is known that the wide absorption at wavelength

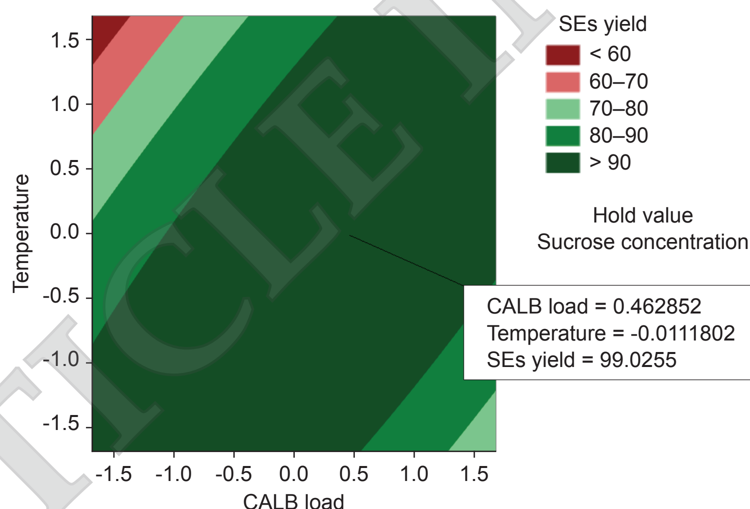


*Figure 7.* *Contour area of interaction between* Candida antarctica *lipase B (CALB) load ($X_2$) and reaction temperature ($X_3$), with sucrose concentration ($X_1$) at actual level = 400 mg mL$^{-1}$ (coded level = 0).*

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**TABLE 5. RECOMMENDATION CONDITION OF LIPASE-TRANSESTERIFICATION REACTION BASED ON RESPONSE SURFACE METHODOLOGY ANALYSIS USING THREE INDEPENDENT VARIABLES**

| Recommendation | Independent variables | | | | | | SEs yield (%) |
|---|---|---|---|---|---|---|---|
| | Sucrose concentration (mg mL$^{-1}$) | | CALB load (wt%) | | Reaction temperature (°C) | | |
| | Coded | Actual | Coded | Actual | Coded | Actual | |
| Condition 1 | 0.147708 | 407.39 | 0.456844 | 0.42 | 0 | 40 | 99.29 |
| Condition 2 | 0.0982056 | 404.91 | 0 | 0.38 | -0.435050 | 37.82 | 99.00 |
| Condition 3 | 0 | 400 | 0.462852 | 0.42 | -0.0111802 | 39.94 | 99.03 |

Note: CALB - *Candida antarctica* lipase B; SEs - sucrose esters.

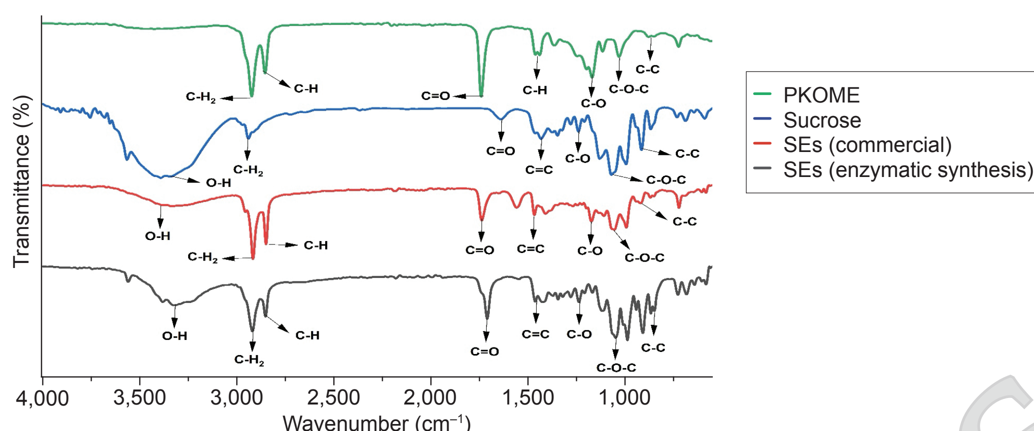


*Figure 8. Comparison of the infrared spectra between sucrose esters (enzymatic), sucrose ester [commercial, palm kernel oil methyl ester (PKOME)] and sucrose.*

3,391.19 cm$^{-1}$ shows the absorption of O-H (hydroxyl) found in sucrose. Sucrose is a disaccharide consisting of glucose and fructose units and has eight O-H groups. The band at wavelength 2,955.24–2,724.01 cm$^{-1}$ shows the absorption of C-$H_2$ bonds and C-H. Sucrose has an aromatic ring characterised by the presence of a C=C group at 1,638.15 cm$^{-1}$, which shows a characteristic cyclic structure. The C-O group at 1,171.82 cm$^{-1}$ indicates the presence of carbonyl groups in the form of ketones that cannot be oxidised. The fingerprint region of sucrose is characterised by the appearance of C-O-C and C-C groups at 1,088.12 and 865.93 cm$^{-1}$, respectively. PKOME shows band spectra for the carbon chain group of fatty acids at 1,197.29 cm$^{-1}$. The largest fatty acid content in PKOME is medium-chain fatty acids, namely lauric and myristic acid. Therefore, the absorption for hydrocarbon bonds in C-$H_2$ and C-H groups has high absorption at wavelengths 1,460.79 and 2,922.70–2,853.76 cm$^{-1}$. The absorption band at 1,197.29 cm$^{-1}$ shows the C-O carbonyl group of fatty acids, and the C=O absorption band at 1,739.38 cm$^{-1}$ shows the ester bond of PKOME. The fingerprint region of PKOME is characterised by the appearance of C-O-C and C-C groups at 1,029.49 and 876.86 cm$^{-1}$, as a unique absorbance. One of the main functional groups of SEs is the O-H, which exhibits a short curve and is a typical characteristic of sucrose. The presence of a sharp curve region is indicative of a low water content in SEs. This finding is consistent with the hypothesis that the use of PKOME as a substrate inhibits the presence of $H_2O$ formation (by-product) as evidenced by the low $H_2O$ content of SEs. The hydroxyl group is always in the first absorption region, which is detected at 3,600–2,500 cm$^{-1}$. The hydroxyl group (O-H) of SEs was detected at 3,323.47 cm$^{-1}$. The subsequent functional groups are C=O and C-O, which indicate the presence of carboxylic and ester bonds. The wavelength of the C=O group exhibited a pronounced intensity at 1,708.65 cm$^{-1}$. The C-O wavelength was detected with great intensity at 1,278.30 cm$^{-1}$. The C-O group exhibits an extensive absorption range, which is indicative of the elevated degree of esterification exhibited by SEs. The presence of carbonyl groups also indicates that SEs are synthesised from triglycerides or fatty acid derivatives. The aliphatic group of C-$H_2$ indicates the presence of carbon atoms bonded together in the form of a branched straight chain, linked to sucrose. SEs have an aromatic ring group detected as C=C at 1,545.93 cm$^{-1}$ and a C-O group at 1,278.30 cm$^{-1}$, which is derived from ketone compounds. Both groups indicate that SEs are sucrose-derived compounds. The fingerprint region is characterised by the presence of C-O-C and C-C groups at 1,047.82 and 847.56 cm$^{-1}$, which show different absorbance from the substrate and are characteristic of SEs. The comparison of wave numbers between products and substrates is presented in *Table 6*.

The findings of the present study align with those of previous research that employed enzymatic synthesis to produce SEs (Enayati et al., 2018; Fernandes et al., 2021). The results of the functional group analysis in *Table 6*, show that the functional groups of SEs (enzymatic) have a main functional group agreement with SEs (commercial). The different functional group intensities between SEs (enzymatic) and SEs (commercial) indicate the different carbon chain lengths of the fatty acids or triglycerides used. But overall, the functional group analysis showed that CALB was able to convert the substrate into SEs.

***Physical and chemical properties.*** To support the functional group analysis, the identification of the physical, chemical, and functional properties of SEs was carried out. The physical, chemical and functional properties analysed were compared to SEs commercial. The analytical results for SEs (enzymatic) and SEs (commercial) based on the FAO-JECFA Compendium of Fatty Acid Sucrose Esters are presented in *Table 7*.

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TABLE 6. FUNCTIONAL GROUP OF SUCROSE ESTER AND SUBSTRATES

| Functional group | Wavenumber ($cm^{-1}$) | | | |
|---|---|---|---|---|
| | SEs (Enzymatic) | SEs (Commercial) | Sucrose | PKOME |
| O–H | 3,323.47 | 3,329.16 | 3,391.19 | - |
| C–H | 2,919.26–2,851.93 | 2,915.97 | 2,955.24–2,724.01 | 2,922.70–2,853.76 |
| C–$H_2$ | 1,461.59 | 1,431.31 | 1,431.31 | 1,460.79 |
| C=O | 1,708.65 | 1,736.89 | - | 1,739.38 |
| C=C (aromatic) | 1,545.93 | 1,557.57 | 1,638.15 | - |
| C–O | 1,278.30 | 1,267.00 | 1,171.82 | 1,197.29 |
| C–O–C | 1,047.82 | 1,053.00 | 1,088.12 | 1,029.49 |
| C–C | 847.56 | 867.92 | 865.93 | 876.86 |

Note: SEs - sucrose esters; PKOME - palm kernel oil methyl ester.

TABLE 7. PHYSICAL AND CHEMICAL PROPERTIES OF SUCROSE ESTERS

| Properties | Analysis result | | |
|---|---|---|---|
| | SEs (enzymatic) | SEs (commercial) | FAO-JECFA |
| Yield (%) | 98.25 | ≥ 98–99 | ≥ 80 |
| Acid value (mg KOH $g^{-1}$) | 2.93 | 3.181 | Max 6 |
| Methanol content (%) | 0.10 | 0.29 | 1.0 |
| HLB | 16.13 | 15.21 | - |
| Solubility | Oil, ethanol, $H_2O$ | Oil, ethanol, $H_2O$ | - |

Note: HLB - hydrophilic lipophilic balance; SEs - sucrose esters; $H_2O$ - dihydrogen oxide.

Based on the analysis, SEs synthesised from PKOME by enzymatic reaction have the same characteristics as commercial SEs synthesised by chemical reaction and have met FAO standards. SEs from PKOME have a lower acid value than commercial SEs synthesised from fatty acids. This proves that the use of methyl ester as a substrate can produce products with low acid value. Analysis of the functional properties showed that SEs from PKOME had an HLB value of 16.13, meanwhile commercial SEs had an HLB of 15.21. SEs with HLB values of 15–16 are classified as oil-in-water (O/W) emulsifiers, which are widely used in the foods, cosmetics, and household industries (Ye & Hayes, 2014).

## CONCLUSION

Optimisation of SEs enzyme has been successfully carried out using PKOME and sucrose as substrates in a solvent-free system, and CALB as the biocatalyst. The main advantage of using PKOME over free fatty acids is the inhibition of $H_2O$ formation as a by-product, which is a known factor that can complicate purification and trigger browning reactions (Maillard reaction). Optimisation of lipase-catalysed transesterification conditions using response surface methodology resulted in a high SEs yield of 98.29% under mild conditions, with a reaction time of 12 hr, CALB load of 0.4 wt%, and temperature of 40°C in a solvent-free system using PKOME and sucrose as the main substrates. The optimal SEs product showed characteristics in accordance with commercial SEs and met the FAO-JECFA standard. SEs (enzymatic) had a lower acid value of 2.93 mg KOH $g^{-1}$ (max 6 mg KOH $g^{-1}$), lower methanol content of 0.10% (max 1%), and a high HLB value of 16.13 (HLB range 1–20), classifying them as effective oil-in-water (O/W) emulsifiers. Functional group analysis confirmed the successful formation of substrates into products with the appearance of structures of O-H, C=O, C-O and $CH_2$ (aliphatic chain) structures as the main functional groups of SEs. This study has demonstrated significant progress in SEs production through a sustainable, economical, and efficient lipase-transesterification synthesis method.

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**REFERENCES**

Abdulmalek, E., Hamidon, N. F., & Rahman, M. B. A. (2016). Optimization and characterization of lipase catalyzed synthesis of xylose caproate ester in organic solvents. *Journal of Molecular Catalysis B: Enzymatic*, *132*, 1–4. https://doi.org/10.1016/j.molcatb.2016.06.010

Adnani, A., Basri, M., Chaibakhsh, N., Ahangar, H. A., Salleh, A. B., Rahman, R. N. Z. R. A., & Rahman, M. B. A. (2011). Chemometric analysis of lipase-catalyzed synthesis of xylitol esters in a solvent-free system. *Carbohydrate Research*, *346*(4), 472–479. https://doi.org/10.1016/j.carres.2010.12.023

American Oil Chemists' Society (AOCS). (1998). *Official Methods and Recommended Practices of the American Oil Chemist's Society* (4th ed.). AOCS Press.

Arsalan, A., & Younus, H. (2018). Enzyme and nanoparticles: Modulation of enzymatic activity via nanoparticles. *International Journal of Biological Macromolecules*, *118*, 1833–1847. https://doi.org/10.1016/j.ijbiomac.2018.07.030

Bouzaouit, N., & Bidjou-Haiour, C. (2016). Response surface methodological study of glucose laurate synthesis catalyzed by immobilized lipase from *Candida cylindracea*. *Biological Forum – An International Journal*, *8*(1), 420–427.

Brockman, H. L. (2013). Lipases. In *Encyclopedia of Biological Chemistry* (pp 729–732). https://doi.org/10.1016/B978-0-12-378630-2.00118-3

Carley, K. M., Kamneva, N. Y., & Reminga, J. (2004). *Response surface methodology* (Technical Report No. CMU-ISRI-04-136). Carnegie Mellon University.

Chansanroj, K., & Betz, G. (2010). Sucrose esters with various hydrophilic-lipophilic properties: Novel controlled release agents for oral drug delivery matrix tablets prepared by direct compaction. *Acta Biomaterialia*, *6*(8), 3101–3109. https://doi.org/10.1016/j.actbio.2010.01.044

Chen, J. Q., Hai, Y. W., Qing, C., Liang, M. Y., Banwell, M. G., & Lan, P. (2023). The synthesis of certain fatty acid ester derivatives of trehalose and an investigation of their emulsifying properties and bioactivities. *LWT, 187*, 115369. https://doi.org/10.1016/j.lwt.2023.115369

Choi, J., & Nidetzky, B. (2022). Ionic liquid as dual-function catalyst and solvent for efficient synthesis of sucrose fatty acid esters. *Molecular Catalysis*, *526*, 112371. https://doi.org/10.1016/j.mcat.2022.112371

De Witte, F., Penagos, I. A., Moens, K., Skirtach, A. G., Van Bockstaele, F., & Dewettinck, K. (2024). Multiscale assessment of the effect of a stearic-palmitic sucrose ester on the crystallization of anhydrous milk fat. *Food Research International*, *197*, 115243. https://doi.org/10.1016/j.foodres.2024.115243

Delforce, L., & Tcholakova, S. (2024). Alkyl sucrose esters vs. Brijs: How chain length and temperature impact surface and foam properties. *Journal of Molecular Liquids*, *416* (Part B), 126491. https://doi.org/10.1016/j.molliq.2024.126491

Dian, N. L. H. M., Sahri, M. M., Ping, T. C., Ying, L. Y., & Ming, L. O. (2018). Optimization of enzymatic directed interesterification of palm oil for high yield of triunsaturated triacylglycerol using response surface methodology. *Journal of Oil Palm Research*, *30*(4), 602–616. https://doi.org/10.21894/jopr.2018.0050

El-Naggar, M. E., Soliman, R. A., Morsy, O. M., & Abdel-Aziz, M. S. (2020). Nanoemulsion of *Capsicum* fruit extract as an eco-friendly antimicrobial agent for the production of medical bandages. *Biocatalysis and Agricultural Biotechnology*, *23*, 101516. https://doi.org/10.1016/j.bcab.2020.101516

Enayati, M., Gong, Y., Goddard, J. M., & Abbaspourrad, A. (2018). Synthesis and characterization of lactose fatty acid ester biosurfactants using free and immobilized lipases in organic solvents. *Food Chemistry*, *266*, 508–513. https://doi.org/10.1016/j.foodchem.2018.06.051

Fernandes, R. N., Simiqueli, A. A., Vidigal, M. C. T. R., Minim, V. P. R., & Minim, L. A. (2021). Kinetic stability of the oil-in-water emulsions and dynamic interfacial properties of mixtures of sucrose esters and polysaccharides. *Food Chemistry*, *357*, 129693. https://doi.org/10.1016/j.foodchem.2021.129693

Fernandes, S. A., Natalino, R., Silva, M. J. D., & Lima, C. F. (2012). A comparative investigation of palmitic acid esterification over

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*p*-sulfonic acid calix[4]arene and sulfuric acid catalysts via $^{1}$H NMR spectroscopy. *Catalysis Communications*, *26*, 127–131. https://doi.org/10.1016/j.catcom.2012.05.007

Food and Agriculture Organization-Joint FAO/WHO Expert Committee on Food Additives (FAO-JECFA). (2007). *Compendium of food additive specifications (FAO-JECFA monographs 4) sucrose esters of fatty acids*.

Gumel, A. M., Annuar, M. S. M., Heidelberg, T., & Chisti, Y. (2011). Lipase-mediated synthesis of sugar fatty acid esters. *Process Biochemistry*, *46*(11), 2079–2090. https://doi.org/10.1016/j.procbio.2011.07.021

Guo, D. H., Jin, Z., Xu, Y. S., Wang, P., Lin, Y., Han, S. Y., & Zheng, S. P. (2015). Scaling-up the synthesis of myristate glucose ester catalyzed by a CALB-displaying *Pichia pastoris* whole-cell biocatalyst. *Enzyme and Microbial Technology*, *(75–76)*, 30–36. https://doi.org/10.1016/j.enzmictec.2015.04.002

Gutiérrez, M. F., Orjuela, Á., Rivera, J. L., & Suaza, A. (2018). Production of sucrose esters using solvent-free reactive systems containing emulsifiers. *Ingenieria e Investigacion*, *38*(1), 16–23. https://doi.org/10.15446/ing.investig.v38n1.61432

Hidayat, C., Fitria, K., Supriyanto, & Hastuti, P. (2016). Enzymatic synthesis of bio-surfactant fructose oleic ester using immobilized lipase on modified hydrophobic matrix in fluidized bed reactor. *Agriculture and Agricultural Science Procedia*, *9*, 353–362. https://doi.org/10.1016/j.aaspro.2016.02.150

Huang, D., Jiang, X., Zhu, H., Fu, X., Zhong, K., & Gao, W. (2010). Improved synthesis of sucrose fatty acid monoesters under ultrasonic irradiation. *Ultrasonics Sonochemistry*, *17*(2), 352–355. https://doi.org/10.1016/j.ultsonch.2009.08.009

Hvidsten, I. B., & Marchett, J. M. (2020). Novozyme® 435 as bio-catalyst in the synthesis of methyl laurate. *Energy Conversion and Management: X*, *10*, 100061. https://doi.org/10.1016/j.ecmx.2020.100061

Inprakhon, P., Wongthongdee, N., Amornsakchai, T., Pongtharankul, T., Sunintaboon, P., Wiemann, L. O., Durand, A., & Sieber, V. (2017). Lipase-catalyzed synthesis of sucrose monoester: Increased productivity by combining enzyme pretreatment and non-aqueous biphasic medium. *Journal of Biotechnology*, *259*, 182–190. https://doi.org/10.1016/j.jbiotec.2017.07.021

Indonesian Oil Palm Research Institute (IOPRI). (2004). *Oil chemical physics testing work instruction-viscosity*.

Kleijnen, J. P. C. (2014). *Response surface methodology*. (CentER Discussion Paper; No. 2014–013). Tilburg University.

Krishnaiah, D., Nithyanandam, R., & Sarbatly, R. (2014). A critical review on the spray drying of fruit extract: Effect of additives on physicochemical properties. *Critical Reviews in Food Science and Nutrition*, *54*(4), 449–473. https://doi.org/10.1080/10408398.2011.587038

Kumar, R., Kumar, V., Mathur, D., Kumar, R., Kumar, A., & Prasad, A. K. (2019). Biocatalyst CALB catalyzed synthesis of modified nucleosides: An overview. *Synthetic Communications*, *49*(13), 1659–1678. https://doi.org/10.1080/00397911.2018.1554745

Kurniasih, E., & Fachraniah, F. (2019). Performance of *Rhizomucor miehei* in amidation reaction for fatty monoethanolamide synthesis based on distilled palm fatty acid. *IOP Conference Series: Materials Science and Engineering*, *536*, 012084. https://doi.org/10.1088/1757-899X/536/1/012084

Kurniasih, E., Rahmi, R., Darusman, D., & Supardan, M. D. (2023). Synthesis of sucrose ester through enzymatic esterification and stability analysis as a food emulsifier. *E3S Web of Conferences*, *373*, 04014. https://doi.org/10.1051/e3sconf/202337304014

Kurniasih, E., Rahmi. R., Supardan, M. D., & Darusman, D. (2025). Two-step reaction for biodiesel synthesized from a high-free fatty acid crude palm kernel oil. *Applied Science and Engineering Progress*, *18*(2), 7570. https://doi.org/10.14416/j.asep.2024.09.009

Lee, S. H., Ha, S. H., Hiep, N. M., Chang, W. J., & Koo, Y. M. (2008). Lipase-catalyzed synthesis of glucose fatty acid ester using ionic liquid mixtures. *Journal of Biotechnology*, *133*(4), 486–489. https://doi.org/10.1016/j.jbiotec.2007.11.001

Leow, J. W. H., & Chan, E. C. Y. (2019). A typical Michaelis-Menten kinetics in cytochrome P450 enzyme: A focus on substrate inhibition. *Biochemical Pharmacology*, *169*, 113615. https://doi.org/10.1016/j.bcp.2019.08.017

Li, L., Ji, F., Wang, J., Li, Y., & Bao, Y. (2015). Esterification degree of fructose laurate exerted

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by *Candida antarctica* lipase B in organic solvents. *Enzyme and Microbial Technology*, *69*, 46–53. https://doi.org/10.1016/j.enzmictec.2014.12.003

Maghraby, Y. R., El-Shabasy, R. M., Ibrahim, A. H., & Azzazy, H. M. E. S. (2023). Enzyme immobilization technologies and industrial applications. *ACS Omega*, *8*(6), 5184–5196. https://doi.org/10.1021/acsomega.2c07560

Mangiagalli, M., Diletta, A., Marcella de, D., Stefania, B., Tiziano, C., Antonino, N., & Marina, L. (2022). Short-chain alcohols inactivate an immobilized industrial lipase through two different mechanisms. *Biotechnology Journal*, *17*(6), 2100712. https://doi.org/10.1002/biot.202100712

Manley, C., & Mayer, J. (2012). Lipase. In *Clinical veterinary advisor: Birds and exotic pets* (pp. 624–625). https://doi.org/10.1016/B978-1-4160-3969-3.00364-4

Marathe, S. J., Shah, N. N., & Singhal, R. S. (2020). Enzymatic synthesis of fatty acid esters of trehalose: Process optimization, characterization of the esters and evaluation of their bioactivities. *Bioorganic Chemistry*, *94*, 103460. https://doi.org/10.1016/j.bioorg.2019.103460

Mardani, M., Farmani, J., & Kenari, R. E. (2015). Efficacy of some commercial lipases in hydrolysis of palm olein for the production of free fatty acids and diacylglycerol oil. *Journal of Oil Palm Research*, *27*(3), 250–260.

Nagai, Y., Kawano, S., Motoda, K., Tomida, M., Tatebe, C., Sato, K., & Akiyama, H. (2017). Solubility testing of sucrose esters of fatty acids in international food additive specifications. *Biological and Pharmaceutical Bulletin*, *40*(3), 284–289. https://doi.org/10.1248/bpb.b16-00738

Neta, N. S., Peres, A. M., Teixeira, J. A., & Rodrigues, L. R. (2011). Maximization of fructose esters synthesis by response surface methodology. *New Biotechnology*, *28*(4), 349–355. https://doi.org/10.1016/j.nbt.2011.02.007

Ortiz, C., Luján, F. M., Barbosa, O., Santos, J. C. S. D., Rodrigues, R. C., Berenguer-Murcia, Á., Briand, L. E., & Fernandez-Lafuente, R. (2019). Novozyme 435: The "perfect" lipase immobilized biocatalyst? *Catalysis Science & Technology*, *9*, 2380–2420. https://doi.org/10.1039/c9cy00415g

Pérez, B., Anankanbil, S., & Guo, Z. (2017). Synthesis of sugar fatty acid esters and their industrial utilizations. In *Fatty Acids* (pp. 329–354). AOCS Press. https://doi.org/10.1016/b978-0-12-809521-8.00010-6

Pyo, S. H., Chen, J., Ye, R., & Hayes, D. G. (2019). Sugar esters. In D. G. Hayes, D. K. Y. Solaiman, & R. D. Ashby (Eds.) *Biobased Surfactants* (2nd Edition pp. 325–363). AOCS Press. https://doi.org/10.1016/B978-0-12-812705-6.00010-1

Rakmi, A. R., Tjahjono, H., & Othman, O. (1997). Preparation of biodegradable and vegetable-based surfactant from sugar and palm fatty acid catalyzed by *Mucor miehei lipase*. *Journal Oil Palm Research*, *9*(2), 100–110.

Reddy, V. K.T., Rani, G. S., Prasad, R. B. N., & Devi, B. L. A. P. (2015). Green recyclable $SO_3H$-carbon catalyst for the selective synthesis of isomannide-based fatty acid monoesters as non-ionic bio-surfactants. *RSC Advances*, *5*(51), 40997–41005. https://doi.org/10.1039/c5ra03605d

Ren, K., & Lamsal, B. P. (2017). Synthesis of some glucose-fatty acid esters by lipase from *Candida antarctica* and their emulsion functions. *Food Chemistry*, *214*, 556–563. https://doi.org/10.1016/j.foodchem.2016.07.031

Sasayama, T., Kanezawa, A., Hiromori, K., Takahashi, A., & Shibasaki-Kitakawa, N. (2021). Controlling reaction selectivity for sugar fatty acid ester synthesis by using resins with different basicities. *Food Chemistry*, *340*, 128100. https://doi.org/10.1016/j.foodchem.2020.128100

Shin, D. W., Mai, N. L., Bae, S. W., & Koo, Y. M. (2019). Enhanced lipase-catalyzed synthesis of sugar fatty acid esters using supersaturated sugar solution in ionic liquids. *Enzyme and Microbial Technology*, *126*, 18–23. https://doi.org/10.1016/j.enzmictec.2019.03.004

Shishir, M. R. I., & Chen, W. (2017). Trends of spray drying: A critical review on drying of fruit and vegetable juices. *Trends in Food Science and Technology*, *65*, 49–67. https://doi.org/10.1016/j.tifs.2017.05.006

Sindhu, R., Shiburaj, S., Sabu, A., Fernandes, P., Singhal, R., Mathew, G. M., Nair, I. C., Jayachandran, K., Vidya, J., De Souza Vandenberghe, L. P., Deniz, I., Madhavan, A., Binod, P., Sukumaran, R. K., Kumar, S. S., Anusree, M., Nagavekar, N., Soumya, M., Jayakumar, A., . . . Pandey, A. (2021). Enzyme technology in food processing: Recent developments and

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future prospects. In *Innovative Food Processing Technologies* (pp. 191–215). Elsevier. https://doi.org/10.1016/b978-0-12-815781-7.00016-0

Sukkathanyawat, H., & Wichianwat, K. (2023). Optimization of FAME production from waste cooking palm oil with KOH catalyst supported on palm kernel shells ash (PKSA) using response surface methodology (RSM). *Journal of Oil Palm Research*, *35*(4), 668–681. https://doi.org/10.21894/jopr.2023.0005

Szuts, A., & Szabó-Révész, P. (2012). Sucrose esters as natural surfactants in drug delivery systems – A mini-review. *International Journal of Pharmaceutics*, *433*, 1–9. https://doi.org/10.1016/j.ijpharm.2012.04.076

Tadros, T. F. (Ed.) (2009). *Emulsion science and technology*. Wiley-VCH Velag GmbH & Co.KGaA.

Teng, Y., Stewart, S. G., Hai, Y. W., Li, X., Banwell, M. G., & Lan, P. (2021). Sucrose fatty acid esters: Synthesis, emulsifying capacities, biological activities, and structure-property profiles. *Critical Reviews in Food Science and Nutrition*, *61*(19), 3297–3317. https://doi.org/10.1080/10408398.2020.1798346

Tongtummachat, T., Jaree, A., Kaewchada, A., Sunorakum, B., Ignacio, P., & Akkarawatkhoosith, N. (2023). Sucrose monolaurate production from lauric acid through a two-stage process. *Scientific Reports*, *13*(1), 11218. https://doi.org/10.1038/s41598-023-38461-7

Vargas, J. A. M., Ortega, J. O., Santos, M. B. C. D., Metzker, G., Gomes, E., & Boscolo, M. (2020). A new synthetic methodology for pyridinic sucrose esters and their antibacterial effects against *Gram*-positive and *Gram*-negative strains. *Carbohydrate Research*, *489*, 107957. https://doi.org/10.1016/j.carres.2020.107957

Vassilev, D., Petkova, N., Koleva, M., & Denev, P. (2021). Optimization of ultrasound synthesis of sucrose esters by selection of a suitable catalyst and reaction conditions. *Journal of Chemical Technology and Metallurgy*, *56*(2), 268–274.

Wardoyo, F. A., & Hidayah, F. F. (2019). Thermal and reused stability of immobilized lipase in carrageenan. *IOP Conference Series: Earth and Environmental Science*, *292*(1), 012028. https://doi.org/10.1088/1755-1315/292/1/012028

Ye, R., & Hayes, D. G. (2014). Recent progress for lipase-catalysed synthesis of sugar fatty acid esters. *Journal of Oil Palm Research*, *26*(4), 355–365.

Yu, J., Zhang, J., Zhao, A., & Ma, X. (2008). Study of glucose ester synthesis by immobilized lipase from *Candida* sp. *Catalysis Communications*, *9*(6), 1369–1374. https://doi.org/10.1016/j.catcom.2007.11.036

Zhang, Q., Fan, W., Shi, Y., Tu, Z., Hu, Y., & Zhang, J. (2023). Interaction between soy protein isolate/whey protein isolate and sucrose ester during microencapsulation: Multi-spectroscopy and molecular docking. *LWT, 188*, 115363. https://doi.org/10.1016/j.lwt.2023.115363

Zhang, S., Xiong, J., Lou, W., Ning, Z., Zhang, D., & Yang, J. (2019). Antimicrobial activity and action mechanism of triglycerol monolaurate on common foodborne pathogens. *Food Control*, *98*, 113–119. https://doi.org/10.1016/j.foodcont.2018.11.017

Zhang, Y., Chen, M., Lu, J., Li, W., Wolynes, P. G., & Wang, W. (2022). Frustration and the kinetic repartitioning mechanism of substrate inhibition in enzyme catalysis. *Journal of Physical Chemistry B*, *126*(36), 6792–6801. https://doi.org/10.1021/acs.jpcb.2c03832

Zhao, L., Zhang, H., Hao, T., & Li, S. (2015). *In vitro* antibacterial activities and mechanism of sugar fatty acid esters against five food-related bacteria. *Food Chemistry*, *187*, 370–377. https://doi.org/10.1016/j.foodchem.2015.04.108

Zheng, Y., Zheng, M., Ma, Z., Xin, B., Guo, R., & Xu, X. (2015). Sugar fatty acid esters.In M. U. Ahmad & X. Xu (Eds.) *Polar Lipids: Biology, Chemistry, and Technology* (pp. 215–243). https://doi.org/10.1016/B978-1-63067-044-3.50012-1

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