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Synthesis of fructose laurate: optimization and thermodynamic study

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Abstract

In this present study, enzymatic esterification synthesis of fructose laurate was performed in organic media using lipase from *Candida antarctica* immobilized on acrylic resin. Screening phase of esterification reaction was done using two commonly used commercial immobilized lipases, in three different solvents, molecular sieve loadings, and reaction time to observe their effect on the yield of fructose laurate. The highest yield was obtained by using ethanol as the solvent, 0.02 g lipase loading, 0.10 g molecular sieves loading, and 24 hr of reaction time. By using response surface methodology (RSM) with central composite design (CCD), it was found that the optimum operational conditions were 40°C, 0.13 g lipase loadings, 200 rpm agitation rate, and without molecular sieve needed for the system. The optimized esterification conditions could give the yield of fructose laurate about 93.21 ± 0.13% with 92.91% confidence level.

Introduction

Sugar fatty acid ester (SFAE) is principally known as non-toxic, biodegradable, and environmentally friendly surfactant that is widely used in the food, detergent, pharmaceutical, and cosmetic industries (Cao *et al*., 1996; Nakamura, 1997; Watanabe *et al*., 2001; Ferrer, 2005). This surfactant can be synthesized either by using chemical catalyst or biocatalyst. Enzyme is a biocatalyst which is highly specific for certain reactants (Brennan, 2013) and able to produce only one form of desired product (Plou *et al.*, 2002). Whereas, chemical catalyst usually run in high temperature esterification with poor selectivity, and later forms colored by-product (Polat and Linhardt, 2001; Yan *et al.,* 2001). Therefore, enzyme is more favorable compared to chemical catalyst. In this study, lipase was chosen as catalyst for the esterification reaction and used for the optimization in the synthesis of fructose laurate via immobilized lipase. Lipases (E.C. 3.1.1.3) catalyze the hydrolysis of esters bond or shifting the equilibrium reaction towards synthesis or known as esterification reaction in nonaqueous solvents with minimum amount of water (Gumel *et al.*, 2011). The immobilized lipase has its own advantages due to its stability, reusability, and easy to handle in term of separation process compared to free lipase.

In synthesis of fructose laurate ester, there are many factors i.e. reaction time, type of solvent, type of lipase, and lipase loading, that can affect the yield and the rate of esterification reaction. Therefore, it was difficult to get the optimum condition for each parameter and their interactions (Lundstedt *et al*., 1998). In this work, the experimental data were divided into two parts; screening and optimization. The screening part would determine the range input for different parameters in optimization study using Design Expert® software via response surface methodology with central composite design. Using the optimum conditions obtained from the optimization part, thermodynamic studies were carried out to investigate the thermal resistance of immobilized lipase towards temperature variations in producing fructose laurate.

Materials and methods

Materials

D(-)-fructose was purchased from HmbG® Chemicals, Germany. Commercial immobilized lipase from Candida antarctica immobilized on acrylic resin, Lipozyme® from Mucor meihei and phenolphthalein indicator 1% in ethanol was from Sigma Aldrich. Molecular sieve type 3Å was purchased from Prolabo, Belgium. Ethanol (99.7%), 2-methyl-2-butanol, acetone, lauric acid and other reagents were purchased from RM Marketing, UK

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and used directly without further treatment.

Enzymatic esterification of sugar ester

Fructose fatty acid ester was produced by esterification reactions. The experiments were conducted in 25 mL conical flasks by adding lauric acid (0.10 g), fructose (0.09 g), immobilized lipase (0.02 g), with molecular sieves (0.10 g) in ethanol (10 mL) and agitates in incubator shaker at 37°C, 200rpm for 24hr unless otherwise stated. In addition, control experiments were performed using the same procedures described in this section, without the incorporation of the lipase and molecular sieves. This method has previously discussed by Šabeder *et al.* (2006) with some modifications. All the experiment was done in triplicate.

Quantification of sugar ester

The sugar ester content was quantified by calculating the residual fatty acids amount in the reaction mixture, which was determined by the volumetric method by Leitgeb and Knez (1990). Aliquots were withdrawn at periodic intervals and diluted with 0.1% phenolphthalein solution in absolute ethanol, and titrated with 0.02M sodium hydroxide.

Optimization

After the factors screening, a central composite design of four parameters (reaction temperature, lipase loading, molecular sieve loading, agitation rate) was employed to obtain the optimal conditions for enzymatic esterification. Series of experimental runs was suggested and the responses were analyzed by using Design Expert® software. The experiments were running in random sequence and duplicate readings were taken for each run. The obtained experimental data were fitted into second-order polynomial equation (6) to generate the response surface, which was used to assess the optimum esterification conditions. Validation runs were carried out to confirm the predicted optimal condition.

Thermodynamic study

The optimal conditions obtained from the optimization part will be further analyzed for its thermodynamic properties represented by the changes in enthalpy (ΔH°), Gibbs free energy (ΔG°), entropy (ΔS°), and thermal deactivation constants (kd). To obtain these thermodynamic constants, the rate constant on variation of temperature were studied and this temperature-dependent rate was determined using Arrhenius law as given below in equation (1):

$$k = A_0.e^{-E_a/RT} \quad (1)$$

$$\ln k = \ln A_0 + (-E_a/RT) \quad (2)$$

Where, k = rate constant; A_0 = pre-exponential factor; Ea = activation energy (J/mol); R = gas constant and T = temperature (Kelvin). Equation (2) shows the linear form of Arrhenius equation. For the esterification reaction to occur, the change in enthalpy (ΔH°) is determined to quantify amount of heat adsorbed by the system. On the other hand, the change in Gibbs free energy (ΔG°) defined the available energy to make the reaction proceed from the substrates (reactants) and products to chemical equilibrium. The change for the entropy (ΔS°) is obtained from change of enthalpy over the temperature (ΔH°/T) and positive value of ΔH°/T means the increase of entropy in system, whilst negative value means increase of entropy in surroundings. Both changes in enthalpy (ΔH°) and entropy (ΔS°) will be determined from the slope between ln K with respect to the reciprocal temperature (T) using the Equation (3) and the change in Gibbs free energy (ΔG°) will be defined from Equation (4). As the immobilized enzyme tends to lose its catalytic activity over time and the value of its catalytic deactivation is represented by kd Equation (5) at optimal maximum reaction temperature.

$$ln k = \Delta H/RT - \Delta S/R \quad (3)$$

$$\Delta G = \Delta H - T.\Delta S \quad (4)$$

$$k_d = (E_a + R.T_{max})/(\Delta H - (E_a/R.T_{max}) \quad (5)$$

Results and discussion

Screening of process parameter

Reaction time

Figure 1 shows the influence of reaction time on yield of fructose laurate. The highest yield was obtained at 24hr (90.99%) reaction time and began to reduce after 24hr. The thermal deactivation that occurred after 24hr has resulted in poor conversion of reactants to product (fructose laurate) (Šabeder *et al*., 2006). Hence, for the subsequent experiments, the esterification reaction will be carried out for 24hr.

Solvent

A suitable organic solvent used in the esterification process is very important to develop a stable enzymatic

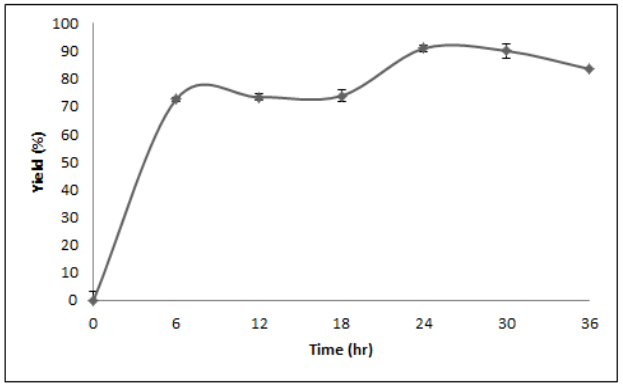

Figure 1. Influence of reaction time to yield of esterification

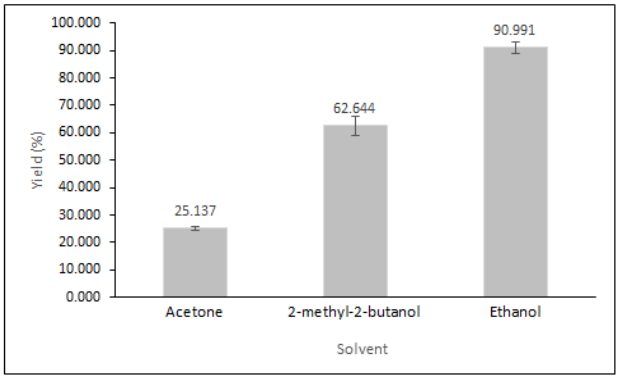

Figure 2. Influence of organic solvent to yield of esterification

reaction. A good organic solvent will not interfere the enzyme activity and selectivity, and at the same time, allow the lipase-catalyzed esterification to occur without the need to be in excessive amount. The low stability of product in the presence of solvent, will ease the crystallization process that eventually attain a favored equilibrium for ester formation (Yan *et al*., 1999; Šabeder *et al*., 2006). In screening part, different solvent selected based on previous study was used as the reaction media (acetone, ethanol, 2-methyl-2-butanol) (Šabeder *et al*., 2006; Miranda *et al*., 2014). The differences in the fructose laurate yield by using these solvents were illustrated in Figure 2.

The highest yield was obtained for esterification in ethanol at 24hr reaction time with 90.99% yield. In addition, as reported by Thelwall (1982), the monoacylation (e.g. fructose) is favored in the less hydrophobic solvents (log P<0.8) since the value of log P for ethanol is -0.58. Therefore, ethanol shown to be a better solvent compared to the others. The esterification rate was the lowest in acetone. Lower solubility of fructose in both acetone and 2-methyl-2-butanol might be the reason of this lower formation rate of sugar ester. This finding was opposed with the study done by Šabeder and coworkers (2006), who has compared the esterification of fructose palmitate in several different solvents and found that acetone gave higher conversion compared to 2-methyl-2-butanol.

Lipase loading

The amount of lipase used in the reaction should be sufficient to obtain high yield of fructose laurate. This study compared the effect of different lipase loadings at 0.02, 0.06, 0.10, and 0.14 g to the yield of sugar ester. Lipase loading 0.02 g gives the highest yield (92.21%). The yield decreased when more lipase was added, which is due to increase of water formation. Water is the by-product of esterification reaction and the system with high water content could trigger a reversible reaction (Arcos *et al.* 2001). In addition, excessive loading of lipase into the system can cause agglomeration and limit the accessibility between substrate and immobilized lipase, thus decrease the reaction efficiency (Duan *et al*., 2010). When the enzyme is loading more than the available substrate to react with, it will stack up onto one another in multilayer forming large particle. Typically, enzyme exists in powder form and soluble in water in homogeneous mixture (Homaei *et al.* 2013). However, in excess, these dispersed particles tend to be held together by weak physical interactions that resulting in reversible phase separation by precipitates of larger particles, called agglomeration (McNaught and Wilkinson *et al*., 2007).

Molecular sieve loading

Water generated during esterification reaction was removed by adding molecular sieve to circumvent excessive water content. The influence of different molecular sieve loading (0.0, 0.1, 0.2 and 0.3 g) towards the yield of fructose laurate was investigated. The yield abruptly increases when molecular sieves were added to the system but the range of molecular sieve loading showed no significant changes in fructose laurate yield. Similar trend also happened with Tarahomjoo and Alemzadeh (2003) for synthesizing glucose palmitate in hexane, where they found the substrate conversion with and without molecular sieve are 29.17% and 6.86%, respectively.

Optimization using RSM

Following the preliminary experiments, a statistical software for the prediction of optimal reaction temperature, lipase loading, molecular sieve loading, and agitation rate was run to maximize the percentage yields of the ester. The design matrix for the factors being investigated required a total of 62 experimental runs (data not shown). Application of

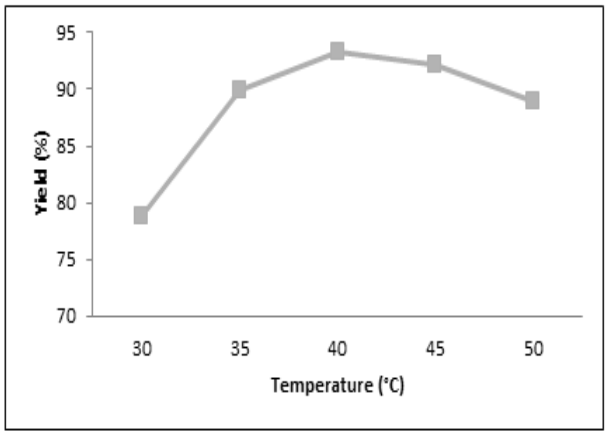

Figure 3. Influence of temperature towards yield of esterification

the response surface methodology and analysis of the experimental data with Design Expert Software represented with a quadratic polynomial correlation equation (6). In this study, the predictive functions representing the yield of fructose laurate (Y), A, B, C, and D were coded terms for temperature (ºC), lipase loading (g), molecular sieve loading (g), and agitation rate (rpm), respectively. The experimental data that was fitted into various models (linear, two factorial, quadratic, and cubic) showed the quadratic equation was best fitted because it exhibited higher R-squared (R^2 = 0.9291). Based on the R^2 value, the variation in the percentage yields can be described by the equation with certainty of 92.91%. The correlation between parameters obtained as follows:

Yield = 86.88 - 7.33 A + 5.59 B - 1.47 C + 2.51 D - 16.33 A2 4.39 B2 + 1.55 AB + 3.18 AC - 4.11 AD + 1.48 BD (6)

The optimization by Design Expert® software gave the result with desirability of 1.00 suggested running the esterification process at 40ºC, 0.012 g lipase loading, 200 rpm agitation rate with zero addition of molecular sieves. Under these optimum conditions, the fructose laurate yield was predicted to be 93.37%. The experiment was run triplicate to validate the condition predicted by design of experiment (DOE). The value of percentage yields obtained from experiment was then compared. The validation runs obtained 93.21±0.13% yield with 92.91% confidence level and this indicates that the suggested optimal conditions was validated and optimized.

Thermodynamic study

Enzyme stability study was conducted to investigate the effect of temperature to the reaction based on study conducted by Khor *et al.* (2010). The esterification reaction was performed at temperature ranges from 30°C to 50°C. Figure 3 present the effect of temperature to the yield of fructose laurate. It was clearly shown that the yield was increased with the increasing of reaction temperature, but started to decrease when the temperature exceeds 40°C. The estimation of thermodynamic constants provides important information on mechanism of enzyme inactivation. All the thermodynamic properties were tabulated in Table 1. Enzyme stability decreases with increasing rate constant of lipase deactivation, kd, at particular temperature. This implies that at elevated temperatures from 40 to 50^{O}C, the immobilized lipase stability decreased with indication of increasing kd value from 0.0021 to 0.0069. The positive value of enthalpy of activation (ΔH) indicated the endothermic nature of enzymatic esterification reaction, where heat is needed for the reaction to occur. These values also showed that lower enthalpy of activation was needed as the temperature raised and this is probably due to active collision occur between the substrate molecules at high temperature. The entropy of activation (ΔS) is the measure of disorder in a system and for this study, positive entropy implies an increase in disorder in the system as the temperature increases. The Gibbs free energy of activation (ΔG) at each reaction temperature showed negative value, and these values confirmed that the enzymatic esterification reaction could proceed spontaneously (Khor *et al*., 2010).

Table 1. Thermodynamic properties

Constants \ Temperature (°C)	40	45	50
k_d (min^{-1}) ($x10^{-3}$)	2.05	2.22	6.88
ΔH° (kJ/mol)	98.70	98.65	98.61
ΔG° (kJ/mol)	-241.50	-245.17	-246.02
ΔS° (kJ/mol.K)($x10^{1}$)	10.86	10.81	10.67

Conclusion

Synthesis of fructose fatty acid ester using lipase from Candida antarctica immobilized on acrylic resin was performed. The influences of reaction time, solvent, lipase loading and molecular sieve loading were studied as screening for optimization using RSM. The optimization study obtained were 40°C, 0.13 g lipase loading, 200rpm, without molecular sieve needed for the system and produced about 93.21% of fructose laurate.

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