IJST EXPLORE International Journal of Science and Technology
CENTRE OF PROFESSIONAL RESEARCH PUBLICATIONS
ISSN 2049-7318

International Journal of Science and Technology Volume 3 No. 7, July, 2014

Kinetic Investigation of Enzymatic Synthesis of Sucrose Fatty Acid Esters from Sucrose and Non-edible Rice Bran Fatty Acid

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ABSTRACT

Enzymatic synthesis of sugar fatty acid esters were performed in an organic solvent using Immobilized *Rhizomucor miehei* (*RMIM*) lipase with sucrose and non-edible rice bran fatty acid as substrates. The maximum conversion of sucrose esters up to 78% obtained when sucrose and non-edible rice bran fatty acid were reacted in molar ratio of 1:8, using n-butanol as a solvent and 1% molecular sieves with 10% enzyme by wt of substrates at 50°C for 16 hours. This sucrose ester, have hydrophile-lipophile balance (HLB) 15.3. This HLB value is in the range suitable for use in detergency and solubilizers.

Keywords: *Sucrose esters, Non-edible rice bran fatty acid Rhizomucor miehei (RMIM), hydrophile-lipophile balance (HLB)*

1. INTRODUCTION

Sugar fatty acid esters, usually called sugar esters, are nonionic and biodegradable surfactants, having very good emulsifying, stabilizing, or conditioning effects. They are widely used in food, cosmetic, pharmaceutical, and detergent industries. Sugar esters are synthesized by esterification of sugars or sugar alcohols with fatty acids[1] (Akoh,c.c. 1994). Synthesis of the esters can be carried out either chemically or enzymatically. The chemical process occurs with low selectivity and leads to a mixture of sugar esters with different degree of esterification. It is carried out at high temperatures, which causes coloration of the products. These problems can be overcome by the use of biological catalyst, such as lipase, for the synthesis of sugar esters. The main advantage of enzymatic synthesis is high regioselectivity leads mainly to monoesters production. In addition, the enzymatic method can be performed under mild reaction conditions; thus, denaturation of substrates and/or products can be avoided[2] (Sarney,D.B. 1995).

Direct enzymatic esterification of sugar with fatty acids in aqueous media was attempted in the early eighties, but the products formed in low yield[3] (Park 2004). More recently, enzymatic reactions have been carried out in organic media. In this case major problem is the low solubility of the sugars in organic solvents. To solve this problem, activated fatty acids in polar solvents[4] (Therisod (1986), or activated sugars in apolar solvents [5] (Oguntimein 1993), have been used. However, these methods require substrate derivatization step that increase production costs. As another approach, the partial solubilization of both substrates in intermediate-polarity solvents was reported to be effective for sugar ester synthesis.

Enzymatic sugar esters synthesis is based on esterification reaction catalyzed by hydrolases. Because esterification is a reversible reaction, the esterification reaction products such as water in media should be removed to shift the equilibrium of the reaction away from hydrolysis to obtain a maximum yield of sugar ester[3]. To remove the water librated by the reaction, evaporation under reduced pressure[6] and azeotropic distillation[7] during the reaction were performed. Selective C–H activation remains a challenge for synthetic chemists, who often rely on differences in the steric and electronic properties of bonds to achieve regioselectivity [6]. The preparative-scale generation of hydroxylated intermediates can often provide synthetically useful derivatives as well as pharmaceutically important drug metabolites and lead compounds[7].

This paper reports synthesis of sucrose esters surfactants via determining the effect of several parameters affecting on enzymatic synthesis of sugar esters. This process of synthesis giving maximum conversion in shorter time and shows some physic-chemical properties of biodegradable & vegetable oil based surfactant.

Although the enzymatic synthesis sucrose esters surfactants are reported under different experimental conditions, no attempt has been made to study the kinetics of synthesis and to observe the effect of solvent, temperature etc. on synthesis. It is therefore thought worthwhile to investigate the kinetics of enzymatic sugar synthesis catalyzed by an immobilized lipase and to observe the effect of solvent, temperature, molecular sieves, enzyme molar ratio etc. from mechanistic view point of synthesis.

2. EXPERIMENTAL

2.1 Materials

All materials used in this experiment are purchased from NATH CHEMICALS PVT. LTD Mumbai India and non-edible rice bran fatty acids were purchased from local market and Lipozyme RMIM, supplied by NOVODISD, all organic solvents, including n-butanol are of

Physico-chemical properties of raw materials (non-edible rice bran fatty acid and Sucrose) for the synthesis of sucrose ester were analyzed. The results of analysis are depicted in **Table 2.1**

2.1 Analysis of raw materials

S.No.	Raw material	Analytical details	Value
1.	Non-edible rice bran fatty acid	Acid Value	175.0 ±5
		Saponification Value	195.5 ±5
		Iodine Value	105.1 ±02
2.	Sucrose Melting Point		175^0C
		Solubility	10% (in water)

2.2 Reaction Procedure

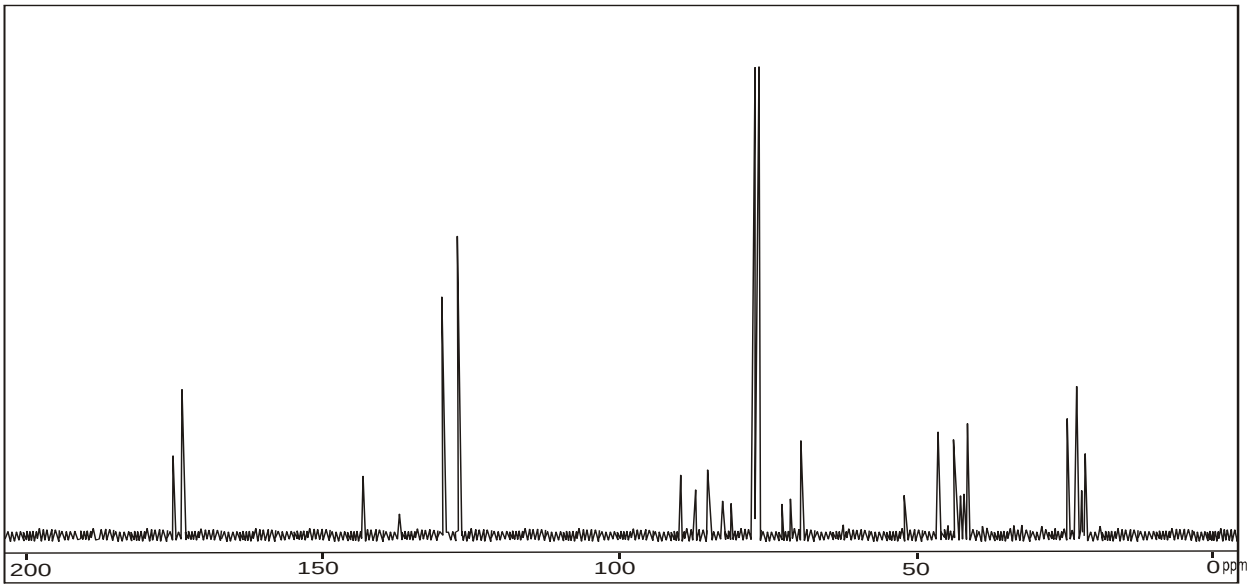
Reaction were carried out by mixing the desired amount of sugar or sugar alcohol and rice bran fatty acid with chosen solvent in stoppered glass bottles, which were shaken at 200 rpm in an orbital shaker. Activated molecular sieves were added in the reaction mixture and esterification was initiated by adding the enzyme. A reaction mixture consist 1:8 molar ratio of sucrose and non-edible rice-bran fatty acids and 10% w/w of lipozyme RMIM, 50ml-butanol, and 1% molecular sieves at 50°C for 16hr. At the end of each batch reaction, the enzyme was removed by filtration and the solvent evaporated. Ester formation was calculated based on acid value of the reaction mixture measured before and after the incubation time with a procedure suggested by Novo Nordisk A/S[8]. The product was identified by thin layer chromatography (TLC), using kiesilgel 60 and a mobile phase of Chloroform/methanol/water (64/10/1v/v/v) in agreement with the Ducret's methods[9].

2.3 Purification of synthesized product

The unreacted materials and the product were dissolved in ethyl acetate, unreacted sugar removed by filtration. The ethyl acetate was removed by evaporation and unreacted free fatty acid and the product was mixed with about 20g of silica gel 60 the material was eluted with chloroform to separate the unreacted free fatty acid from product, and soon after the ester of sugar was separate from the silica with mobile phase of solvent acetone/water 64/10 v/v). Finally, the solvent was evaporated under reduced pressure.

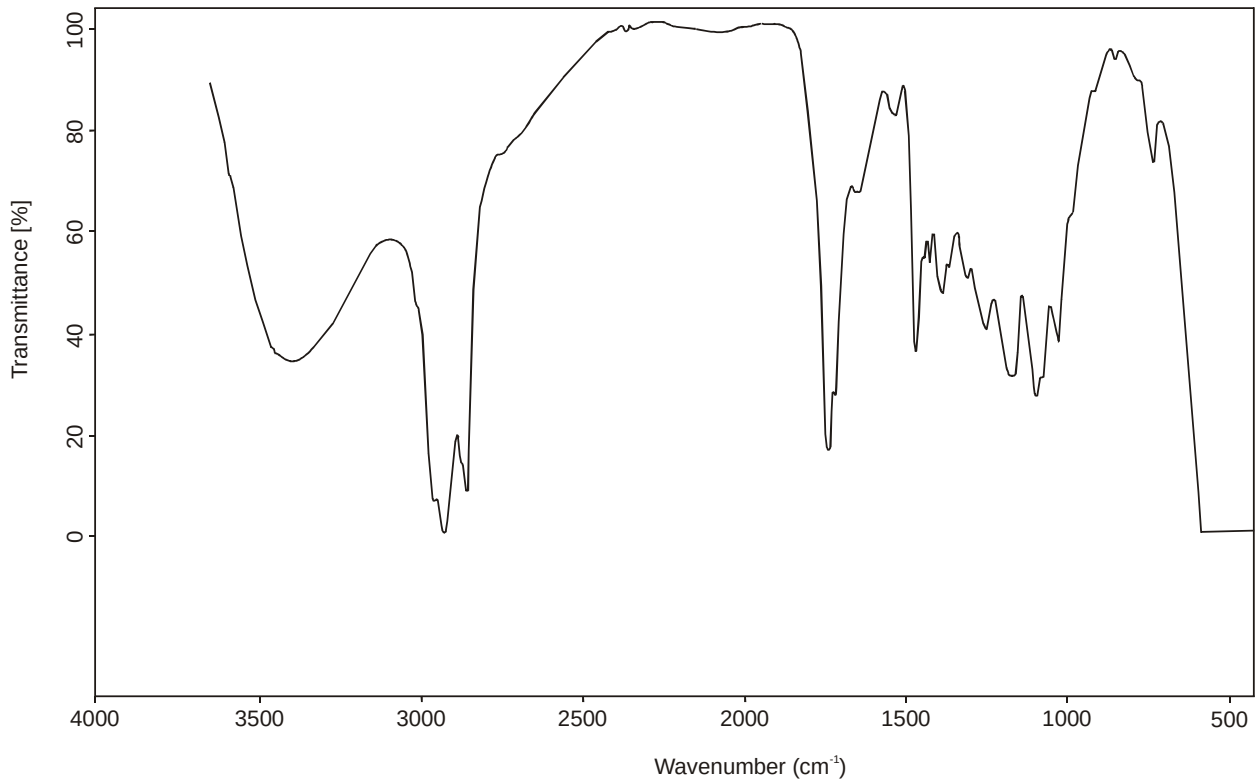
2.4 Identification of sucrose esters

1) ^{13}C *NMR* (400 MHz) ($CDCl_3$): δ 21.9,22.6,22.7,42.6,43.4,46.2,127.1,129.7,143.5,170.6



2) FTIR Spectra of Sucrose Ester

The presence of ester groups by infra red spectra which were recorded on FT-IR spectrophotometer (Perkin Elmer Spectrum BX, which showed that product had absorption bands at wavernumber 3380,90cm characteristic for OH, 2853,91 - 2924,26cm for C-H in CH2 or CH3 and 1724,16cm for ester C=O, 1468 (for -CH,-CH,), 1055-1183 (for the C-O, ester bond), and 723 [for the (CH), bond].



3) Hydrophilic- Lipophilic Balance (HLB)

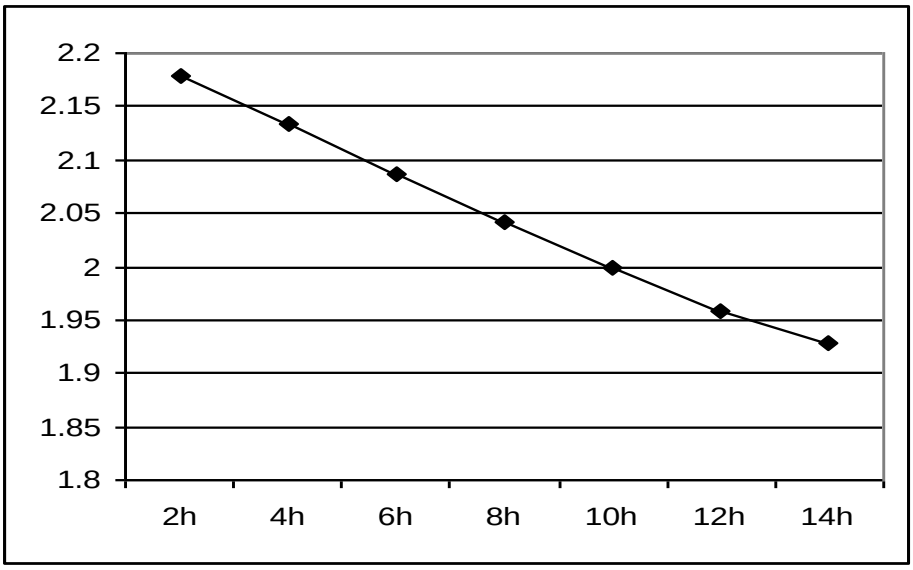
The hydroplilic and lipophilic balance (HLB) value of sucrose esters was also obtained using the Griffin equation. The HLB value of sucrose esters was calculated as 15.3 according this equation[10]. This result indicated that it was used in detergency and as solubilizers.

2.5 Kinetic Measurement

Reaction were carried out by mixing the desired amount of sucrose and non-edible rice bran fatty acid with chosen solvent in the stoppered bottles, which were shaken at 200 rpm in an orbital shaker. The reaction mixture was kept in a thermostatic bath at desired temperature ± 0.1°C. Activated molecular sieves were added in reaction mixture and esterification was initiated by adding enzyme.

The kinetics of synthesis was followed by determining the acid value of the non-edible rice bran fatty acid at different time intervals. It was observed that log (Acid Value) verses time plot was always linear with a negative slope upto 14-15hours of the kinetic runs, suggesting that rate of disappearance of non-edible rice bran fatty acid followed first order kinetics with respect to the non-edible rice bran fatty acid. Therefore first order rate constant (k_{obs}) were determined from the slopes of straight lines plotted between log (acid value) versus time for 1:8 molar ratio at a temperature 45^0C using 5 % enzyme and hexane as solvent show in figure 1.

Fig. 1 shows the slopes of straight lines plotted between log (acid value) versus time for 1:8 molar ratio.



The rate constant (k_{obs}) were reproducible within ± 5% in replicate kinetic runs.

3. RESULT AND DISCUSSION

Synthesis of the sucrose esters has been studied at different initial conditions of reagents, catalyst, temperature and solvent.

3.1. Effect of Molar Ratio

Study the effect of molar ratio of sucrose to fatty acid on esterification, the synthesis has been studied at different molar ratio and at a temperature (45°C) using 5% enzyme and hexane as solvent. The results are reported in Table 1.

Table 1. Effect of molar ratio

Molar Ratio(Sucrose to Fatty acid)	k_{obs} x 10^{-4} (min^{-1})	Conversion (%)
1:6	8.2	65.0
1:8	8.7	69.8
1:10	8.0	67.1

It is clear from the table 1 that 1:8 molar ratio is the optimum molar ratio for the maximum esterification of sucrose.

3.2. Effect of Solvent

Although enzymes work efficiently in organic solvents the more appropriate solvent should be determined as a specific solvent depending on the type of enzyme, substrate and product. The synthesis of the sucrose ester have been carried out in presence of three solvents viz- hexane, n-butanol and t-butanol unless the similar conditions. The yield of the product and observed rate constant are respected in Table-2.

Table 2. Effect of solvent on yield of product and k_{obs} for synthesis of sucrose esters with 1:8 molar ratio and 5% enzyme at 45°C.

Solvent	k_{obs} x 10^{-4} (min^{-1})	% Conversion
Hexane	8.7	69.8
n-butanol	9.7	73.2
t-butanol	9.6	72.1

It is clear from Table 2 that the n-butanol is the most effective solvent in the synthesis of sucrose ester.

3.3. Effect of Temperature

The synthesis was studied at different temperatures viz- 45, 50 and 55°C. The reaction was carried out for 16h. The observed rate constant (k_{obs}) and yield of the product at different temperatures are given in Table 3.

Table 3- Effect of temperature on k_{obs} and yield of product in n-butanol with 5% enzyme and 1:8 molar ratio.

Temperature (ºC)	k_{obs} x 10^{-4} (min^{-1})	Conversion (%)
45	9.7	73.2
50	14.6	75.9
55	11.6	74.8

It is observed in table 3 that the optimum temperature for the synthesis is 50°C

3.4. Effect of enzyme percentage

Due to the nature of the highly concentrated and mainly solid reaction mixtures, it is obvious that effective mixing of reactants and enzyme is important to provide good transport and contact of the reaction partners. The optimum enzyme concentration highly depends on the stirring status. The synthesis of sucrose ester has been studied at different initial concentration of enzyme at 50°C using 1:8 molar ratio of sucrose and non-edible rice bran fatty acid and n-butanol as solvent. The % conversion and rate constant k_{obs} for the synthesis at different enzyme concentrations are given in Table 4.

Table 4- Effect of enzyme on % conversion and k_{obs} when molar ratio is 1:8, temperature 50°C in n-butanol solvent

Enzyme %	k_{obs} x 10^{-4} (min^{-1})	Yield (%)
5%	2.40	76.9
10%	7.213	78.9
15%	5.190	77.2

3.5. Effect of agitation speed on enzyme activity

To investigate possible mass transfer effect on the reaction rates, a number of experiments were performed using different agitating speeds while keeping all other variables at standard values. The effect of the agitating speed was studied in the range of 100-1000 rpm. It was observed that the initial reaction rate remains essentially constant from 200 to 1000 rpm agitating speed.

3.6.Effect of internal and external diffusion limitations

In order to measure the intrinsic kinetics, the mass transfer of the substrates to the locus of the reaction should be much faster than chemical reaction. Both the diffusion of the substrate in the dispersed phase (internal mass transfer) as well as diffusion of enzyme and substrate in the continuous phase (external mass transfer) may effect the over all reaction rate.

The experiments performed at different agitation speeds indicates that the external mass transfer effects do not affect the over all reaction rate provided that the stirrer speed exceeds 200 rpm. Therefore, all experiments to determine the kinetics of the reaction were performed at the stirring speed of 200 rpm. The possible effect of internal mass transfer limitations may be estimated by using the approach of characteristic times for reaction and mass transfer. The characteristic times for internal mass transfer in our case was calculated by considering diffusion coefficients of fatty acid and n-butanol and was found to in order of nearly 0.02s. The characteristic time reaction may be defined as the time required for 60% of the reaction and is about 10 hours for the fastest reaction in present studies. This estimation indicates that the internal mass transfer effect may also be neglected in our experiments.

Mechanism:

The mechanism for the synthesis may be suggested as follows,

Fatty Acid + Enzyme $\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}$ X …….. (i)
(Slow) (Intermediate)

X + Sucrose $\xrightarrow{K_2}$ Sucrose Esters …….. (Fast) (ii)

According to above mechanism the rate of disappearance of fatty acid may be given as,

$$-\frac{d[\text{Fatty acid}}{dT} = k_2[x][\text{Sucrose}] \quad \ldots\ldots(1)$$

By appearing steady state approximation with respect to [X], we get,

$$[X] = \frac{k_1[\text{Fatty acid}][\text{Enzyme}]}{k_{-1} + k_2[\text{Sucrose}]} \quad \ldots\ldots(2)$$

By substitution the value of [X] in equation (1), the rate law becomes,

$$-\frac{d[\text{Fatty acid}]}{dT} = \frac{k_1 k_2[\text{Fatty acid}][\text{Enzyme}][\text{Sucrose}]}{k_{-1} + k_2[\text{Sucrose}]} \quad \ldots\ldots(3)$$

Since the step (ii) is a fast step,

k_2 [Sucrose] >> k_{-1}

may be taken as suitable approximate, the rate law (3) thus became,

$$-\frac{d[\text{Fatty acid}]}{dT} = k_1[\text{Fatty acid}][\text{Enzyme}] \quad \ldots\ldots$$

According to the rate law (4), the reaction seems to be first order with respect to [Fatty acid], which has been observed experimentally.

4. CONCLUSION

In this study, the effect of several parameters on enzymatic synthesis of sugar esters such as molar ratio, solvent, temperature and enzyme percentage has been investigated. It appeared that a suitable organic solvent should dissolve enough of the substrate to allow the lipase catalyzed esterification. The optimal sugar to fatty acid ratio was 1:8 for high conversion in the presence of 10% enzyme (w/w) at 50°C in n-butanol. The HLB of product is 15.3. The results are in favoured, that the synthesized sucrose fatty acid ester may be used in detergency and also as solubilizers.

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