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LIPASE CATALYZED SYNTHESIS OF SUCROSE ESTERS FROM PALM FATTY ACID

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Abstract: Sucrose esters have been successfully synthesized from sucrose and palm fatty acid with *Candida antarctica (NOVOZYME 435)* lipase as catalyst in an organic solvent. The maximum conversion (84.7%) of sucrose esters obtained when sucrose and palm fatty acid were reacted in molar ratio of 1:3, using hexane as a solvent and 1% molecular sieves with 5% enzyme by wt of substrates at 55°C for 14 hours. This process for synthesis is economical and in this experiment we used *Candida antarctica (NOVOZYME 435)* for giving maximum conversion in shorter time.

Key words: Sucrose ester, Lipase, Palm fatty acid, Organic solvent system, Esterification

1. Introduction

Sucrose fatty acid esters (sugar esters) are nontoxic compounds that may be produced from renewable sources. Sucrose esters (SE) have been commercially produced for food industry, which are widely used in foods, cosmetics, agriculture and pharmaceuticals. Apart from their emulsifying properties, they are completely biodegradable, harmless to environment, nontoxic, skin-compatible, odorless and tasteless. Since the SE products are non-toxic to humans, crops and higher animals, fully biodegradable and hydrolyzed to readily metabolizable sucrose and fatty acid, and appear to be good insecticide candidate (Zi-juan, 2006).

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 which 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 (Sarney et al., 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 (Park et al., 2004)

More recently, enzymatic reactions have been carried out in organic media. However in this case, the major problem is the low solubility of the sugars in organic solvents. To solve this problem, activated fatty acids in polar solvents (Therisod et al.,1986), or activated sugars in apolar solvents (Oguntimein et al., 1993), have been used. However, these methods required substrate derivatization step that would increase production costs. In another approach, partial solubilization of both substrates in intermediate-polarity solvents was reported to be effective for sugar ester synthesis.

On the other hand 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 in order to shift the equilibrium of the reaction away from hydrolysis which could lead to a maximum yield of sugar ester ((Park et al., 2004). To remove the water librated by the reaction, evaporation under reduced pressure (Ducret et al., 1995) and azeotropic distillation (Yan et al.,1999) during the reaction were performed.

In this study, the key parameters of enzymatic sugar ester synthesis catalyzed by an immobilized lipase were investigated, including the solvent, molecular sieves, molar ratio and effect of enzyme percentage with reaction time and rpm (revolution per minute).

The aim of this study was to investigate the enzymatic esterification of palm fatty acid and sucrose by using NOVOZYME 435 and to examine the affect.

2. Experimental

2.1 Materials

All materials used in this experiment were purchased from CDH CHEMICALS PVT. LTD. and palm fatty acid

was purchased from local market and *Candida antarctica (NOVOZYME 435)*, supplied by Novo Nordisk. All organic solvents were of HPLC or analytical grades.

2.2 Reaction Procedure

Reaction was carried out by mixing the desired amount of sugar or sugar alcohol with palm fatty acid in selected solvent. The mixtures were placed in stoppered glass bottles and they were shaken at 200 rpm in an orbital shaker. Activated molecular sieves were added to the mixture and esterification was initiated by adding the enzyme. A typical reaction mixture consist of 1:3 molar ratio of sucrose to palm fatty acid and 5% (w/w) of *Candida antarctica (NOVOZYME 435),* 50ml hexane, and 1% molecular sieves placed at 60^{o}C for 14h. The reaction was terminated by removing the enzyme and molecular sieves by centrifugation. 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 (Novozyme 435 Product sheet, 1999). At the end of each batch of reaction, the enzyme was removed by filtration and the solvent evaporated. The product was identified by thin layer chromatography (TLC), using kiesilgel (Silica gel) 60 and a mobile phase of chloroform/methanol/water (64/10/1, v/v/v) in agreement with the Ducret's methods, (1995).

2.3 Purification

About 20 g silica gel 60 were added to the mixture of unreacted materials and the product were dissolved in chloroform .The chloroform was then evaporated. The silica gel containing palm fatty acid and product was eluted with chloroform to separate the palm fatty acid from product, and then eluted with chloroform/methanol/water (64/10/1 v/v/v) to isolate the sucrose esters from the silica gel according to Ducret's methods (Ducret et al., 1995). Finally, the solvent was evaporated under reduced pressure to obtain the product.

2.4 Analysis

Separation of the products mono and diesters of sucrose was done using column chromatography by adopting QUINILIN WEISER METHOD Cocks, L.V., 1966. A silica gel column Borocil LC 18,5 µm (250× 4.6mm) was used at a flow rate 0.7 ml/min.. After separation of products the fractions were monitored using thin layer chromatography and were further identified using reference standards.

The presence of ester was confirmed by inrfa red spectra which was recorded on FT-IR spectrometer (Perkin Elmer Spectrum BX). The FFA content was determined by titrating the material in an alcoholic medium with aqueous potassium hydroxide solution. Indian Standards (IS) 1964.

2.5 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

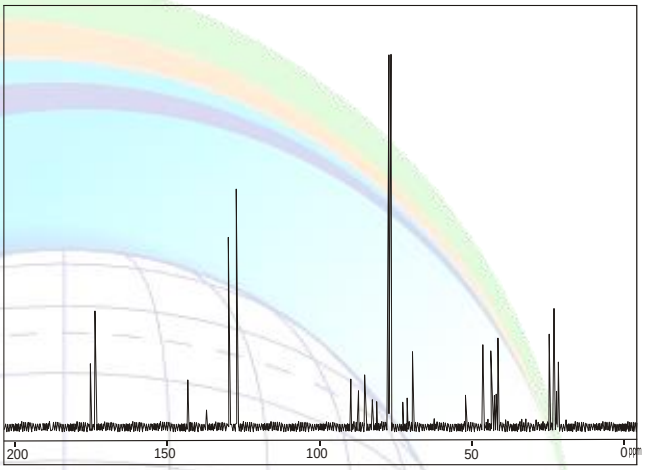


Fig.1. shows the effect of temperature on esterification reaction

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].

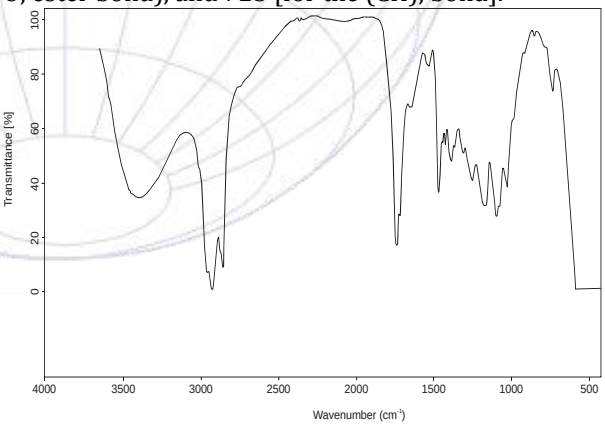


Fig.2. shows the effect of organic solvent on esterification reaction

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.

3. Results and discussions

Physico-chemical properties of raw materials (palm fatty acid and sucrose) for the synthesis of sucrose ester were analyzed. The results of analysis are depicted in **Table 1**.

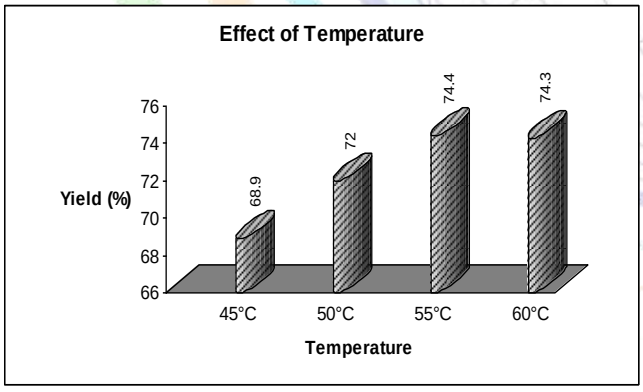
TABLE 1: Analysis of raw materials

S.No.	Raw material	Analytical details	Value
1.	Palm fatty acid	Acid Value	187.0
		Sap. Value	201.5
		Iodine Value	42.6
2.	Sucrose	Melting Point	175^{0}C
		Solubility	10% (in water)
		Apperance	White solid
		Density	1.513 g/cm^3

3.1 Effect of Temperature on Esterification

Reaction temperature was the main factor that influenced the maximum esterification. The reaction rate increased when the temperature was raised, but the enzyme used had low optimum reaction temperature. When synthesis was carried out at 50^{o}C, 55^{o}C and 60^{o}C respectively, maximum yield of Monoesters and Diesters was found at 55^{o}C. (**Fig. 1**)

Fig.1. shows the effect of temperature on esterification reaction



3.2 Effect of Stirring (RPM)

The effect of rpm with a molar ratio of 1:2, at 55^{0}C and reaction time 14 hour at 150 to 300 rpm, acid value of reaction mixture was decreases respectively; from Table **2** it is clear that the 250 rpm is the best condition for synthesis. When we increase rpm 300, there is very little difference in acid value.

Table 2. shows the effect of Stirring on esterification reaction

	RPM (revolution per minute)	Yield (%)
	150	67.8
	200	74.4
201.5	250	75.6
	300	75.7

3.3 Effect of solvent

The organic solvent affects the reactivity of enzyme, solubility of substrates in water and other components of hydrophobic nature. Organic solvents of different type affect the rate of the reaction differently including physicochemical properties of substrate and enzyme. In the present study n-hexane and acetone were used as solvent. The maximum yield was observed when n-hexane was used as solvent.

3.4 Effects of molecular sieves (MS)

The amount of water present in non-aqueous media and some bound to enzymes influenced the dynamic and catalytic properties of enzymes (Bell et al., 1995). Molecular sieves were often introduced to remove water accumulated during esterification, and many research groups reported that the use of molecular sieves enhanced the reaction rate. {Tsuzuki et al., (1999) and Torres et al., (2000)}

To obtain the maximum yield, the esterification product like water from the media should be removed by shifting the equilibrium of reaction away from hydrolysis. When the reaction mixture contained 1%molecular sieves (a dehydrating agent), reaction rate and the final conversion was found to be better than the mixture containing 0 % molecular sieves shows in (**fig. 2**).

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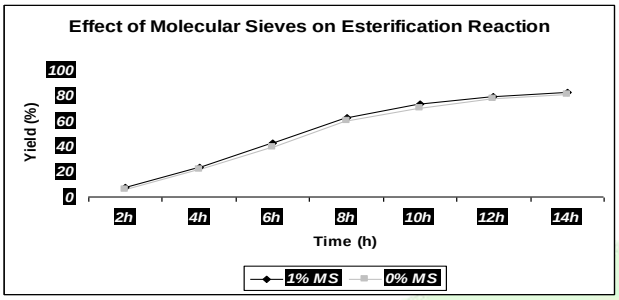


3.5 Effect of molar ratio

To study the effect of molar ratio of sucrose to fatty acid on esterification, the temperature and catalyst concentration was fixed at 55°C and 5% respectively in presence of 1 % molecular sieves and n-hexane as solvent. Change in the composition of lipid in 50ml n-hexane observed during the course of esterification reaction by lipase. The increase in yield of esterified products with respect to increase in molar ratio of sucrose to fatty acid is shown in The increase in yield of esterified products with respect to increase in molar ratio of sucrose to FA's is shown in **Fig.3 and Fig.4** respectively for molar ratio 1:2 and 1:3. The optimum molar ratio was found to be 1:3 for maximum esterification of 84.7% in 14hr.

Fig.3. shows the effect of 1:2 molar ratio of sucrose to palm fatty acid on esterification

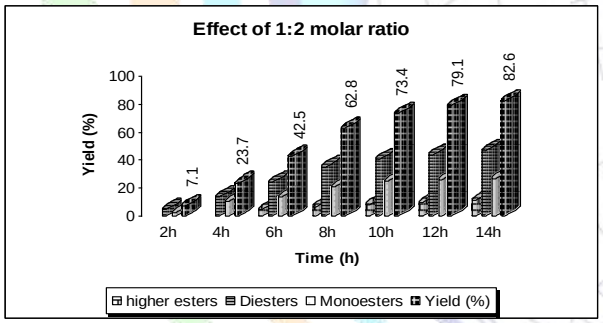
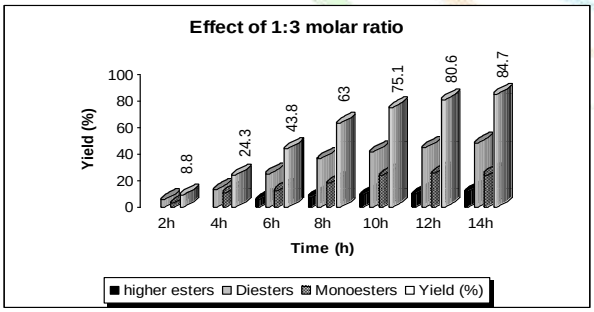


Fig.4. shows the effect of 1:3 molar ratio of sucrose to palm fatty acid on esterification



4. Conclusion

In this study, we determined the effects of several parameters affecting enzymatic synthesis of sugar esters. The influence of solvent, molecular sieves, molar ratio with reaction time and rpm each investigated. It appeared that a suitable organic solvent able to dissolve enough of the substrate in order to allow the lipase catalyzed esterification to take place. The optimal sugar to fatty acid ratio was found to be that would give 1:3 high conversions of di and mono-esters.

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