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PREPARATION OF BIODEGRADABLE AND VEGETABLE BASED SURFACTANT FROM SUGAR AND PALM FATTY ACID CATALYZED BY *Mucor miehei* LIPASE

Keywords: Palm fatty acid, sugar ester, lipozyme IM

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Sugar ester was prepared from palm fatty acid distillate (PFAD) with Lipozyme IM (immobilized Mucor miehei) as catalyst. Four sugars were used sucrose, sucrose octaacetate, glucose and fructose. Fructose was esterified by PFAD to produce 17.70 mg/ml solvent of product while glucose produced 13 mg/ml solvent. It was found that a mol ratio of fructose/PFAD of 1/10, 10% lipase concentration and a temperature of 55°C gave the highest yield. Analyses of its physical and chemical properties showed that fructose ester had a melting point of 49°C - 52.3°C. The surfactant, a fructose ester, also reduced the surface tension of water from 74 dyne/cm to 38.3 dyne/cm.

Even though the yield was still quite low compared to what had previously been obtained using other substrates, this study showed that enzymatic preparation of surfactant from PFAD is possible.

INTRODUCTION

Surfactants or surface active agents, usually also known as emulsifiers, constitute an important class of industrial chemicals widely used in almost every sector of modern industry, especially in the manufacture of household detergents, personal-care products, cosmetics and in food industries. In the United States, the total surfactant demand is expected to increase by 3% annually to 4 million MT in 1997 (Branna, 1995). Europe, Japan and other Asian countries also use surfactants in large volumes.

Today, even though both oleochemicals and petrochemicals can be used to make surfactants, only about 21% of surfactants are made from oleochemicals (Anon, 1993). With increasing

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environmental and health consciousness there is increasing demand for biodegradable (or vegetable based) surfactants. The biodegradability of household and industrial detergents has become almost as important as their functional performance to consumers. Also, greater consumer awareness of potential adverse allergic effects caused by artificial food additives, including food emulsifiers, and new environmental legislation are forcing the food industry to search for more natural alternatives.

There are two main ways to biosynthesis surfactants – whole-cell biotransformation and enzymatic synthesis (Sarney and Vulfson, 1995). Biosurfactants produced by microorganisms usually can not be manipulated much in their structure and are difficult and costly to purify (Chapineau *et al.*, 1988). Enzymatic synthesis or biotransformation, on the other hand, offers some flexibility to modify the chemical structure of the product (Sarney and Vulfson,1995). There is therefore increasing interest in enzymatic esterification of carbohydrates and fatty acids due to the specificity of the products and mild conditions of reaction.

Carbohydrate esters are used as surfactants in the food, detergent, and cosmetic industries (Jansenn *et al.*, 1990). Several papers have reported on the esterification of carbohydrates and fatty acids or modified fatty acids such as octanoic (Ljunger *et al.*, 1994), oleic (Khaled *et al.*, 1992), palmitic, stearic (Oguntimein *et al.*, 1993), and vinyl acetate (Sharma and Chattopadhyay, 1993) catalyzed by lipases or proteases with solvents such as pyridine, dimetyl formamide and tert-butyl alcohol.

From the economic view point, it would be particularly advantageous to prepare surfactants from sugar and palm fatty acid distillate (PFAD) as both are produced from cheap and renewable sources. PFAD is a by-product from palm oil refining (generated at about 4% of the volume of palm oil refined). Currently, it is only used as a raw material for soap.

This paper reports on a preliminary study of esterifying sugar and PFAD using lipase (from *Mucor miehei*) as biocatalyst, and tert-butyl alcohol as solvent to produce sugar fatty acid ester. Lipozyme IM was used as biocatalyst because it was cheaper than other enzymes although Khaled *et al.*(1992) showed that a 1-3 specific lipase was better. Tert-butyl alcohol was used because of its non toxic nature. It is a food solvent, capable of partially solubilizing sugars, and is the only alcohol that does not react with fatty acids in the presence of lipase (Novo, 1992; Khaled *et al.*, 1993).

MATERIALS AND METHODS

Materials

Materials used in this experiment were fructose, glucose, sucrose and sucrose octaacetate (Merck), raw PFAD and Lipozyme IM (brand name of immobilized lipase from *Mucor miehei,* activity 6 BAUN/gram) supplied by Novo Malaysia. All organic solvents, including tert-butyl alcohol, were of analytical grade.

METHODS

Preparation

Sugar (fructose, glucose, sucrose, and sucrose octaacetate) at 2 mmol was mixed with PFAD in a mol ratio of 1:10, 10% Lipozyme IM and 12.5 ml tert-butyl alcohol (2-methyl-2-propanol). The reaction was carried out in a 50 ml flask on a mechanical shaker incubator (250 rpm) at 55°C for 24 hours. At the end of each batch reaction, the enzyme was filtered out and the solvent evaporated. The mixture of unreacted sugar, PFAD and product was dissolved in chloroform. The unreacted sugar was removed by filtration. The effects of molar ratio of reactants, lipase concentration and temperatures were studied using fructose:PFAD mol ratios of 1:1, 1:2, 1:5 and 1:10, lipase concentrations of 5%, 10% and 20%, and temperatures of 28°C - 30°C, 40°C and 55°C.

Purification

About 5g silica gel 60 was added to the mixture of unreacted PFAD and the product dissolved in chloroform. The chloroform was then vacuum evaporated. The silica gel containing PFAD and product was eluted with chloroform to wash away the PFAD and then eluted with chloroform/methanol/water (64/10/1 v/v/v) to dissolve out the fructose ester according to Ducret *et al.*

(1995). Finally the solvent was evaporated under partial vacuum to leave the product.

Analysis

The qualitative estimation of the product was done by thin layer chromatography (TLC) in a chloroform/methanol/acetic acid/water (80/10/8/2 v/v/v/v) solvent according to Khaled *et al.* (1993). Quantitative analysis was done using a Scan-Densitometer (Bio-Rad Model GS-670).

Qualitative analysis was carried out by HPLC (Gilson model 714). Separation was achieved on Supelcosil LC18, 5 µm column (250 mm x 4.6 mm) at a flow rate 0.7 ml/min and detected by a Gilson 116 UV detector at 280 nm. The mobile phase consisted of methanol and acetic acid (99.7 % : 0.3 % v/v) as reported by Oguntimein *et al.* (1993).

The presence of an ester was confirmed by infra red spectra recorded on a FT-IR spectrophotometer (Bio-Rad model FTS 165) from a film of surfactant between the potasium bromida plates.

The surface tension of distilled water as modified by surfactant, was measured at room temperature (28°C) with du-Nouy's ring method (Fisher Surface Tensiomat model 21). The hydrophile-lipophile balance was also analysed by the method of Gupta *et al.* (1983) using pyridine/benzene (95%/5% v/v) as solvent and titrated using distilled water. The melting point of the surfactant was observed on Electrothermal. Solubility of the surfactant in various organic solvents was also investigated.

RESULTS AND DISCUSSION

TLC analysis of the products from esterification of PFAD with different sugars, using Lipozyme IM in tert-butyl alcohol, showed that the product from fructose gave spots at R_f values of 0.53, 0.88, and 0.97. With glucose as the carbohydrate, R_f values of 0.41, 0.88 and 0.97 were noted, whilst sucrose and sucrose octaacetate gave spots only at R_f 0.88 and 0.97, as did the controls (mixture of sugar and PFAD without enzyme). Using the same eluent, Khaled (1992) showed that a sugar ester has an R_f value of 0.5, that the sugar remained in deposit and that fatty acids gave an R_f value of 0.9. The results showed that there were significant esterification between fructose or glucose and PFAD, but no reaction with sucrose and sucrose octaacetate. The yields of the products are shown in *Table 1.*

TABLE 1. YIELDS OF ESTERS FROM DIFFERENT SUGARS

Sugar	Ester Production (a)		RF Value
	mg/ml solvent	mg/mg enzyme	
1. Sucrose	ND	ND	-
2. Sucrose octaacetate	ND	ND	-
3. Glucose	13.1	0.55	0.41
4. Fructose	17.7	0.74	0.53

(a) Before purification, detected by scan densitometer
ND: Not detectable

Theoretically, all the sugars used can be esterified by PFAD because they all, except sucrose octaacetate, have a primary hydroxyl group. Glucose, however, was only slightly esterified because the proximity of the primary hydroxyl group to the oxygen of the pyranic ether bridge interaction from the hydrogen makes the hydroxyl group less available and more difficult for the lipase to access. Logically, sucrose can be esterified because it has two primary hydroxyl groups on the fructose ring. However, its low solubility and that of sucrose octaacetate in tert-butyl alcohol were probably the reasons for its inertness.

Fructose gave a better yield than glucose so it was used as the sugar source in further studies.

Effects of reaction temperature on the production of surfactant

The production of surfactant improved when the reaction temperature was increased, especially up to 40°C. The differences in yields

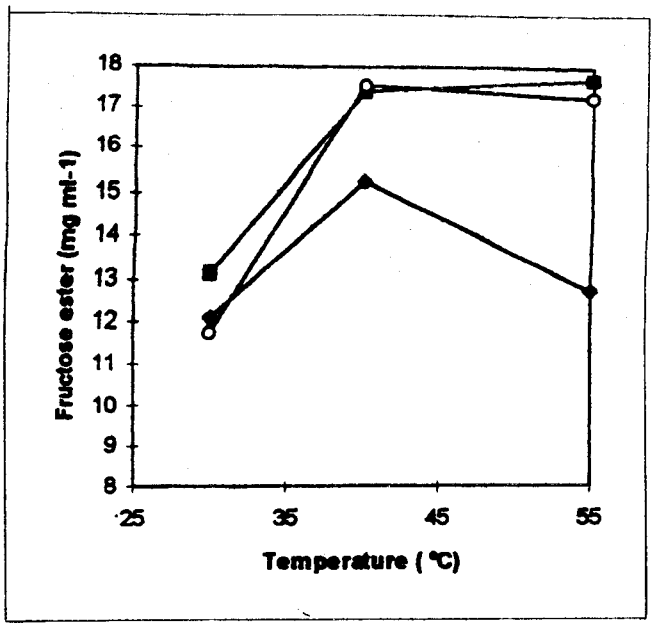


Figure 1. Effect of reaction temperature on the yield of fructose ester at molar ratio of fructose-PFAD of 1/10 and (◆) 5% lipase, (■) 10% lipase, (○) 20% lipase

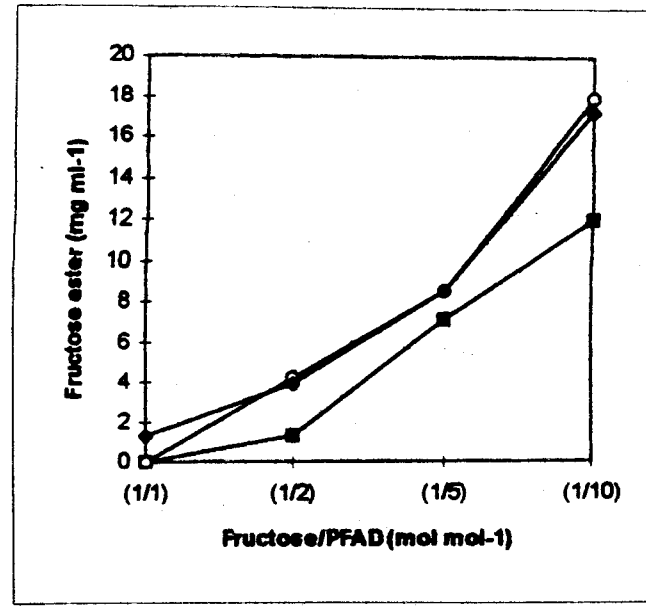


Figure 2. Effect of molar ratio of fructose: PFAD on the yield of fructose ester at 55°C for 24 hours, Lipase concentrations of (■) 5%, (○) 10%, and (◆) 20% (w/w substrate)

between reaction temperatures of 40°C and 55°C, except for 5% Lipozyme, were small *(Figure 1)*. Three reaction temperatures were tried as the temperature for interesterification or ester synthesis recommended by Novo (1992) is 30°C -70°C. Generally, the lower the reaction temperature the higher is the productivity of the enzymes. Also, solubility of the sugar in the organic solvent influenced the production of surfactant. As sugar is only slightly soluble in tert-butyl alcohol at room temperature, the production of surfactant at room temperature (28-30°C) was less than at higher temperatures. At 40°C, the yield was 17 mg/ml solvent as compared to 13 mg/ml solvent at room temperature.

Effects of molar ratio on the production of surfactant

A molar ratio of fructose:PFAD of 1:10 gave the highest yield (about 17.70 mg/ml solvent at lipase concentrations of 10% and 20%), showing that esterification occured better with excess PFAD *(Figure 2)*. Excess PFAD is required because water formed from the reaction is then proportionately minimal. The reaction is a reversible one and, under a low water regime, the enzyme functions "in reverse", that is, it synthesises ester bonds instead of hydrolysing (Miller *et al,* 1988). If the water formed is not removed, excess PFAD is required to create a low water regime to minimize hydrolysis.

Effects of lipase concentration on the production of surfactant

The lipase concentration also influenced the product yield. In the experiment, the yield increased until a lipase concentration of 10% of substrate (w/w), then decreased when the lipase concentration further increased to 20% *(Figure 3)*. This could be because fructose has two hydroxyl groups which can react with PFAD to produce fructose diester, and that the reaction is reversible and excess lipase acts as a catalyst for hydrolysis.

Effects of reaction time on the production of surfactant

Using a 10% lipase concentration at 55°C, the effect of reaction time on the yield of ester was

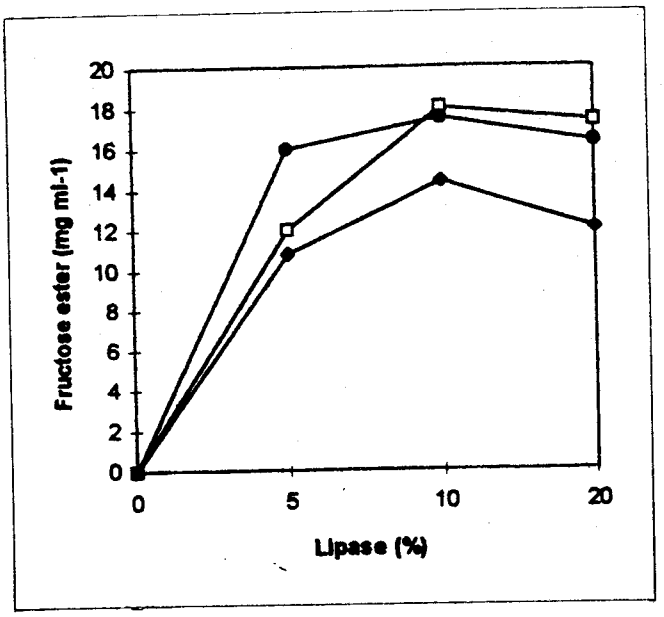


Figure 3. Effect of lipase concentrations on the yield of fructose ester in the molar ratio of fructose:PFAD of 1/10 at different temperature (◆) Ambient (28°C-30°C), (●) 40%°C, and (□) 55°C.

studied. The yield increased to 17.70 mg/ml solvent after 24 hours. Longer reaction times did not further increase the yield *(Figure 4).*

Comparison of surfactants produced in this study and previous studies

Previous studies on the preparation of sugar esters using chemical catalysts gave high yields of 87.6% - 99.6% (Akoh, 1994). The yield using a biocatalyst (this study) compared with the yields from previous studies are shown in *Table 2.*

In this study, the low yield could be due to water released during reaction as the reaction was done in batches and at atmospheric pressure. The yield may have been higher if the water formed was continuously removed during reaction, or by using a continuous process in which the contact time between substrate and catalyst is longer than in a batch process.

Even though this study produced a lower yield than previous studies, it showed that it is possible to prepare a surfactant from sugar, especially fructose, and PFAD catalyzed by *Mucor miehei.* The process can be further developed.

This study also tried to use the HPLC to separate out fructose ester from excess PFAD using a mixture of methanol and acetic acid as described by Oguntimein (1993) but was not

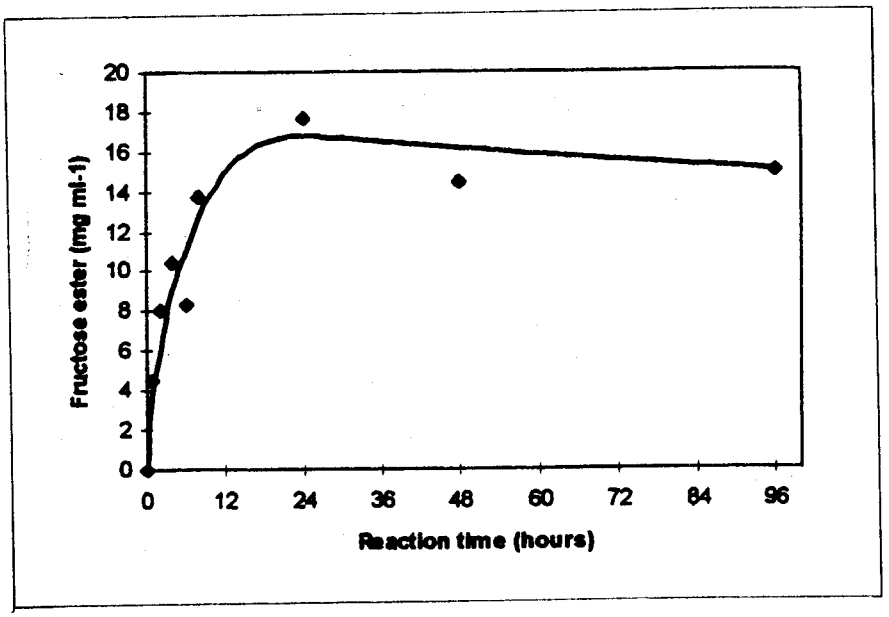


Figure 4. Effect of reaction time on the yield of fructose ester at 55°C and 10% lipase (w/w substrate)

TABLE 2. COMPARISON OF SURFACTANT YIELDS IN THIS AND PREVIOUS STUDIES

No.	Substrate		Lipase	Surfactant Production				Reference
	Sugar	Fatty acid		mg ml^{-1} solvent	mg mg^{-1} enzyme	mg mg^{-1} sugar	mg mg^{-1} FA	
			Candida cylindracea					
	(mg ml^{-1} solvent)		(mg ml^{-1} solvent)					
1.	Glucose (3.6)	Oleic (22.0)	4	10.7	2.67	2.97	0.49	Seino *et al.* (1984)
2.	Fructose (3.6)	Oleic (22.0)	4	12.2	3.05	3.39	0.55	Seino *et al.* (1984)
3.	Sucrose (4.0)	Oleic (11.3)	4	7.7	3.84	1.93	0.68	Seino *et al.* (1984)
4.	Sorbitol (3.6)	Oleic (22.5)	4	7.5	3.74	2.08	0.33	Seino *et al.* (1984)
			Porcine pancreatic					
	(L L^{-1} solvent)		(mg/ml)					
5.	Sorbitol (25.5)	Corn oil (0.15)	200	8.4	0.04	0.33		Chopineau *et al.* (1984)
6.	Sorbitol (25.5)	Olive oil (0.15)	200	6.4	0.03	0.25		Chopineau *et al.* (1984)
7.	Sorbitol (25.5)	Soybean Oil (0.15)	200	5.1	0.02	0.2		Chopineau *et al.* (1984)
8.	Sorbitol (25.5)	Triolein (0.15)	200	11	0.06	0.43		Chopineau *et al.* (1984)
			Candida antartica					
	mg (without solvent)		(% w/w)					
9.	Ethyl D-Gluco pyranoside (50)	Decanoic .. (55.7)	2.5	76.9	29.13	1.54	1.38	Bjorkling (1989) Lang *et al.* (1993)
10.	Ethyl D-Gluco pyranoside (50)	Tetradecanoic ..(73.9)	2.5	89.5	28.89	1.79	1.21	Bjorkling (1989) Lang *et al.* (1993)
11.	Ethyl D-Gluco pyranoside (50)	Octadecanoic ..(92.0)	2.5	131.8	37.12	2.64	1.43	Bjorkling (1989) Lang *et al.* (1993)
12.	Ethyl D-Gluco pyranoside	Octadecanoic ..(91.4)	2.5	126.2	35.7	2.52	1.38	Bjorkling (1989) Lang *et al.* (1993)
			Mucor miehei					
	mg ml^{-1} solvent)		(mg/mg solvent)					
13.	Fructose (10)	Oleic (156.8)	9	20	2.22	2	0.13	Khaled N (1993)
14.	Fructose (14.4)	Palm fatty acid Distillate (153.2)	17	17.7	0.74	1.23	0.12	This study

successful. This could be due to unsuitability of the column used and the insignificant difference in molecular weight between the ester and PFAD. A better solvent system is being sought to improve the separation. Using a mobile phase of methanol and acetic acid (99.7%/0.3%, v/v), the chromatograms are shown in *Figure 5a, b* and *c*. The standard used was sucrose monocaprate. The chromatograms showed that the product (fructose ester) had main peaks at retention times of 1.5 minutes (0.80%), 2.25 minutes (70.65 %), 2.61 minutes (7.70%) and 3.36 minutes (11.31%), compared to the sucrose monocaprate standard which had main peaks at 2.14 minutes (27.00 %), 2.27 minutes (36.70 %) and 3.34 minutes (18.06 %). The raw material, PFAD, had main peaks at 2.13 minutes (35.38%), 2.53 minutes (11.21%) and 3.27 minutes (22.68%). The results showed that the ester emerged at around 2.25 minutes.

The presence of ester was also confirmed by FT-IR Spectrophotometry which showed a product with absorption bands at wavenumber

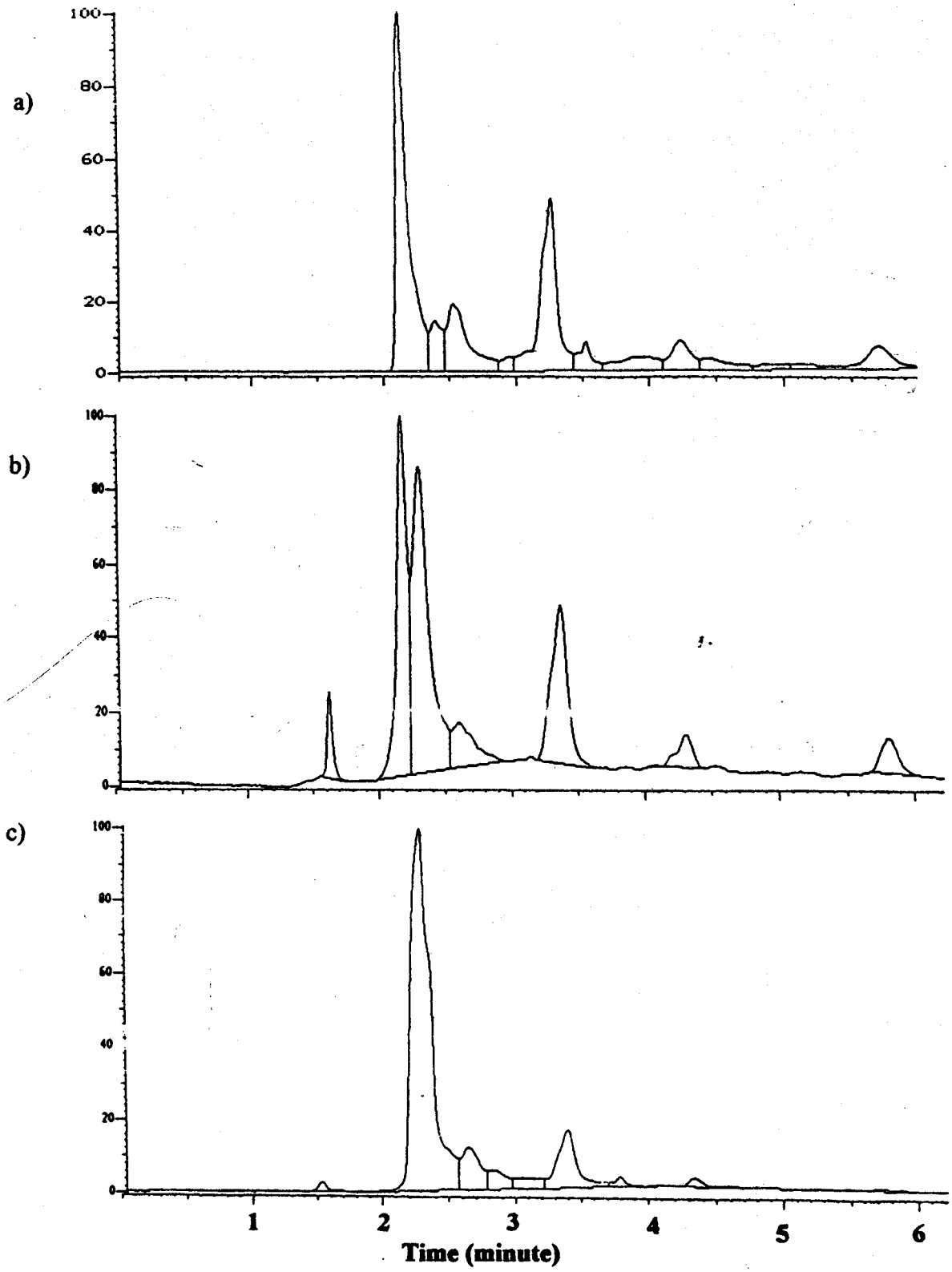


Figure 5. HPLC separation of (a) PFAD (raw material), (b) sucrose monocaprate standard, and (c) fructose ester (after purification) on Supelcosil LC18, 5µm column (250 mm x 4.6 mm) at a flow rate 0.7 ml/min and detected by a Gilson 116 UV detector at 280 nm. The mobile phase consists of methanol:acetic acid (99.7%:0.3% v/v).

3381 - 3407 cm^{-1} (for the OH bond), 2851 - 2919 cm^{-1} (for the C-H bond in $-CH_2$ or $-CH_3$), 1735 cm^{-1} (for the C=O ester bond), 1468 cm^{-1} (for $-CH_2$, $-CH_3$), 1055 - 1183 cm^{-1} (for the C-O ester bond), and 723 cm^{-1} (for the $(CH)_2$ bond) as shown in *Figure 6.*

One of the most important characteristics of a surfactant is its ability to reduce interfacial

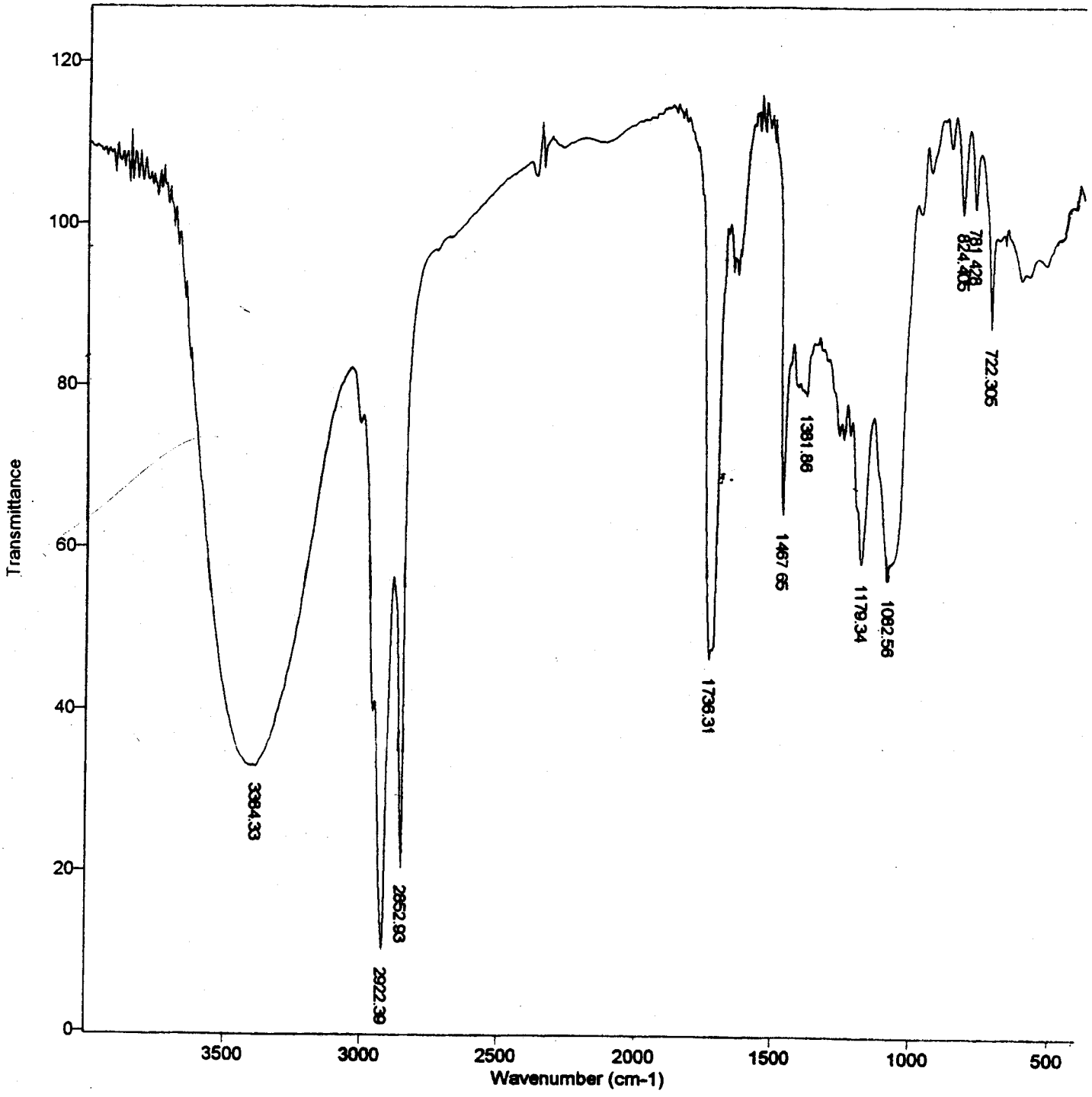


Figure 6. Infra red spectrum of fructose ester derived from fructose and palm fatty acid distillate after purification by column chromatography using solvent chloroform:methanol:water (64%:10%:1% v/v/v).

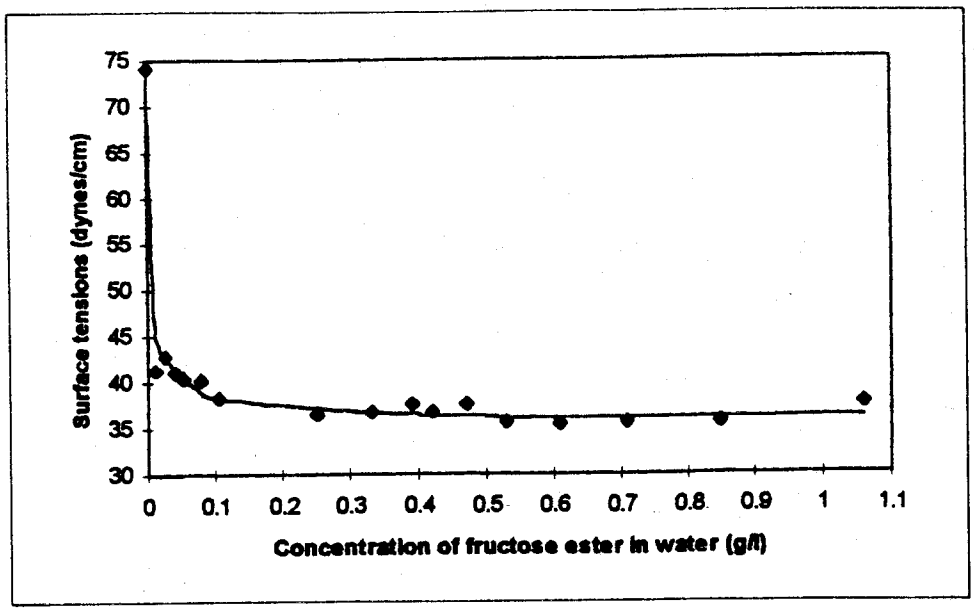


Figure 7. Reduction of surface tension of water by enzymatically prepared fructose esters.

and surface tensions of liquids. The fructose ester prepared reduced the surface tension of water from 74.2 dynes/cm to 38.3 dynes/cm. The optimum concentration of surfactant used was 1.06 g/l because of its low water solubility. The reduction of water surface tension shown in *Figure 7* was quite similar to that in a previous report which showed that fructose monooleate reduces the surface tension of water by up to 31.6 dynes/cm (Ducret, 1996).

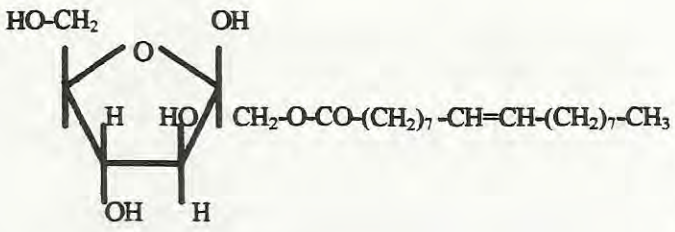
As mentioned above, the HLB is also an important characteristic of a surfactant. This study shows that biosurfactant derived from fructose and palm fatty acid distillate has an HLB value of 16+. This value indicates that the surfactant prepared could be utilized as solubilizing agent, detergent and oil in water (o/w) emulsifier.

Other physico chemical properties are shown in *Table 3*. Comparison with a chemically

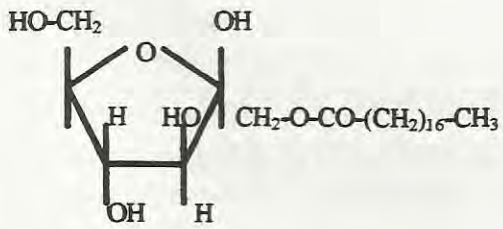
TABLE 3. PHYSICAL AND CHEMICAL PROPERTIES OF SURFACTANTS

No	Fructose ester (a)	Span 60 (b)
1. Melting point (°C)	49.2-52.3	54.2-55.3
2. HLB (c)	16+	5.0
3. Physical form	White-yellow wax	White powder
4. Saponification value (d)	459	
5. Solubility in:		
- Water (28°C)	Slightly soluble	Not soluble
- Water (60°C)	Emulsion	Not analyzed
- Chloroform	Soluble	Soluble
- Acetone (40°C)	Soluble	Soluble
- Methanol	Soluble	Slightly soluble

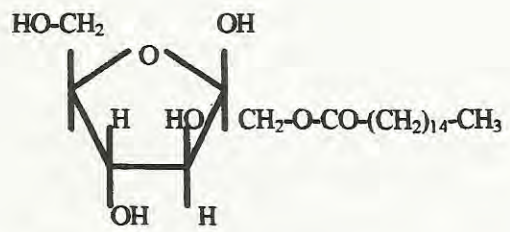
a) Derived from fructose and PFAD by enzymatic process.
b) Commercial sorbitan monostearat with HLB value of 4.7, synthesised by chemical process.
c) Hidrophyle-lipophyle balance, as analysed by the Gupta(1983) method
d) Analysed by PORIM standard method



a. Fructose mono-oleate



b. Fructose mono-stearate



c. Fructose mono-palmitate

Figure 8. Chemical structure of fructose esters of PFAD

prepared surfactant showed that fructose ester synthesized by enzymatic process has a HLB value higher than surfactant synthesized chemically and is more soluble in organic solvent. The data showed that fructose esters of palm fatty acids have a potential to be upgraded for use as biodegradable surfactants.

Depending on the fatty acid composition of PFAD, the chemical structure of the fructose esters produced may be varying mixtures of fructose mono-oleate, fructose mono-palmitate and fructose mono-stearate as shown in *Figure 8.*

CONCLUSION

PFAD, a by-product from palm oil refining, can be used to make sugar ester, a biodegradable surfactant for food, cosmetic and other industries. Fructose was esterified by PFAD using *Mucor miehei* as biocatalyst to produce about 17.70 mg/ml solvent of fructose monoester, while glucose esterified by PFAD produced 13.1 mg/ml solvent of product. The fructose ester biosurfactant had a melting point of 49-52.3 °C and HLB of 16+ and reduced the surface tension of water from 74 dynes/cm to 38.3 dyne/cm. With its characteristics, the surfactant can be used as a solubilizing agent, detergent and oil in water emulsifier. The yield of the product can be further increased by optimizing the process, such as by removing the water formed during reaction or by using a continuous process.

REFERENCES

AKOH, C C (1994). Enzymatic synthesis of acetylated glucose fatty acid esters in organic solvent. *J. Am. Oil Chem. Soc., 71*: 319-323.

ANON, (1993). Oleochemical provide 20% of U.S surfactants, *INFORM, 4*:1166.

BRANNA, T (1995). Surfactants '95. Household and personal product industry (Happi), 32: 91-103, 192.

CHAPINEAU, J; MCCAFFERTY, F D; THERISOD, M and KLIBANOV, A M (1988). Production of biosurfactants from sugar alcohols and vegetable oils catalyzed by lipase in nonaqueous medium. *Biotech. and Bioeng., 31*: 208-214.

DUCRET, A; GIROUX, A; TRANI, M and LORTIE, R (1995). Enzymatic preparation of biosurfactants sugars or sugar alcohols and fatty acids in organic media under reduced pressure. *Biotech. and Bioeng., 48*: 214-221.

DUCRET, A; GIROUX, A; TRANI, M and LORTIE, R (1996). Characterization of enzymatically prepared biosurfactants. *JAOCS, 73*: 109-113.

GUPTA, R K; JAMES, K and SMITH, F J (1983). Sucrose esters and sucrose ester/ glyceride blends as emulsifier. *JAOCS, 60*: 862-869.

JANSENN, A E M; LEFFERTS, A G and RIENT, K (1990). Enzymatic synthesis of carbohydrate esters in aqueous media. *Biotechnology Letters, 12*: 711-712.

JASPERS, M E A P; LEEUWEN, F F; NIEUWENHUIS, H J W and VIANEN, G M (1987). High Performance Liquid Chromatographic separation of sucrose fatty acid esters. *J. Am. Oil Chem. Soc., 64*: 1020-1025.

KHALED, N; MONTET, D; FARINES, M; PINA, M and GRAILLE, J (1992). Syntheses de mono-esters de sucre par biocatalyse, *Oleagineux. 47*: 181-189.

KHALED, N; MONTET, D; PINA, M and GRAILLE, J (1993) Fructose oleate synthesis in a fixed catalyst bed reactor. *Biotechnology letters, 13:* 167-172.

KOSARIC, N (ed.), (1993). *Biosurfactant*. Marcel Dekker, Inc., New York, pp. 483.

LANG, S and WAGNER, F (1993). Bioconversion of oil and sugar to glycolipids. *Biosurfactant* (ed: Kosaric, N.). Marcel Dekker, Inc., New York, pp. 483.

LJUNGER, G; ADLERCREUTZ, P and MATTIASSON, B (1994). Lipase catalyzed acylation of glucose. *Biotechnology Letters, 16*: 1167-1172.

MILLER, C; AUSTIN, H; POSORSKE, L and GANZIES, J (1988). Characteristic of an immobilized lipase for the commercial synthesis esters. *J. Am. Oil Chem. Soc., 65*: 927-931.

NOVO NORDISK, (1992). Use of Immobilized Lipases for Interesterification reactions and ester synthesis, Application sheet, Enzyme Process Division, pp. 4.

OGUNTIMEIN, G B ERDMANN, H and SCHMIDT, R D (1993). Lipase catalysed synthesis of sugar esters in organic solvent. *Biotechnology Letters, 15*: 175-180.

SARNEY, D B and VULFSON, E N (1995). Application of enzymes to the synthesis of surfactant. *Trend in Biotechnology, 13*:164-172.

SEINO, H and UCHIBORI, T (1984). Enzymatic synthesis of carbohydrate esters of fatty acids. *J. Am. Oil Chem. Soc., 61*: 1761-1765.

SHARMA, A and CHATTOPADHYAY, S (1993). Lipase catalysed acetylation of carbohydrates. *Biotechnology Letters. 13*: 1145-1146.