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PROPERTIES OF BIOSURFACTANT ENZYMATICALLY PREPARED FROM FRUCTOSE AND PALM FATTY ACID

Keywords: Biosurfactant, palm fatty acid, fructose ester, lipozyme.

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A biosurfactant has been successfully synthesized from fructose and palm *fatty acid distillate (PFAD) with Lipozyme IM as biocatalyst. Analysis of its* physical *and* chemical *properties showed that this surfactant* had *a melting point of 49°C-523°C* and *a hydrophile-lipophile* balance *(HLB) of 16+. This HLB value is in the* range *suitable for use in cosmetics, detergents and foods. This surfactant, which* was *a fructose ester, also reduced the surface tension of water from 74 dynes* cm^{-1} *to 38.3 dynes* cm^{-1}.

INTRODUCTION

Sugar esters, known since the fifties, are usually synthetic molecules which are rarely encountered in this form in nature. A sugar esterified with long-chain fatty acids can serve as a surfactant (Kosaric, 1993). This type of surfactant constitutes an important class of nonionic surfactants because of its good emulsifying, solubilizing and foaming characteristics. Besides, this surfactant is environmentally friendly (as it can be completely biodegraded under aerobic or anaerobic conditions), nontoxic, non-skin irritant, odourless and tasteless. Sugar esters can be used in many industrial applications, especially in food, personal-care products and household/laundry detergents (Khaled *et al.*, 1992). However, only the monoester and diester are of interest for cosmetic applications (Kosaric, 1993).

Sugar esters can be prepared by the transesterification of methyl esters with sugar in the presence of a basic catalyst in dimethylformamide or without a solvent (Osipow *et* al., 1956; Feuge *et al.*, 1970). However, the products from such a process are a mixture of mono-, di-, and tri-, polyesters of sugar, and specific products can only be obtained after extensive or expensive separation.

There is a growing interest in enzymatic esterification of sugar and fatty acids or fatty

acid derivatives, due to its specificity and mild conditions of reaction. From the economic viewpoint, it would be particularly advantageous to prepare sugar esters from fructose and PFAD which are both renewable resources. PFAD is a by-product of palm oil refining (generated at about 4% of the total palm oil refined). Currently, it is only used as a raw material for soap manufacturing (Rakmi et al., 1997).

The physico-chemical properties of a surfactant are very important in determining its economic value. One of the most important characteristics of a surfactant is its ability to reduce the surface and interfacial tensions of oil-water interfaces, as measured by the classical du-Nouy ring method (Kosaric, 1993; Lin, 1996). One of the most widely used index for evaluating surfactant activity is the critical micelle concentration (CMC). The CMC is in effect the minimum surfactant concentration required for reaching the lowest interfacial or surface tension value (Lin, 1996).

Another parameter frequently used for predicting surfactant behaviour is the HLB value. Generally, surfactants with HLB values of less than 6 are more soluble in the oil phase and act as W/O (water in oil) emulsifiers. Those with HLB values between 9 and 18 have the opposite characteristics, the surfactants are hydrophilic and act as solubilizing agents, detergent and O/W (oil in water) emulsifier (Attwod and Florence, 1983; Lin, 1996).

This paper reports some physico-chemical properties of a biodegradable and vegetable oil-based surfactant, fructose ester, which is prepared from fructose and PFAD via an enzymatic process using Lipozyme IM as biocatalyst.

MATERIALS AND METHODS

Materials

The materials used in this experiment are fructose from Merck, PFAD (without purification) and Lipozyme IM (brand name of the immobilized lipase of *Mucor miehei*, activity 6 BAUN g^{-1}) supplied by Novo, Malaysia. All organic solvents, including tert-butyl alcohol, are of HPLC or analytical grade.

Methods

Preparation. Fructose [1.44 g (8 mmol)] was mixed with 22.64 g (about 80 mmol) of PFAD and 10% w/w Lipozyme IM and 100 ml tert-butyl alcohol (2-methyl-2-propanol). The experiments were carried out in 250 ml flasks shaken by a mechanical shaker incubator (at 250 rpm) at 55°C for 24 hr. At the end of each batch reaction, the enzyme was removed by filtration and the solvent evaporated. The mixture of unreacted palm fatty acids and products were dissolved in chloroform. The unreacted sugar was removed by filtration and the products were identified by thin layer chromatography (TCL) using Kieselgel 60 and a mobile phase of chloroform/methanol/acetic acid/water (80/10/8/2 v/v/v/v) according to Khaled's method (Khaled et al., 1992).

Purification. About 20 g silica gel 60 were added to the mixture of unreacted palm fatty acid and the product dissolved in chloroform. The chloroform was then vacuum evaporated. The silica gel containing palm fatty acid and product was eluted with chloroform to separate the palm fatty acid from the product, and then eluted with chloroform/methanol/water (64/10/1 v/v/v) to isolate the fructose ester 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. The crude product was crystallized by adding warm acetone (40°C) and left overnight in a refrigerator.

Analysis. Qualitative analysis was carried out by HPLC (Gilson, model 714). Separation was achieved on a Supelcosil LC18, 5 µm column (250 x 4.6 mm), at a flow rate 0.7 ml min^{-1} 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 in the Oguntimein's methods (Oguntimein *et al.*, 1993).

The presence of ester was confirmed by infra red spectra which was recorded on FT-IR spectrophotometer (Bio-Rad model FTS 165), using a film of test surfactant between potasium bromide plates.

The surface tension was measured in distilled water at room temperature (28°C) using the du-Nouy ring method (Fisher surface

Tensiomat model 21). The hydrophile and lipophile balance was also analyzed by the Gupta's method, using pyridine/benzene (95/5 v.v) as solvent and titrated by using distilled water (Gupta et al., 1983). The melting point of surfactant was electrothermally determined. The solubilities of the surfactant in various organic solvents were also measured.

RESULT AND DISCUSSION

The HPLC method for separation of fructose ester and excess fatty acid with a mixture of methanol and acetic acid was carried out as described by Oguntimien et al. (1993). Using a mobile phase consisting of methanol and acetic acid (99.7/0.3, v/v), the chromatograms are shown in Figures la, *lb* and *lc.* The standard used was sucrose monocaprate. The chromatogram showed that the products (fructose ester) had main peaks at the retention times of 1.5 min (0.8%), 2.25 min (70.65%), 2.61 min (7.7%) and 3.36 min (11.31%). Comparatively, the sucrose monocaprate standard had main peaks at 2.14 min (27%), 2.27 min (36.70%) and 3.34 min (18.06%), while the raw material, palm fatty acids had main peaks at 2.13 min (35.38%), 2.53 min (11.21%) and 3.27 min (22.68%). The results showed that the ester emerged at around 2.25 min.

The presence of ester was also confirmed by FT-IR spectrophotometer analysis which showed that the product had absorption bands at wavenumber 3381– 3407 cm (for the OH bond), 2851-2919 (for the C-H bond in $-CH_2$ or $-CH_3$), 1735 (for the C=O, ester bond), 1468 (for $-CH_2$, $-CH_3$), 1055-1183 (for the C-O, ester bond), and 723 [for the $(CH)_2$ bond] as shown in ***Figure 2.***

One of the most important characteristics of a surfactant is its ability to reduce interfacial and surface tensions. The fructose ester prepared in this experiment could reduce the surface tension of water from 74.2 to 38.3 dynes cm-i. The optimum concentration of surfactant used was 1.06 g l^{-1} because of its low water solubility. The reduction of water surface tension shown in ***Figure*** 3 is quite similar to that in a previous report which showed that fructose monooleate could reduce the surface tension by up to 31.6 dynes cm^{-1} (Ducret et al., 1996).

As mentioned above, the HLB is an important characteristic of a surfactant. This study shows that the biosurfactant derived from fructose and PFAD had an HLB value of 16+. This value indicates that the surfactant prepared was hydrophilic and could be utilized as a solubilizing agent, detergent and oil in water (O/W) emulsifier.

Other physico-chemical properties are shown in ***Table 1.*** Comparison with a chemically prepared surfactant showed that the fructose

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES OF RESULTANT SURFACTANT

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

Notes:

[a] Derived from fructose and PFAD by enzymatic process.

[b] Commercial sorbitan monostearate with HLB value of 4.1, synthesized by chemical process.

[c] Hydrophile-lipophyle balance, as analyzed using the Gupta's method.

[d] Analyzed using PORIM standard method.

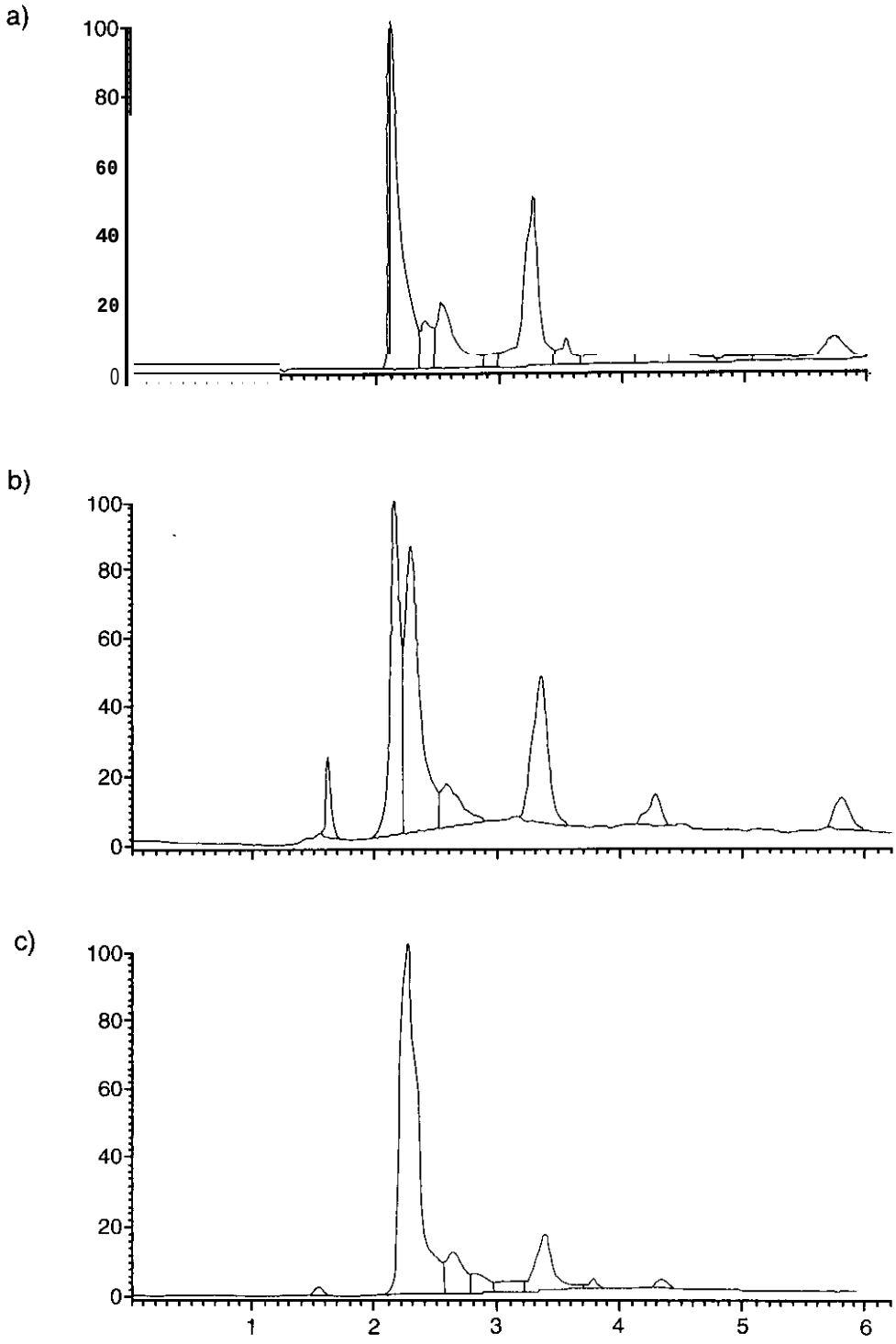

Figure I. HPLC separation of a) PFAD (raw material), b) sucrose monocaprate standard, and c) fructose ester (after purification) on Supelcosil C18, 5 μm column (250 x 4.6 mm) at a flow rate 0.7 ml min^{-1} and detected by Gilson 116 W detector at 280 nm. The mobile phase consisting methanol:acetic acid (99.7:0.3 v/v).

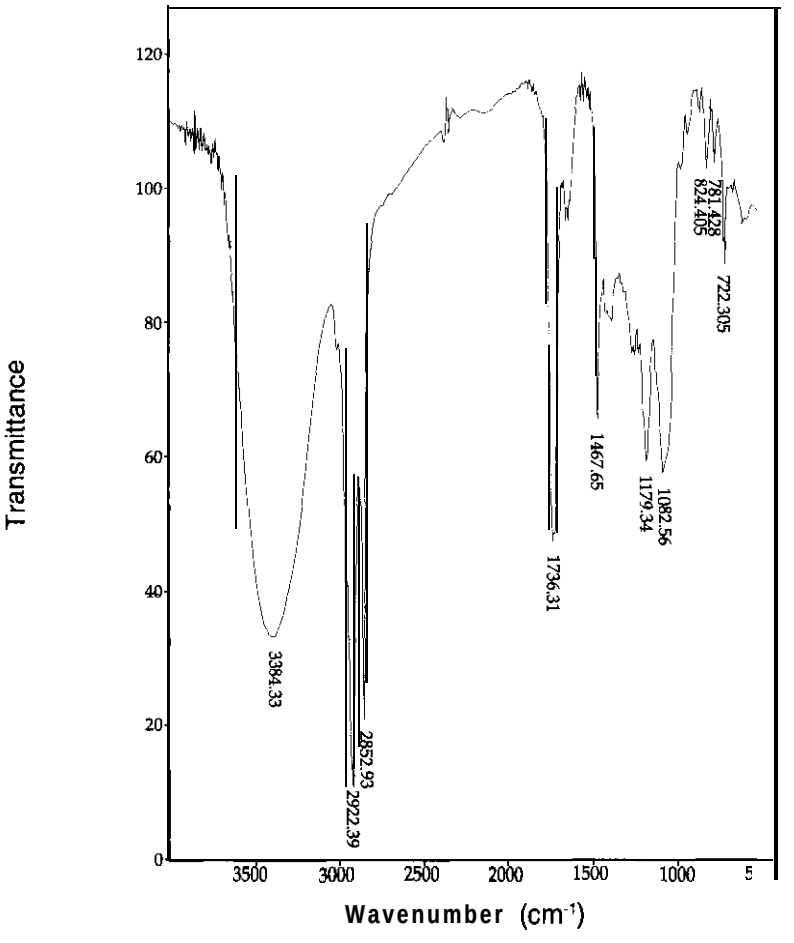

Figure 2. Infra red spectrum of fructose ester derived from fructose and PFAD after purification.

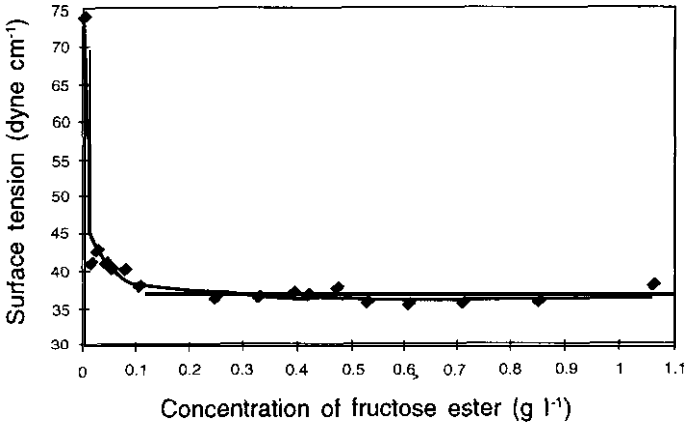

Figure 3. Reduction of surface tension of water product by enzymatically prepared fructose esters.

ester synthesized by an enzymatic process had a higher HLB value and was more soluble in organic solvents. The data showed that fructose esters of palm fatty acid origins have a potential to be upgraded for utilization as biodegradable surfactants.

CONCLUSION

A biosurfactant can be enzymatically synthesized from fructose and PFAD using Lipozyme as biocatalyst. The biosurfactant synthesized by this process had a melting point of 49°C-52.3°C and a HLB of 16+ and reduced the surface tension of water from 74 to 38.3 dynes cm^{-1}. With these characteristics, this surfactant can be used as solubilizing agent, detergent and oil in water emulsifier and can be promoted as a higher added value product derivable from palm oil.

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