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Mango kernel fat: A novel lipid source for the fermentative production of sophorolipid biosurfactant using *Starmerella Bombicola NRRL*-Y 17069

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ABSTRACT

It is found that the fat extracted from mango seed kernel is edible and is rich in stearic and oleic acid, which could be used as surfactant hydrophobe for the synthesis of bio-surfactants. This article describes downstream processing of mango kernel fat (MKF) to isolate its fatty acid fractions. Further the unsaturated fatty acid fraction (mango kernel olein) has been exploited as a surfactant hydrophobe for the fermentative production of sophorolipids by solid state fermentation as well as submerged fermentation at a shake flask scale using starmerella bombicola NRRL-Y 17069. Yield of 17.48 grams of sophorolipids per 100g of substrate (17.48% conversion) was obtained by solid state fermentation, while the submerged fermentation resulted in a yield of 5.8 grams of sophorolipids per 100 grams of substrate (5.8 % conversion). Thus here we have successfully carried out simple downstream process to obtain stearin fraction and olein fraction from mango kernel fat and its olein fraction was successfully used as a novel lipid source for the production of sophorolipids. Further, to the best of our knowledge we have obtained highest yield of a microbial bio-surfactant by solid state fermentation technology. This is also the first report on the possibility of production of sophorolipids by solid state fermentation technique.

Key words: Sophorolipids, biosurfactant, Mango kernel fat, Mango kernel olein, Downstream processing, Solid state fermentation.

INTRODUCTION

India's production of Mango (*Mangifera indica*) is 7-8 million tons per year which can be a used as a novel renewable source of fatty acids since processing of the fruit leaves a considerable amount of seeds, which represents up to 25% of the weight of the fruit and which goes to the waste. It has been estimated that in India alone, 30,000 tons of Mango kernel fat (MKF), which is found to have high content of stearic and oleic acid could be industrially obtained each year [1]. Further MKF being a non traditional and renewable resource and because it is produced from the seed portion of the fruit that generally goes to waste makes MKF a potentially cheap lipid source as a surfactant hydrophobe for the synthesis of bio-surfactants. Thus the objective of the present work was to carry out downstream processing of the MKF to obtain an oleic acid rich fraction, which could be used as a cheaper alternative to the oleic acid, which is usually used for the fermentative production of sophorolipids. This paper describes a simple method for the downstream processing of MKF to obtain stearic acid rich saturated fraction of fatty acids (referred as stearin fraction) and oleic acid rich unsaturated fraction (referred as olein fraction). Further this paper describes the fermentative production of a glycolipid class of microbial biosurfactant named "sophorolipid" from the olein fraction of MKF.

Sophorolipids are surface-active glyco-lipid class of microbial biosurfactants, synthesized by few of the non-pathogenic yeast species like *Candida bombicola* (Starmerella bombicola)[2,3], *Wickerhamiella domericqiae* [4], *Rhodotorula bogoriensis* [5], *Candida Tropicalis* [6] etc. Microbial bio-surfactants are biodegradable and posses excellent interfacial properties [7], thus they have been focused as advanced surfactants for the use in food, cosmetics, pharmaceutical and environmental industries. [8,9] The emulsifying property of sophorolipids can be used in the food industry to improve the quality of wheat flour products and in the cold storage transportation in air conditioning systems for the prevention of ice particle formation.[10] Further it has been reported that the sophorolipid alkyl esters, enhances the characteristics of the prepared food products such as bakery and oily emulation. [11]

MATERIALS AND METHODS

Materials

Potassium hydroxide and pre-coated thin layer plates of silica gel G was procured from Merck (India) Ltd. Mumbai. All the solvents used (Ethyl alcohol, Ethyl acetate, Diethyl ether, n-Hexane, petroleum ether 60-80^0 C and acetic acid glacial) were of AR grade and were purchased from s.d.fine chem. Ltd. Mumbai. Sulfuric acid and oleic acid were purchased from s.d.fine chem. Ltd. Mumbai. Stearic acid was obtained from Fine Organics Ltd. MKF sample used was a kind gift from Charbhuja Trading and Agencies Pvt. Ltd. Mumbai.

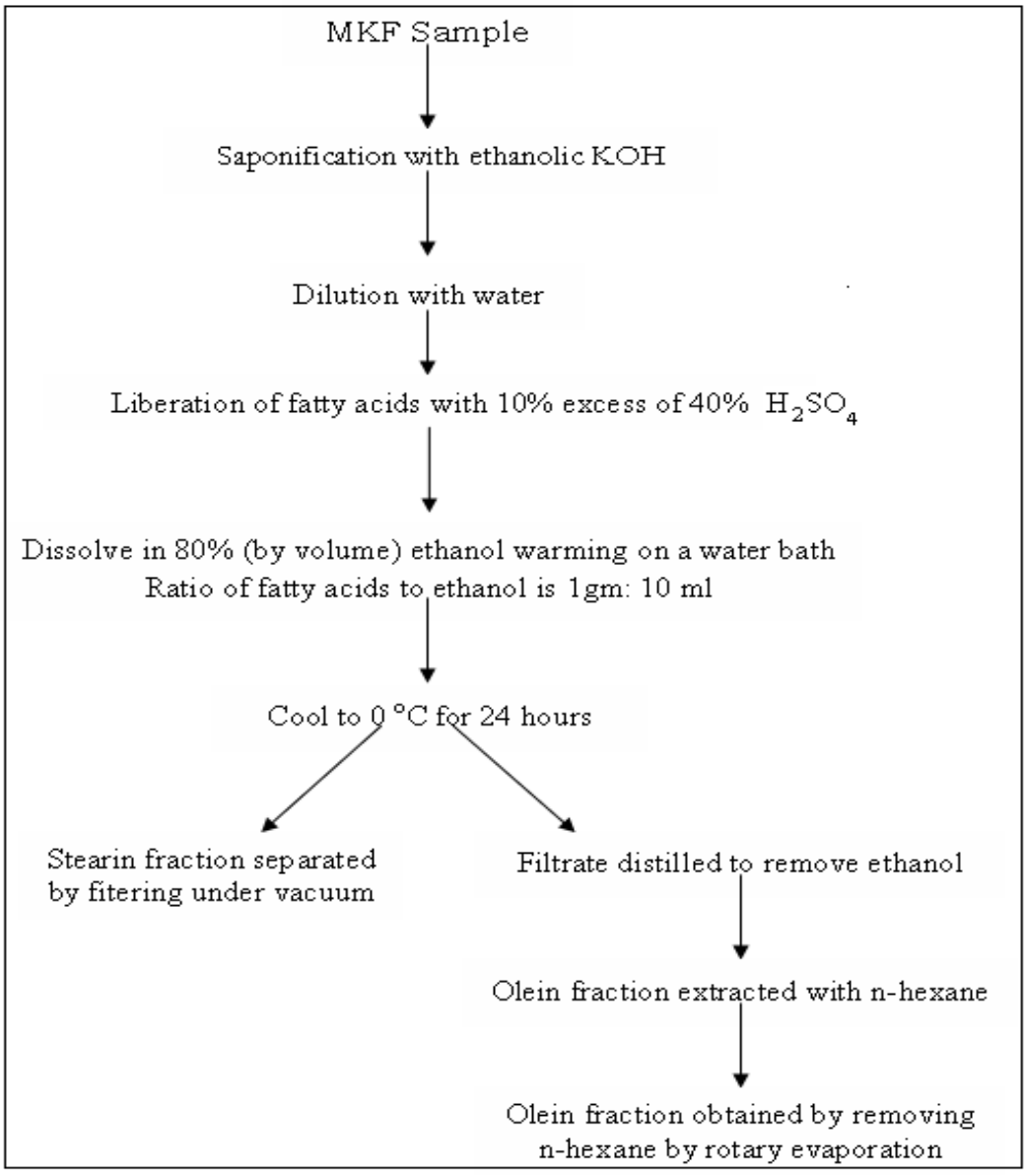

Figure 1: Downstream processing of MKF

Downstream processing of MKF:
A simple approach was used for obtaining stearin and olein fraction from MKF. A schematic representation of downstream processing of MKF is depicted in Figure 1. 20 g of MKF sample was dissolved in 100 ml of 1 Molar solution of Potassium hydroxide (KOH) in ethanol and the resultant mixture was refluxed for 3-4 h in order to carry out complete saponification of fat. This was followed by the removal of approximately half the alcohol (50 gm) by distillation. The sample from the previous step was diluted with 40 ml of water. The mixture of fatty acids was liberated from their soaps by the addition of a 10% excess of 40% solution of sulfuric acid .The separated fatty acids (mixture) were then filtered under vacuum using cold filtration unit. The fatty acid layer was washed with distilled water to remove entrained sulfuric acid. An accurately weighed quantity of the separated fatty acid mixture was dissolved in required quantity of 80% ethanol to obtain 10% w/v solution. The solution was cooled to 4^{o} C and kept for 24 h. The stearin fraction consisting of saturated fatty acids which remained solid was then filtered under vacuum using previously cooled filtration assembly. The stearin fraction was washed with 20 ml of 80 % chilled ethanol (at 4^{o} C). The filtrate obtained, which contained the solution of the olein fraction in ethanol, was distilled off to remove the ethyl alcohol. The unsaturated fatty acid fraction was then extracted with n-hexane. Finally the olein fraction was obtained by removing n-hexane by rotary evaporation.

Characterization of isolated fatty acid fractions:
The presence of stearic and oleic acids in the individual fractions was confirmed using thin layer chromatography, with commercial stearic and oleic acids as standard compounds. The details of the TLC procedure adopted are as follows: Sample: Stearin fraction, Olein fraction, Standard: Stearic acid, Oleic acid, Mobile Phase: Petroleum ether: Diethyl ether: Glacial acetic acid (90:10:1 v/v), Method for detection: Charring with Anisaldehyde solution on a hot plate.

Microorganism:
Starmerella bombicola NRRL Y-17069, was obtained from ARS Culture Collection, USA. The organism was maintained on Potato Dextrose Agar (PDA) slants and was sub-cultured monthly.

Preparation of the pre-culture and inoculum:
The 250-ml Erlenmeyer flasks containing 50 ml of the medium (100 g/L of glucose, 10 g/L of yeast extract and 1 g/L of urea) were inoculated with one loop from a 48 h slant and were incubated in a rotary shaker for 48 hr at 30 °C and 180 rpm to produce the inoculum.

Sophorolipid production by submerged fermentation:
The media for the production of sophorolipid contained 40g/L of glucose, 5g/L of yeast extract and 20g/L of various lipid sources (Mango kernel fat, mango kernel olein, oleic acid, mango stearin, stearic acid). pH of the fermentation media was maintained at pH of 3 by using 0.1 M citrate buffer. Two ml of the inoculum was added to the 50ml of the autoclaved media in a 250ml Erlenmeyer flask and was incubated in a rotary shaker at 30ºC and 180 rpm. Lipid substrate was added aseptically on the 48^{th} h after inoculation and the fermentation was allowed to take place further for a total period of 240 h.[12]

Sophorolipid production by solid state fermentation technique:
A total of 4g of substrate containing 2g of glucose and 2g of lipid substrate was blended with 6g of wheat bran powder (which was used as a solid support) in a 250 ml Erlenmeyer flask and was moistened with 8 ml of 0.1M citrate buffer of pH 4 and was sterilized by autoclave. After cooling, the flasks were inoculated with 2 ml of the inoculum; further a very small additional quantity of buffer was added to increase the moisture content up to 50% w/w. And then the contents, after mixing, were incubated at 30°C for 240 h.

Isolation of sophorolipids:
Sophorolipids were extracted by mixing fermented substrate of each flask with 40 ml ethyl acetate and then shaking the mixture in an orbital shaker 250 rpm for 1h. The suspension was then centrifuged at 5000×g for 15 min for separating the ethyl acetate extract from the fermented substrate. The solvent was then removed from the extract by rotary evaporation. The amber colored, honey like semi-crystalline sophorolipids was washed twice with 20 ml of n-Hexane to remove the unused lipid residue.

Structural characterization of synthesized sophorolipids:
Structural identity of the synthesized sophorolipids was confirmed by FTIR spectroscopy.

RESULTS AND DISCUSSION

Downstream processing of MKF:

MKF contains a high proportion of stearic acid (40-45%) and oleic acid (40-50%) while other saturated and unsaturated fatty acids are present in very small concentrations. Thus due to relative simplicity and the uniqueness of the fatty acid profile of the MKF made it possible to employ a simple method for the downstream processing of MKF to obtain high yields of stearin fraction (8.2 ± 0.5 g) and olein fraction (10.0 ± 0.6 g) from 20 g of MKF. The method reported here for the downstream processing of MKF can be easily adopted on a lab scale; however the economics and the scalability of the same for the industrial application will require further work

Characterization of isolated fatty acid fractions

The presence of stearic acid in the separated stearin fraction and oleic acid in separated olein fraction was confirmed by TLC. The R_f values of the standard stearic acid, oleic acid and separated stearin and olein fractions from MKF is depicted in Table 1.

Table 1: R_f values of Stearin and Olein Fractions

Compound	R_f value
Stearic acid	0.61
Oleic acid	0.54
Stearin fraction	0.62
Olein fraction	0.53

Sophorolipid production by submerged fermentation and solid state fermentation:

Comparitive yields of sophorolipids produced under submerged fermentation and under solid state fermentation conditions with different lipid sources are depicted in Figure 2.

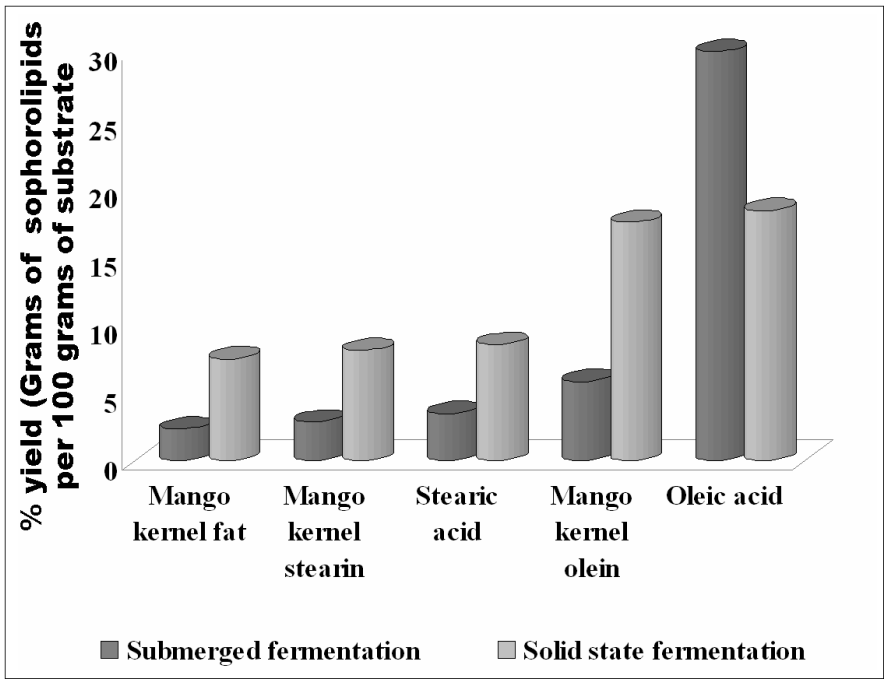

Figure 2: Comparative yields of sophorolipids obtained under solid state and submerged state fermentating conditions using different lipid sources.

It was observed that the mango kernel fat and stearin fraction of mango kernel fat as a lipid source resulted in a lower sophorolipid yields under both submerged state and solid state fermentation conditions compared to olein fraction of mango kernel fat. The olein fraction of mango kernel fat was found to be useful as a novel lipid source for the fermentative production of sophorolipids by both submerged and solid state fermentation. Further, under the solid state fermentation conditions, the yields of sophorolipids obtained by mango kernel olein as a lipid substrate was comparable to that of yields obtained by conventional oleic acid as a lipid source. Use of oleic acid as the standard lipid source yielded the highest sophorolipid yields under both solid state as well as submerged condition but only oleic acid as lipid source gave higher yields under submerged condition then under solid sate fermentation condition, while all the other lipid sources gave relatively better yields under solid state fermenting conditions than

under submerged state fermenting conditions. The yield of sophorolipids obtained by various lipid sources under different fermentation conditions is depicted in Table 2.

Table 2: Production of sophorolipids using various lipid sources

Lipid source	Sophorolipid yield (Grams of sophorolipids obtained per 100g of substrate)	
	Submerged fermentation	Solid state fermentation
Mango kernel fat	2.32	7.48
Mango kernel stearin	2.84	8.1
Stearic acid	3.46	8.5
Mango kernel olein	5.80	17.48
Oleic acid	30.0	18.32

Structural characterization of synthesized sophorolipids:

The FTIR spectra of both the sophorolipids synthesized by solid state fermentation and sophorolipids synthesized by submerged fermentation showed mostly identical absorption bands. FTIR Spectrum of the sophorolipids synthesized by Solid state fermentation is depicted in Figure 3(a). The broad band at 3396.2cm−1 corresponds to the OH stretch. The asymmetrical stretching and symmetrical stretching of methylene (CH2) was observed at 2929.6 and 2855.9 cm−1 respectively. The C=O absorption band at 1744.4 cm−1 includes contributions from that of lactones, esters, or acids, while the C=O absorption band from acetyl esters was observed at 1243.4 cm−1. C-O stretch from C-O-H groups of sugar (sophorose moiety) is observed at 1036.8 cm−1. The band at 1460.2 cm−1 that corresponds to the C-O-H in-plane bending of carboxylic acid (COOH) which indicates the presence of small quantities unused olein fraction that was left after the hexane washings. FTIR Spectrum of the sophorolipids synthesized by submerged fermentation method is depicted in Figure 3(b). The broad band at 3398.5cm−1 corresponds to the OH stretch. The asymmetrical stretching and symmetrical stretching of methylene (CH2) was observed at 2927.8 and 2855.2 cm−1 respectively. The C=O absorption band at 1742.8 cm−1 includes contributions from that of lactones, esters, or acids, while the C=O absorption band from acetyl esters was observed at 1242.5 cm−1. C-O stretch from C-O-H groups of sugar (sophorose moiety) is observed at 1036.8 cm−1. The band at 1460.2 cm−1 that corresponds to the C-O-H in-plane bending of carboxylic acid (COOH) which indicates the presence of small quantities unused olein fraction that was left after the hexane washings.

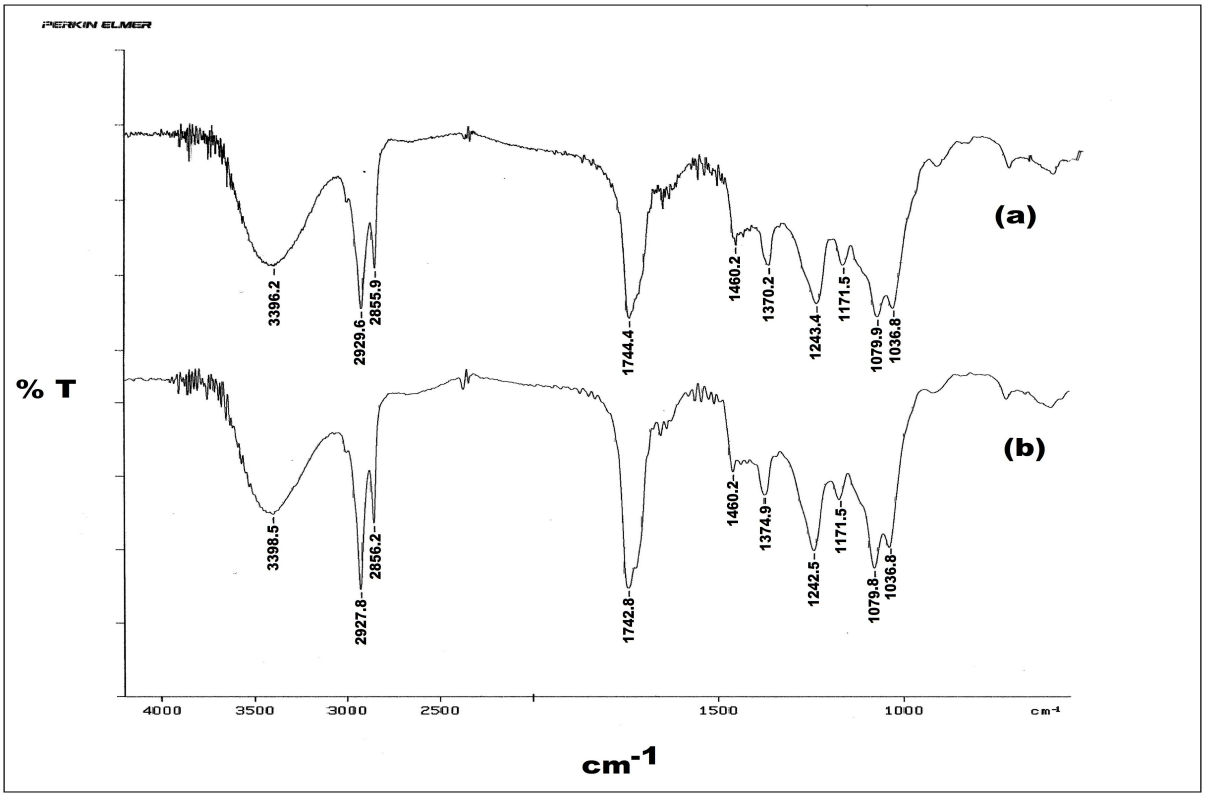

Figure 3: FTIR spectra of sophorolipids synthesized using mango kernel olein. (a): by solid state fermentation; (b): by submerged fermentation.

CONCLUSION

Considering the fact that, the mango kernel fat is obtained from mango seeds, which is the portion of the fruit which usually goes to the waste and thus the mango kernel fat can be obtained very cheaply. It has been estimated that in India alone, 30,000 tons of Mango kernel fat, could be industrially obtained each year. Further the mango kernel fat is found to be edible and is found to have high content of stearic and oleic acid which can be obtained easily from Mango kernel fat by a simple downstream process. Thus here we have successfully carried out simple downstream process to obtain stearin fraction and olein fraction from mango kernel fat and its olein fraction was successfully used as a novel lipid source for the production of sophorolipids. Further, to the best of our knowledge we have reported highest yield of a microbial bio-surfactant by solid state fermentation technique. Also this is the first report on the possibility of production of sophorolipids by solid state fermentation technique.

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REFERENCES

[1] PP Dandekar; VB Patravale, *Indian Journal of Chemical Technology*, **2009**, 16(4), 317-321.
[2] PA Felse; V Shah; J Chan; KJ Rao; RA Gross, *Enzyme Microb Technol*, **2007**, 40: 316-323.
[3] G Perkin; FV Sukan; N Kosaric, *Eng Life sci*, **2005** , 5(4): 357-362.
[4] C Jing; S Xin; Z Hui; QU Yinbo, *Enzyme Microb Technol*, **2006**, 39: 501–506.
[5] A Nunez; R Ashby; TA Foglia; DKY Solaiman, *Biotechnol Lett*, **2004**, 26: 1087–1093.
[6] S Sudha; R Kumanan; K Muthusamy, *Der Pharmacia Lettre*, **2010**, 2(2): 155-158.
[7] S Lang, Curr. Opin. *Colloid & Interface Sci*, **2002**, 7, 12-20.
[8] D Kitamoto; H Isoda; T Nakahara, *J. Biosci. Bioeng*, **2002**, 94, 187-201.
[9] INA Van Bogaert; K Saerens; CD Muynck; D Develter; W Soetaert; EJ Vandamme, *Appl. Microbiol. Biotechnol*, **2007**,76, 23-34.
[10] K Masaru, N Takashi, A Yoji, N Kazuo, N Tatsu, T Sumiko, N Jotaro, Japanese patent, 2001131538 **(2001).**
[11] RP Allingham, US patent, 3622344 **(1971).**
[12] VJ Parekh; AB Pandit, *Int. J. Pharm.Bio.sci*, **2011**, 1(3): 103-116.