SHORT COMMUNICATION

Soy Lecithin–Monoester Interchange Reaction by Microbial Lipase

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ABSTRACT: Modification of the fatty acid composition of soy lecithin, principally at its 1-position, was investigated by interchange reaction with the methyl ester of individual fatty acids and a lipase as the catalyst. The consequent effect on the surface activity of soy lecithin was also examined. The interchange reaction was carried out by heating a mixture of soy lecithin and methyl ester of a fatty acid at 60°C for 48 h with 10% (by weight of the reactants) *Mucor miehei* lipase. The lipase was filtered from the reaction mixture, and the product was isolated by combination of acetone extraction, which removed the methyl ester fraction, and by preparative thin-layer chromatography separation. The soy lecithin showed distinct change in its fatty acid composition in the *sn*-1 position. Capric acid was incorporated by 8.4%, while lauric acid and myristic acid were introduced at 14.1 and 15.7%, respectively. The linolenic acid percentage was increased by about 10 units. The interfacial tension of soy lecithin changed significantly after incorporation of various saturated fatty acids.

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KEY WORDS: Interchange reaction, interfacial tension, *Mucor miehei* lipase, soy lecithin.

Phospholipids are well recognized for their surface-active properties, and soy phospholipids from soybean oil constitute a major natural source of phospholipids in commerce. They find extensive use because of their composition and surface-active properties in food, cosmetics, pharmaceutical, medicinal, and industrial applications (1–3). Considerable interest is growing to alter their existing fatty acid profile to extend their scope of applications. Soy phospholipids are receiving renewed interest for modifications by chemical and biochemical reactions. Modified phospholipids could have better surface-active properties and nutritional quality than the original compounds. Work done so far on modification of phospholipids is not extensive, and there is opportunity for more research to meet more specific utilization of phospholipids.

A report on the lipase-catalyzed regiospecific 1-position transesterification of fatty acids in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was published by Brockerhoff *et al.* (4). They used *Rhizopus delemar* lipase in buffer with PC and oleic acid as substrates. Yoshimoto *et al.* (5) used ethylene glycol-modified *Candida cylindracea* lipase, dissolved in benzene, to incorporate polyunsaturated fatty acids into PC. A two-phase water/oil system was used by Yagi *et al.* (6) for transesterification of PC and PE with different fatty acids. Yoichiro and Setsuko (7) also used a water/oil system but used sardine oil as a source of polyunsaturated fatty acids for incorporation into soy phospholipid. Incorporation of heptadecanoic acid into egg PC by an immobilized lipase was accomplished by Svensson *et al.* (8). They also investigated the controlled water activity during the transesterification reaction of PC and heptadecanoic acid with various lipases (9). Mutua and Akoh (10) successfully incorporated n-3 polyunsaturated fatty acids in the phospholipid moiety with the aid of *Mucor miehei* lipase.

Transesterification of a fatty acid in the 2-position should be possible with phospholipase A_2, but so far no practical method for doing this has been presented. It has been easier to carry out esterification between lysophosphatidylcholine and free fatty acid, but the yield of PC was only in the range of 6–7% (11).

The phospholipid–monoester interchange reaction with the help of a specific lipase also can be utilized in fortifying phospholipids in their fatty acid profile at the 1-position. By this type of ester-ester interchange, it may be possible to introduce a specific fatty acid molecule in the phospholipid moiety. The advantage of using 1,3-specific lipases for transesterification of phospholipid is to protect the natural fatty acid composition in the 2-position, which is mainly enriched with the unsaturated fatty acids.

The present study aimed at introducing fatty acids, such as capric ($C_{10:0}$), lauric ($C_{12:0}$) and myristic ($C_{14:0}$), to enhance the surface-active properties of soy lecithin. An effort has also been made to increase the content of polyunsaturated fatty acids, such as linoleic and linolenic acid, in soy lecithin by transesterification of soy lecithin with the methyl ester of linseed oil.

MATERIALS AND METHODS

Substrate and enzyme. Crude soy phospholipid was supplied by M/s. Dyechem (Indore, M.P., India) and was deoiled by extraction of neutral lipids with acetone. To obtain lecithin,

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i.e., PC, the deoiled soy phospholipid was fractionated by refluxing it with six times the amount of ethanol at 40°C for 30 min (12). The ethanol solution, which was rich in lecithin, was concentrated in a rotary evaporator to get lecithin concentrate.

The immobilized lipase *M. miehei* (lipozyme IM 20) was a gift of NOVO A/S (Copenhagen, Denmark). Fatty acids (C_{10}, C_{12}, C_{14}) were purchased from E. Merck (India) (Worli, Bombay, India). Methanol, ethanol, and all other solvents and chemicals were reagent-grade. Refined and bleached linseed oil was obtained from M/S. V.K.V.K. Oil Industries (Calcutta, India).

Methyl ester of fatty acids, i.e., methyl caprate, methyl laurate, methyl myristate and methyl ester of linseed oil, were prepared in the laboratory.

Ester-interchange reaction. Soy lecithin (about 1.0 g) and different methyl esters in 1:5 molar ratio (to solubilize PC) were taken in a 50-mL round-bottomed flask and stirred by a magnetic stirrer at 60 ± 2°C for 48 h with 10% (by weight of the reactants) of *M. miehei* lipase (lipase initially contained 10% w/w water). A typical example of the proportion of reactants used was 1 g (1312 µmoles) and 1.2 g (6452 µmoles) of methyl caprate. After the reaction, the product mixture was filtered to remove the enzyme. Transesterified PC was isolated by separation from the mixed methyl esters through extraction with acetone. The final separation was done on a preparative thin-layer chromatography (TLC) plate.

TLC. A sample from the reaction product was dissolved in chloroform, and aliquots of 50 µL were applied as bands on TLC plates (Silica Gel G, 0.2 mm). The solvent used to separate PC from the methyl esters and fatty acids, if any, was hexane/diethyl ether (80:20, vol/vol). Bands on the TLC were visualized by iodine vapor. The PC band was scraped off and eluted by Folch solvent (methanol/chloroform, 2:1).

Fatty acid analysis of transesterified PC. The solvent was removed from the eluted PC product by evaporation under vacuum. The recovered product was methylated by adding 1 mL diethyl ether and 2 mL of 0.5 N methanolic KOH, followed by shaking the mixture for 10 min. HCl (2 mL, 1 N) was added, and the methyl esters of fatty acids were extracted with petroleum ether (40–60°C) (13). Gas-chromatographic analysis followed.

Gas chromatography. Fatty acid methyl esters were analyzed on a Hewlett-Packard gas chromatograph (HP 5890A) (Palo Alto, CA), equipped with a flame ionization detector (FID). The column was packed with Chromosorb-WHP (Supelco, Bellefonte, PA), coated with DEGS (6′ × ⅛″ i.d.). The column temperature was programmed between 100–190°C. The different fatty acid methyl esters and the standard sample were separated on the same column under identical conditions. N_2 as a carrier gas was used at 30 mL/min.

Interfacial tension measurement. Interfacial tensions of different transesterified lecithins were measured by the drop volume method with an Agla Micrometer Syringe (Burroughs Wellcome and Co., London, England) (14). The surfactants were dissolved in chloroform at different concentrations, and the interfacial tensions were measured against water at 26.5°C.

RESULTS AND DISCUSSION

Lipase-catalyzed soy lecithin–monoester interchange was sought as a means to modify soy lecithin to improve its surface properties and nutritional qualities. Lipozyme was used because of its advantage that short- and medium-chain fatty acids (C_4 and above) could be successfully incorporated into oils (15). Also, the specificity of this enzyme can prevent the fatty acids at the *sn*-2 position of PC, which are mainly unsaturated, from undergoing transesterification. So, only the fatty acid present in the 1-position of PC will be exchanged in the presence of this lipozyme catalyst. This enzyme can be used in the presence of solvent and higher temperature (up to 70°C). In the present study, a temperature of 60°C was used as recommended by the manufacturer.

In the present study, the methyl esters of pure fatty acids ($C_{10:0}$, $C_{12:0}$, $C_{14:0}$) and of linseed oil (to get methyl esters of linoleic and linolenic acid) were used as monoesters for the interchange reaction. The fatty acid compositions of the methyl esters of the different fatty acids and of linseed oil are shown in Table 1.

Table 2 reveals the extent of incorporation of fatty acids into the lecithin moiety. These new fatty acids were incorporated mainly in place of palmitic and linoleic acid, which is supported by the fact that the total incorporation of the new fatty acids is in an amount equal to the elimination of the total content of palmitic and linoleic acids. The extent of incorporation of a particular fatty acid evidently depends on its molecular weight; those with the higher molecular weights were incorporated to a large extent.

The transesterified soy lecithin products with noticeable quantities of the incorporated saturated fatty acids were examined for interfacial tension values (Table 3) against water at 26.5°C. The interfacial tension measurements definitely

TABLE 1
Fatty Acid Composition of the Monoesters Used for the Interchange Reaction with Soy Phosphatidylcholine

	Composition of fatty acid (% w/w)							
Ester	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Methyl caprate	99.2	0.8	—	—	—	—	—	—
Methyl laurate	—	99.8	—	—	—	—	—	—
Methyl myristate	—	—	99.9	—	—	—	—	—
Methyl ester of linseed oil	—	—	—	6.5	5.2	20.6	14.2	53.5

TABLE 2
Fatty Acid Composition of Soy Lecithin Transesterified with Methyl Caprate, Methyl Laurate, Methyl Myristate and Linseed Oil Methyl Ester in the Presence of *Mucor miehei* Lipase for 48 h at 60° ± 2°C

Samples of phospholipid	Composition of fatty acid (% w/w)							
	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Original soy phosphatidylcholine	—	—	—	18.8	2.8	13.4	59.3	5.2
Phospholipid transesterified with methyl caprate	8.4	—	—	14.5	2.3	12.5	56.5	5.4
Phospholipid transesterified with methyl laurate	—	14.1	—	12.7	2.1	12.8	53.5	4.5
Phospholipid transesterified with methyl myristate	—	—	15.7	12.2	2.4	11.4	53.2	4.5
Phospholipid transesterified with methyl ester of linseed oil	—	—	—	8.3	1.9	12.3	61.6	15.4

TABLE 3
Interfacial Tension of Chloroform Solutions of Different Transesterified Soy Lecithins Against Water at 26.5°C[a]

Samples	Interfacial tension in dynes/cm	
	0.1% Solution	0.2% Solution
Original soy phosphatidylcholine	25.67	14.57
Phosphatidylcholine transesterified with methyl caprate	14.17	10.30
Phosphatidylcholine transesterified with methyl laurate	19.82	12.37
Phosphatidylcholine transesterified with methyl myristate	19.61	11.98

[a]Interfacial tension of $CHCl_3$ against water at 26.5°C is 33.2 dynes/cm.

showed that soy lecithin modified with new saturated fatty acids lowers the interfacial tension against water, and the degree of lowering the interfacial tension depends on the nature and amount of fatty acid incorporated. This behavior makes for better utilization of soy lecithin as an emulsifier in the food, cosmetic, and pharmaceutical industries.

Soy phospholipid is an established emulsifier, but it has some limitations over some areas of emulsification. Therefore, the above modifications of soy lecithin may have useful application in surfactants.

Transesterification of soy lecithin with the help of microbial lipase can produce a new soy lecithin without changing its original composition at the *sn*-2 position by only altering the *sn*-1 position with desired fatty acids. The ease of reaction and utilization of the products have influenced us to carry out these types of reactions.

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REFERENCES

1. Nieuwenhuyzen, W.V., The Industrial Uses of Special Lecithins—A Review, *J. Am. Oil Chem. Soc. 58*:886–888 (1981).
2. Schneider, M., Phospholipids, in *Proceedings of World Conference on Emerging Technologies in the Fats and Oils Industry,* edited by A.R. Baldwin, American Oil Chemists' Society, Champaign, 1986, pp. 160–164.
3. Dashiell, G.L., Sources, Processing Methods, and Commercial Uses of Lecithin, *Proceedings of World Conference on Edible Fats and Oils Processing,* edited by D.R. Erickson, American Oil Chemists' Society, Champaign, 1990, pp. 396–401.
4. Brockerhoff, H., P.C. Schmidt, J.W. Fong, and L.J. Tirri, Introduction of Labeled Fatty Acid in Position-1 of Phosphoglycerides, *Lipids 11*:421–422 (1976).
5. Yoshimoto, T., M. Nakata, S. Yamaguchi, T. Funada, Y. Saito, and Y. Inada, Synthesis of Eicosapentaenoyl Phosphatidylcholines by Polyethylene Glycol-Modified Lipase in Benzene, *Biotechnol. Lett. 8*:771–775 (1986).
6. Yagi, T., T. Nakanishi, Y. Yoshizawa, and F. Fukui, The Enzymic Acyl Exchange of Phospholipids with Lipases, *J. Ferment. Bioeng. 69*:23–29 (1990).
7. Yoichiro, T., and H. Setsuko, Preparation of Polyunsaturated Phospholipids by Lipase Catalysed Transesterification, *J. Am. Oil Chem. Soc. 68*:848–851 (1991).
8. Svensson, I., P. Adlercreutz, and B. Mattiasson, Interesterification of Phosphatidylcholine with Lipase in Organic Media, *Appl. Microbiol. Biotechnol. 33*:255–258 (1990).
9. Svensson, I., P. Adlercreutz, and B. Mattiasson, Lipase-Catalysed Transesterification of Phosphatidylcholine at Controlled Water Activity, *J. Am. Oil Chem. Soc. 69*:986–991 (1992).
10. Mutua, N.L., and C.C. Akoh, Lipase-Catalysed Modification of Phospholipids: Incorporation of n-3 Fatty Acids into Biosurfactants, *Ibid. 70*:125–128 (1993).
11. Pernas, P., L.J. Olivier, M.D. Legoy, and G. Bereziat, Phospholipid Synthesis by Extracellular Phospholipase-A_2 in Organic Solvent, *Biochem. Biophy. Res. Commun. 168*:644–646 (1990).
12. Hanras, C., and J.L. Perrin, Gram-Scale Preparative HPLC of Phospholipids from Soyabean Lecithins, *J. Am. Oil Chem. Soc. 68*:804–808 (1991).
13. Litchfield, C., Extraction, Isolation, Measurement, and Fatty Acid Analysis, *Analysis of Triglycerides,* Academic Press, New York, 1972, pp. 17–35.
14. Chattoraj, D.K., and A.K. Chatterjee, Cohesive Pressure in Ionized Monolayer and Thickness of the Interfacial Phase, *J. Colloid Interface Sci. 21*:159–169 (1966).
15. Sridhar, R., G. Lakhminarayana, and T.N.B. Kaimal, Modification of Selected Indian Vegetable Fats into Cocoa-Butter Substitutes by Lipase-Catalysed Ester Interchange, *J. Am. Oil Chem. Soc. 68*:726–730 (1991).

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