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Enzymatic Interesterification of Blend of Soyphospholipid and Coconut Oil

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Simultaneous modification of the fatty acid composition of both soyphospholipid (SPL) and coconut oil (CO) was performed by enzymatic interesterification. In modified soyphospholipid (MSPL), the extent of incorporation of caprylic acid, capric acid and lauric acid were 5.735 ± 0.31, 14.39 ± 0.15 % and 7.945 ± 0.13 %, respectively and in modified coconut oil (MCO) the extent of incorporation of linoleic acid and linolenic acid were 6.625 ± 0.3 % and 1.58 ± 0.35 %. The antioxidant properties of the modified products were determined and significant increase in case of MSPL and decrease with MCO were observed compared to the original soyphospholipid and coconut oil. The surface active property such as surface tension was measured in case of modified soyphospholipid product that got reduced by about 4 units. This lowering of surface tension for MSPL can be useful as an emulsifier effective for food use.

Keywords: Soyphospholipid, Coconut oil, Interesterification.

INTRODUCTION

Phospholipid modification by interesterification reaction involving fatty acyl groups interchange with lipase catalyst has been extensively studied involving the incorporation of individual fatty acid in phospholipid molecule. Some of the investigations are cited as the participation of medium chain fatty acid phospholipids and long chain PUFA incorporated phospholipids. These products are already reported to be surface active and nutritionally beneficial.

Medium chain fatty acids (capric acid 8.4 %, lauric acid 14.1 %, myristic acid 15.7 %) were incorporated into soy lecithin in its SN-1 position [1]. n-3 PUFAs such as eicosapentaenoic acid (EPA) (20:5) and docosahexaenoic acid (DHA) (22:6) were introduced into phospholipids for modification by lipase-catalyzed transesterification. The maximum incorporation of EPA (20:5) was 17.7 mol% [2]. The incorporations of dibasic acids such as adipic acid and sebacic acid were 4-13 % and 9-20 % in soyphospholipids by transesterification reaction with alkyl ester of dibasic acid with both lipase and alkoxide as a catalyst [3]. Capric acid containing soyphospholipid significantly decreases the total cholesterol (TC), triglycerides (TG), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol level [4]. Similarly, EPA containing soyphospholipid significantly decreases the total cholesterol (TC), triglycerides (TG), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol level and increases high density lipoprotein (HDL) cholesterol level [5].

Modification of soyphospholipid by interesterification with a vegetable oil for simultaneous modification in respect of fatty acid composition in both soyphospholipid and natural oil has not been reported so far. The interesterification reaction of blend of soyphospholipid and natural oil can offer advantage to carry out reaction and isolate the modified products much more conveniently. Such kind of approach may be helpful in producing modified soyphospholipid and natural oil with better composition and properties.

The present study investigates the extent of changes in the fatty acid composition of both soyphospholipid and coconut oil with different molar ratio and varying time intervals to modify for better surface active property and antioxidant property in case of modified phospholipid and antioxidant property for modified coconut oil.

EXPERIMENTAL

Soyphospholipid (SPL) has been collected from Ruchi Soya Industries Ltd., Nagpur, India while the coconut oil has been procured from the local market. Enzyme RM IM (*Rhizomucour miehei*) has been received as a gift from Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark.

Deoiling of soyphospholipid: Crude soyphospholipid was deoiled by acetone (in 1:7 w/v ratio) treatment repeatedly. Acetone was added to crude soyphospholipid and the total solution was allowed to stand at 4 ºC for 2 h. The solution was filtered and distilled to isolate acetone. This treatment was

repeated until the acetone layer was colourless. Acetone was removed under vacuum pressure and soyphospholipid was isolated [6]. The yield of deoiled soyphospholipid was 86 % with 95.56 % purity based on phosphorus content of soyphospholipid [7].

Enzymatic interesterification: Different molar ratios such as 1:1, 1:2, 1:3 and 1:4 of soyphospholipid and coconut oil were taken for experiments. Desirable ratio was 1:4 for reaction. Blend of soyphospholipid and coconut oil was heated up to 60 ºC to obtain a uniform solution. Enzyme (10 %) was then added as catalyst. After 6, 12 and 24 h of stirring at a constant speed of 300 rpm, samples were collected. The collected mixture was cooled at room temperature. Chloroform was added to it to dissolve the mixture absolutely. Enzyme was separated by filtration process and the chloroform solution was dried under vacuum. Interesterified oil and soyphospholipid were isolated by separation from this mixture through extraction with acetone.

Fatty acid analysis of esterified phospholipid and oil: Isolated products were methylated by adding 1 mL diethyl ether and 1 mL of 0.5 N methanolic KOH, followed by shaking the mixture for 10 min vigorously. HCl (1 mL, 1 N) was added, and methyl esters of fatty acids were extracted with petroleum ether (40-60 ºC) [8].

Gas chromatography: Fatty acid methyl esters were analyzed on a Hewlett-Packard gas chromatograph (HP 5890A) (7890B GC system of Agilent Technologies), equipped with a flame ionization detector (FID) and capillary DB-Wax column (30mL, 0.250 mm I.D, 0.25 μm FT). The column temperature was programmed between 150-240 ºC. 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 25 mL/min.

Antioxidant activity determination by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition assay: Each sample was added to 1 mL of 1mM DPPH in chloroform. The decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The readings were compared with the controls, which contained solvent (chloroform) instead of the extract [9]. The percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

Surface tension measurement: Surface tension of the esterified phospholipid was measured by using Dynamic Contact Angle meter and Tensio meter (DCAT) [10].

RESULTS AND DISCUSSION

Fatty acid composition (FAC): Table-1 shows the fatty acid composition of original soyphospholipid (SPL) and coconut oil (CO) while Table-2 shows the fatty acid composition of both modified soyphospholipid and modified coconut oil.

It is evident from Table-2 that a significant interchange in fatty acid composition has been observed in both soyphospholipid and coconut oil. In modified soyphospholipid (24 h) after reaction 5.735 ± 0.31 % caprylic acid, 14.39 ± 0.15 % capric acid and 7.945 ± 0.13 % lauric acid got incorporated whereas displacing 15.485 ± 0.1% palmitic acid and 14.99 ± 0.05 % linoleic acid. In modified coconut oil 6.625 ± 0.3 % linoleic acid and 1.58 ± 0.35 % linolenic acid were incorporated. Most desirable interchange in fatty acid composition was obtained in 24 h reaction time. In the earlier report, 8.4 % capric acid and 14.1 % lauric acid were incorporated in soyphospholipid whereas in the present study 14.39 ± 0.15 % capric acid and 7.945 ± 0.13 % lauric acid were incorporated. Incorporation of capric acid and lauric acid were better in this study. Modification of both soyphospholipid and coconut oil simultaneously by enzymatic interesterification was obtained significantly in this study. Soyphospholipid has its 1-position available for fatty acyl group interchange while coconut oil has both 1- and 3-positions available for interchange reaction. In view of these facts and the use of selectively much higher molar proportion of coconut oil, the incorporation of the various fatty acids in both modified soyphospholipid (MSPL) and modified coconut oil (MCO) accordingly observed.

TABLE-1
FATTY ACID COMPOSITION OF ORIGINAL SOYPHOSPHOLIPID (SPL) AND COCONUT OIL (CO)

	Sample fatty acid composition (Area %)								
	Caprylic acid	Capric acid	Lauric acid	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
SPL	–	–	–	–	25.14	3.25	16.71	50.48	4.41
CO	7.62	5.99	48.49	20.16	8.49	2.76	6.50	–	–

TABLE-2
FATTY ACID COMPOSITION OF MODIFIED PHOSPHOLIPID (MSPL) AND MODIFIED COCONUT OIL (MCO)

	Fatty acid composition (Area %) Sample					
	MPL (24 h)	MCO (24 h)	MPL (12 h)	MCO (12 h)	MPL (6 h)	MCO (6 h)
Caprylic acid	5.735 ± 0.31	1.295 ± 0.20	1.465 ± 0.58	4.075 ± 0.10	1.215 ± 0.030	6.78 ± 0.53
Capric acid	14.39 ± 0.15	5.14 ± 0.32	8.225 ± 0.17	4.92 ± 0.15	9.435 ± 0.020	5.53 ± 0.21
Lauric acid	7.945 ± 0.13	48.905 ± 0.37	3.975 ± 0.09	47.53 ± 0.24	3.855 ± 0.007	47.3 ± 0.07
Myristic acid	–	19.455 ± 1.03	–	20.025 ± 0.03	–	18.88 ± 0.01
Palmitic acid	9.655 ± 0.10	8.32 ± 0.28	15.205 ± 0.33	7.445 ± 0.23	21.095 ± 0.007	7.92 ± 0.08
Stearic acid	3.46 ± 0.07	2.91 ± 0.11	3.39 ± 0.28	3.17 ± 0.18	3.02 ± 0.020	2.65 ± 0.29
Oleic acid	16.605 ± 0.06	5.77 ± 0.04	16.995 ± 0.17	7.895 ± 0.14	14.97 ± 0.040	6.77 ± 0.04
Linoleic acid	35.49 ± 0.05	6.625 ± 0.30	48.9 ± 0.31	3.32 ± 0.07	43.125 ± 0.160	2.68 ± 0.13
Linolenic acid	6.72 ± 0.14	1.58 ± 0.35	1.85 ± 0.21	1.625 ± 0.41	3.28 ± 0.020	1.48 ± 0.25

Results were mean ± SD (n = 2); MPL = Modified phospholipid; MCO = Modified coconut oil

Antioxidant activity: The significant differences in the antioxidant activity in both modified soyphospholipid and coconut oil with comparison of their original activity are observed (Table-3). In this study, linoleic acid content decreased in soyphospholipid, thereby improved its stability and antioxidant activity significantly while antioxidant activity of coconut oil decreased as a result of the incorporation of linoleic acid from soyphospholipid in it.

TABLE-3
ANTIOXIDANT ACTIVITY RESULTS OF ORIGINAL AND MODIFIED SOYPHOSPHOLIPID AND COCONUT OILS BY DPPH SCAVENGING EFFECT

Sample	Scavenging effect by DPPH (%)
Original soyphospholipid oil	11.268
Modified soyphospholipid oil	28.375
Original coconut oil	23.121
Modified coconut oil	6.154

Surface tension: The modified phospholipid (20.838 ± 0.027 mN/m) shows a lowering in surface tension value by about 4 units, which indicate an improvement in the surface active property of original soyphospholipid (24.589 ± 0.027 mN/m).

Conclusion

The present enzymatic interesterification process involving blend of 1:4 molar proportion soyphospholipid and coconut oil is a convenient approach for incorporating medium chain fatty acids caprylic, capric, lauric in soyphospholipid and the essential fatty acids (EFA) such as linoleic and linolenic in coconut oil. The modified soyphospholipid product shows a significant improvement in its antioxidant properties and a decrease in the surface active property such as surface tension for modified soyphospholipid for better food application.

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