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ω-3 fatty acids in phospholipids: Incorporation and investigation through enzymatic approach

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Abstract : Functional and nutritional foods are becoming more and more important for the benefit of human health and reduction of prevalent diseases. Phospholipids (PLs) modification using Omega-3 fatty acids like eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) is significantly important for this purposetoobtain structured foods by altering the nutritional, physiological and functional properties. Modification processimproves their oxidative stability, emulsification properties, reduces the risk of early death, exhibits various psychological functions in human systems and significantly provides systemic benefits throughout the body. Biotechnological advancement helps the process modification and product characterization.

In the present research investigation, soybean phospholipids (SBPLs), a cheap raw material obtained from soybean oil refinery industry were modified by the introduction of EPA (C20:5) in hexane medium. The bioprocess was conducted using 1, 3- specific Lipozyme TL IM immobilized lipase (*Thermomyces lanuginosus*) at 40^0C and the process continued upto 48 hours. The modified PLs prepared by this bioprocess contain 28.36% EPA along with other fatty acids. The properties of the modified PLs have been analyzed which showed satisfactory results.

Keywords : Phospholipids, Modified Phospholipids, TL IM enzyme, Eicosapentaenoic acid.

Introduction:

Modification of PLsisdone to achieve products with improved technological, physicochemical and nutritional properties. This includes selectivedevelopment of emulsifying properties, enhancement of dispersibility in aqueous systems or for the achievement of nutritionally valuable PL fractions. Modified PLs with medium chain triglycerides (MCT), carotenoids, dietary fiber, omega-3 fatty acids, conjugated linoleic acid (CLA), polyphenols, phytosterols and tocotrienols are already acknowledged [1]. PL modification with different functional ingredients enhances the effectiveness, versatility, lower the risk of premature death [2] and chemo preventive effect [3,4]. Scientific studies show that replacement of existing fatty acids in original PLs with desired fatty acids may develop better physical and chemical properties or even nutritional, pharmaceutical and medical characteristics.Omega-3 fatty acids like EPA and DHA play a pivotal role in this aspect and introduction of these acids in PLs contribute desired functional characteristics, health beneficial effects [5,6] and oxidative stability [7]. It has been claimed that EPA and DHA have been associated with fetal development, cardiovascular function, and Alzheimer's disease. Studies have shown that these acids are important for proper fetal development, including neuronal, retinal, and immune function. They may also affect

many aspects of cardiovascular functions including inflammation, peripheral artery disease, major coronary events, and anticoagulation[8]. EPA and DHA have been linked to promising results in prevention, weight management, and cognitive function in those with very mild Alzheimer's disease. EPA modified PLs also exhibits various psychological functions in human systems [9,10].

Phospholipids modification with the help of long chain fatty acids in the presence of chemical or biological catalyst are appreciablyimportant for obtaining tailor made technological and physiological properties which are different from those of natural products and utilized for specific purposes.Modification with chemical as well as biological catalyst are used by any researchers but enzyme as catalyst has various advantagesover chemical catalytic methoddue to its reusability, specificity, thermo stability and mild reaction conditions[11].But little endeavor has so far been made for the enzymatic acyl exchange of PLs to pilot plant scale or production scale due to mass transfer limitations and low yields. Some researchers studied the modification of PLs in the presence of enzyme and highlighted the pros and cons of the process and product characteristics. Penga*et al.*[12]prepared the structured PLs using lipase-catalyzed acidolysis and optimized the process by response surface methodology. Another study was made by Hosse and Hernandez[13]and they prepared enzyme-catalyzed structured phospholipids with conjugated linoleic acid.Estiasih *et al.*[14]aimed to optimize eicosapentaenoic acid (EPA) incorporation into phospholipids structure by acidolysis reaction using free lipase (EC 3.1.1.3) from *Rhizomucor miehei* and obtained22.81% of EPA content of structured phospholipids. Adlercreutz and Wehtje[15] studied the enzymatic method for thesynthesis of mixed-acid phosphatidylcholine and introduced decanoic acid in the *sn*-1 and hexanoic acid in the *sn*-2 position.Biocatalytic method for the preparation of structured or modified PLs are also studied by Hama *et al.*[16], Reddy *et al.*[17], Vikbjerg*et al.et al.*[18].

In the present research investigation, SBPLs are used as raw material. After deoiling, the deoiled SBPLs are treated with EPA using hexane medium in the presence of 1, 3- specific Lipozyme TL IM immobilized lipase (*Thermomyceslanuginosus*) maintaining a temperature of 40^0C for 48 hrs.. The modified PLs contained considerable amount of EPA along with other fatty acids.EPA introduction during the entire duration of reaction has been investigated and interfacial tension has been compared with the original PLs which showed encouraging results.

Experimental

Crude SBPLs was collected from M/s. Sethia OilMills, Burdwan, West Bengal, India. The enzyme 1, 3-specific LipozymeTL IM immobilized lipase (*Thermomyces lanuginosus)* was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India with catalytic activity 75 Interesterification unit Novo/g (IUN/g). Eicosapentaenoic acid and hexane were purchased from S.D. Fine Chemicals (Mumbai, India). Except otherwise specified all other chemicals used were A.R. Grade.

Results and discussions

The analytical characteristics and fatty acid composition of crude SBPLs are shown in Table 1. Crude SBPLs contains 54±0.284% oil and 39±0.157% PLs. Regarding fatty acid composition, SBPL contains 21.3±0.136% palmitic acid, 3.1±0.049% stearic acid, 19.7±0.242% oleic acid,47.1±0.273% linoleic acid and 4.2±0.011% linolenic acid. Initially, SBPLs is deoiled through acetone fractionation andthe composition of the deoiledSBPLsis determined by high performance liquid chromatography technique which is revealed in Figure 1. It can be estimated fromFigure 1that deoiledSBPLs contains higher amount of phosphatidylcholine (PC) or lecithin (42.4%) and phosphatidylethanolamine (PE) (22.1%) compared to phosphatidic acid (PA) (11.4%) and phosphatidylinositol (PI) (16.4%). It also contains negligible amount of phosphatidylserine (PS) (1.3%).

Table 1. Analytical characteristics and fatty acid composition of crude SBPLs

Oil content (%, w/w)	PL content (%,w/w)	Fatty acid composition (%,w/w)				
		C16:0	C18:0	C18:1	C18:2	C18:3
54±0.284	39±0.157	21.3±0.136	3.1±0.049	19.7±0.242	47.1±0.273	4.2±0.011

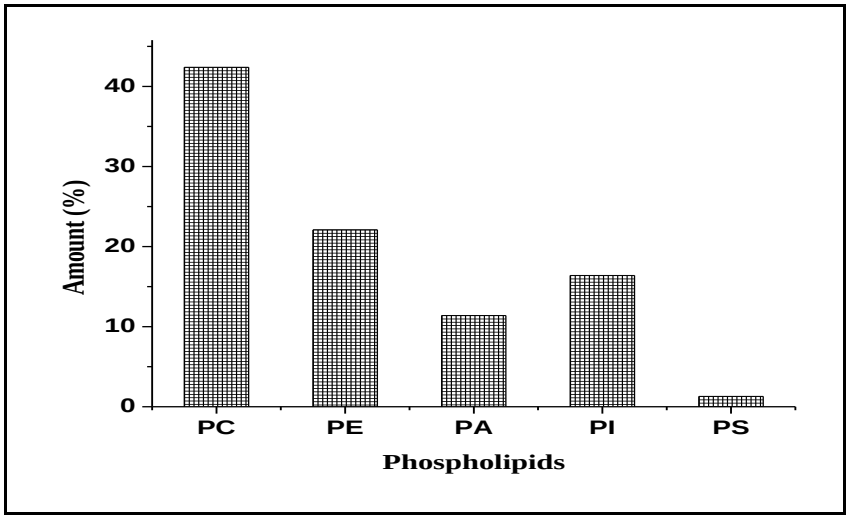

Figure1 Composition of deoiled SBPLs (%, w/w)

For the preparation of modified or structured PLs, deoiled PLs was taken in hexane medium and then treated with EPA (PLs : EPA::1:2 molar ratio) in the presence of 1, 3- specific Lipozyme TL IM immobilized lipase (10% w/w) at 40^0C for 48 hrs. The introduction of EPA in PLs was monitored by analyzing samples through thin layer chromatographic method which was collected periodically. After 48 hrs of reaction, the immobilized enzyme was separated by filtration and after removal of hexane, the product was recovered by acetone fractionation.Figure 2 shows the rate of introduction of EPA in deoiled PLs in the presence of enzyme. It has also been observed that after 48 hrs of reaction, no significant enhancement of introduction of EPAhas occurred during last 6 hrs of reaction.

Table 2 shows the changing pattern of fatty acid compositions of the product during the entire reaction time. It has been observed from the Table that the final product contains 28.36±0.176% EPA along with 11.1±0.107% palmitic acid, 0.8±0.001% stearic acid, 9.9±0.033% oleic acid,49.7±0.222% linoleic acid and 3.0±0.010% linolenic acid. It reveals from Table 2 that palmitic acid content decreased (from 21.3% to 11.1%) significantly during the introduction of EPA along with oleic acid (from 19.7% to 9.9%). This may be due to the continuous breaking and forming of ester bonds during the reaction.

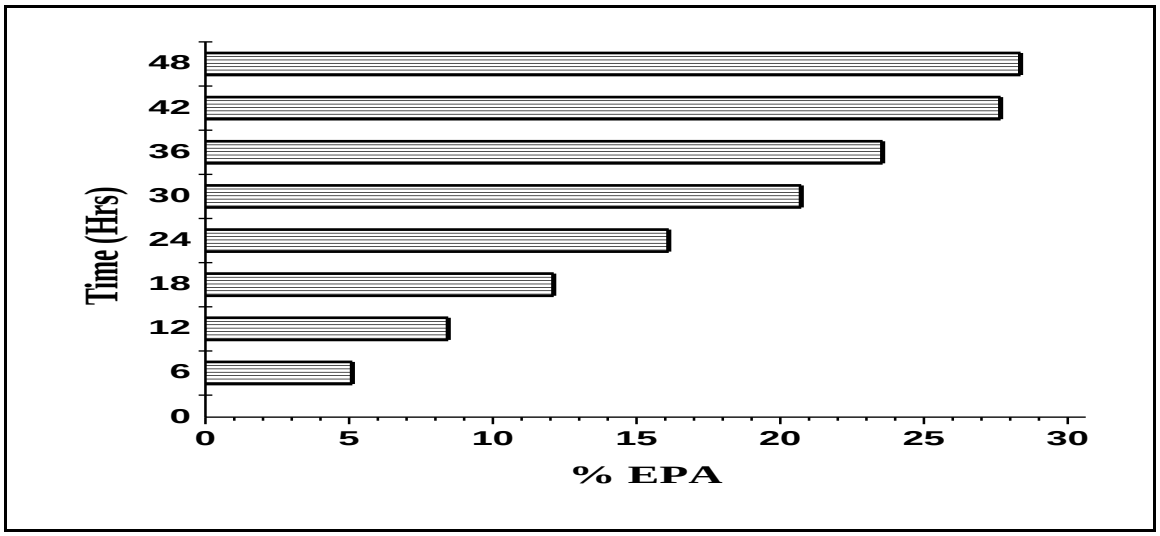

Figure2 Rate of introduction of EPAin PLs(Enzyme: TL IM-10%, Temperature-40^0C, Deoiled PL: Decanoic acid-1:2, Time-48 hrs)

Table 2 Fatty acid composition during synthesis of modified PLs

Time (hrs)	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{22:5}$
0	21.3±0.136	3.1±0.049	19.7±0.242	47.1±0.273	4.2±0.011	0
6	22.5±0.167	2.7±0.011	17.4±0.081	49.4±0.273	4.4±0.031	5.11±0.039
12	23.5±0.159	2.1±0.009	15.2±0.071	48.7±0.254	5.7±0.042	8.45±0.067
18	24.4±0.198	1.9±0.004	13.1±0.067	48.6±0.255	4.3±0.018	12.12±0.106
24	15.4±0.111	1.8±0.006	11.9±0.101	47.5±0.283	4.9±0.022	16.12±0.132
30	13.7±0.088	1.4±0.003	10.7±0.059	48.8±0.218	3.9±0.016	20.73±0.159
36	12.6±0.109	1.1±0.004	10.2±0.044	49.1±0.269	3.7±0.021	23.56±0.166
42	11.7±0.081	0.9±0.001	9.8±0.036	49.3±0.235	3.4±0.016	27.67±0.168
48	11.1±0.107	0.8±0.001	9.9±0.033	49.7±0.222	3.0±0.010	28.36±0.176

Figure 3 shows the comparative study based oninterfacial tension of PLs and EPA modified PLs against water at 27ºC in chloroform solutionat six different concentrations. Itreveals from the Figurethat interfacial tension of EPA modified PLs is less than interfacial tension of PLs at all concentration levels. So the suitability of the process technology is depicted herewith.

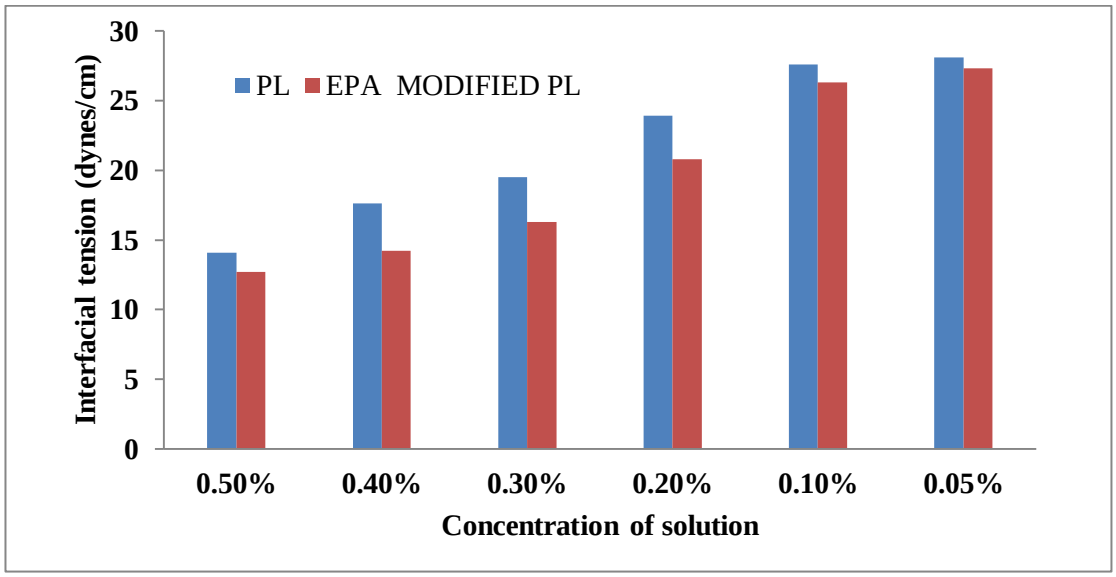

Figure 3 Interfacial tension (dynes/cm) against water at 27ºC in chloroform medium (Interfacial tension of chloroform against water at 27ºC is 33.2 dynes/cm)

Conclusion:

Preparation of modified phospholipids in the presence of 1, 3-specific Lipozyme TL IM immobilized lipase (*Thermomyces lanuginosus)* from cheap raw material like crudesoybean phospholipids is a novel approach. The present bioprocess method requires low temperature for the introduction of eicosapentaenoic acid which showsan energy savingtechnology. Recycling of enzyme may be adopted which encourages future researchers for the exploration of cost effective approach. Modified phospholipids contain considerable amount of eicosapentaenoic acid which is also encouraging for this technology. The present bioprocess technology for the preparation of modified or structured phospholipids may be adopted in industrial scale also. This technology may be adopted for the modification of other phospholipids using important long chain fatty acids for functional applications.

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