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Enzymatic Synthesis and Characterization of Modified Phospholipids using Decanoic Acid

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Abstract

Phospholipids (PLs) are significantly utilized in different areas of food, pharmaceutical and cosmetic products as emulsifiers, stabilizers and antioxidants. The properties of PLs can be modified with a view to improve its inherent properties like emulsification and oxidative properties as well as tailor made technological and physiological properties. Advancement of biotechnology serves as a viable synthetic route in this regard which is helpful for the preparation of modified or structured PLs not only in academic research but also in industry. In the present research investigation, structured PLs are prepared from soybean phospholipids (SBPLs) by the introduction of decanoic acid (C10:0) in hexane medium using 1, 3- specific Lipozyme TL IM immobilized lipase (*Thermomyces lanuginosus*) at 40^0C. The enzymatic modification reaction takes some longer time (10 days) for completion. The modified PLs prepared by this method contain 23.16% decanoic acid along with other fatty acids. The property of the modified PL has been investigated and found good results.

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

INTRODUCTION

Increasing trend for the development of functional and nutraceutical foods improves the disease prevention process. Functional characteristics of different ingredients such as medium chain triglycerides (MCT), carotenoids, dietary fiber, omega-3 fatty acids, conjugated linoleic acid (CLA), polyphenols, phytosterols, and tocotrienols are

already acknowledged [1]. Designing functional and nutritional foods enriched with these ingredients enhances the use of their effectiveness, versatility and chemopreventive effect [2, 3]. PLs play an important role in this aspect as it acts as an efficient carrier to deliver the functional ingredients in dietary fractions. 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. The increasing use of PLs as natural emulsifiers, wetting agents and dispersal agents in foods, cosmetics and pharmaceuticals creates a lot of attention on the structure and modification of its properties. So, PLs with specific fatty acids and/or polar head groups are considered an important viewpoint for its application in different areas in this regard.

Modifications of PLs by the introduction of medium chain or long chain fatty acids through chemical or bio catalytic method to alter emulsifying and dispersing properties are significant and demanding in the present scenario due to the wider application range of modified PLs [4]. The main objective of these modifications is to obtain tailor made technological and physiological properties which are different from those of natural products and utilized for specific purposes. Chemical modification involves different stages like hydrolysis, hydroxylation, acylation and hydrogenation [5] but it needs some scrupulous reaction conditions. Moreover, chemical catalytic method is energy intensive, recovery of by product and the spent catalyst is difficult and the presences of unwanted products greatly interfere with the reaction. On the other hand, bio catalytic method has several advantages over chemical catalytic method due to its reusability, specificity, thermo stability and mild reaction conditions. Present author made a comparative study between chemical catalytic and biocatalytic method for the preparation of biodiesel and showed that biocatalytic method is more advantageous than chemical catalytic method [6]. But little effort has so far been made for the enzymatic acyl exchange of PLs to pilot plant scale or production scale because of mass transfer limitations and low yields. Some researchers studied the PLs modification though enzymatic method and highlighted the advantages and disadvantages of the process conditions and product characteristics. Penga *et al*. [7] prepared the structured PLs using lipase-catalyzed acidolysis and optimized the process by response surface methodology. Another study was made by Hosse and Hernandez [8] and they prepared enzyme-catalyzed structured phospholipids with conjugated linoleic acid. Vikbjerg *et al*. [9] evaluated and recovered the structured PLs by Lipase-Catalyzed Acyl Exchange of Soybean Phosphatidylcholine in Hexane medium. In another study, Vikbjerg *et al*. [10] studied the reaction parameters for the synthesis of structured PLs for the lipase-catalyzed acidolysis in solvent-free system for the introduction of caprylic acid and optimized the process. Biocatalytic method for the preparation of structured or modified PLs are also studied by Hama *et al*. [11], Reddy *et al*. [12], Vikbjerg *et al*. [13].

In the present research investigation, SBPLs are used as raw material. After deoiling, the deoiled SBPLs are treated with decanoic acid using hexane medium in the

presence of 1, 3- specific Lipozyme TL IM immobilized lipase (*Thermomyces lanuginosus*) maintaining a temperature of 400C for ten days. The modified PLs contained considerable amount of decanoic acid along with other fatty acids. Changing pattern of fatty acid composition of the modified PLs has been investigated during the preparation and interfacial tension has been compared with the original Pls which showed good results.

MATERIALS AND METHODS

Crude SBPLs was collected from M/s. Sethia Oil Mills, Burdwan, West Bengal, India. The enzyme 1, 3- specific Lipozyme TL IM immobilized lipase from *Thermomyces lanuginosus* was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India with catalytic activity 75 Interesterification unit Novo/g (IUN/g). Decanoic 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 58±0.512 % oil and 36±0.182 % PLs. Regarding fatty acid composition, SBPL contains 22.4±0.111% palmitic acid, 3.7 ±0.042% stearic acid, 18.4±0.212% oleic acid, 49.2±0.382% linoleic acid and 4.7±0.005% linolenic acid. Initially, SBPLs is deoiled through acetone fractionation and the composition of the deoiled SBPLs is determined by high performance liquid chromatography technique which is revealed in Figure 1. It can be estimated from Figure 1 that deoiled SBPLs contains higher amount of phosphatidylcholine (PC) or lecithin (41.4%) and phosphatidylethanolamine (PE) (23.8%) compared to phosphatidic acid (PA) (12.6%) and phosphatidylinositol (PI) (15.7%). It also contains negligible amount of phosphatidylserine (PS) (1.7%).

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
58±0.512	36±0.182	22.4±0.111	3.7 ±0.042	18.4±0.212	49.2±0.382	4.7±0.005

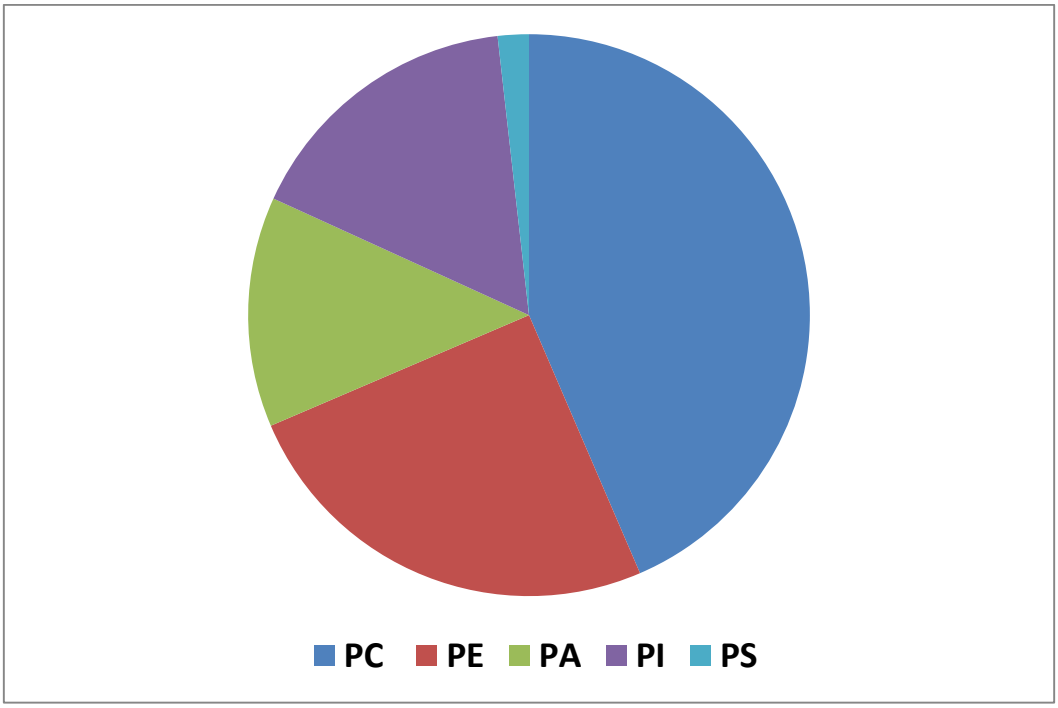

Figure 1: Composition of deoiled SBPLs (%, w/w)

For the preparation of modified or structured PLs, deoiled PLs was taken in hexane medium and then treated with decanoic acid (PLs : decanioc acid::1:4 molar ratio) in the presence of 1, 3- specific Lipozyme TL IM immobilized lipase (10% w/w) at 400C. The reaction was continued for 10 days. The progress of reaction is monitored by taking samples from the reaction system and analysed through thin layer chromatographic method. After completion of reaction, the immobilized enzyme was separated by filtration and the product was recovered by acetone fractionation after hexane removal from the system. Introduction of decanoic acid in the deoiled PLs takes longer time indicating slower interaction between the PLs and decanoic acid. Figure 2 shows the rate of introduction of decanoic acid in deoiled PLs in the presence of enzyme. It has also been observed that after 10 days of reaction, no significant enhancement of introduction of decanoic acid has occurred during the last two days.

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 23.16±0.116% decanoic acid along with 12.1±0.101% palmitic acid, 1.4±0.004% stearic acid, 10.7±0.021% oleic acid, 49.1±0.243% linoleic acid and 3.7±0.012% linolenic acid. It reveals from Table 2 that palmitic acid content decreased (from 25.1% to 12.1%) significantly during the introduction of decanoic acid along with oleic acid (from 15% to10.7%). This may be due to the continuous breaking and forming of ester bonds during the reaction.

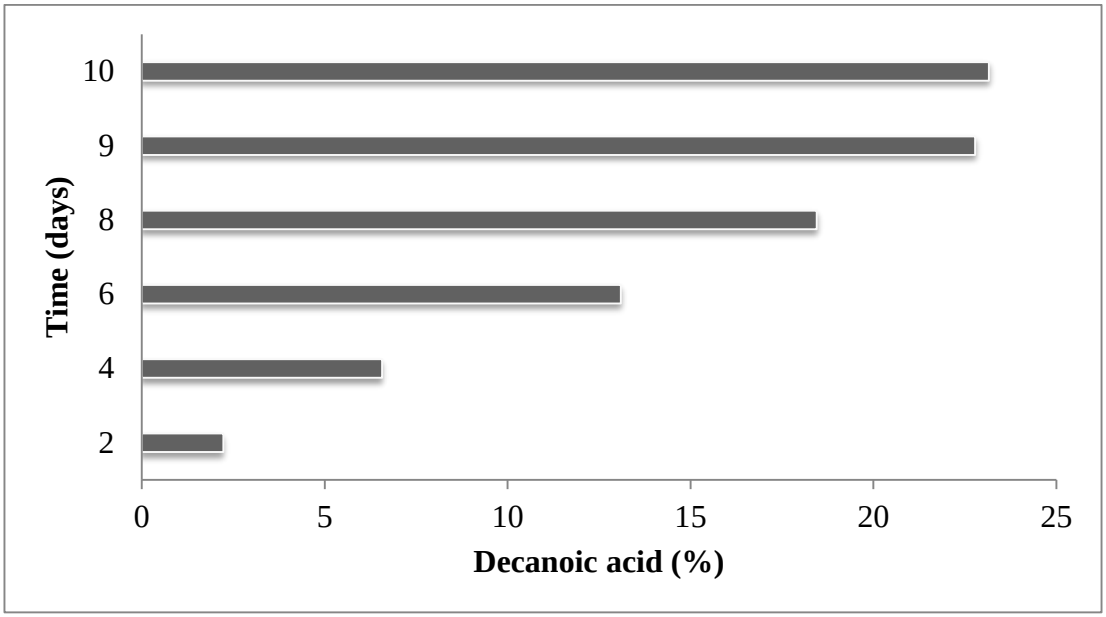

Figure 2: Rate of introduction of decanoic acid in PLs

(Enzyme: TL IM-10%, Temperature-400C, Deoiled PL: Decanoic acid-1:4, Time-10 days)

Table 2: Fatty acid composition during synthesis of modified PLs

Time (days)	C16:0	C18:0	C18:1	C18:2	C18:3	C10:0
0	25.1±0.159	2.8±0.003	15.0±0.073	51.4±0.276	4.5±0.004	0
2	21.8±0.122	2.1±0.008	16.4±0.084	51.8±0.259	5.4±0.011	2.23±0.004
4	23.2±0.173	1.9±0.005	14.8±0.056	48.0±0.233	5.3±0.032	6.57±0.016
6	24.3±0.108	1.6±0.002	11.1±0.043	45.5±0.294	4.5±0.010	13.1±0.034
8	16.5±0.102	1.8±0.008	10.9±0.031	47.5±0.270	4.9±0.009	18.45±0.094
9	13.2±0.094	1.1±0.006	10.6±0.023	48.3±0.258	3.9±0.013	22.78±0.111
10	12.1±0.101	1.4±0.004	10.7±0.021	49.1±0.243	3.7±0.012	23.16±0.116

Figure 3 shows the comparative study based on interfacial tension of PLs and C10 modified PLs against water at 27ºC in chloroform solution at six different

concentrations. It reveals from the Figure that interfacial tension of C10 modified PLs is less than interfacial tension of PLs at all concentration levels. So the suitability of the process technology is depicted herewith.

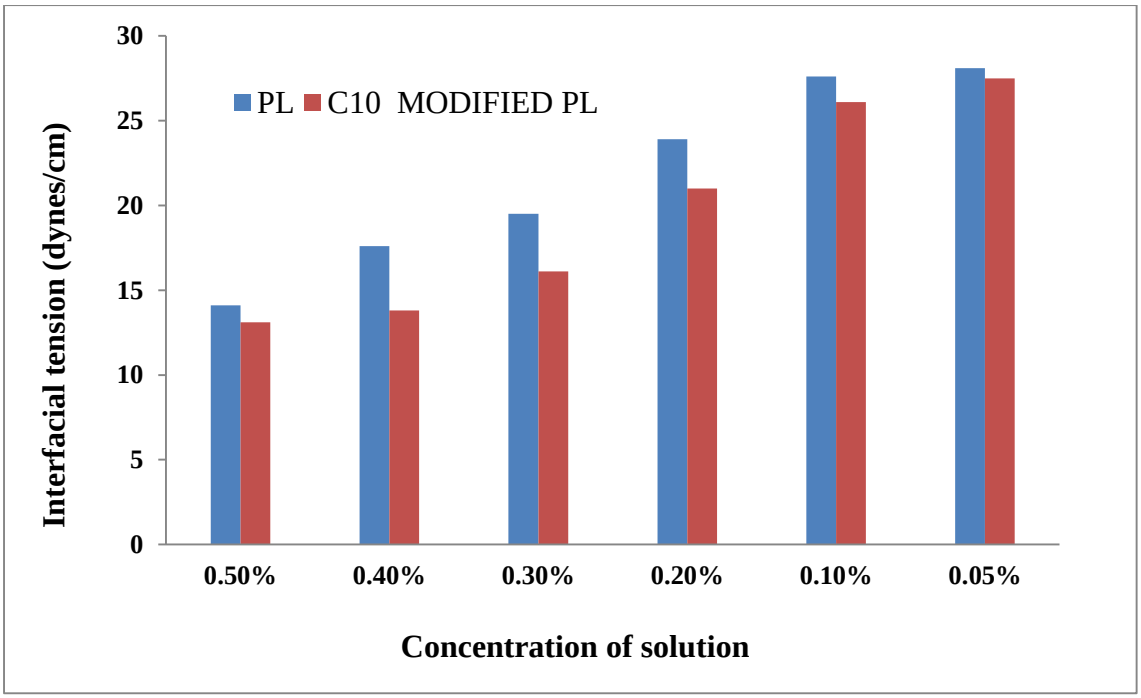

Figure 3: Interfacial tension (dynes/cm) against water at 27ºC in chloroform medium

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

CONCLUSION

Enzymatic method for the preparation of modified or structured phospholipids from cheap raw materials is an innovative approach. Present study deals with the modification of crude soybean phospholipids with decanoic acid in hexane medium in the presence of 1, 3-specific Lipozyme TL IM immobilized lipase (*Thermomyces lanuginosus*). Temperature requirement is very less during the reaction indicating the energy saving through this process. Introduction of decanoic acid into the phospholipids takes ten days which is much slower rate of reaction and encourages finding out more suitable biocatalyst for the process technology which may reduce reaction time. Modified phospholipids contain considerable amount of decanoic acid which is also encouraging. The present bioprocess technology for the modification of crude soybean phospholipids may be applied in industrial sector also. Future researchers may be encouraged and adopted this process technology for the modification of different phospholipids using other fatty acids.

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