Generation of functional structural phospholipids from silkworm pupa oil and soybean phospholipids catalysed by lipases

Lu Tan [1], Jin-Zheng Wang [1], Xin-Ying Wang [1], Shuai You [1, 2], Lu-Chan Gong [1, 2], Jun Wang [1, 2, *]

[1] Jiangsu Key Laboratory of Sericultural and Animal Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212100, China.
[2] Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agriculture and Rural Affairs, Sericultural Scientific Research Center, Chinese Academy of Agricultural Sciences, Zhenjiang 212100, China.
Keywords: Structural phospholipids; lipases; silkworm pupa oil; soybean phospholipids.
Presenting author email: wangjun@just.edu.cn.

With the increasingly serious shortage of resources in the world today, people are paying more and more attention to edible insect resources, such as silkworm pupa. Massive unsaturated fatty acids (UFAs) contained in silkworm pupa oil, such as oleic acids, linoleic acid and α-linolenic. Among them, α-linolenic, which could be metabolized to generate DPA and DHA in the human body, has great application prospects due to its various physiological functions (Wei, 2009). To improve the utilization of UFAs in silkworm pupae oil, Soya phospholipid was employed as a carrier. The use of phospholipid skeleton will effectively improve the absorption of unsaturated fatty acids in the human body (Chojnacka, 2017). Moreover, Soya phospholipids are an essential component of biological membranes and have an important regulatory function in the normal metabolism of the organism, which is determined by their fatty acid composition and the composition of their polar ends. But the natural phospholipids have a limited diversity of fatty acid compositions, which limited its applications. Hence, more and more studies are focusing on the modification of phospholipids to obtain more functional modified phospholipids. Therefore, Soybean phospholipids as a carrier of unsaturated fatty acids in silkworm pupa oil will be expected to expand the utilization of UFAs in silkworm pupa oil.

Enzymatic methods have significant advantages in terms of safety, environmental protection and economy. Relevant studies have shown that fatty acids and phospholipids undergo esterification reaction catalysed by enzymes to obtain modified phospholipids, whose physicochemical properties and biological activities are altered to varying degrees compared with those of the original phospholipids, which can effectively improve the absorption and utilisation of fatty acids in vivo (Gamal, 2016). PUFA-rich lysophosphatidylcholine was efficiently synthesised by MASI lipase-catalysed esterification of n-3 PUFA with sn-glycero-3-phosphatidylcholine under vacuum (Wang, 2019).

In order to efficiently synthesise structural phospholipids rich in special physiological functions, oils and fats rich in functional unsaturated fatty acids and cost-effective soybean phospholipids are usually chosen as reaction substrates (Zhang, 2024). Therefore, constructing an ester exchange reaction between silkworm pupa oil and soybean phospholipids can not only improve the utilisation of silkworm pupa oil, but also prepare structural phospholipids with stronger functionality. However, most of the studies have focused on the optimisation of the process of enzyme-catalyzed synthesis, and fewer studies have been conducted on the structural features of the modified phospholipids.

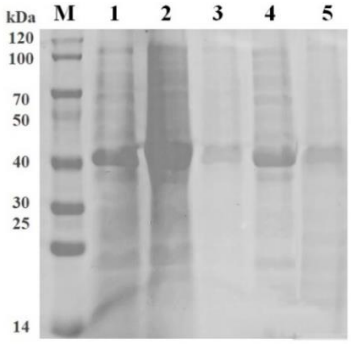

Figure 1 SDS-PAGE analysis of lipase 6A12 and mutants

Lanes: M, Protein Maker of 120 kDa; 1, Lipse 6A12; 2, Lipase mutant G15R;3, Lipase mutant T17R; 4, Lipase mutant D357L; 5, Lipase mutant L359H

To be able to improve the binding of linolenic acid to phospholipids as much as possible, the wild-type lipase 6A12 was modified in this study. Figure 1 shows SDS-PAGE analysis of lipase 6A12 and the mutant with modified affinity for its channel substrate. The lipase 6A12 wild-type band was 43 kDa, and the size of the mutant was consistent with that of the wild type. Since only one amino acid site differs between the lipase 6A12 wild type and the mutant, there is no difference in size between the two.

Figure 2 shows the optimum temperature for hydrolysis of both C18 and C16 substrates by lipase 6A12 and mutants. It can be found that the optimal temperature for hydrolysis of C18 substrate by mutant D357L became 40 °C, and the optimal temperature of other mutants was consistent with that of wild type; the optimal temperature for hydrolysis of C16 substrate by mutant D357L was 50 °C in agreement with that of the wild type, and the other mutants were increased by 10 °C compared with that of the wild type.

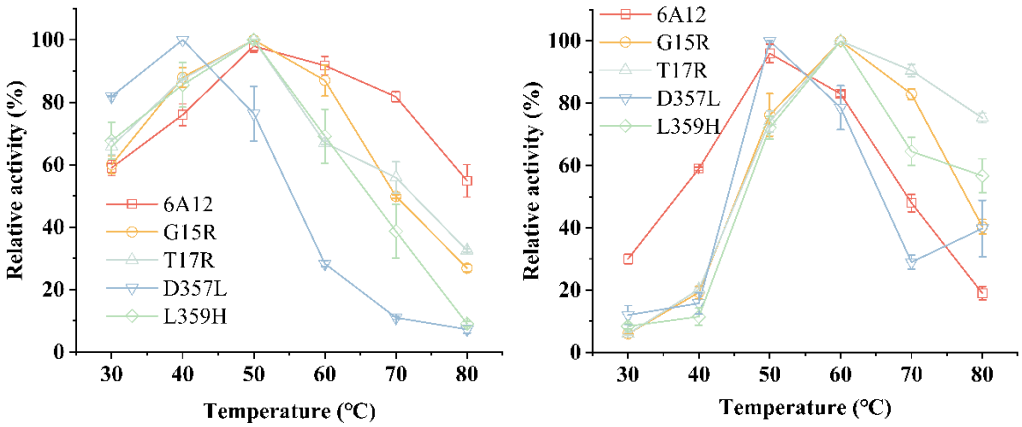

Figure 2. The optimum temperature of lipase 6A12 and mutants G15R, T17R, D357L, L359H. (A) The optimum temperature for hydrolysis of C18 substrate by lipase 6A12 and mutants G15R, T17R, D357L, L359H; (B) Optimum temperature for hydrolysis of C16 substrate by lipase 6A12 and mutants G15R, T17R, D357L.

In summary, in addition to altering the optimum temperature for hydrolysis of the substrate, the mutant also increased its affinity for the C18 substrate compared to the wild type. Therefore, the use of the mutant to catalyse the synthesis of structural phospholipids with higher linolenic acid content using silkworm pupa oil and soybean phospholipids is likely.

Acknowledgements: This study was financially supported by the Key Research and Development Program (Modern Agriculture) of Jiangsu Province (BE2019358), the Jiangsu Agricultural Science and Technology Innovation Fund (CX(20)2029).

References:

[1] Wei Z J, Liao A M, Zhang H X, Liu J, Jiang S T. Optimization of supercritical carbon dioxide extraction of silkworm pupal oil applying the response surface methodology. *Bioresource Technology*, 2009, 100(18): 4214- 4219.

[2] Chojnacka A, Gładkowski W, Grudniewska A. Lipase-catalyzed transesterification of egg-yolk phophatidylcholine with concentrate of n-3 polyunsaturated fatty acids from cod liver oil [J]. *Molecules*, 2017, 22(10): 1771.

[3] Gamal A, El-Nattat W. S, El-Sheshtawy R. I, El-Maaty A. M. A. Substitution of egg yolk with different concentrations of soybean lecithin in tris-based extender during bulls'semen preservability. *Asian Pacific Journal of Reproduction*, 2016, 5(6): 514-518.

[4] Zhang T T, Wang J, Zhao Y K, Wang Z L, Hu D, Liu Y Y, Zhang X L, Li H N, Zhao B X, Li B L. Green biosynthesis of DHA-phospholipids in tailor-made supersaturated DHA aqueous solution and catalytic mechanism study. *Food Chemistry*, 2024, 431: 137164.

[5] Soni S. Trends in lipase engineering for enhanced biocatalysis. *Biotechnology and Applied Biochemistry*, 2022, 65(1): 265-272.

[6] Wang X, Qin X, Li X, Zhao Z, Yang B, Wang Y. An efficient synthesis of lysophosphatidylcholine enriched with n-3 polyunsaturated fatty acids by immobilized MAS1 lipase [J]. *Journal of Agricultural and Food Chemistry*, 2019, 68(1): 242-249.