Supplementary materials for

Nutritional targeting modification of silkworm pupae oil catalyzed by a smart hydrogel immobilized lipase

Jin-Zheng Wang [a], Cheng-Kun Wu [a], Cheng-Hai Yan [a], Huan Chen [a], Shuai You [a, b], Sheng Sheng [a, b], Fu-An Wu [a, b], Jun Wang [a, b, c, *]

[a] *Jiangsu Key Laboratory Sericutural Biology and Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang, Jiangsu 212100, China;*

[b] *Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agricultural and Rural Affairs, Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang, Jiangsu 212100, China;*

[c] *Attached silkworm medicine factory, Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang, Jiangsu 212100, China.*

To whom all correspondence should be addressed.

*Corresponding author. *Phone:* +86-511-84448290, *Fax:* +86-511-84448290.

E-mail: wangjun@just.edu.cn (Prof. Dr. Jun Wang).

Results of the modified SPO preparation process

The immobilized lipase-catalyzed triglyceride transesterification reaction was carried out in a solvent-free system to prepare the modified SPO. The relative content of UFAs in modified SPO was designed as the inspection index, and the effects of three single factors of fluid flow rate, reaction temperature, and substrate molar ratio on the relative content of UFAs in the product were investigated.

Figure S1 shows the effects of flow rate on FAs composition in the modified SPO. The heat transfer enhancement mechanism could be attributed to the interaction of main flow separation, recirculation, eddy currents, and interrupted boundary layer [1]. While PNIPAAm the kind of gel layer with porous structure [2]. When the fluid flow rate is in the range of 2 μL/min~10 μL/min, the relative content of palmitic acid in the total fatty acids shows a general downward trend and drops to the lowest (22.84±0.00%) when the flow rate is 10 μL/min. The relative content of oleic acid and linoleic acid showed an overall upward trend and rose to the highest when the flow rate was 10 μL/min (46.48±0.31%, 9.50±0.32%). While the relative content of UFAs did not change significantly with the fluid flow rate in Figure S2B at the same flow rate. This phenomenon is mainly caused by the uneasily hydrolyzed property of the long-chain fatty acids at the *sn*-2 position [3]. The selection was mainly based on the highest relative content of oleic acid, linoleic acid, and linolenic acid in figure S2D. 10 μL/min of flow rate was still a suitable condition. Finally, combined with the detection of the total content of UFAs in Figure S1D, the relative content of UFAs was the highest when the flow rate was 10 μL/min, and the content of UFAs at the three positions was 77.16 ± 0.00. %, 77.31±4.36% and 77.08±2.18%, respectively. Due to the fixed size of the microchannel, the reaction residence time and the fluid morphology in the microchannel are most affected by the flow rate. The intensity of turbulence near the hole formed

by the photopolymer PNIPAAM in the microchannel increased with the flow rate, which improved the mass and heat transfer characteristics in microchannel [4] and helped increase the interaction between the substrate molecule and the active center of the enzyme molecule.

Figure S2 shows the effect of temperature on FAs composition in the modified SPO. The highest relative content of oleic oil (58.86±11.72%), linoleic acid (11.33±2.10%), and linolenic acid (28.69±5.30%) at 60°C in figure S2A, while the relative content of palmitic acid was the lowest (23.49±0.05%). And there was an observed increase of oleic oil and linolenic oil in figure S2B, which might lead to the increase in figure S2A. While the temperature has no significant effect on FAs composition at the sn-1,3 position, the relative content of UFAs could be the only results to confirm the suitable temperature. Due to the sensitivity of enzymes to temperature, an appropriate increase in temperature is very important to maximize the immobilized lipase activity and product yield in microchannel [5]. In addition, the photopolymer PNIPAAM is used as a temperature-sensitive material. When the temperature rises appropriately, its pore structure is affected, making the lipase molecules more firmly adsorbed on the inner surface of the microchannel [6]. And the temperature rise is conducive to conduct heat and mass transfer in the microchannel, thereby increasing the reaction rate [7]. Therefore, 60°C was determined as a suitable reaction temperature based on the same principle.

Figure S3 shows the effect of substrate ratio on FAs composition in the modified SPO. The relative content of oleic oil reached the highest value at 1/8 of substrate ratio in figure S3A, B, and C, indicating the microchannel reactor enhanced the binding effect of oleic acid in the transesterification reaction, and the highest relative content was 56.56±0.29%, 44.97±4.92%, and 62.36±2.89%, respectively. According to the results in figure S3D, the relative content of UFAs at total FAs and sn-1,3 positions reached the highest content, which was 80.98±0.29% and

82.65±3.0%, respectively. Different molar ratios of substrates may affect the reaction balance, thereby affecting the formation of transesterified products [8]. Properly increasing the content of oleic acid could shift the reaction balance to the product side and promote the transesterification reaction. But high substrate concentration may inhibit enzyme activity, leading to enzyme instability and inactivation, thereby affecting catalytic performance [9]. When the substrate concentration was properly controlled, it could be beneficial to the prepared products. Therefore, the suitable substrate ratio was selected as 1/8.

Figure captions

Figure S1 Effect of flow rate on fatty acids composition in the modified SPO. (A) Total fatty acids composition; (B) *sn*-2 fatty acids composition; (C) *sn*-l,3 fatty acids composition; (D) changes in unsaturated fatty acids content in different positions.

Figure S2 Effect of temperature on fatty acids composition in in the modified SPO. (A) Total fatty acids composition; (B) *sn*-2 fatty acids composition; (C) *sn*-l,3 fatty acids composition; (D) changes in unsaturated fatty acids content in different positions.

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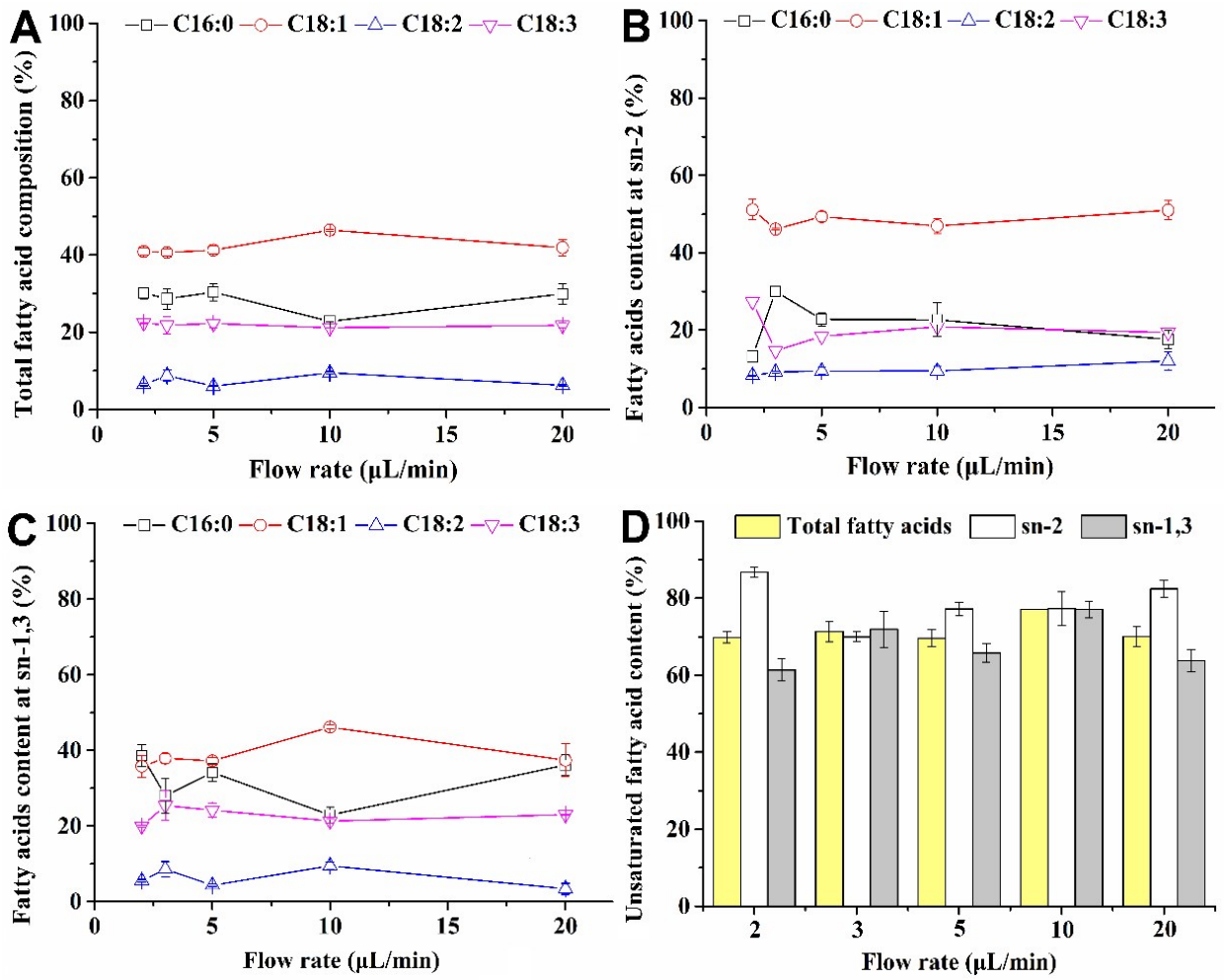
Figure S4 Standard curve of p-NP concentration.

Figure S5 TLC profile after removal of free fatty acids. Sodium hydroxide (0.5M) and n-hexane were used to mix with SLs in a volume ratio (20:30:3), and the mixture was placed at 60°C for 1 hour. The developing agent was a mixture of n-hexane, ether, and formic acid with a volume ratio of 60:40:1.6.



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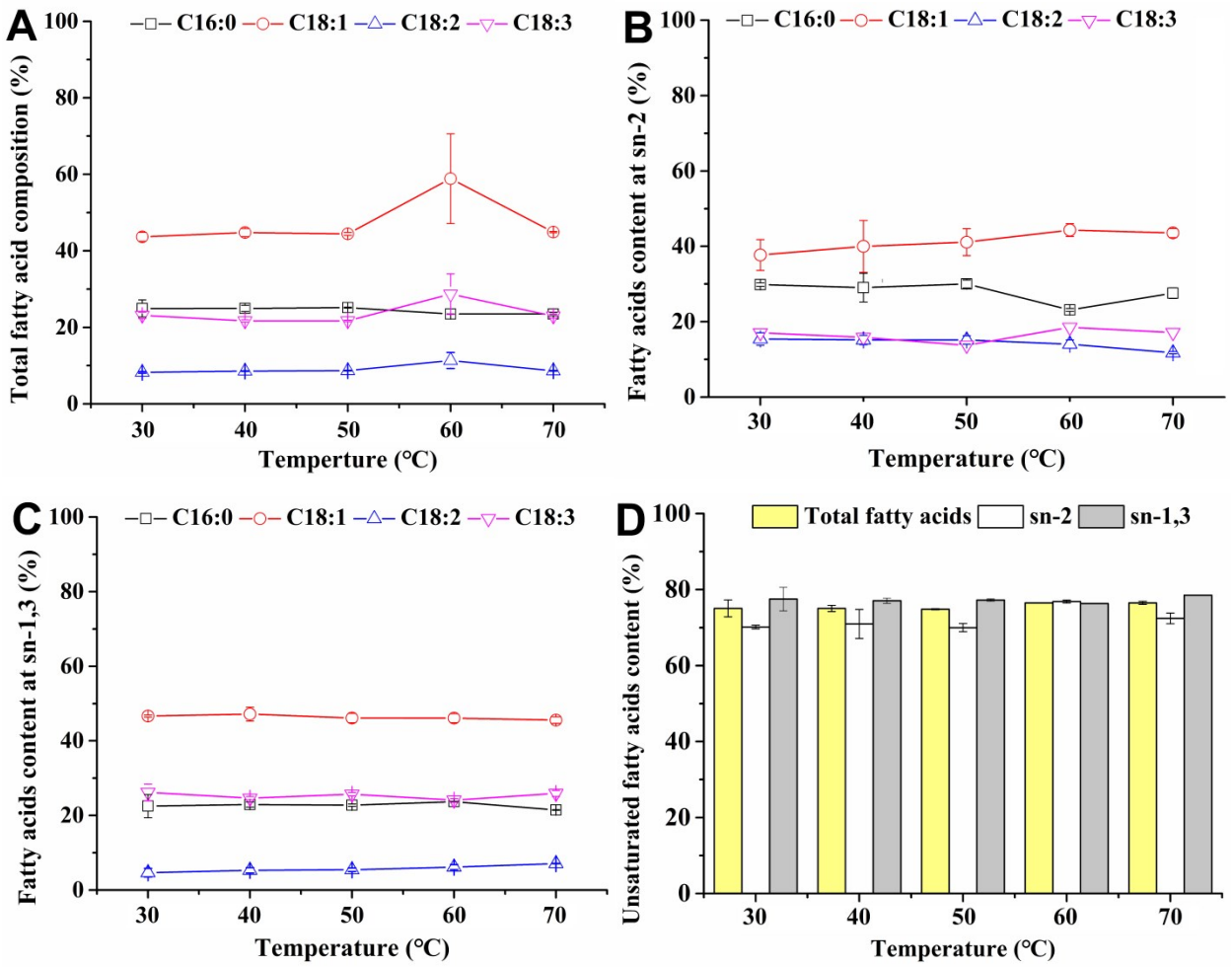

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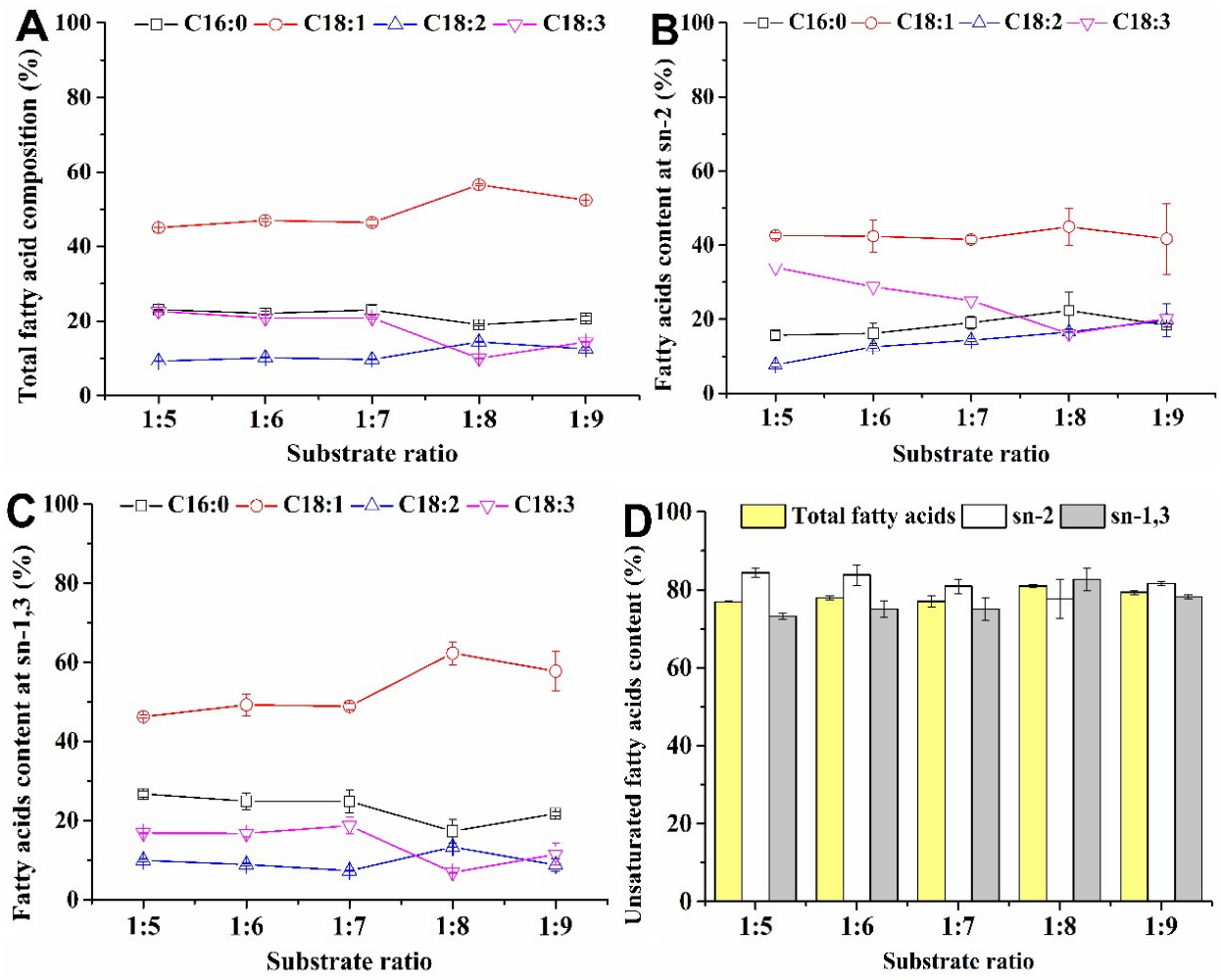

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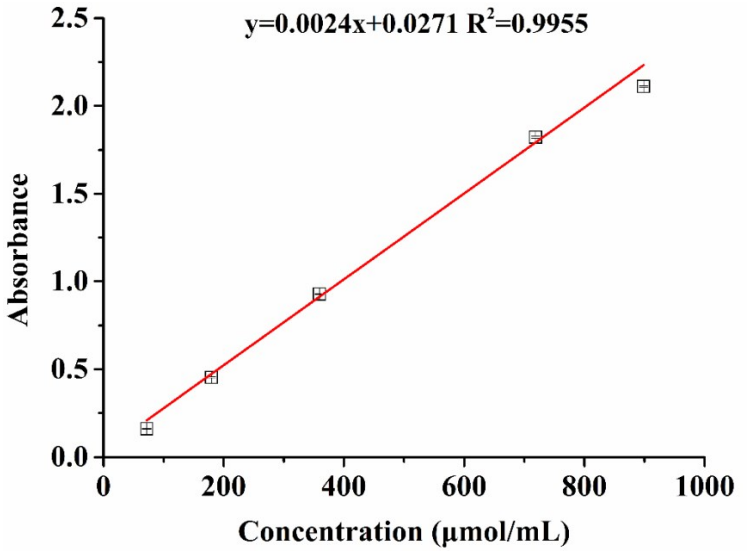

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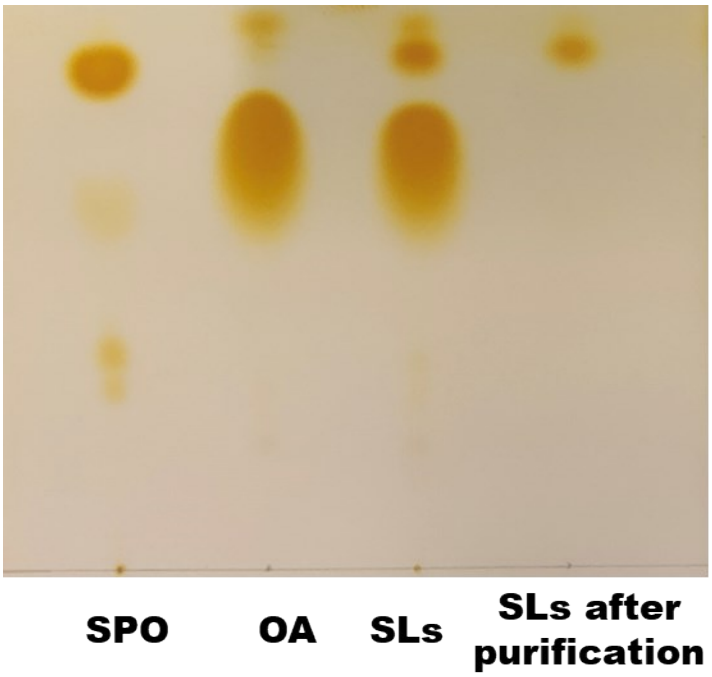

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