Enzymatic preparation of structural lipids from mulberry seed oil and *α*-linoleic acid

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Mulberry seed is an agricultural residue of mulberry juice, and has high nutritional value. In mulberry seeds, there are 33.4% of oil, 25% of crude protein, 19.9% of total amino acids and 5.71% of essential amino acids. Mulberry seed oil is a dark brown fat extracted from mulberry seeds at low temperature. Its unsaturated fatty acid content accounted for 81.2% of total fatty acids, and the content of essential fatty acid-linoleic acid content up to 69.93%, has high economic value and nutritional value. Silkworm pupae oil is extracted from silkworm pupae and contains a variety of high-grade fatty acid glycerides mixture, the appearance of yellow to red transparent oily liquid. Silkworm chrysalis contains more than 80% of unsaturated fatty acids, of which the main ingredient is *α*-linolenic acid, oleic acid and so on. In the present study, the use of mulberry seeds oil and silkworm pupae oil as raw material, the use of mulberry seeds oil and silkworm pupa oil linoleic acid to prepare a new ratio of structural lipids. The use of these raw materials can not only bring good economic benefits to farmers, but also improve the efficiency of mulberry seeds and comprehensive benefits of sericulture (Bhagat and Das, 2015).

Linoleic acid in mulberry seeds oil belongs to the *ω*-6 family of polyunsaturated fatty acids (PUFAs), and linolenic acid in silkworm pupa oil belongs to *ω*-3 PUFAs. WHO and FAO have proposed that a suitable dietary ratio of *ω*-6: *ω*-3 PUFAs is (5-10):1, but many studies have found that different proportions were beneficial for different diseases (Singh et al., 2013). A large number of epidemiological data show that diet *ω*-6: *ω*-3 PUFAs ratio is too high and is closely related to the high incidence of certain diseases, such as: coronary heart disease, diabetes, breast cancer (Mccusker and Grant-Kels, 2010), etc. For example, *ω*-6: *ω*-3 ≤ 4:1 in the elderly diet can reduce cholesterol and low density lipoprotein, and clear blood vessels; when PUFAs ratio is *ω*-6: *ω*-3 = 2.5: 1, the rectal cell proliferation of patients with colorectal cancer can be reduced; when PUFAs ratio is *ω*-6: *ω*-3 = 2-3:1, inflammation in the body of rheumatoid arthritis can be inhibited. So, the optimum ratio range of *ω*-6: *ω*-3 is 1:1- 4:1 depending on the diseases (Muriuki et al., 2011), so how to regulate the balance of *ω*-6 and *ω*-3 PUFAs becomes one of the hot topics in the field of fatty acid food application (Heidmann and Marília, 2003).

In this study, lipase was used to catalyze the transesterification of mulberry seeds oil and *α*-linolenic acid to synthesize structural lipids. The reaction conditions including time, substrate ratio, enzyme amount and temperature are selected (Ketsa et al., 2016). And plan to use response surface methodology to design and synthesize structural lipids containing different proportions of PUFAs and to examine their effects on chronic diseases (Singh et al., 2011).

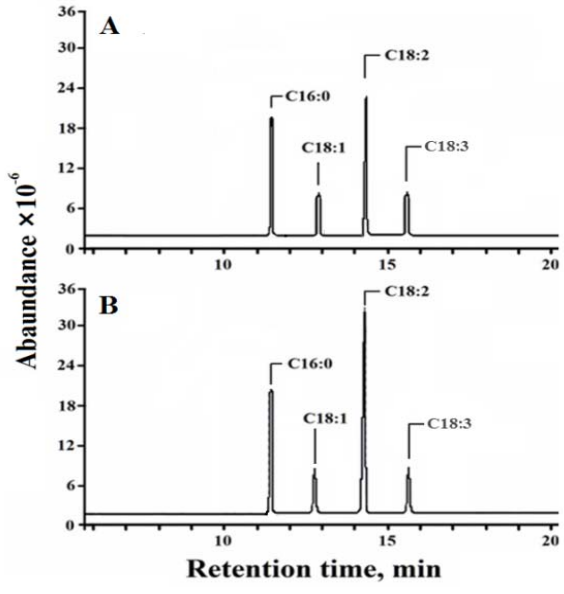

Fig. 1 GC chromatogram of fatty acid methyl esters (FAMEs) from mulberry seed oil TAGs.
(A) FAMEs standards; (B) FAMEs sample of mulberry seed oil.

After the esterification of mulberry oil triglyceride, GC chromatography was used to analyze the composition of all components after comparison with standard products (Fig. 1). The main components shown in Fig. 1 were as follows: C16:0 represents palmitic acid, C18:1 represents oleic acid, C18:2 represents linoleic acid, C18:3 represents α-linoleic acid. The results show that the substances contained before and after the reaction basically the same, but the content has changed.

The optimal reaction time and substrate ratio were experimentally tested, respectively. As shown in Fig. 2, the optimum reaction time was 20 h, and the optimum substrate ratio was 1:7. Fig. 2A shows that samples with different substrate ratios reacted for the same time at 24 h and samples were taken every 4 h to determine the fatty acid content ratio that is ω-6: ω-3 PUFAs ratio, when the ratio reached the lowest, the content of α-linolenic acid reached the highest. And the esterification rate was the largest, it was shown that the most appropriate reaction time is 20h; After 20 h reaction, different substrate and the ratio of PUFAs was shown in Fig. 2B, when the substrate ratio was 1:7, α-linolenic acid content reached the maximum. Thus, the optimal reaction time and substrate ratio were experimentally tested, respectively. The results provide the basic conditions for subsequent experiments.

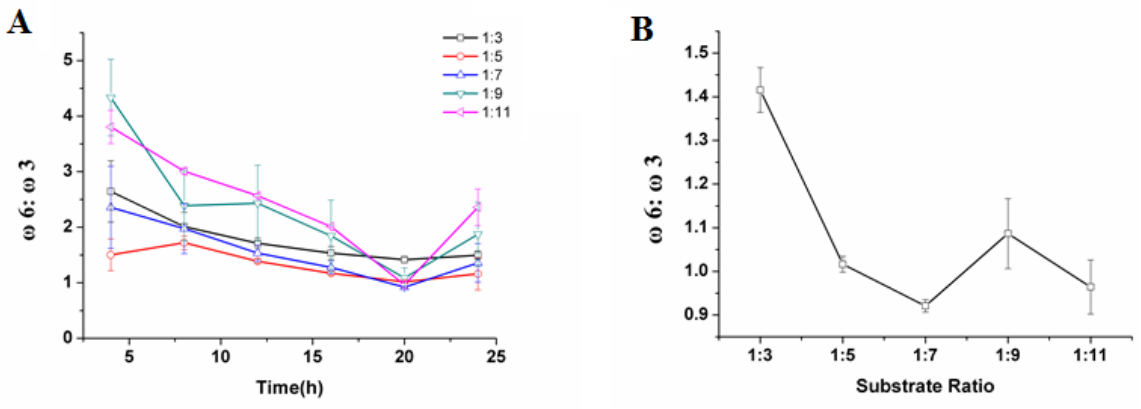

Fig. 2 The optimal reaction time and the optimal substrate ratio on PUFAs ratio (ω-6: ω-3)
(A) PUFAs ratio change trend; (B) PUFAs ratio after different substrate ratios reacted 20h.

Other conditions of reaction are still being exploed. This experiment is expected to be designed to inhibit the proliferation of tumor cells by using the response surface technology.

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References

[1] Okuyama, H., 2001. High n-6 to n-3 ratio of dietary fatty acids rather than serum cholesterol as a major risk factor for coronary heart disease. *European Journal of Lipid Science & Technology*, 103, 418-422.

[2] Bhagat, U., Das, U.N., 2015. Potential role of dietary lipids in the prophylaxis of some clinical conditions. *Archives of Medical Science*, 11, 807-818.

[3] Heidmann, S.M.C., Marília, O., 2003. Seafood as functional food. *Brazilian Archives of Biology & Technology*, 46, 443-454.

[4] Ketsa, O.V., Shmarakov, I.O., Marchenko, M.M., 2016. Lipid peroxidation in cardiac mitochondrial fraction of rats exposed to different supplementation with polyunsaturated fatty acids. *Biochemistry Supplement*, 10, 50-55.

[5] Mccusker, M.M., Grant-Kels, J.M., 2010. Healing fats of the skin: the structural and immunologic roles of the ω-6 and ω-3 fatty acids. *Clinics in Dermatology*, 28, 440.

[6] Muriuki, M.W., Dumancas, G.G., Purdie, N., Reilly, L., 2011. Quantification of total ω-6 and ω-3 fatty acids and ω-6/ω-3 ratio in human serum using GC-MS. *LC-GC North America*, 29, 60.

[7] Singh, R., Wilcynska, A., De, M.F., Wilson, D.W., Hungin, A.P.S., 2011. Can high ω-6/ω-3 ratio of fatty acids in the neuronal cell membrane phospholipids predispose fear behaviour? *Open Nutraceuticals Journal*, 4, 1-2.

[8] Singh, R.B., Singh, A.K., Alam, S.E., Meester, F.D., Fedacko, J., Dharwadkar, S.M., Juneja, L.R., Wilson, D.W., 2013. Effects of omega-3 fatty acids on genetic expressions. *Humana Press*, 2013:27-50.