Microfluidic production of structural lipids from silkworm pupa oil prepared by low-temperature enzymatic extraction

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Insects are the most powerful, most diverse, large biomass, high food conversion rate, and fast reproduction biological populations on the earth. Since ancient times, humans have used them as an important resource for food and medicine. But in contemporary life, this kind of resource has been ignored for years due to its terrible smell or crappy shape, despite that it has a promising potential of nutritional value. Such as silkworm pupa which was considered as co-products of silk industry was discarded, while it is rich in a large amount of essential unsaturated fatty acids and essential amino acids which could be discovered as nutritional supplements. Furthermore, in recent years, due to the increasing demand of human for nutrient, there is a lack of resource to produce those nutrients. Thereby, a novel method for utilizing the insect resource might be a promised path.

There are two important resources in silkworm pupa, one is the silkworm pupa oil (SPO) which is rich in unsaturated fatty acids (Liu et al. 2015) such as α-linolenic acid that could not be synthesized by the human body, and the other is a protein resource, which is rich in various essential amino acids (Table 1). Among them, Fats and oils are one of the most important components in food. In the metabolic system, they not only provide energy for life action, but also play a structural role in cells. There are a lot of researches indicates that high-fat diet has associated with hyperlipidemia, fatty liver, hypertension, cerebral thrombosis, and some cancers increased. As people become more aware of the nutritional nature of lipids, people are beginning to realize that consuming certain fats has a positive effect. Structured lipids (SLs) has the function of health care (Tecelao et al. 2010), has become a hot topic in the field of oil industry.

Table 1 The amino acid content of silkworm pupa

Amino acid species	Asp	Glu	Cys	Ser	Gly	His	Arg	Thr
Content (mg/mL)	19.39±22.02	49.41±0.86	2.43±0.23	19.92±2.13	22.90±1.29	14.18±9.69	24.44±5.04	18.85±1.15
Amino acid species	Ala	Pro	Tyr	Val	Met	Ile	Leu	-
Content (mg/mL)	20.27±2.78	58.33±5.93	25.23±2.24	20.52±1.06	10.38±4.23	16.12±1.18	25.40±2.56	-

Generally, these silkworm pupae can be extracted by organic solvents to conveniently obtain silkworm pupae oil and defatted silkworm pupae powder rich in high protein. However, there is an issue here. Organic solvents and high temperatures were involved in this defatted process (Zhao et al. 2015), jeopardizing the quality of silkworm pupa oil and protein. Thus, enzymatic-assisted extraction might be a beneficial method.

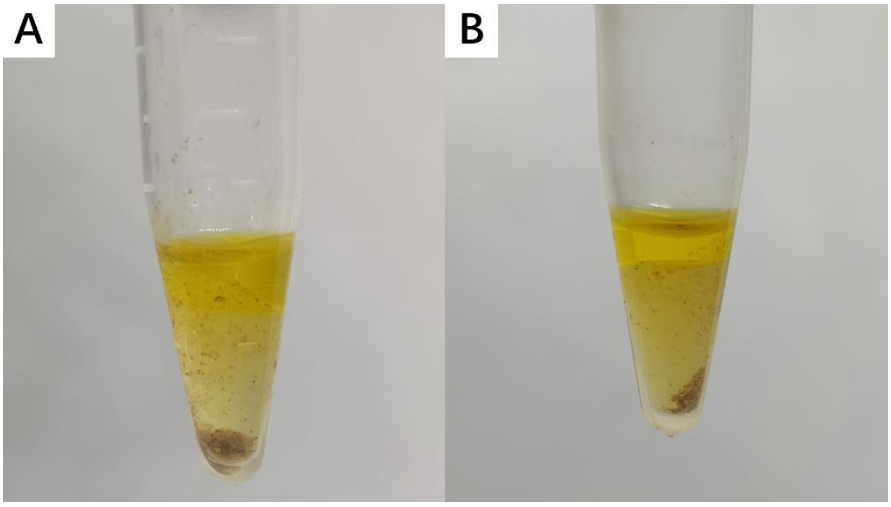

Figure 1 Silkworm pupa dregs treated with papain (A) and complex protease (B) at water bath under 40 °C for 6 h without shaking.

The enzymatic-assisted method is an efficient method for extracting insect fat (Su et al. 2019), compared with ultrasonic-assisted aqueous extraction and microwave-assisted extraction which shows low extraction efficiency (Sun et al. 2018) or required a high amount of solvent (Wang et al. 2017). Due to those issue, these physical approaches are hard to scale up. Figure 1 shows that enzymatic-assisted extraction can easily obtain two-phase products, and the upper layer is SPO and the lower layer is an aqueous solution containing a large amount of protein. An HPLC instrument with an evaporative light scattering detector (HPLC-ELSD) was employed to analysis the triglyceride composition. Figure 2 shows that in the composition of silkworm pupa oil extracted by enzymatic hydrolysis treatment (SPOE) and silkworm pupa oil extracted by solvent method (SPO), there is little difference in component types, but the content of individual TAGs is different.

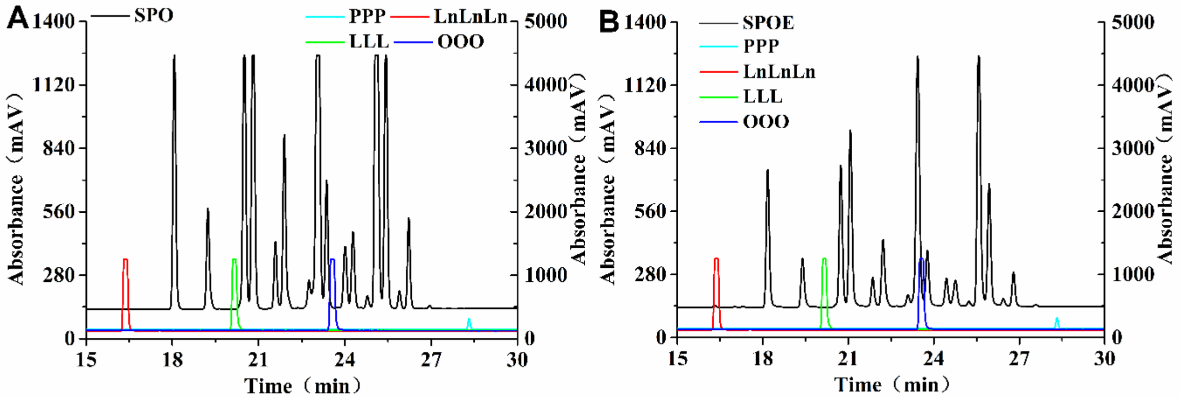

Figure 2 The HPLC-ELSD chromatogram of SPO (A) and SPOE (B). The ordinate on the left is the absorbance of the standard triglyceride (PPP, LLL, OOO, LnLnLn).

Furthermore, the reaction system also should be considered. The traditional reactor shows a lot of disadvantages, such as long reaction time or lower quality. While microfluidics devices have been praised as next-century technology or just a fashionable trend (Daw &Finkelstein 2006), and the features reduced sample consumption, rapid molecular diffusion, precise reaction control and high separation efficiency (Wang et al. 2019).

Thus, a novel method combining enzymatic-assisted extraction and microfluidics devices will be constructed in this study to utilize the insect resource.

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