Preparation and digestion characteristics of silkworm pupae oil microemulsion

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Silkworm pupae oil is rich in *α*-linolenic acid, which has a variety of health functions such as protecting the brain, promoting retinal development and preventing cardiovascular diseases. Previous studies have shown that silkworm pupae oil is a high-quality lipid based on its higher digestibility characteristics *in vitro* (Yan, 2021). However, silkworm pupae oil is unstable, easily rancid, and has peculiar smell, which severely limits its application in food. Therefore, the focus of high value-added product development is to improve the stability of silkworm pupae oil and give full play to its physiological function. The microemulsion system has a good solubilization effect on hydrophobic nutrients, and nutrients are encapsulated in the microemulsion to isolate from the environment and improve the antioxidant capacity (Chen, 2017).. At present, there is no research on the microemulsion system loaded with silkworm pupae oil. Therefore, in this study, the silkworm pupae oil microemulsion system was constructed to protect its biological activity and broaden the application of silkworm pupae oil.

Pseudo-ternary phase diagram is used in the study of microemulsion system loaded with silkworm pupae oil. The microemulsion preparation process was optimized based on the microemulsion area and safety, and the physical and chemical properties of the microemulsion were characterized by the particle size, electrical conductivity, and phase transition temperature. Meanwhile, the stability of silkworm pupae oil microemulsion was investigated through different treatments under storage conditions. Finally, studies have shown that the use of microemulsion as a delivery vehicle for nutrients can not only improve its solubility, but also can be mixed with meals during the digestion process to form a new type of micelles, and promote the bioavailability of nutrients (Hu, 2012). Therefore, the *in vitro* digestion process of silkworm pupae oil microemulsion was monitored through the pH-stat model, which helps to make a comprehensive nutritional value evaluation of silkworm pupae oil microemulsion.

The preparation process of the silkworm pupae oil microemulsion were optimized by the pseudo-ternary phase diagram. The results showed that the pseudo-ternary phase diagram has the largest microemulsion area, when the surfactant was EL-35, the co-surfactant was ethanol, the Km value was 2 and the oil ratio was 1:2, Figure 1 shows the size change of silkworm pupae oil microemulsion with the different ratio of oil phases and mixed surfactants. The droplet sizes at ratios of 1:9, 2:8, 3:7, 4:6, and 5:5 are 15.29 nm, 16.25 nm, 19.05 nm, 29.69 nm, and 44 nm, respectively. The particle size is much smaller than that of human red blood cells, which is conducive to improving the absorption and utilization of silkworm pupa oil (Park, 1999). As shown in Figure 1B, the silkworm pupae oil microemulsion is evenly distributed under the transmission electron microscope. After microemulsification, the particle size of silkworm pupa oil is less than 100 nm, which helps to improve its stability and solubility.

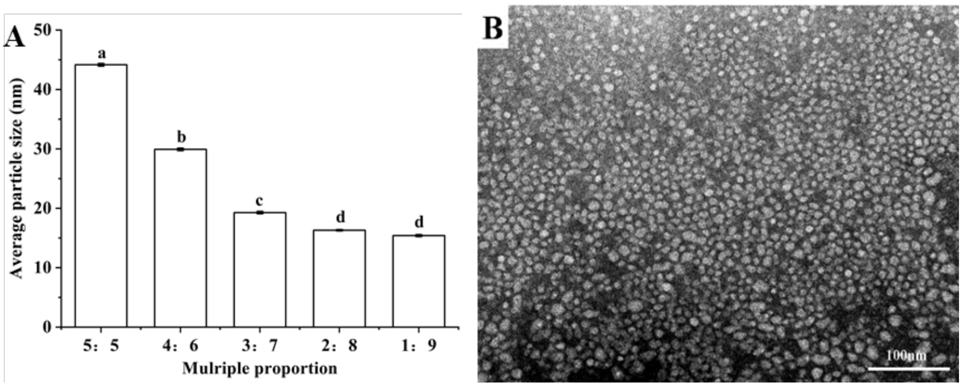

Fig. 1 The particle size (A) and morphology (B) of silkworm pupae oil microemulsion.

Figure 2 shows the total antioxidant capacity of silkworm pupa oil and its microemulsion. The antioxidant capacity of silkworm pupa oil and its microemulsion are 0.0399 mM Trolox and 0.3074 mM Trolox, respectively. The total antioxidant capacity of the microemulsion was significantly higher than that of silkworm pupa oil ($p<0.05$), and the active ingredients in silkworm pupa oil showed better stability.The reason may be that silkworm pupa oil accumulates in the inner core of the O/W microemulsion droplets and is surrounded by closely arranged surfactant and co-surfactant molecules(Sun, 2015). The silkworm pupa oil inside the droplet has less contact with light, heat and oxygen, which makes it difficult to be oxidized and destroyed.

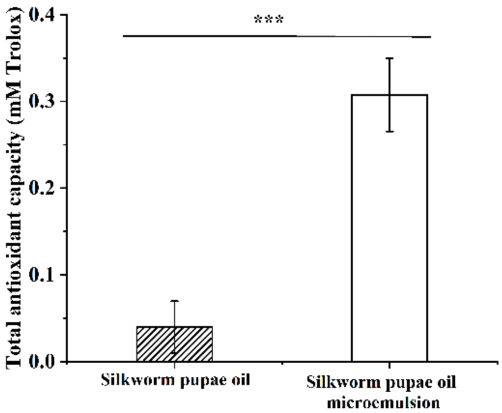

Fig. 2 Total antioxidant capacity of silkworm pupae oil and its microemulsion.

Table 1 shows the *in vitro* digestion characteristics of silkworm pupae oil and its microemulsion. Within 0-30 min, the hydrolysis rates of both have a good linear relationship, and R^2 is greater than 0.9992. At the same time, the maximum free fatty acid release level of silkworm pupae oil was 59.49±8.74%, and the digestion rate was 0.26266 s^{-1}; while the maximum free fatty acid release level of silkworm pupae oil microemulsion was 72.91±6.85%, and the digestion rate was 0.32507 s^{-1}. Results show that the free fatty acid release level and digestion rate of silkworm pupae oil microemulsion are higher than silkworm pupae oil. This may be because silkworm pupae oil microemulsion has a better emulsification effect and is more conducive to the binding reaction with enzymes and bile salts (Ye, 2018). Therefore, microemulsification can significantly improve the *in vitro* digestibility of silkworm pupae oil.

Table 1 The Free fatty acid release profiles and the kinetics results..

Lipids	Free fatty acid released	Kinetic parameter	
	Maximum extent (%)	Apparent rate constant (s^{-1})	Linear $RAdj^2$
Silkworm pupae oil	$59.49±8.74^b$	0.263±0.01	0.9929
Silkworm pupae oil microemulsion	$72.91±6.85^a$	0.325±0.01	0.9909

Lowercase letters indicate significant difference between groups ($p<0.05$). Mean ± SD (standard deviation) of triplicate determinations.

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