

Research Article

Isolation of Phospholipid from Egg Yolk with Ultrasonic Separation Technology

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Abstract: This study presented a new solution of isolation for phospholipid from egg yolks by ultrasonic wave. Degradation of phospholipid was discussed with the aggregation of micro-particles. The frequency of ultrasonic wave was 20 kHz. Lubricant was treated for 9 min under 0, 200, 400, 600W, respectively. It was showed that concentration of phospholipid reduced as ultrasonic power and time increased. Ultrasonic wave was useful for degradation of high molecular protein. Phospholipid secondary structure transforming was also observed, which was affected by ultrasonic wave. Suspension particles aggregated under the different ultrasonic wave condition. Content of the aggregation increased and volume of the aggregate reduced as ultrasonic treatment time increased.

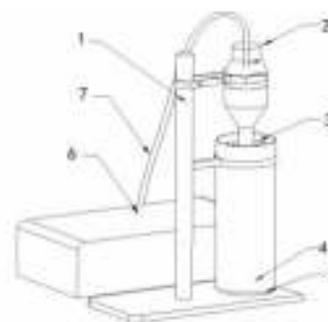
Keywords: Isolation, nutrition, phospholipid, ultrasonic wave separation

INTRODUCTION

Egg yolk is a key ingredient in many food products (Yan *et al.*, 2010). Recently scientists have been paid more attention to the phospholipid separation of egg yolk, which was ample and not well developed. Effective isolation of phospholipid became a key issue for nutrition research. Phospholipid was generated from egg yolks and used for this research. Other components such as a variety of Low-Density Lipoproteins (LDL), High-Density Lipoprotein (HDL) in granules etc (Folch *et al.*, 1957; Kevin and Kinsella, 1978), would inhibit the separation process of phospholipid in the egg yolks. The collected egg yolks must be isolated to analyze the size and morphology of phospholipid. Compared with many extraction methods of phospholipids from egg yolk, such as enzymatic hydrolysis and so on, which performed with a low efficiency, a new separation method was therefore urgently needed to achieve. We isolate fresh egg yolk by ultrasonic separation technology, aimed to find a safe, cheap and effective way.

MATERIALS AND METHODS

Materials and equipments: Scanning Electron Microscopy (SEM), S-3000N, Japan Hitachi. UV-Vis spectrophotometer, EV-60, American Thermo. Infrared microscopy system, VERTEX80VFT-IR HYPERION 2000, Germany Bruker. Real-time online particle analyzer, Lasentec®, Swiss Mettler-Toledo.



1 bracket 2 amplitude transformer 3 lubricating fluid 4 plexiglass container
5 stainless steel 6 ultrasonic power 7 conductor

Fig. 1: Experimental device sketch

Experimental device was shown in Fig. 1. It could be seen that the experimental apparatus for phospholipid isolation and separation was enhanced by ultrasonic wave. This equipment mainly consisted of ultrasonic power, amplitude transformer, plexiglass container with bottom of stainless steels, bracket and a number of conductors. Ultrasonic wave was generated by ultrasonic power and amplitude-end output. Collected lubricant was placed in the square plexiglass container with a stainless steel bottom. By adjusting the height of amplitude transformer, ultrasonic wave could do well with the treatment of suspension inside the container.

Experimental procedures: Egg yolk was fractionated by a low speed centrifugation into granules and plasma

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(Causeret *et al.*, 1991). Fresh egg yolk was collected and placed in the square plexiglass container of the experimental apparatus. Ultrasonic wave was generated by ultrasonic power and amplitude-end output. The height of the container should be adjusted to make sure that the head of amplitude transformer was under the liquid level and the length was more than 20 mm. Then the sound chemical processing system power was opened to treat the lubricating fluid for 9 min under 0, 200, 400, 600 W, respectively and for 0, 3, 6, 9 min under 600 W, respectively. The samples were collected to be preprocessed for analysis and the following tests.

Infrared microscopy system (VERTEX80VFT-IR HYPERION 2000, Germany Bruker) was used to analyze egg yolks prepared after the processing of different parameters by using the KBr pellet method. The scanning frequency of infrared spectrum was 4 cm^{-1} with a scanning number of 1762.

Analytical methods: Scanning electron microscope (S-3000N, Japan Hitachi) was used to investigate the stripping effect of phospholipid. Ultrasonic degradation effect of phospholipid in the egg yolks was characterized by the value of absorption at 280 nm of UV curves (EV-60, American Thermo). The aggregation of phospholipid in the egg yolks was studied to obtain the distribution of particles by using real-time online particle analyzer (Lasentec®, Swiss Mettler-Toledo).

RESULTS AND DISCUSSION

Degradation of phospholipid: Protein molecule had the typical absorbing ultraviolet light ranged from 260 to 300 nm wave length and the value of absorption was increased as the protein concentration increased. The

egg was treated by ultrasonic wave with a frequency of 20 kHz. Absorption peaks ranged from 260 to 300 nm in Fig. 2 indicated that there were proteins remained in the egg. But protein concentration became lower in the lubricant as the ultrasonic power increased. It indicated that the greater ultrasonic power would induce the more degradation of phospholipid.

It could be seen that there were absorption peaks at the wavelength of 280 nm induced by ultrasonic wave. It indicated that there were proteins remained in the lubricating liquid. But protein concentration became lower in lubricant as the ultrasonic power increased at the wavelength of 280 nm. The longer working time of the ultrasonic wave would induced, the lower concentration of phospholipid.

From the above it could be seen that ultrasonic power and time played an important role in the degradation of phospholipid. It should be the reason of the locally high temperature and pressure generated by ultrasonic cavitation function, which would accelerate the denaturation and degradation of serum protein. Protein degradation would be accelerated when the various ultrasonic wave exhibited the different acoustic characteristics.

Structure disruption of phospholipid with ultrasonic wave: Alteration and destruction of spatial structure was the direct reason of denaturalization and degradation of phospholipid. Infrared spectra characteristics of the I-amino band ranged from 1600 to 1700 cm^{-1} was a fairly useful information for the identification of the protein spatial structure (Xie and Liu, 2003). The influence of ultrasonic wave on the structure of phospholipid could be determined by analyzing the correspondence relationship of protein secondary structure with each sub-peak.

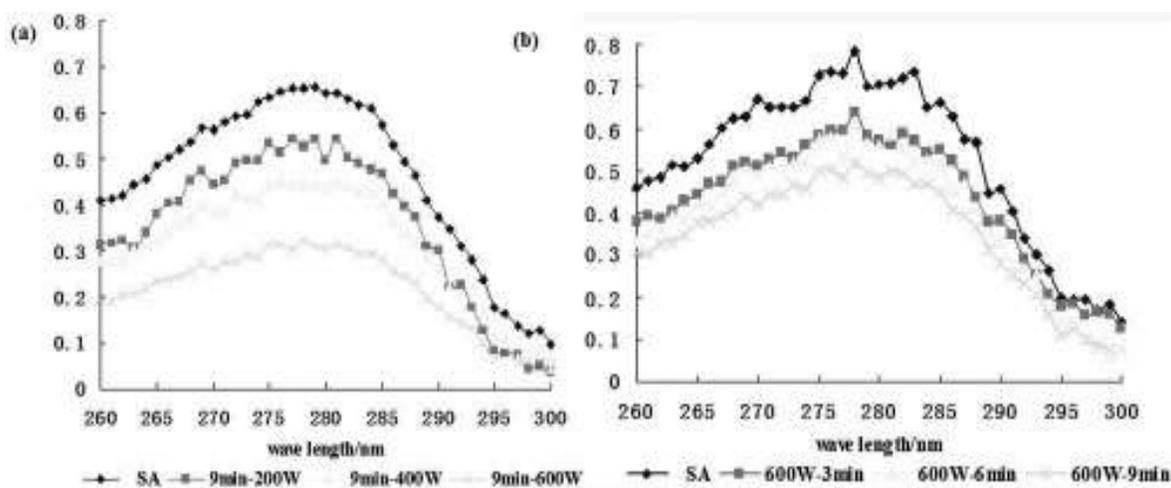


Fig. 2: Phospholipid wavelength-absorbance curves, (a) SA wavelength-absorbance curves with input power, (b) SA wavelength-absorbance curves with time

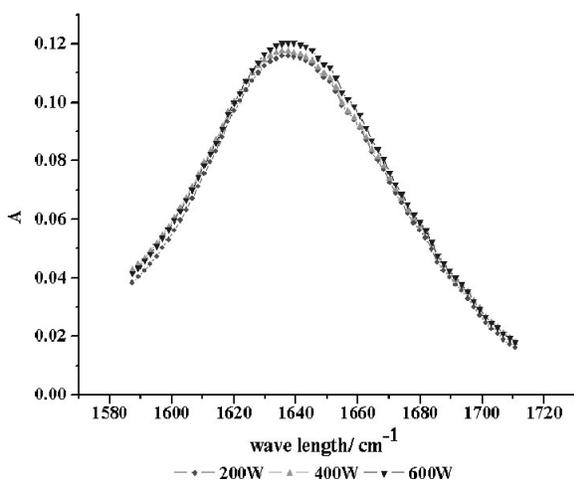


Fig. 3: Infrared spectra of I-amino band

It could be seen from Fig. 3 that α -helix band ($1650\text{-}1658\text{ cm}^{-1}$) and coils ($1650\text{-}1640\text{ cm}^{-1}$) of I-amino band reduced significantly as ultrasonic power increased. Ultrasonic treatment with different power induced β -folded ($1640\text{-}1610\text{ cm}^{-1}$) content to be increased. It could be deduced that ultrasonic wave could convert α -helix and random coil to β -sheet, which was consistent with the result obtained by Liu *et al.* (2010). Meanwhile, the peak positions tended to move towards the lower wave number because the α -helix and other conformations were relative to hydrogen. Hydrogen of the polypeptide chain disconnected for the reason of the synergistic effect of ultrasonic cavitation and jet. Then the number of hydrogen bonds between small molecule and peptide-chain molecules increased and the number of hydrogen bonds between the peptide chain and the peptide chain molecules reduced, thus protein structure changed.

Effects of ultrasonic wave on phospholipid remove process: Phospholipid had complex shape and different size. The density of the egg yolks was more than phospholipid. Due to the close density to fresh egg yolks, phospholipid could easily flow and redistribute in the egg yolks. It induced the concentration of phospholipid changed difficultly by the sedimentation function and phospholipid with different shape and size were coated by egg yolks.

Phospholipid was stripped from egg yolks by ultrasonic treatment. Ultrasonic treatment was an ongoing process. Due to factors such as thermal effects, mechanical shear force, the formation of the gas-liquid interface as well as the production of free radicals, macromolecular molecules exhibited a wide range of effects, including degeneration, degradation and the formation of aggregation (Coakley and Dunn, 1971; Machova *et al.*, 1999; Tian *et al.*, 2004; Howard *et al.*, 1997). Firstly, local high temperature and pressure generated by ultrasonic cavitation would accelerate the

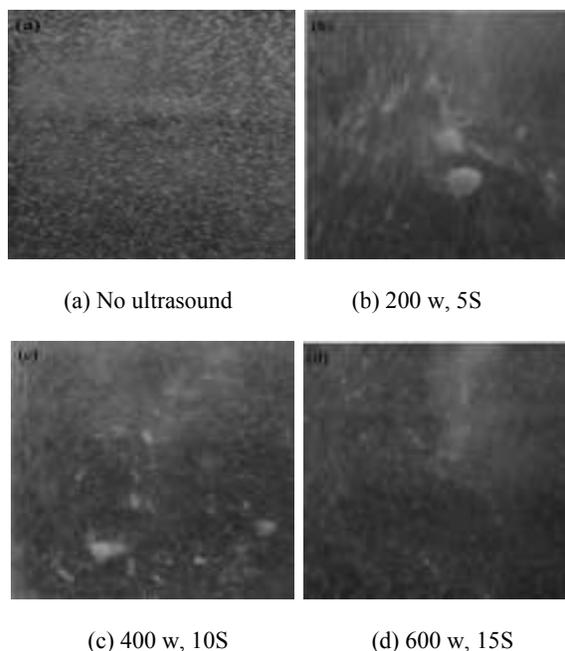


Fig. 4: Procedure of aggregation of phospholipid with different ultrasonic conditions

denaturation and degradation of the serum protein. Secondly, the shock waves and micro-jet generated by the ultrasonic cavitation had crushing effect on phospholipid, including denaturation and secondary structure disruption. Thirdly, phospholipid moved to the acoustic pressure nodes or loops induced by effects of acoustic radiation force and gravity. At the same time, phospholipid group settled easily in the form of acoustic secondary force. All reasons above would help us isolate and separate phospholipid from egg yolks successfully.

Aggregation of phospholipid in egg yolks: Figure 4 was the procedure of aggregation of phospholipid under different conditions. Phospholipid coated with egg yolks and formed a quantity of aggregations under ultrasonic power of 200, 400 and 600 W, respectively. The aggregations were different sizes and numbers. It could be seen from Fig. 4a that phospholipid were uniformly distributed in the egg yolks when no ultrasonic wave existed. Seen from Fig. 4b, phospholipid moved strenuously in the container and aggregated to form large balls, which were induced by ultrasonic wave of 200 W for 5s. Seen from Fig. 4c, phospholipid moved more strenuously in the container and formed more aggregations. At the same time, large particle aggregations formed in Fig. 4b were broken into small aggregations, which were induced by ultrasonic wave of 400 W for 10s. Seen from Fig. 4d, phospholipid moved so intensively that a large number of bubbles and little aggregations could be found. Aggregations formed in Fig. 4b and c was broken into a large amount of smaller ones, induced by ultrasonic wave of 600 W for 15s. It could be concluded that the

aggregation quantity increased as the ultrasonic power increased and the volume decreased as aggregation degree became weak.

Aggregation of phospholipid was caused by the following reasons, such as the thermal effects caused by ultrasonic cavitation, which were local high temperature and pressure produced by instantaneous blasting of cavitation. A large number of bubbles were generated and new interface was formed during the ultrasonic wave working. The new interface of gas-liquid was one of the reasons for aggregation. Furthermore, the mechanical effects caused by ultrasonic vibration enabled the suspended particles to move with different velocities and directions in liquid medium. There was more opportunity for two particles to get a collision. As the ultrasonic output power increased, ultrasonic agglomeration effects were enhanced in the gas-containing suspension by ultrasonic wave. And most of the aggregations were formed at the upper half space of the container. This was because of the intense acoustic streaming effect in the vicinity of the transducer, which made the inclusions form the aggregations instead of sneaking into the bottom of the container.

CONCLUSION

Concentrations of phospholipid were reduced by ultrasonic treatment under different power conditions and reduced as the ultrasonic power increased. It was concluded that ultrasonic disruption had influence on the degradation of high molecular protein. It was useful to isolate phospholipid from the egg yolks. It was found that the ultrasonic wave played an important role in phospholipid secondary structure transformation. Aggregation of phospholipid in egg yolks was caused by ultrasonic wave. As the ultrasonic treatment time increased, the number of aggregations increased and the volume decreased while the aggregation degree became weak. Aggregation and stripping of phospholipid suspension caused by ultrasonic wave could enhance the isolation and separation of the phospholipid.

ACKNOWLEDGMENT

This study was supported by the National Natural Science Foundation of China (Grant No. 51075387) and Fund of Chinese Ministry of Health LW201004.

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