Absorption characteristics of silkworm pupae peptides treated by ultrasound and irradiation based on Caco-2 cell model

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The sericulture industry is a traditional advantage industry in China. Silkworm pupae is one of the most important by-products of the sericulture industry, in which protein accounts for more than 60% of the total mass. Silkworm pupae protein is rich in 18 amino acids(Altomare, 2020). Therefore, silkworm pupae is a high-quality protein resource. However, the lower solubility and bioavailability limited the application of silkworm pupae protein. Past studies have shown that physical modification (such as ultrasound, high pressure and irradiation) could change the structure and spatial conformation of proteins, and improved the bioavailability of proteins(Zhou, 2017). At present, there are few studies on the bioavailability of silkworm pupae protein modified (Foong, 2015). In this study, ultrasound and irradiation were used to modify silkworm pupae protein to improve its solubility and bioavailability. At the same time, Caco-2 cell model was used to monitor the absorption process of modified silkworm pupae protein, and make a comprehensive assessment of the nutritional value of the modified silkworm pupae protein.

The silkworm pupae protein was treated by ultrasound (320 W, 54°C, 50 min) and irradiation (Electron beam, 10 kGy), which was hydrolyzed into polypeptide under optimal conditions with alkaline protease (200U/mg). 5 kDa filter membrane was used to filter out other impurities and proteases. The nutritional value of the obtained peptide has been verified by Caco-2 cells. Caco-2 cells are derived from human colon cancer cells and their morphology and structure are similar to small intestinal epithelial cells (Lin, 2017). Therefore, Caco-2 cells was used to effectively survey the absorption of nutrients in the body in this study (Scheers, 2014). 0.5mL of cell suspension with a concentration of 2×10^5 cells/mL was seeded on the transwell chamber, the cells are fully differentiated after 21d. Cell transmembrane resistance measurement and fluorescent yellow transport test were performed to verify the integrity of the cell membrane. Different treated peptides were inoculated on the transwell chamber to investigate the absorption characteristics. Finally, the transport status is determined by HPLC.

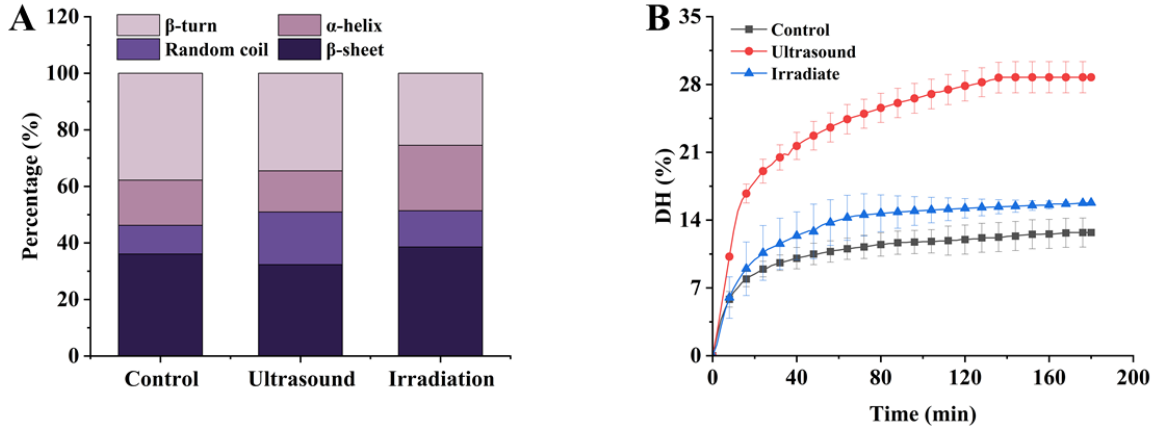

Fig. 1 The characteristics of silkworm pupae protein. (A) Protein secondary structure with different physical treatments; (B) The degree of hydrolysis of silkworm pupae protein by ultrasonic and irradiation treatment.

In this study, the silkworm pupae protein extracted without physical auxiliary pretreatment was used as the control group. Figure 1 shows that compared with the protein without physical treatment, the proportion of β-turns in the secondary structure of silkworm pupae protein ultrasound and irradiation treated was significantly reduced, and the proportion of irregular curling increased; the proportion of α-helix and β-sheet of silkworm pupae protein after ultrasonic treatment decreases, the proportion of irregular curls increases. On the contrary, the proportion of α-helix and β-sheets of silkworm pupae protein after irradiation increases, and the proportion of irregular curls decreases. The silkworm pupae protein obtained without physical treatment was hydrolyzed by alkaline protease and its degree of hydrolysis measured by pH-stat was 12.72%. In addition, the degree of hydrolysis of silkworm pupae protein ultrasound and irradiation treated has increased. After irradiation, the degree of hydrolysis of silkworm pupae protein reached 15.79%, and the degree of hydrolysis after ultrasonic treatment reached 28.75%. This may be because the protein structure was changed due to the cavitation effect produced by ultrasound, which

made the protein looser, exposing more enzyme cleavage sites in contact with the protease, thereby increasing the degree of hydrolysis (Li, 2021). Similarly, the sulfhydryl groups and disulfide bonds of the protein were exposed through the irradiation treatment, which changed the secondary structure of the protein, reduced the surface flatness of the protein molecule, and made it easier for enzymatic hydrolysis.

The trans-epithelial electrical resistance value of this experiment reached 471 $\Omega \cdot cm^2$, and the cell fluorescence yellow permeability value showed $0.26\text{-}0.65\times10^{-6}$ cm/s, indicating that the cells have formed a tightly connected monolayer structure, which could be used for transport experiments. Cytotoxicity determination determined that the optimal concentration of the three polypeptides inoculated on the transwell chamber was 0.5 mg/mL. At this concentration, the samples treated by ultrasound and irradiation assisted treatment had no inhibitory effect on the cells and could be used for absorption experiments. The absorption rate of the control group reached 10.4% at 2 h. Compared with the control group, the cell absorption rate of the peptides after ultrasonic treatment was 8.8%, which was slightly lower than the control group. However, after irradiation, the absorption rate of the peptide increased to 15.7%. This may be due to the uneven surface of the protein caused by irradiation, which reduced the molecular weight of the peptide after enzymatic hydrolysis, which was more conducive to absorb.

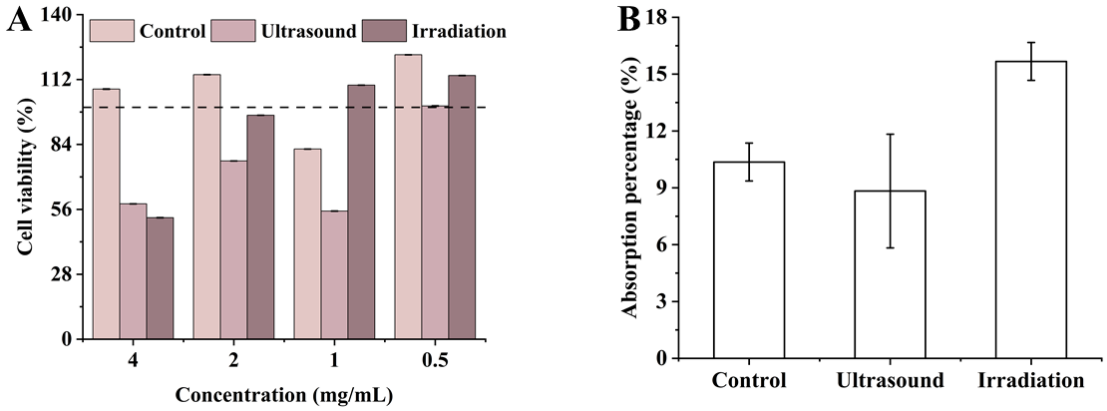

Fig.2 Caco-2 cells model establishment. (A) Toxicity test of different concentrations of hydrolysate on Caco-2 cells; (B) The absorption rate of the polypeptide by ultrasound and radiation treatment for 2 h.

In this study, ultrasound and irradiation were used to physically modify silkworm pupae protein to improve its bioavailability. The results showed that compared with the control group, ultrasound and irradiation changed the secondary structure of silkworm pupae protein, resulting in an increase in the degree of hydrolysis by 1.3 times and 2.3 times, respectively. Meanwhile, the results of *in vitro* absorption showed that the absorption rate of silkworm pupae protein after irradiation was 15.7%, which was significantly higher than that of the control group ($p<0.05$). Therefore, irradiation treatment can effectively improve the hydrolysis and bioavailability of silkworm pupae protein, and provides a new solution for improving the nutritional value and comprehensive utilization of silkworm pupae protein.

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