Isolation and activity of antioxidant peptides from silkworm pupae pretreated by irradiation technology

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The silkworm pupae protein has a variety of effects such as lowering blood pressure, lowering blood sugar and antioxidant, and is a high-quality natural protein (Altomare 2020). However, silkworm pupae protein is only used as feed in the traditional sericulture industry, and has low utilization (Li, 2020). The traditional scheme cannot completely hydrolyze silkworm pupae protein, and the conventional chemical extraction would lead to serious protein damage, many by-products and low extraction rate (Li, 2020). Irradiation is a safe and non-residual treatment of silkworm pupae, which can open the secondary structure of the protein so that the hydrophobic groups are exposed,and effectively improve extraction rate of silkworm pupae protein (Gulcin, 2020). Excessive free radicals in human body can cause damage to the body, and natural antioxidants are safer and more efficient. Therefore, the treatment of silkworm pupae protein by irradiation and the preparation of natural antioxidant peptides are beneficial to the high-value development of silkworm resources.

Fig. 1 shows that the solubility of untreated and irradiated silkworm pupae proteins. After irradiation treatment, the solubility of silkworm pupae proteins increased significantly, which was 2.9 times higher than that of untreated group. Compared with the control group, β-turn in the irradiation group decreased by 32.61%, and α-helix, β-sheet and random coil increased by 44.91%, 26.32% and 6.87%, respectively. Changes in protein conformation lead to exposure of more hydrophilic groups, hydrophobic groups, and polar groups (Wongsrangsap, 2021). The exposed polar groups have more charges on the surface, which further enhances the water interaction and increases the solubility of silkworm pupa protein (Li, 2019). Therefore, irradiation treatment can significantly improve the solubility of silkworm pupae protein.

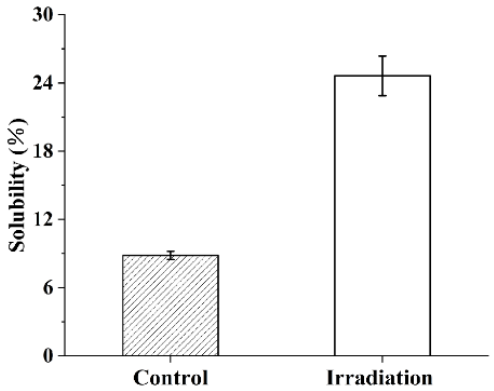

Fig. 1 The solubility of untreated protein and irradiated protein

Table 1 The antioxidant activity of polypeptides from untreated and irradiated group after ultrafiltration

Ultrafiltration (<5 kDa)	Antioxidant activity			
	Scavenging rate of DPPH (%)	Inhibition of hydroxyl radical (U/mg)	$ABTS^{+}$ scavenging assay (Mm Trolox)	Chelating rate of Fe^{2+} (%)
Undisposed	46.42±2.99	8.35±0.88	0.72±0.011	51.81±2.87
Irradiation	46.62±14.06	20.52±1.14	1.33±0.007	93.38±2.10[a]

Table 1 shows that the antioxidant activity of polypeptides from untreated and irradiated group after ultrafiltration (<5 kDa). The results show that the antioxidant capacity of polypeptides in the irradiation group was significantly increased compared with the control group, among which the ability to inhibit hydroxyl free radicals, reduce power, and chelate Fe^{2+} increased by 2.5, 1.8, and 1.8 times, respectively. Therefore, the irradiation treatment group can be used for further separation of antioxidation peptides.

Fig 2 shows the isolation and identification of antioxidants in the irradiated group. As shown in Fig 2A, sephadex G-15 gel column were used to further isolate silkworm pupae peptides, and three components were divided, namely, peak 1, peak 2 and peak 3. The DPPH free radical scavenging ability of three component was shown in Figure 2B, which were 74.84 %, 60.71 % and 71.97 %, respectively. Among them, peak 1 had the best scavenging ability of DPPH, so component one is further separated by high performance liquid chromatography. As shown in Fig 2C, peak 1 was divided into two component s. These two sub-components were collected, and identified.

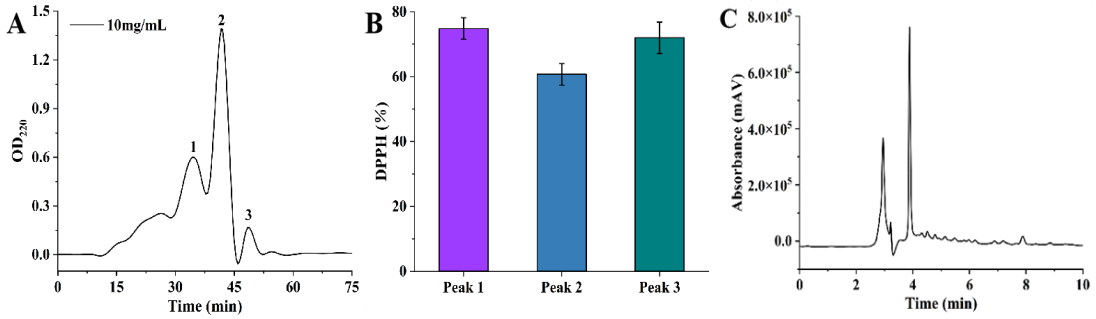

Fig. 2. Separation and determination of antioxidant peptides from silkworm pupae. (A) Sephadex G-15 gel chromatogram; (B) DPPH determination of peak 1, peak 2 and peak 3; (C) HPLC chromatograms.

The above results indicated that the spatial structure of silkworm pupae protein was changed after irradiation treatment, and the solubility was increased by 2.9 times compared with the control group. At the same time, the anti-oxidation ability of the enzymatic hydrolysate of the irradiated protein was significantly improved, in which the ability to inhibit hydroxyl radicals, the reducing power, and the ability to chelate Fe^{2+} were increased by 2.5 times, 1.8 times, and 1.8 times, respectively. After the hydrolysate was separated by ultrafiltration, gel chromatography, and reverse phase chromatography, the antioxidant capacity was further improved. Therefore, irradiation treatment of silkworm pupae is an effective way to prepare natural antioxidants, and promotes the utilization of resources in the sericulture industry.

Acknowledgements

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