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UV-C Photochemistry of β-Lactoglobulin in Deep Eutectic Solvents: Structural Changes and Impact on Trypsin Digestibility

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Palavras Chave: *DES, whey, protein, beta-lactoglobulin, bioactive peptides*

Highlights.

This study investigates the impact of UV-C irradiation in deep eutectic solvents (DES) on β-lactoglobulin structure to enhance its digestibility and promote the formation of bioactive peptides during trypsinolysis.

Resumo/Abstract

The growing demand for high-quality protein products is driving the search for alternatives that combine superior nutritional value, functionality, and food safety. Whey protein, a byproduct of the dairy industry, is widely used in protein concentrates for protein-based beverage production. It primarily consists of β-lactoglobulin (β-LG), α-lactalbumin, and lactoferrin, with β-LG being the most abundant, representing about 60% of whey protein. Although β-LG has significant bioactive potential due to numerous bioactive peptides within its sequence, its compact barrel-shaped structure and disulfide bonds confer resistance to digestion, limiting its bioavailability. Recently, our Group demonstrated the potential of UV-C light as a non-thermal processing technology to induce structural changes in β-LG, enhancing its digestibility and promoting the release of bioactive peptides. Deep eutectic solvents (DES), composed of a quaternary ammonium salt and a polyalcohol, offer a promising eco-friendly alternative due to their unique interactions with proteins that can potentially induce structural changes. In this study, we investigated the effect of non-thermal UV-C (266 ± 4 nm) treatment on β-LG in DES to improve digestibility and production of bioactive peptides. β-LG solutions were prepared at a final concentration of 2 $mg \cdot mL^{-1}$ in two different solvents: DES mixture (choline chloride and glycerol at a 1:2 w/w ratio with 40% PBS 0.01 M, pH 7.4) and a traditional PBS buffer (0.01 M, pH 7.4) for comparison. Both solutions were irradiated with UV-C light for 2 hours at 25°C under continuous stirring. Following irradiation, samples were digested with trypsin (enzyme-to-protein ratio of 1:20 w/w, pH 7.8, 37°C, 300 rpm) for 4 hours. HPLC-UV profile analysis probed at 214 nm revealed distinct peptide profiles for UV-C treated protein in DES and PBS solutions. For PBS solution, β-LG was almost completely hydrolyzed within one hour, with only 3% of intact protein remaining. In contrast, in DES solution, 80% of the intact β-LG persisted after 4 hours of proteolysis. Circular dichroism (CD) analysis indicated slightly changes in the secondary structure of β-LG UV-C treated in DES, with a 2.2% increase in β-sheet and a 2.3% increase in random coil content, accompanied by a 1.8% decrease in α-helix content. These findings demonstrate that UV-C photolysis in DES induces structural modifications in β-LG and, unlike photolysis in aqueous PBS, significantly impairs its trypsinolysis. This modulation of rate of protein hydrolysis and alteration of the peptide profile could pave the way for developing novel functional protein products. Furthermore, the use of choline chloride-based DES aligns with dietary supplementation strategies, presenting exciting opportunities for innovation in health and nutrition.

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