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Effect of Whey Proteins and Caffeic Acid Interactions on Antioxidant Activity and Protein Structure

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Abstract : Proteins and phenolic compounds can interact mainly by hydrophobic interactions. Those interactions may lead to structural changes in both molecules, which in turn could affect positively or negatively their functional and nutritional properties. Here, the structural changes of whey proteins (WPI) due to interaction with caffeic acid (CA) were investigated by intrinsic and extrinsic fluorescence. The effects of protein-phenolic compounds interactions on the total phenolic content and antioxidant activity were also assessed. The WPI-CA complexes were obtained by mixture of WPI and CA stock solutions in deionized water. The complexation was carried out at room temperature during 60 min, using 0.1 M NaOH to adjust pH at 7.0. The WPI concentration was fixed at 5 mg/mL, whereas the CA concentration varied in order to obtain four different WPI:CA molar relations (1:1; 2:1; 5:1; 10:1). WPI and phenolic solutions were used as controls. Intrinsic fluorescence spectra of the complexes (mainly due to Trp fluorescence emission) were obtained at λex = 280 nm and the emission intensities were measured from 290 to 500 nm. Extrinsic fluorescence was obtained as the measure of protein surface hydrophobicity (S0) using ANS as a fluorescence probe. Total phenolic content was determined by Folin-Ciocalteau and the antioxidant activity by FRAP and ORAC methods. Increasing concentrations of CA resulted in decreasing of WPI intrinsic fluorescence. The emission band of WPI red shifted from 332 to 354 nm as the phenolic concentration increased, which is related to the exposure of Trp residue to the more hydrophilic environment and unfolding of protein structure. In general, the complexes presented lower S0 values than WPI, suggesting that CA hindered ANS binding to hydrophobic sites of WPI. The total phenolic content in the complexes was lower than the sum of two compounds isolated. WPI showed negligible AA measured by FRAP. However, as the relative concentration of CA increased in the complexes, the FRAP values enhanced, indicating that AA measure by this technique comes mainly from CA. In contrast, the WPI ORAC value (82.3 ± 1.5 μM TE/g) suggest that its AA is related to the capacity of H+ transfer. The complexes exhibited no important improvement of AA measured by ORAC in relation to the isolated components, suggesting complexation partially suppressed AA of the compounds. The results hereby presented indicate that interaction of WPI and CA occurred, and this interaction caused a structural change in the proteins. The complexation can either hide or expose antioxidant sites of both components. In conclusion, although the CA can undergo an AA suppression due to the interaction with proteins, the AA of WPI could be enhanced due to protein unfolding and exposure of antioxidant sites.

Keywords : bioactive properties, milk proteins, phenolic acids, protein-phenolic compounds complexation
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