Studies on the Binding of Iron and Zinc to Milk Protein Products

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ABSTRACT

The principal objective of this study was to characterize the binding of iron and zinc to three commercial milk protein products; namely sodium caseinate, whey protein isolate (WPI) and milk protein concentrate (MPC).

The mineral–protein mixtures were prepared by mixing either iron ($FeSO_4.7H_2O$) or zinc ($ZnSO_4.7H_2O$) at a range of concentrations with 1% protein solutions (e.g. sodium caseinate), in 50 mM HEPES buffer at pH 6.6. The mineral–protein mixtures were then centrifuged (10,800 *g*, 20 min) to separate the soluble protein and soluble minerals from the insoluble protein and insoluble minerals. The supernatant, which contained the soluble fractions, was carefully removed and passed through an ultrafiltration membrane to separate "free" minerals from the minerals bound to the soluble proteins.

Under the experimental conditions used in the study, aqueous solutions of ferrous sulphate were relatively insoluble. This was due mainly to the oxidation of ferrous sulphate to the insoluble ferric hydroxide. The addition of a 1% sodium caseinate solution markedly improved the solubility of ferrous sulphate due to the binding of iron to the caseins. The casein molecules were able to bind up to 8 moles Fe/mole protein. Addition of iron above a certain critical concentration (approximately 4 mM) caused the aggregation and precipitation of casein molecules. The loss of solubility was due mainly to the neutralisation of the negative charges on the casein molecules by iron with a consequent decrease in the electrostatic repulsions between the protein molecules.

In contrast to the behaviour of the sodium caseinate, the interactions of iron with the whey protein molecules in WPI did not cause significant precipitation of the iron–WPI mixtures. Whey proteins remained soluble up to a concentration of 20 mM added iron and were able to bind up to approximately 7 moles Fe/mole of protein.

Analysis of the binding curves by Scatchard plots showed that sodium caseinate has a higher binding affinity for iron (log K_{app} = 5.3) than WPI (log K_{app} = 3.6). This confirmed the experimental observation that in sodium caseinate solutions, up to the critical concentration of iron, virtually all iron was bound to the protein molecules whereas in WPI solutions, a small amount of free iron was present. The strong affinity for iron shown by the casein molecules is due mainly to the presence of clustered phosphoserine residues, which are absent in whey proteins.

The binding characteristics of iron to MPC were broadly similar to those for sodium caseinate. However, soluble MPC was able to bind greater amount of iron (45 mg Fe/g protein) than soluble sodium caseinate (20 mg Fe/g protein). In MPC, casein molecules exist in the micellar form and iron was likely to be bound to both the caseins and the colloidal calcium phosphate, probably displacing calcium ions in the process.

The binding properties of proteins were significantly affected by changes in pH. As the pH was decreased from about 6.5 to 5.0, there was a marked decrease in the ability of proteins to bind cations. For example, the amount of iron bound to WPI decreased from approximately 8 to 1 mg Fe/g soluble protein as the pH dropped from 6.5 to 5.0. This decrease was presumably due to the change in the ionisation state of the negatively charged residues. In the case of sodium caseinate and MPC, the situation was complicated by the marked loss of protein solubility at pH values ≤ 5.0.

The binding characteristics of zinc to the three milk protein products were broadly similar to those for iron. For sodium caseinate and MPC, there was a critical concentration of added zinc above which proteins lost solubility. Sodium caseinate showed a greater binding affinity for zinc than WPI (Log K_{app} values were 4.8 and 3.3 respectively), while MPC was able to bind more zinc (25 mg Zn/g protein) than sodium caseinate (14 mg Zn/g protein). However, there was one distinctive difference between the binding behaviour of iron and zinc. In the case of WPI, addition of zinc caused precipitation of whey proteins at a concentration above 4 mM added zinc. This was due to the specific binding sites for zinc in the α-lactalbumin fractions.

Oxidation tests, using linoleic acid as the substrate, showed that iron–protein mixtures were able to markedly suppress the rate of oxidation compared to free iron. Among the iron–protein mixtures, iron–sodium caseinate and iron–MPC mixtures suppressed the oxidation rate to a greater extent than iron–WPI mixtures. In iron–sodium caseinate and iron–MPC mixtures, the iron was completely bound to the protein whereas in iron–WPI mixtures, there was still a small amount of unbound iron, which could cause oxidation.

The data obtained from this study will provide valuable information for the production of mineral–protein complexes with good functional properties, which could be used as a source of ingredients in other food products.

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