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**IRON BINDING PROPERTIES OF
WHEY PROTEIN, CASEIN, SOYA PROTEIN AND
EGG ALBUMEN**

A THESIS PRESENTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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ABSTRACT

Iron binding properties of whey protein, casein, soya protein and egg albumen were investigated in aqueous dispersions using centrifugation and ultrafiltration techniques. Protein-iron mixtures were centrifuged at 10,800 *g* for 20 min and iron that co-sedimented with protein was considered to be bound to the insoluble protein fraction. The supernatants were ultrafiltered to obtain iron bound to the soluble protein fraction.

Both the soluble and insoluble fractions of each protein were shown to bind substantial quantities of iron from ferrous sulphate. The amount of iron bound/g to the insoluble fraction of the protein was highest for casein (87 mg) followed by albumen (80 mg), soya protein (66 mg) and whey protein (63 mg). A similar trend was observed for the soluble fraction; casein bound 74 mg iron/g protein followed by albumen (68 mg), soya protein (54 mg) and whey protein (12 mg). This binding was markedly influenced by pH of the protein-iron mixtures in the range 2 - 7.

The binding data was analyzed using the Scatchard equation to obtain binding constants (*k*) and the number of binding sites (*n*). The *n* values obtained were ~ 2 (whey protein), 13 (casein), 200 (soya protein) and 42 (albumen). The values obtained for the binding constants were ~ 11 (whey protein), 5 (casein), 3 (soya protein) and 1 (albumen). Thus soya protein had the highest number of binding sites and whey protein had the greatest affinity for iron.

Solubility of each protein was dependent on pH and it generally decreased with increase in iron concentration.

The effects of chelating agents (citric acid and ascorbic acid) on the iron binding properties of the four proteins were also examined. Addition of citric

acid and ascorbic acid increased the solubilities of both protein and iron. The solubilizing effect of these two acids was dependent on the protein source, pH and acid concentration. Iron binding by both the insoluble and soluble fractions decreased in the presence of citric acid and ascorbic acid, with no significant differences between the effects of the two acids.

The effects of proteins and protein digestion products on *in vitro* iron availability were studied. Ferrous iron complexes with protein were prepared and subjected to simulated gastrointestinal digestion followed by measurement of soluble iron. The *in vitro* availability of iron was in the order of 26% (soya protein), 16% (casein), 14% (albumen) and 10% (whey protein). When citric acid and ascorbic acid were added prior to enzymatic digestion the availability of iron increased to 63% (soya protein), 36% (albumen), 31% (casein) and 22% (whey protein).

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