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
MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY
AT MASSEY UNIVERSITY

NETRA MANVIKAR

1993

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 soys 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).

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr. Harjinder Singh and Dr. Juliet Wiseman, for their guidance, advice and continued encouragement during the course of this investigation.

I would also like to thank all the staff of the Department of Food Technology, especially the following:
Mr Hank Van Til, Mrs Margaret Bewley, Mr Steve Glasgow, and Mr Alistair Young for their technical assistance.
Mrs Lesely James, Ms June Latham and Miss Rebecca Baxter for their encouragement and help during the course of this work.

I am very grateful to Mr Ranjan Sharma, Ms Janet Weber and Mr Tang Qingnong for their valuable advice and help.

I would also like to thank all my fellow graduate students, particularly Gayathri, Wibha, Pinthita, David and Sarathy for their encouragement, assistance and friendship.

I would like to extend my thanks to Rani, Bente, Marijke, Claudia and Joost for their friendship and help without which my stay here would not have been so enjoyable.

Special thanks is extended to my 'Hosts' - Mr and Mrs Lakshmanan, and other friends in Palmerston North for making my stay in a foreign land comfortable.

Finally, my greatest appreciation is extended to my sister Mrs Veda Sattur and her husband Mr Avinash Sattur, and my family for their continued moral support and encouragement and this thesis is dedicated to them.

TABLE OF CONTENTS

List of figures

List of tables