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

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN FOOD TECHNOLOGY

BY

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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 (FeSO₄·7H₂O) or zinc (ZnSO₄·7H₂O) 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.
Abstract

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 $\leq 5.0$.

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 $\alpha$-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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# TABLE OF CONTENTS

## ABSTRACT

i

## ACKNOWLEDGMENTS

iv

## TABLE OF CONTENTS

vi

## CHAPTER 1. INTRODUCTION

1

## CHAPTER 2. LITERATURE REVIEW

4

2.1. Introduction 4

2.2. Milk Proteins 4

2.2.1. Caseins 5

2.2.2. Whey Proteins 12

2.3. Milk Protein Products 15

2.3.1. Caseinate 15

2.3.2. Whey Protein Concentrates (WPI) and Whey Protein Isolate (WPI) 18

2.3.3. Milk Protein Concentrate (MPC) 20

2.4. Metal-Protein Interactions 22

2.4.1. Properties of Metal Ions 22

2.5. Binding of Metal Ions to Proteins 24

2.5.1. Determining binding sites and binding constants 24

2.6. Iron and Zinc Deficiencies 26

2.7. Iron Binding Studies 27

2.7.1. Iron in bovine and human milk 27

2.7.2. Sources of added iron to milk or milk products 28

2.7.3. Binding of iron to casein in milk 32

2.7.4. Binding of iron to caseinate 34

2.7.5. Iron binding to whey protein 39

2.7.6. Problems with oxidation due to addition of iron 40
Table of Contents

2.7.7. Application of iron–protein complexes 41
2.8. Zinc Binding Studies 43
  2.8.1. Zinc in bovine and human milk 43
  2.8.2. Binding of zinc to casein in milk 45
  2.8.3. Binding of zinc to caseinate 46
  2.8.4. Binding of zinc to individual caseins 46
  2.8.5. Binding sites 48
  2.8.6. Effect of pH, ionic strength, chelating agents on zinc binding 48
  2.8.7. Binding of zinc to whey protein 50
2.9. Oxidation 51
  2.9.1. Measurement of lipid oxidation 54

CHAPTER 3. MATERIALS AND METHODS 58

3.1. Materials 58
  3.1.1. Chemicals 58
  3.1.2. Protein Powders 58
3.2. Methodology 60
  3.2.1. Binding of iron and zinc to proteins 60
  3.2.2. Mass balance 63
  3.2.3. Analysis of samples 65
  3.2.4. Oxidation activity of mineral–protein mixtures 68

CHAPTER 4. BINDING OF IRON TO MILK PROTEIN PRODUCTS 74

4.1. Introduction 74
4.2. Solubility of ferrous sulphate in HEPES buffer 75
4.3. Solubility of iron and sodium caseinate in iron–sodium caseinate mixtures 79
| 4.3.1.  | Solubility of individual caseins in iron–sodium caseinate mixtures | 86 |
| 4.4.   | Binding of iron to sodium caseinate | 88 |
| 4.4.1. | Binding of iron to sodium caseinate in the soluble fraction | 90 |
| 4.4.2. | Binding of iron to sodium caseinate in the insoluble fraction | 93 |
| 4.4.3. | Binding sites and binding constants | 93 |
| 4.4.4. | Effect of pH on the binding of iron to sodium caseinate | 95 |
| 4.4.5. | Effect of ionic strength on the binding of iron to sodium caseinate | 100 |
| 4.5.   | Solubility of iron and WPI in iron–WPI mixtures | 101 |
| 4.5.1. | Solubility of individual whey proteins in iron–WPI mixtures | 104 |
| 4.6.   | Binding of iron to WPI | 104 |
| 4.6.1. | Binding of iron to WPI in the soluble fraction | 105 |
| 4.6.2. | Binding of iron to WPI in the insoluble fraction | 107 |
| 4.6.3. | Binding sites and binding constants | 108 |
| 4.6.4. | Effect of pH on the binding of iron to WPI | 109 |
| 4.6.5. | Effect of ionic strength on the binding of iron to WPI | 112 |
| 4.7.   | Solubility of iron and MPC in iron–MPC mixtures | 113 |
| 4.7.1. | Solubility of individual proteins of MPC in iron–MPC mixtures | 116 |
| 4.8.   | Binding of iron to MPC | 119 |
| 4.8.1. | Binding of iron to the proteins in MPC in the soluble Fraction | 119 |
| 4.8.2. | Binding of iron to the proteins in MPC in the insoluble Fraction | 122 |
| 4.8.3. | Effect of pH on the binding of iron to MPC | 122 |
| 4.8.4. | Effect of ionic strength on the binding of iron to MPC | 125 |
Table of Contents

5.8. Binding of zinc to MPC
   5.8.1. Binding of zinc to MPC in the soluble fraction 170
   5.8.2. Binding of zinc to MPC in the insoluble fraction 172
   5.8.3. Effect of pH on the binding of zinc to MPC 173
   5.8.4. Effect of ionic strength on the binding of zinc to MPC 176
5.9. Discussions 176

CHAPTER 6. EFFECT OF BINDING ON MINERAL–CATALYSED LIPID OXIDATION 184

6.1. Introduction 184
6.2. Oxidation induced by iron–protein mixtures 185
6.3. Oxidation induced by zinc–protein mixtures 189
6.4. Discussions 190

CHAPTER 7. CONCLUDING REMARKS AND RECOMMENDATIONS 194

REFERENCES 203