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# **Study of the interactions between milk proteins and hydroxyapatite particles**

A thesis presented in partial fulfilment of the requirements for the degree of

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## ABSTRACT

Hydroxyapatite (HA) and other insoluble calcium salts added to calcium-fortified milks are often described as inert, as they do not cause any protein aggregation and heat instability during heat treatment of the milk. However, it is well-known that proteins can interact with HA. The adsorption of milk proteins on HA has been demonstrated in many systems, for example in chromatography, bioceramic and dentistry applications, and has been shown to have consequence on the colloidal stability of HA, but has never been studied in food systems.

The main objective of the present study was therefore to explore the adsorption of milk proteins onto HA particles under a range of physico-chemical conditions. The consequences of these interactions on the colloidal properties of the HA particles and on the stability of the milk proteins were investigated.

It was shown that the five individual milk proteins  $\alpha_s$ -casein,  $\beta$ -casein,  $\kappa$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin adsorbed onto the HA particles. A Langmuir model was used to fit the adsorption data and determine the affinity constant and maximum surface loads of the different proteins. The adsorption of the different milk proteins onto HA particles was found to be of competitive nature.  $\beta$ -casein and  $\alpha_s$ -casein were always preferred for adsorption over  $\kappa$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin. This was attributed to the presence of phosphoserine clusters in  $\beta$ -casein and  $\alpha_s$ -casein, forming many anchor points capable of binding to the calcium sites of HA.  $\beta$ -casein and  $\alpha_s$ -casein also adsorbed to higher maximum levels compared to  $\kappa$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin. Both  $\beta$ -Casein and  $\alpha_s$ -casein were considered to self-associate or associate together in the adsorbed layer, therefore forming a thick layer onto the HA surface. Conversely,  $\kappa$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin adsorbed to lower maximum amounts and had lower affinities for HA, which was attributed to adsorption in a monolayer through their carboxyl groups binding to the calcium sites of HA.

The amount of protein adsorbing to the HA surface was affected by the physico-chemical properties of the solution such as pH and ionic strength, for all proteins. Decreasing pH and increasing ionic strength decreased the electrostatic repulsive forces between HA and the proteins and the electrostatic repulsive forces within the protein molecules, which allowed more protein to adsorb onto the HA surface. Milk serum ions such as calcium, phosphate

and citrate bound specifically onto HA particles, therefore competing with the milk proteins for adsorption.

In milk, it was shown the addition of HA in milk disrupted the mineral equilibrium and the milk protein phase. When HA particles were added to milk, the milk serum ions bound to the HA surface. This caused the colloidal calcium phosphate to be released from the casein micelles and the casein micelles to dissociate. Therefore the casein micelles did not bind as intact micelles but as individual molecules or small aggregates onto the HA particles.

The adsorption of milk proteins onto HA particles affected the colloidal properties of the HA particles in suspension. The adsorption of both caseins and whey proteins onto HA particles resulted in the particles becoming negatively charged, thus improving their suspension stability. Whey protein adsorption probably provided only electrostatic stabilisation, whereas casein adsorption also provided steric stabilisation.

Overall, this work has provided a detailed understanding of the interactions between milk proteins and HA particles. Calcium fortification of milk using insoluble calcium salts such as HA should be approached using an awareness of these interactions, as they may have consequences on the stability of calcium fortified milks.

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## TABLE OF CONTENTS

<b>ABSTRACT</b> .....	<b>I</b>
<b>TABLE OF CONTENTS</b> .....	<b>IV</b>
<b>LIST OF FIGURES</b> .....	<b>VIII</b>
<b>LIST OF TABLES</b> .....	<b>XIV</b>
<b>LIST OF ABBREVIATIONS</b> .....	<b>XVI</b>
<b>CHAPTER 1 Introduction</b> .....	<b>1</b>
<b>CHAPTER 2 Literature review</b> .....	<b>4</b>
2.1 Calcium supplementation of food .....	4
2.1.1 Calcium role and metabolism in the human body.....	4
2.1.2 Calcium deficiency problems .....	5
2.1.3 Recommended daily intake for calcium .....	6
2.1.4 Factors to take into account in the selection of calcium fortificants to add in milk.....	7
2.2 Milk composition .....	8
2.2.1 Milk: an overview .....	8
2.2.2 Protein phase.....	9
2.2.3 Mineral phase.....	26
2.3 Calcium supplementation of cows' milk: an industrial challenge .....	35
2.3.1 Possible calcium sources: advantages and drawbacks.....	35
2.3.2 Ways of improving suspension of HA in products.....	37
2.4 Interactions between hydroxyapatite and milk proteins.....	44
2.4.1 Hydroxyapatite properties.....	44
2.4.2 Protein adsorption on HA .....	49
2.4.3 Milk protein binding to calcium phosphate and HA.....	60
2.5 Methods and models to measure and characterise protein adsorption on HA.....	65
2.5.1 Milk protein ingredients.....	65
2.5.2 Measurement methods.....	66
2.5.3 Adsorption modelling.....	66
2.6 Conclusion and positioning of the study .....	69
<b>CHAPTER 3 Material and methods</b> .....	<b>71</b>
3.1 Materials .....	71
3.1.1 Sources.....	71

3.1.2	Characterisation .....	72
3.2	Adsorption experiments.....	74
3.2.1	Preparation of stock protein solutions .....	74
3.2.2	Preparation of the suspending solutions.....	76
3.2.3	Preparation of suspensions of HA particles in protein solutions .....	77
3.2.4	Determination of surface concentration and composition .....	78
3.3	Characterisation of HA particles .....	80
3.3.1	Particle size .....	80
3.3.2	Zeta-potential.....	81
3.3.3	Microscopy .....	81
3.3.4	Suspension stability.....	83
3.4	Characterisation of WPD-SM and SMP solutions.....	83
3.4.1	Casein micelle size.....	83
3.4.2	Non-micellar casein content .....	84
3.4.3	Transmission electron microscopy .....	84
3.5	Protein quantification methods.....	84
3.5.1	Absorbance at 280 nm .....	84
3.5.2	Kjeldahl method.....	85
3.5.3	Sodium dodecyl sulphate polyacrylamide gel electrophoresis .....	85
3.5.4	Microfluidic chip electrophoresis.....	87
3.6	Mineral analysis .....	91
3.6.1	Total calcium .....	91
3.6.2	Ionic calcium concentration .....	92
3.6.3	Inorganic phosphate.....	92
3.6.4	Citrate.....	92
3.7	Preparation of modified milk protein ingredients.....	93
3.7.1	Preparation of whey protein-depleted skim milk powder.....	93
3.7.2	Preparation of EDTA-treated skim milk .....	94
3.8	Statistical analysis.....	95
3.8.1	Statistical tests.....	95
3.8.2	Modelling.....	95
<b>CHAPTER 4 Adsorption of caseins and whey proteins onto hydroxyapatite particles.....</b>		<b>96</b>
4.1	Abstract.....	96
4.2	Introduction.....	97
4.3	Material and methods.....	98
4.4	Results and discussion .....	99
4.4.1	Protein adsorption .....	99

4.4.2	Characterisation of the protein coated HA particles.....	123
4.5	Conclusions.....	131
<b>CHAPTER 5</b>	<b>Adsorption of individual milk proteins onto hydroxyapatite particles and competitive adsorption between milk proteins.....</b>	<b>134</b>
5.1	Abstract.....	134
5.2	Introduction.....	135
5.3	Material and methods.....	137
5.3.1	Adsorption of individual milk proteins.....	137
5.3.2	Adsorption of milk proteins from solutions containing equal amounts of different isolated proteins.....	137
5.3.3	Displacement of proteins.....	138
5.3.4	Calculation of protein net charge.....	139
5.4	Results and discussion.....	140
5.4.1	Adsorption of individual milk proteins.....	140
5.4.2	Adsorption from mixtures of individual proteins.....	148
5.4.3	Protein displacement.....	157
5.5	Conclusions.....	159
<b>CHAPTER 6</b>	<b>Effect of pH, ionic strength and milk serum composition on the adsorption of milk proteins onto hydroxyapatite particles.....</b>	<b>161</b>
6.1	Abstract.....	161
6.2	Introduction.....	162
6.3	Material and methods.....	163
6.3.1	Protein adsorption onto HA particles, under different physico-chemical conditions.....	163
6.3.2	Characterisation of HA particles.....	163
6.4	Results and discussion.....	164
6.4.1	Effect of the pH and composition of the suspending solution on colloidal properties of HA particles.....	164
6.4.2	Effect of pH and composition of the suspending solution on milk protein adsorption onto HA particles.....	173
6.4	Conclusions.....	199
<b>CHAPTER 7</b>	<b>Interactions of casein micelles with hydroxyapatite particles.....</b>	<b>202</b>
7.1	Abstract.....	202
7.2	Introduction.....	203
7.3	Material and methods.....	204
7.3.1	Adsorption experiments.....	204
7.3.2	Transmission electron microscopy and light microscopy.....	205
7.3.3	Dialysis experiments.....	205
7.3.4	Mineral quantification.....	206

7.4	Results and discussion .....	206
7.4.1	Adsorption of caseins and whey proteins from WPD-SM and SM.....	206
7.4.2	Adsorption of caseins and whey proteins from EDTA-treated SM .....	221
7.4.3	Mechanism of casein micelle dissociation by HA particles .....	226
7.5	Conclusions .....	234
<b>CHAPTER 8</b>	<b>Overall summary and recommendations .....</b>	<b>237</b>
8.1	Key summary points.....	238
8.2	Recommendations for future work .....	244
<b>References.....</b>	<b>.....</b>	<b>248</b>
<b>APPENDIX 1</b>	<b>Synthesis of HA particles in the presence of milk proteins: a preliminary study.....</b>	<b>272</b>
<b>APPENDIX 2</b>	<b>Poster.....</b>	<b>282</b>

## LIST OF FIGURES

<b>Figure 2.1:</b> Representation of a phosphoserine residue; the phosphate is esterified to serine as a monoester (from Fox & McSweeney, 1998).....	11
<b>Figure 2.2:</b> Schematic illustration of the distribution of charged, phosphoserine, and hydrophobic residues in the (A) $\alpha_{S1}$ - and (B) $\alpha_{S2}$ -casein primary sequences. ....	13
<b>Figure 2.3:</b> Schematic illustration of the distribution of charged, phosphoserine, and hydrophobic residues in the $\beta$ -casein primary sequence.....	15
<b>Figure 2.4:</b> Schematic illustration of the distribution of charged, phosphoserine, and hydrophobic residues in the $\kappa$ -casein primary sequence.....	17
<b>Figure 2.5:</b> Sub-micelle model of casein micelles (Schmidt, 1982)..	19
<b>Figure 2.6:</b> Calcium phosphate nanocluster model, as first proposed by Holt & Horne (1996).....	20
<b>Figure 2.7:</b> Illustration of the calcium phosphate nanocluster formation and the growth of the protein network in the casein micelles (Horne, 2006). ....	20
<b>Figure 2.8:</b> Two most recent models of casein micelles involving the concept of calcium phosphate nanoclusters surrounded by $\alpha_s$ - and $\beta$ -caseins.....	22
<b>Figure 2.9:</b> Schematic representation of a monomer of $\beta$ -lactoglobulin; modified from Kraulis (1991). ....	24
<b>Figure 2.10:</b> Schematic representation of a monomer of $\alpha$ -lactalbumin; modified from Kraulis (1991). ....	25
<b>Figure 2.11:</b> Schematic representation of mineral equilibrium in milk (from Brule, 1981 in Gaucheron, 2004). ....	30
<b>Figure 2.12:</b> Typical SEM pictures of HA ingredients used for calcium fortification. SEM pictures kindly provided by Chemische Fabrik Budenheim KG (Germany).....	39
<b>Figure 2.13:</b> Structural properties of HA crystals.....	45
<b>Figure 2.14:</b> Schematic illustration of the binding interactions between HA and proteins.....	51
<b>Figure 2.15:</b> Schematic illustration of the interactions between HA and (A) acidic and (B) alkaline proteins.....	54
<b>Figure 2.16:</b> Schematic representation of a calcium phosphate nanocluster. ....	64
<b>Figure 3.1:</b> FTIR spectrum for HA powder used in this thesis (TCP-53-83).....	74
<b>Figure 3.2:</b> Layout of the wells and channels in a typical microfluidic electrophoresis chip.....	89
<b>Figure 3.3:</b> Typical elution profile obtained from MF-electrophoresis technique for reduced skim milk.....	90

<b>Figure 3.4:</b> Computer-generated gel image from the elution profile of reduced skim milk obtained by MF-electrophoresis.....	91
<b>Figure 3.5:</b> Preparation of whey protein-depleted skim milk by microfiltration; the obtained solution was then spray-dried to obtain whey protein-depleted skim milk powder (WPD-SMP).....	94
<b>Figure 4.1:</b> SDS-PAGE pattern of initial protein solutions and supernatants obtained for adsorption experiments carried out with sodium caseinate (SC) solutions at initial protein concentrations (w/w) of: A, 3.6%; B, 2.7%; C, 1.8%; D, 0.9%.....	100
<b>Figure 4.2:</b> SDS-PAGE pattern of initial protein solutions and supernatants obtained for adsorption experiments carried out with whey protein isolate (WPI) solutions at initial protein concentrations (w/w) of: A, 3.72%; B, 2.79%; C, 1.86%; D, 0.93%. ....	101
<b>Figure 4.3:</b> Surface protein concentration (mg/m <sup>2</sup> ) of (■) caseins from SC and (●) whey proteins from WPI on HA particles. ....	102
<b>Figure 4.4:</b> SDS-PAGE pattern of the adsorption supernatants obtained after different stirring times. ....	103
<b>Figure 4.5:</b> Isotherms of milk proteins adsorbed onto HA and the different best-fit model curves for (A) SC and (B) WPI. ....	105
<b>Figure 4.6:</b> Estimated surface protein concentrations (mg/m <sup>2</sup> ) of (A) individual caseins from SC and (B) individual whey proteins from WPI, as a function of initial protein concentration. ....	111
<b>Figure 4.7:</b> SDS-PAGE gels of the supernatants of samples containing (A) 0.1% (w/w) SC and (B) 0.1% (w/w) WPI and different HA concentrations. ....	116
<b>Figure 4.8:</b> Surface protein concentration (mg/m <sup>2</sup> ) of (A) caseins from SC and (B) whey proteins from WPI on HA particles. ....	118
<b>Figure 4.9:</b> Estimated surface protein concentrations (mg/m <sup>2</sup> ) of (A) individual caseins from SC and (B) individual whey proteins from WPI, as a function of initial HA concentration. ....	120
<b>Figure 4.10:</b> Confocal micrographs obtained for hydroxyapatite pellets prepared with water, SC or WPI solutions (0.5%, w/w, total solids), rinsed and re-suspended in water (0.05%, w/w) and stained with fast green. ....	125
<b>Figure 4.11:</b> Effect of protein concentration on the zeta-potential of HA particles suspended in (A) SC or (B) WPI solutions of different initial concentrations. ....	127
<b>Figure 4.12:</b> Linear relationship between the surface protein coverage of the protein-coated particles and the zeta-potential of the corresponding particles suspended in water. ....	128
<b>Figure 4.13:</b> Suspension stability of SC-coated HA particles prepared with increasing concentrations of SC. ....	129
<b>Figure 4.14:</b> Variation of absorbance (% of initial absorbance) as a function of time of suspensions of HA particles (0.125%, w/w) made with water or solutions of different (A) SC or (B) WPI concentrations.....	130

<b>Figure 4.15:</b> Proposed mechanism explaining the adsorption of whey proteins and caseins onto HA particles. ....	132
<b>Figure 4.16:</b> Proposed mechanism explaining the improvement of suspension stability observed for HA particles when caseins and whey proteins were adsorbed on the surface of the particles. ....	133
<b>Figure 5.1:</b> Surface protein concentration of individual milk proteins as a function of initial protein concentration, in 50 mM HEPES buffer (pH 6.8) of 7 mM ionic strength (panel A) and 100 mM ionic strength (panel B). ....	141
<b>Figure 5.2:</b> Surface protein concentration of individual milk proteins as a function of protein concentration at equilibrium, in 50 mM HEPES buffer (pH 6.8) of 7 mM ionic strength (panel A) and 100 mM ionic strength (panel B). . ....	141
<b>Figure 5.3:</b> Zeta-potential of protein-coated HA particles, as a function of the initial protein concentration used in the adsorption experiments. ....	145
<b>Figure 5.4:</b> Linear relationship between the surface protein concentration of (●) $\alpha_S$ -casein, (▲) $\beta$ -casein, (■) $\kappa$ -casein, (◆) $\beta$ -lactoglobulin and (▼) $\alpha$ -lactalbumin, and the zeta-potential of the corresponding particles. ....	146
<b>Figure 5.5:</b> Integrated data of the MF electrophoresis gel patterns (inserts) of the supernatants of samples containing different concentrations of HA particles (0.1% to 4%, w/w) and 0.2% (w/w) total protein comprising equal amounts of $\alpha_S$ -casein ( $\alpha_S$ -CN; $\alpha_{S1} + \alpha_{S2}$ ), $\beta$ -casein ( $\beta$ -CN) and $\kappa$ -casein ( $\kappa$ -CN). ....	149
<b>Figure 5.6:</b> Estimated surface protein concentrations (mg/m <sup>2</sup> ) of total protein and individual caseins from an initial solution containing 0.2% (w/w) total protein comprising equal amounts of $\alpha_S$ -casein ( $\alpha_{S1} + \alpha_{S2}$ ), $\beta$ -casein and $\kappa$ -casein, in 50 mM HEPES buffer, pH 6.8, at 7 mM (panel A) and 100 mM ionic strengths (panel B), as a function of HA concentration. ....	151
<b>Figure 5.7:</b> Integrated data of the MF electrophoresis gel patterns (inserts) of the supernatants of samples containing different concentrations of HA particles (0.1% to 4%, w/w) and 0.08% (w/w) total protein comprising equal amounts of $\beta$ -lactoglobulin and $\alpha$ -lactalbumin. ....	152
<b>Figure 5.8:</b> Estimated surface protein concentrations (mg/m <sup>2</sup> ) of individual whey proteins from an initial solution containing 0.08% (w/w) total protein comprising equal amounts of $\beta$ -lactoglobulin and $\alpha$ -lactalbumin, in 50 mM HEPES buffer, pH 6.8, at 7 mM (panel A) and 100 mM ionic strength (panel B), as a function of HA concentration. ....	154
<b>Figure 5.9:</b> Integrated data of the MF electrophoresis gel patterns of the supernatants (inserts) of samples containing different concentrations of HA particles (0.1% to 8% w/w) and 0.08% (w/w) total protein comprising equal amounts of $\beta$ -lactoglobulin and $\alpha$ -lactalbumin. ....	155
<b>Figure 5.10:</b> Estimated surface protein concentrations (mg/m <sup>2</sup> ) of total protein and individual milk proteins from an initial solution containing 0.2% (w/w) total protein comprising equal amounts of $\alpha_S$ -casein ( $\alpha_{S1} + \alpha_{S2}$ ), $\beta$ -casein, $\kappa$ -casein, $\beta$ -lactoglobulin and $\alpha$ -lactalbumin, in 50 mM HEPES buffer (pH 6.8) at 100 mM ionic strength. ....	157

<b>Figure 5.11:</b> MF electrophoresis gel patterns of $\beta$ -casein initial solution and supernatants obtained from adsorption experiments carried out with $\beta$ -lactoglobulin-covered HA particles added to $\beta$ -casein solutions at different initial concentrations. ....	158
<b>Figure 5.12:</b> MF electrophoresis gel patterns of initial solutions and respective supernatants obtained for adsorption experiments carried out with $\beta$ -casein-covered HA particles added to $\beta$ -lactoglobulin solutions at different initial concentrations. ....	159
<b>Figure 6.1:</b> Effect of (A) NaCl concentration and (B) pH on the zeta-potential of suspensions of HA particles (0.05%, w/w). ....	164
<b>Figure 6.2:</b> Effect of (A) NaCl concentration and (B) pH on the suspension stability of HA particles. ....	167
<b>Figure 6.3:</b> Variation of absorbance (expressed in percentage of initial absorbance) as a function of time of suspensions of HA particles (0.125%, w/w) in solutions of different NaCl concentrations. ....	168
<b>Figure 6.4:</b> Effect of various ions on the zeta-potential of suspensions of HA particles (0.05%, w/w). ....	169
<b>Figure 6.5:</b> Effect of (A) phosphate ions, (B) citrate ions, and (C) calcium ions on the suspension stability of 5% (w/w) HA particles. ....	171
<b>Figure 6.6:</b> SDS-PAGE pattern of initial protein solutions (lanes 1-4) and supernatants (i.e., containing the unadsorbed protein; lanes 5-9) obtained for adsorption experiments carried out with (A) 4% (w/w) SC solutions and (B) 2% (w/w) WPI solutions prepared with different concentrations of added NaCl. ....	175
<b>Figure 6.7:</b> Effect of NaCl concentration on the surface protein concentration of (A) SC and (B) WPI onto HA particles. ....	176
<b>Figure 6.8:</b> Changes in the estimated surface concentration ( $\text{mg}/\text{m}^2$ ) of (A) individual caseins from 4% (w/w) SC and (B) individual whey proteins from 2% (w/w) WPI, as a function of NaCl concentration. ....	179
<b>Figure 6.9:</b> Suspension stability of (A) SC-coated HA particles and (B) WPI-coated particles, suspended in solutions of varying ionic strength. ....	183
<b>Figure 6.10:</b> SDS-PAGE pattern of initial 4% (w/w) SC solutions and supernatants (i.e., containing the unadsorbed protein) obtained for adsorption experiments carried out at (A) pH 6, (B) pH 6.8, and (C) pH 8, with different levels of NaCl addition. ....	185
<b>Figure 6.11:</b> SDS-PAGE pattern of initial 2% w/w WPI solutions and supernatants (i.e., containing the unadsorbed protein) obtained for adsorption experiments carried out at (A) pH 6, (B) pH 6.8, and (C) pH 8, with different levels of NaCl addition. ....	185
<b>Figure 6.12:</b> Effect of pH on the surface protein concentration of (A) SC and (B) WPI onto HA particles at three different NaCl concentrations. ....	186
<b>Figure 6.13:</b> Changes in the estimated surface concentration ( $\text{mg}/\text{m}^2$ ) of individual caseins from SC as a function of pH in (A) water, no NaCl and (B) 0.1 M NaCl. ....	188

<b>Figure 6.14:</b> SDS-PAGE pattern of initial protein solutions and supernatants obtained for adsorption experiments carried out with SC and WPI solutions in water and in SMUF: (A) SC in water; (B) SC in SMUF; (C) WPI in water; (D) WPI in SMUF. ....	189
<b>Figure 6.15:</b> Effect of milk serum composition using SMUF on the surface protein concentration of (A) SC and (B) WPI, compared with adsorption in water. ....	191
<b>Figure 6.16:</b> Comparison between Langmuir isotherms of (A) SC and (B) WPI adsorption onto HA particles. ....	191
<b>Figure 6.17:</b> Comparison between estimated surface concentration (mg/m <sup>2</sup> ) of (A) individual caseins from SC and (B) individual whey proteins from WPI, on HA particles in water (closed symbols, solid lines) and in SMUF (open symbols, dashed lines) as a function of initial protein concentration. ....	194
<b>Figure 6.18:</b> SDS-PAGE pattern of initial 2% (w/w) SC solutions (lanes 1–5) and supernatants (i.e., containing the unadsorbed protein; lanes 6–10) obtained for adsorption experiments carried out in SMUF containing different amounts of citrate. ....	196
<b>Figure 6.19:</b> Effect of initial protein concentration on the zeta-potential of HA particles that were suspended in solutions of SC (■) or WPI (●) reconstituted in SMUF at different initial concentrations.....	197
<b>Figure 6.20:</b> Suspension stability of (A) SC-coated HA particles and (B) WPI-coated particles, prepared in SMUF with increasing concentrations of protein. ....	199
<b>Figure 6.21:</b> Graphical summary of the effect of ionic strength, pH, and SMUF on milk protein adsorption on HA. ....	201
<b>Figure 7.1:</b> MF-electrophoresis gel pattern of initial protein solutions and supernatants obtained for adsorption experiments carried out with (A) WPD-SM and (B) SM solutions at different initial protein concentrations. ....	207
<b>Figure 7.2:</b> Surface protein concentration of (●) caseins and (▲) whey proteins adsorbed onto HA particles (mg/m <sup>2</sup> ) and (○) unadsorbed protein concentration (% w/w) as a function of initial protein concentration: (A) WPD-SM; (B) SM. ....	208
<b>Figure 7.3:</b> Adsorption isotherms of caseins onto HA particles: (A) WPD-SM; (B) SM. ....	210
<b>Figure 7.4:</b> TEM micrographs obtained from: (A and B) WPD-SM solution reconstituted from WPD-SMP in water (10%, w/w); (C and D) suspension of HA particles in SMUF; (E and F) suspension of HA particles in WPD-SM solution, corresponding to a stage of adsorption at which the maximum amount of adsorbed protein was reached. ....	212
<b>Figure 7.5:</b> Light microscopy picture obtained from a suspension of HA particles in (A) SMUF and (B) WPD-SM solution. ....	213
<b>Figure 7.6:</b> MF-electrophoresis gel pattern of supernatants containing the total unadsorbed protein and their respective serum phase containing the non-micellar unadsorbed caseins, obtained from adsorption experiments carried out with WPD-SM at different initial protein concentrations.....	215
<b>Figure 7.7:</b> (A) Distribution of unadsorbed caseins between (white bar) micellar and (grey bar) non-micellar forms and (B) diameters of unadsorbed caseins. ....	216

- Figure 7.8:** Composition of the supernatants containing the unadsorbed protein after adsorption of WPD-SM onto HA particles as a function of the initial protein concentration. .... 217
- Figure 7.9:** Estimated amount of adsorbed caseins ( $\text{mg}/\text{m}^2$ ) on HA particles as a function of initial protein concentration of WPD-SM. .... 218
- Figure 7.10:** Zeta-potential, surface protein concentration and visual turbidity of HA particles prepared with WDP-SM at different initial protein concentrations. .... 221
- Figure 7.11:** SDS-PAGE patterns of the initial solutions of EDTA-treated SM and the adsorption supernatants of the EDTA-treated SM and HA particles mixed to different ratios SM to HA powder. .... 224
- Figure 7.12:** Langmuir-type representation of the surface protein concentration obtained in adsorption experiments carried out with HA particles added to different EDTA-treated SM. . 225
- Figure 7.13:** Visual turbidity of SM samples after dialysis against SMUF or against SMUF containing 10% (w/w) HA particles. .... 227
- Figure 7.14:** Effect of addition of HA particles in (■) SMUF and in (▲) SM on ionic calcium concentration. .... 229
- Figure 7.15:** Effect of addition of HA particles in (A) SM and (B) SMUF on (▼) total calcium, (■) total citrate, and (●) inorganic phosphate concentrations..... 230
- Figure 7.16:** MF-gel electrophoresis showing the dialysed SM samples and their respective serum phase (i.e., containing the dissociated casein micelles), for SM samples dialysed against different concentrations of HA particles (0, 0.1, 0.5 and 5%, w/w, lanes A to D) and for different dialysis times (1, 2, 3 and 7 days, columns 1 to 4). .... 233
- Figure 7.17:** Proposed mechanism explaining the dissociation of casein micelles upon addition of HA particles in milk and the subsequent binding of caseins to the particle..... 236

## LIST OF TABLES

<b>Table 2.1:</b> Recommended daily calcium intakes by age group.....	6
<b>Table 2.2:</b> Principal characteristics of casein molecules (from Walstra and Jenness, 1984, and Fox, 2003).....	10
<b>Table 2.3:</b> Salt partitioning in cows' milk (from Gaucheron, 2005). .....	28
<b>Table 2.4:</b> Effect of physico-chemical parameters of the suspending solution on zeta-potential and aggregation and crystal growth behaviour of HA particles .....	47
<b>Table 2.5:</b> Summary of the main studies looking at the effect of various factors on protein adsorption on HA.....	55
<b>Table 3.1:</b> Protein composition of the blends of milk proteins.....	72
<b>Table 3.2:</b> Summary of the adsorption experiments carried out in this thesis (protein sources, concentration and suspending solution used for the preparation of the stock solutions) .....	75
<b>Table 3.3:</b> SMUF recipe.....	76
<b>Table 4.1:</b> Parameters for the adsorption of SC and WPI onto HA particles calculated according to the Langmuir, Langmuir–Freundlich and Freundlich models .....	106
<b>Table 4.2:</b> Relative proportions of individual caseins adsorbed onto HA particles at different initial protein concentration of SC.....	112
<b>Table 4.3:</b> Relative proportions of individual whey proteins adsorbed onto HA particles at varying initial protein concentration of WPI.....	112
<b>Table 4.4:</b> Relative proportion of individual caseins adsorbed onto HA particles at different initial HA concentration, with a constant initial SC protein concentration (0.1%, w/w).....	121
<b>Table 4.5:</b> Relative proportion of individual caseins adsorbed onto HA particles at different initial HA concentrations, with a constant initial WPI protein concentration (0.1%, w/w).....	123
<b>Table 5.1:</b> Affinity constant and maximum surface concentration for the adsorption of $\alpha_S$ -casein ( $\alpha_{S1} + \alpha_{S2}$ ), $\beta$ -casein, $\kappa$ -casein, $\beta$ -lactoglobulin ( $\beta$ -Lg) and $\alpha$ -lactalbumin ( $\alpha$ -La) onto HA particles in 50 mM HEPES buffer (pH 6.8) of 7 mM and 100 mM ionic strengths (I), calculated according to the Langmuir model.....	142
<b>Table 5.2:</b> Estimated calculated net charge at pH 6.8 and in water of the individual caseins and whey proteins used in this chapter.....	147
<b>Table 6.1:</b> Effect of NaCl concentration on zeta-potential of HA particles coated with SC or WPI, and calculated change between the initial zeta-potential (uncoated particles) and the final zeta-potential (coated particles).....	182
<b>Table 6.2:</b> Parameters for the adsorption of SC and WPI onto HA particles in SMUF and in water, calculated according to the Langmuir model.....	192

<b>Table 6.3:</b> Relative proportions of individual caseins adsorbed onto HA particles when the adsorption experiment was carried out from 2% (w/w) SC in SMUF containing different amounts of citrate.....	196
<b>Table 7.1:</b> Parameters for the adsorption of SC and WPI onto HA particles in SMUF and in water, calculated according to the Langmuir model.....	210
<b>Table 7.2:</b> Total surface protein concentration and relative proportions of individual caseins adsorbed onto HA particles after different adsorption times.....	219
<b>Table 7.3:</b> Non-micellar casein content and casein micelle diameter of EDTA-treated skim milks.....	222
<b>Table 7.4:</b> Non-micellar casein content and casein micelle size of the dialysed milks.....	228
<b>Table 7.5:</b> Percentage of non-micellar caseins in SM, after dialysis of SM against SMUF containing different concentrations of HA particles, for different times.....	234

**LIST OF ABBREVIATIONS**

°C	Degree(s) Celsius
%	Percent
$\alpha$ -CN	$\alpha$ -Casein
$\alpha$ -La	$\alpha$ -Lactalbumin
$\beta$ -CN	$\beta$ -Casein
$\beta$ -Lg	$\beta$ -Lactoglobulin
$\kappa$ -CN	$\kappa$ -Casein
$\gamma$ -CN	$\gamma$ -Casein
$\mu$ L	Microlitre(s)
$\mu$ m	Micrometre(s)
BSA	Bovine serum albumin
ACP	Amorphous calcium phosphate
ANOVA	Analysis of variance
Asn	Asparagine
Asp	Aspartic acid
BSA	Bovine Serum Albumin
Ca	Calcium
CaCl <sub>2</sub>	Calcium chloride
CCP	Colloidal calcium phosphate
Cit <sup>3-</sup>	Citrate ions
Cl	Chlorine
Cl <sup>-</sup>	Chloride ions
CMC	Carboxymethylcellulose
COO <sup>-</sup>	Carboxyl group(s)
CPP	Caseinophosphopeptide(s)
C-site	Calcium site

DIC	Differential interference contrast
DF	Dilution factor
EC	Extinction coefficient ( $\text{cm}^2/\text{g}$ )
EDTA	Ethylenediaminetetraacetic acid
FTIR	Fourier transform infrared
g	Gram(s)
<i>g</i>	Centrifugal force
Glu	Glutamic acid
h	Hour(s)
H <sup>+</sup>	Protons
HA	Hydroxyapatite
HCl	Hydrochloric acid
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
<i>K</i>	Langmuir equilibrium constant (100g/g)
K	Potassium
kDa	Kilodalton(s)
$K_{LF}$	Langmuir-Freundlich equilibrium constant $((100\text{g/g})^{1/n})$
$K_F$	Freundlich affinity constant $((100\text{g/g})^N)$
kJ	Kilojoule(s)
kV	Kilovolt(s)
L	Litre(s)
Lys	Lysine
$m_{\text{abs}}/S$	Mass of protein per unit area ( $\text{mg}/\text{m}^2$ )
MCC	Microcrystalline cellulose
MF	Microfluidic or Microfiltration
Mg	Magnesium
$\text{m}^2$	Square metre(s)

mg	Milligram (s)
min	Minute(s)
mL	Millilitre(s)
mM	Millimolar (mmol.L <sup>-1</sup> )
mmol	Millimole(s)
mol	Mole(s)
n	Surface heterogeneity parameter of the Langmuir-Freundlich model
Na	Sodium
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH <sub>3</sub> <sup>+</sup>	Amino groups
nm	Nanometre(s)
OH <sup>-</sup>	Hydroxyl ions
[P]	Protein concentration at equilibrium
Pi	Inorganic phosphate
pI	Isoelectric point
pK	Dissociation constant
pKa	Acid dissociation constant
PO <sub>4</sub>	Phosphate
P-site	Phosphate site
q <sub>m</sub>	Maximum surface coverage
s	Second(s)
SEM	Surface electron microscopy
Ser	Serine
Ser-P	Phosphoserine groups
SC	Sodium caseinate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SM	Skim milk
SMUF	Simulated milk ultrafiltrate
T	Temperature
TCP	Tricalcium phosphate
TEM	Transmission electron microscopy
TN	Total nitrogen
TS	Total solids
UHT	Ultra-high temperature
UV	Ultraviolet
V	Volt(s)
WDP-SM	Whey protein-depleted skim milk
WDP-SMP	Whey protein-depleted skim milk powder
WPI	Whey protein isolate
w/w	Weight/weight
ZP	Zeta-potential

