# Study of the interactions between milk proteins and hydroxyapatite particles

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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, β -casein, κ-casein, β-lactoglobulin and α-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 competive nature. β-casein and $\alpha_S$-casein were always preferred for adsorption over κ-casein, β-lactoglobulin and α-lactalbumin. This was attributed to the presence of phosphoserine clusters in β-casein and $\alpha_S$-casein, forming many anchor points capable of binding to the calcium sites of HA. β-casein and $\alpha_S$-casein also adsorbed to higher maximum levels compared to κ-casein, β-lactoglobulin and α-lactalbumin. Both β-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, κ-casein, β-lactoglobulin and α-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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| | |
|---|---|
| °C | Degree(s) Celsius |
| % | Percent |
| α-CN | α-Casein |
| α-La | α-Lactalbumin |
| β-CN | β-Casein |
| β-Lg | β-Lactoblobulin |
| κ-CN | κ-Casein |
| γ-CN | γ-Casein |
| µL | Microlitre(s) |
| µm | 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 |
| $CaCl_2$ | Calcium chloride |
| CCP | Colloidal calcium phosphate |
| $Cit^{3-}$ | Citrate ions |
| Cl | Chlorine |
| $Cl^-$ | Chloride ions |
| CMC | Carboxymethylcellulose |
| $COO^-$ | Carboxyl group(s) |
| CPP | Caseinophosphopeptide(s) |
| C-site | Calcium site |

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

| | |
|---|---|
| mg | Milligram (s) |
| min | Minute(s) |
| mL | Millilitre(s) |
| mM | Millimolar (mmol.$L^{-1}$) |
| mmol | Millimole(s) |
| mol | Mole(s) |
| n | Surface heterogeinity parameter of the Langmuir-Freundlich model |
| Na | Sodium |
| NaCl | Sodium chloride |
| NaOH | Sodium hydroxide |
| $NH_3^+$ | Amino groups |
| nm | Nanometre(s) |
| $OH^-$ | Hydroxyl ions |
| [P] | Protein concentration at equilibrium |
| Pi | Inorganic phosphate |
| pI | Isoelectric point |
| pK | Dissociation constant |
| pKa | Acid dissociation constant |
| $PO_4$ | Phosphate |
| P-site | Phosphate site |
| $q_m$ | 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 |