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 α_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 α_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 α_S-casein, forming many anchor points capable of binding to the calcium sites of HA. β-casein and α_S-casein also adsorbed to higher maximum levels compared to κ-casein, β-lactoglobulin and α-lactalbumin. Both β-Casein and α_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