Comparative study of the effects of added calcium on the heat-induced changes in three complex whey protein systems

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ABSTRACT

Because of their superior functional properties and nutritional quality, whey proteins are widely used in the food industry. Some of the important functional properties of whey proteins include gelation, water-binding, emulsification and foaming. Heat-induced gelation of whey proteins is particularly important in many food applications, and it involves a series of complex changes to protein structures (denaturation, aggregation). However, recent clinical studies on health promoting properties of whey proteins (e.g. weight management, muscle mass retention) has prompted the food industry to develop foods with high levels of whey proteins, such as high protein beverages. In these products, the heat-induced aggregation and gelation functionality of whey proteins becomes a major limiting factor.

The main objective of the present study was to determine the effects of added calcium on heat-induced denaturation, aggregation and gelation of whey proteins in three different whey protein products: whey protein isolate (WPI), acid whey protein concentrate (AWPC) and cheese whey protein concentrate (CWPC). The results were interpreted to assess the suitability of different whey protein systems as influenced by the effects of added calcium on their properties for making new denatured whey protein products.

The effects of added calcium chloride on heat-induced changes in whey protein solutions prepared from WPI, AWPC and CWPC were investigated using polyacrylamide gel electrophoresis (PAGE), high-performance liquid chromatography (HPLC), differential scanning calorimetry (DSC), circular dichroism (CD), nuclear magnetic resonance (NMR), small deformation oscillatory rheometry, large deformation compression testing and transmission electron microscopy (TEM). The loss of native proteins in 4% (w/w) protein solutions increased with increase in added calcium levels up to an optimum level (varying between 20 – 110 mM depending on the whey protein product), but then decreased with further increase in added calcium levels. The firmness of gels was maximal at 4 mM added calcium for WPI solutions, 20 mM for AWPC, and 80 mM for CWPC. These results showed that a certain level of added calcium maximally enhanced the heat-induced aggregation and gelation of whey proteins, and

these levels were different for the different whey protein systems. The effects of added calcium appeared to be related to the initial calcium contents of the three systems (2.1, 8.4 and 11.2 mM for 4.8% w/w protein solutions of WPI, AWPC and CWPC). It was considered that the addition of calcium changed the types of protein interactions leading to the formation of protein aggregates during heating. Increasing levels of calcium caused dramatic decreases in the fracture stress of whey protein gels due to the formation of increasingly larger protein aggregates; the gels became softer and, to an extent, mushier depending on the whey protein system. The TEM micrographs showed that on addition of calcium, the gels became coarser. WPI (12%, w/w protein) gels without the addition of calcium had a very fine structure (< 0.1 μm). With 60 mM of added calcium, 0.5 μm bead-like aggregates formed, and with further increase in added calcium levels, the aggregate size increased to 2 μm. AWPC (12%, w/w protein) gels without addition of calcium also showed a relatively fine structure (< 0.2 μm), and with the addition of 60 mM of calcium, the aggregate size increased to 0.1 – 0.2 μm. In the case of CWPC (12%, w/w protein) gels, the aggregate size increased from 0.05 to 0.3 μm on the addition of 60 mM of calcium.

The kinetics study showed that the mechanism of denaturation and aggregation of whey proteins in AWPC (but not in WPI or CWPC) was not affected by protein concentration in the range 4 – 28% (w/w). The orders of reaction were found to be 1.7 for β-lactoglobulin and 1 for α-lactalbumin at all protein concentrations. Without addition of calcium, the transition temperature decreased from 85 to 80ºC with increasing total solids for both proteins, whereas with 20 mM added calcium the transition temperature remained constant (~ 80ºC) over the total solids range (5 – 35%, w/w) for β-lactoglobulin and α-lactalbumin. The effects of added calcium on the aggregation kinetics appeared to be related to the calcium to protein ratio. The addition of 20 mM of calcium to low total solids solutions (5 – 10%, w/w) increased the rate constants, whereas addition of 20 mM of calcium to high total solids solutions (25 – 35%, w/w) decreased the rate constants.

These findings contribute to knowledge of the effects of added calcium on changes in whey proteins during heat treatments, and the relevance of the initial mineral content of whey protein products. AWPC appeared to be potentially the most suitable of the three systems studied for use as a feed material for manufacturing denatured whey proteins

with the aid of added calcium. The addition of calcium to AWPC solutions decreased the fracture stress and fracture strain of the gels formed, making them softer and mushier, and possibly more “processable”. Further, at high protein concentrations (20 – 28%, w/w), which correspond to desired feed material concentrations in a processing plant, the addition of 20 mM of calcium to AWPC solutions optimally slowed down the aggregation rate, which might help to decrease plant fouling during the manufacture of denatured whey protein products.

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LIST OF ABBREVIATIONS

ºC	Degree(s) Celsius
%	Per cent
2D	Second dimension
α-La	α-Lactalbumin
Asn	Asparagine
Asp	Aspartic acid
AWPC	Acid whey protein concentrate(s)
β-Lg	β-Lactoglobulin
BSA	Bovine serum albumin
Ca^{2+}	Calcium ion
$[Ca^{2+}]$	Concentration of ionic calcium (mM)
$CaCl_2$	Calcium chloride
CD	Circular dichroism
Cl^-	Chloride ion
cm	Centimeter(s)
CWPC	Cheese whey protein concentrate(s)
Cys	Cysteine
DF	Diafiltration
DSC	Differential scanning calorimetry
E_a	Activation energy
F_p	Fracture point
F_{sn}	Fracture strain
F_{ss}	Fracture stress
g	Gram(s) or centrifugal force
G'	Storage modulus
G'_{final}	Final storage modulus
G'_{max}	Maximum storage modulus
h	Hour(s)
H^+	Hydrogen ion
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography

Hz	Hertz
IE	Ion-exchange
Ig	Immunoglobulin
Ile	Isoleucine
K^+	Potassium ion
kDa	Kilodalton(s)
kg	Kilogram(s)
kHz	Kilohertz
kJ	Kilojoule(s)
k_n	Rate constant(s) at the order of reaction *n*
kV	Kilovolt(s)
L	Litre(s)
Leu	Leucine
Lys	Lysine
μL	Microlitre(s)
μm	Micrometre(s)
M	Molar (mol L^{-1})
mA	Milliampere(s)
mdeg	Millidegree(s)
MeCN	Acetonitrile
Met	Methionine
MF	Microfiltration
mg	Milligram(s)
Mg^{2+}	Magnesium ion
MHz	Megahertz
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar (mmol L^{-1})
mmol	Millimole(s)
mol	Mole(s)
ms	Millisecond(s)
mV	Millivolt(s)
MW	Molecular weight

n	Order of reaction or number of replicates
N	Newton(s)
Na^+	Sodium ion
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NEM	N-ethylmaleimide
nm	Nanometre(s)
NMR	Nuclear magnetic resonance
P	Significance level
Pa	Pascal(s)
PAGE	Polyacrylamide gel electrophoresis
Phe	Phenylalanine
PO_4^{3-}	Phosphate ion
PP	Proteose peptones
ppm	Part(s) per million
RP-HPLC	Reverse-phase high performance liquid chromatography
s	Second(s)
SDS	Sodium dodecyl sulphate
Ser	Serine
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
Thr	Threonine
TOCSY	Total correlation spectroscopy
Tris	Tris(hydroxymethyl)methylamine
Trp	Tryptophan
ts	Total solids
T_t	Transition temperature
UDP	Uranyl diphosphate
UF	Ultrafiltration
UV	Ultraviolet
V	Volt(s)
Val	Valine
vs.	Versus
v/v	Volume/volume

w/v	Weight/volume
w/w	Weight/weight
WPC	Whey protein concentrate(s)
WPI	Whey protein isolate(s)