Food Research International 62 (2014) 128–136

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Tuning heat-induced colloidal aggregation of whey proteins, sodium caseinate and gum arabic: Effect of protein composition, preheating and gum arabic level

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ARTICLE INFO

Article history:
Received 20 September 2013
Accepted 22 February 2014
Available online 1 March 2014

Keywords:
Colloidal aggregation
Whey protein
Casein
Gum arabic
Heating
Disulfide bonding

ABSTRACT

Heating can drive the colloidal complexation of negatively-charged proteins and polysaccharides by strengthening hydrophobic interactions and denaturing proteins, thereby exposing reactive sites for covalent and noncovalent bonding. We have previously shown that stable colloidal aggregates comprising whey protein, sodium caseinate and gum arabic can be produced by careful selection of heat treatment, pH and protein type. Here we tested how the size, composition, charge and morphology of colloidal aggregates are affected by the amounts of whey protein, sodium caseinate and gum arabic, as well as the thermal history of the proteins. Increasing amounts of whey protein resulted in larger particles, which were more prone to precipitate. Preheating whey protein slightly enhanced aggregation, and this effect was mitigated when sodium caseinate was present during preheating (chaperone effect). Increasing amounts of gum arabic produced larger particles with less charge, but the gum arabic effect was statistically confounded with ionic strength. We believe that both covalent (disulphide) and noncovalent interactions among protein molecules are required to overcome electrostatic repulsion at pH 7 and form stable aggregates.

1. Introduction

Complexation between polysaccharides and proteins is a well-known phenomenon that finds application in protein purification (Wang, Gao, & Dubin, 1996), nutrient delivery (Zimet & Livney, 2009), and food structure design (Dickinson, 2012; Paquin, 1999). Both proteins and polysaccharides are polyelectrolytes whose charge depends on pH, and attractive or repulsive electrostatic interactions have a strong influence on phase behaviours in mixed aqueous systems. Most food polysaccharides have very low isoelectric pH (pI), so they are negatively charged in conditions relevant to food systems. By contrast, food proteins often have isoelectric points (pI) within the operating pH range of food manufacturing processes, so they may have either a net negative charge (pH > pI) or a net positive charge (pH < pI) depending on the pH.

Complexation often occurs in the pH range where protein and polysaccharide are oppositely charged, and may result in colloidal aggregates or a precipitate. The nature of aggregates depends on biopolymer type and concentration, ionic strength, pH, and temperature. Aggregates that are colloidally stable may be useful in foods as Pickering emulsifiers (Dickinson, 2012), fat mimetics (Paquin, 1999) or nutrient delivery vehicles (Zimet & Livney, 2009).

Earlier work has shown that heating at neutral pH drives reversible hydrophobic association between sodium caseinate (SC) and gum arabic (GA) (Ye, Edwards, Gilliland, Jameson, & Singh, 2012). We have shown that adding whey protein isolate (WPI) to mixtures of SC and GA promotes covalent aggregation, particularly at pH 7 but also to a limited extent at pH 3.5 (Loveday, Ye, Anema, & Singh, 2013). Here we extend this work to examine how heat-induced denaturation of milk proteins prior to mixing with GA affects the nature of aggregation.

Heating whey proteins at 80 °C and neutral pH leads to unfolding of globular proteins and exposure of the reactive free thiol groups of β-lactoglobulin and bovine serum albumin (BSA), leading to complete denaturation within 12.5 min for β-lactoglobulin (Manderson, Creamer, & Hardman, 1999) or even faster for BSA (Baier, Decker, & McClements, 2004; Boye, Alli, & Ismail, 1996). Subsequent intermolecular disulphide bonding among whey proteins of the same or different type drives the formation of oligomers and polymers, whose size depends strongly on protein concentration, the proportions of different whey proteins, and ionic strength (Anema, 2009b; Livney, Corredig, & Dalgleish, 2003). α-Lactalbumin contains four disulphide bonds and no free thiol groups, but it nevertheless polymerises via disulphide bonds in the presence of other proteins with free thiol groups, which appear to catalyse

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http://dx.doi.org/10.1016/j.foodres.2014.02.018
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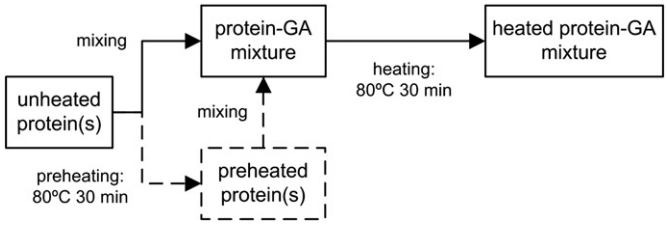

Fig. 1. Schematic of the experimental protocol for sample preparation.

sulphydryl–disulphide interchange in α-lactalbumin (De la Fuente, Singh, & Hemar, 2002).

The casein fraction of milk protein comprises four gene products, namely α_{s1}-, α_{s2}-, β-, and κ-caseins. Caseins are phosphoproteins in which the clustering of hydrophilic residues (particularly phosphoserine) and hydrophobic residues imparts a strongly amphiphilic character (Horne, 2009). This gives them a tendency to form micelles by hydrophobic association, and this tendency is enhanced with increasing temperature due to a strengthening of thermodynamic driving forces for hydrophobic association, relative to other effects opposing association (Schellman, 1997). α_{s1}- and β-casein contain no cysteine residues, whereas α_{s2}-casein contains two, which may form an intramolecular disulphide bond or may bond with Cys residues on another α_{s2}-casein molecule, forming a dimer (Zhang & Vardhanabhuti, 2014). The two Cys residues of κ-casein make it highly susceptible to polymerisation (Farrell et al., 2004; Hamilton-Brown, Bekard, Ducker, & Dunstan, 2008). The caseins are classed as 'intrinsically unstructured' due to their low level of secondary structure and lack of well-defined tertiary structure (Farrell, 2011).

In previous work we fixed the ratio of SC to WPI at 1:1 and fixed the mass ratio of protein to polysaccharide (Pr:Ps) at 1:5. Here we tested SC to WPI ratios from 1:4 up to 4:1, as well as Pr:Ps from 0.2 to 1.0. The results show how colloidal protein–polysaccharide aggregation can be sensitively modulated at neutral pH by varying the protein type and thermal history, as well Pr:Ps.

2. Materials and methods

2.1. Chemicals

Fonterra Cooperative Ltd. (Auckland, NZ) supplied WPI and SC, and Bronson and Jacobs Ltd. (Auckland, NZ) supplied GA. Sigma-Aldrich (St. Louis., MO, USA) supplied all other chemicals, which were of analytical grade.

2.2. Preparation of solutions

SC, WPI, and GA were dissolved in Milli-Q® water and neutralized to pH 7.0, as previously reported (Loveday et al., 2013). Where required, solutions of 1% w/w protein in 50 mL plastic tubes were preheated in a water bath (Lab Companion BS-11, Jeio Tech, Seoul, Korea) at 80 ± 0.2 °C for 30 min and then cooled to room temperature. After mixing,

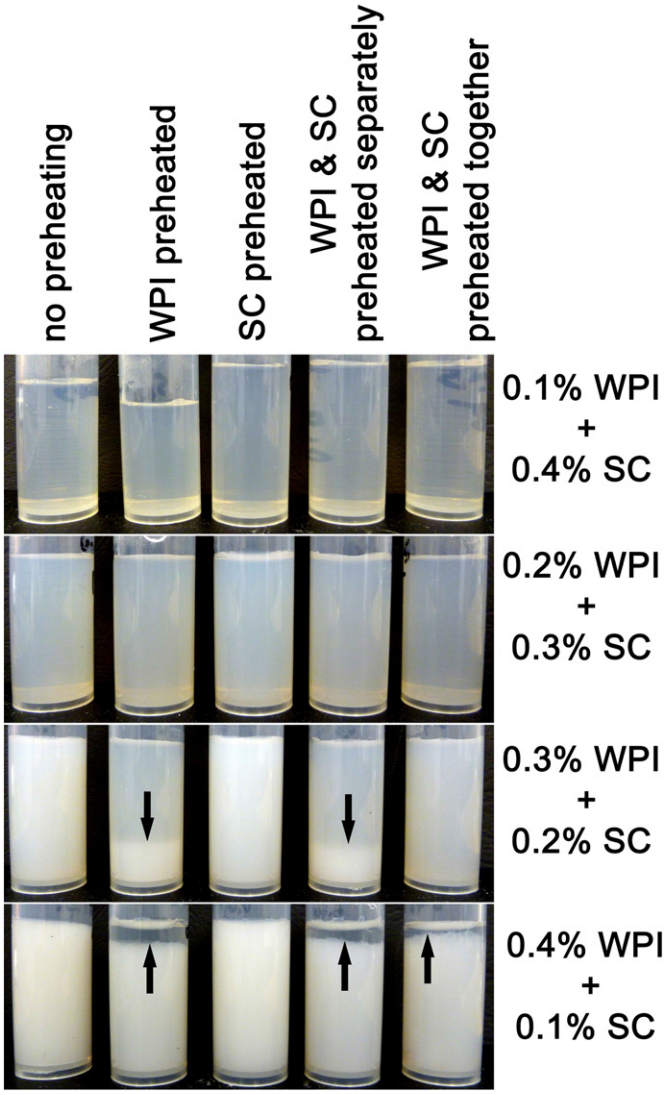

Fig. 2. Appearance of heated mixtures of WPI, SC and GA, showing the effect of preheating WPI and/or SC and the effect of the WPI:SC ratio. All samples contained 2.5% GA. Arrows indicate precipitation.

solutions of protein(s) and GA in small screw-top plastic bottles were heated in a water bath at 80 ± 0.2 °C for 30 min and then cooled to room temperature. The experimental protocol is depicted schematically in Fig. 1, which shows that protein solutions were either mixed with GA then heated, or preheated alone, mixed with GA then heated again.

Where specified, heated mixtures were centrifuged at 4000 ×*g* for 15 min at room temperature to sediment aggregates. All percentages given in the text are % w/v unless otherwise specified, and the '%' sign is omitted when referring to protein–polysaccharide mixtures, e.g.

Table 1
Effect of preheating (30 min at 80 °C) on the size of aggregates and derived count rate in 1% WPI, 1% SC or a 0.4% WPI + 0.6% SC mixture. Figures are the mean from 2 to 4 replicates, where variation among replicates was on the order of 1% of the mean.

WPI (% w/v)	SC (% w/v)	Z-average diameter (nm)		Derived count rate (kilo counts per second)	
		Unheated	Heated	Unheated	Heated
1	0	150 (0.40)[a]	116 (0.39)	18,307	129,848
0	1	182 (0.22)	199 (0.21)	25,140	53,256
0.4	0.6	–[b]	192 (0.26)	–	59,992

[a] Figures in brackets are polydispersity indices.
[b] Not tested.