Ca^{2+}-Induced Cold-Set Gelation of Whey Protein Isolate Fibrils

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Abstract:
In this paper we describe the rheological behaviour of Ca^{2+}-induced cold-set gels of whey protein mixtures. Cold-set gels are important applications for products with a low thermal stability. In previous work [1], we determined the state diagram for whey protein mixtures that were heated for 10 h at pH 2 at 80°C. Under these conditions, the major whey protein, β-lactoglobulin (β-lg), forms fibrils. When whey protein mixtures are heated at protein concentrations in the liquid solution regime of the state diagram, cold-set gels can be formed by adding Ca^{2+} ions at pH 7. We studied the rheological behaviour of cold-set gels for various sample compositions for whey protein mixtures. When keeping the total whey protein concentration constant, the elastic modulus, *G'*, for the cold-set gels decreased for increasing α-lactalbumin and bovine serum albumin ratios, because less material (β-lg fibrils) was available to form a gel network. In the cold-set gels the interactions between the β-lg fibrils induced by the calcium ions are dominant. The β-lg fibrils are forming the cold-set gel network and therefore determine the gel strength. α-Lactalbumin and bovine serum albumin are not incorporated in the stress-bearing structure of the gels.

Zusammenfassung:
In diesem Artikel wird das rheologische Verhalten von Ca^{2+}-induzierten, kaltgehärteten Gelen aus Mischungen von Molke-Protein beschrieben. Kaltgehärtete Gele sind wichtige Anwendungen für Produkte mit einer geringen thermischen Stabilität. Schon in früheren Arbeiten wurde von uns das Zustandsdiagramm von Molke-Protein-Mischungen, die für 10 Stunden bei einem pH 2 auf 80°C erhitzt wurden, erstellt [1]. Unter diesen Bedingungen formt das häufigste und daher wichtigste Molke-Protein, β-Lactoglobulin (β-lg), Fibrillen. Die flüssige Phase des Zustandsdiagramms von Molke-Protein kann, bei einem pH-Wert von 7, durch Erhitzten und Zugabe von Ca^{2+}-Ionen kaltgehärtete Gele bilden. Dies wird für verschiedene Mischungen von Molke-Proteinen untersucht. Bei gleich bleibender totaler Proteinkonzentration und bei steigendem α-Lactalbumin Gehalt und Bovin Serum Albumin Gehalt wird der elastische Modul für kaltgehärtete-Gele kleiner, da weniger Material (β-Lactoglobulin Fibrillen) zur Formung eines Gelnetzwerkes vorhanden ist. In kaltgehärteten Gelen sind die durch Kalziumionen induzierten Wechselwirkungen zwischen den β-lg Fibrillen dominant. Die β-lg Fibrillen formen das kaltgehärtete Gel-Netzwerk und bestimmen daher die Gelstärke. α-Lactalbumin und BSA wurden nicht in diese Strukturen eingebaut.

Résumé:
Dans cet article, nous décrivons le comportement rhéologique de gels formés à partir de mixtures de protéines du petit lait, et induits à froid par l'addition de Ca^{2+}. Les gels formés à froid sont utilisés de façon importante dans les produits montrant une faible stabilité thermique. Dans un travail précédent [1], nous avons déterminé le diagramme de phase pour les mixtures de petit lait qui ont été chauffées durant 10 heures à 80°C et à pH 2. Sous ces conditions, la protéine majoritaire, la β-lactoglobuline (β-lg), forme des fibrilles. Quand les mixtures sont chauffées avec des concentrations en protéines correspondant au régime de solution liquide du diagramme de phase, des gels peuvent être formés à froid par addition d'ions Ca^{2+} à pH 7. Nous avons étudié le comportement rhéologique des gels formés à froid pour des échantillons comprenant des compositions variées de mixtures de protéines. Lorsque la concentration totale en protéines est maintenue constante, le module élastique G' des gels formés à froid décroît avec l'augmentation des ratios de α-lactalbumine et de d'albumine de sérum bovin, parce que moins de matériau (fibrilles de β-lg) est disponible pour former un réseau de gel. Dans les gels formés à froid, les interactions entre les fibrilles de β-lg forment le réseau du gel et donc déterminent la rigidité du gel. L'α-lactalbumine et l'albumine de sérum bovin ne sont pas incorporées dans la structure du gel qui supporte la contrainte.

Key words: cold-set gelation, whey protein isolate, β-lactoglobulin, fibrils

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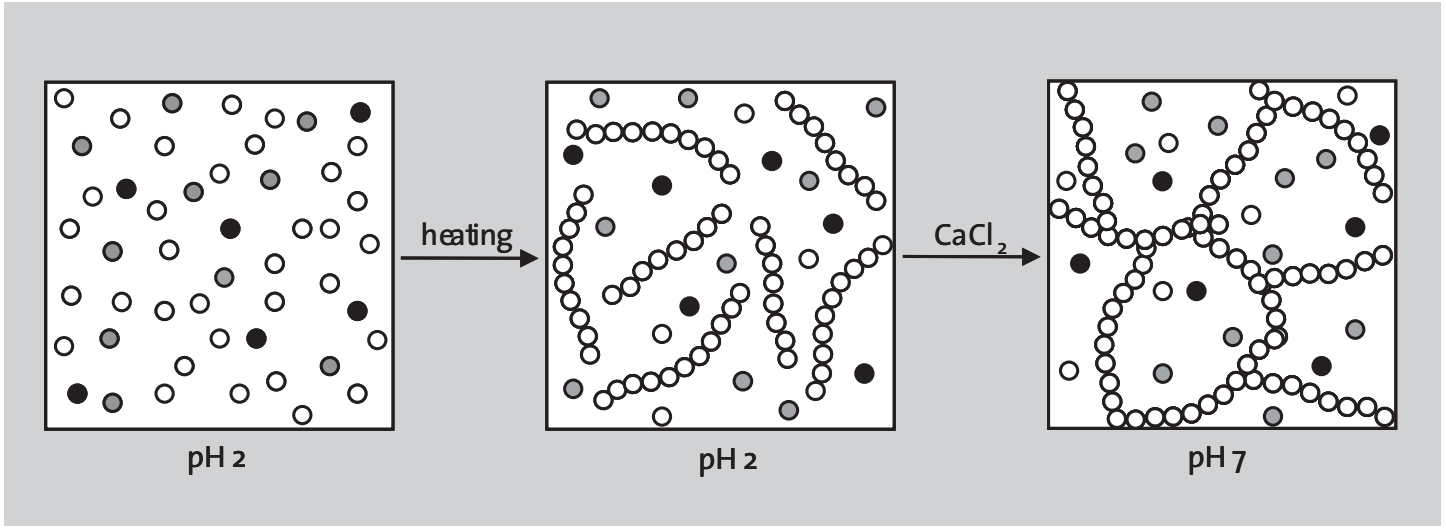

ples (see the right vertical planes in Figs. 3a and 3c). Therefore we can conclude that β-lg is dominant, and that α-lac and BSA do not contribute to the cold-set gel network. Under the conditions used in our experiments, α-lac and BSA do not form fibrils and form at most small aggregates [1], whereas β-lg forms long fibrils upon heating at pH 2. In heated whey protein samples depletion interactions between the long β-lg fibrils can be induced by monomers or small aggregates. This can result in attractive interactions between β-lg fibrils, causing a decrease in the minimum gelation concentration of β-lg for heat-set gels at pH 2 [1]. The small α-lac and BSA aggregates apparently are too small to contribute to the Ca^{2+}-induced cold-set gels at pH 7. The β-lg fibrils form the Ca^{2+}-induced cold-set gel network and therefore determine the gel strength. The attractive depletion interactions that play a role in the heat-set gelation at pH 2 do not contribute as much in the cold-set gels as in the heat-set pH 2 gels. In the cold-set gels the interactions between the β-lg fibrils, induced by the calcium ions, are dominant. Therefore a lower G' is found when the α-lac or BSA concentration in the samples increases, and the total protein concentration is kept constant, because less material (β-lg fibrils) is available to form a gel network. A schematic representation of the Ca^{2+}-induced cold-set gelation method for a mixture of whey proteins is shown in Fig. 5.

From the values of G' obtained from the various concentration curves, the critical percolation concentration, c_p, and the scaling component, t, were calculated. Here we define the minimal gelation concentration, based on the total protein concentration, as the critical percolation concentration, c_p. The scaling relation that we used is $G' \sim (c - c_p)^t$, where c is the concentration of monomers. Both c_p and t were determined using the method described by Van der Linden and Sagis [25]. This method is a graphical method that uses plots of $(G')^{1/t}$ versus c and extrapolates these plots to $(G')^{1/t} = 0$. In this procedure we make use of the fact that independent of the value of t the curves must all intersect the concentration axis at the same value. When the assumed value for t is close to the actual value, the plot will be linear. If t is too small or too large, the lines are curved. From the plots of $(G')^{1/t}$ versus c for various t, we selected those t values that give an approximately straight line. From these plots, an average value of c_p was determined. We plotted log G' versus log $(c - c_p)$, using the different values for c_p obtained from the estimated t values. For each of the values for c_p, we determined a new value for t and averaged these values. In Tab. 2 the calculated values for c_p and t are shown. The c_p values are all in a narrow range between 0.45 and 0.94 and do not show a systematic dependence on composition of the samples. This confirms that α-lac and BSA do not contribute to the gel network. For almost all samples the calculated values for t were between 1.8 and 2.0, which indicates isotropic force percolation. Isotropic force percolation assumes that nearest neighbours in the network interact through an isotropic force.

Sample	c_p [wt%]	t [-]
	total protein concentration	
β-lg	0.57 ± 0.04	2.24 ± 0.12
β-lg/α-lac = 90/10	0.48 ± 0.07	2.18 ± 0.18
β-lg/α-lac = 80/20	0.45 ± 0.08	1.94 ± 0.21
β-lg/BSA = 90/10	0.53 ± 0.07	1.97 ± 0.15
β-lg/BSA = 83/17	0.50 ± 0.04	1.75 ± 0.12
β-lg/BSA = 70/30	0.94 ± 0.04	1.80 ± 0.11
WPI	0.75 ± 0.15	1.97 ± 0.44
purified WPI	0.58 ± 0.12	1.91 ± 0.33

Samples were first heated at 2.5 wt % for 10 h at pH 2 and 80°C, subsequently cooled to room temperature. The values for G' obtained from Ca^{2+}-induced cold gelation with the addition of 0.01 M $CaCl_2$ at pH 7 was measured at 25°C after 3 h of gelation.

Figure 5:
Schematic representation of the Ca^{2+}-induced cold-set gelation method for a mixture of whey proteins; (○) β-lg; (●) α-lac; (●) BSA. First we have whey proteins in solution at pH 2. This solution is heated at a temperature above the denaturation temperature of the protein, and at a total protein concentration below the heat-induced gelation concentration. Under the conditions used β-lg fibrils are formed during heating. The fibril solution is cooled and the pH is adjusted from pH 2 to pH 7. Gelation is induced by addition of $CaCl_2$.

Table 2:
Calculated values for c_p and t for the total whey protein concentrations.

4 CONCLUSION

For the Ca^{2+}-induced cold-set gelation, performed with whey protein samples in the liquid solution regime of the state diagram, the minimal gelation concentration, c_p, was not affected by the addition of α-lac or BSA to β-lg (within the accuracy of the experiments). However, the G' for cold-set gels at equal total protein concentra-

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tions was found to decrease for increasing α-lac or BSA concentrations, because less material (β-lg fibrils) was available to form a gel network. The attractive depletion interactions that appear to play a role in the heat-set gelation at pH 2 do not contribute as much in the cold-set gels. In the cold-set gels the interactions between the β-lg fibrils, induced by the calcium ions, are dominant. The β-lg fibrils are forming the cold-set gel network and therefore determine the gel strength.

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