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Influence of κ-carrageenan on the aggregation behaviour of proteins in heated whey protein isolate solutions

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Abstract

The influence of the addition of κ-carrageenan to 2% whey protein isolate (WPI) at pH 7 and heated at 75 °C for different times, has been investigated by size-exclusion chromatography with multi-angle laser light scattering and gel electrophoresis. Under the conditions used, the addition of κ-carrageenan to WPI did not influence the loss of native β-lactoglobulin or α-lactalbumin. However, κ-carrageenan addition affected the molecular weight (M_w) of the aggregates formed by the heat treatment. The M_w of the whey protein aggregates formed in the early stages of heating in the presence of κ-carrageenan were lower than the aggregates formed in WPI solutions heated in the absence of κ-carrageenan. At longer heating times, the M_w of the aggregates in the WPI/κ-carrageenan mixtures were similar to the M_w of the aggregates in the WPI solutions heated alone.
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1. Introduction

Because of their high nutritional value and functionality, whey protein products, e.g., whey protein concentrates (WPCs) and whey protein isolates (WPIs), have become an important source of functional ingredients in many formulated foods. These products are commonly used in food products to increase viscosity, to form gels, to stabilize emulsions or foams or as water-holding agents. Whey proteins have the ability to form a gel after heat-induced denaturation and aggregation. The mechanisms involved in the denaturation process have been extensively studied and the aggregates and intermediates produced as a consequence of various heat treatments have been well characterised in pure β-lactoglobulin solutions (Hoffman, Sala, Olieman, & De Kruif, 1998; Schokker, Singh, Pinder, Norris, & Creamer, 1999). The influences of pH and ionic environment on heat-induced denaturation and aggregation have also been studied (Boye, Kalab, Alli, & Ma, 2000; Hoffman & Van Mil, 1999; Mleko & Foegeding, 2000; Xiong, 1992).

The incorporation of polysaccharide ingredients into food products increases the viscosity of the aqueous phase or results in the formation of gels. In this way, the shelf-life of food products, such as emulsions, can be improved. κ-Carrageenan, the polysaccharide most commonly added to dairy products, has been the subject of many studies. This product can interact with native β-lactoglobulin at or below pH 2.5, but not at pH 6 or higher (Hidalgo & Hansen, 1969). Nevertheless, when heated at neutral pH, β-lactoglobulin becomes denatured, thus exposing some positively charged amino groups, which could become available for interaction with anionic κ-carrageenan and could lead to complex formation.

Although the interactions of κ-carrageenan with the caseins are well documented (Bourriot, Garnier, & Doublier, 1999; Mora-Gutierrez, Kumosinski, & Farrell, 1998; Snoeren, Payens, Jeunink, & Both, 1975), the information available on whey protein and κ-carrageenan

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interactions is very limited. In addition, most studies have used purified β-lactoglobulin in model solutions (Capron, Nicolai, & Durand, 1999; Croguennoc, Durand, Nicolas, & Clark, 2001a, 2001b; Ould Eleya & Turgeon, 2000). Mleko, Li-Chan, and Pikus (1998) studied the interactions of κ-carrageenan with WPI in gels formed at different pH values. At pH 6–7, where the optimal gelation of mixtures was observed, a mixed structure gel with attractive inter-particle interactions was detected. Fernandes (1998), using differential scanning calorimetry, detected a synergistic interaction in heated mixtures of WPI/κ-carrageenan. Although this effect was attributed to the phase separation between globular protein and polysaccharides, local cross-linking between denatured whey proteins and κ-carrageenan could not be ruled out.

The aim of this study was to investigate the effects of the presence of κ-carrageenan, during heat treatments of WPI solutions, on the aggregation of whey proteins. The use of size-exclusion chromatography, coupled with multi-angle laser light scattering, allowed characterisation of the aggregates formed during heating.

2. Materials and methods

2.1. Materials

κ-Carrageenan used in this study was extracted from *Euchema cottonii* and was donated by Woods and Woods (Auburn, Australia, P.O. Box 98, product no. KK-104). WPI (ALACEN 895) was supplied by Fonterra Cooperative, New Zealand. All reagents were of the highest possible purity and of analytical grade. Milli-Q water with 18 MΩ cm resistivity was used in the preparation of all samples.

2.2. Sample preparation

WPI powder (4 g/100 ml) was dissolved in Milli-Q water containing 0.1 M added NaCl and the pH was adjusted to 7.0. These solutions were ultracentrifuged at 90,000*g* for 1 h in a temperature-controlled ultracentrifuge (Sorvall RC5C, DuPont Company, USA). Supernatants were collected carefully and used for the experiments.

κ-Carrageenan solutions (0.2 g/100 ml) were prepared by dispersing κ-carrageenan in water with moderate stirring for 30 min at 20 °C. In order to prevent clumping, κ-carrageenan was added slowly into the vortex of the stirring water. An appropriate amount of 0.5 M NaCl was then added to give a final concentration of 0.1 M NaCl and pH was adjusted to 7.0. The κ-carrageenan dispersion was then heated at 75 °C for 30 min with moderate stirring to fully dissolve the polysaccharide.

WPI and κ-carrageenan solutions were mixed in appropriate proportions to give a final concentration of 2% WPI and a range of κ-carrageenan concentrations. The mixture was stirred at 20 °C for 60 min and aliquots (2 ml) were removed, sealed in glass tubes, and then heated at different temperatures in a thermostatically controlled water bath for various times, ranging from 1 to 60 min. After heating, the samples were cooled in cold water and kept at 20 °C until analysis.

2.3. Size exclusion chromatography combined with multi-angle laser light scattering (SEC-MALLS)

Samples were filtered through 0.22 μm filters (Millipore) and 50 μl applied to a high-performance gel chromatography system, consisting of a model LC 1150 HPLC pump, a model LC 1200 UV/VIS detector operating to 280 nm, and a differential refractive index (DRI) detector (Waters, model R401, Milford) a model LC 1440 system organizer, and WinChrom software (GBC Scientific Equipment, Dandenong, Victoria 3175, Australia). Superdex 75 HR 10/30 (Pharmacia, Uppsala, Sweden, S-75182) or Shodex Ohpak SB-806 HQ (Shoko Co. Ltd., Japan) columns, equilibrated with 20 mM imidazole and 50 mM NaCl at pH 7.0, at a flow rate of 0.5 ml/min, were used in the analysis. The exclusion limits of these columns were 5–5000 and 3–20,000 kDa, respectively. UV absorption was measured at 280 nm. The weight average molecular weight (M_w) of the eluting material was determined by integrated light scattering using a DAWN DSP laser photometer, consisting of a linearly polarized He–Ne laser ($\lambda = 632.8$ nm; 5 mW), a K5 flow cell, 18 detectors at various angles and Astra (version 4.50) software (Wyatt Technology, Santa Barbara, CA 93103).

2.4. Electrophoresis

Samples were also analysed by polyacrylamide gel electrophoresis (PAGE), using Mini Protean II equipment (Bio-Rad Laboratories, Hercules, CA 94547, USA). Alkaline (native)-PAGE and sodium dodecyl sulphate (SDS)–PAGE of reduced and non-reduced samples were used, as described by Havea, Singh, Creamer, and Campanella (1998). The PAGE gels were scanned and the gel bands quantified using a Molecular Dynamics Personal Densitometer SI and ImageQuant (version 5.0) software (Molecular Dynamics, Sunnyvale, CA 94086, USA).

2.5. Confocal scanning laser microscopy

Confocal scanning laser microscopy (CSLM) was performed on a Leica TCS 4D confocal microscope (Leica Lasertechnik GmbH, Heidelberg, Germany) with a 100 mm oil immersion objective lens. CSLM was used in a

fluorescent mode using Fast Green CFC dye (Merck, Darmstadt, Germany). The excitation using an air-cooled Ar/Kr laser was performed at 488 nm. The sample was placed in a glass slide with cavity and a cover slip was placed on it. The glass slides where kept overnight at 4 °C, and the micrographs were taken the next day.

3. Results and discussion

3.1. SEC (Superdex 75)-MALLS experiments

Typical UV elution profiles of a 2% (w/w) WPI solution heated at 75 °C for 5, 15, 30 and 60 min on a Superdex 75 column are shown in Fig. 1(a). The unheated sample showed two major peaks at 10 and 12 ml, corresponding to β-lactoglobulin and α-lactalbumin, respectively. The RI elution profiles were similar to the UV profiles (data not shown), but the light-scattering (LS) profiles showed the presence of some large aggregates at about 7.0 ml (close to the void volume of the column) (Fig. 1(b)). The apparent M_w of β-lactoglobulin and α-lactalbumin, calculated from the combined RI and LS signals (Wyatt, 1992), were found to be ~30 and ~15 kDa, respectively, in agreement with the results of Schokker, Singh, and Creamer (2000). Upon heating at 75 °C, the quantities of native α-lactalbumin and β-lactoglobulin decreased with heating time and a broad aggregate peak, at elution volumes of 5–8 ml, emerged at longer heating times (Fig. 1(a)).

To determine the kinetics of heat-induced aggregation in more detail, WPI solutions were heated at temperatures between 65 and 80 °C for various times and analysed using SEC on a Superdex 75 column (Fig. 2). The quantities of both native α-lactalbumin and native β-lactoglobulin decreased with heating time at each temperature. As expected, the higher the temperature, the greater the loss of native proteins. Upon heating at 65 °C for up to 60 min, there was only a slight decrease in native β-lactoglobulin, but α-lactalbumin showed a considerable decrease under these conditions. At all temperatures and times used in this study, native α-lactalbumin, unexpectedly in view of its apparently unreactive nature, was found to disappear rather more rapidly than β-lactoglobulin from the heated WPI solution. This result is in agreement with the findings of Hollar, Parris, Hsieh, and Cockley (1995) and Havea et al. (1998) who reported greater denaturation of α-lactalbumin than β-lactoglobulin, at temperatures between 66 and 75 °C, in whey protein concentrates samples. Schokker et al. (2000) also found that when 1:1 mixtures of α-lactalbumin and β-lactoglobulin were heated at 75 °C, α-lactalbumin aggregated faster than β-lactoglobulin.

By selecting a suitable temperature–time condition, it is possible to study how the addition of κ-carrageenan

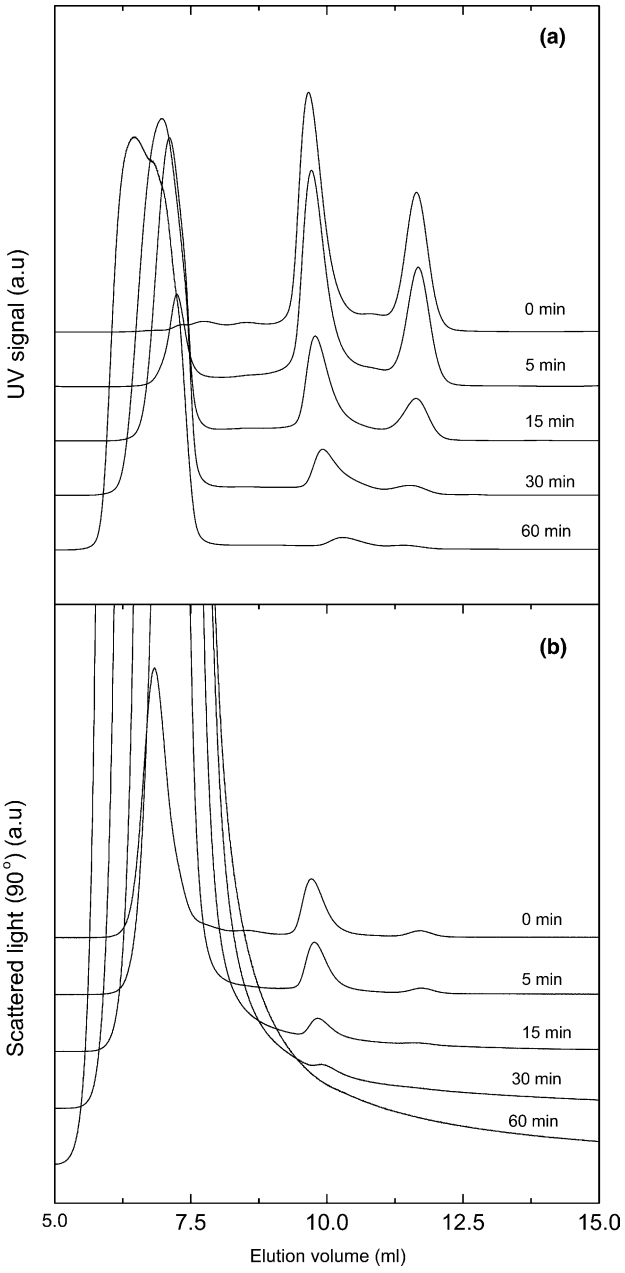

Fig. 1. Size-exclusion chromatography combined with multi-angle laser light scattering (Superdex 75). UV absorption (a) and light scattering at 90° (b) elution profiles of WPI solution heated for 0, 5, 15, 30 or 60 min at 75 °C.

influences the aggregation behaviour of α-lactalbumin and β-lactoglobulin in heated WPI solutions. We selected 75 °C for up to 15 min, because it gave kinetics on a suitable time scale for the experiments and it is above the denaturation temperatures of both β-lactoglobulin and α-lactalbumin. All the subsequent experiments were carried out under these conditions. Addition of κ-carrageenan between 0% and 0.15% to the WPI solution had no significant effect on the rate of loss of native β-lactoglobulin and α-lactalbumin during heat treatment

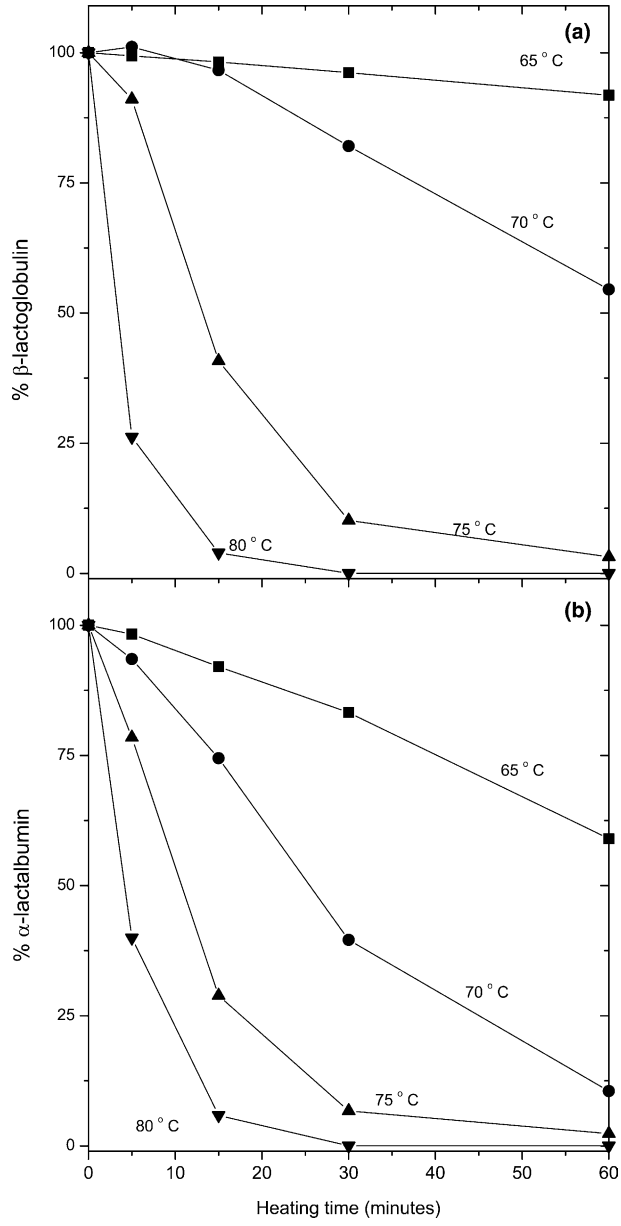

Fig. 2. Amounts of native β-lactoglobulin (a) and α-lactoglobulin (b) in heated 2% WPI solution as a function of heating time at 65 (■), 70 (●), 75 (▲) 80 °C (▼) (determined from SEC, Superdex 75, results).

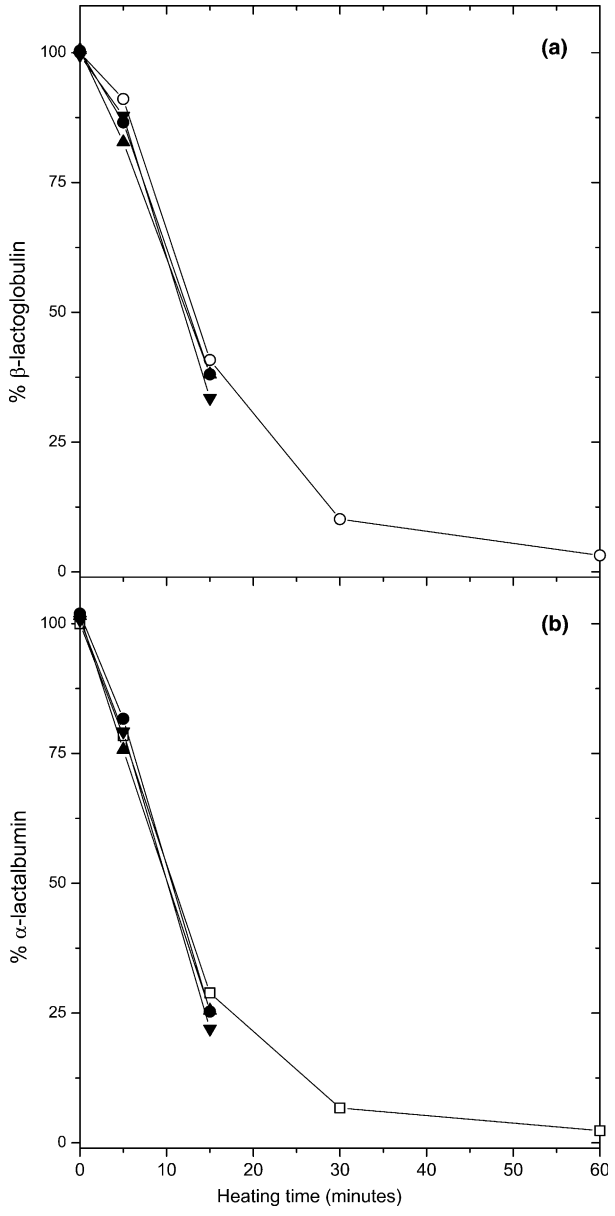

Fig. 3. Amounts of native β-lactoglobulin (a) and α-lactalbumin (b) in heated mixture of 2% WPI and 0 (○), 0.05 (▲), 0.10 (●) and 0.15 (▼) κ-carrageenan as a function of heating time at 75 °C (determined from SEC, Superdex 75, results).

at 75 °C (Fig. 3). Experiments conducted at 65 °C showed no effect of added κ-carrageenan on the rate of loss of these proteins (data not shown).

Previous studies (Capron et al., 1999; Croguennoc et al., 2001a, 2001b) have demonstrated that the addition of κ-carrageenan to pure β-lactoglobulin solutions does not affect the rate of loss of native protein. DSC measurements showed that the denaturation temperature of β-lactoglobulin is not modified by the presence of κ-carrageenan (Ould Eleya & Turgeon, 2000). From our results, it appears that the behaviour of β-lactoglobulin in the heated WPI solution was similar to that observed in pure protein solutions.

The UV elution profiles of heated mixtures of WPI and κ-carrageenan are shown in Fig. 4. It was observed that the shape of the protein aggregate peak, present at 7 ml, in heated WPI sample, was modified considerably when κ-carrageenan was added before heat treatment. The aggregate peak became broader and shifted to higher elution volumes with increasing concentration of added κ-carrageenan, indicating greater polydispersity and smaller size of "soluble" aggregates in the heated mixtures.

Unfortunately, due to the exclusion limit of the Superdex 75 column, it was not possible to separate the

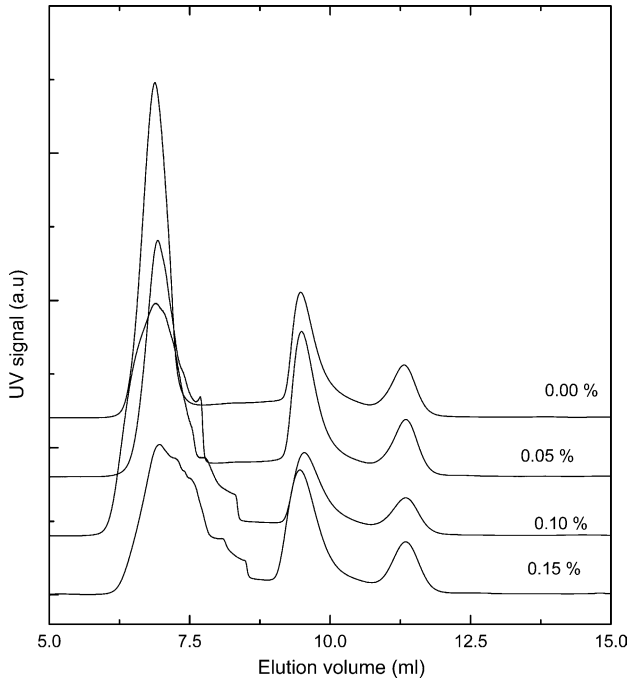

Fig. 4. UV absorption elution profiles (Superdex 75) of mixtures of 2% WPI and 0%, 0.05%, 0.10% or 0.15% κ-carrageenan heated at 75 °C for 15 min.

various intermediates and aggregates. Because the scattered intensity of the aggregates was very high, beyond the linear range of MALLS detectors, it was not possible to know, in detail, the molar masses of the different aggregates present in the heated mixtures. To better understand the characteristics of these intermediates and aggregates, the Shodex column with an exclusion limit of 20,000 kDa, was also used to separate different aggregates.

3.2. SEC (Shodex)-MALLS experiments

The DRI and LS elution profiles of heated and unheated κ-carrageenan are shown in Fig. 5. Both heated and unheated κ-carrageenan eluted in the same position with a broad peak between 7 and 9 ml. The unheated sample had a slightly lower maximum DRI signal compared to the heated samples. The estimated M_w of heated and unheated κ-carrageenan was in the range from 100 to 1500 kDa.

Unheated WPI eluted as a single protein peak (shown as the UV signal) between 9.5 and 10.5 ml (Fig. 6(a)). The estimated M_w of this peak from LS was 34 kDa. Upon heating, this peak decreased in size and a broad peak, representing large aggregates, also appeared; this peak increased gradually in size and shifted to slightly lower elution volumes with increase in heating time. These aggregates were clearly detected by LS (Fig. 6(b)). After heating for 1 min, this peak represented aggregates ranging in M_w from 500 to 2000 kDa; further increase in

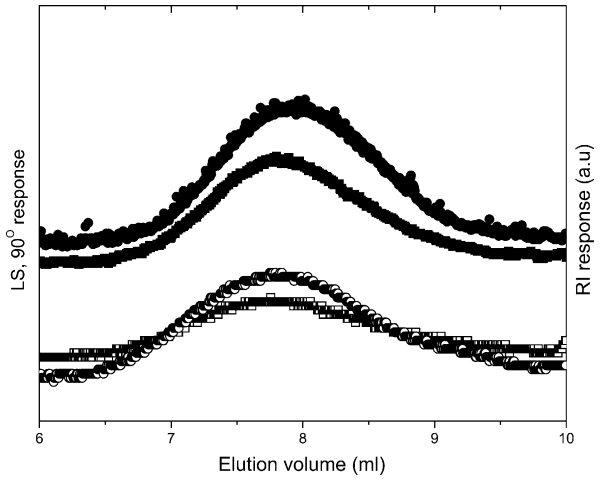

Fig. 5. DRI and light scattering at 90 °C elution profiles of 0.10% unheated (■) or heated (75 °C for 15 min) κ-carrageenan solution (●). Solid symbols are for light scattering and open symbols for DRI.

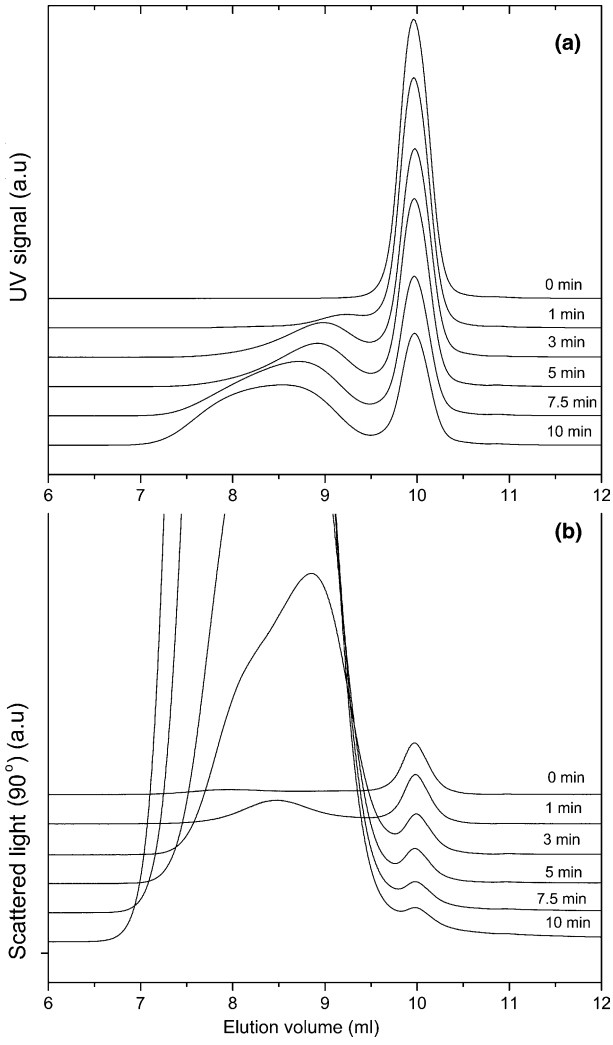

Fig. 6. Size exclusion chromatography (Shodex), combined with multi-angle laser light scattering: UV absorption (a) and light scattering at 90° (b) elution profiles of WPI solution heated at 75 °C for 0, 1, 3, 5, 7.5 and 10 min.

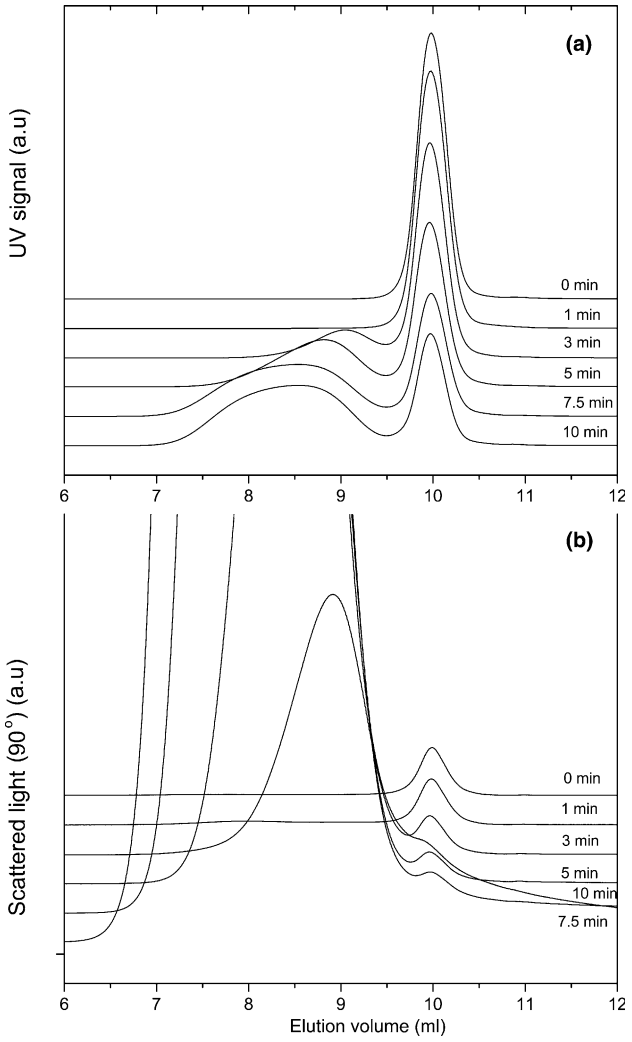

Fig. 7. Size exclusion chromatography (Shodex) combined with multi-angle laser light scattering: UV absorption (a) and light scattering at 90° (b) elution profiles of WPI (2%) and κ-carrageenan (0.1%) mixture solution heated at 75 °C for 0, 1, 3, 5, 7.5 and 10 min.

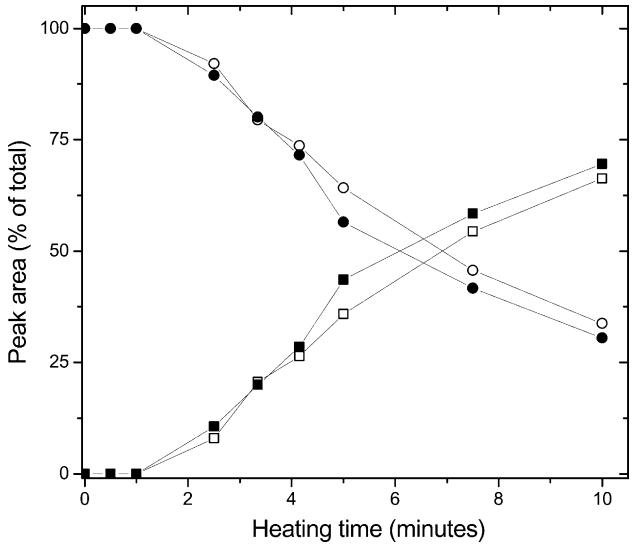

Fig. 8. Areas of aggregate (□, ■) and monomeric protein peaks (○, ●) as a function of heating time at 75 °C. WPI heated alone (○, □) and heated WPI/κ-carrageenan mixture solution (●, ■).

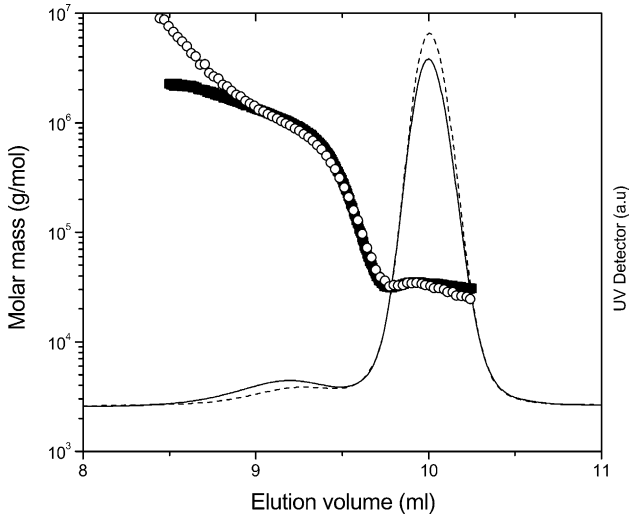

Fig. 9. Estimated molar mass profile of WPI solution (●) or 2% WPI and 0.01% κ-carrageenan solution (○) heated at 75 °C for 5 min. UV absorption profiles for heated WPI (——) and the heated mixture (- - - -) are included.

heating time resulted in aggregates with higher M_w values.

The UV and LS elution profiles of heated WPI (2%)/κ-carrageenan (0.1%) mixtures were qualitatively similar to that of the WPI heated alone (Figs. 7(a) and (b)). However, the aggregate peak in the mixture appeared to be slightly smaller in the early stages of heating, but became considerably broader in the later stages of heating compared with WPI solution heated alone. For both WPI and WPI/κ-carrageenan mixtures, the area of aggregate peak increased with increase in heating time, with a corresponding decrease in the area of the peak representing monomeric proteins (Fig. 8). After heating for $\geqslant 5$ min, the proportion of aggregates in heated mixtures appears to be slightly greater than for WPI heated alone.

A typical molar mass distribution plot is shown in Fig. 9, and the estimated M_w of the aggregate peaks at different heating times are shown in Table 1. In WPI solutions heated in the absence of κ-carrageenan, large aggregates with M_w 2–3 $\times 10^6$ Da were formed, and the aggregate size did not appear to change much with heating time. In contrast, the M_w of the aggregates in WPI/κ-carrageenan mixtures were lower for heating times up to 2.5 min, but were similar to the WPI solutions heated alone at longer heating times.

3.3. Electrophoresis experiments

Mixtures of WPI (2%)/κ-carrageenan (0.1%) and WPI (2%) solutions were heated at 75 °C and analysed

Table 1
Estimated molecular weight (from Shodex column) of aggregates formed during heat treatment of WPI solutions or WPI/κ-carrageenan mixtures

Heating time at 75 °C (min)	WPI (2%) (g/mol)	WPI (2%)/ κ-carrageenan (0.01%) (g/mol)
0.5	2.28×10^6	4.48×10^5
1	2.88×10^6	4.20×10^5
2.5	3.20×10^6	7.85×10^5
3.3	1.81×10^6	1.52×10^6
5	3.30×10^6	3.75×10^6

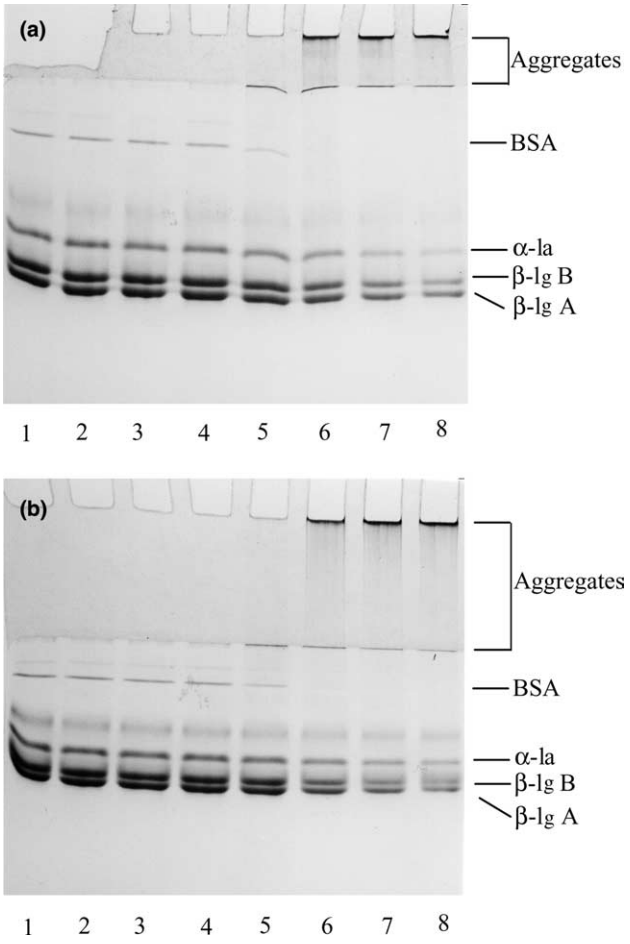

Fig. 10. Alkaline native-PAGE of (a) WPI (2%) solution heated at 75 °C (b) WPI (2%) and κ-carrageenan (0.1%) mixture heated at 75 °C for 0 (lane 1), 0.5 (lane 2), 1 (lane 3), 2 (lane 4), 5 (lane 5), 7 (lane 6), 10 (lane7) or 15 min (lane 8).

using alkaline PAGE (Fig. 10) and SDS–PAGE (Fig. 11). The unheated samples separated into four major protein bands containing native β-lactoglobulin A, β-lactoglobulin B, α-lactalbumin and bovine serum albumin (BSA), respectively. Heating at 75 °C resulted in a decrease in these protein bands; BSA disappeared first, followed by α-lactalbumin and then β-lactoglobulin, essentially confirming the SE-HPLC results. The

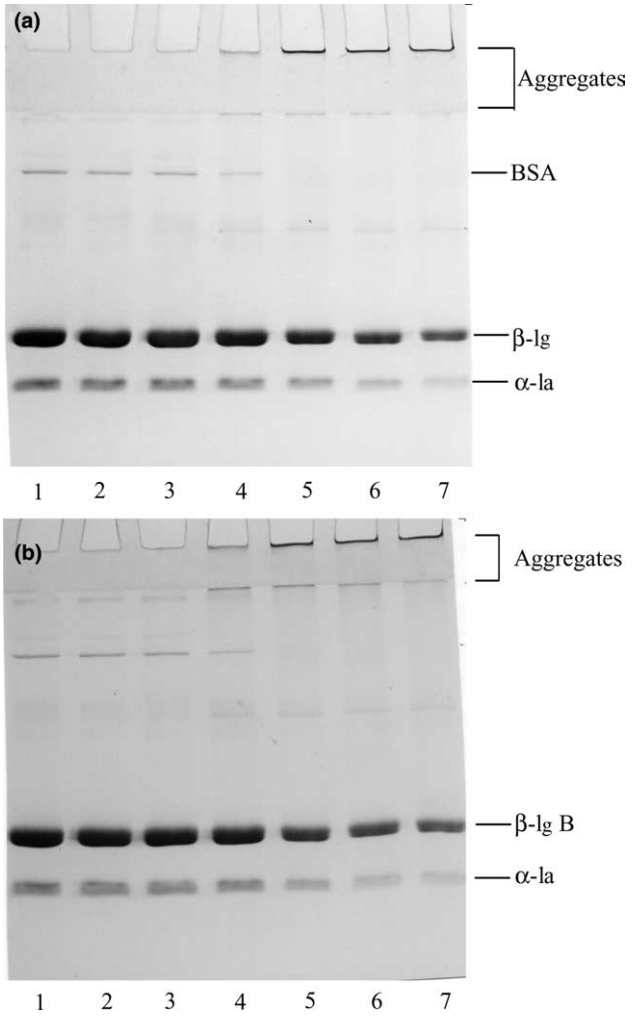

Fig. 11. SDS–PAGE (non-reduced) of (a) WPI (2%) solution heated at 75 °C (b) WPI (2%) and κ-carrageenan (0.1%) mixture heated at 75 °C for 0 (lane 1), 0.5 (lane 2), 1 (lane 3), 2 (lane 4), 5 (lane 5), 7 (lane 6), 10 (lane7) or 15 min (lane 8).

heat treatment also resulted in the formation of aggregates with lower electrophoretic mobilities and the large aggregates could not enter the stacking gel. There were no obvious differences between the native PAGE patterns of heated WPI/κ-carrageenan mixtures and WPI solutions without κ-carrageenan.

In the SDS–PAGE, the non-reduced, unheated samples separated into three major bands, containing α-lactalbumin, β-lactoglobulin and BSA, respectively. As expected, heating decreased the intensity of the major protein bands, and increased the proportion of protein aggregates that failed to migrate into the resolving stacking gel. In addition to the native proteins, the monomeric protein bands observed in this PAGE system included those protein molecules that were dissociated from aggregates by SDS (i.e., non-covalently bonded to the aggregates) (Gezimati, Singh, & Creamer, 1996). The rate of disappearance of SDS-monomeric

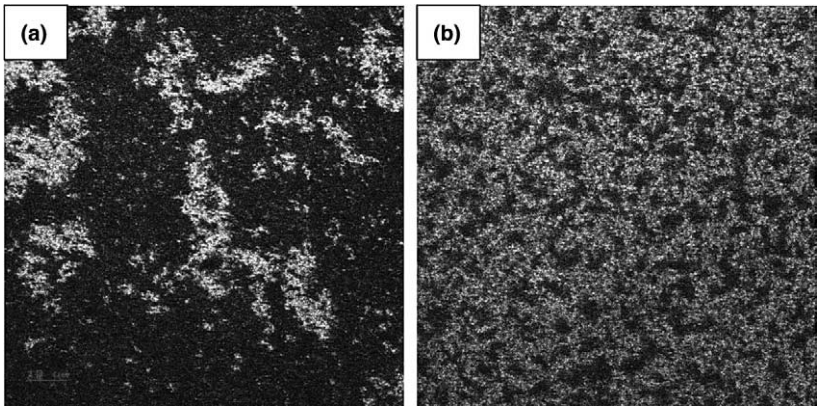

Fig. 12. Confocal laser micrographs of WPI (2%) solution (a) and WPI (2%)/κ-carrageenan (0.1%) mixture (b) heated at 75 °C for 15 min.

β-lactoglobulin and α-lactalbumin (from SDS–PAGE) was lower than that of native β-lactoglobulin and α-lactalbumin (from Native-PAGE), indicating that some of the aggregates were held together by non-covalent bonds. However, the rates of disappearance of SDS-monomeric and native proteins were similar in the presence or absence of κ-carrageenan.

3.4. Microstructures

The microstructures of WPI (2%) and WPI (2%)/κ-carrageenan (0.1%) mixtures heated at 75 °C were determined using CSLM. WPI samples heated in the absence of κ-carrageenan at 75 °C for up to 5 min remained homogeneous. Samples heated for longer times showed large clusters of protein aggregates on the length scale of tens of micrometers (Fig. 12(a)). WPI/κ-carrageenan mixtures heated under the same conditions showed somewhat smaller clusters and more uniform pore size distributions (Fig. 12(b)).

Both the SEC and PAGE results confirmed that the presence of κ-carrageenan during heat treatment of WPI solutions at 75 °C did not significantly influence the loss of native β-lactoglobulin and α-lactalbumin under the conditions used. LS results indicated that the M_w of the whey protein aggregates formed in the early stages of heating in the presence of κ-carrageenan were smaller than the aggregates formed in WPI solutions heated in the absence of κ-carrageenan. This may be related to the increase in the viscosity of the system with the addition of polysaccharide, which would slow down the growth of the aggregates. These findings could be compared with the observations made on pure β-lactoglobulin and κ-carrageenan heated solutions (Capron et al., 1999), which showed that the initial aggregation of β-lactoglobulin into small aggregates was not affected by the presence of κ-carrageenan, but further aggregation of small aggregates into large, fractal particles was accelerated by κ-carrageenan. Croguennoc et al. (2001a, 2001b) reported that the presence of κ-carrageenan accelerated the growth of β-lactoglobulin aggregates, but the structure of the aggregates was not modified. These effects could be ascribed mainly to volume exclusion effects, originating from incompatibility between unlike biopolymers. When the β-lactoglobulin aggregates reach a certain size, flocculation of aggregates may occur by a depletion mechanism, caused by the κ-carrageenan molecules. This phenomenon could lead to an increase in local concentration of aggregates and consequently to greater interactions between the protein aggregates. In these studies, κ-carrageenan and β-lactoglobulin mixtures were heated at relatively low temperatures (65–70 °C) for very long times. It is not clear whether similar changes would occur at higher temperatures (i.e., well above the denaturation temperature of β-lactoglobulin). If the aggregation is very fast, phase separation may not fully develop. In the complex mixture of whey proteins, such as WPI used in our study, there was no evidence of phase separation, as rapid cooling of heated WPI/κ-carrageenan solutions did not result in macroscopic phase separation. Confocal microscopy showed no evidence of phase separation in these mixtures.

Acknowledgements

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References

Bourriot, S., Garnier, C., & Doublier, J. L. (1999). Micellar-casein-kappa-carrageenan mixtures. I. Phase separation and ultrastructure. *Carbohydrate Polymers, 40*, 145–157.

Boye, J. I., Kalab, M., Alli, I., & Ma, C. Y. (2000). Microstructural properties of heat-set whey protein gels: Effect of pH. *Lebensmittel Wissenschaft und Technology, 33*, 165–172.

Capron, I., Nicolai, T., & Durand, D. (1999). Heat induced aggregation and gelation of β-lactoglobulin in the presence of κ-carrageenan. *Food Hydrocolloids, 13*, 1–5.

Croguennoc, P., Durand, D., Nicolas, T., & Clark, A. (2001a). Phase separation and association of globular protein aggregates in the presence of polysaccharides. 1. Mixtures of preheated β-lactoglobulin and κ-carrageenan at room temperature. *Langmuir, 17*, 4372–4379.

Croguennoc, P., Durand, D., Nicolas, T., & Clark, A. (2001b). Phase separation and association of globular protein aggregates in the presence of polysaccharides. 2. Heated mixtures of native β-lactoglobulin and κ-carrageenan. *Langmuir, 17*, 4380–4385.

Fernandes, P. B. (1998). Interactions in whey protein polysaccharide mixtures at pH 7. In R. H. Walters (Ed.), *Polysaccharides Association Structures in Food* (pp. 257–261). New York: Marcel Dekker Inc.

Gezimati, J., Singh, H., & Creamer, L. K. (1996). Heat-induced interactions and gelation of mixtures of bovine β-lactoglobulin and serum albumin. *Journal of Agricultural and Food Chemistry, 45*, 1130–1136.

Havea, P., Singh, H., Creamer, L. K., & Campanella, O. H. (1998). Electrophoretic characterisation of the protein products formed during heat treatment of whey protein solutions. *Journal of Dairy Research, 65*, 79–91.

Hidalgo, J., & Hansen, P. T. M. (1969). Interactions between food stabilizers and β-lactoglobulin. *Journal of Agricultural and Food Chemistry, 17*, 1089–1992.

Hoffman, M. AM., Sala, G., Olieman, C., & De Kruif, K. (1998). Molecular mass distributions of heat-induced β-lactoglobulin aggregates. *Journal of Agricultural and Food Chemistry, 45*, 2949–2957.

Hoffman, M. AM., & Van Mil, P. J. J. M. (1999). Heat-induced aggregation of β-lactoglobulin as a function of pH. *Journal of Agricultural and Food Chemistry, 47*, 1898–1905.

Hollar, C. M., Parris, N., Hsieh, A., & Cockley, K. D. (1995). Factors affecting the denaturation and aggregation of whey proteins in heated whey protein concentrate mixtures. *Journal of Dairy Science, 78*, 260–267.

Mleko, S., & Foegeding, E. A. (2000). pH-induced aggregation and weak gel formation of whey protein polymers. *Journal of Food Science, 65*, 139–143.

Mleko, S., Li-Chan, E. C. Y., & Pikus, S. (1998). Interactions of κ-carrageenan with whey proteins in gels formed at different pH. *Food Research International, 6*, 427–433.

Mora-Gutierrez, A., Kumosinski, T., & Farrell, H. M., Jr. (1998). κ-Carrageenan interaction with bovine and caprine caseins as shown by sedimentation and nuclear magnetic resonance spectroscopic techniques. *Journal of Agricultural and Food Chemistry, 46*, 4987–4996.

Ould Eleya, M. M., & Turgeon, S. L. (2000). Rheology of κ-carrageenan and β-lactoglobulin mixed gels. *Food Hydrocolloids, 14*, 29–40.

Schokker, E. P., Singh, H., & Creamer, L. K. (2000). Heat-induced aggregation of β-lactoglobulin A and B with α-lactalbumin. *International Dairy Journal, 10*, 791–800.

Schokker, E. P., Singh, H., Pinder, D. N., Norris, G. E., & Creamer, L. K. (1999). Characterisation of intermediates formed during heat-induced aggregation of β-lactoglobulin AB at neutral pH. *International Dairy Journal, 9*, 791–800.

Snoeren, T. H. M., Payens, T. A. J., Jeunink, J., & Both, P. (1975). Electrostatic interactions between κ-carrageenan and κ-casein. *Milchwissenschaft, 30*, 393–396.

Xiong, Y. L. (1992). Influence of pH and ionic environment on thermal aggregation of whey proteins. *Journal of Agricultural and Food Chemistry, 40*, 380–384.

Wyatt, P. J. (1992). Combined differential light scattering with various liquid chromatographic separation techniques. In S. E. Harding, D. B. Satelle, & Bloomfield (Eds.), *Laser light scattering in biopolymers* (pp. 35–58). Cambridge: Royal Society of Chemistry.