

# Effect of the addition of CMC on the aggregation behaviour of proteins

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## Abstract

The effect of carboxymethylcellulose (CMC) on the aggregation of formulation based on calcium caseinate, commercial whey protein (WPC), and a 1:1 mixture of soy protein isolate (SPI) and whey protein isolate (WPI) was investigated. Protein aggregation could be observed upon addition of CMC, as demonstrated by size-exclusion chromatography. This aggregation behaviour was enhanced by means of physical treatments, such as heating at 90°C for 30 min or gamma-irradiation at 32 kGy. A synergy resulted from the combination of CMC to gamma-irradiation in Caseinate/CMC and SPI/WPI/CMC formulations. Furthermore, CMC prevented precipitation in irradiated protein solutions for a period of more than 3 months at 4°C.

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## 1. Introduction

The last decade has been witnessed of several works devoted to the development of biodegradable edible films (Kester and Fennema, 1986; Krochta and De Mulder-Johnston, 1997). Polymers derived from natural monomers, like milk and soy proteins, offer the greatest opportunities since their biodegradability and environmental compatibility are assured. The challenge for the successful use of biodegradable polymer products lies in achieving a controlled lifetime. Milk and soy proteins can be formulated into edible coatings for improving food shelf life or made into biodegradable films (Lacroix

et al., 2002; Ouattara et al., 2002; Vachon et al., 2000). The highly hydrophilic nature of the proteins limits however, their ability to provide desired edible films functions. Previous works reported that  $\gamma$ -irradiation produce protein cross-links and reduced the solubility of the protein-based films (Brault et al., 1997; Mezgheni et al., 1998; Ressouany et al., 1998; Lacroix et al., 1998; Vachon et al., 2000; Le Tien et al., 2000; Letendre et al., 2002). Sabato et al. (2001) have demonstrated that the addition of carboxymethylcellulose (CMC) to soy protein isolate (SPI) and to a mixture of SPI:whey protein isolate (WPI) resulted in an improvement of the mechanical and barrier properties. These findings motivated us to investigate further the benefit of CMC with several protein systems, in combination to physical treatments such as heating and  $\gamma$ -irradiation. Among the systems investigated, there are commercial whey protein (WPC), caseinate and a 1:1 mixture of SPI and WPI.

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## 2. Material and methods

### 2.1. Formulation preparation

Soy protein isolate (Dupont Campbell Protein Technologies, St-Louis, MO, USA) and WPI (Food Research and Development Centre, St-Hyacinthe, Quebec, Canada) produced from permeate obtained by tangential membrane microfiltration (St-Gelais et al., 1995) were solubilised in distilled water, under stirring, at 90°C for 30 min, to obtain an SPI/WPI ratio of (1/1), with a total protein concentration of 5% (w/v) in the film forming solution. The pH was adjusted at 8.5 with 1 M Na<sub>2</sub>CO<sub>3</sub>. CMC (Sigma Chemicals Co., St-Louis, MO, USA), 0.25% (w/v) was added. WPC (Saputo Cheeses Ltd., Montreal, Qc, Canada) and caseinate (New Zealand Milk Products Inc., Santa Rosa, CA, USA) formulations were obtained as described above. After complete solubilisation, the solution was degassed under vacuum to remove dissolved air (Brault et al., 1997). Heating treatment was done at 90°C for 30 min under agitation. Irradiation of the solution was done at the Canadian Irradiation Centre (CIC) at a dose of 32 kGy and a mean dose rate of 31.24 kGy/h, using a <sup>60</sup>Co source UC-15A (MDS-Nordion Int. Inc., Kanata, Ont., Canada).

### 2.2. Size-exclusion chromatography

Size-exclusion chromatography was performed using a Varian Vista 5500 HPLC. Detection of the protein

solution was done using a standard UV detector set at 280 nm. Two Supelco Progel TSK PWH and GMPW columns followed by two Waters Hydrogel columns (2000 and 500) were used for the molecular weight determination of the cross-linked proteins. The total molecular weight (MW) exclusion limit was  $25 \times 10^6$  Da based on linear polyethylene glycol (PEG). The eluant (80% v/v aqueous and 20% v/v acetonitrile) was flushed through the columns at a flow rate of 0.8 ml/min. The aqueous portion of the eluant was 0.02 M tris buffer (pH = 8) and 0.1 M NaCl. The MW calibration curve was established using a series of protein MW markers (Sigma, MW-GF-1000, USA) ranging from  $2 \times 10^6$  Da to 29 kDa. All soluble protein solutions (0.5% w/v) were filtered on 0.45 μm prior to injection.

## 3. Results and discussion

### 3.1. Wpc

The addition of CMC to WPC resulted in a significant shift of the MW toward higher values (Fig. 1). Indeed, a shift (20-fold) of the main Mw peak could be observed from 10 to 200 kDa with a shoulder at 10 kDa (Fig. 1). This enhancement of the MW is attributed to the complex formed between the CMC and WPC. The protein with and without CMC is presented in Fig. 1b. In both cases, that are in the presence and in the absence of CMC, the SEC chromatograms exhibit a bimodal pattern (Fig. 1b). In presence of CMC the aggregation

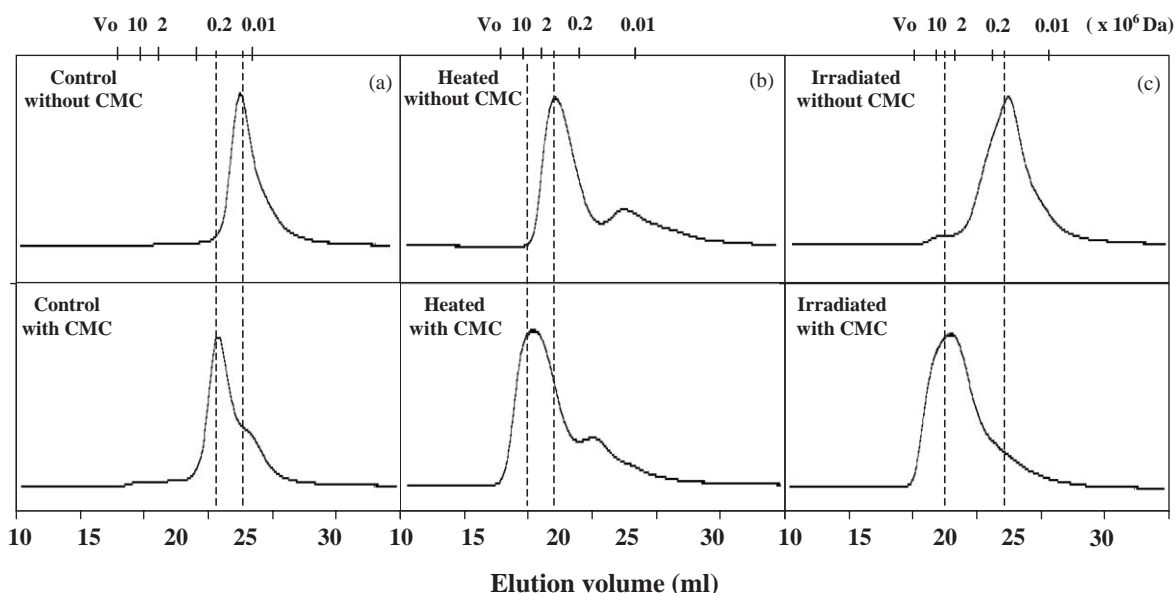


Fig. 1. Effect of carboxymethylcellulose (CMC) on the aggregation of commercial whey protein (WPC): (a) elution pattern of WPC (Control) and WPC/CMC formulation; (b) elution pattern of WPC and WPC/CMC following heating at 90°C for 30 min; (c) elution pattern of WPC and WPC/CMC following irradiation at 32 kGy.

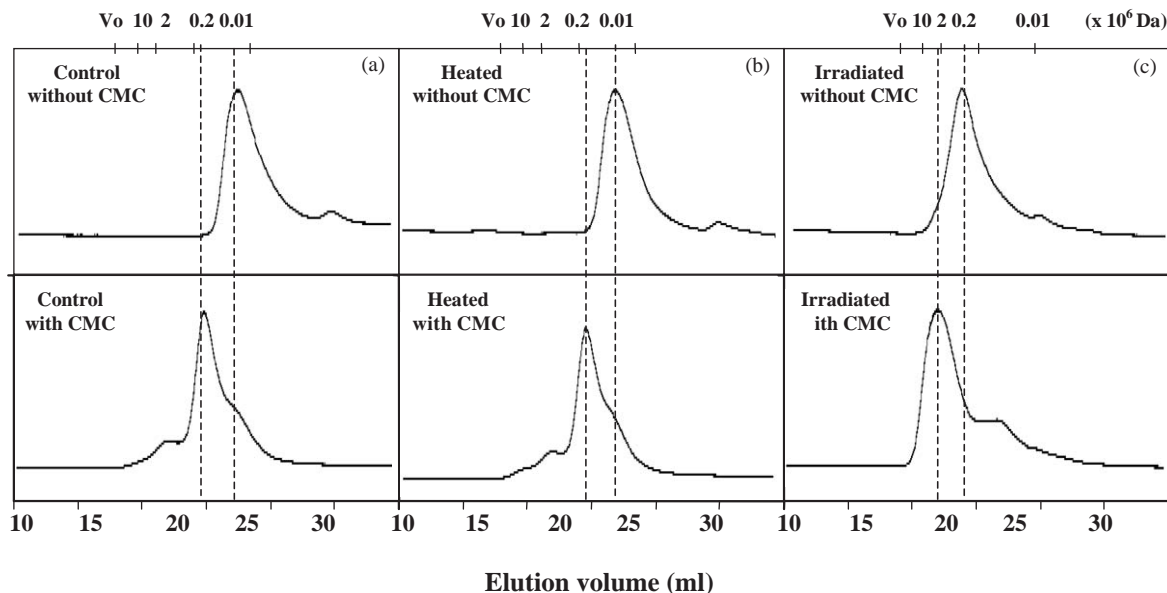


Fig. 2. Effect of carboxymethylcellulose (CMC) on the aggregation of calcium-caseinate: (a) elution pattern of caseinate (Control) and caseinate/CMC formulation; (b) elution pattern of caseinate (Control) and caseinate/CMC following heating at 90°C for 30 min; (c) elution pattern of Caseinate (control) and caseinate/CMC following irradiation at 32 kGy.

of heated WPC was even more important, as inferred from the improvement of 5-fold of the main peak in the SEC elution pattern: ca.  $10 \times 10^3$  vs.  $2 \times 10^3$  kDa (Fig. 1b), and 20-fold for the peak of unheated WPC: ca.  $0.2 \times 10^3$  vs.  $0.01 \times 10^3$  kDa (Fig. 1a). The chromatogram obtained upon irradiating the WPC/CMC formulation (Fig. 1c) shows some similarities with the elution pattern resulting from heated WPC/CMC formulation (Fig. 1b).

### 3.2. Caseinate

As observed with WPI, the presence of CMC in a formulation constituted of caseinate affected significantly the aggregation of the protein as confirmed by the SEC elution pattern (Fig. 2a). In absence of CMC, a main peak corresponding to a MW of 10 kDa could be observed, while the addition of CMC generated a more complex elution pattern with a main peak at 200 kDa and two shoulders, one at 10 kDa and the other at  $2 \times 10^3$  kDa respectively (Fig. 2a). Heat treatment did not have any significant influence on the generation of cross-links and, therefore, on the protein aggregation (Fig. 2b) as compared to the control formulation (Fig. 2a). However, when caseinate formulation was irradiated,  $\gamma$ -irradiation improved significantly the MW of the caseinate, from ca. 10 to 200 kDa, and Caseinate/CMC system, from ca. 200 to  $2 \times 10^3$  kDa (Fig. 2a,c).

### 3.3. Spilwpi mixture

When SPI was mixed to WPI, the elution pattern exhibited a main peak at ca. 200 kDa, with a shoulder at ca.  $2 \times 10^3$  kDa, independently of the presence or the absence of CMC (Fig. 3a). Irradiation in combination with heating treatment enhanced the protein aggregation, as confirmed by the shift of low MW peak from 200 to ca.  $1 \times 10^3$  kDa (Fig. 3b).

## 4. Discussion

CMC is known to form charge–charge electrostatic complexes with proteins (Imeson et al., 1977). The interaction between the polysaccharide and the protein likely involved the negatively charged carboxyl group in the CMC and the positively charged side chains of the amino acids in the WPC (Ledward, 1979). Calcium ions are also known to favour electrostatic interactions between polypeptide chains (Hongsprabhas and Barbut, 1997), which contribute to a more dense structure of the proteins. A similar behaviour was reported in gels and films based on caseinate, plasticizers, and salt (Mezgheni et al., 1998). It is well known that heating generate stable disulphide bridges within lactoserum proteins (Bernal et al., 1987; Voet and Voet, 1990), resulting in the formation of a three-dimensional network (Gennadios

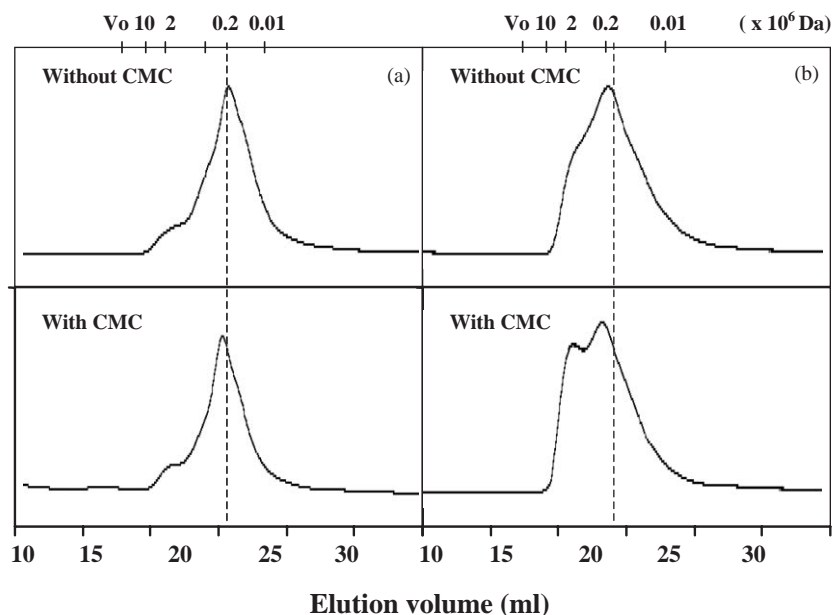


Fig. 3. Effect of carboxymethylcellulose (CMC) on the aggregation of 1:1 mixture of soy protein isolate (SPI) and whey protein isolate (WPI): (a) elution pattern of SPI/WPI (control) and SPI/WPI/CMC formulation; (b) elution pattern of SPI/WPI and SPI/WPI/CMC following heating at 90°C for 30 min and irradiation at 32 kGy.

and Weller, 1991). Previous investigations have demonstrated that the irradiation of protein solution resulted in the formation of bityrosine (Brault et al., 1997; Ressouany et al., 1998; Mezgheni et al., 1998; Lacroix et al., 1998; Vachon et al., 1999). However, in this study, it was rather believed that  $\gamma$ -irradiation would not affect the structure of WPC, since the tyrosine residues in WPC is quite low, 2.5% (Stuchell and Krochta, 1994). This assumption was further confirmed by the slight increase of the Mw of the irradiated formulation without CMC: 20-fold after the irradiation treatment vs. 200-fold after the heating treatment (Fig. 1c). However, the presence of CMC had a major impact on the aggregation of WPC, as confirmed by the enhancement of the MW main peak (Fig. 1c):  $10 \times 10^3$  kDa after the irradiation treatment in the presence of CMC vs.  $0.2 \times 10^3$  kDa after the irradiation treatment without CMC. Similar results obtained with the elution pattern of heated and irradiated WPC/CMC formulation suggest that CMC interact with WPI in such a way that it compensates for the lack of effect coming from the irradiation treatment. The Mw enhancement observed in caseinate MW in presence of CMC is assigned to the formation of the electrostatic complex between the both compounds.  $\gamma$ -irradiation induce the formation of bityrosine, responsible for the Mw shift toward higher values. Also, SPI contains more tyrosine residues than WPC, 3.3% vs. 2.5%, respectively (Stuchell and Krochta, 1994). Moreover, CMC seemed to interact with the protein mixture in

such a way to enhance the aggregation. It is assumed that upon  $\gamma$ -irradiation SPI and WPI orient themselves in a systematic fashion to favour the electrostatic interaction of CMC with both of them yielding thus, to a more complex three-dimensional network or aggregate with a higher molecular weight. The impact of CMC could be also attributed to its characteristic to form electrostatic complexes with proteins (Shih, 1994). However, the effect of CMC can also be explain by one of the basic function of CMC that is to impart viscosity to the aqueous phase, thereby stabilising the other ingredients.

## 5. Conclusions

Our results showed that  $\gamma$ -irradiation treatment and the addition of CMC to proteins, enhance their aggregation. A synergistic effect was noted between the addition of CMC and  $\gamma$ -irradiation on the protein aggregation. Moreover, the addition of CMC in protein media, seem to prevent proteins precipitation during storage (3 months at 4°C).

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