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# Isothermal Calorimetry Study of Calcium Caseinate and Whey Protein Isolate Edible Films Cross-Linked by Heating and $\gamma$-Irradiation

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The contribution of thermal and radiative treatments as well as the presence of some excipients, namely glycerol, carboxymethylcellulose (CMC), pectin, and agar, on the formation of protein−protein interactions as well as the formation and loss of protein−water interactions was investigated by means of differential scanning calorimetry in an isothermal mode. Protein−water interactions were assessed through measurement of the heat of the wetting parameter. Isothermal calorimetry measurements pointed out that $\gamma$-irradiation does not favor protein−water interactions, as reflected by its endothermic contribution ($P \leq 0.05$) to the heat of wetting values. Although significant ($P \leq 0.05$), the effect of the thermal treatment on endothermic responses using isothermal calorimetry was found to be somewhat lower. Among excipients added to biofilm formulations, glycerol generated the most important losses of protein−water interactions, as inferred by its significant ($P \leq 0.05$) endothermic impact on the heat of wetting values.

**KEYWORDS: Milk protein; isothermal calorimetry; heat of wetting; cross-links; glycerol; carboxymethylcellulose; polysaccharides; edible films**

## INTRODUCTION

Increased consumer demand for both higher quality and longer shelf life foods in combination with environmental needs for reduction of disposable packaging amounts have led to an increased interest in edible film research (*1*). Among the films investigated, edible films based on proteins showed the best mechanical properties (*2*). Moreover, the biological nature of protein-based films confers biodegradability properties and environmental compatibility (*3*).

Biofilms obtained from heating and/or $\gamma$-irradiating milk proteins have been extensively studied and have shown promising results. These investigations have demonstrated that heating and/or $\gamma$-irradiating were responsible for cross-linking the proteins and improving the mechanical stability, the resistance to proteolysis, and the water vapor barrier (*4*−*7*). Moreover, a recent microstructural study revealed a direct correlation between mechanical properties and film porosity (*8*). Likewise, according to a further work (*9*), protein conformation is related to the protein cross-linking and seems also to play a major role in the films' properties. However, none of the works reported so far have investigated existing interactions between constituents of biofilms, at the molecular level.

Differential scanning calorimetry (DSC) is a thermoanalytical technique that has been widely used to monitor changes as well as thermodynamic properties of proteins (*10*−*13*). A recent isothermal calorimetric investigation pointed out the impact of heat on amylose−water interactions through the measurement of the heat of wetting (*14*). The heat of wetting is a thermodynamic property that reflects the number of substrate−solvent interactions. Thus, the formation of protein−protein interactions as well as the loss of protein−water interactions could be monitored by measuring the heat of wetting. A previous report concluded that the formation of a network induced by thermal treatment results essentially from protein−protein and protein−solvent interactions (*15*). Such phenomena, such as the formation and loss of protein−water interactions, are assumed to occur in both calcium caseinate and WPI upon heating and/or $\gamma$-irradiation treatments. Furthermore, the biofilm excipients are also expected to influence protein−water interactions. Therefore, an isothermal calorimetric study was undertaken with the aim of rationalizing the contribution of excipients within biofilms, namely glycerol, caroboxymethylcellulose, pectin, and agar, as well as the impact of both heat and $\gamma$−irradiation treatments, at the molecular level.

## MATERIALS AND METHODS

**Materials.** Calcium caseinate (CC) was provided by New Zealand Milk Products Inc. (Santa Rosa, CA). Commercial whey protein concentrate (WPC) (Sapro-75, 76.27% w/w protein) was purchased from Saputo Cheeses Ltd. (Montreal, Quebec, Canada). Whey protein

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**Table 1.** Composition of Calcium Caseinate and Whey Protein Isolate

| | protein (%) | ash (%) | fat (%) | lactose (%) |
|---|---|---|---|---|
| calcium caseinate[a] | 91.8 | 3.8 | 0.7 | 0.1 |
| whey protein isolate[b] | 89.9 | 2.15 | traces | traces |

[a] Technical sheet, New Zealand Milk Protein Inc., Santa Rosa, CA, 1999. [b] Centre de recherche et de développement sur les aliments, Agriculture et agro-alimentaire Canada, Saint-Hyacinthe, Québec, Canada, 1999.

isolate (WPI; 90.57% w/w protein) was prepared at the Centre de Recherche et de Développement sur les Aliments (Saint-Hyacinthe, Québec, Canada). WPI was produced from permeate obtained by tangential membrane microfiltration. Fresh skim milk was microfiltered 3-fold at 50 °C using an MF pilot cross-flow unit as described previously by St-Gelais et al. (*16*). The proteins contained in the permeate were concentrated 25-fold at 50 °C using a UF pilot unit equipped with a Romicon membrane (PM 10, total surface area 1.3 $m^2$). The concentrate was diafiltered 5-fold by constant addition of water and freeze-dried before use in order to obtain WPI. Low-viscosity carboxymethylcellulose sodium salt (CMC), glycerol (99.5%), and agar were purchased from Sigma Chemicals Co. (St. Louis, MO). Pectin (Certo) was supplied by Kraft Canada (Toronto, Ontario, Canada). Acetonitrile (99.95%) was obtained from Anachemia Chemicals (Montreal, Québec, Canada).

**Film Formation.** The formulations are based on 5% w/w total protein, 2.5% glycerol, and 0.25% CMC, as previously reported by Ressouany et al. (*6*). Different protein sources were used for the film formulations. Moreover, different polysaccharides were added: 0.1% pectin and 0.1% agar. The content in protein, fat, lactose, and ashes in CC and WPI is summarized in **Table 1**. The components were solubilized in distilled water, with stirring, and the solutions were heated to 90 °C for 30 min. The solution was then degassed with agitation to remove dissolved air and flushed with nitrogen according to the method of Brault et al. (*4*). Irradiation of the solution was carried out at the Canadian Irradiation Centre (CIC; Laval, Québec, Canada) at a dose of 32 kGy and a mean dose rate of 17.33 kGy/h, using a UC-15 $^{60}$Co underwater calibrator unit (MDS-Nordion International Inc., Kanata, Ontario, Canada). Films were then cast by pipetting 5 mL of the solution onto smooth-rimmed 8.5 cm (i.d.) Petri dishes that were sitting on a leveled surface. Solutions were spread evenly and allowed to dry overnight at room temperature (20 ± 2 °C) in a climatic chamber (45−50% RH). Dried films could be peeled intact from the casting surface.

**Film Thickness Measurements.** Film thickness was measured using a Digimatic Indicator (Mitutoyo, Tokyo, Japan) at five random positions around the film, by slowly reducing the micrometer gap until the first indication of contact. Depending on the formulation, the average film thickness was in the range of 45−65 ± 2 $\mu$m.

**Isothermal Calorimetry.** Measurements were obtained using a Setaram C80 calorimeter (Lyon, France) in an isothermal mode (heats of swelling). The great sensitivity and stability of this calorimeter are due to several features of its construction. This calorimeter measures the voltage difference between the working cell and the reference using an array of 1000 thermocouples. The two large cells (12 $cm^3$) are placed at the center of a well-isolated well which contains also a temperature sensor, voltage detector, and heating parts. A known weight of dried sample (30 mg) was introduced in a homemade thin glass bulb and sealed under vacuum. The bulb was placed in a stainless steel cell filled with water. The cell is closed with a top well isolated from the exterior by Teflon joints to prevent water evaporation, and then the cell was placed into the calorimeter. During thermal equilibrium between the sample and the calorimeter, the sample in the evacuated glass bulb is not in contact with water. When thermal equilibrium is reached, as seen by a constant and small signal between the two cells, contact is made between sample and water by breaking the glass bulb. This can be done by a special device which avoids the perturbation of the system thermal equilibrium. The bulb was broken by pushing gently, from the top of the calorimeter, a stem going across the cork of the cell. Due to the vacuum in the bulb, water filled the entire glass bulb once it was broken and interacted with the sample. After the cell was broken, an exothermic heat was evolved that lasted about 1 h. The integration of the heat flow leads to the experimental heats ($\Delta H_{exptl}$). This value obtained after integration of the heat flow change is the sum of three contributions:

$$\Delta H_{exptl} = \Delta H_{interaction} + \Delta H_{glass-breaking} + \Delta H_{vaporization} \quad (1)$$

The last two terms can be measured by control experiments. $\Delta H_{glass-breaking}$ is obtained without water: ca. −150 to −200 mJ. $\Delta H_{vaporization}$ depends on the temperature of the water: i.e., of the calorimeter. It corresponds to the endothermic heat of vaporization of water in the volume of the bulb, which was not saturated by water vapors because it was under vacuum. $\Delta H_{vaporization}$ can be calculated from the volume of the bulb and the equilibrium water pressure at a given temperature. By subtracting these values from $\Delta H_{exptl}$, $\Delta H_{interaction}$ is obtained (*14*). The values of $\Delta H_{interaction}$ can also be obtained for each component of the mixture so that, in the case of a three-component mixture, three values of $\Delta H_{interaction}$ are measured from three different binary systems.

In the case of multicomponent systems, an equation similar to eq 1 can be written where $\Delta H_{interaction}$ is a combination of the values for the binary systems. As a first approximation, one can write

$$\Delta H_{interaction}(\text{multicomponent, expected}) = \sum[\varphi(\text{i})][\Delta H(\text{i})]$$

where $\varphi$(i) are the weight or volume fractions of each component. Since the values of $\varphi$(i) and $\Delta H$(i) are known, a value of $\Delta H_{interaction}$ (multicomponent) can be calculated from the results of the analysis of the three binary solutions. This is $\Delta H$(expected).

An eventual difference between the experimental value of $\Delta H_{interaction}$ (multicomponent) and that given by eq 2, $\Delta H_{interaction}$(multicomponent, expected) is interesting. It gives some information about the changes in swelling properties or hydrophilic character due to the preparation of a multicomponent film. An example of calculation of $\Delta H_{interaction}$-(expected) for a multicomponent system is presented. As will be seen below (**Table 3**), the values of $\Delta H_{interaction}$ in J/g are −87.9 for the calcium caseinate, −65.3 for WPI, and −57.1 for glycerol. For a mixture of the three components having an equal volume or weight fraction of each, $\varphi(1) = \varphi(2) = \varphi(3) = 0.33$, so that $\Delta H_{interaction}$(expected) is represented by the calculation

$$\Delta H_{interaction}(\text{expected}) = -0.33(87.9 + 65.3 + 57.1) = -70 \text{ J/g}$$

$\Delta H_{interaction}$(expected) represents the expected value if each constituent had the original ability to bind water molecules, as represented in **Table 3**. Moreover, the calculated values will provide insights on the effect of both heating and $\gamma$-irradiation treatments on the biofilm constituents. The difference between $\Delta H_{interaction}$(expected) (−70 J/g) and $\Delta H_{interaction}$ (−23 J/g) is outside the experimental uncertainty and has a physical meaning, as will be discussed below.

**Table 2.** Formulations Investigated[a]

| formulation | ratio |
|---|---|
| CC:WPI | 1:1 |
| CC:WPI:Gly | 1:1:1 |
| CC:WPI:Gly:CMC | 10:10:10:1 |
| CC:WPI:Gly:CMC:pectin | 25:25:25:2.5:1 |
| CC:WPI:Gly:CMC:pectin:agar | 25:25:25:2.5:1:1 |

[a] Abbreviations: CC = calcium caseinate; WPI = whey protein isolate; Gly = glycerol; CMC = carboxymethylcellulose.

**Table 3.** Interaction between Water and Main Film Constituents[a]

| sample | $\Delta H$ (J/g) |
|---|---|
| calcium caseinate | −87.9 ± 0.5[a] |
| whey protein isolate | −65.4 ± 0.2[b] |
| glycerol | −57.2 ± 0.2[c] |

[a] Means followed by the same letter in each row are not significantly different at the 5% level.

**Statistical Analysis.** Analysis of variance and Duncan multiple-range tests with $P \leq 0.05$ were employed to analyze statistically all results. For mechanical properties, 5 replicates of 10 samples were analyzed. For isothermal calorimetry, 5 replicates of 6 samples were analyzed. The Student *t* test was utilized at the time of the analysis of the variance and paired-comparison with $P \leq 0.05$ (*17*).

## RESULTS AND DISCUSSION

**Table 3** exhibits the average results for the heats of wetting of calcium caseinate, WPI, and glycerol. All values indicate that the dispersion of these constituents in water results in an exothermic reaction, as inferred from the negative values. In other words, the dispersion of proteins and glycerol are all thermodynamically favorable and, thus, release heat. Negative values of $\Delta H$ are associated with the formation of hydrogen bonds between the constituents and water. When the constituents are compared among them, calcium caseinate has the most exothermic heat of wetting, −87.9 J/g. The values of $\Delta H_{\text{interaction}}$ are, in absolute value, 26% and 35% lower for, respectively, WPI and glycerol than those for calcium caseinate representing more endothermic values. Since a more negative heat of wetting value corresponds, in this investigation, to more protein−water interactions, values represented in **Table 3** suggests that more caseinate−water interactions take place upon dissolution, while WPI induces fewer interactions with water and glycerol even fewer. The higher heat of wetting value, in absolute value, for calcium caseinate can be related to a more flexible structure of this protein due to the presence of proline residues that are evenly distributed along the polypeptide chain. Such flexibility would favor H-bonds between calcium caseinate and water molecules. The structure of globular proteins such as WPI is known to be more compact and more hydrophobic as well (*18*). The heats of wetting of CMC, pectin, and agar were not measured, owing to their very low concentration in our biofilm formulation.

Isothermal calorimetry investigations have shown the ability of calcium caseinate, WPI, and glycerol to interact with water. However, mixing these constituents together and treating this solution by heating or by $\gamma$-irradiation might alter their ability to interact with water molecules, leading to the formation of protein−protein interactions. It is well-known and accepted that milk proteins undergo cross-linking to yield a three-dimensional network upon heating, due to the formation of disulfides, and/or $\gamma$-irradiation, owing to the formation of bityrosine (*4*, *19*−*23*). These processes are expected to reduce the interactions between milk proteins and water molecules, since fewer sites become available, and also because cross-linking might also modify the proteins structure, as previously reported (*9*).

On the basis of the proportion of each constituent, the heats of wetting expected for five formulations were calculated (**Table 4**). Then, the average heats of wetting of the same formulations were measured after a thermal treatment was applied at 90 °C for 30 min and after heating under the same conditions and irradiation at 32 kGy (**Table 4**). Therefore, comparing the experimental heat of wetting to the expected value can reveal the role of each constituent in the formulation, at the molecular level.

The experimental value of the heated 1:1 mixture of calcium caseinate and WPI was 16% more endothermic than the theoretical value: ca. −64.2 J/g vs ca. −76.6 J/g (**Table 4**). A less negative heat of wetting (lower absolute value) denotes a loss of protein−water interactions upon heating. The thermal treatment is well-known to generate cross-links in WPI via the oxidation of sulfhydryl groups into disulfide linkages (*8*, *23*).

**Table 4.** Effect of Heating and $\gamma$-Irradiation on Loss of Protein–Water Interactions Present in Film Formulations[a]

| | Δ | | |
|---|---|---|---|
| formulation | $\Delta H$ (J/g) | $\Delta H$(expected) (J/g) | $\Delta + \gamma$ $\Delta H$ (J/g) |
| CC:WPI | $-64.2 \pm 3.2^{a(2)}$ | $-76.6 \pm 0.3^{a(1)}$ | $-56.2 \pm 1.6^{a(3)}$ |
| CC:WPI:Gly | $-23.4 \pm 0.4^{b(2)}$ | $-70.1 \pm 0.3^{b(1)}$ | $-20.1 \pm 1.0^{b(3)}$ |
| CC:WPI:Gly:CMC | $-16.5 \pm 0.1^{cd(2)}$ | $-70.1 \pm 0.3^{b(1)}$ | $-14.7 \pm 0.3^{c(3)}$ |
| CC:WPI:Gly:CMC: pectin | $-19.5 \pm 0.5^{c(2)}$ | $-70.1 \pm 0.3^{b(1)}$ | $-12.2 \pm 0.2^{d(3)}$ |
| CC:WPI:Gly:CMC:pectin: agar | $-15.1 \pm 0.2^{d(2)}$ | $-70.1 \pm 0.3^{b(1)}$ | $-13.1 \pm 0.5^{cd(3)}$ |

[a]Legend and abbreviations: Δ = heating; $\gamma$ = $\gamma$-irradiation; CC = calcium caseinate; WPI = whey protein isolate; Gly = glycerol; CMC = carboxymethylcellulose. Means followed by the same number in each row are not significantly different at the 5% level. Means followed by the same letter in each column are not significantly different at the 5% level.

The formation of disulfides favors protein−protein interactions at the expense of protein−water interactions, thus accounting for a less exothermic heat of wetting.

When the same biofilms were then irradiated, the experimental heat of wetting was 26% more endothermic than the theoretical value: ca. −56.2 J/g vs ca. −76.6 J/g (**Table 4**). The contribution of $\gamma$-irradiation to the loss of protein−water interactions can be measured by subtracting the heat of wetting obtained following irradiation to the values for the same biofilms that were submitted to heat only. Thus, $\gamma$-irradiation induces a further loss of protein−water interactions equivalent to ca. 8 J/g (13%), due to the formation of protein cross-links (*4*−*6*, *19*, *20*). Recent reports confirmed that the combination of thermal and radiative treatments enhances protein−protein interactions, since further cross-links are formed: disulfide bonds in addition to bityrosine (*7*, *8*). As a result, protein chains are closer and, thus, fewer sites are available to interact with water molecules, which accounts for the experimental heat of wetting values obtained.

The addition of glycerol to the previous formulation followed by heating treatment generated an even more endothermic heat of wetting in comparison to the biofilms made from an equal mixture of both proteins. A significant difference ($P \leq 0.05$) of 64% was observed: ca. −23.4 J/g vs ca. −64.2 J/g (**Table 4**). A similar difference was obtained when the formulations were heated and irradiated: −20.1 J/g vs −56.2 J/g (**Table 4**). These results suggest that the addition of glycerol induced a greater loss of protein−water interactions. Similar results were recently reported upon heating and irradiation of whey protein based biofilms (*9*). It is assumed that such a dramatic gap between the expected exothermic heat of wetting and the more endothermic experimental values could be due to the concentration used or to the hydrophilic structure of glycerol, which might be involved in association with the hydrophilic sites of proteins (H-bonding), thus limiting protein hydration: i.e., protein−water interactions.

Comparisons with the expected heat of wetting value point out a significant difference of 67% for the heated formulation, ca. −23.4 J/g vs ca. −70.1 J/g (**Table 4**), and 71% for the formulations that were both heated and irradiated: ca. −20.1 J/g vs −70.1 J/g (**Table 4**). The impact of $\gamma$-irradiation is equivalent to 14%: ca. −20.1 J/g vs −23.4 J/g (**Table 4**). As discussed previously, the combination of the thermal and radiative treatments increases the amount of cross-links, thus enhancing protein−protein interactions at the expense of protein−water interactions, accounting for a greater heat of wetting value when compared to that of the heated biofilm.

Furthermore, previous investigations reported that the presence of glycerol in caseinate-based formulations enhances the production of bityrosine, resulting in a higher number of cross-links between tyrosine units (*4*, *5*). This phenomenon was related to the preferential binding concept elaborated by Gekko and Timasheff (*24*).

Addition of CMC induced further loss of protein−water interactions, as explained below. The heat of wetting obtained for thermally treated biofilms containing CMC in addition to calcium caseinate, WPI, and glycerol was even higher than the value obtained for biofilms without CMC, representing a more endothermic heat of wetting value ($P \leq 0.05$). A 29% difference ($P \leq 0.05$) was found: ca. −16.5 J/g vs ca. −23.4 J/g (**Table 4**). When the formulations containing CMC were heated and then irradiated, the heat of wetting was 27% ($P \leq 0.05$) more endothermic than the formulation without CMC: ca. −14.7 J/g vs −20.1 J/g (**Table 4**). These results revealed that the presence of CMC induces further losses of protein−water interactions of about 6−7 J/g.

Comparing the experimental data to the expected value shows the impact of both thermal and radiative treatments. As mentioned previously, the difference is greater when the formulations are heated and irradiated. Indeed, the heat of wetting was 79% more endothermic for heated and irradiated biofilms, ca. −14.7 J/g vs −69 J/g (**Table 4**), whereas heated formulations showed a 76% more endothermic value: ca. −16.5 J/g vs −70.1 J/g (**Table 4**). $\gamma$-Irradiation contributed to lower protein−water interactions by 11% (ca. 2 J/g): ca. −14.7 J/g vs −16.5 J/g (**Table 4**). These results demonstrate once again that the combination of $\gamma$-irradiation with heating favors more cross-links, which results in an enhancement of protein−protein aggregation and, thus, reduces site availability for protein hydration. Moreover, a preliminary experiment pointed out a synergistic effect when CMC was combined with $\gamma$-irradiation at 32 kGy (unpublished data). In fact, it was demonstrated that such a combination favors protein aggregation in caseinate-based films and mixtures of soy and whey protein based films (*7*). Since biofims investigated in this work contained both caseinate and whey proteins, it is, therefore, assumed that protein aggregation occurred at the expense of protein−water interactions, thus accounting for the heat of wetting values obtained.

When pectin was added and the formulation thermally treated, no significant impact ($P > 0.05$) on the heat of wetting, and thus protein−water interactions, could be noticed with respect to the biofilms without pectin. Protein−water interactions were respectively −16.5 and −19.5 J/g for these formulations (**Table 4**). However, the contribution of pectin seemed to be significant ($P \leq 0.05$) when the formulation underwent both thermal and radiative treatments, with a loss of protein−water interactions of ca. 2.5 J/g (**Table 4**): −12.2 J/g vs −14.7 J/g (+17%). The combination of $\gamma$-irradiation with the heating treatment allowed the further weakening of protein−water interactions. In fact, $\gamma$-irradiation gave rise to a heat of wetting value that was 37% more endothermic: ca. −12.2 J/g vs −19.5 J/g (**Table 4**). This difference, due to $\gamma$-irradiation, reflects the loss of protein−water interactions.

The presence of agar, a third polysaccharide, in the biofilm formulations did not show such a great impact on the heat of wetting value. No significant difference ($P > 0.05$) appeared between heated formulations made of a 1:1 mixture of calcium caseinate and WPI, glycerol, and CMC and those containing in addition pectin and agar. Protein−water interactions were respectively −16.5 and −15.1 J/g for these formulations (**Table 4**). Likewise, the addition of agar to formulations made of calcium caseinate, WPI, glycerol, CMC, and pectin combined with heating and irradiation treatments did not produce significant changes ($P > 0.05$). Protein−water interactions were respectively −12.2 and −13.1 J/g (**Table 4**). However, the irradiation of formulations containing agar contributed to further break down the protein−water interactions, as suggested by the 13% difference from the heat of wetting value for formulations that have been only heated: −13.1 vs −15.1 J/g (**Table 4**). The same observations could be made for formulations containing agar and pectin: −12.2 vs −19.5 J/g (**Table 4**).

Although the presence of CMC in our biofilm formulation had a significant endothermic impact on the heat of wetting value (**Table 4**), not as great as the one generated by glycerol (**Table 4**), this behavior is somewhat surprising. It was believed that the addition of CMC would have an exothermic effect on the heat of wetting and, thus, strengthen protein−water interactions. Indeed, CMC is comprised of many hydrophilic sites, among them hydroxyl groups, that can bind water molecules through H-bonds. Our experimental findings (**Table 4**) suggest that other molecular phenomena might take place. It is assumed that CMC might interact preferentially with calcium caseinate and WPI proteins. This would result in a decrease of available water-binding sites on polysaccharide molecules. Consequently, protein−polysaccharide interactions occurred at the expense of protein−water interactions, accounting for a more endothermic heat of wetting value.

The ability of glycerol to reduce protein−water interactions could be related to its hydrophilic properties in combination with its smaller size with respect to CMC. It is assumed that the bulky structure of CMC hampers some contacts with protein sites. However, being a smaller molecule, glycerol is more labile. This would result in increased contacts with several protein sites. It is also possible that the available interaction sites of the proteins had been filled due to the high glycerol concentration used. This would result in a decrease of protein−polysaccharide interactions when pectin or agar were added in the presence of CMC.

## CONCLUSION

The use of isothermal calorimetry allowed the monitoring of protein−water interactions such as calcium caseinate−water and WPI−water, via the measurement of the heat of wetting thermodynamic parameters. All experimental heat of wetting results differ from the expected heat of wetting values, confirming the complexity of the molecular phenomenon that occurred within biofilm constituents.

In general, protein−water interactions are not thermodynamically favorable in the presence of glycerol, CMC, and pectin, as inferred from the endothermic impact of these constituents on the heat of wetting values. Therefore, other interactions are assumed to take place within biofilms. For example, protein−glycerol and protein−CMC interactions can be formed, although the impact of CMC was not found to be as important as that of glycerol. The higher influence of glycerol could be explained by the concentration used, by its hydrophilic structure and by its smaller size with respect to CMC, which might favor more contacts with both calcium caseinate and WPI.

This investigation also pointed out the endothermic contribution on the heat of wetting values of both thermal and radiative processing treatments for all five formulations. Cross-links resulting from these treatments did not favor protein−water interactions but, rather, protein−protein interactions. Obviously, the combination of both treatments enhanced the generation of cross-links, which resulted in a greater endothermic impact on

the heat of wetting values. Experiments under different conditions (e.g. thermal history and duration of $\gamma$-irradiation of biofilms) are necessary to compare more closely the effects of both thermal and radiative treatments.

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## LITERATURE CITED

(1) Peyron, A. L'enrobage et les produits filmogènes: un nouveau mode d'emballage.*Viandes Prod. Carnés* **1991**, *12*, 41−46.
(2) Chen, H. Functional properties and applications of edible films made of milk proteins. *J. Dairy Sci.* **1995**, *78*, 2563−2583.
(3) Krochta, J. M.; De Mulder-Johnston, C.; Edible and biodegradable polymer films: challenges and opportunity. *Food Technol.* **1997**, *51*, 61−74.
(4) Brault, D.; D'Aprano, G.; Lacroix, M. Formation of free-standing sterilised edible films from irradiated caseinates. *J. Agric. Food Chem.* **1997**, *45*, 2964−2969.
(5) Mezgheni, E.; D'Aprano, G.; Lacroix, M. Formation of sterilised edible films based on caseinates: effects of calcium and plasticisers. *J. Agric. Food Chem.* **1998**, *46*, 318−324.
(6) Ressouany, M.; Vachon, C.; Lacroix, M. Irradiation dose and calcium effect on the mechanical properties of cross-linked caseinate films. *J. Agric. Food Chem.* **1998**, *46*, 1618−1623.
(7) Sabato, S. F.; Ouattara, B.; Yu, H.; D'Aprano, G.; Lacroix, M. Mechanical and barrier properties of cross-linked soy and whey protein based films. *J. Agric. Food Chem.* **2001**, *49*, 1397−1403.
(8) Vachon, C.; Yu, H. L.; Yefsah, R.; Alain, R.; St-Gelais, D.; Lacroix, M. Mechanical and structural properties of cross-linked milk protein edible films. *J. Agric. Food Chem.* **2000**, *48*, 3202−3209.
(9) Le Tien, C.; Letendre, M.; Ispas-Szabo, P.; Mateescu, M. A.; Delmas-Patterson, G.; Yu, H. L.; Lacroix, M.; Development of biodegradable films from whey proteins by cross-linking and entrapement in cellulose. *J. Agric. Food. Chem.* **2000**, *48*, 5566−5575.
(10) Relkin, P. Differential scanning calorimetry: a useful tool for studying protein denaturation. *Thermochim. Acta* **1994**, *246*, 371−386.
(11) Ma, C. Y.; Harwalkar, V. R. Effects of medium and chemical modification on thermal characteristics of $\beta$-lactoglobulin. *J. Therm. Anal.* **1996**, *47*, 1513−1525.
(12) Barbeau, J.; Gauthier, S. F.; Pouliot, Y. Thermal stabilisation of $\beta$-lactoglobulin by whey peptide fractions. *J. Agric. Food Chem.* **1996**, *44*, 3939−3945.
(13) John, A.; Shastri, P. N. Studies on food macromolecules by DSC: a critical appraisal. *J. Food Sci. Technol.* **1998**, *35*, 1−14.
(14) Le Lay, P.; Delmas-Patterson, G. Investigation of the physical network of amorphous amylose by slow calorimetry. *Carbohydr. Polym.* **1998**, *37*, 49−59.
(15) Arntfield, S. D.; Murray, E. D. Heating rate affects thermal properties and network formation for vicilin and avalbumin at various pH values. *J. Food Sci.* **1992**, *3*, 640−646.
(16) St-Gelais, D.; Piette, M.; Bélanger, G. Production of cheddar cheese using milk enriched with microfiltered milk retentate, a preliminary study. *Milchwissencschaft* **1995**, *50*, 614−619.
(17) Snedecor, G. W.; Cochran, W. G. One-way classifications. Analysis of variance. In *Statistical methods*; The Iowa State University Press: Ames, IA, 1978.
(18) Kinsella, J. E.; Whitehead, D. M. Protein in whey chemical, physical and functional properties. *Adv. Food Nutr. Res.* **1989**, *33*, 343−438.
(19) Prütz, W. A.; Butler, J.; Land, E. J. Phenol coupling initiated by one-electron oxidation of tyrosine units in peptides and histone. *Int. J. Radiat. Biol.* **1983**, *44*, 183−196.
(20) Davies, J. A.; Delsignore, M. E.; Lin, S. W. Protein damage and degradation by oxygen radicals, II. modification of amino acids. *J. Biol. Chem.* **1987**, *262*, 9902−9907.
(21) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis: London, 1987.
(22) Kinsella, J. E.; Damodaran, S.; German, B. Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. In *New Protein Foods*; Altachul, A. M., Wilcke, H. L., Eds.; Academic Press: Orlando, FL, 1985; Vol. 5, pp 107−179.
(23) Gennadios, A.; Weller, C. L. Edible films and coatings from soymilk and soy protein. *Cereal Foods World* **1991**, *36*, 1004−1009.
(24) Gekko, K.; Timasheff, S. Mechanism of protein stabilization by glycerol: Preferential hydration in glycerol-water mixtures. *Biochemistry* **1981**, *20*, 4667−4676.

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