INFLUENCE OF MODERATE ELECTRIC FIELDS ON THE FORMATION AND PROPERTIES OF WHEY PROTEIN NETWORK STRUCTURES

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Abstract

Gelation plays a major role in enhancing textural properties of foods (substituting fats), once it provides unique textural properties that gives pleasant mouth feel, and enables holding water and other ingredients in one matrix. The general objective of this study was to assess the effects of MEF on properties of whey protein hydrogels. Results show that MEF originated a weaker gel structure than conventional heating treatment. Significant decreases in storage and loss moduli were observed upon application of MEF. Aggregation and cross-linking patterns of whey proteins during MEF was not sufficiently high to form a true elastic gel network. As conclusion, MEF may provide a novel method for production of a protein matrix with distinctive properties. However a larger body of research is needed to fully address the role of the MEF on protein electrostatics and protein-protein interactions.

Key words : electric field, whey protein hydrogels, aggregation, viscoelastic behavior

1 Introduction

Whey protein ingredients, such as WPC and WPI, are being widely employed in food formulations due to its high nutritional value, desirable sensory characteristics and excellent emulsifying, gelling, foaming and water-binding properties. Moreover, physicochemical properties of the whey proteins suggest that they may be suitable not only for novel food applications but also perform important roles in pharmaceuticals and biological systems [1]. Recent studies on the use of WPCs and WPIs as functional ingredients have highlighted the formation heat-induced gels (fine-stranded and particulate). Gelation plays a major role in enhancing textural properties of foods (substituting fats), once it provides unique textural properties that gives pleasant mouth feel, and enables holding water and other ingredients in one matrix. Gelling systems can be used in preparation of several foods, such as dairy food products, coagulated egg whites, gelatin gels, comminuted meat or fish products, bread dough [2, 3]. Heat treatments modify technological and functional characteristics of whey ingredients in the manufacture of the whey derived products, such as gels and films, for variety of applications [1]. The rates and pathways of gel systems are determined by several factors such as heating conditions, protein concentration, pH, ionic strength, and solvent condition [4]. Application of moderate electric fields (MEF) during thermal processing

influences denaturation rates and aggregation behavior of whey proteins in WPI. Therefore, modifications of whey protein preparations induced by MEF treatment could result in products with distinctly different functional characteristics from those of the conventional preparations. The general objective of this study was to assess the effects of MEF on properties of whey protein hydrogels.

2 Materials & Methods

2.1 Whey Protein Dispersions

WPI powder (Lacprodan DI-9212) was kindly supplied by Arla Foods Ingredients (Viby, Denmark). WPI was essentially free of lactose (max 0.5 %) and fat (max 0.2 %) and had β-Lg content of approximately 87 %, in a total protein content of 91 % (of dry weight). WPI solutions at 10 % (w/v) of protein were dispersed in 0.02 M phosphate buffer. After overnight rehydration the final pH was adjusted to 3.0 with 1 M of HCl (Merck, Germany).

2.2 Gel Formation

When concentrated whey protein solutions (e.g., > 8 % w/v protein) are heated at a sufficiently high temperature the protein molecules unfold and interact to form intermediate aggregates prior to the formation of a gel network [5]. WPI solutions were then heated through conventional indirect heating (COV) and with application of moderate electric fields (MEF) at 85ºC for 30 minutes to induce gel formation. Heat-induced gels were cooled in an ice water bath for 30 min and held in the tubes overnight at 4 °C prior to rheology analysis. A close coincidence of the temperature profiles during the sample in both treatments (COV and MEF) was achieved in order to evaluate non-thermal effects.

2.3 Dynamic Rheological Measurements

Viscoelastic properties of the produced WPI gels were accessed through small amplitude oscillatory dynamic rheological measurements (SAOS), conducted on an AR-G2 rheometer (TA Instruments, New Castle, DE) at 25 ºC, using parallel plate geometry with a gap of 1 cm. In these experiments, the samples were subjected to a sinusoidal deformation, the storage (*G′*) and loss (*G″*) moduli were recorded as a function of increasing frequency of oscillation. The ratio of energy dissipated to energy stored per cycle (*G″/G′*) allowed the calculation of tan-δ. After preliminary tests, the strain value of 0.3 % was selected in order to keep the linear viscoelastic behavior. The strain sweep experiments, which were conducted at a constant frequency of 1 rad/s, served to provide the limit of linear viscoelasticity that could be used in the frequency experiments.

3 Results & Discussion

The network structure in a heat-induced globular protein gel is strongly dependent on the balance between attractive and repulsive forces among denatured protein molecules during aggregation [6]. In this study, WPI fine-stranded and transparent gels structures were formed at pH 3.0 in the presence of 0.02 M of phosphate buffer by heating at 85 ºC for 30 minutes (results not shown). This behavior was expected, once at acidic pH (< 4) far from the isoelctric point of the protein and low ionic strength, a gel network is formed composed of fine strands in the order of nanometers in size [6, 7]. Figures 1 and 2 show the frequency sweep of the viscoelastic moduli and tan-delta, respectively, of WPI gels formed by COV and MEF treatments.

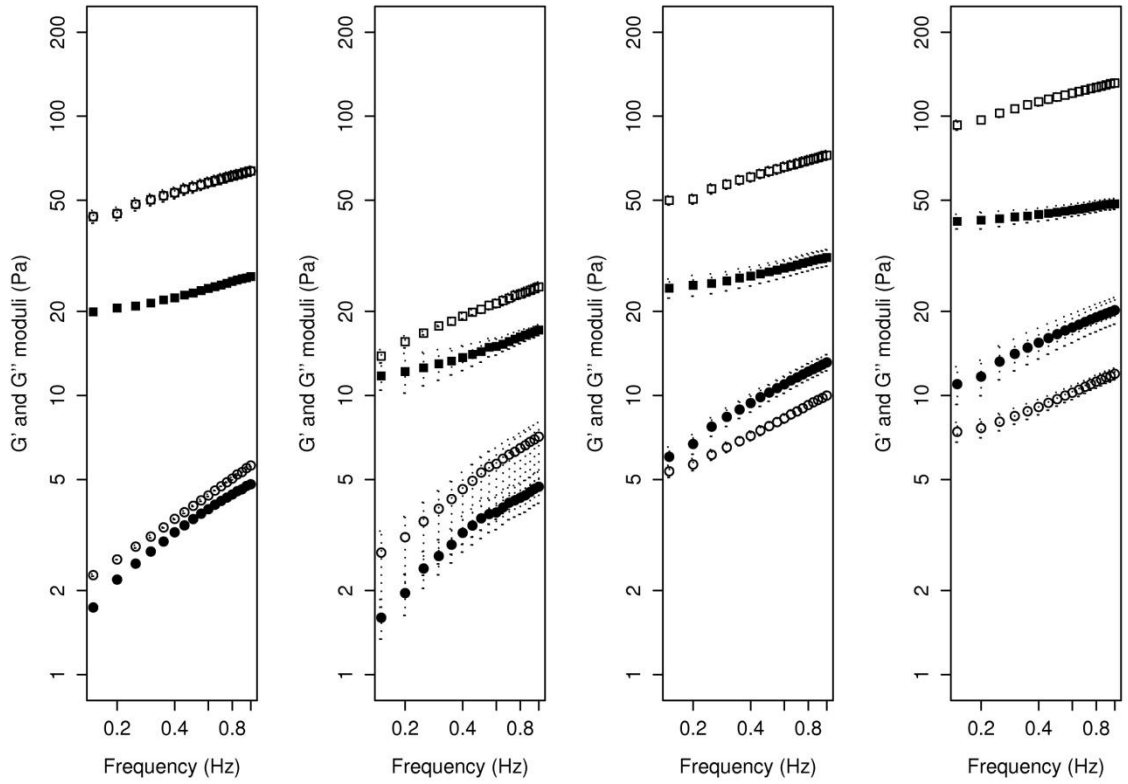

Figure 1 : Effects of MEF and COV treatments on the storage (G') and loss moduli (G'') of WPI gels heated at 85 ºC for 30 minutes. Opens symbols correspond to G' and closed symbols to G''. Squares correspond to COV gels while circles to MEF gels.

For COV and MEF treatments, *G'* and *G''* exhibited a very weak frequency dependence. However, COV heating have determined gels with a lager *G'* than *G''* over the entire frequency range observed. Such features are characteristic of a true gel [8]. MEF treatment originated a weaker gel structure, once decreases in both *G'* and *G''* were observed. In addition, MEF resulted in nearly identical values for *G'* and *G''* or alternatively higher *G''* than *G'*, thereby demonstrating two important points: 1) the number of disulfide linkages formed during the polymerization step is small; and 2) these new disulfide linkages have a negligible effect on gel rheology. Since G' is related to the degree of cross-linking, this suggests the existence of additional cross-links in the COV gel [8] when compared with MEF one. Through tan-δ calculation it was possible to evaluate the dynamic character of the protein-protein bonds in the gel network. MEF

samples presented always a higher tan-δ than COV ones ($p < 0.05$), indicating that MEF gel reacted to a stress in a relatively more viscous and less elastic manner [9]. During the initial stages of heating gelation, a transition from the protein native state to a "progel" state occurs, and this is associated with unfolding and denaturation of the protein.

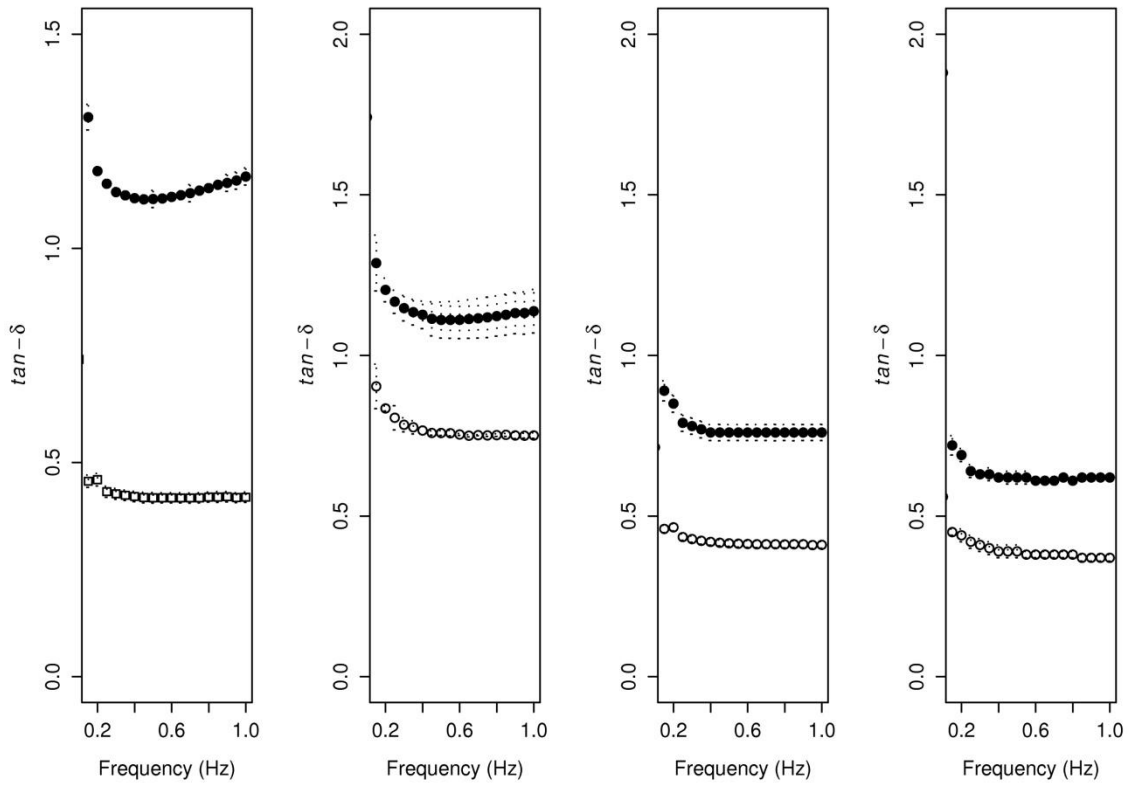

Figure 2 : Effects of MEF and COV treatments on tan-δ (G''/G') of WPI gels heated at 85 ºC for 30 minutes. Closed symbols correspond to MEF while open symbols to COV treatments.

The progel state is usually a viscous liquid state in which some degree of protein polymerization has already occurred [2]. Therefore, it can be suggested that a progel state was achieved at the end of MEF treatment. This progel or liquid-like state was evidenced by the fact that *G''* dominated *G'* during SAOS measurements at 25 ºC. *Erro! A origem da referência não foi encontrada.* shows the effects of heating on complex viscosity of whey gels. In both treatments the complex viscosity decreased with increasing frequency over the frequency region measured, confirming the weaker nature of fine-stranded gels produced. Despite this decrease being more accentuated in COV, gels formed through MEF exhibited lower range of complex viscosity than COV gels ($p < 0.05$). These events also support the lower degree of MEF gels structure.

The formation of a network gel or film structure is a physical manifestation of protein denaturation mechanisms, which consists of the following sequential three steps: 1) denaturation; 2) aggregation; and 3) cross-linking between aggregates. This aggregation and polymerization into a gel or film matrix involves covalent bonding and non-covalent bonding. The former consists of inter- and intramolecular disulfide bonds formed via sulfhydryl-disulfide interchange or sulfhydryl oxidation reactions. The latter are considered to be relatively weak hydrophobic-driven interactions [10, 11]. The results obtained in this present study are in accordance with our previous studies [12, 13], showing the influence that different patterns of denaturation/aggregation have on physical properties of protein network structures; MEF induces less denaturation of β-Lg

and α-Lac and less aggregation during early stages of thermal processing. Therefore, the level of protein polymerization during MEF was not sufficiently high to produce a true elastic gel. Weaker gels with different dynamic viscoelastic behavior can be obtained under MEF. Different protein compositions due to different levels of denaturation or protein interactions, may also influence the viscoelastic behavior of gels. For example, it has been reported that increasing the proportion of α-Lac results in gels characterized by an open microstructure and reduced elastic and viscous moduli [14]. The effect of MEF on denaturation of whey proteins is not significant compared to that of heat, but may interfere with inter- and intra-molecular protein interactions producing a marked reduction in whey protein aggregation [15].

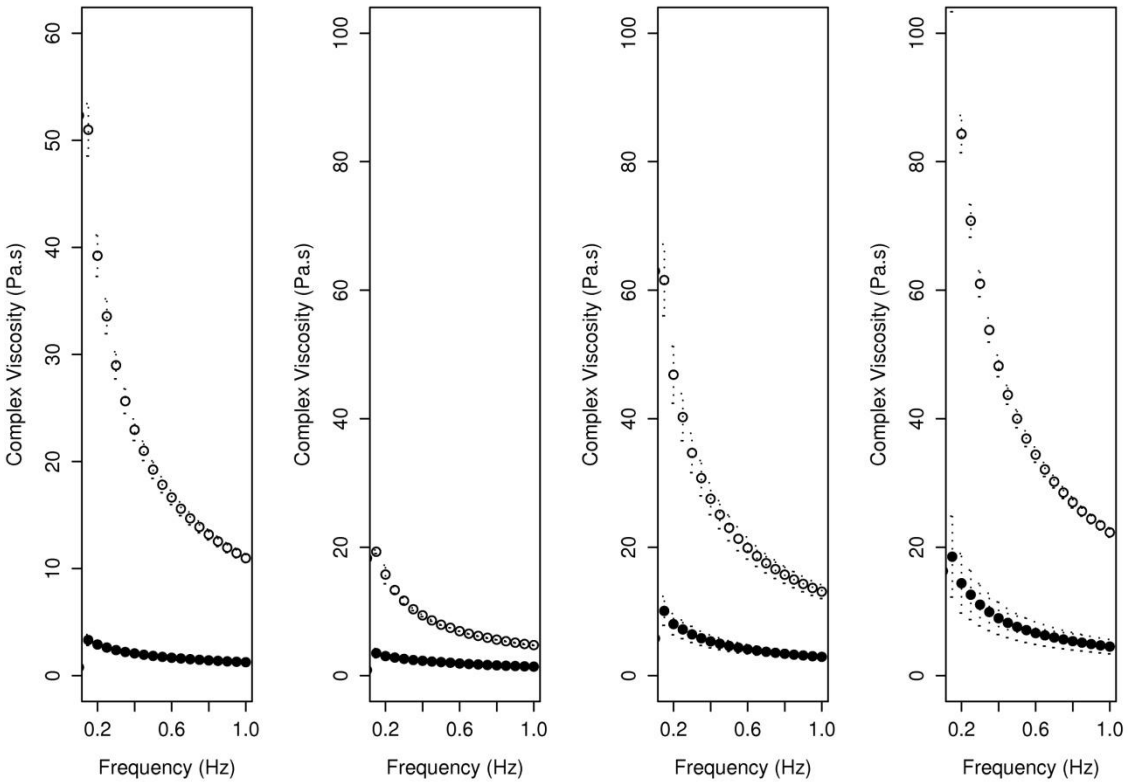

Figure 3 : Effects of MEF and COV treatments on complex viscosity of WPI gels heated at 85 ºC for 30 minutes. Closed symbols correspond to MEF treatments while open symbols to COV treatments.

This behavior can be related to conformational disturbances on tertiary protein structure due to rearrangement of hydrogen bonds, hydrophobic interactions, and ionic bonds; non-covalent interactions may be impaired by reorientation of hydrophobic clusters occurring in the proteins' structure during application MEF, thus affecting physical aggregation. MEF treatment may also affect ionic movement in the medium and modify the molecular environment due to the increased number of ions and their different distributions around the protein molecules [16]. Alternatively, the combined effects of MEF and sinusoidal frequency may promote splitting of large aggregates induced by thermal processing thus enhancing the formation of small particles.

4 Conclusions

The present study clearly shows that MEF interferes with the mechanisms of gelation or at least at the level of the protein-protein interactions. MEF can modify the structure of whey proteins in order to achieve specific and/or desired functional properties in a

manner similar to the use of controlled heat treatments. MEF treatment offers a great potential to the development of hydrogels with a diverse mechanical and microstructural features and hence textural properties. Despite the influence of MEF on biochemical structure of whey proteins, the reasons behind of this peculiar behavior are still unknown. A more complete study should be developed for a clearer understanding of the importance of these changes on real food systems applications and to better understand how MEF interacts at a molecular level with individual whey proteins in order to clarify the events occurring during unfolding and aggregation steps.

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