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A comparative study of heat and high pressure induced gels of whey and egg albumen proteins and their binary mixtures

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Abstract

Large deformation rheological studies of either egg albumen or whey protein isolate (15% protein w/w) gels induced by heating at 90 °C for 30 min were compared to those induced by a range high pressures (400–800 MPa for 20 min). Gels made by heating indicated higher gel strength and Young's modulus values for whey protein from pressures of 400–600 MPa for 20 min but similar values at 650–800 MPa. In contrast, egg albumen showed no gelation below 500 MPa for 20 min, but there was an increase in both gel strength and Young's modulus with increasing pressure, although values remained lower than those of the heat-induced gels. A mixture of 10:5 whey/egg albumen showed the highest gel strength and Young's modulus for both heated and high pressure-treated (400–600 MPa) gels, although, the heated mixture had the highest values. Electron micrographs indicated that high pressure-treated gels had a porous aggregated network for egg albumen while whey proteins showed a continuous fine stranded network. The heated mixtures of whey:egg albumen (7.5:7.5) showed large dense aggregates whereas high pressure-treated mixtures produced smaller aggregates. Raman spectroscopy of both heated and high pressure-treated whey and egg albumen (15% w/w in D_2O pD7) and their binary mixtures (7.5:7.5, protein w/w) indicated changes in β-sheet structures in the Amide 111′ region (980–990 cm^{-1}); however, peak intensity was reduced for high pressure-treated samples. β-Sheet structure (1238–1240 cm^{-1}) present in heated whey was absent in high pressure-treated whey and in egg albumen. Involvement of hydrophobic regions was reflected by changes in the CH (1350 cm^{-1}) and CH_2 (1450 cm^{-1}) bending vibrations. In addition to the Trp residues at 760 cm^{-1}, there were broad peaks at 874–880 cm^{-1} and tyrosine 1207 cm^{-1} in the high pressure-treated samples. Disulphide bands (500–540 cm^{-1}) in heated whey and egg albumen proteins showed higher peak intensities compared to high pressure-treated samples. Differences in the experimental and theoretical spectra indicated changes in the hydrophobic regions, tyrosine (1207 cm^{-1}) and tryptophan (880 cm^{-1}) and CH_2 bending in high pressure-treated samples, whereas heated samples indicated marked changes in β-sheet structures (987 and 1238 cm^{-1}) as well as hydrophobic regions CH (1350 cm^{-1}) and CH_2 (1450 cm^{-1}) bending vibrations.

Keywords: Egg albumen; Whey; Protein; Rheology; Raman spectroscopy; High pressure; Heat gelation; Transmission electron microscopy

1. Introduction

Protein gelation is used to obtain or improve the consistency of a food product. The use of whey and egg albumen proteins in food systems as gelling and thickening agents and texture modifiers has been extensively reviewed in the literature (Howell & Lawrie, 1985; Kinsella & Whitehead, 1989; Morr & Ha, 1993; Nakamura & Doi, 2000; Ngarize, Adams, & Howell, 2004).

Traditionally, gel formation is induced by application of heat to the protein solution. However, foods are rarely homogenous and are composed of a mixture of proteins with carbohydrates and fats. Heat treatment of proteins and other nutrients can induce various chemical reactions such as Maillard browning which may lead to nutritional, sensory and safety deterioration in certain foods (DeMan, 1996; Hayashi & Balny, 1996; Hayashi, Kawamura, Nakasa, & Okinaka, 1989; Knorr, Ade-Omowaye, & Heinz, 2002; Tauscher, 1995). Consequently, there is now an increasing trend towards the application of non-thermal technologies which may prolong shelf-life without these detrimental effects. Non-thermal technologies such as high pressure

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processing are of interest to the food industry, because they not only provide alternatives to conventional methods of thermal processing but also offer opportunities for creating new ingredients and textures.

High pressure processing, involves subjecting food material to a high hydrostatic pressure and holding it isobarically for a specified period of time before release. Typical commercial processes are in the order of 100–1000 MPa and holding times vary from 10 to 30 min. This is sufficient to bring about the necessary level of processing in terms of microbial inactivation, pasteurisation, extension of shelf-life and induction of molecular changes (Galazka & Ledward, 1998; Mertens & Deplace, 1993).

High pressure technology is popular because it can be used to obtain stable products with minimal effects on flavour, colour and nutritional value or to create novel texture and taste (Cheftel, 1995). Although the effect of high pressure on individual proteins have been reviewed (Balny & Masson, 1993; Galazka & Ledward, 1998; Gross & Jaenicke, 1994; Heremans, 2002), the way high pressure affects protein interactions in mixed systems has only been studied by few researchers (Galazka, Sumner, & Ledward, 1996; Walkenstrom & Hermansson, 1997). Therefore, in this present study, whey and egg albumen protein–protein interactions were investigated using both heat and pressure denaturation. Our previous studies (Ngarize et al., 2004) revealed synergistic interaction in the treated binary mixtures of whey and egg albumen proteins especially when mixed in the ratio of 10%whey/5% egg albumen. In this study, heated individual whey and egg albumen protein gels and the mixed gels were compared with pressure-treated gels using a range of pressures (400–800 MPa).

Both heat and pressure are known to denature proteins resulting in aggregation and gelation (Hayakawa, Kajihara, Morkawa, Oda, & Fujio, 1992; Hayakawa, Linko, & Linko, 1996; Ngarize et al., 2004; Sikorski, 2001; Van Camp & Huyghebaert, 1995). The denaturation process results in changes in protein conformation, structure and hence functionality. The mechanism of heat and pressure protein denaturation is reported to differ and consequently affects the type of gels formed (Galazka & Ledward, 1998; Hayashi et al., 1989; Ledward, 2000). Pressure denatured proteins are reported to involve rupture of hydrophobic and electrostatic interactions which result from a decrease in volume of the protein solution while heat denaturation of the protein is caused by violent movement of molecules that can destroy heat labile hydrogen bonds. In addition, heat denaturation is accompanied by destruction and formation of covalent bonds which enhances the production of off-flavours or toxic compounds (Knorr et al., 2002; Okamoto, Kawamura, & Hayashi, 1990). In pressure denaturation, the rearrangement of water molecules surrounding amino acids residues promotes increased glossiness and transparency (Okamoto et al., 1990).

The mechanism involved in heat and high pressure gels has been investigated mostly by chemical modification, chromatography, electrophoresis, solubility, enzymatic digestion, immunology, thermal analysis and microscopy. In recent years, several spectroscopic techniques have been used to obtain a more highly resolved picture of molecular structures of aggregates and gels formed during heating and by application of pressure.

These techniques include nuclear magnetic resonance (NMR) (Zhang, Peng, Jonas, & Jonas, 1995), ultraviolet (UV) spectroscopy (Mombelli et al., 1997), as well as fluorescence and infrared spectroscopy with small angle X-ray scattering (Pannick, Malessa, & Winter, 1999). Circular dichroism (CD) has also been used to study heat denaturation of several proteins (Clark & Lee-Tuffnell, 1986; Clark, Saunderson, & Suggett, 1981; Wang & Damodaran, 1991) but like most of the above methods its application is limited to dilute solutions. Furthermore, CD cannot be used to study denaturation during the high pressure treatment for technical reasons; the correction that might be required to account for pressure-induced birefringence in the windows will be much larger than the expected changes in the protein (Heremans, 2002; Heremans, Van Camp, & Huyghebaert, 1997).

The effect of pressure on protein–protein interactions has been studied by light scattering or turbidity methods (Gorovits & Horowitz, 1998). However, these methods give information on the degree of association and aggregation, but no information on conformational changes or the nature of intermolecular interactions. Recent reports have indicated differences between the structural changes induced by temperature and pressure as judged from Fourier transform infrared spectroscopy (Smeller, Rubens, & Heremans, 1999). The use of Raman spectroscopy to study heat denaturation and to a limited extent pressure denaturation has been reported (Clark et al., 1981; Heremans & Wong, 1985; Howell, Guillermo, Nakai, & Li-Chan, 1999; Howell & Li-Chan, 1996; Nakai, Li-Chan, & Hirotsuka, 1994). FT-Raman spectroscopy allows a detailed investigation of solid gels as well as direct measurement of disulphide bonds. In the present study, a range of complementary techniques including large deformation rheology, FT-Raman spectroscopy and transmission electron microscopy were used to provide a detailed picture of the mechanism of gelation and properties of heat and pressure-treated whey and egg albumen gels. In addition, the hypothesis that globular proteins such as whey and egg albumen can display synergistic interactions under high pressure, similar to those indicated in heat-treated gels was tested (Howell & Lawrie, 1984; Ngarize et al., 2004).

2. Materials and methods

2.1. Materials

Deuterium oxide, (D, 4561, 99.9% atom D) and egg albumen was obtained from Sigma-Aldrich Chemical