Food Chemistry 265 (2018) 32–38

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Modeling water partition in composite gels of BSA with gelatin following high pressure treatment

Carine Semasaka[a], Pranita Mhaske[a], Roman Buckow[b], Stefan Kasapis[a,*]

[a] School of Science, RMIT University, Bundoora West Campus, Plenty Road, Melbourne, VIC 3083, Australia
[b] CSIRO, Food and Nutrition, Werribee, VIC 3030, Australia

ARTICLE INFO

Keywords:
Bovine serum albumin
Gelatin
Phase separated gels
High pressure processing
Water partition

ABSTRACT

Changes in the structural properties of hydrogels made with gelatin and bovine serum albumin mixtures were recorded following exposure to high pressure at 300 MPa for 15 min at 10 and 80 °C. Dynamic oscillation, SEM, FTIR and blending law modelling were utilised to rationalise results. Pressurization at the low temperature end yielded continuous gelatin networks supporting discontinuous BSA inclusions, whereas an inverted dispersion was formed at the high temperature end with the continuous BSA network suspending the discontinuous gelatin inclusions. Lewis and Nielsen equations followed the mechanical properties of the composites thus arguing that solvent partition between the two phases was always in favour of the polymer forming the continuous network. As far as we are aware, this is the first attempt to elucidate the solvent partition in pressurised hydrogel composites using blending law theory. Outcomes were contrasted with earlier work where binary mixtures were subjected only to thermal treatment.

1. Introduction

High pressure processing (HPP) is a valuable tool to industry due to its ability to improve the nutritional quality of perishable foods alongside with preservation of their structural functionality, storage stability and microbial safety (Deliza, Rosenthal, Abadio, Silva & Castillo, 2005). Pressurisation can be implemented up to 1000 MPa and an advantage, as compared to heat transfer in thermal treatments, is that pressure is uniformly applied to materials no matter their size or geometry (San Martin, Barbosa-Cánovas, & Swanson, 2002; Oey, Van der Plancken, Van Loey, & Hendrickx, 2008). Although the original intention of HPP's application was to inactivate enzymes and microorganisms, it is now understood that it can also denature proteins, induce starch gelatinization and promote a plethora of interactions amongst food ingredients (Knorr, Heinz, & Buckow, 2006).

It has been reported that HPP affects the structural properties of globular proteins by altering their secondary, tertiary and quaternary structure leading to a variable functionality (solubility, emulsification, foaming, etc.) (Bouaouina, Desrumaux, Loisel & Legrand, 2006; Kuhn & Cunha, 2012; He, He, Chao, Xingrong, & Aluko, 2014; De Maria, Ferrari & Maresca, 2016). Unlike thermal processing, HPP induced protein denaturation can be reversible or irreversible depending on its concentration, pH, and the magnitude or duration of the applied pressure, which should be monitored carefully in industrial food processing (Acero-Lopez, Ullah, Offengenden, Jung, & Wua, 2012).

The effect of high hydrostatic pressure on the structural properties of globular proteins has been documented in denaturation, aggregation and gelation phenomena due to its ability to disrupt mainly the hydrophobic and disulphide interactions, and salt bridges at the interfaces of protein–protein or protein-peptide complexes. This in turn affects reversibly or irreversibly the conformational and structural characteristics of the molecule (Michel et al., 2001; Patel, Singh, Havea, Considine & Creamer, 2005; Puppo et al., 2011; Manassero, Vaudagna, Anon & Speroni, 2015; Savadkoohi, Bannikova, Mantri & Kasapis, 2016). Commonly, pressurisation below 200 MPa dissociates the quaternary structure of the protein and levels between 300 and 700 MPa alter its secondary structure. The latter range of pressurisation is able to expose inaccessible SH groups from the unfolded globular protein and encourage sulfhydryl-disulphide group exchange leading to successive steps of soluble aggregate, insoluble particle and gel formation (Tang & Ma, 2009).

Bovine serum albumin (BSA) is a globular protein, and a component of bovine whey protein and blood plasma. Hosseini-nia, Ismail & Kubow (2002) stated that due to the high number of disulphide bonds in BSA (17), application of low pressure (< 200 MPa) is unable to cause unfolding and subsequent aggregation of the protein, which remains in the native conformation "protecting" the hydrophobic groups in the interior of the molecule. Pressurisation at 300 MPa and above is able to

---

* Corresponding author.
E-mail address: stefan.kasapis@rmit.edu.au (S. Kasapis).

https://doi.org/10.1016/j.foodchem.2018.05.068
Received 4 March 2018; Received in revised form 14 May 2018; Accepted 14 May 2018

unfold and eventually aggregate BSA thus imparting desirable technofunctionality in food applications (Savadkoohi, Bannikova, Kasapis & Adhikari, 2014). The extent to which changes made on the secondary structure of BSA by pressure are irreversible depends on the physicochemical environment, concentration of the protein and extent of pressure treatment (De Maria, Ferrari & Maresca, 2015).

Gelatin, on the other hand, is a fibrous protein produced by the hydrolysis of cow or pig collagen. The effect of HPP on the structural and functional properties of gelatin depends on several factors including gelatin type/origin, pH, concentration, solvent quality and initial state due to industrial manufacturing. Montero, Fernandez-Diaz & Gomez-Guillen (2002) reported that there are differences in the gelation characteristics of unpressurized and pressurized samples in terms of the specific location on the gelatin chain and extent of non-covalent bond formation that stabilizes the three-dimensional structure. This distinct pattern of hydrophilic and hydrophobic interactions in the two types of networks results, for example, in pressure-induced gels with elevated melting temperature.

The present study applies hydrostatic pressure on preparations of BSA and gelatin in an effort to record their structural properties and relate them to the phase behaviour of their mixtures. The impact of pressurization on the network strength of formed gels, phase continuity in composite systems and water partition between the two polymeric phases that determines technofunctionality are examined and critically discussed with earlier results reported in the literature for binary blends subjected to a variable sequence of thermal tests. It is hoped that the work will assist food manufacturers to respond to the growing demand for safe and fresh-looking high-pressure processed meat products where BSA and gelatin feature as prominent components in formulations.

2. Experimental protocol

2.1. Materials

Type B gelatin was purchased from Sigma-Aldrich (Sydney, Australia). The material has an isoelectric point (*pI*) range of 4.8–5.0 with a bloom value of 210 and a weight average molecular weight of 280 to 310 kDa, as provided by the supplier. BSA was also obtained from Sigma-Aldrich (Sydney, Australia) and was purified using a heat shock fractionation method by the supplier. Its isoelectric point is 4.7, pH value of solutions is 6.9 and the molecular weight is ~66 kDa. Both polymers are negatively charged at the neutral pH of this investigation.

2.2. Sample preparation

BSA solutions (14, 16, 18, 20, 23 and 25% w/w) were prepared by dissolving the powder in Milli-Q water at ambient temperature with constant stirring on a magnetic stirrer for 2 h to ensure the formation of a homogenous dispersion. Samples were left overnight at 5 °C for proper hydration and the removal of air bubbles. Gelatin solutions (8, 10, 12, 14, 16, 18 and 20% w/w) were made by adding the powder to milli-Q water at ambient temperature, stirring with a magnetic stirrer for 30 min and hydrating overnight at 5 °C. That was followed by heating to 55 °C for 10 min to ensure proper dissolution of the polymer. The above polymer concentrations were chosen following preliminary experimentation in this work, which showed that at least 8% (w/w) gelatin and 14% (w/w) BSA were needed to produce cohesive gels under current conditions of pressurization.

Binary mixtures of the two hydrocolloids, on one hand, maintained the gelatin concentration at 8% (w/w) and varied that of BSA (8, 12 and 16%, w/w) and, on the other hand, maintained the BSA concentration at 14% (w/w) and varied that of gelatin (8, 12 and 16%, w/w). Samples were made by preparing initial (stock) solutions of the individual components at concentrations twice those analysed in the binary mixtures of this work and mixed 50:50 at 40 °C, i.e. at a temperature that both polymers remain in solution, and stirred for 15 min prior to pressurization.

2.3. High pressure processing

That was carried out using a Stansted ISOLAB FPG11501 High Pressure 3.6 L unit (Stansted Fluid Power Ltd., Stansted, Essex, UK), as described elsewhere (Knoerzer, Buckow, Sanguansri, & Versteeg, 2010). The vessel has a permitted initial temperature range of −20 to 110 °C for pressure levels up to 800 MPa. The temperature range that can be then reached and maintained inside the vessel during pressure holding extends to 130 °C, which is useful for ensuring a complete microbial kill. A deionised water/propylene glycol mixture (40% propylene glycol) was used as the pressure transmitting medium.

Samples were pressure treated for 15 min at 300 MPa at 10, 40 and 80 °C. The samples, and the water/propylene glycol mixture in the vessel, were conditioned to an initial temperature that would result in the target temperature during compression testing (Knoerzer et al., 2010). Conditioning times of samples were short (< 1 min) but varied slightly depending on the applied temperature conditions. The compression and decompression rates were set to 800 and 1400 MPa/min, respectively. Temperature of the compression fluid and the examined sample was monitored using type T thermocouples, which were attached to the sample carrier (Knoerzer, et al. 2010). All treatments were duplicated on different days to produce consistent outcomes.

2.4. Rheological measurements

Those were performed using a stress controlled rheometer (Paar-Physica MCR 302, Anton Paar GmbH, Graz, Austria), with measurements being conducted at 10, 40 and 80 °C. Parallel plate geometry (25 mm) and a gap of 5 mm were used throughout the experimentation. A thin layer of silicon fluid (50 mPa s) was applied to cover the exposed edges of the sample to minimize moisture loss during the rheological procedure. Following pressure treatment for 15 min at 300 MPa, samples were removed from the vessel, temperature was maintained, and they were immediately subjected to frequency sweeps within 0.1 to 100 rad/s at the target temperature with a constant strain of 0.1%, which is within the linear viscoelastic region (LVR).

Thermal gelation of gelatin samples was implemented by cooling from 40 to 10 °C (required to induce network formation), kept there for 60 min, followed by the standard frequency sweep. Complementary thermal gelation of BSA samples was carried out by heating from 40 to 80 °C (required to induce BSA denaturation), kept there for 10 min, followed by a frequency sweep from 0.1 to 100 rad/s. A heating/cooling rate of 2 °C/min was used and measurements were taken at 0.1% strain. Experiments were carried out in triplicate obtaining essentially overlapping mechanical spectra.

2.5. Fourier transform infrared spectroscopy (FTIR)

FTIR measurements of pressurized and heat-treated preparations were carried out from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and averaged over 64 scans using an instrument from PIKE Technologies, GladiATR™, Fitchburg, USA. To identify the characteristic signals that represent the secondary structure of both proteins, we focused on the absorption spectra at the amide I region (1700–1600 cm^{-1}) and signal deconvolution was implemented with the Opus 7.1.5 software from Bruker Optics Corp (Billerica, MA). All measurements were performed in triplicate yielding effectively identical results.

2.6. Environmental scanning electron microscopy (ESEM)

Micrographs of pressurized and heat-treated samples were obtained, using the FEI Quanta 200 ESEM (Hillsboro, Oregon, USA), in order to visualize the network morphology and phase topology of single and mixed systems. In doing so, they were rapidly cooled to subzero