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Quantitative analysis of the network structure that underlines the transitioning in mechanical responses of pea protein gels

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ABSTRACT

The objective of this study was to analyze quantitatively the network structure that underlines the transitioning in the mechanical responses of heat-induced pea protein gels. To achieve this, gels were prepared from pea proteins at varying pHs from 3.0 to 4.2 at a fixed 100 mg/mL protein concentration. Gels were also prepared by varying the protein concentration from 100 to 150 mg/mL at a fixed pH 3.0. Mechanical deformation properties of the gels were determined. An increase in protein concentration at a fixed pH resulted in an increase in fracture stress and Young's modulus. Variation of the pH at a fixed protein concentration resulted in transitioning in mechanical responses such as fracture stress, fracture strain, and the recoverable energy. The network structures were visualized by the use of confocal laser scanning and scanning electron microscopy, and the characteristic length scales of these structures were quantitatively analyzed in terms of the pair correlation function. Variation of the protein concentration at a fixed pH did not significantly alter the microstructure of the gels, whereas variation of the pH at a fixed protein concentration resulted in significant changes in the gel structure. Structural transitioning was shown to occur around pH 3.7. The findings from this study show transitioning in rheological responses of pea protein gels occur as a result of structural changes. The results from this study offer opportunities to broaden the application of pea proteins in food products, as products with desirable rheological (textural) and structural properties can be designed from pea proteins.

1. Introduction

Storage proteins of pea (*Pisum sativum* L.) have a considerable potential to be used in the food industry for the formulation of new food products as a better accessible and sustainable alternative to animal proteins supplies (Gharsallaoui, Saurel, Chambin, & Voilley, 2012; Pimentel & Pimentel, 2003). However, the inability to structure plant proteins such as from pea into desirable textures can limit their application (Manski, van der Goot, & Boom, 2007).

Gel formation by globular proteins is of great importance in the generation of texture in food. Heating globular proteins above their denaturation temperature results in partial unfolding of the proteins. This exposes interaction sites, giving rise to intermolecular interactions, eventually resulting in clustering of protein aggregates to form a spatial gel network (Clark, Kavanagh, & Ross-Murphy, 2001). At sufficiently high protein concentrations, these gels can be self-supporting, and large deformation and fracture behavior can be studied (Munialo, van der Linden, & de Jongh, 2014). Large deformation and fracture properties are important quality characteristics that determine the functional properties of gels such as cutting, handling, oral processing, and sensory/mouthfeel perception (van den Berg et al., 2008; van Vliet, 1996).

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The fracture and macroscopic breakdown properties of gels can be explained by the energy balance in materials (Çakir et al., 2012). When gels are subjected to deformation, all supplied energy is either stored (van Vliet, 1996) or dissipated during deformation (van Vliet, Luyten, & Walstra, 1991). Some of the processes that lead to dissipation of energy are via fracturing events at length scales larger than that of microstructures that make up the spatial network, the de-bonding of physical contacts that form the network, friction between microstructural elements during applied deformation, viscous serum flow of the liquid that is entrapped by the network, plastic deformation, and the relaxation of the structure elements. The detailed explanation of how these dissipation modes contribute to energy loss can be found elsewhere (de Jong, van Vliet, & de Jongh, in press). The energy that is stored elastically during deformation is referred to as the recoverable energy. The recoverable energy is one of the main characteristics that define the fracture characteristics of food gels by controlling energy balance within the gel structure during deformation (Çakir et al., 2012; Munialo, van der Linden, et al., 2014). The recoverable energy has been correlated to crumbly perception of food gels (van den Berg et al., 2008).

The properties of protein gels are influenced by a number of interrelated factors such as: protein concentration, pH, and ionic strength during gel formation (Vardhanabhuti, Foegeding, McGuffey, Daubert, & Swaisgood, 2001). The strength of protein gels has been shown to increase with an increase in protein or solid matter content (Thompson, Boland, & Singh, 2009). Whereas, depending on the gelling conditions, globular proteins such as pea proteins form gels varying in appearance from transparent to opaque (Munialo, van der Linden, et al., 2014). With increasing the pH towards the isoelectric point (IEP) of the protein, the formation of gels with coarse-strands is promoted. Coarse-stranded gels are composed of particulate aggregates (Ikeda & Morris, 2002), and have an opaque appearance (Munialo, van der Linden, et al., 2014). Contrarily, gels formed at a low ionic strength, and at pH away from IEP are fine-stranded with transparent appearance (Langton & Hermansson, 1992).

The mechanical deformation properties of protein gels at varying conditions have been reported before. A significant increase in stiffness of gels made from whey protein was reported with an increase in protein concentration from 60 to 100 g/L (w/v) (Hongsprabhas, Barbut, & Marangoni, 1999). Changes in fracture properties of protein gels with changes in pH have also been reported. At pH < 4.5, whey proteins were shown to form particulate gels that had a rather adhesive texture (Gwartney, Larick, & Foegeding, 2004). Whey protein gels prepared at pHs 2–4 were reported as being weak, and brittle, whereas gels that were formed around neutral pH were described as being strong and elastic (Errington & Foegeding, 1998). Pea protein gels formed at varying pH and ionic strength that showed differences in the rheological behavior have also been reported (Gueguen, Chevalier, And, & Schaeffer, 1988; Munialo, van der Linden, et al., 2014; Shand, Ya, Pietrasik, & Wanasundara, 2007; Sun & Arntfield, 2010, 2011). However, the precise pH range at which structural transitioning of pea protein gels occurs, and the effect of varying pH and protein concentration on the resultant rheological responses of the formed gels has not yet been reported.

The structural changes between fine and coarse-stranded gels have been reported to occur over a range of pH and ionic strength in systems containing globular proteins such as whey proteins (Ako, Nicolai, Durand, & Brotons, 2009; Mehalebi, Nicolai, & Durand, 2008). A sharp transition from transparent to opaque gels was observed in 10% w/v beta-lactoglobulin (β-lg), when pH was increased from pH 4.1 to 4.3 or decreased from 5.7 to 5.3 (Ako, Nicolai, et al., 2009). However, studies on the structural transitioning of pea protein gels over a narrow range of pH, and at varying protein concentrations have not yet been reported.

To be able to determine the transitioning in mechanical responses of pea protein gels as a result of changes in the network structure, various microstructures were generated in this study. The aim of this study was to quantitatively characterize the network structure that underlines the transitioning in the mechanical responses of heat-induced pea protein gels. To realize the structural changes, two approaches were chosen to affect the morphology, (i) via pH variations from pH 3.0 to pH 4.2 at a fixed protein concentration (100 mg/mL) (where electrostatic interactions are changed as a result of changes in the pH), and (ii) via changes in the protein concentration from 100 to 150 mg/mL at a fixed pH 3.0 (where kinetics of gel formation may be altered due to changes in protein content). The variation of the pH prior to gelation was chosen based on a preceding study where gels prepared at pH 3.0 were reported to be translucent, and to have somewhat low fracture stress, high fracture strain values, and high recoverable energy, characteristics of a fine-stranded network (Munialo, van der Linden, et al., 2014). Subsequently, the rheological and structural properties of the formed gels were evaluated.

2. Material and methods

2.1. Material

Pre-dried de-hulled commercial green pea seeds with a protein content of 22 g/100 g (dry weight basis) were purchased from a local supermarket. Rhodamine B, acetone, dimethyl sulfoxide (DMSO), glutaraldehyde, NaOH pellets, HCl, and NaCl were all purchased from Sigma–Aldrich (Steinheim, Germany). Paraffin oil was purchased from Merck (Darmstadt, Germany). Omnifix® syringes were obtained from Braun (Melsungen, Germany). All materials were of analytical grade and used without further purification. All solutions were prepared with MilliQ water.

2.2. Pea protein extraction

Pea protein isolate was extracted from commercial green peas by isoelectric precipitation according to a previously described procedure (Munialo, Martin, van der Linden, & de Jongh, 2014; Munialo, van der Linden, et al., 2014). The pea protein isolate extracted in this way will be referred to as 'native' pea protein. The protein content of native pea protein was determined using the Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25, and found to be 123.9 ± 1.3 mg/mL. The dry matter content of native pea protein was 12.6 ± 1.2%. Conductivity measurements showed that native pea protein isolate at pH 7.0, following the washing steps during protein extraction had an ionic strength comparable to that of about 0.02 M NaCl. Stock solutions of pea protein were prepared by concentrating native pea protein isolate to a final concentration of 150 mg/mL by ultrafiltration using a Merck Millipore Amicon stirred cell fitted with a 30 kDa molecular weight cut-off membrane (Darmstadt, Germany).

2.3. Characterization of pea proteins

The thermal denaturation of the proteins in native pea protein solutions was evaluated by a TA Q100-Tzero™ differential scanning calorimetry thermal analyzer (TA Instruments, New Castle, DE, USA) according to a previously described procedure (Munialo, van der Linden, et al., 2014). The calorimetric analysis of native pea proteins showed a single peak denaturation temperature (T_d) of 84.9 ± 1.6 °C, on-set temperature of denaturation (T_m) of 74.3 ± 2.1,