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Interaction of selected volatile flavour compounds and salt-extracted pea proteins: Effect on protein structure and thermal-induced gelation properties

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ABSTRACT

Characterization of the impact of protein–flavour interactions on protein structure and thermally-induced salt-extracted pea protein gels with selected volatile flavours was studied using a fluorometric method (intrinsic) and small-strain dynamic oscillatory rheology. By monitoring intrinsic fluorescence of protein–flavour mixtures, conformational changes in proteins due to flavour binding were noted with long chain aldehydes resulting in higher degrees of protein unfolding in comparison with ketones. Protein gel forming properties were significantly altered as a function of flavour class (aldehyde and ketone), chain length (6–8 carbon number) and flavour concentrations (0, 250, 500, 1000 ppm). Addition of homologues aldehydes and ketones at 250 ppm decreased gel storage (G′) and loss (G″) moduli with long chain aldehydes possessing more prominent effects. Interestingly, protein gel strength was restored with increasing concentration and chain length of aldehydes accompanied by gradual decreases of gelling points, while elasticity and gelling points of protein–ketone mixtures remained constant. The additional protein denaturation observed in the fluorometric study could account for the formation of stronger gels during thermally-induced gel formation. A flavour-induced protein structure/function relationship was presented. In addition, flavour binding produced changes in G′ during both heating and cooling phases of gelation. For aldehydes, the change in G′ during heating had a more predominant effect, whereas the cooling phase was more responsible for the decrease in G′ for ketone flavours.

1. Introduction

Pulse proteins including those from dried peas, edible beans, lentils and chickpeas have been recognized as potential ingredients in manufacturing novel protein foods and natural health products (Boye & Maltais, 2011). In Canada, about 5.7 million tonnes of pulses were produced in 2010 and a record of 4.7 million tonnes of pulses was exported in 2011 (Pulse Canada, 2014a). From a nutritional perspective, pulses contain high amount of proteins ranging from 17 to 30% which is twice the amount of protein found in whole grain cereals (Boye, Zare, & Pletch, 2010; Pulse Canada, 2014b). More than that, some functional properties of pulse proteins have shown promise and were comparable to those of animal and plant proteins such as whey and soy proteins (Boye et al., 2010).

Although various benefits of pulse proteins have been recognized, it has been shown that when adding volatile flavouring compounds to plant protein-based food matrices, proteins could interact with flavouring components causing changes in desirable flavour profiles often leading to reductions in aroma intensity (Gremli, 1974; Heng et al., 2004; Suppavorasatit & Cadwallader, 2012; Suppavorasatit, Lee, & Cadwallader, 2012; Wang & Arntfield, 2014, 2015a, 2015b, in press; Zhou, Lee, & Cadwallader, 2006) and generation of potential volatile flavour by-products (Kühn, Considine, & Singh, 2008; Wang & Arntfield, 2014).

When looking at the interactions between protein and flavours, most researchers focused on exploring retention and release of flavours as affected by a number of intrinsic (e.g. structure and composition of proteins and flavours) and extrinsic (e.g. heat, pH, salt and high pressure treatment) factors (Tromelin, Andriot, & Guichard, 2006; Suppavorasatit & Cadwallader, 2010). Little work has been conducted on how protein–flavour interactions affect protein structure and functionality. As protein

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functionality strongly impacts how proteins can be utilized in the food systems, the potential influence of flavouring components on protein behaviour and resulting functionality must not be overlooked.

Considering the nature of protein–flavour interactions, the mechanisms underlying the phenomena are still not explicit (Wang & Arntfield, 2014). One perspective to gain further insight into the nature of these interactions is to monitor protein–-flavour binding using small hydrophobic ligands and spectroscopic techniques. As spectrofluorometric method has been extensively used in protein–ligand binding studies (Damodaran & Kinsella, 1980; Dufour & Haertlé, 1990; Liu, Powers, Swanson, Hill, & Clark, 2005; Meynier, Rampon, Dalgalarrondo, & Genot, 2004; Muresan, van der Bent, & der Wolf, 2001), the wavelength shifts (λ_{max}) and changes in fluorescence intensity (FI) of emission peak of protein tryptophan residues can be used to monitor the environmental changes of these residues in proteins, consequently providing information on protein structure (Cho, Batt, & Sawyer, 1994). To date, no systematic comparison of the influence of aldehydes and ketones on protein structure has been made before using this spectrofluorometric technique.

Protein thermal gelation is an important functionality for plant proteins and is influenced by protein structure (Moure, Sineiro, Domínguez, & Parajó, 2006; Sun & Arntfield, 2010). Limited information has been found on performance of proteins in gel formation in the presence of volatile flavour compounds. It was shown that protein–flavour interactions involve a wide range of linkages, including reversible interactions (hydrogen and ionic bonds, van der Waals forces and hydrophobic interactions) and irreversible covalent linkages (−NH_2 and −SH groups of proteins) (Kim & Min, 1989; Kühn, Considine, & Singh, 2006; Reineccius, 2006). Hydrophobic interactions and hydrogen bonding are the major forces responsible for gelation of pea proteins (O'Kane et al., 2004; Sun & Arntfield, 2012). In thermally induced protein gel formation with flavour added, it is possible that interactions between flavour molecules and proteins could disturb the intermolecular hydrophobic interactions between protein molecules that contribute to gel formation. As a result, a weaker gel could be formed. In addition, it can be further speculated that the irreversibly bound aldehyde flavours would be less likely to be affected by the heating process and would therefore have a greater chance of influencing protein gel formation than the reversibly bound ketone flavours.

The objective of this research was to evaluate the rheological characteristics of heat-induced PPIs gels in a mixed system with selected volatile flavour compounds. Both aldehydes and ketones were chosen to address the potential reversible and irreversible binding effect. In addition, protein conformational changes as a function of flavour added were monitored using a spectrofluorometric method. The potential correlation between the effect of flavour binding on protein structure and associated changes in protein thermal gelation properties were evaluated and discussed.

2. Materials and methods

2.1. Source of materials

Homologous series of aldehyde (hexanal, heptanal, and octanal) and ketone (2-hexanone, 2-heptanone, and 2-octanone) flavours were selected and purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Commercial yellow pea (*Pisum sativum* L.) flour was kindly supplied by Best Cooking Pulses Inc. (Portage la Prairie, MB). All chemicals used were of reagent grade if not stated otherwise.

2.2. Preparation of salt-extracted pea protein isolates (PPIs)

Following the method previous described by Wang and Arntfield (2014, 2015a), salt-extracted pea protein isolates (PPIs) was extracted from sieved (500 μm opening, USA Standard NO. 35) yellow pea flour using 0.3 M NaCl (pea flour: sodium chloride solution = 3:10, w/v) under constant stirring for ½ hour. After the first centrifugation (4260 *g*, 4 °C, 15 min), pea protein was recovered by diluting the supernatant in two times its volume of cold distilled H_2O. After leaving in a refrigerator (3 °C) for 2 h, the precipitated protein sediment was collected after a second centrifugation (680 *g*, 4 °C, 15 min) and re-suspended in a small amount of distilled H_2O. The resulting protein suspension was then dialysed against 20 times cold distilled H_2O using 12–14,000 Da molecular weight cut-off dialysis tubing (Spectra/Por Dialysis Membrane, Rancho Dominguez, CA) for 72 h in a refrigerator. Distilled H_2O was changed every 24 h. The desalted protein isolates were stored at −30 °C until freeze dried (Genesis SQ Freeze Dryer, Gardiner, NY, USA).

By using an N to protein conversion factor of 5.7 (Sun & Arntfield, 2010), the freeze dried PPIs contained 82.68% protein using a Dumas method and an FP-528 Nitrogen/Protein Determinator (LECO Corporation, St. Joseph, MI, USA). The PPIs contained 3.53% (w/w) of crude fat content determined using the AOAC Official Method 2003.06 (2003) using Soxhlet apparatus.

2.3. Amino acids composition of PPIs

Prior to spectrofluorometric analysis, the amino acid composition of PPIs was determined to ensure the existence of hydrophobic amino acids such as tryptophan residues; this was done using an amino acid analyser (Sykan Germany, Model S2100, S4300, Gewerbering, Eresing). After samples were hydrolysed in 6 M HCl, the amino acid profiles of PPIs were determined following AOAC Official Method (Method 994.12; AOAC, 1995). Contents of cysteine and methionine were obtained by an oxidized hydrolysis procedure with performic acid (Andrews & Baldar, 1985). Tryptophan content was measured after alkaline hydrolysis (Hugli & Moore, 1972).

2.4. Preparation of flavour stock solutions

Stock solutions of each volatile flavour compound (hexanal, heptanal, octanal, 2-hexanone, 2-heptanone and 2-octanone) were prepared in 0.3 M NaCl solution at 1500 ppm (0.15 mL/100 mL) and sealed in amber gastight glass bottles to prevent decomposition. Following the method of Gkionakis, Taylor, Ahmad, and Heliopoulos (2007), flavour stock solutions were put in an ultrasonic water bath (Branson 3200, 50 Hz, Bransonic Ultrasonic Cleaner, Shelton, CT, USA) for 1 h to ensure a thorough mixing before each use. Previous headspace GC/MS analysis (Wang & Arntfield, 2014, 2015a, 2015b, in press) found that no change of flavour structure/composition was detected after this ultrasonication step while a significant and stable amount of flavour was retained in aqueous phase of flavour stock solutions.

2.5. Spectrofluorometric measurement

Potential conformational changes to PPIs upon flavour binding were followed by monitoring intrinsic fluorescence of tryptophan residues. Following the reaction conditions previously described by Wang and Arntfield (2014), 10% (w/v) PPIs suspension was prepared in 0.3 M NaCl at the desired flavour concentration, followed by completely mixing the protein–flavour mixture on an RKVSD rotary shaker (Appropriate Technical Resources, Inc., Laurel, MD) for 1 h at speed 40 to allow an interaction between protein and