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Microstructure of canola protein-guar gum gels: A bench note

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ABSTRACT

Plant proteins contribute to structure of food products via the formation of three-dimensional matrix which can entrap water and other food components. Establishing the structural factors responsible for gelation of canola protein isolate (CPI) in food systems will enhance the understanding of texture development in foods containing plant protein ingredients. Microstructural assessment was performed using scanning electron microscope. The influence of pH (6, 10) and 0.15 M dithiothreitol on the molecular forces responsible for structural alignment of CPI-guar gum gels was examined. CPI-guar gum gels prepared at optimum gelling condition (20 % w/v CPI, 1.5% w/v guar gum, 0.05 M NaCl, pH 10) had strong beaded strands. Evidence from gel micrographs showed improved network structure when CPI and guar gum are mixed (due to protein-protein and protein-polysaccharide interactions) and of complete breakdown of CPI-guar gum network structure (seen as excessive disrupted gel microstructure) in mixed gels containing dithiothreitol. Canola protein-guar gum microstructure may provide insight on the mechanisms and basis of texture formation in mixed foods containing proteins. These findings would allow newer protein sources such as canola protein to compete effectively with other predominant plant proteins such as soy proteins.

KEYWORDS

Guar gum;
Canola protein;
Gel microstructure.

INTRODUCTION

Recent interest in protein gelation studies has been on the microstructure and fracture properties of gels. However, appearance, water-holding attributes and intragel diffusion (textural properties of gels) have also been studied because these aspects have direct application to food quality. Food products can be augmented by the addition of proteins as gelling agents, e.g. the addition of milk proteins to water-added hams as a means of increasing water-holding and rheological properties[7]. Some food products that rely on gelation of denatured proteins to produce desirable attributes are processed meats, baked goods, cooked eggs and some cheese. Understanding the physical/chemical basis for the attainment of quality characteristics of food protein gels is a valuable tool when adding proteins in multicomponent food formulations.

Microscopy can be a useful tool when describing the microstructure of a system at the end of the gelation process. The use of scanning electron micros-

copy (SEM) to examine the structure of globular protein products have been reported[5,9,16,17]. A review article by Foegeding[7] stated that heat-induced globular protein gels can be transparent, turbid or opaque depending on the gelation conditions. Gels that are transparent or translucent are generally classified as fine-stranded (also termed fibrillar, 'strings of beads' or 'true' gels). They are formed under conditions of pH greater or less than the isoelectric point (IEP), and at low ionic strength. Fine-stranded (a term that describes microstructures as consisting of strands of various lengths but with diameters corresponding to the length of one to several molecules) gels formed at low and high pH have different textural properties. Foegeding[7] made the following observations: 1) gels formed at low pH are weak and brittle; 2) those formed at high pH are strong and elastic; while 3) opaque gels display particulate gel structure (also termed coarse-aggregated, random-aggregated or coagulated). Particulate gels are formed under conditions where there is minimal charge repulsion, such as at pH close to IEP or at high ionic strength. The microstructure of particulate gels consists of spherical particles (with diameters in the micrometer range) that are associated into a gel network. There exist conditions that lie between the two cases that produce gels with structures that can be considered as either (i) a combination of stranded and particulate or (ii) distinct and intermediate between these extremes[7,8]. The type of gel formed depends on conditions during gel formation. Generally, gels become coarser as the pH approaches the IEP or when the ionic strength increases[6]. The use of SEM to examine the network structure of CPI and CPI-guar gum gels is a valuable application of this technique in characterizing the network properties of CPI in a mixed food system.

The purpose of this inquiry is to characterize CPI-guar gum gels using SEM to assess the network structure as influenced by pH and denaturant. It is hypothesized that high alkaline pH (e.g., pH 10) will cause excessive disruption and breakdown of CPI-guar gum gel network. In the assessment of CPI-guar gum mixture, structures developed during gelation and type of network arrangement was used to evaluate the effect of denaturant.

MATERIALS AND METHODS

Source of materials & sample preparation

Commercial canola protein isolate (CPI) was purchased from BMW Canola, Winnipeg, Canada and used without further purification. Proximate analysis[1] of the CPI sample indicated a protein content of 87% (N × 5.7), 0.7% oil, 2% ash, 5.9% moisture and 4.4% total carbohydrate (determined by difference). Food grade guar gum (G-4129; Lot No. 95H0653) and dithiothreitol (DTT; D-0632; Lot No. 61K16571) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals such as NaCl (BP358-212; Lot No. 028091), HCl (Lot No. 296220; A144-225) and NaOH (BP359-212; Lot No. 974661) were certified reagent grade (Fisher Scientific Co., NJ).

Dispersions of CPI (15% w/v CPI, protein-basis; 0.05 M NaCl; pH 6), guar gum (1.5%, w/v guar gum; 0.05 M NaCl; pH 10) and CPI-guar gum (20%, w/v CPI; pH 10; 0.05 M NaCl; 1.5%, w/v guar gum) mixtures were prepared separately at optimum gelling conditions established in a previous study[15]. Macromolecular dispersion was stirred for approximately 1 hr at room temperature or until a complete dispersion of mixture was achieved. Dispersions of CPI or guar gum sample in NaCl solution were prepared separately in similar manner to serve as control. Interaction assessment was performed by treating CPI-guar gum mixture (20%, w/v CPI; 1.5%, w/v guar gum; pH 10) with 0.15 M DTT (a disulfide blocker) prior to heat treatment. Samples were allowed to equilibrate for 30 min at room temperature (22-25°C) to maintain accurate pH.

Mixed macromolecular gel preparation and microstructural analysis

Design-Expert® Software (Stat-Ease Inc., MN) was used to generate model experimental design with factorial and response surface optimization. Factors examined were pH (6, 8, 10), CPI (10, 15, 20% w/v), NaCl (0.05, 0.15, 0.25 M) and guar gum (1, 2, 3% w/v) concentrations. Model fitting was performed using optimization model (Design-Expert® Software) to determine optimum conditions that produced improved gel properties for CPI-guar gum mixtures: 20 % (w/v) CPI, 1.5% (w/v) guar gum, 0.05 M NaCl, pH 10[15]. Confirmatory analysis was performed to verify the opti-

mum gelling conditions obtained. Influence of molecular interactions on gel formation was assessed by treating CPI-guar gum mixture with 0.15 M DTT at established optimum conditions stated above. Control tests were run with CPI (20%, w/v + 0%, w/v guar gum) or guar gum (1.5%, w/v + 0%, w/v CPI) dispersion at optimum pH and NaCl concentration. All experimental parameter measurements were done in duplicates.

Gel networks were prepared as described by Léger and Arntfield[10] with slight modification. The optimum gelling conditions established for CPI-guar gum gels were used to prepare gels used for microstructural assessments. Heat-set CPI, guar gum, and CPI-guar gum gel networks were prepared by heating sample dispersions in closed stainless steel vials from 25 to 95ºC at a rate of 2ºC/min. At the end of the heating regime, samples were held at 95ºC for 5 min and cooled to room temperature in an ice bath. Gels were removed from the vials, frozen and freeze dried. Dried gel samples were fractured and mounted on a specimen stub with double-sided adhesive tape. Samples were sputter-coated (Edwards Sputter Coater, model S150B) under vacuum with gold/palladium (75/25). Scanning electron microscopy (SEM) measurements were done on a JEOL JSM-5900LV SEM (JEOL SEM, Japan) operating at an acceleration voltage of 10 kV. Micrographs of each gel sample were obtained at ×3000 magnification. All experimental measurements were done in duplicates.

RESULTS AND DISCUSSIONS

Structure of canola protein-guar gum networks and effect of disulfide blocker

Canola protein gel prepared at pH 6 displayed a fluffy, honey-comb microstructure (Figure 1A). The structural arrangement of CPI-guar gum gel prepared at optimum conditions (20% canola protein, 1.5% guar gum, 0.05 M NaCl, pH 10) showed an elastic crosslink with strong bead-like strands (Figure 1B). Although pH 10 is unconventional in food manufacture, some food systems are either prepared at or have alkaline pH; e.g., egg albumin which has a pH of 9.0[2] and Asian alkaline noodles which are prepared at high pH conditions (to produce texture and color desired by consumers).

Variation in pH affects the overall net charge on the protein molecule as well as its molecular conformation and structural arrangement. CPI gel prepared at pH 6 (Figure 1A) had a soft, spongy network with large openings between loosely-linked mesh-like structure. The large openings produced gave some insight on the water-holding capacity of canola proteins. Water and other components may have been immobilized in those pockets during gelation. This is an important mechanism of gel formation in food systems. The canola protein-guar gum gel prepared at pH 10 formed a firm, fine-stranded and elastic structure (Figure 1B). Photomicrographs of laboratory prepared CPI gels showed improved gel properties at alkaline pH[10]. The irreversible, heat-set gelation or coagulation of proteins (alone or in mixed form) often controls the sensory (texture, mouthfeel) acceptability of most cooked food products.

The pH and ionic strength of a protein environment can alter the charge distribution between amino acid side chains which may decrease or increase the protein-protein interaction. At pH 6, below the isoelectric point (IEP) of canola proteins (IEP=6.8-7.2)[13], net positive charge was dominant. As a result, attractive intermolecular electrostatic interaction should be maximized in the presence of 0.05 M NaCl. At low salt concentrations, ions interact with proteins via nonspecific electrostatic interactions; indicating that electrostatic neutralization of protein charges usually stabilizes protein structure[14]. The weak, spongy structure of CPI gel seen at pH 6 (Figure 1A) was not as strongly-knit as that observed in CPI-guar gum gels at pH 10 (Figure 1B). It is possible that the range for the attractive-repulsive electrostatic interaction balance necessary for a tightly-crosslinked three dimensional gel was maximized at alkaline pH to produce tight-beaded strands.

A balance between the charge repulsion and the potential for interaction, mainly via inter-protein and/or inter-biopolymer hydrogen bonds and hydrophobic interactions, is critical to the formation of network characteristics. Excessive attractive forces or insufficient charge repulsion results in coagulation rather than network formation.

The CPI-guar gum gel treated with DTT (Figure 1C) had an amorphous structure and lacked the characteristic strands and crosslinks usually depicted in gel systems. This probably confirms the disruption of disulfide linkages by DTT. As a reducing agent, DTT is

capable of disrupting existing disulfide linkages, thus causing destabilization and modification of protein's native conformation[4]. Reports on the effect of DTT on protein gelation have been variable. Some findings showed enhanced gel network[10,12] while some indicated poor network formation[11]. The findings on CPI-guar gum gel treated with DTT show formation of an amorphous mass that support the contribution of covalent and noncovalent forces in gelation of mixed macromolecular system. Evidence of significant structural modification in micrograph of CPI-guar gum gel treated with DTT shows involvement of disulfide bonds in network formation of this system.

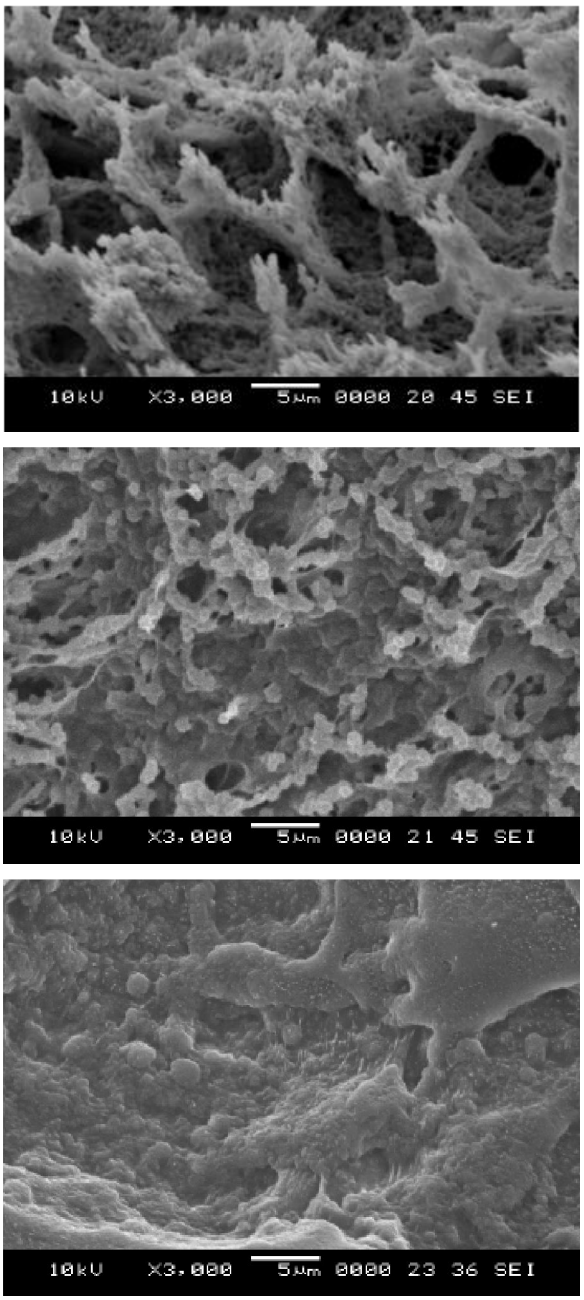

Figure 1 : Scanning electron micrographs of gels made from canola protein isolate (CPI) and its mixture. Figure 1A depicts CPI gel at pH 6 (15% w/v CPI, 0.05 M NaCl) while 1B shows CPI-guar gum gel prepared under optimum conditions for the mixture (pH 10; 1.5%, w/v guar gum; 20%, w/v CPI; 0.05 M NaCl). Figure 1C shows CPI-guar gum gel treated with 0.15 M DTT (pH 10; 20%, w/v CPI; 1.5%, w/v guar gum); Magnification ×3000.

CONCLUSION

Microstructural data on CPI-guar gum gel showed improved network structure at high pH due to protein-protein and protein-polysaccharide interaction. This does not support the hypothesis of this study. Mixed macromolecular gels treated with disulfide blocker showed disrupted network structures, indicating that covalent and noncovalent interactions are important factors in CPI-guar gum gelation. Mixed gels such as canola protein-guar gum network can enhance the texture and structural integrity of multicomponent food systems.

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