Evaluation of Canola Protein Functionality in Mixed Food Systems

By

Florence Ojiugo Uruakpa

A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirement
for the Degree of

DOCTOR OF PHILOSOPHY

Food and Nutritional Sciences
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Oilseed proteins contribute useful functionality to food systems. Knowledge of the molecular interactions between plant proteins such as commercial canola protein isolate (CPI) and hydrocolloids such as κ-carrageenan (κ-CAR) and guar gum will allow the manipulation of physical and textural properties of mixed biopolymers by adjusting the interaction in a desirable way. This research investigates the molecular interactions involved in gelation and emulsification of these CPI-hydrocolloid systems. The objectives were to: a) determine the functional and physicochemical properties of CPI-κ-CAR and CPI-guar gum mixtures; b) determine the type and degree of interactions between CPI and κ-CAR or guar gum based on protein conformational changes assessed by differential scanning calorimetry, fluorescence spectroscopy and microscopy; and c) establish optimum conditions for gelation and emulsification of these systems. Gelling characteristics and emulsifying properties were evaluated using dynamic rheology and spectroturbidimetry, respectively. This study has 1) characterized the gel properties of CPI-κ-CAR and CPI-guar gum mixtures using dynamic rheology and microscopy; 2) optimized conditions for specific functional properties such as network and emulsion formation. Optimum conditions for gelation of CPI-κ-CAR mixtures were pH 6, 3% (w/v) κ-CAR, 0.05 M NaCl, 15% (w/v) CPI; whereas those for CPI-guar gum mixtures were pH 10, 1.5% (w/v) guar gum, 0.05 M NaCl, 20% (w/v) CPI. Electrostatic complexing and synergistic interaction contributed to the formation of strong and elastic CPI-κ-CAR gels. The synergistic behaviour was supported by the microstructural data of gel networks. Furthermore, optimum conditions for CPI-κ-CAR-stabilized emulsions were pH 6, 1% (w/v) κ-CAR, 0.25 M NaCl, 10% (w/v) CPI; whereas those for CPI-guar

gum-stabilized emulsions were pH 10, 3% (w/v) guar gum, 0.25 M NaCl, 10% (w/v) CPI; 3) confirmed that hydrophobic interactions, hydrogen bonding and disulfide linkages are the main molecular forces involved in the formation and stabilization of CPI-κ-CAR and CPI-guar gum gels and emulsions; 4) reported improvements in the emulsifying properties and surface hydrophobicity of these CPI-hydrocolloid mixtures, when compared to CPI alone. Protein-polysaccharide interactions give a realistic indication of plant proteins behaviour in food systems and provide useful information for the development of functional canola protein products.

INTRODUCTION

Plant protein functionality in food systems can be affected by other components in that system. Studies on protein-polysaccharide interactions have shown that under some conditions, these interactions can result in improved gelation (Samant et al., 1993) and emulsification properties (Ledward, 1994). Understanding the interactions between components in food systems is a prerequisite for the incorporation of plant proteins such as CPI into conventional foods or the development of new foods. Interactions between proteins and polysaccharides may result in complex formation, homogeneous solutions and incompatibility (Tolstoguzov, 1998). Industrial significance of interactions between proteins and polysaccharides for protein recovery, protein stabilization and food texture modification has been documented (Ledward, 1979). The mechanisms of forming protein gelling systems by these interactions (Stainsby, 1980) and designing of new food systems (Tolstoguzov, 1995) based on these interactions have been reviewed. The type of association does not necessarily determine the contribution to the functional properties of the food. For example, thermally stable protein gels have been produced through electrostatic interactions between sodium alginate and sodium caseinate (Tolstoguzov, 1991). However, studies with canola protein revealed that the addition of low concentrations of guar gum improved protein gel characteristics; but not when methylcellulose or sodium alginate was included (Arntfield and Cai, 1998).

Canola is the term registered and adopted in Canada to describe the oil obtained from the seed cultivars *Brassica napus* and *Brassica campestris*. In 1986, the term canola was amended to refer to *B. napus* and *B. campestris* (now *B. rapa*) cultivars containing <2% erucic acid in the oil and <30 μmol/g glucosinolates in the air-dried, oil-

free meal (OECD, 2001). Canola seeds, the main sources of vegetable oil in Canada (Rubino et al., 1995), contain about 40% edible oil and the meal produced after oil extraction contains 40% protein (Shahidi, 1990). Canola protein has a well-balanced amino acid profile (Ohlson, 1978) and high protein efficiency ratio, PER (Jones, 1979). It does not contain the enzyme lipoxygenase that promotes oxidative rancidity in other oilseeds such as soybean. However, this meal with a well-rounded amino acid composition is predominantly used as animal feed due to the inherent antinutritional factors such as glucosinolates, phytates, phenolics and erucic acid (Ismond and Welsh, 1992). Nevertheless, canola meal has great potential as a protein source for human consumption.

So far, several studies have focused on isolation methods and evaluation of laboratory-prepared canola proteins. A number of protein isolation procedures, some of which avoid the use of extreme pH and organic solvents, have been developed for canola (Ismond and Welsh, 1992, Murray et al., 1981). Thermal gelation of the 12S canola globulin derived from commercial canola meal has been studied by Leger and Arntfield (1993). Dispersions of 12S rapeseed glycoprotein at pH $\geq$ 4 readily self-associated on heating with gelation at 4.5% protein and measurable thickening at 1% protein (Gill and Tung, 1978). Since it is necessary to move findings from the laboratory to the industry, there is a need to assess the functional properties of commercial CPI in multicomponent systems. This will enable CPI to compete effectively with other commercially available and widely used plant proteins such as soy protein products. In a food system containing proteins and other components, the interactions between the different components need to be well balanced so that a stable system evolves.

This study examines the structure and functionality of commercial CPI-κ-CAR and CPI-guar gum mixtures. The long-term objective was to determine the structure-function properties of protein-polysaccharide complexes using model food systems. The short-term objectives were to:

1. Determine the functional properties of CPI-κ-CAR and CPI-guar gum mixtures.
2. Assess the type of molecular interactions that contribute to the functionality of these mixed biopolymer systems.
3. Characterize these CPI-hydrocolloid systems with respect to gel formation, emulsifying ability, structural thermal stability, surface hydrophobicity and microstructure.
4. Examine the effects of pH and salt concentration on these functional properties.
5. Establish the optimum conditions responsible for enhanced network formation and emulsification of these systems.

REVIEW OF LITERATURE

Canola/rapeseed proteins and their preparation

Canola is the name adopted by the rapeseed industry in Canada in 1978 to identify rapeseed cultivars which are genetically low in both erucic acid and glucosinolates (McCurdy, 1990). Canola is widely grown, particularly in Canada, because it is a rich source of high quality edible oil (Shahidi, 1990). The meal obtained after oil extraction of rapeseed contains about 35-40% protein with a good balance of essential amino acids (Sosulski, 1983; Finnigan and Lewis, 1985; Klockeman et al., 1997) and a high protein efficiency ratio (Jones, 1979). Canola meal, predominantly used as an animal feed ingredient, has immense potential as a source of protein for human consumption. Interest in rapeseed as a protein source materialized in the sixties when it became generally appreciated that the essential amino acid composition of rapeseed protein compared favourably with that of soybean and the FAO/WHO protein reference pattern (FAO, 1992); and was corroborated by the findings of Klockeman et al. (1997). However, the presence of antinutritional factors (e.g. glucosinolates, phytic acid, phenolic compounds) in canola protein limits the possibility of using this protein as a food ingredient (Ismond and Welsh, 1992).

In a review on rapeseed, Mieth et al., 1983 classified protein products from *Brassica* seeds into three types which differ from one another in their composition, properties and possible applications: 1) seed meals (hull-rich products with <45% protein and dehulled products with >45% protein) are extracted from seeds and industrial meals; 2) protein concentrates (dehulled products with >60% protein and hull-rich products with <60% protein) are obtained by extracting antinutritive components of seeds or seed meal with aqueous and/or non-aqueous solvents; and 3) isolates (>90%

protein) are obtained by extracting proteins from seeds or seed meals and precipitation or ultrafiltration.

By modern processing, canola seeds are flaked, cooked and pressed to remove as much oil as possible. The press-cake is extruded to produce porous rope-like segments of material for solvent extraction. After the oil has been extracted, the solvent is removed, and the meal is dried, resulting in defatted canola meal (Pickard, 1997). Canola protein products are mostly made from defatted canola meal. There are three major types of canola protein products: protein fractions, concentrates and isolates.

Canola protein fractions

A study (Schwenke et al., 1983) on the structure of rapeseed globulin revealed that the 12S globulin (a main storage protein in the seeds of *Brassica species*) represents an oligomeric protein with a molecular weight of 300,000. It is composed of 6 subunits and each subunit contains smaller units (polypeptide chains) with molecular weights in the range of 18500 to 31200. The protein contains 4 polypeptide chains differing by their molecular weights in the sodium dodecyl sulfate (SDS)-electrophoresis: 18500 ± 800; 21100 ± 500; 26800 ± 900; 31200 ± 1600. According to its quaternary structure the protein dissociates under environmental (i.e. solvent) conditions.

The secondary structure of the protein is characterized by a low (11%) content of α-helix and a relatively high (31%) content of β-conformation. In a more recent study on the solubility profile of canola proteins, Klockeman et al. (1997) characterized the major proteins in canola seed as glutelins (91.8% soluble in 0.4% w/v NaOH) and globulins (50.9% soluble in 5% w/v NaCl), while prolamins (33.9% soluble in 60% w/v

ethanol) and albumins (31.2% soluble in distilled deionized water) were the minor proteins.

Canola protein concentrate

By analogy to soybean technology, rapeseed protein concentrate may be defined as the product prepared from high quality, sound, cleaned, dehulled rapeseed by removal of the oil and water-soluble nonprotein components. A water extraction process was developed (Jones, 1979) which removes over 90% of the glycosinolates and results in a protein concentrate having an essential amino acid balance that has been shown by nutritional evaluation to be superior to any other known oilseed. In this process, the dehulled seed fraction is subjected to a series of treatments: a) boiling water treatment to inactivate enzymes in the cotyledons capable of hydrolyzing glucosinolates to toxic compounds; b) water-leaching to extract water-soluble components, glucosinolates, problem sugars such as raffinose and stachyose, and phenol-like compounds such as sinapine; c) the extracted cotyledons are recovered, dried and hexane-extracted to yield a protein concentrate with 59.7-69.6% protein and 5.2-7.7% fiber, depending on rapeseed variety.

Canola protein isolate

The extraction and isolation of canola protein have been the focus of several investigations (Gillberg and Tornell, 1976; Diosady et al., 1984; Tzeng et al., 1988; Deng et al., 1990; Rohani and Chen, 1993; Xu and Diosady, 1994). Other studies on canola protein isolation are highlighted below. A process designed (Tzeng et al., 1990) to characterize rapeseed and canola proteins consists of extraction of oil-free meal (50-

52% protein) at pH 10.5-12.5, isoelectric precipitation to recover proteins and ultra-filtration followed by diafiltration to concentrate and purify the remaining acid-soluble proteins. Isoelectric and soluble protein isolates (with low phytate, light colour and bland taste) containing 87-104% protein (N × 6.25) and a meal residue were obtained from canola meal. A method termed protein micellar mass (PMM) procedure (Murray et al., 1978) was applied to the isolation of protein from canola (Ismond and Welsh, 1992). Meal samples were mixed in a buffer characterized by pH 5.5, 0.1 M NaCl/0.1 M NaH_2PO_4 (most successful environment in removing anti-nutritional factors), centrifuged, filtered, concentrated by ultrafiltration, diluted to 15 times its volume with cold (4°C) distilled water and left for at least 16 hr in cold storage (4°C). Insoluble protein micelles were collected by centrifugation and freeze-dried and the isolate has 79% protein.

The 12S globulin from canola meal was isolated (Léger and Arntfield, 1993; Arntfield and Cai, 1998) using a modified PMM technique. The meal was mixed with 0.1 M NaCl (pH 6.2) at a ratio of 1:10, and the isolate was obtained as outlined above. Klockeman et al. (1997) extracted more than 99% protein from defatted canola meal when a 5% w/v suspension in 0.4% w/v NaOH was at room temperature using baffled flasks on an orbital shaker at 180-200 rpm for 60 min. The extract was precipitated with acetic acid (pH dropped to 3.5) and the precipitate was washed and freeze-dried. Protein recovery was 88% upon precipitation with acetic acid. Aluko and McIntosh (2001) reported the preparation of acid- and calcium-precipitated protein isolates from canola seeds, using modified protocols of Klockeman et al. (1997) and Soetrisno and Holmes (1992). Defatted meals were mixed with 10 volumes (w/v) of 0.1 M NaOH solution and

stirred on a magnetic stirrer for 20 min at 23°C. The slurry was centrifuged (30 min at 8°C and 10000 × *g*) and the supernatant was filtered and precipitated as follows: For the acid-precipitated isolate (76-82% protein), pH was adjusted to 4.0 with 0.1 M HCl solution followed by washing to remove salts. For the calcium-precipitated isolate (73-83% protein), pH was adjusted to 6.0 with dilute HCl followed by addition of 1 M calcium chloride. The precipitate was recovered after centrifugation and freeze-dried.

Properties of selected polysaccharides

Guar gum

Galactomannans are seed polysaccharides of which guar gum, locust bean gum and tara gum are well known as food thickeners (Tuinier et al., 2000b). They have a main chain of 1,4-β-D-mannose residues bearing single 1,6-α-D-galactose residues (Whistler and BeMiller, 1997) as shown in Figure 1. Guar gum is the ground endosperm of guar seeds. It has its galactosyl units fairly evenly placed along the chain, and its mannose to galactose ratio is 2:1 (Lapasin and Pricl, 1995). Hence there are few locations on the chain that are suitable for significant junction zone formation. Further, it produces the highest viscosity among natural commercial gums, with a 1% solution having a viscosity of 6,000-10,000 mPas. Guar gum, a neutral molecule, is compatible with most food substances. However, it exhibits interactions with starches, cellulose, agar, κ-carrageenan and xanthan (Whistler and BeMiller, 1997). For instance, the interaction results in binding in the case of cellulose and a synergistic increase in viscosity in the case of water-soluble polysaccharide.

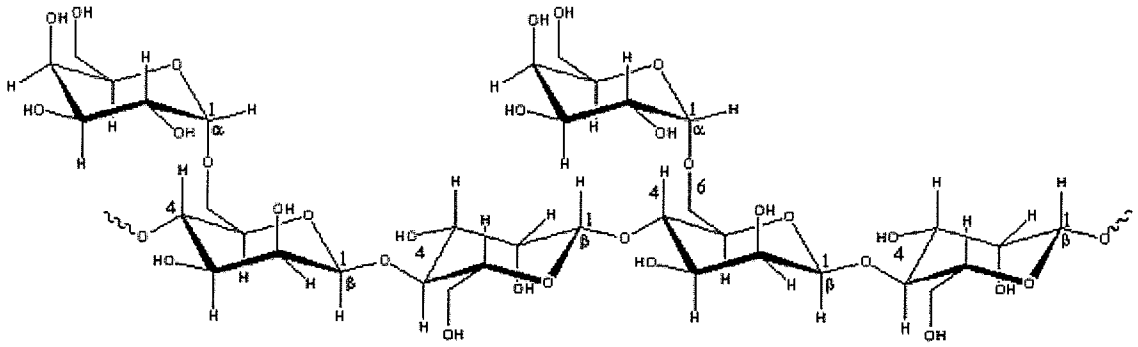


Figure 1. Structural unit of guar gum (Petkowicz et al., 1998; www.sbu.ac.uk/water)

Carrageenan

Kappa-carrageenan (κ-CAR) is an anionic sulfated polysaccharide extracted (with alkaline) from certain species of red seaweed (*Rhodophycae*, mostly of genus *Chondrus*, *Eucheuma*, *Gigartina* and *Iridaea*) and widely used as a thickening, gelling and stabilizing agent in the food industry (Clark and Ross-Murphy, 1987). They are linear polymers of about 25,000 galactose derivatives with regular but imprecise structures, dependent on the source and extraction conditions (Falshaw et al., 2001). Carrageenan consists of alternating 3-linked-β-D-galactopyranose and 4-linked-α-D-galactopyranose units (Fig. 2). Its gelation occurs on cooling and is generally considered a two-step process, characterized by a coil-helix transition followed by aggregation and network formation (Oakenfull et al., 1999; Morris et al., 1980). The gelling behaviour is strongly influenced by the nature and concentration of cations present in the solution as well as by the biopolymer concentration (Hermansson et al., 1991; Rochas and Rinaudo, 1980). The pH is not an important factor for κ-CAR gelation since it has no effect on gel transition temperature (Drohan et al., 1997) and has only a small effect on κ-CAR

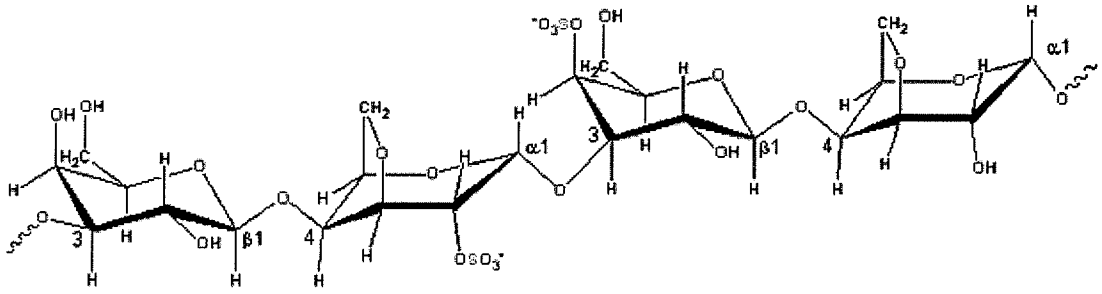


Figure 2. Structural unit of carrageenan (Falshaw et al., 2001; www.sbu.ac.uk/water)

gel strengths over a broad pH range (5-11) because the sulfate half-ester groups are always ionized, giving the molecules a negative charge (Whistler and BeMiller, 1997).

However, at more acidic pHs (below pH 4), it may prevent κ-CAR gelation due to acid hydrolysis (Mleko, Li-Chan and Pikus, 1997). Traditionally, Greek letters have been assigned to carrageenans comprised of certain idealized carrageenan disaccharide repeating units (Rees, 1972). Kappa (κ), iota (ι), mu (μ) and nu (ν) carrageenans are found in the gametophytic life phase of various seaweed species in the family *Gigartinaceae* and Figure 3 shows the disaccharide repeat units of these carrageenans. As shown in Figure 3, μ- and ν-carrageenans are the biochemical precursors of κ- and ι-carrageenans, respectively. They both contain a sulfate ester group at position-6 of a 4-linked α-D galactosyl unit which affects the overall properties of the carrageenan by creating 'kinks' in the polymer chain that reduce its ability to gel (Falshaw et al., 2001). Kappa-carrageenan is characterized by its repeating disaccharide units of 3-linked β-D-galactose 4-sulfate and 4-linked 3,6-anhydro-α-D-galactose (Dunstan et al., 2001). The tetrasporic life phase of *Gigartinacean* seaweeds contains a different type of carrageenan, mostly lambda (λ)-carrageenan (Matulewicz et al., 1989). Lambda is the precursor of theta (θ)-carrageenan but θ-carrageenan does not occur predominantly.

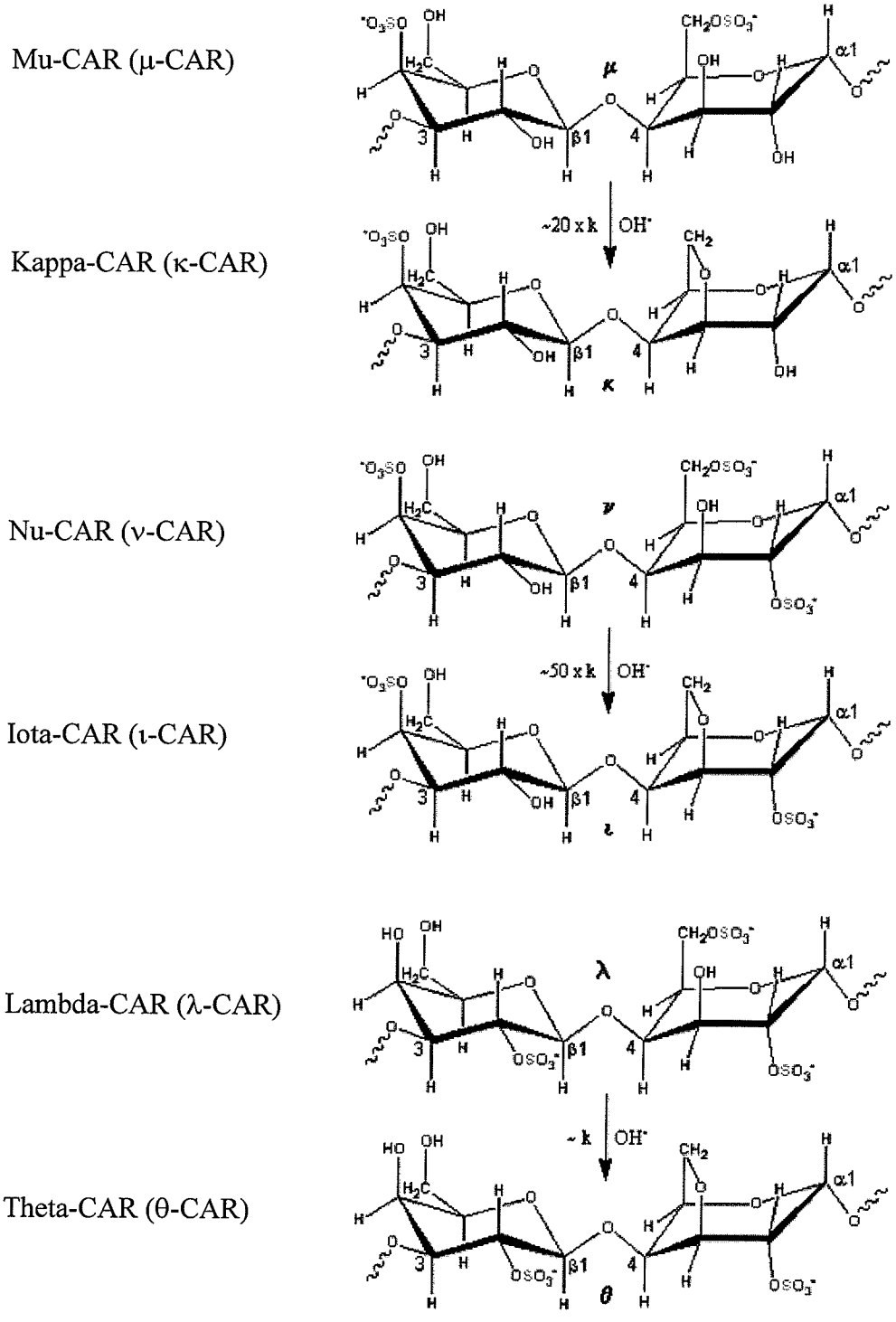


Figure 3. Idealized structures of carrageenan (CAR) showing conversions that occur on alkaline treatment. Major CAR types: κ-, ι- and λ-CAR differ in the number of sulfate groups on the polygalactose backbone. Whereas ι-CAR carries about 32% ester sulfate groups by weight, κ-CAR has ≈25% (Enriquez and Flick, 1989). λ-CAR is non gelling and lacks the $^{1}C_{4}$ 3,6-anhydro-link required for gelling (www.sbu.ac.uk/water).

Gel formation of proteins

Proteins are industrially important because of their gel-forming ability (Clark, 1998) and surface activity (Dickinson, 1998). Network formation is vital to the development of texture within foods. It provides structural matrices for holding moisture, flavours, sugars and other food ingredients, and it also lends to the stabilization of dispersed phases (Clark, 1998). Proteins capable of forming network can be classified as either fibrous or globular. Gelation can be defined as the aggregation of protein (usually denatured) into an ordered matrix in which there is a balance between protein-protein and protein-solvent interactions, and is maintained by a balance of attractive and repulsive forces (Hermansson, 1979). Since proteins are generally stable in aqueous solution, denaturation/destabilization is a prerequisite for gelation. Protein gelation refers to transformation of a protein from the sol (fluid molecular dispersion) state to a gel-like state (Damodaran, 1996). Denaturation/ destabilization is usually induced by thermal treatment while other techniques such as denaturant- (Katsuta et al., 1997; Ikeda et al., 2000) or pressure-induction (Hosseini-nia et al., 1999; Funtenberger et al., 1997) can also be employed. Enzymatic crosslinking (Ote et al., 1999; Dickinson, 1997) and treatment with divalent cations under appropriate conditions (Fennema, 1996) would be other ways to form protein gels. All these agents induce (by denaturation) the formation of structural networks. During thermogelation, the protein in a sol state is first transformed into a progel state (a viscous liquid state in which some degree of protein polymerization has already occurred) following denaturation. Globular proteins from milk (casein), egg white (ovalbumin), soybean (7S, 11S globulins) and muscle (myosin) can also form gels through various intermolecular forces when denatured. In the gelation of a globular protein (Catsimpoolas and Meyer, 1970), the protein sol is converted to a

high viscosity progel by heat which then sets to a gel of higher viscosity upon cooling (Fig. 4). The initial heating causes an irreversible dissociation of the globulin polypeptides (Kinsella, 1976). Once the sol has been activated to a progel, it can only be converted to a gel or metasol. When the progel or gel is excessively heated (125°C), a metasol is formed which does not form a gel on cooling. A metasol is also obtained by the action of chemical denaturants (e.g. 6 M Urea, sulfite, mercaptoethanol) which causes the chemical modification of functional groups. The most common way to produce protein gels is with heat treatment hence this review will focus on thermally-induced gelation.

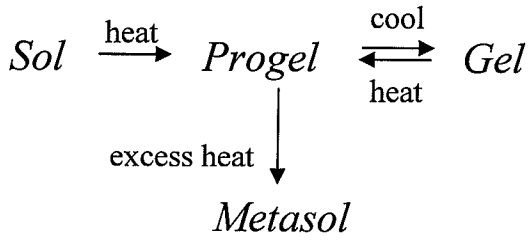


Figure 4. Scheme of globular protein gelation (Catsimpoolas and Meyer, 1970)

Mechanisms for network formation by globular protein

Gelation process is normally carried out in several stages including conformational changes of protein molecules, their aggregation and formation of a 3-D gel network from interacting aggregates. The first and critical step in thermogelation of globular protein is denaturation (at least partially). Ferry (1948) proposed the following two-step process of globular protein gelation:

Native state $\Rightarrow$ *Denatured state* $\Rightarrow$ *Aggregation*

The picture outlined in Ferry's review is that on heat-induced denaturation, a protein completely unfolds, and then intermolecular interactions lead to the formation of a fine-meshed "macromolecular" gel. This principle is still quite appropriate for gelatin (Gilsenan and Ross-Murphy, 2000) and for some chemically denatured globular proteins, but for heat-set globular proteins the picture is rather different. According to Ferry's theory, the type of network formed depends on the relative rates of the two steps. More recently, it has been shown that this theory was not entirely accurate. Departure from Ferry's view resulted after evidence suggested that interaction of partially unfolded, rather than fully extended, polypeptide chains were responsible for network formation in globular proteins (Clark and Lee-Tuffnell, 1986). Problems (i.e. conclusions that protein gelation involves a two-step process and complete unfolding of a protein) with Ferry's theory were particularly noticeable in some electron microscopy studies where the widths of the protein strands in the networks were actually measured.

As a result, Ferry's theory has been replaced with the "corpuscular theory", a three-step mechanism proposed for protein network formation (Clark and Lee-Tuffnell, 1986). In the corpuscular theory, the associating strands in protein networks are not polypeptide chains but a linkage of spherical bead-like structures. The following steps (Fig. 5) described the production and interaction of these beads:

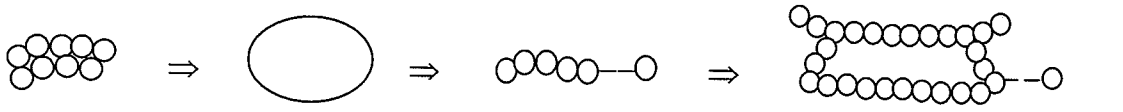


Figure 5. Schematic representation of the corpuscular theory

The articles (Clark 1996, 1998) published on globular protein gelation serve as an invaluable contribution. For instance, it is now appreciated that in simple heat-induced denaturation, the protein size and shape is only mildly perturbed. Some of the hydrophobic groups, which at ambient temperatures remain buried in the protein core, become exposed above some minimum unfolding or denaturation temperature, T_u. (Here, this value corresponding to the onset of denaturation determined from a differential scanning calorimetry (DSC) endotherm, was distinguished from T_m, the temperature of maximum heat flow from the DSC). This effect leads to aggregation to form either fine stranded networks or amorphous particulate structures of a "physical gel" (Ross-Murphy, 1998). Globular proteins partially unfold to expose internal hydrophobic regions on heating. Heat-induced gelation is thus considered to be achieved mainly through hydrophobic interactions among proteins (Relkin, 1998).

However, proteins are not totally unfolded when heated; a loss of α-helix fraction and an increase in β-sheet are usually observed by circular dichroism (CD), infrared (IR) or nuclear magnetic resonance (NMR) spectroscopy (Qi et al., 1997; Belloque and Smith, 1998, Clark, 1998). Bouraoui et al. (1997) conducted the first Raman spectroscopic analysis on structural changes in fish muscle protein, suggesting decreasing α-helical fraction and increasing β-sheet fraction during gelation. If the secondary structures are totally lost, proteins behave as random coils and the gel-forming ability is lost (Nakamura et al., 1997). Thus an intermolecular β-sheet formation seems to be involved in protein aggregate formation (Clark, 1998). Disulfide bridging (not all globular proteins contain disulfide crosslinks) is involved at pH values above 7.0 (Eloffson et al., 1997). At alkaline pH, increase in repulsive charges causes proteins to

unfold; and for globular proteins containing disulfide bonds, the structural unfolding results to disulfide bridging. For example, β-lactoglobulin which has two disulfide bonds, is characterized by a tighter, less elastic conformation at acidic pH and a highly flexible, more hydrophobic molecule at pH values above 7.5 (Phillips et al., 1994). Gelling mechanisms are fundamentally controlled by the balance between attractive hydrophobic interactions and repulsive electrostatic interactions.

Gelation mechanisms and properties of canola protein

The gelation mechanisms and characteristics of soy proteins and other related plant proteins have been extensively studied, whereas insight into the gelation of canola protein is limited. Results of studies on the gelation properties of canola protein have been variable. The ability of canola protein to form gels was first demonstrated by Gill and Tung (1978). It was concluded that gelation occurred through a complex phenomenon involving covalent and noncovalent interactions, though the role of ionic and disulfide bonding were considered minor. Some studies have found the gelation properties of canola protein to be similar to those of soy protein (Gwiazda and Rutkowski, 1983; Dev and Mukherjee, 1986). Working with a hexametaphosphate-extracted protein and heating to 80°C for 30 min, Thompson et al. (1982) demonstrated an increase in viscosity, but were unable to form gels. Work done by Paulson and Tung (1989) which involved heating to 72°C produced canola protein gels only at high pH values or when the protein had been succinylated. The properties of the gels were dependent on degree of succinylation, NaCl concentration and pH. The major forces responsible for gelation in the study by Paulson and Tung (1989) were believed to be

hydrophobic interactions and hydrogen bonding. In a study on the gelation properties of canola protein isolated under mild conditions (using the PMM methodology of Murray et al., 1978), Léger and Arntfield (1993) established that gels prepared with 6% protein at alkaline pH values were superior to gels prepared under acidic conditions. The rheological characteristics of canola protein gels prepared at alkaline and acidic conditions (as observed by Léger and Arntfield, 1993) are outlined as follows:

1. gels prepared under alkaline conditions showed the following properties:
    - at pH 8: G'=2560 Pa and tan δ=0.16
    - at pH 9: G'=1660 Pa and tan δ=0.11
    - at pH 10: G'=1040 Pa and tan δ=0.09
2. while gels prepared under acidic conditions exhibited the following properties:
    - at pH 4: G'=10 Pa and tan δ=0.20
    - at pH 5: G'=214 Pa and tan δ=0.19
    - at pH 6: G'=169 Pa and tan δ=0.20

The development of superior network structures at high pH values has been attributed to the extension of polypeptide chains and the creation of homogeneous structures (Paulson and Tung, 1989). In comparison, gels prepared at lower pH values are heterogeneous in nature. These heterogeneous networks are composed of compact molecules, which aggregate to form regions of high and low protein concentrations. As a result, regions with low protein concentrations act as weak points, causing a drop in overall gel strength. As observed by Léger and Arntfield (1993), treating canola dispersions with denaturants prior to heat treatment showed that hydrophobic forces and electrostatic interactions were responsible for the establishment of canola gel networks,

while gel stabilization and strengthening were attributed to disulfide bonding, electrostatic interactions and hydrogen bonding. In research involving network formation by canola protein, the development of structure was followed by monitoring the storage modulus (G′) during the heating and cooling phases (Arntfield and Cai, 1998). Their observations are as follows: For canola protein at pH 8.8 heated to 95°C, there was little sign of structure development prior to temperature above the denaturation temperature (81-82°C). The first increase in G′ did not occur until temperatures >90°C, and even then, the increase in G′ during the heating phase was relatively minor.

On the other hand, the changes in G′ during cooling indicate a gradual increase in structure as the temperature was lowered and the extent of the increase was highly dependent on protein concentration. Further, at the final cooling stage, the G′ value for 15% canola protein reached a value of ≈500 Pa, 10% canola protein system reached a value of ≈170 Pa; whereas 5% canola protein system did not seem to form a network (G′≈20 Pa). This corroborates a previous finding that a critical concentration of 6% canola protein was required for network formation (Léger and Arntfield, 1993). These scientists indicated that the increase in G′ during cooling was related to an increase in the level of hydrogen bonding. The effects of phytic acid and phenolic compounds on the functionality of canola proteins have been studied by Wong (1995) and Rubino (1995). It was noted that binding between these antinutritional factors and protein was detrimental to the gelation properties of the mixture. The above results on the gelation properties of canola protein have been with laboratory-prepared canola proteins (concentrate or isolate). Currently, progress is being made by commercial firms to

produce canola proteins (concentrate or isolate) which can be used as a food ingredient.

Salt influences on network association

The influence of solvent components, such as salt, on protein network properties can be either by direct interaction with the protein, indirect interaction through modification of the solvent environment or a combination of both (Eagland, 1975). Stabilizing or non-chaotropic salts (e.g. NaCl) at moderate to high salt concentrations tend to promote hydrophobic interactions between protein molecules, thereby impeding network formation (Leger and Arntfield, 1993; Arntfield et al., 1990b). On the other hand, chaotropic salts (e.g. $CaCl_2$) tend to promote the formation of well cross-linked networks (Leger and Arntfield, 1993; Arntfield et al., 1990b).

For some proteins (e.g. ovalbumin), divalent cations contribute to network structure through the formation of salt bridges (Savoie and Arntfield, 1996). However, the amount of divalent cation and the type of anion associated with the cation are critical to optimizing this contribution (Arntfield, 1994; Savoie and Arntfield, 1996). For example, sodium salts, which promoted protein stability, had adverse effect on gelation (Leger and Arntfield, 1993). Similarly, studies (Arntfield et al., 1990a) with ovalbumin and vicilin showed that higher $CaCl_2$ levels resulted in increased vicilin network solubility and promoted ovalbumin aggregation.

Molecular forces responsible for network formation

The interactions involved in network formation of proteins are primarily electrostatic interactions, hydrogen bonds and hydrophobic interactions. The relative contributions of these forces vary with the type of protein, heating conditions, the extent

of denaturation, and environmental conditions. Disulfide bonds contribute more to networks formed from ovalbumin than those formed from plant proteins (Léger and Arntfield, 1993). The study by Arntfield et al. (1991) supported disulfide bond involvement in ovalbumin networks but not in vicilin networks. Although disulfide bonds may contribute to the elasticity and strength of a protein network, non-covalent forces are critical to initial network formation. Gill and Tung (1978) implicated covalent and non-covalent forces as factors involved in the elasticity of canola protein gels. Similarly, Léger and Arntfield (1993) established that hydrophobic and electrostatic interactions as well as disulfide bonds were responsible for the formation of canola protein gel networks.

A balance between the charge repulsion and the potential for interaction, mainly through hydrogen bonds and hydrophobic interactions, is critical to the determination of network characteristics (Arntfield et al., 1990b; Arntfield, 1994). Excessive attractive forces or insufficient charge repulsion results in aggregation rather than network formation. If proteins of opposite charge are present during network formation, as in the case with egg proteins ovalbumin and lysozyme, charge repulsion is low. While the strength of the network formed by this combination is increased compared to ovalbumin alone, the cross-linking within the structure is reduced (Arntfield and Bernatsky, 1993). A good understanding of these interactions would help to predict the optimum conditions in multicomponent food systems.

Gelation mechanisms of polysaccharides

As a general rule, regular linear segments of polysaccharide molecules are in the form of a helix. Intermolecular interactions result either in simple associations or in the form of a

double (in some cases, a triple) helix. The relatively stiff, linear double helical segments may then interact (pack together) to form a super junction and the three-dimensional gel network (Whistler and BeMiller, 1997). Typical gelling polysaccharides include locust bean gum, κ- and ι-carrageenans, alginate, pectin, agarose and xanthan gum. The gelation mechanisms of typical polysaccharides can be divided into three types according to the nature of the junction zones: ribbon junctions, multiple helices and micelle junctions. The term junction zone is used to describe the crosslink because each crosslink involves aggregates of ordered molecular chains like helices. Junction zones between molecules or particles must be of limited size, and are segment interactions.

Ribbon junction

Alginates and low methoxy pectin follow the ribbon junction gelation mechanism. This type of interaction involves simple ionic bridging of two carboxyl groups on adjacent polymer chains with calcium ions. Alginates (from brown seaweed) have linear polyuronic backbone with three types of block structure namely: poly-β-D-mannuronic acid (M), poly-α-L-guluronic acid (G), and mixed (MG) block containing both uronic acids. It has been suggested that alginate gels are formed by ionic bridging of two carboxyl groups on adjacent polymer chains with calcium ions. This takes place between guluronic acid chain segments. From circular dichroism and conformation studies done on isolated M and G segments, large transitions were observed for polyguluronic blocks and insignificant changes for the polymannuronic blocks. The "egg box" association is considered to involve cooperative binding of Ca^{2+} ions between aligned polyguluronic ribbons (Dea, 1982). Oakenfull (1987) revealed that alginate gels are not

thermoreversible. Pectin (partial methyl ester of 1,4-linked poly-α-D-galacturonic acid) can form two types of gels depending on their degree of esterification (Glicksman, 1982). Low methoxy pectin is similar to alginate in gelling ability, and requires Ca^{2+} ion to form a gel network.

Micelle junction

The gelling association between two different polysaccharides which usually can not form gels as individuals is termed micelle junction (Dea, 1982). For example, polysaccharide pairs: xanthan-galactomannan and agarose-galactomannan mixtures will follow the micelle junction gelation mechanism. Xanthan gum and galactomannan will react to form a gel with a very elastic, resilient texture and very high rupture strength. The gels are thermally reversible, and show sharp melting and setting behaviour over a narrow temperature range.

Multiple helices

Associations between chains where two or more chains interact in a certain helical direction is termed multiple helices. Polysaccharides that form junction zones by this mechanism are carrageenans, agarose and amylose. Carrageenans are alternating copolymers of 1,3-linked β-D-galactose and 1,4-linked 3,6 anhydro-α-D-galactose with varying amounts of sulfate ester. These copolymers contribute to the helical junction formation. Double helical conformation was characterized in solid state by X-ray fiber diffraction (Dea, 1982) as a three fold right-handed double helix. The forces involved in the formation of helices are mainly hydrogen bonds.

Protein polysaccharide interactions

Two types of macromolecules, proteins and polysaccharides occur widely in food systems. Both biopolymers act as texture modifiers controlling the rheological properties and mouthfeel (Nishinari, 1997), as well as contributing to the surface properties of foods. Much is known at the molecular level about the functional properties of individual biopolymers. However, knowledge of the role of protein-polysaccharide interactions in terms of their functionality in complex multiphasic systems (such as mixed food solutions, emulsions or gels) is still limited. Protein-polysaccharide interactions are studied with the objective of clarifying the mechanisms behind the formation of structure and properties of food systems, both liquid and gel-like ones. As foods are almost always multicomponent systems, it is of great interest to consider the properties of protein-polysaccahride mixtures in addition to the properties of the separate components. Here the focus is on the structure and physicochemical properties of protein-polysaccharide-water mixtures.

Consequences of mixing biopolymers

On mixing aqueous solutions of proteins and polysaccharides, three situations may arise (Tolstoguzov, 1991, 1998; Kruif and Tuinier, 2001; Ledward, 1994): incompatibility, co-solubility, and complexing (Fig. 6).

1. Incompatibility: A liquid two-phase system can be obtained in which the two macromolecules are mainly in different phases. This is due to thermodynamic incompatibility of proteins and polysaccharides in aqueous media.
2. Co-solubility: Homogeneous stable solutions can be obtained in which the two biopolymers do not interact or alternatively exist as soluble complexes. Hydrogen

bonding and electrostatic interaction are involved in the stabilization of such complexes.

3. Complexing: A two-phase system may be obtained where both macromolecules are largely in the same single concentrated phase. This phenomenon known as complex coacervation is attributed to the formation of an insoluble electrostatic protein-anionic polysaccharide complex.

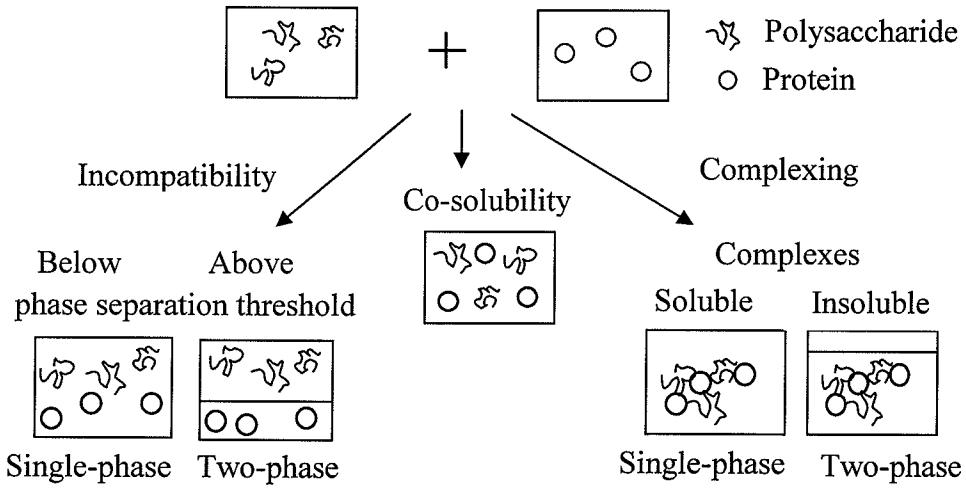


Figure 6. Main trends in the behaviour of protein-polysaccharide mixtures (de Kruif and Tuinier, 2001; Tolstoguzov, 1991)

All three cases are encountered when a protein or polysaccharide is added to a multicomponent food system. As a result, the functional properties of the added material may be altered because of the presence of the biopolymers in the food system. Co-solubility is rather rare due to the polymeric nature of the macromolecules and the presence of various functional groups (Tolstoguzov, 1998). Thus, attention is focused on complexing and incompatibility of proteins and polysaccharides in aqueous media and how these phenomena influence the functional properties of their mixtures. For very dilute solutions the system is stable since the mixing entropy dominates and proteins and

polysaccharides are co-soluble. Upon increasing the concentration of the polymers, the system may be unstable depending on the type of interaction. As a rule, biopolymer mixtures tend to segregate (Tolstoguzov, 1991; Polyakov et al., 1997). Exceeding a certain polymer concentration leads to a phase separation into protein-enriched and polysaccharide-enriched phases.

Complex coacervation: At pH values below the protein isoelectric point (IEP), the protein carries a positive charge while the anionic polysaccharide (with –COOH or $-SO_3H$ groups) has a negative charge. In this pH region, insoluble complexes can be formed, even on mixing very dilute solutions ($\approx 10^{-2}$ mg ml^{-1}) provided the ionic strength is sufficiently low (generally <0.1-0.2) and the ratio of positive to negative charges in the system is close to zero. When interactions between unlike macromolecules are dominant, the two biopolymers may associate into a liquid or gel-like phase by the process termed complex coacervation. When such a system separates into two phases, one of them contains higher concentrations of the two reagents and it has been termed a coacervate phase. The other, an equilibrium liquid, is a dilute solution of the reagents (Tolstoguzov, 1986). Insoluble complexes of globular protein with anionic polysaccharide generally contain an excess of protein and thus possess a net positive charge. It has been established that such interactions are mainly electrostatic in nature and at higher ionic strengths complexing is inhibited (Samant et al., 1993). This energetically favoured reaction has been seen most extensively for proteins with acidic polysaccharides (e.g. alginate, pectate) or sulfated polysaccharides such as carrageenan (Tostoguzov, 1991). For instance, Tolstoguzov (1995) showed that at a low ionic strength and pH value below the IEP of gelatin, the interaction of positively charged

macro-ions of gelatin and the negatively charged macro-ions of gum arabic can give rise to insoluble electrostatic complexes, thus both components concentrate into a single complex coacervate phase.

Soluble complexes: The interaction of protein with anionic polysaccharide is a particular case of an interaction between oppositely charged macromolecules in a solution. However mixtures of protein and anionic polysaccharide show some unusual properties. One of the most interesting of these is the non-equilibrium nature of protein-anionic polysaccharide complexes (Gurov et al., 1977). The non-equilibrium nature of the complexes was shown by the dependence of their solubility and other properties on the formation conditions. This is represented schematically in Figure 7. Here A-B and C-B correspond to the nephelometric titration of a protein-dextran sulfate mixture by an alkali, whereas B-C corresponds to titration by an acid. On mixing solutions of a protein and a sulfated polysaccharide at a pH value below the protein IEP (point A), i.e. under the conditions where the biopolymers carry opposite charges, an insoluble complex is formed, which has been called a mixing complex or an M-complex. However if the same solutions are mixed at a pH value around 9, i.e. when complexing is inhibited (point B) and if the resulting mixture is subsequently acid-titrated down to a pH value below the protein IEP (point C), a soluble complex is formed (Ledward, 1994; Tolstoguzov, 1986) which has been termed a titration complex or T-complex (Tolstoguzov, 1986).

Similar results can be obtained by varying the ionic strength of the mixture. On mixing protein and anionic polysaccharide solutions at a low ionic strength and at pH values below the protein IEP, one can produce insoluble M-complexes. At higher ionic strength, complex formation is inhibited and subsequent dialysis against an acid solution

at the same pH value produces soluble T-complex. Soluble complexes of globular protein with anionic polysaccharide are generally far from being saturated with protein and therefore have a net negative charge. Polysaccharides containing carboxyl groups do not complex with globular protein at ionic strengths above 0.2 or at pH values above the protein IEP. Sulfated polysaccharides, because they have a higher charge density are capable of forming soluble complexes with globular protein at pH values above the protein IEP. For example, it has been observed that the complexes of serum albumin and dextran sulfate can exist in solution at an ionic strength of 0.1 and a pH value of up to 8.5 which is well above the protein IEP of 5 to 5.3 (Tolstoguzov, 1986).

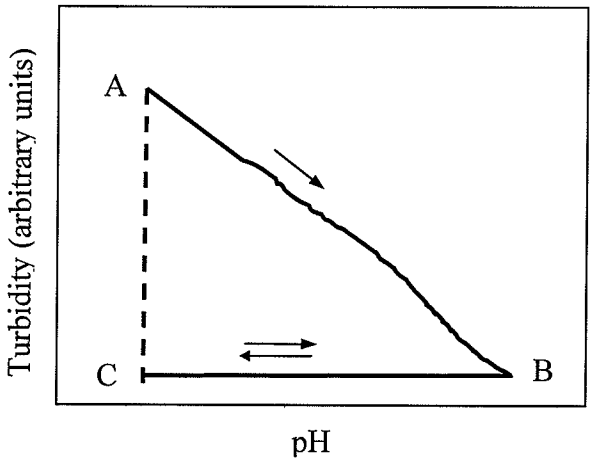


Figure 7. Scheme of nephelometric acid-base titration of a mixture of protein and dextran sulfate solutions (Tolstoguzov, 1986).

Incompatibility of biopolymers: Thermodynamic incompatibility is opposite to the phenomenon of biopolymer complex formation. Tolstoguzov (1986) reported that protein-polysaccharide mixtures can undergo a liquid-phase separation with the macromolecular components concentrated primarily in the different phases. This author further stated that it takes place only under certain conditions (i.e. at certain pH values and ionic strengths when the total concentration of the macromolecular components

exceeds 4%). It increases under conditions that enhance self-association and enlargement of macromolecules (e.g. under a statistical coil-helix conformational transition) and favour inhibition of protein-polysaccharide complexing (Tolstoguzov, 1991). Tolstoguzov (1995) in a review on multicomponent gels cited the study by Bungenberg de Jong (1949) who showed that thermodynamic incompatibility takes place in solutions containing mixtures of gelatin and gum arabic with a sufficiently high ionic strength and at pH values above the IEP of gelatin. Under these conditions, biopolymer macro-ions have like net charges. The mixed solution of biopolymers breaks down into two liquid phases and each of the biopolymers concentrates into one of the phases. Higher salt concentrations screen the electrostatic interaction between polymers and encourage the self-association of gelatin molecules. Incompatibility is observed when the salt concentration is 0.2 M and higher (Tolstoguzov, 1991).

The most common consequence of thermodynamic incompatibility is the phenomenon of mutual exclusion. Macromolecules cannot occupy the same volume in the solution. This means that each biopolymer can only use some part of the volume of the mixed solution. In solutions, incompatible biopolymers mutually concentrate each other. Thus, each incompatible biopolymer will behave as if it was more concentrated (Tolstoguzov, 1991, 1986; Tolstoguzov and Braudo, 1983). Exclusion volume effects depend on the flexibility, shape and size of macromolecules as well as their bulk concentration (Tolastoguzov, 1991; Semenova et al., 1991). Since the number of energetically unfavourable contacts that a chain can make increases with increasing flexibility and size, conformationally mobile biopolymers are more effective in excluding other polymers than are more constrained ones (Ledward, 1994). Further,

exclusion effects are strongly concentration dependent, becoming undetected in very dilute polymer solutions, where the individual chains are widely separated.

Multicomponent emulsifying systems

Emulsifiers/stabilizers that belong to the macromolecular class include protein, polysaccharide and gums (Phillips et al., 1994). In oil-in-water type of food emulsions, the primary emulsifying and stabilizing agent that protects droplets against coalescence is typically a multicomponent mixture of adsorbed proteins. Various polysaccharides may also be present as thickening and gelling agents. The interaction with protein and polysaccharides (e.g. κ-CAR, galactomannan) has been widely studied, especially for the case of caseins (Langendorff et al., 2000; Tuinier et al., 2000b; Bourriot et al., 1999a, 1999b; Schorsch et al., 1999; Syrbe et al., 1998; Keogh et al., 1995; Lynch and Mulvihill, 1994), as well as other protein such as soy protein (Kampf and Nussinovitch, 1997). Caseins can be stabilized against Ca^{2+} precipitation in the presence of carrageenan, and the effect is apparently more important for ι-CAR than κ-CAR (Hansen, 1993). Also, the presence of κ-CAR has been found to protected milk protein-stabilized oil droplets from aggregating at low pH (Dalgleish and Hollocou, 1997). For casein micelles mixed with low concentration of ι-CAR at high temperature, depletion interaction does not lead to instability (Langendorff et al., 2000; Syrbe et al., 1998). On cooling the mixture, one observes a sudden increase in the apparent size of the casein micelles. A similar behaviour was observed with pectin-casein micelle mixtures; if the pH is lowered pectin adsorbs onto the casein micelles (Marozeine and de Kruif, 2000). On increasing the pH the pectin desorbs and the apparent size of the casein micelles

returns to its original value at neutral pH in absence of pectin. In a study on the effect of polysaccharides on stability of model milk protein-based emulsions, Dalgleish and Hollocou (1997) observed that very low concentrations of pectin can protect sodium caseinate-coated droplets against aggregation at pH $\leq$ 5, and that pectin binds to the surface of emulsion droplets even at pH values above the protein's IEP where both biopolymers carry a net negative charge.

Emulsion capacity and stability have been shown to be higher for protein-polysaccharide complex than protein alone (Gurov et al., 1983; Larichev et al., 1983). For instance, addition of equal weight of pectin to a solution of legumin increases the emulsion stability and also decreases the emulsifying threshold of legumin by a factor of 3 (Samant et al., 1993; Tolstoguzov, 1991). This is the case under both incompatibility (pH 7.6) and complexing (pH 4.2) conditions (Tolstoguzov, 1991). Therefore, the kinetic stabilities of emulsions stabilized by a solution containing equal weights of legumin and pectin with a 1% bulk concentration differ very much in the acid (pH 4.2) and neutral (pH 7.6) media. The stable concentrated legumin-pectin emulsions are 96 and 84% for pH 4.2 and pH 7.6 respectively, while for legumin-stabilized emulsions for the same pH values these percentages are 67 and 40% respectively (Tolstoguzov, 1991). Hence, a greater stability of emulsions stabilized by legumin-pectin complexes in an acidic (pH 4.2) medium is observed when compared to that at pH 7.6. Emulsions stabilized by complexes are found at solution concentrations four times lower than protein-stabilized emulsions. The complexing in this system reduces the conformational stability of the protein (Gurov et al., 1978).

Structure of multicomponent gels

Several investigations on protein-polysaccharide systems have been undertaken (Arntfield and Cai, 1998; Ledward, 1994; Ziegler and Foegeding, 1990; Tolstoguzov, 1986) and are leading to a better understanding of the key parameters involved in protein-polysaccharide interactions (Doublier et al., 2000). When looking at the interactions between protein and polysaccharide in terms of network formation, attention must be paid to the potential for both macromolecules to form networks on their own. Also important is the impact of low concentrations of polysaccharide on protein network formation. In some cases, low concentrations of polysaccharide have been shown (Tolstoguzov, 1986) to improve the gelation properties of protein gels.

In many biopolymer mixtures, the entropic contribution is often greater than the enthalpic one, so that phase separation of biopolymers is generally the rule (Doublier et al., 2000). The addition of both polysaccharide and protein in aqueous media has been noted to lead to two types of molecular interactions: complex coacervation and thermodynamic incompatibility (Tolstoguzov, 1995), depending on the affinity between the different biopolymers and the solvent. Thermodynamic incompatibility is generally observed and the general character of incompatibility of protein and polysaccharide has been demonstrated using more than 80 systems (Tolstoguzov, 1986).

Effects of this type of interactions have been variable. For complexes between sodium alginate and sodium caseinate, thermally stable gels were obtained (Tolstoguzov, 1991). Similarly, gelatin-alginate complexes at low salt concentration were thermally stable after 24-hr rest period (Tolstoguzov, 1986). With bovine serum albumin (BSA) and myoglobin at concentrations of 1%, however, gelation of alginate was inhibited, but gelation of pectin was not (Tolstoguzov, 1986). The complexes that

resulted when mixtures of anionic polysaccharide (e.g. sodium alginate and pectin) and canola protein were heated did not form elastic three-dimensional networks (Arntfield and Cai, 1998; Cai, 1996) as has been observed with other proteins such as caseinate (Tolstoguzov, 1991) and gelatin (Tolstoguzov, 1986), but rather produced structures that inhibited the development of three-dimensional networks. This was reflected in decreases in the storage modulus (G′) and increases in the loss tangent (tan δ).

In contrast, neutral polysaccharides and canola protein tended to be incompatible, showing no evidence of interaction at low biopolymer concentrations and phase separation at higher concentrations (Arntfield and Cai, 1998; Cai, 1996). With 5% canola protein (which is below the critical protein concentration for canola protein gelation), this incompatibility led to improved gelation properties with the addition of either methyl cellulose or guar gum. Furthermore, upon closer examination of the gel development, it appeared that the benefits owing to the presence of methyl cellulose reflected the ability of methyl cellulose to form a network upon heating rather than an effect related to the incompatibility of the system. The fact that higher protein levels resulted in network deterioration supported the finding. With guar gum, the benefits seen at low protein levels were also evident at higher protein concentrations although the increase in G′ was slightly offset by the increase in tan δ values so that although stronger gels were obtained, the viscous component of the gel was increased even more (Arntfield and Cai, 1998; Cai, 1996). Associative interactions between pectin and gelatin as well as segregative interactions between starch and pectin or agarose were observed using dynamic viscoelasticity, differential scanning calorimetry and turbidity (Morris, 2000).

Mixed globular proteins-carrageenan systems have been the focus of some investigations. Capron et al., (1999a, 1999b) showed that the first step in the aggregation of β-lactoglobulin was not changed by the presence of κ-carrageenan (κ-CAR), while an acceleration of the gelation process of the protein was experienced in the second step. This was ascribed to a microphase separation; then as soon as the protein network was formed, the separation of the phases was 'frustrated' and the system was 'frozen in'. Other rheological studies confirmed the synergistic effects in κ-CAR-denatured protein systems (Tziboula and Horne, 1999; Ould-Eleya and Turgeon, 2000a, 2000b; Neiser et al., 2000). In detailed rheological studies (Ould-Eleya and Turgeon, 2000a, 2000b) on K^+-κ-CAR in the presence of β-lactoglobulin, these synergistic effects were exhibited in conditions where the two biopolymers gelled at neutral pH. Synergistic interaction was taken as the consequence of two co-continuous networks as a result of a segregative phase separation before gelation takes place. On a similar basis and in combination with transmission electron microscopy (TEM) observations, Neiser et al. (2000) reached the same conclusions in the case of BSA-K^+-κ-CAR systems. In a study of milk gels formed with κ-CAR or low methoxy pectin, it was suggested that κ-CAR acts as molecular velcro to incorporate casein micelles in the network whilst casein acts as a source of calcium ions which promotes the gelation of pectin (Oakenfull et al., 2000). Associative interactions between gelatin-ι-CAR and casein-ι-CAR were demonstrated using dynamic viscoelesticity (Launay et al., 2000). Gels formed using more than one gelling agent can be subdivided into three groups: complex, mixed and filled gels (Tolstoguzov, 1991, 1986). Another classification of gels formed from blends containing more than one gelling agent is as follows: interpenetrating, coupled and

phase-separated (Morris, 1986).

Complex gels

Coupled or complex coacervate networks are formed in the presence of favourable intermolecular interactions between the different types of bioplymers (Ould Eleya and Turgeon, 2000a). A non-gelling component may associate with the primary network in a random fashion via non-specific interactions. Such interactions may reduce the flexibility of the primary network chains and add to the rigidity of the complex gel (Tolstoguzov, 1998). Examples are gels formed by gelatin-sodium alginate and gelatin-low ester pectin complexes. In this case, neither biopolymer formed gels alone under the conditions used for gelation of the complex. This phenomenon is attributed to the occurrence of salt linkages between protein and anionic polysaccharides. Conditions required to produce protein-polysaccharide complex gels and the resultant properties have been discussed (Stainsby, 1980).

Mixed gels

Mixed gels are obtained when the two macromolecular components of a single-phase system form continuous networks separately (Ziegler and Foegeding, 1990; Tolstoguzov, 1986). In a two-phase system, mixed gels filled with dispersed particles are obtained. According to Tolstoguzov (1995), mixed gels are formed when the concentrations of biopolymers in a solution exceed the critical concentration for gelation (Cg) of these biopolymers. Mixed gels can be treated as a particular case of homogeneous interpenetrating polymeric networks (Tolstoguzov, 1986). These gels exhibit properties where one component forms a thermosetting gel and the other a gel which sets by the

action of ions. An example is pectinate and denatured ovalbumin gel (Tolstoguzov, 1995). The ionic gel network has been shown to control the formation of the thermosetting gel.

Filled gels

Filled gels are obtained if one of the components can form a continuous network over the entire system. In this case the other polymeric component serves as gel filler (Tolstoguzov, 1998, 1986). When the bulk concentration (or co-solubility) of a biopolymer in one of the phases is larger than that required for gelation, the biopolymer forms a gel in that phase. In the two-phase area, inside the binodal (i.e. the cosolubility area) a gel filled with gel-like or liquid dispersed particles is obtained (Tolstoguzov, 1995). Several reports (Aguilera, 1992; Ziegler and Foegeding, 1990; Tolstoguzov, 1986) stated that two types of filled gels can be distinguished depending on the phase state of the system: single-phase gels (type I) in which the filler is in a molecularly dispersed state and two-phase gels (type II) where the dispersed phase consists of particles of liquid or gel. In this case, thermodynamic incompatibility causes phase separation resulting in both polymers forming separate segregated networks. An example of type II filled gel is that produced by the addition of wheat or potato starch to red hake surimi (Aguilera, 1992).

Phase-separated gels

This type of gel is formed by incompatible polymers, where interactions between the different polymers are repulsive and/or when the two types of polymers show varying affinity towards the solvent (Picullel and Lindmann, 1992; Tolstoguzov, 1991,

1995). The report of Tolstoguzov (1995) stated that phase separation occurs at concentrations slightly higher than the critical concentration for gelation (Cg) or the gel point. A difficulty arising when dealing with the description of phase-separated systems is related to the fact that phase separation very often competes with the gelation of one or both of the components (Zazypkin et al., 1997). This is classically experienced in the case of globular proteins mixed with a polysaccharide, when the gelation of globular protein is triggered by thermal treatment, or with gelatin-polysaccharide mixed systems, the polysaccharide being gelling or non-gelling (Doublier et al., 2000). In all cases, the resulting system is a gel which may appear homogeneous at the macroscopic level, although heterogeneous at microscopic level. This explains difficulties often encountered in the description of systems to provide evidence that segregative phase separation takes place.

Techniques for assessing network properties

Besides rheological techniques, which are almost systematically used to describe the properties of the systems, some attempts have been made to describe the kinetics of the process as well as the structure of the system. When seeking a description of the protein in the medium, scattering techniques appear suitable. Static light scattering has been applied to β-lactoglobulin/κ-carrageenan mixtures (Capron et al., 1999a, 1999b). In fact, the authors described how β-lactoglobulin denaturation was modified by the presence of κ-carrageenan. Different scattering techniques can be combined: small angle neutron scattering (SANS), dynamic light scattering (DLS) and static light scattering (SLS) to describe the phase separation mechanisms and kinetics of the process (Tuinier

et al., 2000a; de Kruif and Tuinier, 1999). Microscopy is another tool that can be useful to describe the microstructure of the system at the end of the gelation process. Numerous examples can be found based on scanning electron microscopy (SEM), transmission electron microscopy (TEM) or phase contrast microscopy (PCM). Unfortunately, the localization of the macromolecular components in the medium, which could allow a description of the phase diagram in the gel state, cannot be performed. The use of more appropriate methods like Fourier transformation infrared (FTIR) microscopy (Durrani et al., 1993), confocal laser microscopy or confocal Raman microspectrometry could be useful in this respect.

CHAPTER 1

Structural Thermostability of Commercial Canola Protein-Hydrocolloid Systems

Abstract

Proteins in a mixed system with polysaccharides may have their structures modified by disruption of covalent and noncovalent interactions. The structural transition properties (denaturation temperature, T_d; and enthalpy of denaturation, ΔH) of canola protein isolate (CPI) mixed with hydrocolloids (κ-carrageenan, guar gum) were assessed using differential scanning calorimetry (DSC). The effects of salts, hydrocolloids and protein concentrations as well as pH on the conformational stability of CPI were evaluated. Factorial and response surface models were used to examine the effects of these factors on CPI-hydrocolloid mixtures. Salt and CPI concentrations, pH, and hydrocolloid concentration strongly affected structural stability of CPI (Td=86°C, ΔH=16.2 J/g). At 0.5 M salt level, CPI-κ-CAR mixtures treated with sodium acetate ($NaC_2H_3O_2$) had the highest T_d (103.2°C) values whereas those treated with sodium thiocyanate (NaSCN) had the lowest T_d (92.3°C) values. Increases in T_d of CPI-κ-carrageenan mixtures followed the sequence: acetate > sulfate > chloride > thiocyanate, whereas the decrease in ΔH values followed the order: acetate < sulfate = chloride < thiocyanate. For CPI-guar gum mixtures at 0.5 M salt level, biopolymer mixtures treated with acetate had the highest ΔH (18.1 J/g) values whereas those treated with NaSCN had the lowest ΔH (12.3 J/g) values. The degree to which ΔH was decreased by the anions followed the series $C_2H_3O_2^- < Cl^- < SO_4^{2-} < SCN^-$, whereas increases in T_d followed the series: sulphate = acetate > chloride > thiocyanate. The low T_d and ΔH values obtained on treating the

CPI-hydrocolloid mixtures with urea and dithiothreitol support the involvement of noncovalent interactions and disulfide bonds in the structural stability of CPI-hydrocolloid mixtures. This study is relevant because protein-polysaccharide interactions give a more realistic indication of proteins behaviour in multicomponent foods and provide information for improving canola protein functionality.

Introduction

Proteins and polysaccharides contribute to the structure and stability of foods through their interactions. Understanding the way in which these interactions are affected during processing is important when these components are added into foods to improve their functional properties (Stainsby, 1980). Protein structure is greatly influenced by its environment. Minor deviations in pH, ionic strength, temperature, or the presence of stabilizing or destabilizing solutes often results in alteration of protein conformation and functional behaviour (Phillips et al., 1994). Although the mechanisms of protein stabilization are becoming better understood using model protein systems, studies on protein-polysaccharide mixtures such as are encountered in real foods are limited. The influence of solvent components on structure and conformational stability of proteins can either be by direct interaction with the protein, indirect interaction through modification of the solvent environment or a combination of both (Eagland, 1975).

Ranking of salts in terms of ability to stabilize (increase denaturation temperature (T_d) values) or destabilize (decrease T_d values) proteins is termed a lyotropic or Hofmeister series (von Hippel and Schleich, 1969). The effect of salts on structural thermostability of proteins is important in examining the molecular interactions involved

in structural stability. The dependence of T_d values on the position of the salts in the lyotropic series reflects the importance of hydrophobic interactions to the stability of proteins. Damodaran (1996) stated that at low concentrations, salts stabilize protein structure. However, higher concentrations (>1 M) of salts exert an ion specific effect on hydrophobic interactions, which either stabilizes or destabilizes the structure of proteins. The mechanism by which salts affect the structural stability of proteins is not well understood; however, their relative ability to bind to and alter hydration properties of proteins may be involved.

Salts that stabilize proteins enhance hydration of proteins and bind weakly, whereas salts that destabilize proteins decrease protein hydration and bind strongly (Arakawa and Timasheff, 1984). These effects are mainly the consequence of energy perturbations at the protein-water interface (Damodaran, 1996). The relative effectiveness of various anions (at isoionic strength) in maintaining structural stability of proteins generally follows the lyotropic series, with anions promoting structural stability in the order: $F^- > SO_4^{2-} > Cl^- > Br^- > I^- > ClO_4^- > SCN^- > Cl_3CCOO^-$ (Damodaran, 1996). Thus, fluoride (F^-), sulphate (SO_4^{2-}) and chloride (Cl^-) are structure stabilizers (i.e. promoters of hydrophobic interactions), whereas the salts of other anions, such as thiocyanate (SCN^-), are structure destabilizers. In a study on the effect of sodium salts on the thermal stability of canola protein isolate, the degree to which the T_d was increased by the anions depended on the position of the anions in the following sequence: $SO_4^{2-} > C_2H_3O_2^- > Cl^- > SCN^-$ (Léger and Arntfield, 1993). Given that noncovalent interactions (except repulsive electrostatic interactions) and in some cases, covalent (disulfide) bonds, contribute to the native protein structure; this study evaluated the effects of sodium salts (Na_2SO_4, $NaC_2H_3O_2$, NaCl, NaSCN) and denaturants (urea,

dithiothreitol) on the structural stability of commercial canola protein isolate mixed with hydrocolloids (κ-carrageenan, guar gum) using differential scanning calorimetry (DSC).

Materials and Methods

Source of materials

Food grade guar gum (G-4129; Lot No. 95H0653) with a viscosity of 6000-10000 mPas (Whistler and BeMiller, 1997), urea (U-15; Lot No. 863571), DTT (D-0632; Lot No. 61K16571) and κ-carrageenan (No. C-1013; that contains predominantly κ- and lesser amounts of λ-carrageenan) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The composition of κ-carrageenan (κ-CAR) powder as indicated by the manufacturer showed it contained a mixture of the following cations: K^+ (10.4%), Ca^{2+} (2.3%) and Na^+ (0.9%). Commercial canola protein isolate (CPI) was purchased (BMW Canola, Winnipeg, Canada) and used without further purification. Proximate analysis (AOAC, 1990) of the CPI sample indicated a protein content of 87% (N x 5.7), 0.7% oil, 2% ash, 5.9% moisture and 4.4% total carbohydrate (determined by difference). $NaC_2H_3O_2$ (Lot No. 7364 KCLZ) was procured from Mallinckrodt Inc. (Paris, Kentucky, USA). All other chemicals such as NaCl (BP358-212; Lot No. 028091), HCl (A144-225; Lot No. 296220), NaOH (BP359-212; Lot No. 974661), NaSCN (S441-500; Lot No. 987676) and Na_2SO_4 (S421-500; Lot No. 985711) were certified reagent grade (Fisher Scientific Co., NJ, USA).

Sample preparation

Dispersions of CPI (10, 20% w/v; protein-basis), κ-CAR (1,3% w/v) and guar gum (1,

3% w/v) were prepared by mixing in NaCl solution (0.05, 0.25 M) at varied pHs (6, 10). The CPI, κ-CAR, guar gum, pH and NaCl combinations were generated using Design-Expert® Software. The mixture was stirred for approximately 1 hr at room temperature or until a complete dispersion (based on visual examination) of the mixture was achieved. Dispersions of individual CPI, κ-CAR and guar gum samples in appropriate NaCl solutions were prepared in a similar manner to serve as control. To assess the effects of salts on the structural stability of CPI alone and in mixed systems, neutral salts namely sodium sulfate (Na_2SO_4), sodium acetate ($NaC_2H_3O_2$), sodium chloride (NaCl) and sodium thiocyanate (NaSCN) at varied concentrations (0.05, 0.5 M) were used to prepare sample dispersions. Furthermore, to assess the effects of disulfide bonds on the conformation of CPI and its mixtures, denaturants (dithiothreitol, DTT: 0.15 M; urea: 6M) were included in the dispersions prior to heat treatment. For the pH series, samples were adjusted to pH 6, 8, and 10 with 1 M NaOH or 1 M HCl. To ensure that pH was maintained, samples were allowed to equilibrate for 30 min at room temperature and the pH was rechecked prior to further testing.

Experimental design

All experimental parameter measurements were done in duplicates. Design-Expert® Software (Stat-Ease Inc., MN) was used to generate the experimental model for a factorial design. The experiment was carried out in two stages. In the first stage, a series of experiments were carried out to characterize different CPI and hydrocolloids combinations (CPI-κ-CAR, CPI-guar gum) following a full factorial model. A two-level factorial design for 4 factors in 20 experiments including four replicates of the center

point was generated. The factors included pH (6, 10), CPI (10, 20% w/v), NaCl (0.05, 0.25 M), guar gum (1, 3% w/v), and κ-carrageenan (1, 3% w/v) concentrations. The key thermal properties, denaturation temperature (T_d) and enthalpy of denaturation (ΔH) were measured. Using this data, two-dimensional contour plots were generated from the model graph (Design-Expert® Software). In stage two, sodium salts (Na_2SO_4, $NaC_2H_3O_2$, NaCl, NaSCN) at varied concentrations (0.05, 0.5 M) were used to examine the effect of altering hydrophobicity (Phillips et al., 1994) on CPI-hydrocolloids thermal structural stability. Similarly, denaturants were used to assess the involvement of disulfide bonds (0.15 M DTT) and noncovalent interactions (6 M urea) on the structural conformation of CPI-hydrocolloid mixtures. Control runs with individual CPI (15% w/v), κ-CAR (3% w/v) and guar gum (1.5%, w/v) were also carried out.

Statistical analysis

Data used in tables and figures were average values. Data from confirmatory and control tests (phase two) were analyzed by analysis of variance (ANOVA) procedure using SAS® 1999-2001 Proprietary Software Release 8.2 (TS20; SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was used to determine statistical differences ($P \leq 0.05$) between treatment means (Steel et al., 1997).

Calorimetry: Assessment of biopolymer structural thermal stability

Differential scanning calorimetry (DSC) was used to determine the thermal properties

(denaturation temperature, T_d; enthalpy of denaturation, ΔH) of CPI alone, CPI-κ-carrageenan (κ-CAR) and CPI-guar gum mixtures (Arntfield et al., 1991). The effects of chosen conditions (sodium salts, pH, and denaturants) on the structural stability of CPI alone and CPI-hydrocolloid mixtures prior to further treatment were assessed. DSC analysis was conducted using a Dupont 9900 Thermal Analyzer (TA Instruments, DE) with a 910 Cell Base. Samples (10-15 μL) were put in pans and heated (at 10°C min^{-1}) over a temperature range of 25-130°C in a standard DSC cell (calibrated with indium and sapphire standards) with an empty pan as reference. Each sample was analyzed in triplicate and thermal curves were obtained. The T_d (point of maximum heat flow) and ΔH were calculated using the Dupont General Analysis utility software (version 2.2) available for the instrument.

Results and Discussions

Thermal transition characteristics of canola protein-κ-carrageenan systems

Table 1 shows the thermal transition properties (T_d, ΔH) and the model equations from the full factorial design for canola protein-κ-carrageenan mixtures at varied conditions.

Effect of canola protein-NaCl-pH interaction on T_d

Significant factors and interactions were identified using the ANOVA model (Design-Expert® software). CPI concentration, pH, NaCl level, κ-CAR concentration, interaction between pH and NaCl concentration; and interaction between pH and CPI concentration interaction were significant ($P<0.05$; Appendix 1). Also significant was the 3-way interaction between pH, NaCl level and CPI concentration; although the same

Table 1. Structural transition properties (T_d, ΔH) of canola protein-κ-carrageenan (κ-CAR) mixtures under varied conditions.

pH	NaCl (M)	κ-CAR (%)	Protein (%)	T_d (°C)	ΔH (J/g)
6	0.05	1	10	87.3	19.3
6	0.05	1	20	87.9	16.6
6	0.05	3	10	87.1	13.2
6	0.05	3	20	88.9	15.5
6	0.25	1	10	88.8	19.1
6	0.25	1	20	90.8	17.4
6	0.25	3	10	89.5	18.3
6	0.25	3	20	91.3	16.7
8	0.15	2	15	90.8	18.1
8	0.15	2	15	91.1	17.2
8	0.15	2	15	91.2	16.7
8	0.15	2	15	91.1	17.8
10	0.05	1	10	89.3	17.1
10	0.05	1	20	90.5	14.9
10	0.05	3	10	89.7	18.7
10	0.05	3	20	90.4	14.4
10	0.25	1	10	91.1	18.6
10	0.25	1	20	91.5	16.2
10	0.25	3	10	91.5	18.4
10	0.25	3	20	91.9	15.4

$\mathbf{T_d}$ = 88.21 – 0.14 pH – 31.45 NaCl – 2.97 κ-CAR – 0.35 CPI + 4.55 pH*NaCl + 0.35 pH*κ-CAR + 0.053 pH*CPI + 14.97 NaCl*κ-CAR + 3.23 NaCl*CPI + 0.22 κ-CAR* CPI – 1.59 pH*NaCl*κ-CAR – 0.37 pH*NaCl*CPI – 0.025 pH*κ-CAR*CPI – 0.98 NaCl*κ-CAR*CPI + 0.11 pH*NaCl*κ-CAR*CPI (P=0.003).

ΔH = 36.35 – 1.86 pH – 27.36 NaCl - 12.26 κ-CAR – 0.62 CPI + 3.67 pH*NaCl + 1.37 pH*κ-CAR + 0.046 pH*CPI + 21.46 NaCl*κ-CAR + 0.42 κ-CAR*CPI – 2.41 pH*NaCl*κ-CAR – 0.049 pH *κ-CAR*CPI (P=0.021).

range of T_d values was shown at both pH levels (Figure 8). Contour plots of T_d as a function of NaCl and CPI levels at pH 6 and 10 are shown in Figure 8. It was evident from the curves that improved structural thermostability (high T_d values) resulted with increasing concentrations of NaCl and CPI at both pHs (Fig. 8). Increasing the ionic strength of the protein solution masks charged groups that have become newly accessible through heat-induced conformational rearrangements, thus enhancing hydrophobic interactions (Phillips et al., 1994). This electrostatic interaction may explain the increase in T_d values observed in the present study.

Reports on the effect of NaCl on protein structural stability have been varied. NaCl has been described as a stabilizing salt by its ability to increase the thermal stability of plant proteins (Arntfield et al., 1986; Ismond et al., 1986). However, in another study, NaCl at concentrations between 0.1 and 0.5 M was shown to cause a decrease in the enthalpy of denaturation of ovalbumin, indicative of protein denaturation (Arntfield et al., 1990). The general trend in ranking anions in terms of their ability to stabilize proteins depends on the protein in question. Von Hippel and Schleich (1969) observed the anionic series with ribonuclease as follows:

$$\longleftarrow \text{Stabilizing} \qquad \text{Destabilizing} \longrightarrow$$
$$SO_4^{-2} > CH_3COO^- > Cl^- > Br^- > ClO_4^- > CNS^-$$

In a report on the effect of various sodium salts on the T_d of β-lactoglobulin, Damodaran (1996) indicated that at equal ionic strength, Na_2SO_4 and NaCl increased T_d values. DSC analysis on the effect of NaCl on the thermal transition characteristics of β-lactoglobulin

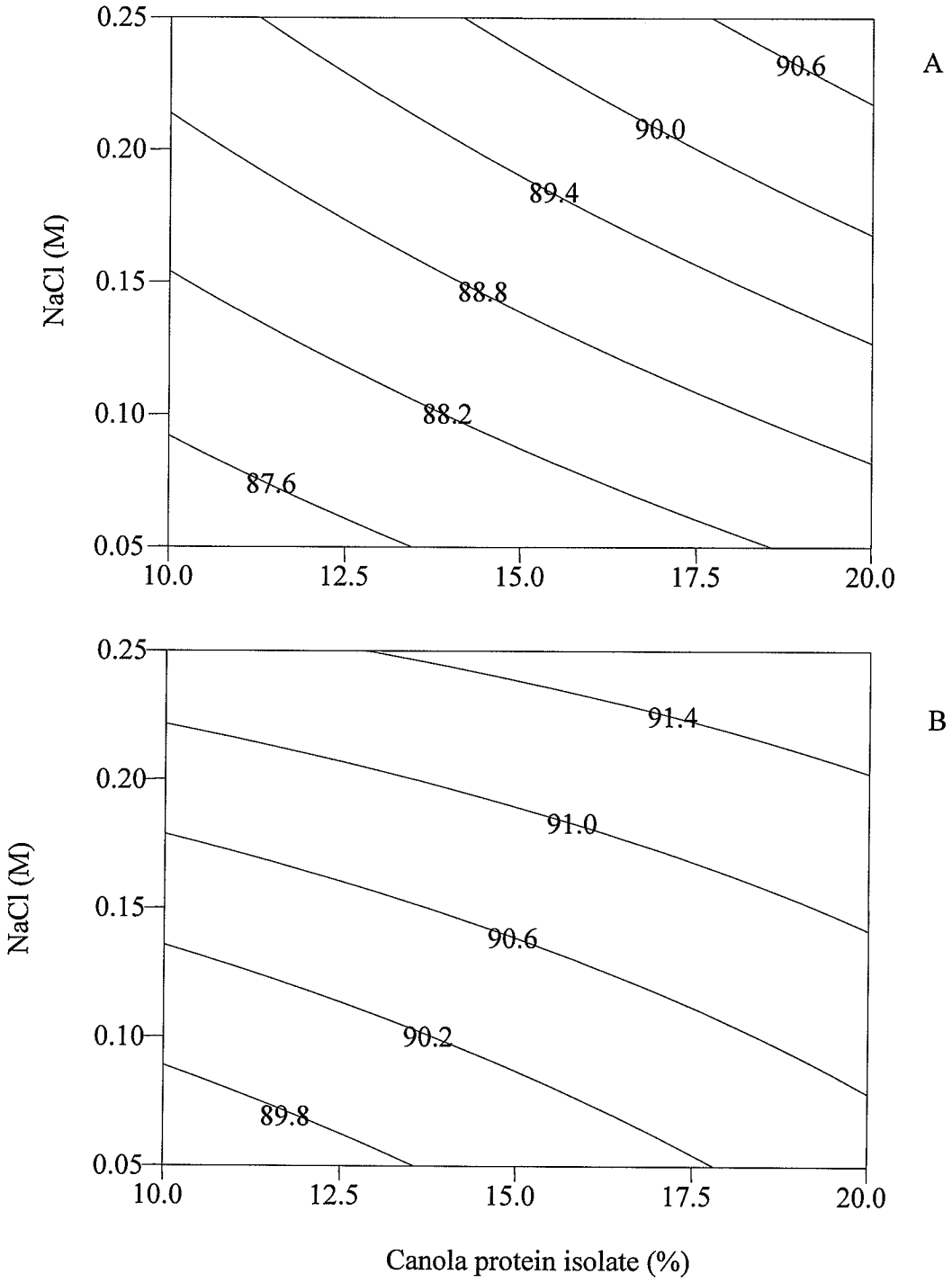


Figure 8. Denaturation temperature (T_d) contour plot showing the influence of CPI and NaCl concentrations on T_d of CPI-κ-CAR mixtures at 3% (w/v) κ-CAR and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given T_d value. For example, in Figure 8B; at ≈15% (w/v) CPI and 0.15 M NaCl, T_d=90.6°C.

showed that the T_d value was increased whereas the ΔH was not significantly affected (Harwalkar and Ma, 1987; 1989). According to the authors, T_d of β-lactoglobulin increased with increasing NaCl concentrations (at 0 - 1 M).

These reports indicate that NaCl could have both stabilizing and destabilizing effects on protein conformation. The results of the present study indicated that NaCl acted as a stabilizing salt at high molar concentration (up to 0.25 M). As a result, the associating structures within the protein-hydrocolloid system remain in a more globular form in which nonpolar groups are buried. This may explain the high T_d values observed for CPI-κ-CAR mixtures at higher NaCl concentration (Fig. 8). Therefore, it is evident that hydrophobic and electrostatic interactions are involved in enhancing the structural thermal stability of CPI-κ-CAR mixtures. Regardless of their chemical makeup and conformation, the structural stability of macromolecules (including DNA) is adversely affected by high concentrations of salts (von Hippel and Schleich, 1969).

Effect of pH-κ-carrageenan interaction on enthalpy of denaturation (ΔH)

Based on the ANOVA model (Design-Expert® software), NaCl concentration, κ-CAR concentration, CPI concentration, and interaction between pH and κ-CAR concentration had significant effect ($P<0.05$) on ΔH (Appendix 2). Thus contour plots of ΔH values of CPI-κ-CAR mixtures as a function of pH and κ-CAR concentration are shown in Figure 9. The ΔH specifically describes the actual heat flow into the macromolecule during the thermal denaturation process. Changes in the ΔH values are a good way to measure major conformational changes. If the solvent (e.g. pH, additives) is causing protein unfolding, then the heat required to unfold it during thermal denaturation

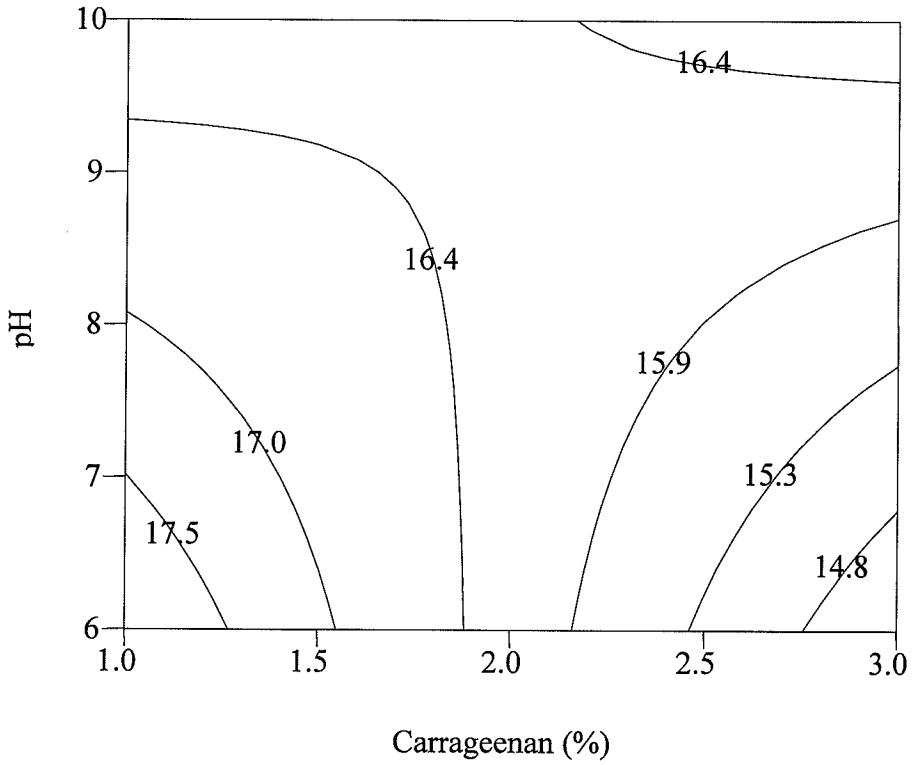


Figure 9. Enthalpy of denaturation (ΔH) contour plot showing the influence of interaction between pH and κ-CAR concentrations on ΔH of CPI-κ-carrageenan mixtures prepared with 0.05 M NaCl and 15% (w/v) CPI. The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given ΔH value. For example, at pH 8.0 and 2.5% (w/v) κ-carrageenan, ΔH =15.9 J/g.

will be less. In turn, the ΔH value will be lower. The greater the heat flow, the greater the structural stability of the biopolymer before the heat treatment. The ΔH values of CPI-κ-CAR mixtures increased at lower pH and lower κ-CAR concentrations; and also decreased at lower pH values with increasing κ-CAR concentrations. In other words, the CPI molecules became denatured with increased concentration of κ-CAR. This effect may be due to the electrostatic interaction between CPI and κ-CAR. According to Tolstoguzov (1986), at pH values below the protein isoelectric point (IEP), the protein

and anionic polysaccharide carry net opposite charges. In this pH region, insoluble complexes can be formed provided the ionic strength is sufficiently low (generally less than 0.1-0.2; such as 0.05 M used in the present study). For instance, at pH 6, CPI (IEP ≈ pH 7.0) carries a net positive charge whereas κ-CAR carries a net negative charge. The formation of an insoluble electrostatic complex between CPI and κ-CAR may have led to the disruption of the protein structure. This may explain the low ΔH values at higher κ-CAR concentrations. However, at lower pHs and lower κ-CAR concentration, ΔH values for these systems increased. It appears that the disruption of protein structure was less at lower κ-CAR concentrations.

Effect of salts on structural properties of CPI-κ-carrageenan systems

Denaturation temperature (T_d): The denaturation of canola protein-κ-carrageenan mixtures was assessed by observing the effects of sodium salts on the structural stability (T_d, ΔH). The influence of salts on the thermal stability could be described as either stabilizing (causing T_d values to increase with increasing concentration) or destabilizing (causing T_d values to decrease). Furthermore, the ability of electrolytes to influence the structural stability of a protein alone or in a mixed system depends on the concentration and/or ionic strength (μ) of the salt. At low ionic strength ($\mu \leq 0.2$), ions associate with proteins via nonspecific electrostatic interactions (Damodaran, 1996; von Hippel and Schleich, 1969). Therefore, the stabilization of CPI or CPI-κ-CAR mixtures at low salt concentration should be independent of the nature of the salt (i.e. no lyotropic effect).

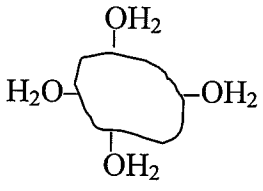
In the present study, there was relatively little difference in the effectiveness of the sodium salts to stabilize CPI-κ-CAR structure at low concentration (Table 2). An exception to this was the stabilization of CPI-κ-CAR mixture by the acetate ion (T_d=95.1°C) which was more effective than the sulfate, chloride and thiocyanate (Table 2). The electrostatic neutralization of protein charges usually stabilizes protein structure (Damodaran, 1996). The present study indicates that the structural stability of CPI-κ-CAR mixtures was higher at the higher salt concentration regardless of salt type. Sodium salts (at 0.5 M level) had a lyotropic effect on the thermal stability (high T_d value) of CPI-κ-carrageenan mixtures. At this salt level, biopolymer mixtures treated with sodium acetate ($NaC_2H_3O_2$) had the highest T_d values (T_d=103.2°C) whereas those treated with sodium thiocyanate (NaSCN) had the lowest T_d values (T_d=92.3°C). Increases in T_d followed the sequence: acetate > sulfate > chloride > thiocyanate. At the higher concentration (0.5 M), the ability of salts (e.g. Na_2SO_4; $NaC_2H_3O_2$ shown to act as a stabilizing salt in the present study) to stabilize protein structures has been attributed to the preferential hydration of the protein molecule as a result of salt-induced alteration of the water structure in the vicinity of the protein (Arakawa and Timasheff, 1982). Non-chaotropic salts influence the order of water such that exposure of nonpolar residues is thermodynamically unfavourable (Fig. 10). Protein-protein interactions in acetate- and sulfate-treated samples probably helped the CPI-κ-CAR mixtures to maintain its folded structure leading to structural stability in these systems.

Table 2. Influence of salt type and concentration on the thermal properties (T_d, ΔH) of canola protein-κ-carrageenan (κ-CAR) mixtures prepared with 15% (w/v) protein and 3% (w/v) κ-CAR at pH 6.

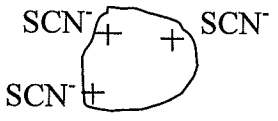
Salt type	T_d (°C)*	
Control (no salt)	89.15 ± 0.51^{b1}	89.15 ± 0.51^{e1}
	0.05 M	**0.5 M**
Na_2SO_4	89.55 ± 0.05^{b1}	96.04 ± 0.26^{b2}
$NaC_2H_3O_2$	95.12 ± 0.16^{a1}	103.16 ± 0.40^{a2}
NaCl	89.84 ± 0.35^{b1}	94.63 ± 0.02^{c2}
NaSCN	89.81 ± 0.02^{b1}	92.25 ± 0.18^{d2}

Salt type	ΔH (J/g)*	
Control (no salt)	14.92 ± 0.09^{b1}	14.92 ± 0.09^{c1}
	0.05 M	**0.5 M**
Na_2SO_4	15.79 ± 0.51^{b1}	15.59 ± 0.79^{bc1}
$NaC_2H_3O_2$	17.44 ± 0.37^{a1}	20.07 ± 0.46^{a2}
NaCl	15.67 ± 0.69^{b1}	16.70 ± 0.57^{b1}
NaSCN	15.67 ± 0.58^{b1}	13.08 ± 0.23^{d2}

*Mean ± SD. For a given parameter (T_d or ΔH), column values with different letters and row values with different numbers are significantly different ($P<0.03$)



Nonchaotropic salts affect the order of water on the protein surface.



Chaotropic salts bind to protein surface.

Figure 10. Schematic representation of the stabilizing and destabilizing effect of salts

Similar lyotropic effect has been reported for vicilin isolated from fababeans (Ismond, 1984; Arntfield et al., 1986). Despite the fact SCN^- is regarded as a destabilizing anion, its T_d value (at 0.05 M) was similar to the T_d values observed for Na_2SO_4- and NaCl-treated biopolymer mixtures. Electrostatic interactions are considered to be the principal noncovalent force responsible for conformational stability at low ionic strength. Although the NaSCN-treated CPI-κ-CAR mixtures had the lowest T_d value (T_d=92.3°C) at 0.5 M salt concentration compared to the T_d values of the other three salt treatments ($NaC_2H_3O_2$, Na_2SO_4 and NaCl), its T_d value was higher than that of the control (T_d=89.15°C). As noted by Arakawa and Timasheff (1982), SCN^- anions can bind to proteins when present at relatively high concentrations; this helps to unfold the protein molecule, exposing buried functional groups and encouraging hydrophobic interactions among neighbouring polypeptide chains. This may explain the higher T_d value of CPI-κ-CAR mixtures treated with SCN^- compared to that of the control. The structural behaviour of CPI-κ-CAR mixtures in the presence of sodium salts is similar to when CPI alone (T_d=86°C, ΔH=16.19 J/g) is examined but the destabilization is not as great in the presence of κ-CAR.

Enthalpy of denaturation (ΔH): The ΔH values of the salt-treated CPI-κ-CAR mixtures were higher than that without salt (ΔH=14.92 J/g; Table 2), indicating increased structural rigidity at 0.05 and 0.5 M salt level. Comparing the salt-treated CPI-κ-CAR mixtures at 0.05 M concentration, the ΔH value for $NaC_2H_3O_2$-treated sample was higher than those for the other three salts; ΔH values of Na_2SO_4-, NaCl- and NaSCN-treated biopolymer mixtures were similar (Table 2). The ΔH values for CPI-κ-CAR mixtures treated with $NaC_2H_3O_2$ at both high and low concentration were higher

than those of the other three salt-treated biopolymer mixtures, suggesting a stabilizing effect at both salt concentrations.

At 0.5 M concentration, decrease in ΔH values followed the series: acetate < sulfate = chloride < thiocyanate. At this salt level, NaSCN-treated CPI-κ-CAR mixture (ΔH=13.08 J/g) had the lowest ΔH value, indicating a structural disruption by NaSCN; and supporting the trend observed in the T_d values of the salt series (Table 2). Damodaran (1988) offered possible explanations for this phenomenon. Since low T_d value reflects low resistance to thermal denaturation, the tertiary and quaternary structures of the NaSCN-treated CPI-κ-CAR mixture may not be the same as that of the native biopolymer mixture. Therefore, a lower ΔH value was obtained for CPI-κ-CAR mixture. Furthermore, the decrease in ΔH value may be due to aggregation of denatured protein, since the exothermic heat effect of such an aggregation process may partly offset the endothermic heat flow as measured by DSC. While high concentrations (>1 M) of anions such as thiocyanate (SCN^-) were required for structure destabilization of β-lactoglobulin (Damodaran, 1996), in the present study, the destabilizing effect of SCN^- anion was evident at 0.5 M.

Effect of urea and DTT on structural thermostability of CPI-κ-CAR systems

Influence of urea: Differences in thermal T_d and ΔH values of CPI-κ-CAR mixtures treated with urea compared to that without urea (control) demonstrate the dependence of CPI alone and CPI-κ-CAR mixture on noncovalent forces for their native structure. Figure 11A shows the impact of urea on the thermal properties (T_d, ΔH) of CPI alone (T_d=74°C, ΔH=2.3 J/g) and CPI-κ-CAR mixtures (T_d=80°C, ΔH=4.8 J/g)

treated with urea as well as CPI-κ-CAR mixture without urea (T_d=89°C, ΔH=14.9 J/g). Samples treated with urea had lower thermal properties than the control (without urea). The data obtained showed that addition of urea had more effect on ΔH than on T_d values. Phillips et al. (1994) stated that urea is one of the standard denaturing agents used to study the transition between the native and unfolded states of proteins (and protein-polysaccharide mixtures). Urea (6 M) induces unfolding of biopolymers by disrupting protein-protein interactions, which promotes protein-solvent interactions. Urea's strong interaction with water alters the structure of the aqueous phase around the protein molecule and increases the solubility of hydrophobic amino acid residues. As a result, urea can disrupt hydrogen bonds and hydrophobic interactions that contribute to the maintenance of protein (and protein-polysaccharide) structure. The disruption of noncovalent forces by urea could explain the low ΔH values obtained in the urea-treated samples. Of note is the fact that the destabilization was not as severe when κ-CAR was present (Fig. 11A).

Effect of DTT: Differences in thermal T_d and ΔH values of CPI-κ-CAR mixtures treated with dithiothreitol (DTT) compared to that without DTT (control) demonstrate the involvement of disulfide bonds in the stabilization and strengthening of the native structure of CPI alone and in a CPI-κ-CAR mixture. Figure 11B displays the influence of DTT on the thermal transition parameters (T_d, ΔH) of CPI alone (T_d=76°C, ΔH=7.7 J/g) and CPI-κ-CAR mixtures (T_d=81°C, ΔH=8.4 J/g) treated with DTT as well as CPI-κ-CAR mixture without DTT (T_d=89°C, ΔH=14.9 J/g). Again, the destabilization was not as severe in the presence of κ-CAR. DTT-treated samples had lower thermal properties than the control (without DTT). The addition of sulfhydryl disrupting reagents

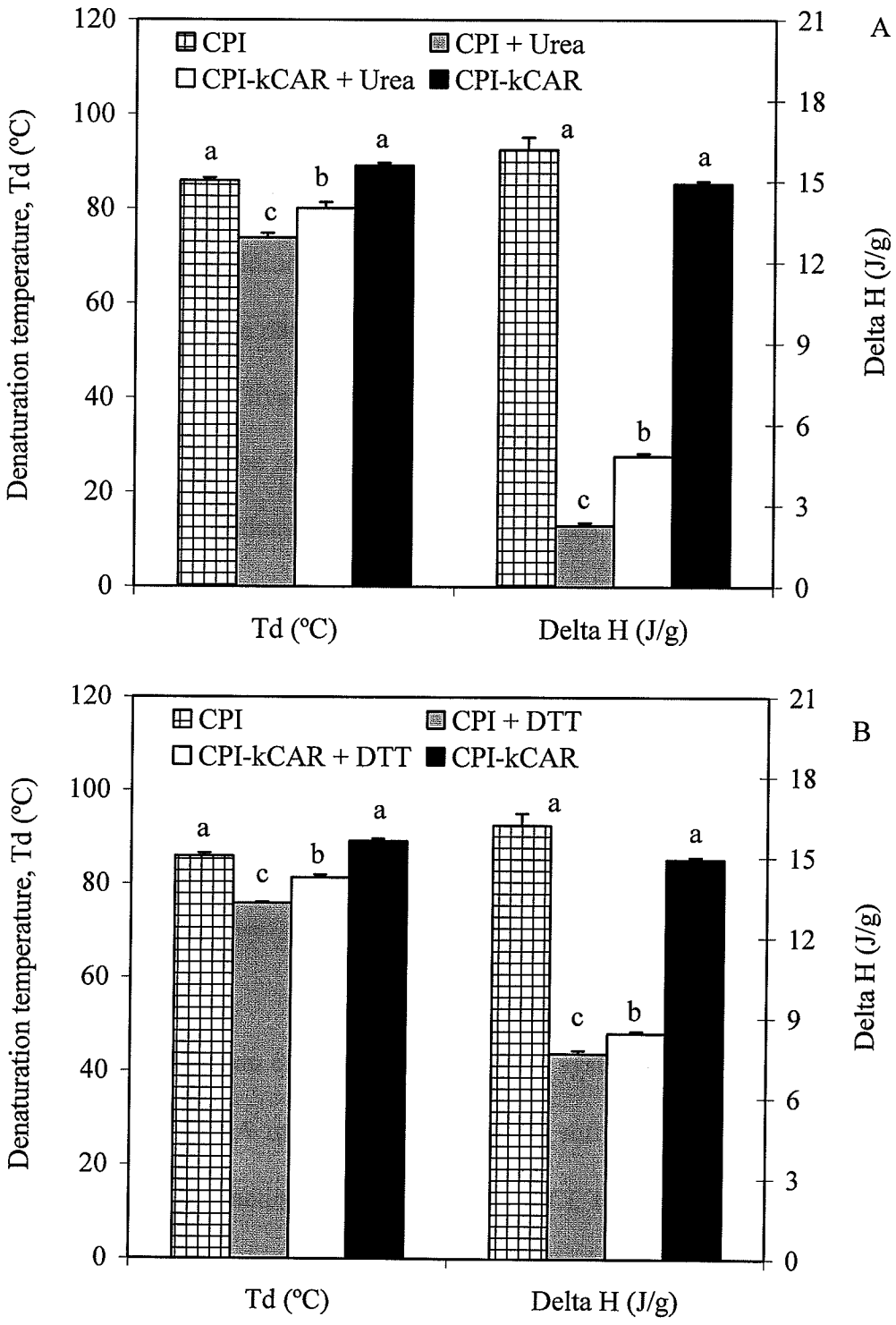


Figure 11. Effect of urea (A) and dithiothreitol (B) on the thermal transition properties (T_d, ΔH) of canola protein isolate (CPI) and CPI-κ-carrageenan (κ-CAR) mixtures (15%, w/v CPI; 3%, w/v κ-CAR; 0.15 M DTT or 6 M urea; pH 6). For T_d or ΔH and within treatments, means with different letters are significantly different ($P<0.05$).

Table 3. Structural transition properties (T_d, ΔH) of canola protein-guar gum mixtures under varied conditions.

pH	NaCl (M)	Guar gum (%)	Protein (%)	T_d (°C)	ΔH (J/g)
6	0.05	1	10	85.0	14.3
6	0.05	1	20	87.4	13.0
6	0.05	3	10	85.2	14.1
6	0.05	3	20	88.0	15.3
6	0.25	1	10	87.6	9.5
6	0.25	1	20	90.8	15.6
6	0.25	3	10	88.6	19.6
6	0.25	3	20	91.2	15.9
8	0.15	2	15	89.8	17.0
8	0.15	2	15	90.1	17.1
8	0.15	2	15	89.7	17.5
8	0.15	2	15	90.5	20.0
10	0.05	1	10	88.0	9.7
10	0.05	1	20	88.3	12.0
10	0.05	3	10	88.2	11.2
10	0.05	3	20	88.6	9.5
10	0.25	1	10	89.5	11.2
10	0.25	1	20	89.6	10.9
10	0.25	3	10	89.3	15.9
10	0.25	3	20	89.4	10.3

Td = 73.46 + 1.42 pH + 11.34 NaCl + 0.048 Ggum + 0.55 CPI – 0.54 pH*NaCl – 0.017 pH*Ggum – 0.056 pH*CPI + 5.24 NaCl*Ggum + 0.86 NaCl*CPI + 5.062×10^{-03} Ggum* CPI – 0.43 pH*NaCl*Ggum – 0.072 pH*NaCl*CPI + 1.688×10^{-03} pH*Ggum *CPI – 0.14 NaCl*Ggum*CPI (P=0.003).

ΔH = 20.54 – 0.88 pH – 126.55 NaCl + 1.40 Ggum – 0.35 CPI + 9.06 pH*NaCl – 0.30 pH*Ggum + 0.036 pH*CPI + 33.85 NaCl*Ggum + 7.36 NaCl*CPI + 0.049 Ggum*CPI – 0.56 pH*NaCl*CPI – 1.70 NaCl*Ggum*CPI (P=0.04).

(1994), increasing the ionic strength of the protein solution masks charged groups that have become newly accessible through heat-induced conformational rearrangements, thus enhancing hydrophobic interactions. In a study using differential scanning calorimetry, the effect of NaCl on the thermal transition characteristics of β-lactoglobulin showed that the T_d was increased at increasing NaCl concentration (Harwalkar and Ma, 1987). In the present study, it is possible that NaCl stabilized the structure of CPI-guar gum systems, thus higher T_d values were obtained as NaCl concentration increased (Fig. 12).

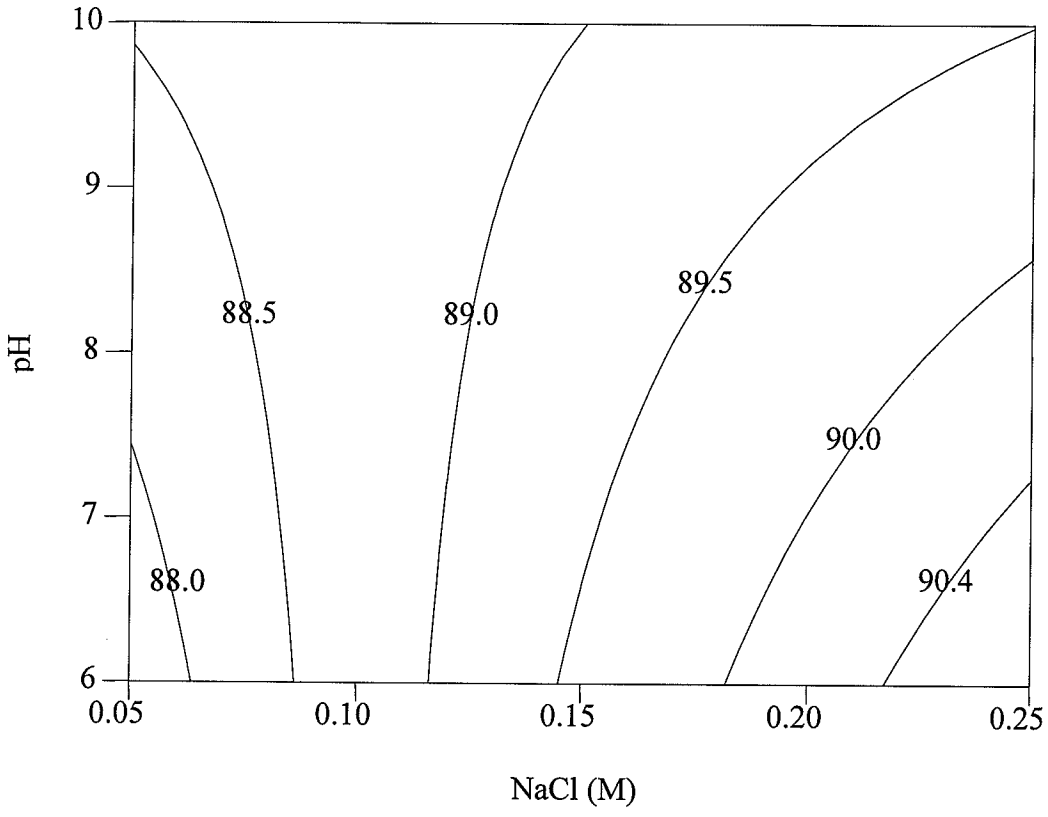


Figure 12. Denaturation temperature (T_d) contour plot showing the influence of NaCl concentration and pH on T_d of CPI-guar gum mixtures prepared with 1.5% (w/v) guar gum and 20% (w/v) CPI. The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given T_d value. For example, at pH 7.0 and 0.20 M NaCl, T_d=90.0°C.

Effect of CPI-pH interaction on T_d of CPI-guar gum systems

The effect of the interaction between pH and CPI concentration on the T_d of CPI-guar gum systems is shown in Figure 13. T_d values of the biopolymer mixture increased as CPI concentration increased. This increase may be due to the extensive interaction (due to quantitative effect) of the unfolded biopolymer molecules. As CPI concentration increased, there is a significant increase in the number of exposed hydrophobic residues involved in protein-protein interaction, thereby stabilizing the CPI-guar gum structure. It is also possible that the biopolymer molecule became more compact in shape as CPI concentration increased, thus improved structural stability was achieved.

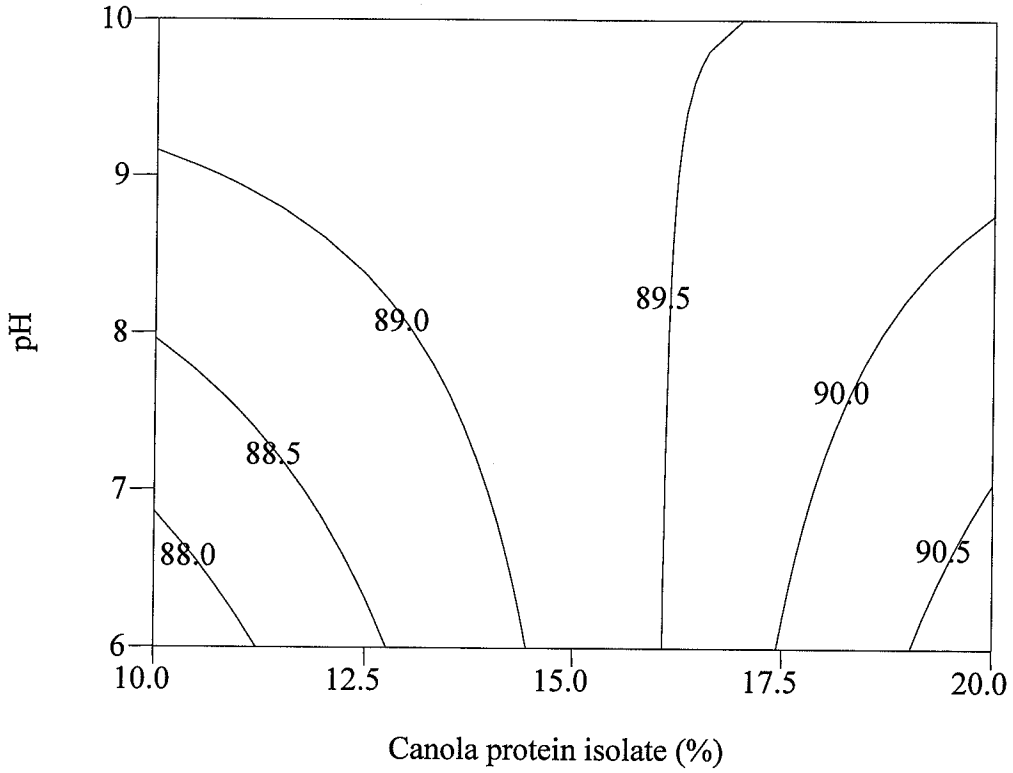


Figure 13. Denaturation temperature (T_d) contour plot showing the effect of interaction between pH and CPI concentration on T_d of CPI-guar gum mixtures prepared with 0.25 M NaCl and 1.0% (w/v) guar gum. The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given T_d value. For example, at pH 7.0 and ≈17.5 % (w/v) CPI, T_d=90.0°C.

Effect of CPI-guar gum interaction on ΔH of CPI-guar gum systems

Based on the ANOVA model (Design-Expert® software), pH, guar gum concentration and interaction between CPI concentration and guar gum concentration were significant ($P<0.05$; Appendix 4). Figure 14 shows the effect of the interaction between CPI and guar gum on the ΔH values of the biopolymer mixtures. At pH 6, the ΔH values increased with increasing guar gum and CPI concentrations; whereas at pH 10, the ΔH values decreased as the guar gum and CPI levels increased. Guar gum is non-ionic and probably does not interact with CPI at pH 6 or 10. According to Arntfield and Cai (1998), neutral polysaccharides (e.g. guar gum) and canola protein tended to be incompatible, showing no evidence of interaction. The stabilization (high ΔH values) of CPI at pH 6 by higher biopolymer concentrations may be due to the incompatibility of the biopolymers such that an increase in the concentration of one or both biopolymers forces the other into a smaller volume of liquid, thus increasing its effective concentration. The decrease in ΔH at pH 10 as biopolymer concentration increased may be due to excessive intramolecular charge repulsion between protein molecules at high pH (such as pH 10), such that the influence on effective concentration seen at pH 6 simply accentuates this repulsion.

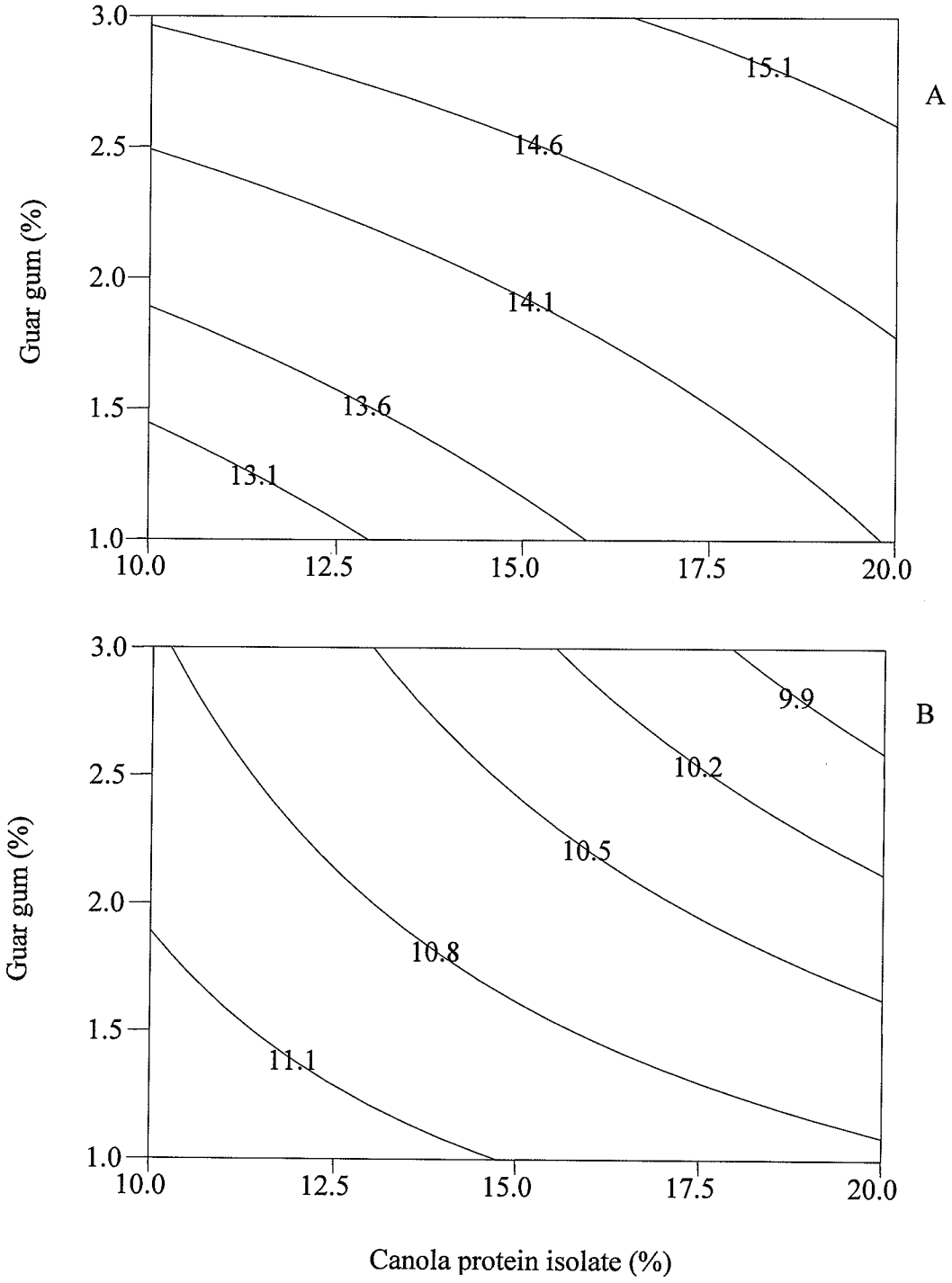


Figure 14. Enthalpy of denaturation (ΔH) contour plots showing the influence of CPI-guar gum interaction on ΔH of CPI-guar mixtures at 0.05 M NaCl and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given ΔH value. For example, in Figure 14B; at 17.5% (w/v) CPI and 2.5% (w/v) guar gum, ΔH =10.2°C.

Impact of sodium salts on thermal characteristics of CPI-guar gum mixtures

Generally, the relative ability of various anions (at isoionic strength) to decrease the structural stability of proteins follows the chaotropic series $F^- < SO_4^{2-} < Cl^- < Br^- < I^- < ClO_4^- < SCN^- < Cl_3CCOO^-$ (Damodaran, 1996). According to this author, NaF, Na_2SO_4 and NaCl increase T_d whereas NaSCN and $NaClO_4$ decrease it. The T_d values of CPI-guar gum mixtures were similar at the 0.05 M salt concentration regardless of salt type (Table 4). At this salt level, the T_d values of all treatments did not support lyotropic effects, thus nonspecific electrostatic interactions may be responsible for the similarity in T_d values observed for all salt treatments. The T_d values for the biopolymer mixtures in the 0.5 M salt series (except for NaSCN-treated samples) were higher than that for the mixture without salt (88.8°C). This reflects an increased resistance to heat denaturation by the CPI-guar gum systems at high salt concentration (0.5 M).

At higher concentrations, neutral salts are known to have ion-specific effects on hydrophobic interactions in addition to nonspecific charge neutralization effects (von Hippel and Schleich, 1969). These ion-specific effects on hydrophobic interactions are thought to be due to salt effects on bulk water structure, which affect protein-protein and protein-water interactions (Damodaran, 1996; von Hippel and Schleich, 1969). At 0.5 M salt level, the ability of the anions to increase T_d of CPI-guar gum mixtures followed the sequence sulfate = acetate > chloride > thiocyanate, whereas the degree to which the anions decreased ΔH followed the order $C_2H_3O_2^- < Cl^- < SO_4^{2-} < SCN^-$. These sequences are different from the lyotropic series observed by Damodaran (1996). This difference may be due to the presence of guar gum. In the present study, $C_2H_3O_2^-$ anion acted as a structure stabilizer (having higher ΔH value at 0.5 M salt

Table 4. Influence of salt type and concentration on the thermal properties (T_d, ΔH) of canola protein-guar gum mixtures prepared with 20% (w/v) protein and 1.5% (w/v) guar gum at pH 10.

Salt type	T_d (°C)*	
Control (no salt)	88.80 ± 0.57^{a1}	88.80 ± 0.57^{c1}
	0.05 M	**0.5 M**
Na_2SO_4	88.65 ± 0.70^{a1}	95.01 ± 0.40^{a2}
$NaC_2H_3O_2$	89.22 ± 0.02^{a1}	95.61 ± 0.10^{a2}
NaCl	88.51 ± 0.34^{a1}	91.90 ± 0.06^{b2}
NaSCN	88.30 ± 0.08^{a1}	88.27 ± 0.05^{c1}

Salt type	ΔH (J/g)*	
Control (no salt)	15.70 ± 0.16^{ab1}	15.70 ± 0.16^{bc1}
	0.05 M	**0.5 M**
Na_2SO_4	14.85 ± 0.91^{bc1}	14.66 ± 0.45^{c1}
$NaC_2H_3O_2$	16.70 ± 0.69^{a1}	18.13 ± 0.24^{a1}
NaCl	16.79 ± 0.45^{a1}	15.93 ± 0.13^{b1}
NaSCN	13.83 ± 0.01^{c1}	12.25 ± 0.83^{d1}

*Mean ± SD. For a given parameter (T_d or ΔH), column values with different letters and row values with different numbers are significantly different ($P<0.05$)

level), whereas SCN- anion probably acted as a structure destabilizer (having lowest ΔH values at the two salt levels). The destabilizing effect of NaSCN (a chaotropic salt) was not evident in the T_d values since the T_d values at the 0.05 M (T_d=88.30° C) and 0.5 M (T_d=88.27° C) salt levels were similar to those of the control. Regardless of salt type, there was no significant change in the biopolymer structure as salt concentration increased to 0.5 M.

In a study on canola protein isolate, the ability for the anions to increase T_d values followed the series sulphate = acetate > chloride = thiocyanate (Léger and Arntfield, 1993). The authors indicated that Na_2SO_4 acted as a promoter of hydrophobic interactions, whereas NaSCN acted as a protein structure destabilizer. In the present study, the stability (higher T_d values) was higher at the higher salt concentration for sulfate-, acetate- and chloride-treated biopolymer mixtures; whereas the T_d of thiocyanate-treated mixtures was similar to that of the control. This suggests that the guar gum may have stabilized the CPI-guar gum mixture in the presence of NaSCN. Whistler and BeMiller (1997) reported that guar gum is used as a stabilizer in food products.

Effect of urea and DTT on thermal transition properties of CPI-guar gum systems

Influence of urea: The thermal properties (T_d, ΔH) of CPI alone (T_d=82°C, ΔH=3.8 J/g) and CPI-guar gum mixtures (T_d=82°C, ΔH=3.1 J/g) treated with urea as well as CPI-guar gum mixture without urea (T_d=89°C, ΔH=15.7 J/g) are shown in Figure 15A. In urea-treated CPI-guar gum mixtures, the effect of urea on the T_d and ΔH values of CPI alone is similar to those of CPI-guar gum mixtures. This indicates that guar gum did not influence the effect of urea on the biopolymer mixtures, suggesting that urea affects proteins alone. However, the urea-treated biopolymer mixtures had lower T_d and ΔH values compared to those of the control (without urea). According to Damodaran (1996), denaturation of proteins by urea is thought to involve the solubilization of hydrophobic amino acid residues in urea solution. Since urea has the potential to form hydrogen bonds, at high concentration the solute breaks down the hydrogen bonded

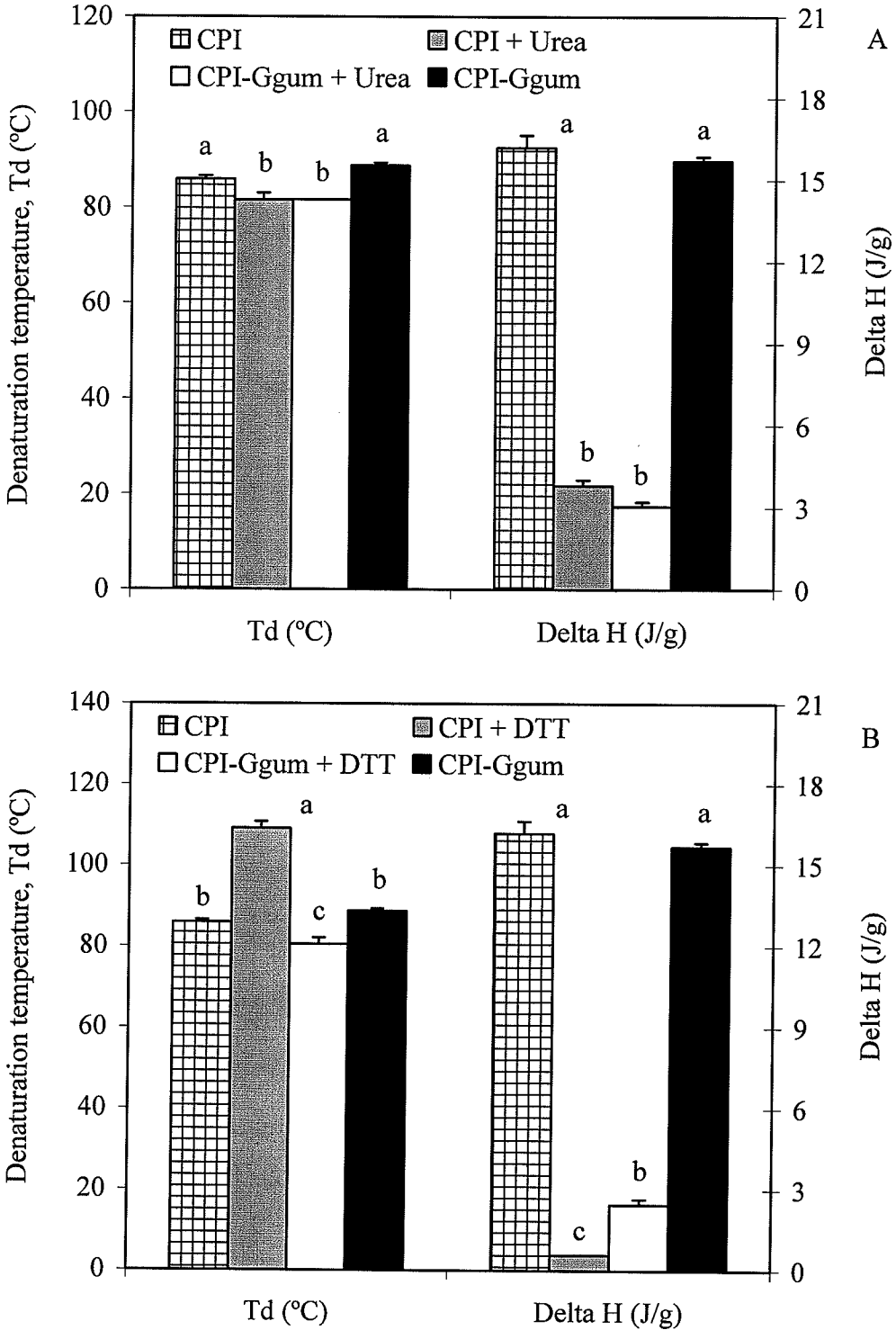


Figure 15. Effect of urea (A) and dithiothreitol (B) on the thermal transition properties (T_d, ΔH) of canola protein isolate (CPI) and CPI-guar gum mixtures (20%, w/v CPI; 1.5%, w/v guar gum; 0.15 M DTT or 6 M urea; pH 10). For T_d or ΔH and within treatments, means with different letters are significantly different ($P<0.05$).

structure of water; and this destructuring of solvent water makes it a better solvent for nonpolar residues. This results in unfolding and solubilization of nonpolar residues from the interior of the protein molecule. Phillips et al. (1994) reported the denaturation of β-lactoglobulin in 6 M urea. In the present study, the denaturing effect of urea explains the lower thermal properties obtained for urea-treated biopolymer mixtures. This supports the involvement of noncovalent interactions in the stability of protein (or protein-hydrocolloid) mixtures.

Influence of DTT: Figure 15B shows the thermal properties (T_d, ΔH) of CPI alone (T_d=109°C, ΔH=0.6 J/g) and CPI-guar gum mixtures (T_d=81°C, ΔH=2.5 J/g) treated with DTT as well as CPI-guar gum mixture without DTT (T_d=89°C, ΔH=15.7 J/g). The ΔH values in DTT-treated CPI-guar gum systems were much lower than those in CPI-κ-CAR systems, indicating pronounced structural disruption of the biopolymer molecules. Elevated pH used (i.e. pH 10) in CPI-guar gum mixtures may have contributed to the very low ΔH values observed in these systems. As seen in the CPI alone treated with DTT, higher T_d and lower ΔH values (T_d=109°C, ΔH=0.6 J/g) were noted as compared to the CPI-guar gum mixture without DTT (T_d=89°C, ΔH=15.7 J/g). This increase in T_d may be due to the cleavage of disulfide bonds in the CPI molecule prior to heating. This exposed the binding sites that otherwise would have remained buried within the molecule, resulting in excessive crosslinking and subsequent structure formation and stability (higher T_d value). As explained by Damodaran (1988), the structure of the stabilized protein molecule may not be the same as that of the native molecule and this may create a net decrease in ΔH. Léger and Arntfield (1993) reported a lower T_d value for canola protein isolate (at pH 9.0) treated with 0.15 M DTT

(T_d=67°C) compared to that of the control (T_d=81°C). In the present study, the addition of DTT to CPI-guar gum mixtures had more effect on the ΔH values than on T_d values. Given that disulfide bonds have high energy requirement for their disruption, it could be that the disruption of these bonds by DTT prior to heat treatment greatly reduces the heat flow required to thermally denature the remaining structure. Since disulfide bonds are thought to be responsible for subunit association (Schwenke et al., 1983), it is possible that the subunits themselves remained relatively intact upon exposure to DTT, thereby requiring temperatures of up to 109°C to denature. Low ΔH values show that disulfide bonding contributes to the stability of canola protein-hydrocolloid structure.

CHAPTER 2

Structural Behaviour of Commercial Canola Protein Mixed with Hydrocolloids

Abstract

The surface activity of a protein is related to its conformation and its interaction with other food components. The number of hydrophobic patches on the protein surface determines how rapidly it can adsorb to an oil-water or air-water interface. This study examined the surface hydrophobicity (S_o) of canola protein isolate (CPI) mixed with hydrocolloids (κ-carrageenan, κ-CAR; guar gum) and the effects of pH, NaCl and hydrocolloid concentrations on this property. The fluorescence intensity of CPI-hydrocolloid mixtures were measured using fluorescence spectrophotometer and the S_o values were determined using 8-Anilino-1-NaphthaleneSulphonate (ANS) as a hydrophobic fluorescent probe. The S_o values of CPI alone were 72 and 88 at pH 6 and 10, respectively. The S_o values of CPI-hydrocolloid mixtures (10% w/v CPI, 1% w/v hydrocolloid, 0.05 M NaCl) increased at high pH, hydrocolloid and NaCl concentrations. Overall, CPI-κ-CAR mixtures had lower S_o values than CPI-guar gum mixtures. High S_o values reflects increased exposure of nonpolar amino acid residues due to changes in protein structure. The results indicate that the surface activity of CPI can be improved when mixed with hydrocolloids and this will enhance its use as a functional food ingredient.

Introduction

In food systems, proteins interact with other food components such as polysaccharides and this modifies proteins or polysaccharides functional behaviour (Damodaran, 1996). The overall structure-function relationships of food systems containing these two biopolymers depends on the individual biopolymers as well as the strength and interaction of the biopolymers (Galazka et al., 1999b). In dairy products, polysaccharides may be used at levels of 0.005-3% to confer required functional properties (Thomas, 1992). For example, polysaccharides such as κ-CAR provide stability to milk products by interacting directly with the milk proteins (Drohan et al., 1997). Canola meal, with a protein content of about 40% (Shahidi, 1990), has a well-balanced amino acid profile (Ohlson, 1978) and a favourable protein efficiency ratio (PER) of 2.64 compared to 2.19 for soy protein (Delisle et al., 1984).

A fundamental understanding of the functional characteristics of canola proteins and the changes these properties undergo during processing is essential for it to compete with traditional food proteins. Surface and emulsifying properties of proteins are strongly correlated to their structure (Dickinson and Stainsby, 1988; Damodaran, 1996). Hydrophobic, steric and attractive forces are important variables that affect the structure of proteins and their interactions with other molecules (Nakai, 1983). Other factors such as pH, temperature, ionic conditions and disulfide bonds may affect molecular flexibility or stability (Harwalker and Ma, 1989; Koning and Visser, 1992), and thus protein hydrophobicity (Alizadeh-Pasdar and Li-Chan, 2001). The addition of polysaccharides to proteins may also alter the functionality of both biopolymers. Galazka et al. (1999b) studied the effect of high pressure treatment on bovine serum albumin (BSA)-sulphated

polysaccharide complexes, and noted that the addition of κ-CAR or ι-CAR to BSA (at pH 7) decreased the surface hydrophobicity (S_o) of the protein. They suggested that the decrease in S_o was mainly due to electrostatic repulsion between the two negatively charged molecules (κ-CAR and BSA), and the blocking of ANS binding sites by the formation of polysaccharide-BSA complexes.

Fluorescent probe methods are simple techniques used to assess protein surface hydrophobicity (Slavic, 1994; Royer, 1995) and a number of fluorescent probes have been used for this purpose. 8-Anilino-Naphthalene-1-Sulfonate (ANS), an anionic hydrophobic probe (Alizadeh-Pasdar and Li-Chan, 2001) is the most popular marker (Horiuchi et al., 1978; Clarke and Nakai, 1972). *Cis*-Parinaric acid ($C_{18}H_{28}O_2$; C18:4), a natural polyene fatty acid was used to assess the S_o of selected proteins (e.g. soy isolate, pea isolate, canola isolate) and it was shown that the hydrophobicity of unfolded proteins (S_e) was better correlated with thickening, coagulation and gelation than the S_o (Voutsinas et al., 1983). An uncharged fluorescent probe PRODAN (6-Propionly-2-N-N-Dimethylamino-Naphthalene) has also been used to examine the hydrophobicity of proteins (whey protein isolate, β-lactoglobulin, BSA) when interacting with κ-CAR (Alizadeh-Pasdar and Li-Chan, 2001). The control or manipulation of protein-polysaccharide interactions is a major factor in the development of novel foods (Tolstoguzov, 1997). The understanding of the factors that control or enhance structural characteristics of plant proteins in the presence of polysaccharides can provide useful information on the utilization of potential functional ingredients (such as canola proteins) to improve the quality of food products. This study examines the surface behaviour of canola protein isolate in multicomponent food systems.

Materials and Methods

Source of materials

Food grade κ-carrageenan (No. C-1013) that contains predominantly κ- and lesser amounts of λ-carrageenan, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The composition of κ-carrageenan (κ-CAR) powder as indicated by the manufacturer showed it contained a mixture of the following cations: K^+ (10.4%), Ca^{2+} (2.3%) and Na^+ (0.9%). Food grade guar gum (G-4129; Lot No. 95H0653) with a viscosity of 6000-10000 mPas (Whistler and BeMiller, 1997), and 8-Anilino-1-NapthaleneSulphonate (ANS; Lot 109C-0640; No. A-5144) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Commercial (BMW Canola, Winnipeg) canola protein isolate (CPI) was purchased and used without further purification. Proximate analysis (AOAC, 1990) 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). All other chemicals such as NaCl (BP358-212, Lot No. 028091), HCl (A144-225, Lot No. 296220), and NaOH (BP359-212, Lot No. 974661) were certified reagent grade (Fisher Scientific Co., New Jersey).

Sample preparation

Dispersions of CPI (10, 20% w/v; protein-basis), κ-CAR (1, 3% w/v), and guar gum (1, 3% w/v) were prepared by mixing in NaCl solution (0.05, 0.25 M) at desired pH (6, 10). The CPI, κ-CAR, guar gum, pH and NaCl combinations were generated using Design-Expert® Software. The mixture was stirred for approximately 1 h at room temperature or until a complete dispersion of the mixture was achieved. Dispersions of individual

CPI samples in appropriate pH and NaCl solutions were prepared in similar manner to serve as a control. To assess the influence of pH, samples were adjusted to pH 6, 10 with 1 M NaOH or 1 M HCl. To ensure that pH was maintained, samples were allowed to equilibrate for 30 min at room temperature and the pH was rechecked prior to further testing.

Experimental design

All experimental measurements were done in duplicates. Design-Expert® Software (Stat-Ease Inc., Minneapolis, MN, USA) experimental designs, factorial and response surface optimization were used in this study. The experiment was carried out in two stages. In the first stage, a series of control experiments with dispersions of canola protein isolate (CPI, 10% w/v) were carried out to characterize CPI under varied conditions (pH and NaCl concentrations). In stage two, another series of experiments were carried out to characterize different combinations of CPI and hydrocolloids (i.e. CPI-κ-carrageenan, CPI-guar gum) following a full factorial model. A two-level factorial design for 4 factors in 20 experiments including four replicates of the center point was generated. The factors included pH (6, 10), CPI (10, 20% w/v), NaCl (0.05, 0.25 M), guar gum (1, 3% w/v), and κ-carrageenan (1, 3% w/v) concentrations. The surface hydrophobicity (S_o) of the samples was measured. Two-dimensional contour plots were generated from the fitted model using Design-Expert® Software.

Fluorometry: Measurements of hydrophobic region on molecular surface

Surface hydrophobicity (S_o) was determined using ANS as a fluorescence probe. Measurements were performed according to the method of Kato and Nakai (1980) with some modifications. The S_o of canola protein isolate (CPI), CPI-κ-carrageenan and CPI-guar gum systems were studied in varied conditions (pH, salt concentration). Each sample was serially diluted with 0.1M phosphate buffer (pH 7) to obtain protein concentrations ranging from 0.01 to 0.4 mg/mL (five concentrations). The fluorescence intensity (FI) of the samples (2 mL each) was measured in the presence and absence of ANS with a Perkin-Elmer LS-5 fluorescence spectrophotometer (Perkin-Elmer Life and Analytical Sciences Inc., Boston, MA, USA) at excitation wavelength of 390 nm and emission wavelength of 470 nm using slit width of 0.5 nm. Samples containing ANS were prepared by adding 10 μL ANS (8 mM) solution to the 2 mL sample dispersion and mixed, then the FI of the samples were measured. The relative fluorescence intensity (RFI) at each protein concentration was calculated by subtracting the FI of each sample without ANS from the FI of the corresponding sample containing ANS. The initial slope (S_o) of the RFI versus protein concentration plot was used as an index of the protein surface hydrophobicity. The initial slope was calculated by linear regression analysis. All determinations were done in duplicate.

Statistical analysis

All analyses were conducted in duplicate and data used in tables and figures are average values. Data were analyzed by analysis of variance (ANOVA) procedure using SAS® 1999-2001 Proprietary Software Release 8.2 (TS20; SAS Institute Inc., Cary, NC, USA).

Duncan's multiple range test was used to determine statistical differences ($P \leq 0.05$) between treatment means (Steel et al., 1997).

Results and Discussions

Visual evaluation

On visual examination, only a single phase was observed at the CPI, κ-CAR (1, 3% w/v), and guar gum (1, 3% w/v) concentrations used for surface hydrophobicity evaluations. There was no visual evidence of separate liquid phases or gel formation. A single-phase mixture can exist in a system of incompatible polymers and this occurs when the protein-polysaccharide concentration is below the phase separation threshold (Tolstoguzov, 1991).

Canola protein isolate-κ-carrageenan systems

The surface hydrophobicity (S_o) values and the model equation from the full factorial design for CPI-κ-carrageenan mixtures at varied conditions are shown in Table 5. Significant factors and interactions were identified using the ANOVA model (Design-Expert® software). Kappa-carrageenan (κ-CAR) concentration, pH, interaction between NaCl and CPI concentrations, a 3-way interaction between pH, NaCl and κ-CAR concentrations, and a 3-way interaction between pH, κ-CAR and CPI concentrations were significant ($P<0.05$; Appendix 5).

Table 5. The surface hydrophobicity (S_0) values of canola protein-κ-carrageenan mixtures under varied conditions.

pH	NaCl (M)	κ-carrageenan (%)	Protein (%)	S_0	R^2 value
6	0.05	1	10	117	0.991
6	0.05	1	20	145	0.9994
6	0.05	3	10	123	0.9992
6	0.05	3	20	131	0.9993
6	0.25	1	10	139	0.9997
6	0.25	1	20	127	0.9996
6	0.25	3	10	139	0.9968
6	0.25	3	20	128	0.9996
8	0.15	2	15	141	0.9995
8	0.15	2	15	141	0.9989
8	0.15	2	15	141	0.9995
8	0.15	2	15	141	0.9997
10	0.05	1	10	126	0.9973
10	0.05	1	20	119	0.9991
10	0.05	3	10	136	0.9995
10	0.05	3	20	143	0.9994
10	0.25	1	10	136	0.9994
10	0.25	1	20	129	0.9998
10	0.25	3	10	124	0.995
10	0.25	3	20	114	0.9991

S_0 = 224.08 – 11.85 pH – 326.88 NaCl – 48.74 κ-CAR + 0.82 CPI + 57.97 pH*NaCl + 5.14 pH*κ-CAR – 0.061 pH*CPI + 233.44 NaCl*κ-CAR – 8.66 NaCl*CPI + 0.11 κ-CAR*CPI – 30.03 pH*NaCl*κ-CAR + 0.037 pH*κ-CAR*CPI (P=0.013)

Effects of pH-NaCl-κ-carrageenan interaction on surface behaviour

The contour plots of S_o as a function of NaCl and κ-CAR concentrations at pH 6 and 10 are shown in Figure 16. S_o is indicative of the number of hydrophobic amino acid residues on protein surface. At pH 6 (Fig. 16A), the interaction between NaCl and κ-CAR affected the protein or protein-κ-CAR conformation. It was evident from the curves that surface structural activity (S_o values) decreased with increasing levels of κ-CAR. At κ-CAR levels below 2% (w/v), higher S_o values were associated with low NaCl level. It is possible that low S_o values indicated that more non-polar amino acid residues were involved in protein-protein or protein-κ-CAR interaction, thus not available as exposed residues for interaction with ANS. It is also possible that the proteins became more compact in shape, resulting in low S_o values.

At pH 10 (Fig. 16B), the interaction between CPI and the other system components (e.g. NaCl and κ-CAR) became more complex. S_o increased at higher salt and lower κ-CAR concentrations and also at lower salt and higher κ-CAR levels, an indication of increased exposure of non-polar amino acid residues in these environments. Higher salt concentrations (>0.2 M) screen the electrostatic interactions between biopolymers (e.g. gelatin and anionic polysaccharide) and encourage the self-association of gelatin molecules (Tolstoguzov, 1986); the self-association may involve hydrophobic interactions. Perhaps binding promoted the unfolding of hydrophobic residues and may explain the high S_o values observed at higher NaCl level in the present study.

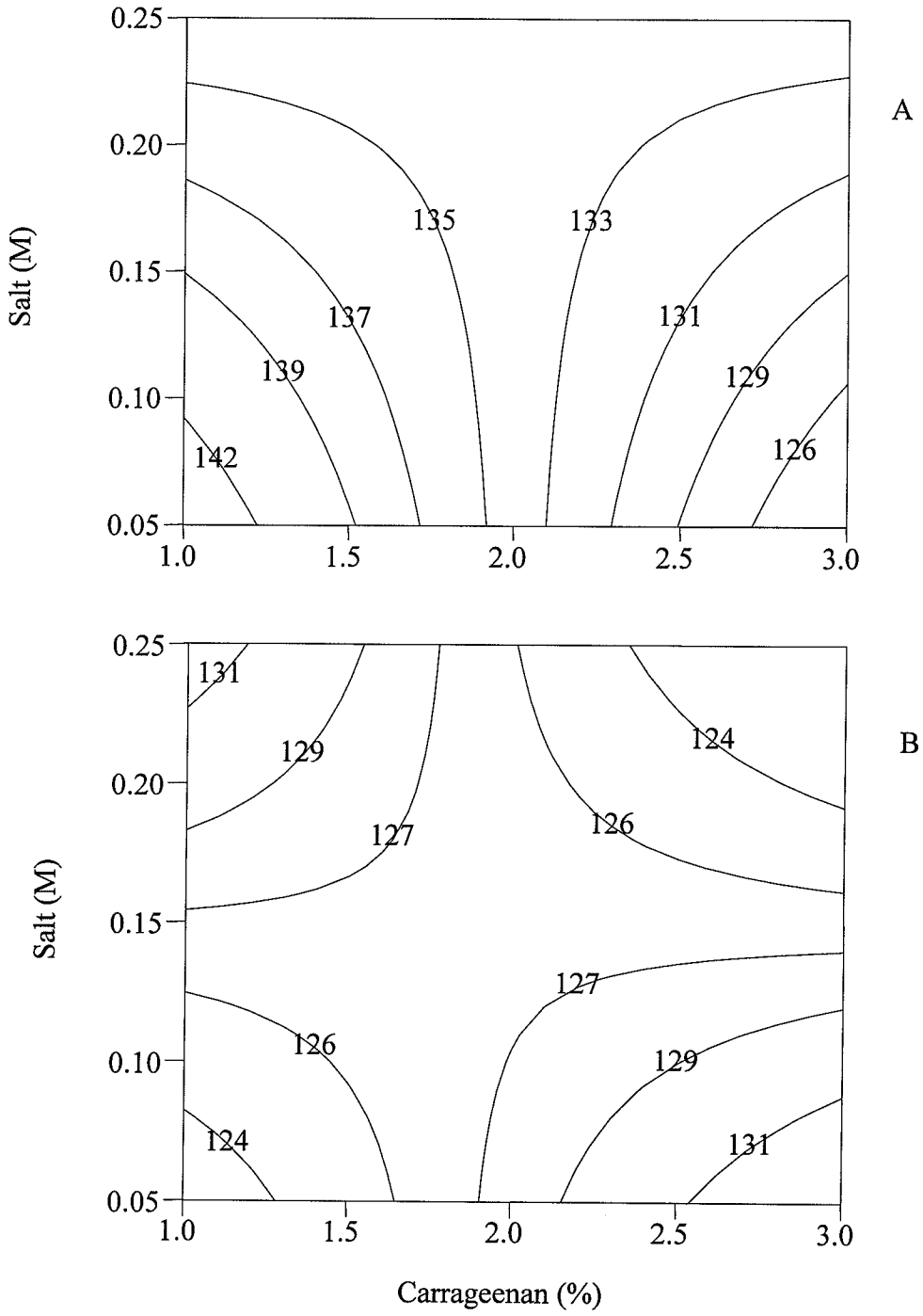


Figure 16. Contour plots showing the influence of κ-carrageenan (κ-CAR) and NaCl concentrations on the surface hydrophobicity (S_o) of CPI-κ-CAR systems at 15% (w/v) CPI and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_o value. For example, in Figure 16B; at 2.5% (w/v) κ-CAR and 0.1 M NaCl, S_o = 129.

Impact of canola protein-pH-κ-carrageenan interaction on surface activity

The contour plots of S_o as a function of κ-CAR and CPI concentrations at pH 6 and 10 are shown in Figure 17. At pH 6, S_o decreased as κ-CAR concentration increased (Fig. 17A). Kappa-CAR is a negatively charged polysaccharide while CPI carries a net positive charge at pH 6 (below CPI's isoelectric point, pH 6.8-7.2); thus an electrostatic interaction results between the two biopolymers (Dalgleish and Hollocou, 1996). This interaction decreased as κ-CAR level increased, thus making sites on the protein molecule inaccessible for binding with ANS. This may explain the low S_o values observed at high κ-CAR levels. In contrast, at pH 10, increased S_o resulted as κ-CAR and CPI levels increased (Fig. 17B). At this pH, CPI and κ-CAR are negatively charged and thus electrostatic repulsion exists. The repulsion may have increased the exposure of functional groups (e.g. hydrophobic residues) resulting in increased binding with ANS, and increased S_o values as CPI and κ-CAR levels increased. The increase in S_o could also be due to quantitative effect (higher protein concentration, higher ANS fluorescence intensity) and not structural changes within the biopolymers.

The affinity of ANS for binding to canola proteins seem to be affected by the presence of κ-CAR in the mixed system, especially at pH 10. Thus under the conditions studied, the binding strength of ANS showed an increase with increasing κ-CAR level (Fig. 17B). This binding of ANS to hydrophobic surfaces on canola protein indicates that these hydrophobic areas are available for other possible reactions. It could be inferred that κ-CAR has an effect on non-covalent interactions (i.e. hydrophobic associations) in mixed CPI systems. With the effect of increasing κ-CAR level at pH 10, the high pH possibly exposed the buried hydrophobic groups in the protein molecule.

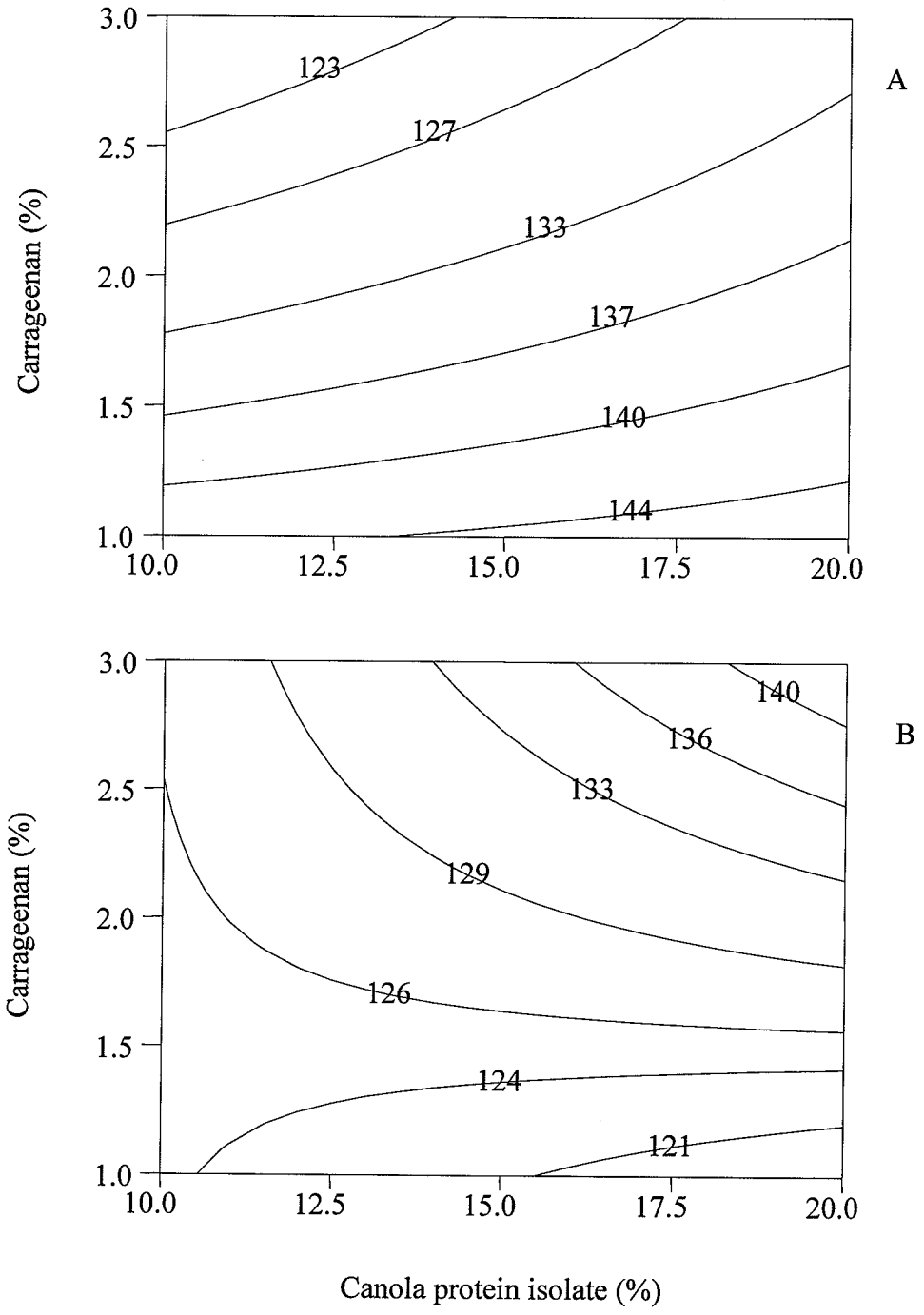


Figure 17. Contour plots showing the effects of canola protein isolate (CPI) and κ-carrageenan (κ-CAR) concentrations on the surface hydrophobicity (S_o) of CPI-κ-CAR systems at 0.05 M NaCl and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_o value. For example, in Figure 17A; at 15% (w/v) CPI and 2% (w/v) κ-CAR, $S_o = 133$.

These unfolded hydrophobic groups probably repositioned themselves in such a way that they could readily interact with ANS. Alizadeh-Pasdar and Li-Chan (2001) reported an increase in S_o of whey protein isolate at high pH (e.g. pH 9) in the presence of κ-CAR, and attributed the increase in S_o to increased exposure of hydrophobic sites resulting from the unfolding of protein molecule in the presence of κ-CAR.

Canola protein isolate-guar gum systems

The surface hydrophobicity (S_o) values and the model equation from the full factorial design for canola protein-guar gum mixtures at varied conditions are shown in Table 6. Based on the ANOVA model (Design-Expert® software), CPI concentration, pH, NaCl concentration, interaction between pH and guar gum concentration, interaction between CPI and guar gum concentrations, a 3-way interaction between pH, NaCl and CPI concentrations, and a 3-way interaction between pH, guar gum and CPI concentrations were significant ($P<0.05$; Appendix 6).

Table 6. Surface hydrophobic (S_o) values of canola protein-guar gum systems under varied conditions.

pH	NaCl (M)	Guar gum (%)	Protein (%)	S_o	R^2 value
6	0.05	1	10	435	0.9975
6	0.05	1	20	449	0.9966
6	0.05	3	10	470	0.9913
6	0.05	3	20	489	0.9961
6	0.25	1	10	540	0.9994
6	0.25	1	20	441	0.9932
6	0.25	3	10	367	0.9959
6	0.25	3	20	461	0.9965
8	0.15	2	15	552	0.9984
8	0.15	2	15	552	0.9959
8	0.15	2	15	552	0.9956
8	0.15	2	15	552	0.9947
10	0.05	1	10	502	0.9973
10	0.05	1	20	432	0.9981
10	0.05	3	10	519	0.9988
10	0.05	3	20	450	0.9894
10	0.25	1	10	490	0.9966
10	0.25	1	20	431	0.996
10	0.25	3	10	518	0.9973
10	0.25	3	20	437	0.9984

S_o = –355.97 + 99.34 pH + 6528.39 NaCl + 404.09 Ggum + 39.79 CPI – 698.99 pH* NaCl – 46.26 pH*Ggum – 5.06 pH*CPI – 3608.04 NaCl*ggum – 327.41 NaCl*CPI – 18.22 Ggum*CPI + 394.57 pH*NaCl*Ggum + 35.18 pH*NaCl*CPI + 2.17 H*Ggum* CPI + 176.59 NaCl*Ggum*CPI – 19.50 pH*NaCl*Ggum*CPI ($P<0.05$)

Effect of CPI-pH-NaCl interaction on molecular conformation

The contour plots of S_o as a function of NaCl and CPI concentrations at pH 6 and 10 are shown in Figure 18. At pH 6 (Fig. 18A), the S_o values increased as NaCl levels increased and CPI concentration decreased. The increase in S_o observed in this study at high NaCl concentration (i.e. 0.25 M) could be described as destabilizing (causing S_o values to increase due to binding of ANS to non-polar residues). Reports on the effect of NaCl on protein structural stability have been varied. NaCl has been described as a stabilizing salt by its ability to increase the thermal stability of plant proteins (Arntfield et al., 1986; Ismond et al., 1986). However, in another study, NaCl at concentrations between 0.1 and 0.5 M was shown to cause a decrease in the enthalpy of denaturation of ovalbumin, indicative of protein denaturation (Arntfiled et al., 1990). Minor variations can occur when ranking anions and cations in terms of their ability to stabilize proteins depending on the protein in question; however, the general trend remains essentially constant (von Hippel and Schleich, 1969); an example (according to the authors) of an anionic series observed with ribonuclease is:

$$\longleftarrow \text{Stabilizing} \qquad \text{Destabilizing} \longrightarrow$$
$$SO_4^{-2} > CH_3COO^- > Cl^- > Br^- > ClO_4^- > CNS^-$$

Based on the above reports, NaCl could be said to have both stabilizing and destabilizing effects on protein conformation. It is possible that NaCl (at high molar concentration) acted as a destabilizing salt in the present study. This may explain the high S_o values observed for CPI-guar gum mixtures at high NaCl concentration (Fig. 18A). As a destabilizing anion, Cl^- may have the ability of binding to protein residues at high levels. This changes the charge profile of the protein by creating an excess negative charge; thus Cl^- could have facilitated unfolding of the protein structure which exposed formerly

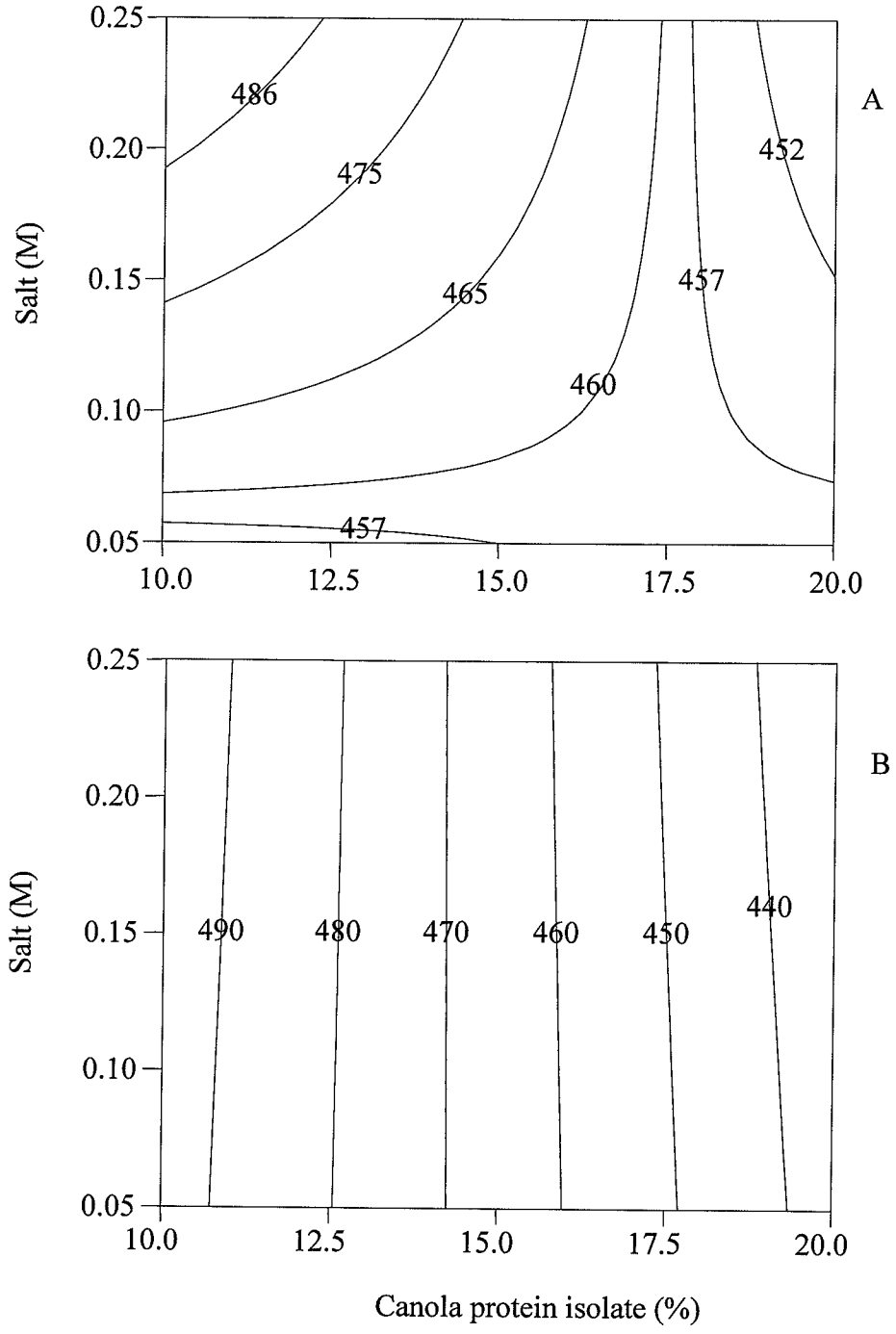


Figure 18. Contour plots showing the influence of canola protein isolate (CPI) and NaCl concentrations on the surface hydrophobicity (S_o) of CPI-guar gum systems at 1.5% (w/v) guar gum and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_o value. For example, in Figure 18A; at 12.5% (w/v) CPI and 0.1 M NaCl, $S_o = 465$.

buried functional groups.

In contrast, at pH 10, S_o values decreased as CPI concentration increased (Fig. 18B). It is possible that the net charge repulsion created by elevated pH led to destabilization of the protein's native structure, exposing reactive groups and thus enhanced protein-ANS interaction over protein-protein association at low protein level. However, the decrease in S_o at increasing CPI level may be due to the unfolding of polypeptide chains as stated by Paulson and Tung (1989), which could have promoted hydrophobic interactions between neighbouring non-polar residues (Samant et al., 1993).

Effects of canola protein-guar gum interaction on surface feature

The 3-way interaction between CPI concentration, guar gum concentration and pH was significant ($P<0.05$). The contour plots of S_o as a function of NaCl and CPI concentrations at pH 6 and 10 are shown in Figure 19. According to Petkowicz et al. (1998), guar gum (being nonionic) is not affected by ionic interaction; an indication that it may not be involved in ionic interaction with CPI. At pH 6 (Fig. 19A), S_o increased as guar gum concentration increased. Perhaps the nonionic guar gum enhances the unfolding of protein structure which exposes hydrophobic residues in the protein so that S_o values are higher. This change in protein conformation was more noticeable at high guar gum concentration. Arntfield and Cai (1998) reported that neutral polysaccharides such as guar gum and canola protein isolate tended to be incompatible. The results of the present study also suggest incompatibility between CPI and guar gum which was more pronounced at high guar gum concentration. The incompatibility may have encouraged

the exposure of hydrophobic residues in the protein resulting in increased interaction with ANS, and increased S_o values as guar gum concentration increased.

However, at pH 10, S_o decreased as CPI concentration increased. Given that proteins carry negative charge at pH above their IEP; this implies that at pH 10, CPI carries a net negative charge. The decrease in S_o with increasing CPI concentration may be due to the electrostatic repulsion between the CPI molecules. The repulsion may have decreased hydrophobic interactions between protein molecules and ANS (Fig. 19B).

Structural activity of canola protein mixed with hydrocolloids

Changes in the conformation of canola protein isolate (CPI) in mixed systems with hydrocolloids (κ-CAR, guar gum) are further illustrated in Figure 20. The S_o values for CPI alone (10%, w/v) were 72 and 88, at pH 6 and 10 respectively (i.e. CPI + 0% κ-CAR in Fig. 20A). Voutsinas et al. (1983) reported a S_o value of 65 for a laboratory prepared CPI using cis-parinaric acid (measures aliphatic amino acid residues) as a fluorescence probe. However, for mixed CPI-κ-CAR treated with 0.05 M NaCl, the S_o values were 117 (at pH 6) and 126 (at pH 10).

These data indicate that the S_o values increased in the presence of κ-CAR. Kato et al. (1981) revealed that most proteins (e.g. ovalbumin, lysozyme) probably increased their S_o values because the hydrophobic residues buried in the interior of proteins were exposed at the molecular surface. It is possible that the electrostatic interaction between CPI and κ-CAR enhanced protein unfolding and the buried hydrophobic residues became available for interaction with ANS, thereby increased S_o values in the presence of κ-CAR. Our results showed that the S_o values were higher at pH 10 than at pH 6.

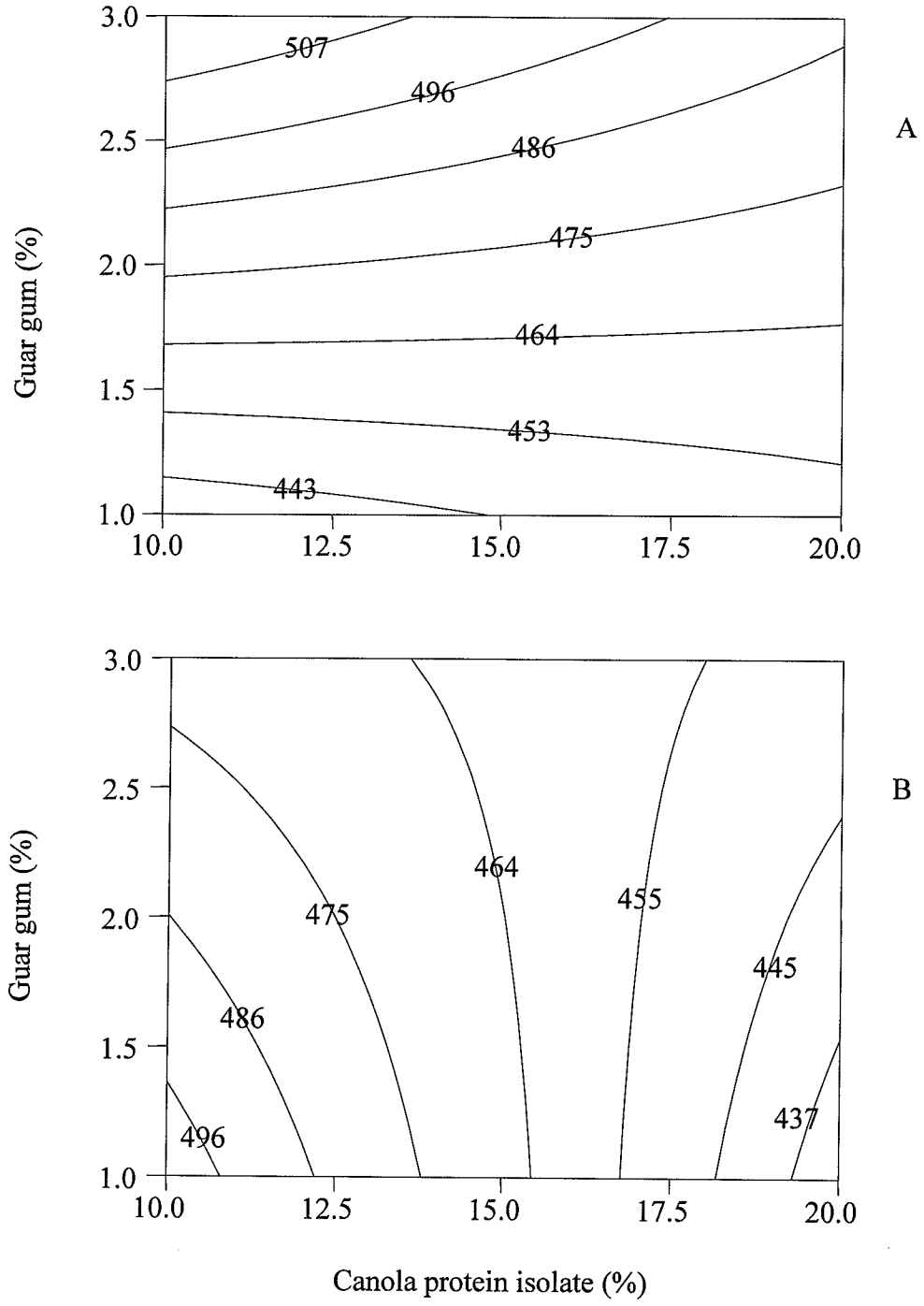
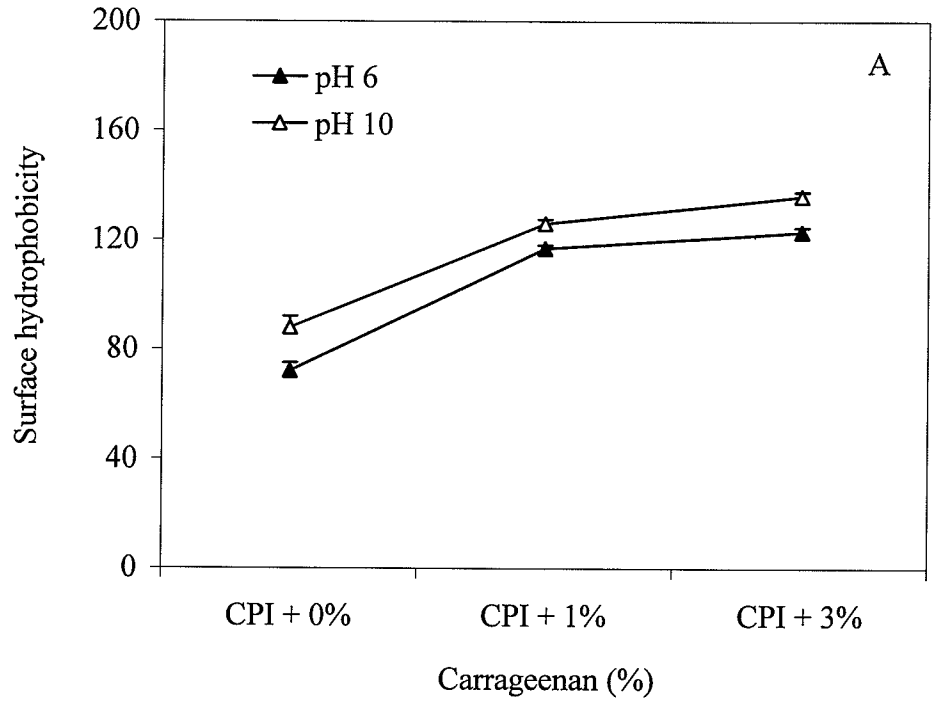


Figure 19. Contour plots showing the influence of canola protein isolate (CPI) and guar gum concentrations on the surface hydrophobicity (S_o) of CPI-guar gum systems at 0.05 M NaCl and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_o value. For example, in Figure 19B; at 12.5% (w/v) CPI and 2% (w/v) guar gum, $S_o = 475$.



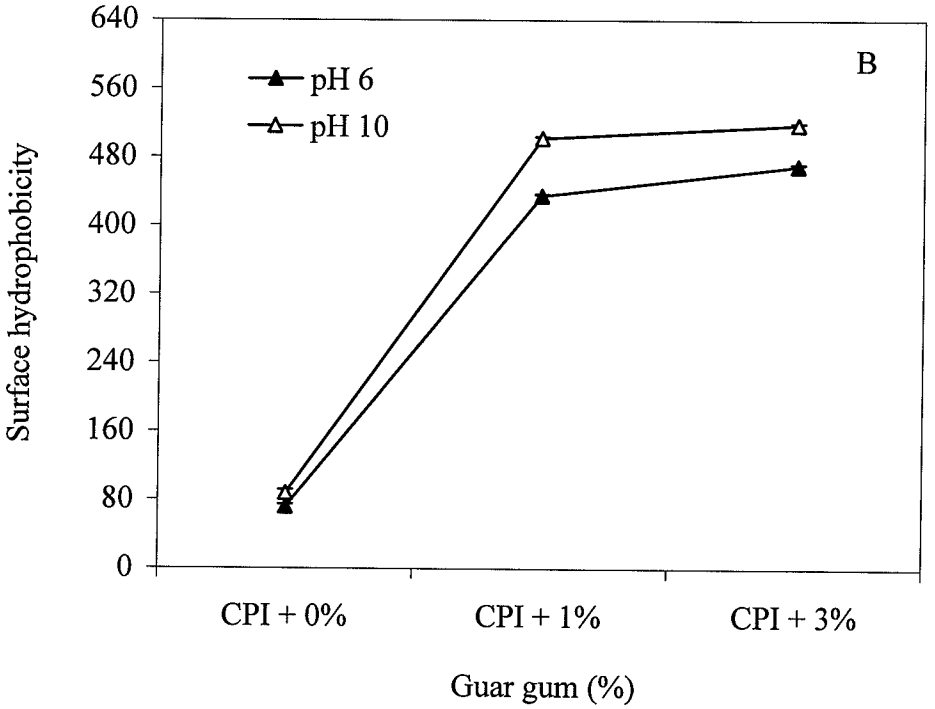


Figure 20. Effects of κ-carrageenan (κ-CAR) and guar gum concentrations on the surface hydrophobicity of canola protein isolate (CPI)-hydrocolloid systems (10%, w/v CPI; 0.05 M NaCl). A: CPI-κ-CAR mixtures; B: CPI-guar gum mixtures.

This finding agreed with the studies cited by Phillips et al. (1994), which reported a compact structure of β-lactoglobulin at low pH and enhanced susceptibility to surface denaturation and a more open flexible structure at high pH (i.e. pH 9.0). A similar observation was made by Alizadeh-Pasdar and Li-Chan (2001) who noted that increases in S_o of whey protein isolate (WPI) in the presence of κ-CAR (i.e. upon addition of high ratio of κ-CAR to WPI at pH 9.0) could be attributed to increased exposure of hydrophobic sites on WPI molecule.

For CPI-guar gum systems; S_o values increased in the presence of guar gum with higher S_o values observed at pH 10 than at pH 6 (Fig. 20B) as was seen with CPI and κ-CAR. However, the S_o values for CPI-guar gum mixtures were significantly ($P<0.05$) higher than those for CPI-κ-CAR systems. This may be attributed to the difference in the chemical structures of the two hydrocolloids.

Surface hydrophobicity of CPI-κ-CAR versus CPI-guar gum systems

It was noted that the S_o values for all CPI-guar gum mixtures (Figs. 18, 19, 20B; Tables 6, 7) were higher than those of CPI-κ-CAR systems (Figs. 16, 17, 20A; Tables 5, 7). One explanation could be the due to the chemical properties of the two hydrocolloids used in this study. Guar gum is nonionic (Whistler and BeMiller, 1997) whereas κ-CAR is a charged sulphated polysaccharide (Tolstoguzov, 1986). This means that κ-CAR can form a soluble or insoluble complex with CPI via electrostatic interaction. This type of interaction will not occur in a system where CPI is mixed with an uncharged polysaccharide such as guar gum. The absence of electrostatic interaction between CPI and guar gum probably made the functional groups in CPI molecules available for

Table 7. Surface hydrophobicity of canola protein-hydrocolloid mixtures prepared with 0.25 M NaCl at varied pH (10%, w/v CPI; 1%, w/v κ-carrageenan or guar gum).

Systems (w/v)	S_o* at pH 6	S_o* at pH 10
CPI (10%)	78 ± 0.75^c	95 ± 0.82^c
CPI + κ-CAR (10 + 1%)	142 ± 0.92^b	134 ± 0.85^b
CPI + Guar gum (10 + 1%)	540 ± 0.14^a	522 ± 0.64^a

*Mean ± SD. Within column, S_o values with different letters are significantly different ($P<0.05$).

interaction with ANS, while disrupting the protein structure.

Galazka et al. (1999b) investigated bovine serum albumin (BSA)-sulphated polysaccharide complexes and showed that adding κ-CAR or ι-CAR to BSA (at pH 7) decreased S_o of the protein. They assumed that the decrease in S_o was mainly due to electrostatic repulsion between the two negatively charged molecules (κ-CAR and BSA). Sulphated polysaccharides, because they have a higher charge density, are capable of forming soluble complexes with globular proteins at pH values above the protein IEP when both polymers carry net negative charge (Tolstoguzov, 1986; Dickinson, 1998). Furthermore, Tolstoguzov (1986) stated that at pH values below the protein IEP, the protein and anionic polysaccharide carry net opposite charge; and insoluble complexes can be formed in this pH region (generally at ionic strength below 0.1-0.2). The complex formation of CPI and κ-CAR (at pH 6 and 10) may have blocked the ANS binding sites, resulting in the lower S_o values observed for CPI-κ-CAR systems when compared to CPI-guar gum mixtures (Fig. 20).

As reported by Alizadeh-Pasdar and Li-Chan (2001), decreases in the S_o values by adding low ratio of κ-CAR to β-lactoglobulin (at pH 5, 7 or 9) may be due to possible blocking of the binding site of the fluorescent probe PRODAN (6-Propionly-2-N,N-Dimethylamino-Naphthalene) by κ-CAR-protein complexing, since PRODAN does not bind to κ-CAR alone. In the present study, evidence of electrostatic interactions between CPI and κ-CAR was demonstrated by changes in S_o of CPI mixed with κ-CAR and NaCl at pH 6 and 10. The lower S_o values observed for CPI-κ-CAR systems (when compared to CPI-guar gum systems) may be attributed to the formation of insoluble complexes (at pH 6) and soluble complexes (at pH 10) in CPI-κ-CAR mixtures in the presence of 0.05 M NaCl; as previously reported for protein and anionic polysaccharides by Tolstoguzov (1986) and Dickinson (1998).

Although electrostatic interactions may not have occurred between CPI and guar gum (since guar gum is nonionic), it is possible that some other interactions (e.g. the formation of hydrogen bonds; electrostatic interactions between CPI and NaCl) occurred in CPI-guar gum systems which resulted in improved surface characteristic (high S_o values) in CPI-guar gum mixtures.

The surface hydrophobicity of CPI-hydrocolloid systems prepared with 0.25 M NaCl at pH 6 and 10 are shown in Table 7. The S_o values for CPI-κ-CAR and CPI-guar gum systems at 0.25 M NaCl (Table 7) were higher than those at 0.05 M NaCl (Fig. 20). It is possible that at the higher salt concentration, destabilization of protein structure exposed previously buried hydrophobic groups. One may argue the rationale for using high hydrocolloid (κ-CAR, guar gum) concentrations (1, 3% w/v) in this study. These hydrocolloid concentrations fall within the range of typical applications using 0.005-3%

polysaccharides in dairy products (Thomas, 1992) based on a 3.5% protein concentration in dairy products such as milk (Alizadeh-Pasdar and Li-Chan, 2001).

CHAPTER 3

Rheological Characteristics of Commercial Canola Protein Isolate-κ-Carrageenan Systems*

Abstract

The thermogelation properties of canola protein isolate (CPI) in a mixed system with κ-carrageenan (κ-CAR) were examined using dynamic rheological testing. The effects of pH, NaCl, κ-CAR and protein concentrations on the gelling ability of CPI were evaluated. Factorial and response surface optimization models were used to identify the processing conditions that would result in CPI-κ-CAR gels with maximized G′ values (≥44000 Pa) and minimized tan δ values (0.01- 0.11). It was found that CPI-κ-CAR gel formation was strongly pH-, NaCl- and κ-CAR concentration-dependent. Results indicated that the optimum conditions for CPI-κ-CAR gels were pH 6, 0.05 M NaCl, 3% κ-CAR and 15% CPI. Samples prepared at pH 6 showed high G′ (97465 Pa) and low tan δ (0.15) values. High G′ values indicate stronger intermolecular network and increased interactions between protein-protein and protein-polysaccharide molecules, while low tan δ values indicate a more elastic network. A synergistic behaviour between CPI and κ-CAR was observed with superior gel strength for the mixed gels at temperatures above 80°C. The mixed gels showed enhanced network structure (low tan δ values) during the heating and cooling phases. The CPI-κ-CAR mixtures exhibited very strong and elastic networks, indicating that CPI can serve as a structuring agent in mixed food systems.

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*Uruakpa, F.O. and Arntfield, S.D. 2004. Food Hydrocolloids 18: 419-427.

Introduction

Oilseed proteins contribute useful functionality to food systems. Functionality can be modified by interactions with other ingredients such as salts and hydrocolloids which are typical food components. An important feature of food systems is their multicomponent character. Proteins and polysaccharides are present together in many kinds of food systems and both macromolecules contribute to the structure, texture and stability of foods. The interactions between these two biopolymers have been investigated (Arntfield and Cai, 1998; Ledward, 1994; Ziegler and Foegeding, 1990; Tolstoguzov, 1986). A review by Doublier et al. (2000) gives an insight on the key parameters involved in protein-polysaccharide interactions. When looking at the interactions between proteins and polysaccharides in terms of network formation, attention must be paid to the potential for both macromolecules to form networks on their own. According to Tolstoguzov (1991), when solutions of two different biopolymers are mixed together, three situations may arise. These are co-solubility, complexing and incompatibility; although co-solubility is rather rare.

The interaction between proteins and anionic polysaccharides is greatest below the isoelectric point (IEP) of the protein but can also occur at pH values above the protein's IEP under certain conditions (Imeson et al., 1977; Tolstoguzov, 1995). Reports (Grinberg and Tolstoguzov, 1997; Syrbe et al., 1998) revealed that the effects of pH on the mixing behavior of protein-polysaccharide solutions differ depending on the nature of polysaccharide (i.e. neutral, carboxylated or sulfated). For protein-neutral polysaccharide solutions, pH affects only the protein self-association. Hence, incompatibility increases under conditions that promote the self-association of biopolymers and favour inhibition of protein-polysaccharide complexing (Tolstoguzov,

1991). For protein and anionic polysaccharide (carboxylated or sulfated) systems, the pH has an effect on protein self-association as well as on protein-polysaccharide cross-association (Grinberg and Tostoguzov, 1997; Syrbe et al., 1998). Complex coacervation is observed at pH below the IEP of the protein, where the macromolecules carry opposite charges. At pH above the IEP of the protein, both biopolymers have similar negative net charges. Thus, complexing is inhibited and incompatibility is promoted (Tolstoguzov, 1991). For sulfated polysaccharides, a soluble protein-polysaccharide complex may be formed at pH above the protein IEP resulting from local segment-segment attraction of protein (NH_3^+) with hydrocolloid (OSO_3^-) residues. Such attraction is very strong and even possible when both protein and sulfated polysaccharide are negatively charged (Grinberg and Tolstoguzov, 1997; Syrbe et al., 1998).

Kappa-carrageenan is an anionic sulfated polysaccharide extracted from certain species of red seaweed (algae), which is widely used as a thickening, gelling and stabilizing agent in the food industry (Clark and Ross-Murphy, 1987). Its gelation occurs on cooling and is generally considered a two-step process, characterized by a coil-helix transition followed by aggregation and network formation (Morris et al., 1980). The gelling behaviour is strongly influenced by the nature and concentration of cations present in the solution as well as by the biopolymer concentration (Hermansson et al., 1991; Rochas and Rinaudo, 1980). The pH is not an important factor for κ-CAR gelation since it has no effect on gel transition temperature (Drohan et al., 1997) and has only a small effect on κ-CAR gel strengths over a broad pH range (pH 5-11) because the sulfate half-ester groups are always ionized, giving the molecules a negative charge (Whistler and BeMiller, 1997). However, at more acidic pH (below pH 4), it may

prevent κ-CAR gelation due to acid hydrolysis (Mleko et al., 1997). Mixed globular proteins-carrageenan systems have been the focus of some investigations. Capron et al., (1999a) and Capron et al. (1999b) showed that the first step of the aggregation of β-lactoglobulin was not changed by the presence of κ-carrageenan, while an acceleration of the gelation process of the protein was experienced in the second step. This was ascribed to a microphase separation; then as soon as the protein network was formed, the separation of the phases was 'frustrated' and the system was 'frozen in'. Other rheological studies confirmed the synergistic effects in κ-CAR-denatured protein systems (Tziboula and Horne, 1999; Ould-Eleya and Turgeon, 2000a, 2000b; Neiser et al., 2000). In detailed rheological studies (Ould-Eleya and Turgeon, 2000a, 2000b) on K^+-κ-CAR in the presence of β-lactoglobulin, these synergistic effects were exhibited in conditions where the two biopolymers gelled at near neutrality. Synergistic interaction was taken as the consequence of two co-continuous networks as a result of a segregative phase separation before gelation takes place. On a similar basis and in combination with TEM observations, Neiser et al. (2000) reached the same conclusions in the case of BSA–K^+-κ-CAR systems. In a study of milk gels formed with κ-CAR or low methoxy pectin, it was suggested that κ-CAR acts as molecular velcro to incorporate casein micelles in the network whilst casein acts as a source of calcium ions which promotes the gelation of pectin (Oakenfull et al., 2000).

Canola is widely grown, particularly in Canada, because it is a rich source of high quality edible oil (Shahidi, 1990). Canola meal that results from oil extraction is predominantly used as an animal feed ingredient though it has immense potential as a source of protein for human consumption. The 12S globulin represents a main storage

protein in the seeds of *Brassica* species (Schwenke et al., 1983), and the isoelectric point (IEP) of 12S canola protein isolate is at pH 6.8-7.2 (Schwenke et al., 1987). The gelling ability of canola protein was first demonstrated in 1978 (Gill and Tung, 1978). It was concluded that gelation occurred through a complex phenomenon involving covalent and noncovalent interactions. One limitation in using CPI is that its gel strength is not comparable to that of egg albumen (Léger and Arntfield, 1993). In a study with canola protein isolate, Arntfield and Cai (1998) revealed that the addition of low concentrations of guar gum improved protein gel characteristics. We hypothesize that the inclusion of κ-CAR in a mixed system with CPI will enhance network formation. The associations between plant proteins and polysaccharides give a more realistic indication of a protein's behaviour in food systems and offer some potential for improving the functionality of the protein. The purpose of this study was to examine the rheological properties of commercial canola protein isolate in a mixed system with κ-carrageenan.

Materials and Methods

Source of materials and sample preparations

Commercial canola protein isolate was purchased from BMW Canola (Winnipeg, Canada) and used without further purification. Proximate analysis (AOAC, 1990) of commercial CPI 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 κ-carrageenan (No. C-1013) that contains predominantly κ- and lesser amounts of λ-carrageenan was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The composition of κ-CAR powder as indicated by the manufacturer showed it contained a

mixture of the following cations: K^+ (10.4%), Ca^{2+} (2.3%) and Na^+ (0.9%). All other chemicals (e.g. NaCl, HCl, NaOH) were certified reagent grade (Fisher Scientific Co., NJ, USA).

Two concentrations of CPI (10, 20% w/v; protein-basis) and κ-CAR (1, 3% w/v) were tested. Dispersions of CPI-κ-CAR mixture were prepared by mixing appropriate amounts of CPI and κ-CAR powders in appropriate portion of NaCl (0.05, 0.25 M) solutions. The CPI, κ-CAR, pH and NaCl combinations were model generated (Design-Expert® Software). The mixture was stirred for approximately 1 h at room temperature or until a complete dispersion of the mixture was achieved. Dispersions of individual CPI and κ-CAR powders in appropriate NaCl solutions were prepared in similar manner to serve as control. The use of dispersions allows monitoring of network formation from the onset of conformational changes. There was no visual evidence of gel formation when CPI-κ-CAR dispersions were prepared at room temperature. However, on mixing individual κ-CAR-salt solution, a very viscous solution was visible. To assess the influence of pH, samples were adjusted to pH 6, 10 with 1 M NaOH or 1 M HCl. To ensure that pH was maintained, samples were allowed to equilibrate for 30 min at room temperature and the pH was rechecked prior to further testing.

Experimental design

All experimental parameter measurements were done in duplicates. Design-Expert® Software (Stat-Ease Inc., MN) experimental designs, factorial and response surface optimization were used in this study. The experiment was carried out in two phases. In phase one, series of experiments were conducted to characterize different combinations

of mixed CPI-κ-CAR gels following a full factorial model. This design contained a 2-level factorial for 4 factors in 20 experiments including four replicates of the center point. The factors included CPI concentration (10, 20% w/v), pH (6, 10), NaCl concentration (0.05, 0.25M) and κ-CAR concentration (1, 3% w/v). Major responses, storage modulus (G′) and loss tangent (tan δ=G″/G′), were measured. Using these data, model fitting was performed using the numerical, graphical and point prediction models (Design-Expert® Software) to determine the optimum conditions for maximizing G′ and minimizing tan δ to obtain improved gel properties (i.e. high G′ and low tan δ values). Two-dimensional contour plots were generated from the fitted model. In phase two, experiments were carried out to verify the optimum conditions obtained in phase one. The model generated conditions for CPI-κ-CAR mixtures were pH 6, 3% (w/v) κ-CAR, 15% (w/v) CPI and 0.05 M NaCl (i.e. verification tests were carried out at these conditions). Control tests of CPI (15%, w/v) and κ-CAR (3%, w/v) at optimum conditions were also carried out.

Statistical analysis

All analyses were conducted in duplicates. Data used in tables and figures were average values. Data from confirmatory and control tests (phase two) were analyzed by analysis of variance (ANOVA) procedure using SAS® 1999-2001 Proprietary Software Release 8.2 (TS20; SAS Institute Inc., Cary, NC, USA); using Duncan's multiple range test to determine statistical differences ($P \leq 0.05$) between treatment means (Steel et al., 1997).

Rheology: Analysis of CPI-κ-carrageenan gel properties

A Bohlin VOR rhoemeter (Bohlin Rheologi, Inc., NJ) was used in phase one while an advanced rheometer 2000 or AR2000 (TA Instruments, New Castle, DE) was used in phase two. The rheometers, operated in a small amplitude oscillatory mode, were used to monitor CPI-κ-CAR gel formation during heating and cooling; and to characterize the rheological properties of resulting gels (Arntfield et al., 1990; Léger and Arntfield, 1993). The Bohlin rheometer was equipped with 30mm parallel plate geometry and sensitivity was established based on a torque bar calibrated to 93.2 gcm. The AR2000 was equipped with 40mm parallel plate geometry and a built-in automated sensitivity. Input strain amplitude for dynamic analysis was 0.02, a value found to be in the linear viscoelastic region in an experiment with canola protein (Arntfied et al., 1990). This strain was used for all rheological measurements.

Approximately 1 mL canola protein-κ-CAR dispersion was placed between parallel plates in the rheometer and the gap between the plates was adjusted to 1 mm when the upper plate was lowered. To prevent drying, samples were surrounded by paraffin oil during the procedure. Samples were heated and cooled over a temperature range of 25° to 95°C at 2°C/min with a 2-min hold time at the final temperature for both the heating and cooling phases. A frequency of 1 Hz was used to collect data during these thermal scans. Sample temperature was controlled by a programmable water bath (for the Bohlin rheometer) and a circulating tap water (for the advanced rheometer 2000). Rheological data were collected at 2 min intervals with a thermal equilibrium time of 10 sec. At the end of each phase, the final temperature was held for 2 min. Frequency sweeps of the final product were measured over a range of 0.01-10 Hz at

25°C. The storage modulus (G′) and loss tangent (tan δ=G″/G′) for representative heating, cooling and frequency sweep curves are reported. Values at 1 Hz were recorded for comparison and statistical analysis. The use of a single frequency (1 Hz) for data comparison has been reported previously (Arntfield et al., 1990; Cai and Arntfield, 1997).

Results and Discussions

Table 8 shows the rheological properties (G′, tan δ) and the model equations from the full factorial design for CPI-κ-CAR mixtures at varied conditions.

Effect of pH-NaCl-κ-carrageenan interaction on storage modulus of gels

Significant factors and interactions were identified using the ANOVA model (Design-Expert® software). NaCl concentration, pH, interaction between pH and NaCl concentration, interaction between pH and κ-CAR concentration, interaction between NaCl and κ-CAR concentrations, and 3-way interaction between pH, NaCl and κ-CAR concentrations were significant ($P<0.05$, Appendix 7). Thus, contour plots of G′ as a function of NaCl and κ-CAR concentrations at pH 6 and 10 are shown in Figure 21. At pH 6 (Fig. 21A), the G′ values increased at lower salt and higher κ-CAR levels, and also at higher salt and lower κ-CAR concentrations. The G′ relates to the compactness of the structures or number of crosslinks within a gel system (Jones, 1979; Murray et al., 1981). Higher G′ values indicate stronger inter-molecular network, possibly due to interactions between CPI and κ-CAR, giving rise to stable high molecular weight complexes. The contribution of electrostatic interactions to gelation of different food

Table 8. Gelation properties (G′, tan δ) of canola protein isolate-κ-carrageenan mixtures under varied conditions.

pH	NaCl (M)	κ-carrageenan (%)	Protein (%)	G′ (Pa)	Tan δ
6	0.05	1	10	42100	0.13
6	0.05	1	20	21700	0.15
6	0.05	3	10	91400	0.10
6	0.05	3	20	80400	0.03
6	0.25	1	10	82300	0.04
6	0.25	1	20	30200	0.09
6	0.25	3	10	2510	0.25
6	0.25	3	20	12000	0.10
8	0.15	2	15	8510	0.09
8	0.15	2	15	6660	0.13
8	0.15	2	15	7490	0.19
8	0.15	2	15	6770	0.11
10	0.05	1	10	21600	0.01
10	0.05	1	20	21200	0.03
10	0.05	3	10	4750	0.22
10	0.05	3	20	4940	0.14
10	0.25	1	10	23400	0.01
10	0.25	1	20	27400	0.01
10	0.25	3	10	4850	0.21
10	0.25	3	20	2490	0.17

G′ = 89813.13 – 9105.31 pH + 1.184×10^{6} NaCl + 54056.88 κ-CAR – 11134.25 CPI – 98818.75 pH*NaCl – 4680.00 pH*κ-CAR + 1308.56 pH*CPI – 7.091×10^{5} NaCl*κ-CAR - 19010.00 NaCl*CPI + 3805.00 κ-CAR*CPI + 61137.50 pH*NaCl*κ-CAR + 816.25 pH *NaCl*CPI – 479.75 pH*κ-CAR*CPI + 5655.00 NaCl*κ-CAR*CPI (P=0.004).

Tan δ = 0.51 – 0.069 pH – 1.89 NaCl – 0.18 κ-CAR + 7.800×10^{-3} CPI + 0.18 pH*NaCl + 0.034 pH*κ-CAR + 1.05 NaCl*κ-CAR – 5.350×10^{-3} κ-CAR*CPI – 0.099 pH*NaCl*κ-CAR (P=0.002).

systems was related to nonspecific ionic attractions at pH below a protein's IEP and specific attraction above the IEP (Stainsby, 1980). At pH 6, CPI will have an overall positive charge (IEP = pH 6.8-7.2) and the anionic polysaccharide (κ-CAR) a net negative charge, hence an electrostatic interaction between the biopolymers is expected. At pH values below CPI's IEP and at low ionic strength, the interaction of the positively charged molecules (such as CPI) and the negatively charged molecules (such as κ-CAR) can result to insoluble electrostatic complexes (Tolstoguzov, 1995; Samant et al., 1993).

In this case the biopolymers are concentrated in the single complex coacervate phase (Tolstoguzov, 1995). This interaction may be responsible for the increased G′ values observed at NaCl concentrations less than 0.15 M and higher κ-CAR levels observed in the present study. However at higher salt concentration, the complexing is inhibited (Samant et al., 1993). Higher salt levels screen the electrostatic interaction between macromolecules and encourage the self-association of protein molecules (Tolstoguzov, 1991). This protein-protein interaction may be responsible for the strong gel network (high G′ values) seen at >0.15M NaCl and lower κ-CAR concentrations (Fig. 21A). Also, the lower G′ values observed at >1.5% κ-CAR and higher salt concentrations could be related to a phase separated network, but this needs to be verified.

Comparing G′ values at pH 6 and 10 showed that G′ values at pH 6 (Fig. 21A) were higher than the values at pH 10 (Fig. 21B). The lower G′ values at pH 10 may be due to thermodynamic incompatibility or phase separation. As stated by Tolstoguzov (1995), at pH values above a protein's IEP and at high ionic strength, the biopolymers macro-ions have like net charges. The mixed solution of biopolymers breaks down into

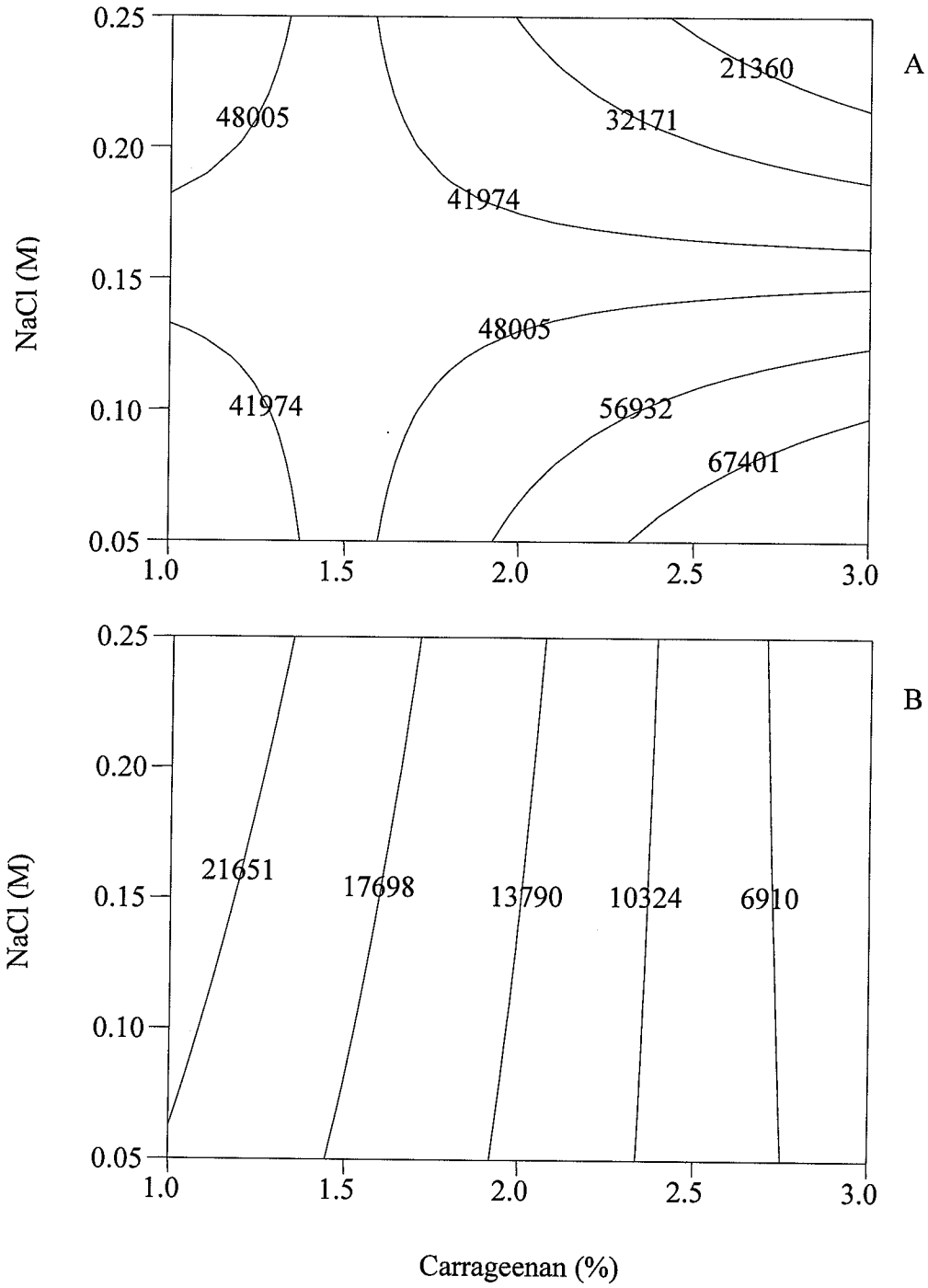


Figure 21. Effect of NaCl and κ-carrageenan concentrations on the storage modulus (G′) of canola protein-κ-carrageenan systems at 15% protein and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given G′ value. For example, in Figure 21B; at 2% (w/v) κ-CAR and 0.15 M NaCl, G′=13790 Pa.

two liquid phases and each of the polymers concentrates into one of the phases. The separated phases could explain our observation in Figure 21B, where at pH 10 (which is well above the IEP of CPI), elastic gels were obtained but with lower G′ values when compared to the G′ values obtained at pH 6. CPI and κ-CAR both have an overall negative charge at pH 10, which increases as the concentration of κ-CAR increases. Similarly, at pH 10 and low ionic strength (such as the 0.05 M NaCl used in this study), relatively linear thin aggregates resulting in the so-called fine-strand gels are formed due to dominant repulsive forces among protein molecules (Aymard et al., 1999) and biopolymers. The increased electrostatic repulsion at this pH may have minimized random aggregation and reduced the degree of protein-protein and protein-polysaccharide interactions. The increase in repulsion between biopolymer molecules may account for the lower G′ values shown in Figure 21B.

Effect of pH-NaCl-κ-CAR interaction on tan delta of CPI-κ-CAR gels

Based on the ANOVA model (Design-Expert® software), the significant ($P<0.05$) factors and interactions were κ-CAR concentration, interaction between pH and κ-CAR concentration, interaction between NaCl and κ-CAR concentrations, interaction between κ-CAR and CPI concentrations, and 3-way interaction between pH, NaCl and κ-CAR concentrations (Appendix 8). Thus, the contour plots of tan δ as a function of NaCl and κ-CAR concentrations at pH 6 and 10 are shown in Figure 22. Tan δ reflects the type of structure or actual arrangement of the networks formed. At pH 6 and 15% CPI, the CPI-κ-CAR gels had lower tan δ values at higher salt and lower κ-CAR concentrations and also at lower salt and higher κ-CAR concentrations (Fig. 22A).

Low tan δ values indicate a more elastic network formation. The networks tend to be less elastic (increased tan δ values) at lower salt and lower κ-CAR concentrations, and also at higher salt and higher κ-CAR concentrations. Increases in tan δ values indicated that the formation of a three dimensional network had been inhibited as was implied by the lower G′ values observed at similar conditions.

At pH 10 (Fig. 22B), lower tan δ values were obtained with decreasing κ-CAR concentration. The lower tan δ values observed here represent the tan δ of pure canola protein gel. Léger and Arntfield (1993) reported lowest tan δ values for canola protein gels prepared at pH 10. The increased electrostatic repulsion at this pH may have minimized random aggregation and reduced the degree of interaction among polypeptides in the system. There is a certain net charge required for gelation rather than coagulation, i.e. some repulsion to prevent excess protein-protein aggregation. The development of superior network structures at high pH values has been attributed to the extension of polypeptide chains and the creation of homogeneous structures (Paulson and Tung, 1989). In comparison, gels prepared at lower pH values are heterogeneous in nature. These heterogeneous networks are composed of compact molecules, which aggregate to form regions of high and low protein concentrations (Léger and Arntfield, 1993). As a result, regions with low protein concentrations act as weak points, causing a drop in overall gel strength and higher tan δ values.

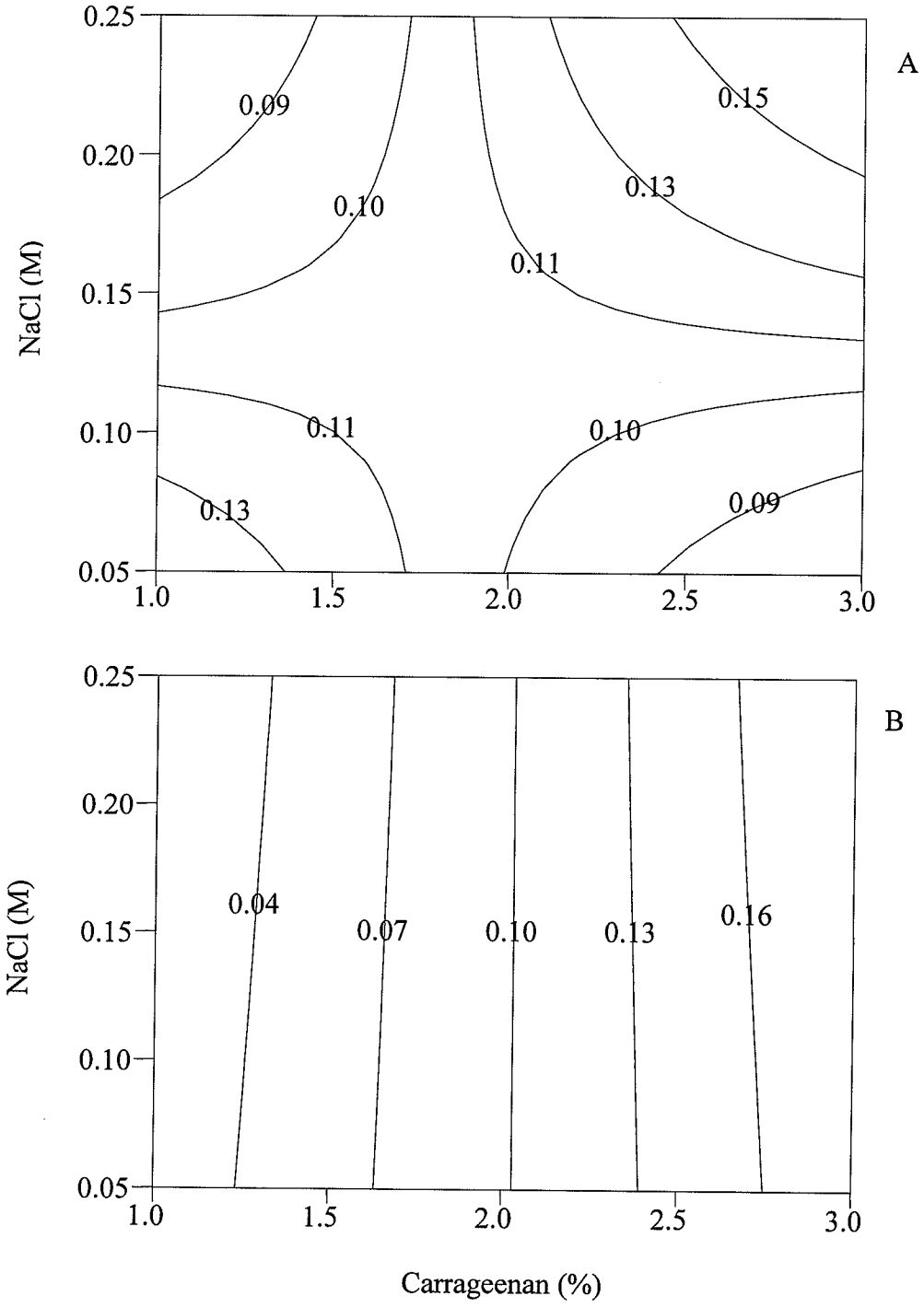


Figure 22. Effect of NaCl and κ-carrageenan concentrations on loss tangent (tan δ) of CPI-κ-carrageenan systems at 15% protein and varied pH 6 (A); pH 10 (B). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given tan δ value. For example, in Figure 22A; at 1.5% (w/v) κ-CAR and 0.1 M NaCl, tan δ=0.11.

Model predictions of optimum conditions for CPI-κ-carrageenan gels

Numerical optimization for CPI-κ-carrageenan systems: It is known that low G′ and high tan δ indicate poor gelation while high G′ and low tan δ indicate enhanced gelation properties. The goal was to obtain CPI-κ-CAR gels with very high G′ and low tan δ values. The numerical and graphical features of response surface optimization model (Design-Expert® Software) were used to identify the optimum conditions that would result in gels with high G′ and low tan δ values. Numerical and graphical optimizations were completed using the combinations from the full factorial design for CPI-κ-CAR systems. Optimization was based on the criteria set for both factors (CPI concentration, pH, NaCl concentration and κ-CAR concentration) and responses (G′, tan δ). Table 9 shows the parameters and criteria set for factors and responses; and summarizes the optimum predicted solutions characterizing improved CPI-κ-CAR gel networks according to desirability. Based on the solutions outlined in Table 9, the model numerical predictions for CPI-κ-CAR gels were:

15% (w/v) CPI, 3% (w/v) κ-CAR, 0.05 M NaCl, and pH 6

At these conditions, the CPI-κ-CAR gel was predicted to have a G′ value of 87082 Pa and tan δ value of 0.11 (i.e. solution No. 1, Table 9). These criteria were selected to achieve the desired goal – maximize G′ and minimize tan δ at the same time. However, using the set parameters at pH 10 (graph not shown) no conditions could give acceptable G′ and tan δ values as obtained at pH 6.

Table 9. Predicted optimum combinations of factors and responses characterizing strong, elastic CPI-κ-CAR gels based on set criteria within numerical optimization model.

Constraints

Parameters	Goal	Lower limit	Upper limit
pH	within range	6	10
NaCl concentration (M)	within range	0.05	0.25
κ-CAR concentration (%)	within range	1	3
CPI concentration (%)	within range	10	20
G′ (Pa)	maximize	44000	91400
Tan δ	minimize	0.01	0.11

Solutions

No.	pH	NaCl (M)	κ-CAR (%)	CPI (%)	G′ (Pa)	Tan δ	Desirability
1	6	0.05	3.0	15.4	87082	0.11	0.91
2	6	0.05	3.0	12.8	86669	0.09	0.90
3	6	0.05	3.0	10.2	85673	0.06	0.88
4	6	0.05	3.0	16.2	85263	0.06	0.87
5	6	0.05	3.0	19.1	84420	0.03	0.85

Graphical optimization for CPI-κ-CAR systems: To maximize these two criteria, the lower limit for G′ was set at 44000 Pa while tan δ was in the range of 0.01 to 0.11. These criteria limits were sufficient to give a rough idea of which combinations of processing conditions should be used in the next experiment to produce very strong gels with elastic structure. Guided by these ranges and using the graphical feature of the optimization model (Design-Expert® Software), corresponding response surface overlay contour plot as a function of κ-CAR and NaCl concentrations were generated at pH 6. Figure 23 shows an overlay plot of the response (G′ and tan δ) surface graph with the optimized regions highlighted. Within the shaded areas, any combinations of NaCl and κ-CAR concentration is predicted to give a gel with G′ of not less than 44000 Pa and a tan $\delta \leq 0.11$; resulting in an adequate product. Figure 23 shows that an adequate gel will be obtained at low salt and high κ-CAR concentrations and even more so at high salt and low κ-CAR concentrations. These criteria were selected to achieve the desired goal which was to maximize the gelation characteristics (i.e. high G′ and low tan δ values). The desired criteria could not be met at pH 8-10 (Figure not shown) for CPI-κ-CAR mixtures.

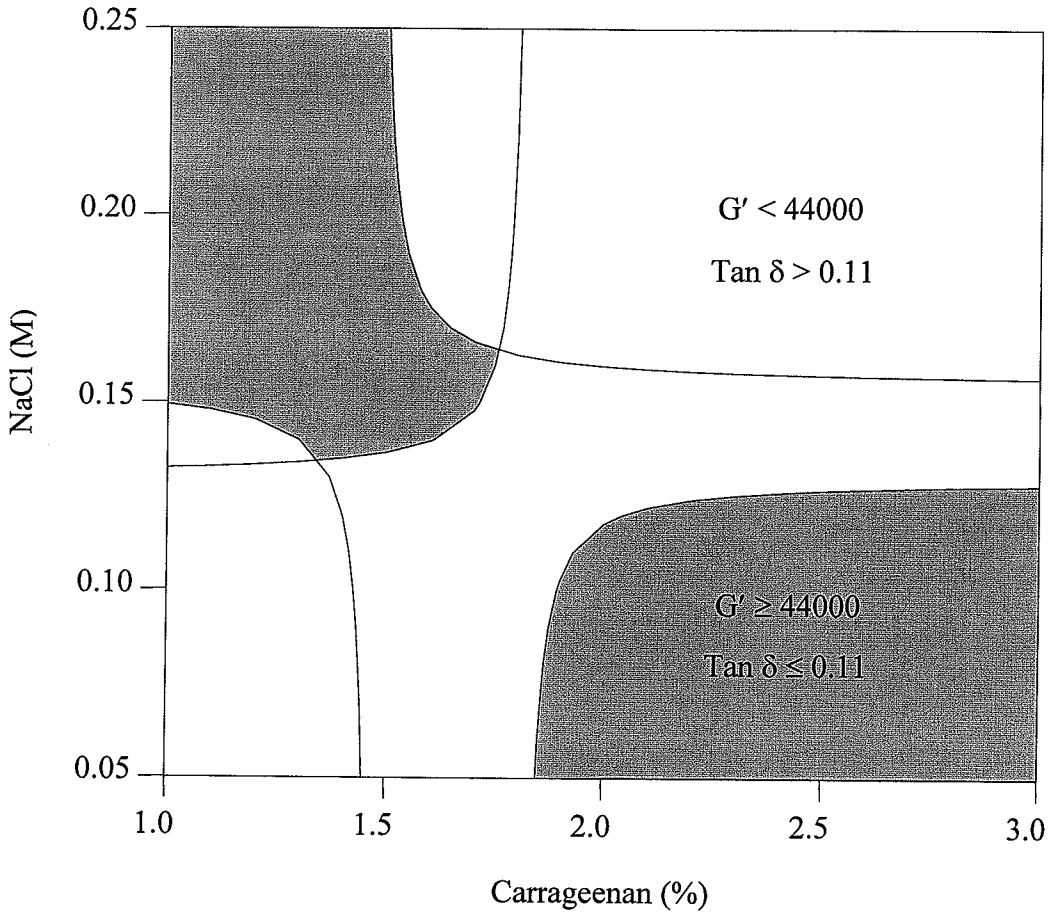


Figure 23. Graphical optimization display of gelation properties (G′, tan δ) as a function of NaCl and κ-carrageenan (κ-CAR) concentrations at pH 6. The clear areas on the plot do not meet the selected criteria. The lines mark the limits on the responses. Shaded areas indicate where factors can be set to satisfy the requirements on both responses. For instance, any point in the shaded region indicates the NaCl and κ-CAR combinations that will give gels with G′ value of not less than 44000 Pa and tan δ ≤ 0.11.

Verification of model predictions for canola protein-κ-carrageenan gels

The model numerical predictions (Design-Expert® Software) for CPI-κ-CAR gels were verified by conducting confirmatory rheological tests at the established optimum conditions for CPI-κ-CAR mixtures (pH 6; 15%, w/v CPI; 3%, w/v κ-CAR; 0.05 M NaCl). Predicted G′ and tan δ values for CPI-κ-CAR gels were established using the point prediction model (Design-Expert® Software). Figure 24 compares the predicted and experimental responses of CPI-κ-CAR gels. As mentioned earlier, the goal was to maximize G′ and minimize tan δ. The experimental G′ values were positively correlated to the predicted values (R^2=0.9985), characterizing the gels as very strong and elastic (G′=97465 Pa; tan δ=0.15) with increased number of crosslinks (Fig. 24). Also, the experimental tan δ values correlated positively to the predicted tan δ values (R^2=0.9745). These results indicate similarity in the model predictions and experimental data.

One may argue that the optimum CPI (15%, w/v) and κ-CAR (3%, w/v) concentrations obtained in this study were high, and thus what could be the economic use of such high concentrations. Arntfield et al. (1990) established that 10% canola protein was sufficiently high to produce well cross-linked heat-induced networks. Based on this finding, we chose the CPI concentrations of 10, 15 and 20% for this study. Commercially (CPKelco Inc., Wilmington, USA; www.cpkelco.com/), carrageenan is used in concentrations ranging from 0.005 to 3%. The κ-CAR concentrations, 1, 3% w/v chosen fall within this range. As reported by Whistler and BeMiller (1997), cold hams and poultry rolls take up 20-80% more brine when they contain 1-2% κ-CAR, and slicing is improved. These authors further stated that tart filling and glazing gels can

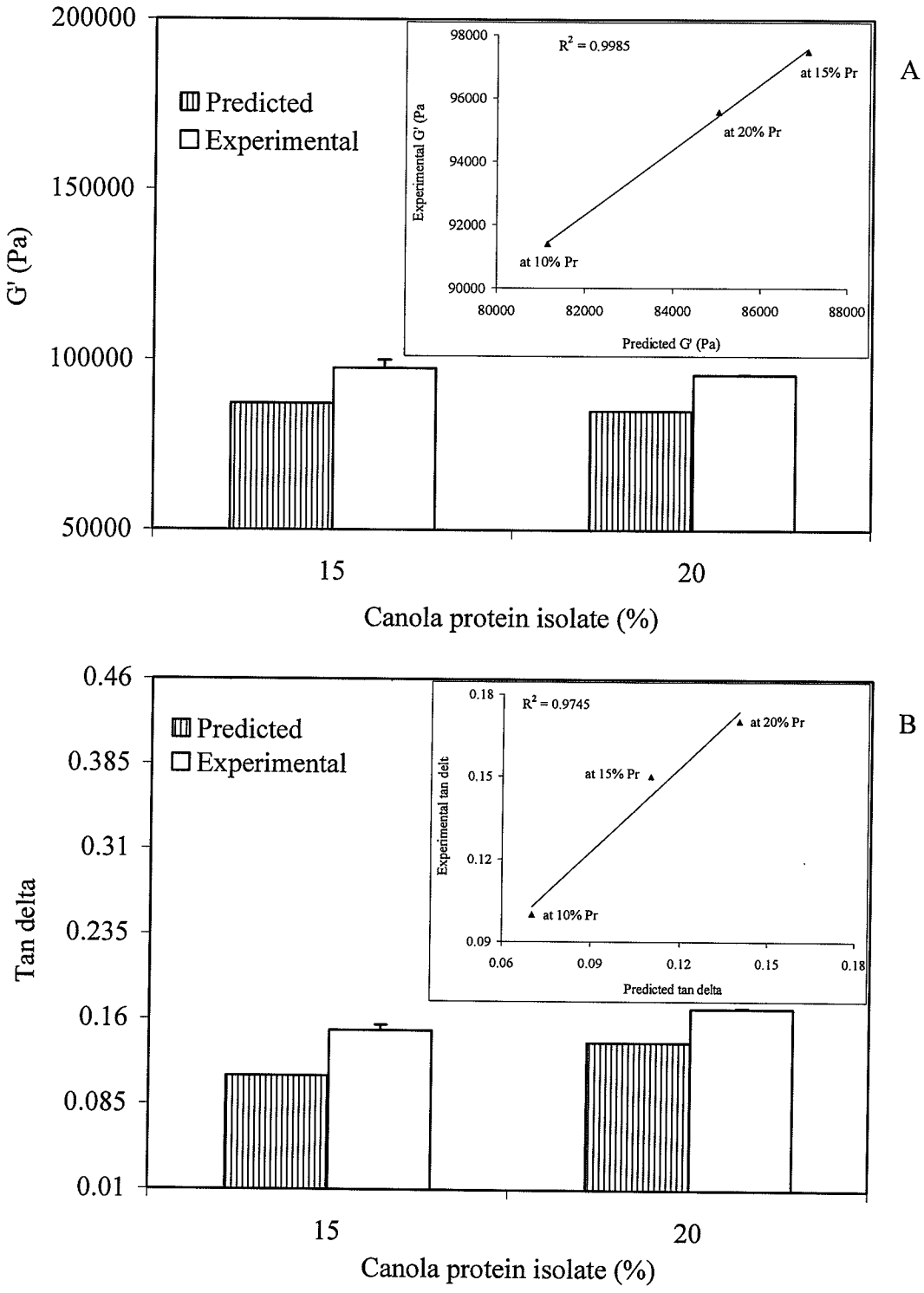


Figure 24. Predicted and experimental gelation properties (A: G′; B: tan δ), of CPI-κ-CAR gels at pH 6, 0.05 M NaCl and 3% (w/v) κ-CAR. Measurements were taken during frequency sweep (after cooling) at 1 Hz. *Insets: Pr = Protein.

contain 1-2% κ-CAR. Based on these reports, 3% κ-CAR may not be too high after all. As stated above, strong networks (G′=56065 Pa) were also obtained when 1% (w/v) κ-CAR was included in the mixture.

Mechanisms for network development of biopolymers and mixtures

Temperature sweep curves in rheological tests for CPI-κ-CAR systems helped to show the course of network formation (G′ and tan δ) as a function of temperature. Generally, the gel network developed in the heating phase, and continued in the cooling phase. Temperature sweep curves for κ-CAR (3% w/v), CPI (15% w/v) and CPI-κ-CAR gels showing the G′ and tan δ values at pH 6, 0.05 M NaCl, 15% CPI and 3% κ-CAR are shown in Figures 25 and 26.

Kappa-carrageenan gels: The onset of aggregation, indicated by the increase in G′ and decrease in tan δ (Fig. 25) occurred below 40°C. Under the conditions used in this study (i.e. pH 6, 0.05 M NaCl, 3% κ-CAR), it was found that heating κ-CAR sol above 50°C resulted in pronounced decrease in G′ and led to a complete meltdown of κ-CAR network at temperatures above 60°C (indicted by low G′ and high tan δ values; Fig. 25). Similar behaviour has been reported earlier for κ-CAR (Ould-Eleya and Turgeon, 2000a). We observed that cooling induced gelation in κ-CAR at temperatures below 50°C.

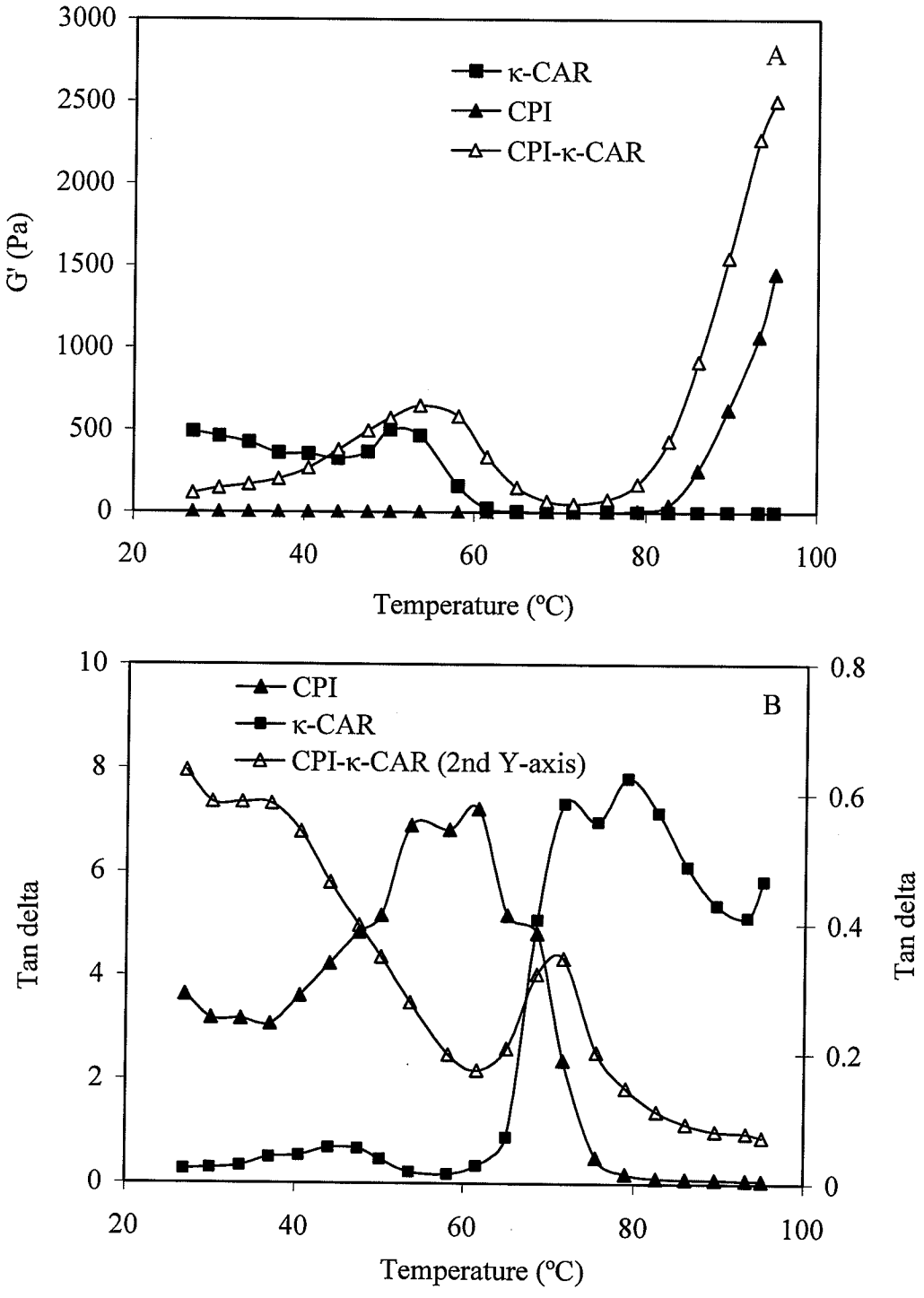


Figure 25. Changes in Rheological parameters (G′ (A); tan δ (B)), of canola protein isolate (CPI), κ-carrageenan (κ-CAR) and CPI-κ-CAR gels during heating (pH 6, 0.05M NaCl, 15% (w/v) CPI, 3% (w/v) κ-CAR). All measurements were taken at 1 Hz.

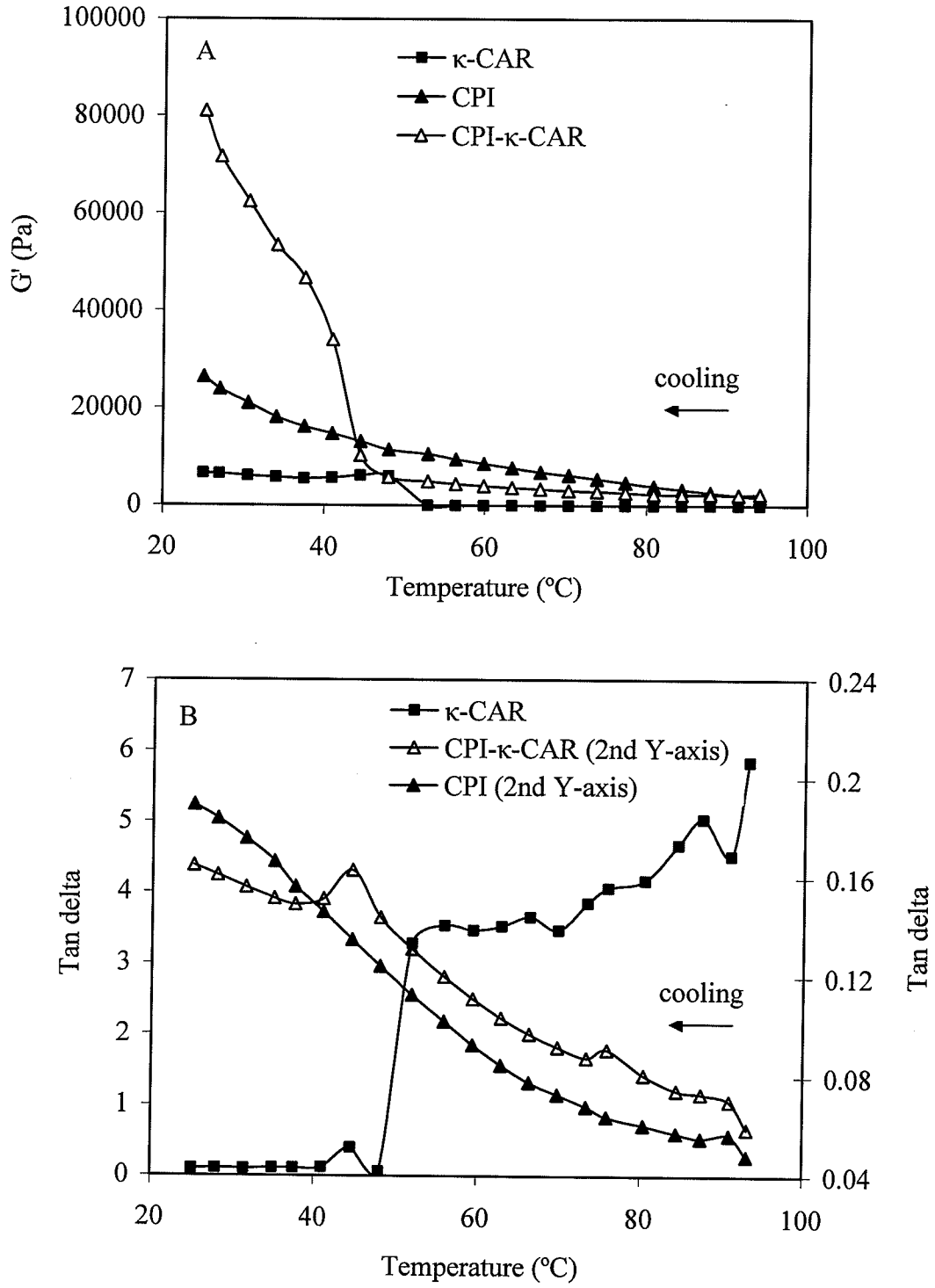


Figure 26. Changes in rheological parameters (G′ (A); tan δ (B)) of canola protein isolate (CPI), κ-carrageenan (κ-CAR) and CPI-κ-CAR gels during cooling (pH 6, 0.05M NaCl, 15% (w/v) CPI, 3% (w/v) κ-CAR). All measurements were taken at 1 Hz.

Canola protein gels: Network formation (deviation from the baseline) for CPI gels started at ≈82°C. It was evident during the heating cycle that the gel strength of CPI was improved at higher temperatures (above 80°C), possibly due to hydrophobic interactions. During the initial heating phase (below 80°C), we observed low G′ and high tan δ values for the CPI gels at temperatures below 80°C. Léger and Arntfield (1993) made similar observation. This is an indication of a weak and less elastic network. Nevertheless, tan δ values decreased while G′ increased above 80°C (Figure 25), an evidence of the formation of strong and elastic network for CPI at higher temperatures.

Canola protein-κ-carrageenan gels: The gelling profiles of mixed κ-CAR and CPI gels are shown in Figures 25 and 26. During the heating phase, it was observed that the onset of aggregation, indicated by the increase in G′ and decrease in tan δ (Figure 25), for the mixed CPI-κ-CAR gels occurred below 40°C, with similar shape of curve for the mixed gel and single κ-CAR gel at temperatures below 70°C. It is evident that κ-CAR was responsible for the network formation of the mixed system at temperatures below 70°C (Figure 25). Also, the shape of curve for the mixed gel and single CPI gel were similar at temperatures above 80°C, an indication that CPI was responsible for network formation of CPI- κ-CAR gels at higher temperatures. A synergistic behavior between CPI and κ-CAR was observed with superior gel strength for the mixed gel at temperatures above 80°C (Figure 25A). The mixed gel showed low tan δ values, indicating enhanced elastic network structure (Figure 25B). Rheological studies confirmed the synergistic effects in κ-CAR-denatured protein systems (Tziboula and Horne, 1999; Ould-Eleya and Turgeon, 2000a, 2000b; Neiser et al., 2000). During the cooling phase, the tan δ values (Figure 26) for the mixed gels was very low when

compared to that of the individual components, indicating that the synergistic effect between canola protein and κ-carrageenan continued during and after cooling. A comparison of the rheological data of the individual components and mixed gels (Table 10) indicated that the gel strength and network structure of the mixed gels were greatly improved (high G′ and low tan δ values) during the heating and cooling phases (Figs. 25, 26), mainly during the final cooling phase at temperatures below 50°C.

Table 10. Gelation properties of canola protein-κ-carrageenan gels prepared with 0.05 M NaCl at pH 6. Measurements were taken during frequency sweep (after cooling) at 1 Hz.

Canola protein (%)	κ-carrageenan (%)	G'^{z}	Tan δ^{z}
0	3	6804 ± 167^{d}	0.13 ± 0.001^{c}
15	0	27755 ± 757^{c}	0.19 ± 0.002^{a}
15	3	97465 ± 2454^{a}	0.15 ± 0.005^{b}
15*	3	$68795 \pm 1082^{*b}$	$0.14 \pm 0.002^{*b}$

[z]Mean ± SD. For G′ or tan δ, column values with the different letters are significantly different ($P<0.05$). *Canola protein-κ-carrageenan gel properties at pH 10.

CHAPTER 4

Physicochemical Properties of Commercial Canola Protein-Guar Gum Gels

Abstract

Biopolymer mixtures impart desirable texture to foods. The gel characteristics of canola protein isolate (CPI)-guar gum mixtures were assessed using dynamic rheological measurements. The effects of salts, guar gum and protein concentrations and pH on the gelling ability of CPI were evaluated. Factorial and response surface optimization models were used to identify the conditions that would result in CPI-guar gum gels with maximized G′ values (≥28000 Pa) and minimized tan δ values (<0.17). These criteria were selected to achieve the desired goal (i.e. maximize G′ and minimize tan δ simultaneously). On using different set of values for G′ and tan δ, no conditions could be obtained or optimized. Protein and guar gum concentration and pH strongly affected gel formation. The model indicated the optimum conditions at which desirable criteria (high G′ and low tan δ) were obtained for CPI-guar gum gels as pH 10, 0.05 M NaCl, 1.5% (w/v) guar gum and 20% (w/v) CPI. Strong and elastic gels resulted at pH 10 (G′=56440 Pa; tan δ=0.18), whereas gels prepared at pH 6 were less elastic (G′=2726 Pa; tan δ=0.2). At the established optimum conditions, CPI formed a stronger gel (G′=64575 Pa) in the absence of guar gum, suggesting that guar gum interfered with protein gelation. The results indicate that canola protein can serve as a structuring agent in food systems when used alone or in combination with guar gum. A better understanding of factors that contribute to improved functionality of canola protein in multicomponent foods will enhance its utilization as a food ingredient.

Introduction

Biopolymers such as proteins and polysaccharides are widely used in food systems because their interactions impart a desirable texture to food products. The formation of these structural networks takes place during processing and is related to the transformation from a liquid or viscous sol into a solid material with elastic properties (Arntfield and Cai, 1998). These networks supply the structural integrity for the food product and also serve to trap other food components such as water, lipids and flavours. The behaviour of mixed gels has earned numerous attentions due to the expected greater flexibility in their mechanical and structural properties compared to those of pure gels (Ould-Eleya and Turgeon, 2000a). Some applications (Cain, 1987; Tolstoguzov and Vincent, 1997) cited by Ould-Eleya and Turgeon (2000b) have shown that such blends can constitute new food ingredients, particularly as fat substitutes.

Gelation is an important functional property of globular proteins (e.g. canola proteins) commonly used in the food industry. However, several factors influence the nature of the protein gel formed. These include environmental conditions (such as pH, ionic strength and mineral content), protein properties/composition (e.g. hydrophobicity, charge potential, level of sulfhydryl groups, electrolytes, phenolic and phytic acids), processing conditions (e.g. heating and cooling rates), extent of denaturation, and concentration (Aguilera, 1995; Hines and Foegeding, 1993; Arntfield et al., 1990a, 1990b, 1991; Arntfield, 1994; Arntfield and Murray, 1992; Savoie and Arntfield, 1996; Rubino et al., 1996). The behaviour of protein-polysaccharide mixtures has been the subject of many investigations. As many as 80 protein-polysaccharide-water ternary systems have been described by Tolstoguzov (1986, 1991). Interesting results were obtained for a mixture of casein micelles with guar gum (Bourriot et al., 1999). A

segregative phase separation phenomenon between the micellar casein and guar gum was reported. Similarly, the ability to improve the network properties of CPI through the inclusion of a small amount of guar gum was demonstrated (Arntfiled and Cai, 1998). However, this improvement was not simply due to the incompatibility of the biopolymers, as similar interaction data for methyl cellulose did not produce the same gelation properties. Thus, great potential remains in mixing polysaccharides with globular proteins (e.g. improving the gel properties of CPI by the addition of gums).

Canola, widely grown in Canada, is highly valued due to its high-quality edible oil content (Shahidi, 1990). The resulting meal after oil extraction finds use as an animal feed ingredient; however, the potential use of canola protein as a human food ingredient is currently being assessed by some commercial firms. The isoelectric point (IEP) of CPI was reported as pH 6.8-7.2 (Schwenke et al., 1987). Since CPI gel is less elastic when compared to those of egg albumin (Léger and Arntfield, 1993), it is expected that the addition of polysaccharides will enhance its gel strength. Guar gum, a neutral molecule, is compatible with most other food substances, including other polymers (Whistler and BeMiller, 1997). It is an economical thickener and stabilizer. For those reasons, guar gum finds application in many food products. On the other hand, guar gum offer some challenges for the food industry, such as poor gelling properties and a propensity to precipitate under certain conditions. The purpose of this research was to understand the physicochemical interactions involved in the network formation of commercial CPI-guar gum mixtures and characterize the gelling behaviour of the mixtures. To assess the role of covalent and noncovalent interactions in gel formation of commercial CPI-guar gum mixtures, fundamental rheological properties have been examined. For this reason, neutral salts such as sodium sulfate (Na_2SO_4), sodium acetate ($NaC_2H_3O_2$), sodium

chloride (NaCl), and sodium thiocyanate (NaSCN) were used as probes (Phillips et al., 1994). Also, two standard denaturants that impact noncovalent interactions (urea) and disulfide bond formation (dithiothreitol, DTT) were included in the protein dispersion prior to heat treatment. Structures developed during gelation as well as properties of the networks formed were used to evaluate the effects of these reagents.

Materials and Methods

Source of materials

Food grade guar gum (G-4129; Lot No. 95H0653), with a viscosity of 6000-10000 mPas (Whistler and BeMiller, 1997), urea (U-15; Lot No. 863571) and DTT (D-0632; Lot No. 61K16571) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Commercial (BMW Canola, Winnipeg, Canada) canola protein isolate (CPI) was purchased and used without further purification. Proximate analysis (AOAC, 1990) of the CPI sample indicated a protein content of 87% (N x 5.7), 0.7% oil, 2% ash, 5.9% moisture and 4.4% total carbohydrate (determined by difference). $NaC_2H_3O_2$ (Lot No. 7364 KCLZ) was procured from Mallinckrodt Inc. (Paris, Kentucky, USA). All other chemicals such as NaCl (BP358-212; Lot No. 028091), HCl (A144-225; Lot No. 296220), NaOH (BP359-212; Lot No. 974661), NaSCN (S441-500; Lot No. 987676) and Na_2SO_4 (S421-500; Lot No. 985711) were certified reagent grade (Fisher Scientific Co., NJ, USA).

Sample preparation

Dispersions of CPI (10, 20% w/v; protein-basis) and guar gum (1, 3% w/v) were prepared by mixing in NaCl (0.05, 0.25 M) at desired pH (6, 10). The CPI, guar gum,

pH and NaCl combinations were generated using Design-Expert® Software. The mixture was stirred for approximately 1 h at room temperature or until a complete dispersion of the mixture was achieved. Dispersions of individual CPI and guar gum samples in appropriate NaCl solutions were prepared in a similar manner to serve as control. The use of dispersions allows monitoring of network formation from the onset of conformational changes. For interaction studies, neutral salts such as sodium sulfate (Na_2SO_4), sodium acetate ($NaC_2H_3O_2$), sodium chloride (NaCl) and sodium thiocyanate (NaSCN) at varied concentrations (0.05, 0.5 M) were used to prepare sample dispersions. Similarly, a disulfide blocker (DTT) or hydrogen bond blocker (urea) at concentrations 0.15 M and 6 M, respectively were included in the dispersion prior to heat treatment. To assess the influence of pH, samples were adjusted to pH 6, 10 with 1 M NaOH or 1 M HCl. To ensure that pH was maintained, samples were allowed to equilibrate for 30 min at room temperature and the pH was rechecked prior to further testing.

Experimental design

All experimental parameter measurements were done in duplicates. Design-Expert® Software (Stat-Ease Inc., MN) experimental designs, factorial and response surface optimization were used in this study. The experiment was carried out in two stages. In stage one series of experiments were carried out to characterize different combinations of mixed CPI-guar gum gels following a full factorial model. A 2-level factorial design for 4 factors in 20 experiments including four replicates of the center point was generated. The factors included CPI concentration (10, 15, 20% w/v), pH (6, 8, 10),

NaCl concentration (0.05, 0.15, 0.25 M) and guar gum concentration (1, 2, 3% w/v). Major responses, storage modulus (G′) and loss tangent (tan δ=G″/G′), were measured. Using these data, model fitting was performed using the numerical, graphical and point prediction models (Design-Expert® Software) to determine the optimum conditions for enhancing gel properties (G′ and tan δ). Two-dimensional contour plots were generated from the fitted model. In stage two, experiments were carried out to verify the optimum conditions obtained in stage one. The model generated conditions for CPI-guar gum mixtures were pH 10, 1.5% (w/v) guar gum, 20% (w/v) CPI and 0.05 M NaCl (i.e. verification tests were carried out at these conditions). Control tests of CPI (20%, w/v) and guar gum (1.5%, w/v) at optimum conditions were also carried out.

Statistical analysis

All analyses were conducted in duplicates. Data used in tables and figures were average values. Data from confirmatory and control tests (stage two) were analyzed by analysis of variance (ANOVA) procedure using SAS® 1999-2001 Proprietary Software Release 8.2 (TS20; SAS Institute Inc., Cary, NC, USA); using Duncan's multiple range test to determine statistical differences ($P \leq 0.05$) between treatment means (Steel et al., 1997).

Rheology: Assessment of CPI-guar gum gel properties

Dynamic rheological testing on an advanced rheometer 2000 (AR2000; TA Instruments, New Castle) was used in stages one and two to monitor CPI-guar gum network formation during heating and cooling as well as to characterize the resulting

networks (Arntfield et al., 1990a, 1991). The AR2000 was equipped with 40mm parallel plate geometry and a built-in automated sensitivity. Input strain amplitude for dynamic analysis was 0.02, a value found to be in the linear viscoelastic region in an experiment with canola protein (Arntfied et al., 1990a). This strain was used for all rheological measurements. Approximately 1 mL canola protein-guar gum dispersion was placed between parallel plates in the rheometer and the gap between the plates was adjusted to 1 mm when the upper plate was lowered. To prevent sample drying during heating, paraffin oil (Mallinckrodt, Paris, Kentucky, USA; Lot No. 6358 KJPC) was placed in the shallow well on top of the upper plate and the edges of the solvent trap were also covered with oil. Samples were heated and cooled over a temperature range of 25° to 95°C at 2°C/min with a 2 min hold time at the final temperature for both the heating and cooling phases. A frequency of 1 Hz was used for the thermal scans. Sample temperature was controlled by a circulating tap water. Rheological data were collected at 2 min intervals with a thermal equilibrium time of 10 sec. Frequency sweeps of the final product were measured over a range of 0.01-10 Hz at 25°C by using the same strain amplitude and sensitivity as in the thermal scans. The rheological properties, G′ (storage modulus), G″ (loss modulus) and loss tangent or tan delta (tan δ=G″/G′) for representative heating, cooling and frequency sweep curves were recorded. The parameters, G′ and G″ represent the elastic and the viscous components of the gel, respectively; while tan δ reflects the relative energy from the viscous and the elastic components. However, the key gel properties, G′ and tan δ have been reported. Values at 1 Hz were recorded for comparison and statistical analysis. The use of a single frequency (1 Hz) for data comparison has been reported (Arntfield et al., 1989, 1990c).

Results and Discussions

Visual evaluation

On visual examination, only a single phase was observed at the CPI and guar gum concentrations (1, 2, 3% w/v) used for rheological evaluations. There was no evidence of separate liquid phases. A single-phase mixture can exist in a system of incompatible polymers and this occurs when the protein-polysaccharide concentration is below the phase separation threshold (Tolstoguzov, 1991). The mixtures were well mixed or agitated prior to rheological measurement and network formation. There was no visual evidence of gel formation when CPI-guar gum and individual guar gum and CPI dispersions were prepared with NaCl at room temperature. Light brown, opaque gels were obtained after the thermal scan. The rheological responses (G′, tan δ) and model equations from the full factorial design for canola protein-guar gum mixtures under varied conditions are shown in Table 11.

Influence of CPI-pH-guar gum interaction on network properties

Storage modulus (G′): Significant factors and interactions were identified using the ANOVA model (Design-Expert® software). CPI concentration, pH, guar gum concentration, interaction between pH and NaCl concentration, interaction between pH and CPI concentration, 3-way interaction between pH, guar gum and CPI concentrations, and 3-way interaction between pH, NaCl and CPI concentrations were significant ($P<0.05$, Appendix 9). Therefore, contour plots of G′ as a function of guar gum and CPI concentrations at pH 6 and 10 are shown in Figure 27. At pH 10 (Fig. 27A), it was evident from the curves that structure development (high G′ values)

Table 11. Rheological properties (G′, tan δ) of canola protein-guar gum mixtures under varied conditions.

pH	NaCl (M)	Guar gum (%)	Protein (%)	G′ (Pa)	Tan δ
6	0.05	1	10	1806	0.18
6	0.05	1	20	23040	0.19
6	0.05	3	10	1107	0.21
6	0.05	3	20	1279	0.22
6	0.25	1	10	3576	0.18
6	0.25	1	20	42280	0.19
6	0.25	3	10	3422	0.55
6	0.25	3	20	1639	0.19
8	0.15	2	15	26920	0.19
8	0.15	2	15	15535	0.20
8	0.15	2	15	28725	0.19
8	0.15	2	15	17120	0.20
10	0.05	1	10	3099	0.16
10	0.05	1	20	48735	0.15
10	0.05	3	10	1300	0.19
10	0.05	3	20	61635	0.16
10	0.25	1	10	5818	0.17
10	0.25	1	20	41820	0.16
10	0.25	3	10	1901	0.19
10	0.25	3	20	1511	0.18

G′ = 1.199×10^{5} – 18395.66 pH – 7.051×10^{5} NaCl – 24747.19 Ggum – 7639.45 CPI + 76313.12 pH*NaCl + 2675.56 pH*Ggum + 1325.42 pH*CPI + 1.787×10^{5} NaCl* Ggum + 53710.50 NaCl*CPI + 281.65 Ggum*CPI – 11560.00 pH*NaCl*Ggum – 5367.12 pH*NaCl*CPI – 8814.50 NaCl*Ggum*CPI (P=0.014).

Tan δ = 0.40 – 0.026 pH – 4.63 NaCl – 0.23 Ggum – 9.438×10^{-3} CPI + 0.48 pH*NaCl + 0.026 pH*Ggum + 9.688×10^{-4} pH*CPI + 4.55 NaCl*Ggum + 0.24 NaCl*CPI + 0.013 Ggum*CPI – 0.46 pH*NaCl*Ggum – 0.024 pH*NaCl*CPI – 1.469×10^{-3} pH* Ggum*CPI – 0.24 NaCl*Ggum*CPI + 0.024 pH*NaCl*Ggum*CPI (P=0.0002).

increased with increasing levels of CPI. Since guar gum alone did not produce a gel network (G′=51 Pa) at pH 10, it appears that the high G′ at pH 10 was due to protein-protein interaction at this pH. The interacting strands (progel state) of CPI may self-aggregate and form a network. This may explain the observed increase in gel strength as the CPI level increased. G′ relates to the compactness of the structures or number of crosslinks within a gel system (Jones, 1979; Murray et al., 1981). Higher G′ values indicate stronger intermolecular network, possibly due to interactions between aggregated protein strands, giving rise to high molecular weight networks stabilized by noncovalent forces. The increase in G′ values at pH 10 probably reflects the contribution from canola protein isolate (Fig. 27A).

At pH 6, the G′ values provided an indication of the effects of CPI-guar gum interactions (Fig. 27B). Improved gel networks (high G′ values) were obtained at low guar gum and high CPI concentrations (i.e. increase in guar gum level decreased the G′ values of the resulting networks). Comparing G′ values at pH 6 and 10 showed that G′ values at pH 10 (Fig. 27A) were higher than the values at pH 6 (Fig. 27B). It has been reported that gels prepared at lower pH values are heterogeneous in nature (Léger and Arntfield, 1993). These heterogeneous networks are composed of compact molecules, which aggregate to form regions of high and low protein concentrations. As a result, regions with low protein concentrations act as weak points, causing a drop in overall gel strength. It is possible that CPI and guar gum produced heterogeneous gels at pH 6 which may account for the reduced gel strength shown in Figure 27B.

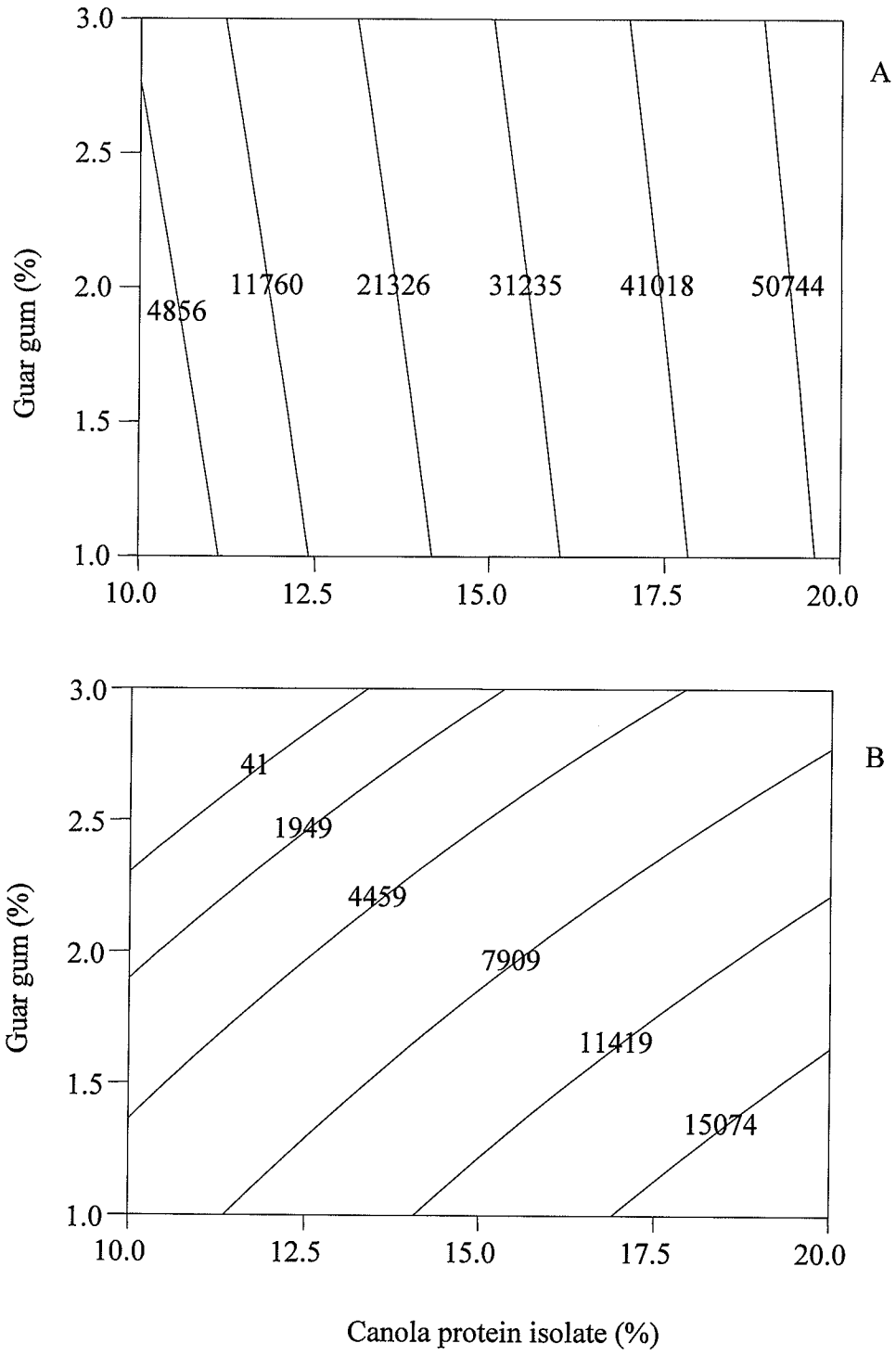


Figure 27. Storage modulus (G′) contour plot showing the influence of CPI and guar gum concentrations on G′ of CPI-guar gels at 0.05 M NaCl and varied pH (A: pH 10; B: pH 6). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given G′ value. For example, in Figure 27A; at ≈20% (w/v) CPI and any guar gum concentration, G′=50744 Pa.

Loss tangent (tan δ): Based on the ANOVA model (Design-Expert® software), the significant ($P<0.05$) factors and interactions were pH, NaCl concentration, guar gum concentration, CPI concentration, 3-way interactions between pH, NaCl concentration and guar gum or CPI concentrations, and 3-way interaction between pH and the biopolymers (Appendix 10). Thus, the contour plots of tan δ as a function of guar gum and CPI concentrations at pH 6 and 10 are shown in Figure 28. A look at the tan δ values gives an indication of the influence of different protein-polysaccharide combinations on the type of network structure formed as tan δ reflects the relative contributions of the viscous (loss modulus) and elastic (storage modulus) components. At pH 10, the CPI-guar gum gels had lower tan δ values at higher CPI and lower guar gum concentrations (Fig. 28A). Arntfield and Cai (1998) in their work with canola protein-guar gum mixture made similar observation and reported that higher protein concentrations resulted in lower tan δ values. They stated that incompatibility of the two macromolecules at higher protein concentration resulted in structures where elasticity was increased, probably due to increased interactions between proteins. The lower tan δ values observed in the present study most likely represented the tan δ of CPI gel. Léger and Arntfield (1993) reported lowest tan δ values for canola protein gels prepared at pH 10. The increased electrostatic repulsion at this pH may have minimized random aggregation resulting in the extension of polypeptide chains and the creation of homogeneous structures (Paulson and Tung, 1989). Low tan δ values indicate a more elastic network formation. On the other hand, the networks tended to be less elastic (high tan δ values) at lower CPI and higher guar concentrations. Increases in tan δ values indicated that the formation of a three-dimensional network had been inhibited as was

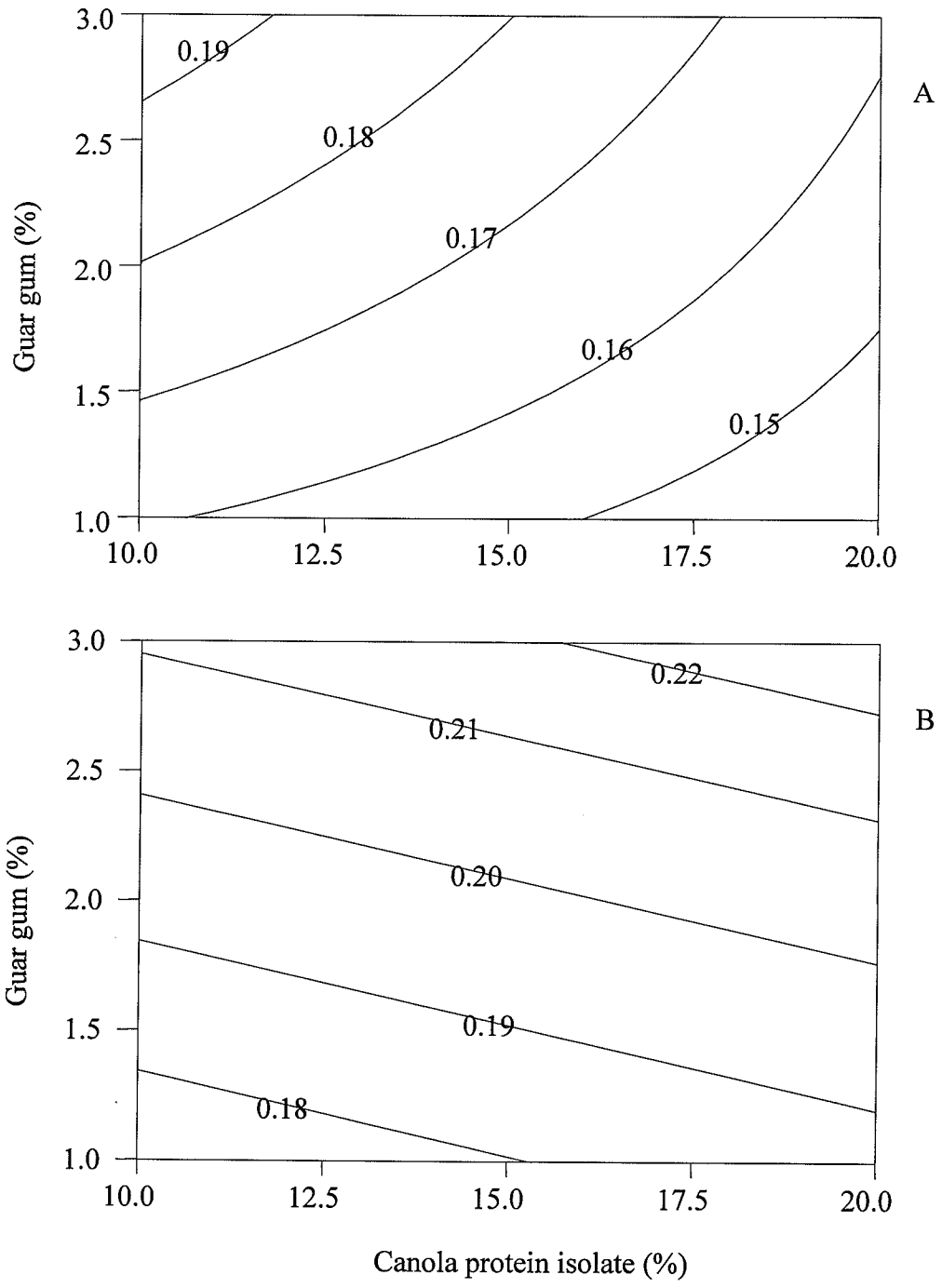


Figure 28. Tan δ contour plot showing the effects of CPI and guar gum concentrations on tan δ of CPI-guar gels at 0.05 M NaCl and varied pH (A: pH 10; B: pH 6). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given tan δ value. For example, in Figure 28A; at 17.5% (w/v) CPI and 1.5 % (w/v) guar gum, tan δ=0.16.

implied by the lower G′ values observed at similar conditions (Fig. 27A).

At pH 6 (Fig. 28B), lower tan δ values resulted at decreasing CPI and guar gum levels. Tan δ values were higher at pH 6 than at pH 10. This may be attributed to the heterogeneous networks formed at lower pH (Léger and Arntfield, 1993). Overall, tan δ values for the CPI-guar gum gels fell below 0.22.

Model predictions of optimum conditions for canola protein-guar gum gels

Numerical optimization for canola protein-guar gum systems: It is known that low G′ and high tan δ indicate poor gelation while high G′ and low tan δ indicate enhanced gelation properties. With this in mind, the goal was to obtain CPI-guar gels with very high G′ and low tan δ values. The numerical and graphical features of response surface optimization model (Design-Expert® Software) were used to identify the optimum conditions that would result in gels with high G′ and low tan δ values. Numerical and graphical optimizations were completed using the combinations from the full factorial design for CPI-guar gum systems. Optimization was based on the criteria set for both factors (CPI concentration, pH, NaCl concentration and guar gum concentration) and responses (G′, tan δ). Table 12 shows the parameters and criteria set for factors and responses; and summarizes the optimum predicted solutions characterizing improved CPI-guar gum gel networks according to desirability. Based on the solutions outlined in Table 12, the model numerical predictions for CPI-guar gum gels were:

20% (w/v) CPI, 1.5% (w/v) guar gum, 0.05 M NaCl, and pH 10

At these conditions, the CPI-guar gum gel was predicted to have a G′ value of 54280 Pa and tan δ value of 0.15 (i.e. solution No. 1, Table 12). These criteria were selected to achieve the desired goal – maximize G′ and minimize tan δ at the same time. However, using the set parameters at pH 6 (graph not shown) no conditions could give acceptable G′ and tan δ values as obtained at pH 10.

Table 12. Predicted optimum combinations of factors and responses characterizing strong, elastic CPI-guar gum gels based on set criteria within numerical optimization model.

Constraints

Parameters	Goal	Lower limit	Upper limit
pH	within range	6	10
NaCl concentration (M)	within range	0.05	0.25
Ggum concentration (%)	within range	1	3
CPI concentration (%)	within range	10	20
G′ (Pa)	maximize	28000	61635
Tan δ	minimize	0.15	0.17

Solutions

No.	pH	NaCl (M)	Ggum (%)*	CPI (%)	G′ (Pa)	Tan δ	Desirability
1	10	0.05	1.5	20.0	54280	0.15	0.81
2	10	0.05	2.1	20.0	54198	0.16	0.78
3	10	0.05	1.2	20.0	53619	0.15	0.76
4	10	0.05	1.4	19.9	53557	0.15	0.76
5	10	0.07	1.0	20.0	52203	0.15	0.72

*Ggum = Guar gum.

Graphical optimization for canola protein-guar gum systems: To maximize these two criteria (i.e. high G′ and low tan δ), the lower limit for G′ was set at 28000 Pa while tan δ ranged from 0.01 to 0.17. These criteria limits were sufficient to give a rough idea of which combinations of processing conditions should be used in the next experiment to produce very strong gels with elastic structure. Guided by these range of parameters and using the graphical feature of the optimization model (Design-Expert® Software), corresponding response surface overlay contour plot as a function of CPI and guar gum concentrations were generated at pH 10. Figure 29 shows an overlay plot of the response (G′ and tan δ) surface graph at pH 10 with the optimized regions highlighted. Within the shaded areas, any combinations of CPI and guar concentration is predicted to give a gel with a G′ value not less than 28000 Pa and tan δ value less than 0.17; resulting in an adequate product. Figure 29 illustrates that an adequate gel will be obtained at high CPI level and any guar gum concentration. These criteria were selected to achieve the desired goal which was to maximize the gelation characteristics (i.e. high G′ and low tan δ values). The desired criteria could not be met at pH 6 for CPI-guar gum mixtures. Although alkaline pHs (above pH 8) are rarely encountered in food systems, the model used in this study indicated pH 10 as the optimum pH for CPI-guar gum gels.

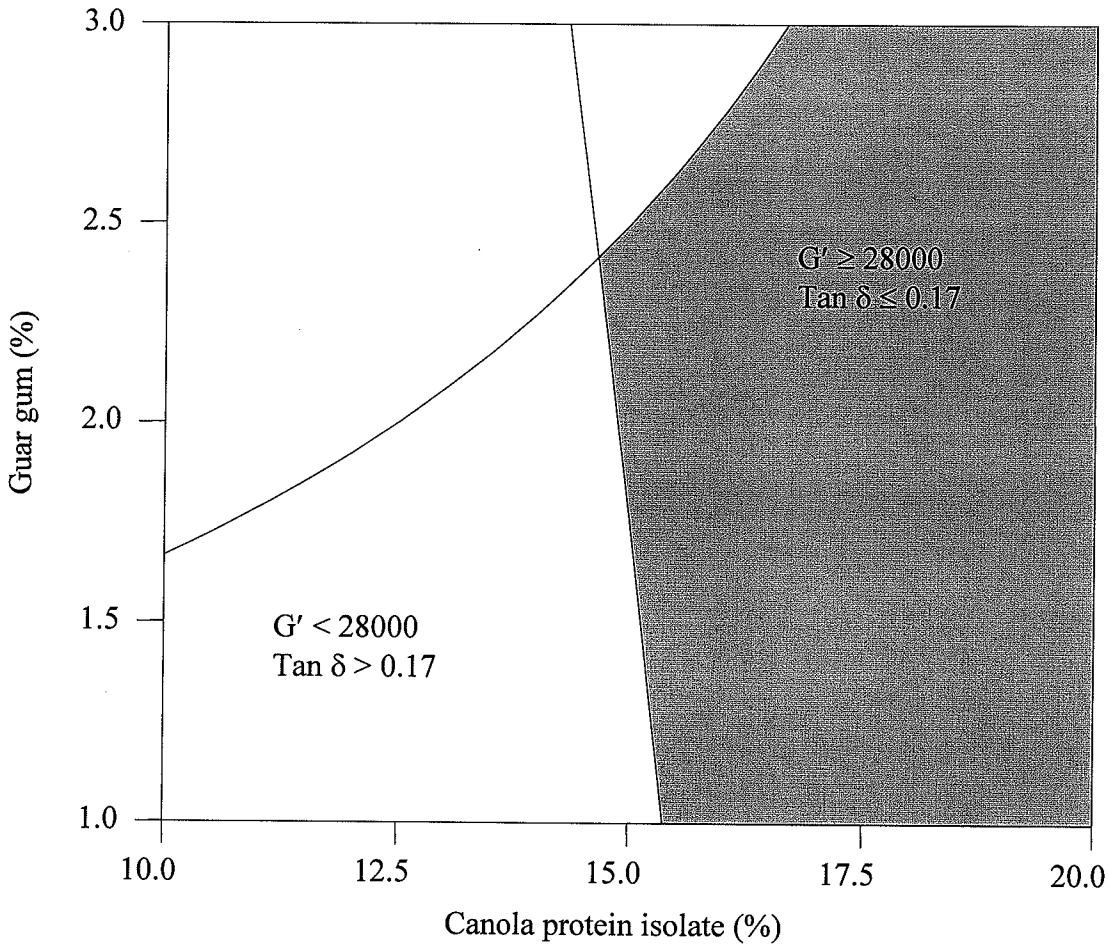


Figure 29. Graphical optimization display of G′ and tan δ as a function of canola protein isolate (CPI) and guar gum concentrations at pH 10 and 0.05 M NaCl. The clear areas on the plot do not meet the selected criteria. The desired criteria were achieved at pH 10 and not at pH 6. The lines mark the limits on the responses. Shaded areas indicate where factors can be set to satisfy the requirements on both responses. For example, any point in the shaded region shows the CPI and guar gum combination that will give a gel with G′ value of not less than 28000 Pa and tan δ value of less than 0.17.

Verification of model predictions for canola protein-guar gum gels

The model numerical predictions (Design-Expert® Software) for CPI-guar gum gels were verified by conducting confirmatory rheological tests at the established optimum conditions for CPI-guar gum mixtures (pH 10; 20%, w/v CPI; 1.5%, w/v guar gum; 0.05 M NaCl). Predicted G′ and tan δ values for CPI-guar gum gels were established using the point prediction model (Design-Expert® Software). Figure 30 compares the predicted and experimental G′ and tan δ values for CPI-guar gum gels. As mentioned earlier, the goal was to maximize G′ and minimize tan δ. Examining Figure 30 shows that G′ values from the experimental trials were not significantly different ($P>0.05$) from the predicted values. This implies that the model predicted values were confirmed and the gels prepared with 20% CPI and 1.5% (w/v) guar gum were characterized as very strong (G′=56440 Pa) with increased number of crosslinks (Fig. 30). Also, the experimental tan δ values were not significantly different ($P>0.05$) from the model predicted tan δ values (Fig. 30). The predicted and experimental tan δ values fell below 0.2, an indication that the gels had elastic structures.

One may argue the rationale for using high CPI (20%, w/v) and guar gum (1.5%, w/v) concentrations in this study. Arntfield et al. (1990a) established that 10% canola protein was sufficiently high to produce properly-crosslinked heat-induced networks. Based on this finding, we chose the CPI concentrations of 10 and 20% (w/v). Another study that analyzed the physical characteristics of canola protein-guar gum gels showed that high protein level (up to 15%) and high guar gum concentration (up to 1.5%) resulted in low tan δ and high G′ values (Arntfield and Cai, 1998). Other reports showed that high levels of guar gum (~1%) are very thixotropic but lower concentrations

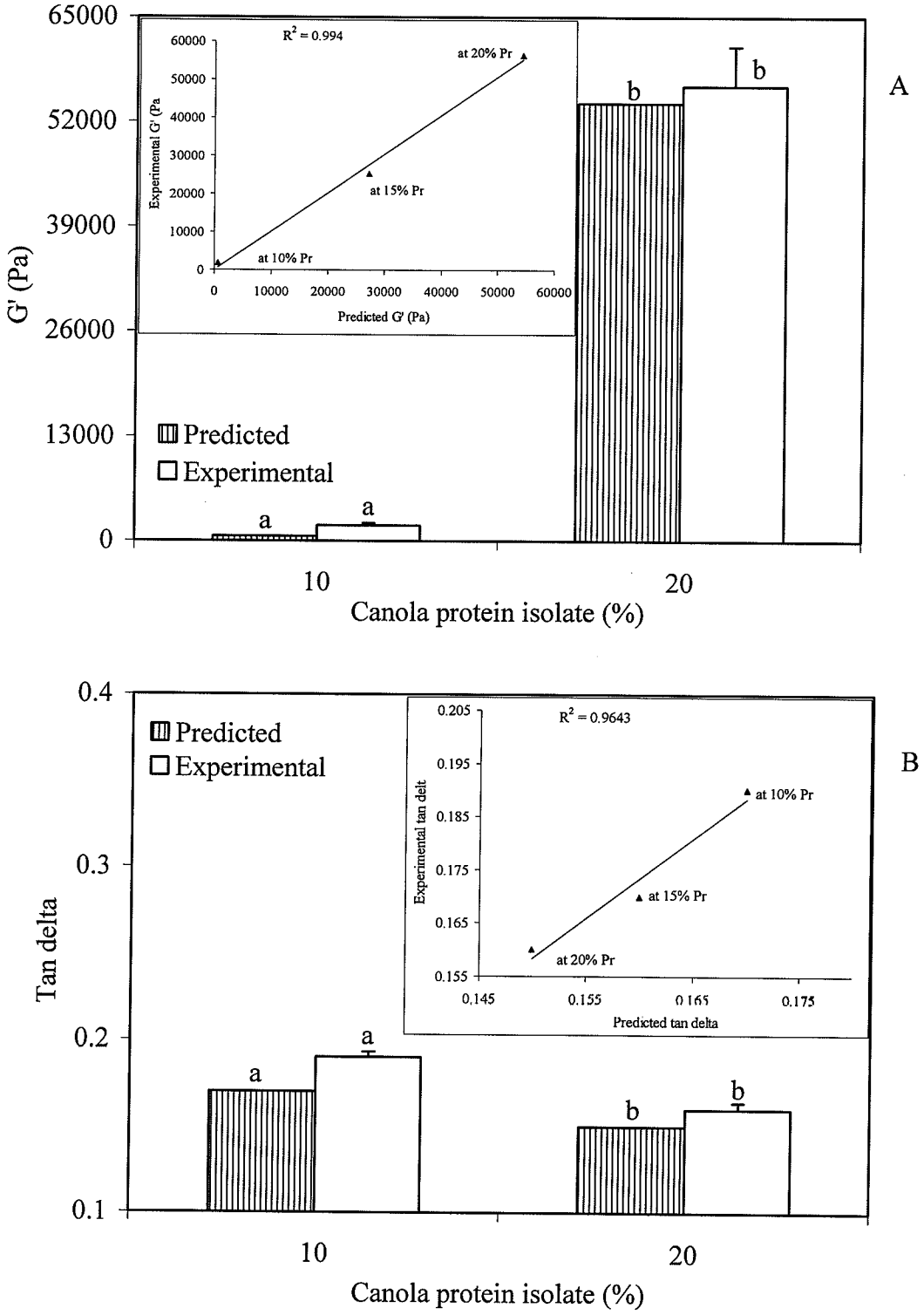


Figure 30. Predicted and experimental response parameters, G′ (A) and tan δ (B), of CPI-guar gum gels at pH 10, 0.05 M NaCl and 1.5% guar gum. Measurements were taken during frequency sweep (after cooling) at 1 Hz. *Insets: Pr = Protein. For each parameter and within each treatment, bars with same letters are not significantly different (P>0.05).

(~0.3%) are far less thixotropic (Petkowicz et al., 1998; http://www.sbu.ac.uk/water). In order to obtain CPI and guar gum combination that will give strong interactions (to produce strong and elastic gels), the optimum CPI (20%, w/v) and guar gum (1.5%, w/v) concentrations were chosen.

Gel formation mechanisms for guar gum, canola protein and their mixture

Thermal sweep curves in rheological measurements for CPI-guar gum mixtures helped to demonstrate the course of network development (key parameters G′ and tan δ) as a function of temperature. Generally, the gel network developed above the denaturation temperature (T_d) in the heating phase, and continued in the cooling phase. As the T_d of CPI alone was determined to be ≈86°C (Chapter 1), the samples were heated to 95°C prior to the cooling stage. Heating curves for guar gum (1.5% w/v), CPI (20% w/v) and CPI-guar gum gels showing the G′ and tan δ values at pH 10, 0.05 M NaCl, 20% (w/v) CPI and 1.5% (w/v) guar gum are shown in Figure 31.

Guar gum gel properties: The onset of aggregation, indicated by the increase in G′ (deviation from the baseline) and decrease in tan δ (Figs. 31A and B) occurred below 50°C. Under the conditions (pH 10, 0.05 M NaCl, 1.5% guar gum) investigated, it was observed that heating guar gum sol resulted in a decrease in G′ overall. A complete breakdown of guar gum network at conditions (pH 10, temperature 25-95°C) used was observed. A slushy mess was obtained, an indication that guar gum did not form a gel (G′=51 Pa, Table 13). The inability of guar gum to form a gel may be attributed to the physical properties of guar gum and the extreme conditions (pH 10, temperature >90°C) used. Guar gum does not form gels (due to its rigid structure) but

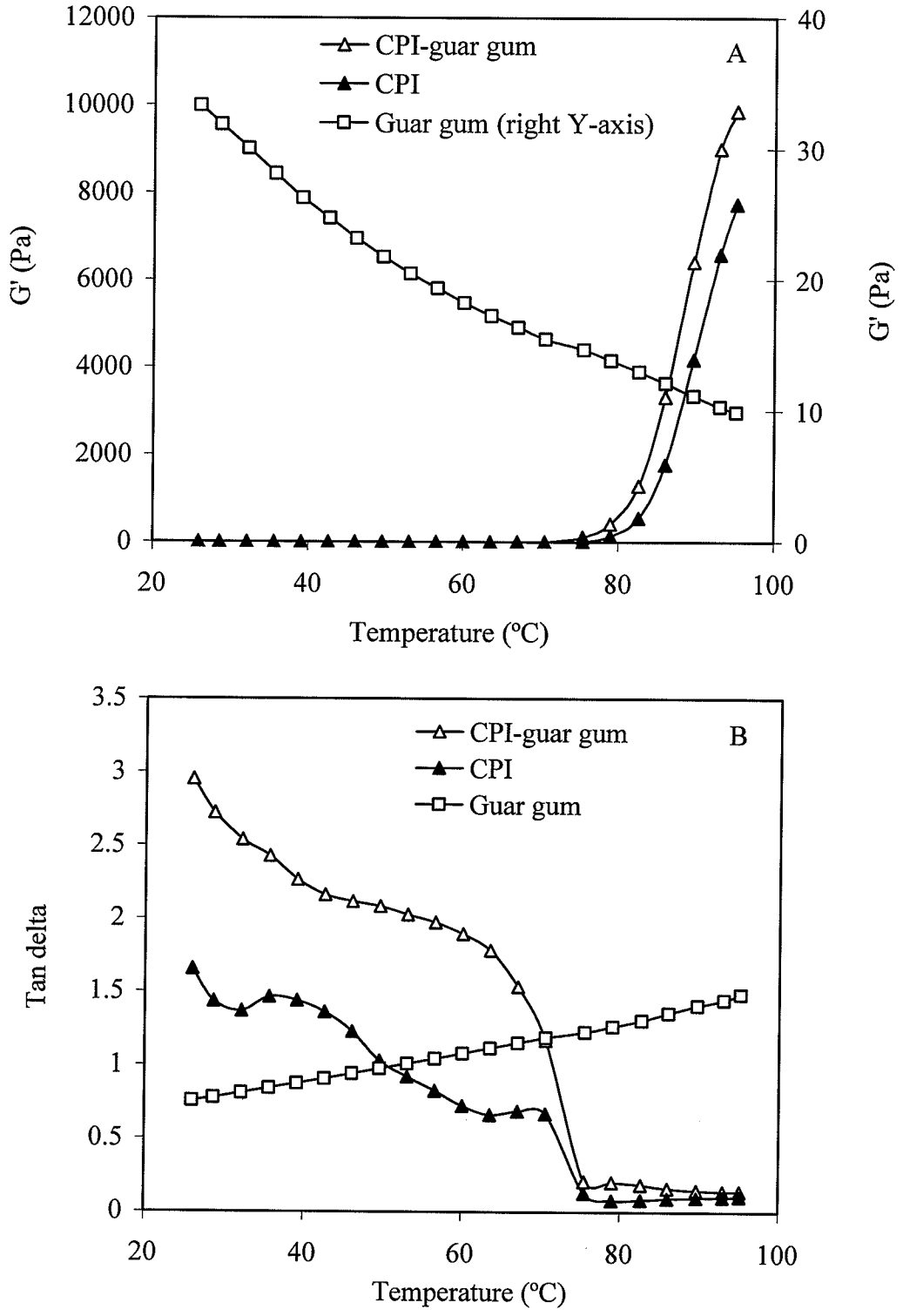


Figure 31. Changes in rheological properties, G′ (A) and tan δ (B) of canola protein isolate (CPI), guar gum and mixed canola protein-guar gum networks during the heating cycle (0.05 M NaCl; 20%, w/v CPI; 1.5%, w/v guar gum; pH 10).

Table 13. Rheological characteristics of canola protein-guar gum gels prepared with 0.05 M NaCl at pH 10. Measurements were taken during frequency sweep (after cooling) at 1 Hz.

Protein + Guar gum (%)	G′[z] (Pa)	Tan δ[z]
00 + 1.5	51 ± 2^{c}	0.67 ± 0.04^{a}
20 + 0.0	64575 ± 856^{a}	0.15 ± 0.01^{b}
20 + 1.5	56440 ± 4879^{b}	0.18 ± 0.00^{b}
10 + 1.5	1914 ± 316^{c}	0.19 ± 0.00^{b}
20 + 1.5*	$2726 \pm 57^{*c}$	$0.2 \pm 0.00^{*b}$

[z]Mean ± SD. For G′ or tan δ, column values with different letters are significantly different ($P<0.05$). *Canola protein-guar gum gel properties at pH 6.

produces high viscous solution (Whistler and BeMiller, 1997). Because guar gum has its galactosyl units fairly evenly placed along the chain, the galactose residues prevent strong chain interactions as few unsubstituted chain areas have the minimum number of residues (about 6) required for the formation of junction zones (Petkowicz et al., 1998; Whistler and BeMiller, 1997). According to Petkowicz et al. (1998), guar gum (being nonionic) is not affected by ionic strength or pH but will degrade at pH extremes when heated. Cooling apparently increased network development of guar gum but this did not result in G′ values that were in the same range as the other samples (Fig. 32A).

Canola protein gel behaviour: For canola protein alone (20%, w/v CPI) at pH 10, there was little sign of structure development (deviation from the baseline) at temperatures below 75°C (Fig. 31A). The first increase in G′ value did not started until temperatures greater than 78°C. It was evident during the heating cycle that the gel strength (G′: 2000-8000 Pa) of CPI was enhanced at higher temperatures (above 78°C,

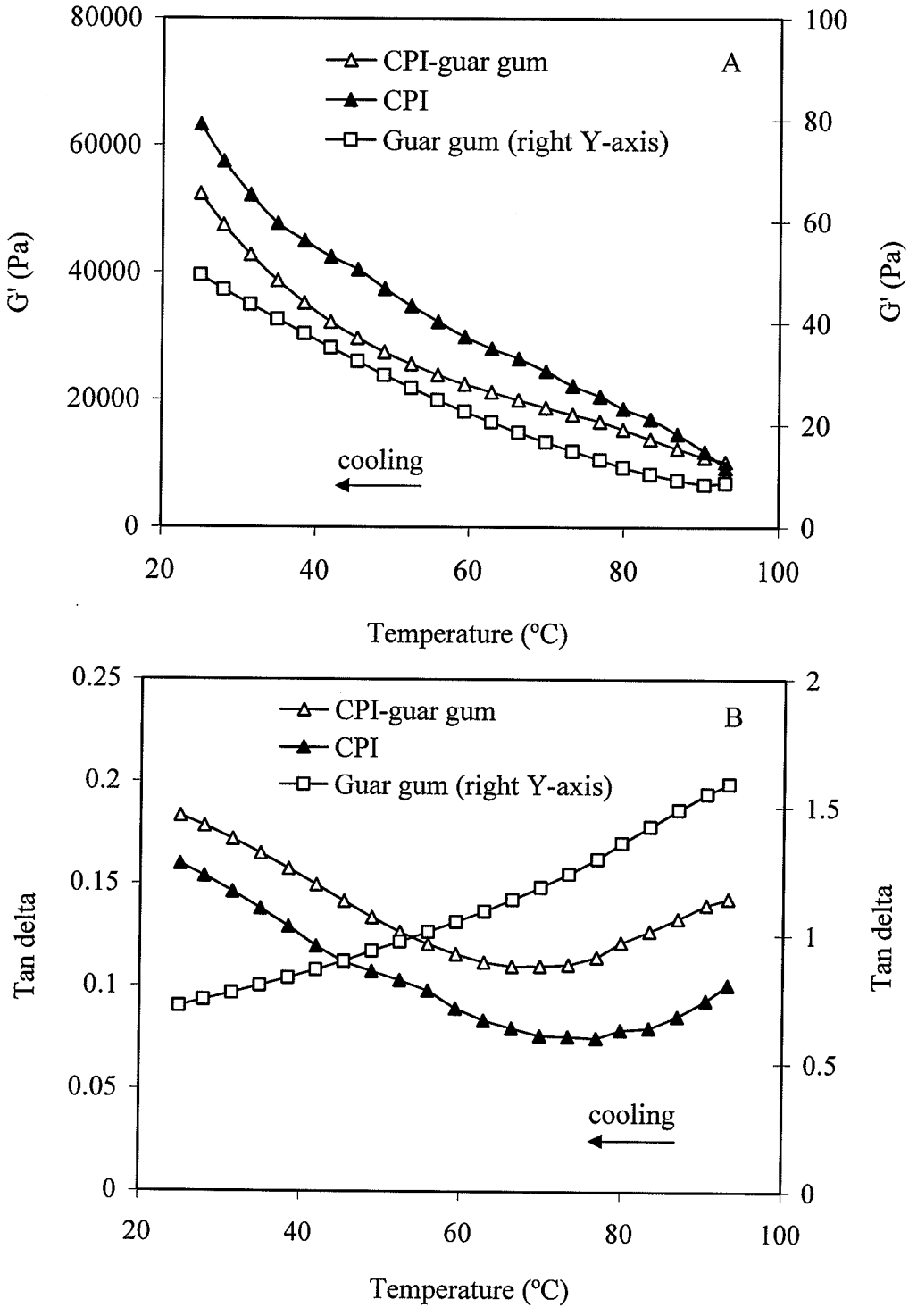


Figure 32. Changes in rheological properties, G′ (A) and tan δ (B) of canola protein isolate (CPI), guar gum and mixed canola protein-guar gum networks during the cooling cycle (0.05 M NaCl; 20%, w/v CPI; 1.5%, w/v guar gum; pH 10).

Fig. 31A), possibly due to unfolding and associations of protein molecules. During the initial heating phase (below 78°C), low G′ and high tan δ values were obtained for the CPI gels at temperatures below 78°C. However, as temperature rose above 78°C, tan δ values decreased while G′ values increased (Figs. 31A and B); evidence of the formation of strong and elastic network for CPI at higher temperatures. Changes in G′ during cooling indicate an increase in structure formation as the temperature was lowered (Fig. 32A). Cooling significantly improved the network strength of canola protein as stronger and more elastic network resulted. Data from frequency sweep (after the cooling cycle) showed that the gel properties improved greatly (G′=64575 Pa, tan δ=0.15) during cooling. G′ value for the 20% (w/v) CPI increased from ≈8000 Pa (at the end of the heating cycle, Fig. 31A) to 64575 Pa (after the cooling cycle as seen in frequency sweep data, Table 13). This increase in G′ during cooling has been related to an increase in the level of hydrogen bonding (Léger and Arntfield, 1993).

Canola protein-guar gum networks: The rheological profiles of guar gum and CPI gels are shown in Figures 31 and 32. During the heating phase, it was observed that the onset of aggregation (increase in G′ and decrease in tan δ, Fig. 31A) for CPI-guar gum gels occurred at approximately 80°C. Strong and elastic networks were formed with increased temperature (>80°C) during the heating cycle (Fig. 31) and continued during the cooling cycle (Fig. 32). At the end of the heating phase (Fig. 31A), the G′ value for the CPI-guar gum gel (G′≈10000 Pa) was higher than that of CPI alone (G′≈8000 Pa), suggesting enhanced gel strength in the presence of guar gum. In contrast, data from the frequency sweep (after the cooling cycle) showed that the G′ value for the CPI-guar gum gel (G′=56440 Pa) was lower than that of CPI alone (G′=64575 Pa). It is possible that

there was considerable interference in protein gelation from the guar gum. The presence of guar gum in a mixed system with CPI was shown to decrease G′ value, and the reduction in gel strength (low G′ value) was significant ($P<0.05$). The interference (by guar gum) in gelation may be due to the physical structure of guar gum; it forms stable solutions because the side-chain galactosyl units along the main chain prevent the intermolecular association necessary for junction zone formation (Whistler and BeMiller, 1997).

The network strength and structure of the mixed CPI-guar gels were significantly affected by protein concentration and pH. The mixed gel prepared with 20% CPI formed a significantly ($P<0.05$, Table 13) stronger network (G′=56440 Pa) than the mixed gel prepared with 10% (w/v) CPI (G′=1914 Pa), indicating that the extent of increase in network strength was dependent on the CPI concentration. This observation agreed with previous studies with canola protein systems where it was reported that the increase in structure development was highly dependent on protein concentration (Arntfield and Cai, 1998; Léger and Arntfield, 1993). For mixed gels prepared with 20% (w/v) CPI at varied pH, the elastic modulus was significantly ($P<0.05$, Table 13) higher at pH 10 (G′=56440 Pa) than at pH 6 (G′=2726 Pa). The increased electrostatic repulsion at pH 10 must minimize random aggregation and reduce the degree of interaction among polypeptides in the system. It is possible that there was a balance between protein-protein and protein-solvent interactions at pH 10 to obtain improved networks.

The development of superior network structures at high pH values has been attributed to the extension of polypeptide chains and the creation of homogeneous structures (Paulson and Tung, 1989). In comparison, gels prepared at lower pH values

are heterogeneous in nature. These heterogeneous networks are composed of compact molecules, which aggregate to form regions of high and low protein concentrations. As a result, regions with low protein concentrations act as weak points, causing a drop in overall gel strength and lower G′ values (Table 13). A comparison of the rheological data of the individual components and the mixed gels indicated that guar gum did not improve the gel strength and network structure of the mixed gels, as was observed in gels formed with CPI and κ-carrageenan (κ-CAR). In that study (Chapter 3), κ-CAR greatly improved the gel strength and structure of CPI, even at pH 10. The difference in the network strength of CPI-based gels prepared with κ-CAR and guar gum could be attributed to the difference in the physical properties of the two hydrocolloids (e.g. κ-CAR is negatively charged while guar gum is nonionic).

CHAPTER 5

Microstructure of Commercial Canola Protein-Hydrocolloid Networks

Abstract

Biopolymer mixtures contribute to the formation of properly crosslinked networks. Effective utilization of these networks depends on the understanding of the type and role of interactions involved in network formation. Establishing the structural factors which control the gelation of canola protein in mixed systems would contribute to a better understanding of texture development in plant protein-based foods. This study examined the molecular forces that affect the structural arrangement of canola protein-hydrocolloid gels. The structural properties of canola protein isolate (CPI), κ-carrageenan (κ-CAR) and CPI-hydrocolloid (κ-CAR or guar gum) networks were examined using scanning electron microscopy (SEM). Visual assessments were made and SEM images of each gel system at three magnifications were obtained. The effects of pH, urea and dithiothreitol (DTT) on the network structure of CPI and CPI-hydrocolloid gels were evaluated. Gels produced from CPI alone showed varied structures at acidic and alkaline pH. At pH 6, a loosely crosslinked protein network with large and small empty cells/pores that entrapped the liquid components was observed, whereas a properly crosslinked network structures were seen at pH 10. CPI-κ-CAR and CPI-guar gum gels prepared at optimum conditions had properly crosslinked networks, whereas those prepared at non-optimal conditions exhibited aggregated network structures. Results showed that network structures of the individual CPI and κ-CAR gels were greatly improved when the two macromolecules were combined. This improved network structure (properly formed network) suggests a synergistic behaviour between

CPI and κ-CAR. Urea and DTT affected the molecular forces that contribute to network formation as well as the structural properties of CPI-κ-CAR gels. Gels treated with urea were excessively crosslinked, demonstrating a marked alteration of the gel structure; whereas systems treated with DTT had an amorphous structure. Disorganization and disruption of gel structures by urea and DTT supports the involvement of disulfide bonds and noncovalent interactions in CPI-κ-CAR and CPI-guar gum gel formation. Understanding the factors responsible for enhancing the physical and textural properties of CPI in food systems can provide useful information for improving plant protein functionality.

Introduction

Microscopy is a tool that can be useful to describe the microstructure of a system at the end of the gelation process. Use of scanning electron microscopy (SEM) to examine the structure of commercially processed soybean protein products has been reviewed (Wolf and Baker, 1981). Studies have shown that the rheological properties of canola protein and canola protein-based gels vary with pH and ionic strength (Arntfield and Cai, 1998; Cai, 1996; Léger and Arntfield, 1993). These differences in rheological properties could be partly explained by the amount of protein incorporated into the network. Soy protein gels were less stiff (lower storage modulus, G′) when more protein remained dissolved after heating instead of being part of the network (Renkema et al., 2000; Renkema et al, 2002). However, this factor cannot explain the whole difference. From work on other proteins (such as casein, β-lactoglobulin, whey protein), it is also known that network structure determines the rheological properties of gels (Bremer et al., 1990; Stading and Hermansson, 1990; Verheul and Roefs, 1998a, 1998b; Mellema et al., 2002). Hermansson (1994) divided network structure roughly into fine-stranded and coarse-aggregated networks. Fine-stranded gels may be completely transparent and are composed of strands with a thickness up to a few times the size of a single protein molecule. Coarse gels are non-transparent and are composed of particles with diameters in the range of 100-1000 times a single protein molecule. Intermediate structures containing fine-stranded and coarse structures simultaneously also exist. The type of gel that is formed depends on conditions during gel formation. In general, gels become coarser as the pH approaches the isoelectric point (IEP) or when the ionic strength is increased (Doi, 1993). Our use of SEM to examine the network structure of CPI and

CPI-hydrocolloid gels is one of the applications of this technique to CPI. The objective was to characterize CPI-hydrocolloid gels in terms of network structure as a function of pH and denaturants using SEM. To evaluate the role of covalent and noncovalent interactions in gel formation of commercial CPI-κ-CAR and CPI-guar gum, two standard denaturants (Phillips et al., 1994) that impact noncovalent interactions (assessed using urea) and disulfide bond formation (assessed using dithiothreitol (DTT) were included in the protein dispersion prior to heat treatment. Arntfield et al. (1991) reported a reduction in protein crosslinking when ovalbumin was treated with 2-mecarptoethanol (20 mM), supporting disulfide bond involvement in ovalbumin networks. DTT has been shown to decrease G′ and increase tan δ values for rapeseed gels; this implicated disulfide interactions as a factor in the elasticity of the network (Gill and Tung, 1978). In the present study, the structures developed during gelation as well as properties of the networks formed were used to evaluate the effects of these reagents.

Materials and Methods

Source of materials

Commercial (BMW Canola, Winnipeg, Canada) canola protein isolate (CPI) was purchased and used without further purification. Proximate analysis (AOAC, 1990) 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 κ-carrageenan (No. C-1013) that contains predominantly κ- and lesser amounts of λ-carrageenan, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The

composition of κ-CAR powder as indicated by the manufacturer showed it contained a mixture of the following cations: K^+ (10.4%), Ca^{2+} (2.3%) and Na^+ (0.9%). Also, food grade guar gum (G-4129; Lot No. 95H0653), with a viscosity of 6000-10000 mPas (Whistler and BeMiller, 1997), urea (U-15; Lot No. 863571), and DTT (D-0632; Lot No. 61K16571) were purchased from Sigma Chemical Co. 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, USA).

Experimental design

All experimental parameter measurements were done in duplicates. Design-Expert® Software (Stat-Ease Inc., MN) was used to generate the model experimental design with factorial and response surface optimization. Appropriate combinations of factors (CPI, κ-CAR, pH and NaCl) and optimum conditions (pH 6; 0.05 M NaCl; 15%, w/v CPI; 3%, w/v κ-CAR) for gelation of CPI-κ-CAR mixtures were established as previously reported (Chapter 3). The procedure described in this previous study was used to generate the optimum gelling conditions for CPI-guar gum mixtures: pH 10; 0.05 M NaCl; CPI (20%, w/v), and guar gum (1.5%, w/v). Since microscopic examination is an additional tool for characterizing the observed rheological properties in terms of network structure, these combinations were used to prepare gels used for microstructural assessments. Control runs with dispersions of individual CPI (15%, w/v + 0%, w/v guar gum), κ-CAR (3%, w/v + 0%, w/v CPI) and guar gum (1.5%, w/v + 0%, w/v CPI) at optimum pH and NaCl concentration were carried out.

Sample preparation

Dispersions of individual CPI (15% w/v CPI, protein-basis; 0.05 M NaCl; pH 6 and 10), κ-CAR (3%, w/v κ-CAR; pH 6; 0.05 M NaCl) and guar gum (1.5%, w/v guar gum; 0.05 M NaCl; pH 10) were prepared. Similarly, dispersions of CPI-κ-CAR (15%, w/v CPI; pH 6; 0.05 M NaCl; 3%, w/v κ-CAR) and CPI-guar gum (20%, w/v CPI; pH 10; 0.05 M NaCl; 1.5%, w/v guar gum) mixtures were prepared. Each mixture was stirred for approximately 1 h at room temperature or until a complete dispersion of the mixture was achieved. Dispersions of individual CPI, κ-CAR and guar gum samples in appropriate NaCl solutions were prepared in similar manner to serve as control. The CPI, κ-CAR, guar gum, pH and NaCl combinations were the optimum conditions generated using Design-Expert® Software; the method of establishing optimum conditions have been reported (Chapter 3). For interaction studies, dispersions of CPI-κ-CAR (15% w/v CPI, 3% κ-CAR, pH 6) and CPI-guar gum (20%, w/v CPI; 1.5%, w/v guar gum; pH 10) mixtures were treated with a disulfide blocker (DTT) or hydrogen bond blocker (urea) at concentrations of 0.15 M and 6 M, respectively prior to heat treatment. To ensure that pH was maintained, samples were allowed to equilibrate for 30 min at room temperature and the pH was rechecked prior to further testing.

Microscopy: Gel microstructural analysis

Gel networks were prepared using previously published protocols of Léger and Arntfield (1993) and Arntfield et al. (1991) with slight modification. Heat-set CPI, guar gum, CPI-κ-CAR and CPI-guar gum gel networks were prepared by heating samples in closed stainless steel vials from 25 to 95°C at a rate of 2°C/min. At the end of the heating

regime, samples were held at 95°C for 5 min and cooled to room temperature in an ice bath. Gels were removed from the vials, frozen and freeze dried. The dried gel samples were fractured and mounted on a specimen stub with a double-sided adhesive tape. The samples were sputter-coated (Edwards Sputter Coater, model S150B) under vacuum with gold/palladium (75/25). The consecutive visual assessments and documentations were done on a JEOL JSM-5900LV SEM (JOEL SEM, Japan), operating at an acceleration voltage of 10 kV. Scanning electron micrographs of each gel sample at three magnifications (X50, X500, and X3000) were obtained.

Results and Discussions

Visual examination

For systems treated with 0.05 M NaCl: CPI, CPI-κ-CAR and CPI-guar gum dispersions formed light brown, opaque networks; whereas κ-CAR dispersion formed a translucent gel. There was no visual evidence of gel formation when CPI-hydrocolloid and individual κ-CAR, guar gum and CPI dispersions were treated with NaCl at room temperature. For systems treated with denaturants: CPI-hydrocolloid dispersions treated with urea formed a gel at room temperature, an indication of a conformational change. Systems treated with DTT formed opaque, yellow gels. As reported by Damodaran (1996), the opaqueness of these gels is due to light scattering caused by the unordered network of insoluble protein aggregates. Guar gum (1.5%, w/v) dispersion prepared with 0.05 M NaCl at pH 10 did not form a gel after heat treatment, suggesting a complete structural breakdown.

Structural arrangements of canola protein isolate networks

The SEM micrographs of 15% w/v canola protein gels treated with 0.05 M NaCl at pH 6 and 10 are shown in Figure 33A and B, respectively. Variation in pH affects the overall net charge on the protein molecule as well as its molecular conformation. The influence of pH on the protein gel was reflected in the differences in network structures. At pH 6 (Fig. 33A), a loosely crosslinked protein network with large and small empty cells/pores that entrapped the liquid components was observable at the three magnifications and a similar but more detailed structure with a porous, spongy texture was seen at higher magnifications (X500, X3000). These empty pores were distributed throughout the network which suggests that the liquid phase was completely entrapped in a continuous three-dimensional network. Also seen in the pH 6 micrographs (Fig. 33A) were regions of good and poor crosslinked structures. At pH 10 (Fig. 33B), the small empty cells (seen in gels prepared at pH 6) were absent in the X50 micrograph and present (but not in the same magnitude as seen in gels prepared at pH 6) in the X500 and X3000 micrographs. Properly crosslinked, tightly-packed network structures were seen in the X500 and X3000 micrographs; where lower tan δ value has been reported (Chapter 3). Rheological data (Chapter 3) showed a stiffer and more elastic network for canola protein gel prepared at pH 10 (G'=60320 Pa, tan δ=0.15) than at pH 6 (G'=27220 Pa, tan δ=0.19). A similar trend in terms of pH response was noted in a study with ovalbumin gels (Arntfield, 1989), and also in a study with CPI gels using a Zeiss Universal Research Microscope (Léger and Arntfield, 1993). The study by Léger and Arntfield (1993) on a laboratory prepared CPI demonstrated that photomicrographs supported the rheological properties that characterized the final gels.

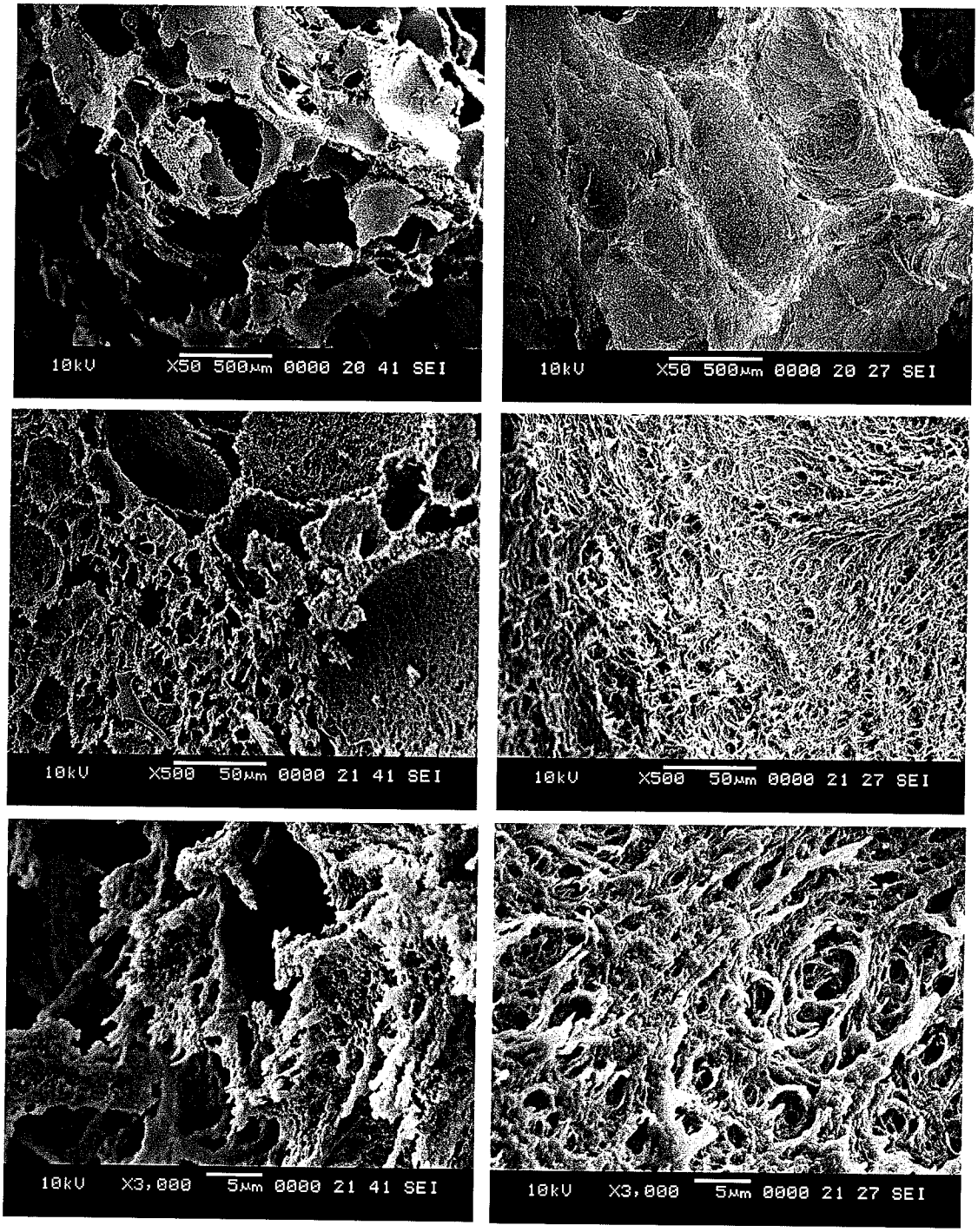


Figure 33. SEM of canola protein isolate (CPI) gels prepared with 0.05 M NaCl and 15% (w/v) CPI at pH 6 (A) and pH 10 (B); showing X50, X 500, X3000 micrographs.

These results may reflect the influence of charge on network structure rather than conformational changes. At pH 6, below the isoelectric point (IEP) of canola proteins (IEP=6.8-7.2; Schwenke et al., 1987), net positive charge was dominant. As a result and in the presence of 0.05 M NaCl, attractive intermolecular electrostatic interaction should be maximized. At low salt concentrations, ions interact with proteins via nonspecific electrostatic interactions (Shih et al., 1992). According to this author, this electrostatic neutralization of protein charges usually stabilizes protein structure. Although a crosslinked network was seen at pH 6, it was not as tightly-packed as that observed at pH 10. The differences in rheological properties at pH 6 and 10 were evident in the microstructural data at the two pH values. According to one school of thought (Arntfield, 1989), it is possible that the range for the attractive-repulsive electrostatic interaction balance necessary for a properly crosslinked three dimensional network formation was maximized at alkaline pH value, so that a good network was observed.

A balance between the charge repulsion and the potential for interaction, mainly through hydrogen bonds and hydrophobic interactions, is critical to the determination of network characteristics (Arntfield et al., 1990; Arntfield, 1994). Excessive attractive forces or insufficient charge repulsion results in aggregation rather than network formation. In another explanation, the development of superior network structures at high pH values has been attributed to the extension of polypeptide chains and the creation of homogeneous structures (Paulson and Tung, 1989). In comparison, gels prepared at lower pH values are heterogeneous in nature. These heterogeneous networks are composed of compact molecules, which aggregate to form regions of high and low protein concentrations. As a result, regions with low protein concentrations act as weak points, causing a drop in overall gel strength. In this study, it is possible that the

regions of low protein concentration described by Paulson and Tung (1989) may be the regions observed as poor crosslinked structures shown in the micrographs (Fig. 33A).

Structure of hydrocolloid (κ-carrageenan and guar gum) networks

The intent was to examine the gel morphology of κ-CAR and guar gum networks at the optimum conditions (see experimental design) established by the model employed in this research. No SEM image was obtained for 1.5% (w/v) guar gum dispersion prepared with 0.05 M NaCl at pH 10 because self-supporting gel could not be obtained under these conditions. Guar gum gel was prepared at pH 10 because this was the optimum pH established for CPI-guar gum gels by the model used in this study. This study does not recommend pH 10 since it is unconventional in food systems (although egg albumin has a pH value of 9.0; Arntfield, 1989). The SEM images for 3% (w/v) κ-CAR gels prepared with 0.05 M NaCl at pH 6 revealing the internal structures are shown in Figure 34. The κ-CAR gels have sheet- or platelet-like structures with flaky particles in the X50 micrograph. The sheet-like structures seen at X50 were observed at X500 and X3000 to be a tight mass of almost cubic structures. A similar structural appearance was reported for the seaweed kelp (McCrone and Delly, 1973). These almost cubic structures were not continuous due to the presence of dissolved patches or structural irregularities in the X500 and X3000 micrographs. As heated solutions of κ-CAR are cooled, the linear molecules form helices, which then associate to form a firm, three-dimensional, stable gel in the presence of appropriate cation (Whistler and BeMiller, 1997). The observed structures in this study are not helical but are almost cubic. This may be due to hydrolysis at high temperature of 95°C used in this study. Whistler and BeMiller (1997)

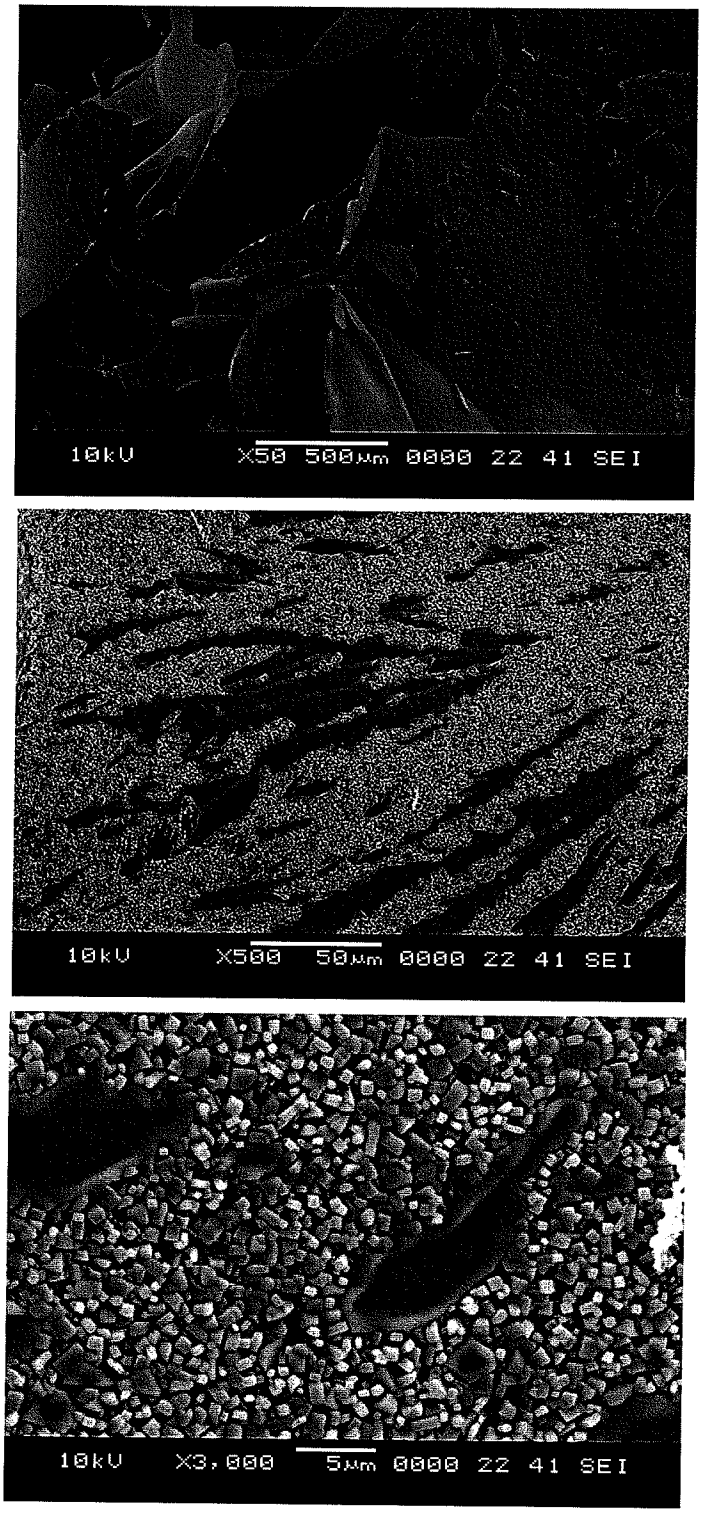


Figure 34. SEM of κ-carrageenan (κ-CAR) gels prepared with 0.05 M NaCl and 3% (w/v) κ-CAR at pH 6. (X50, X500, X3000 micrographs).

reported that carrageenans are quite susceptible to acid-catalyzed hydrolysis; undergoing rapid and extensive degradation when solutions below pH 5 are heated. Although we used pH 6 in this study, the 95°C heat treatment may have caused excessive cleavage of structures.

Structure of canola protein isolate-κ-carrageenan networks

The detailed structural arrangement of CPI-κ-CAR gels prepared at the model-established optimum conditions (pH 6, 3% κ-CAR, 15% canola protein, 0.05 M NaCl) and non-optimal conditions (pH 10, 3% κ-CAR, 20% canola protein, 0.25 M NaCl) are shown in Figures 35A and B, respectively. The rheological data (Chapter 3) for CPI-κ-CAR gel prepared at the established optimum conditions showed a stiffer and more elastic network (G'=97465 Pa, tan δ=0.15), whereas the rheological data for the non-optimal conditions were less elastic and not as strong (G'=12944 Pa, tan δ=0.19). This trend was corroborated by the microstructural images shown in Figures 35A and B. The overall structure of the CPI-κ-CAR gel samples shown at X50 (Fig. 35A and B) were somewhat similar and characterized by large empty cells/pores that looked like properly formed networks. However, there were differences in structures and texture at X500 and X3000. The CPI-κ-CAR gel sample prepared at optimum conditions (Fig. 35A) exhibited a tightly-packed mass of network with a few large pores in the X500 micrograph.

These empty pores may be regions of the gel that entrapped liquid components of the network. The X3000 micrograph showed more details of the network structure; revealing the properly crosslinked, densely-packed CPI-κ-CAR strands.

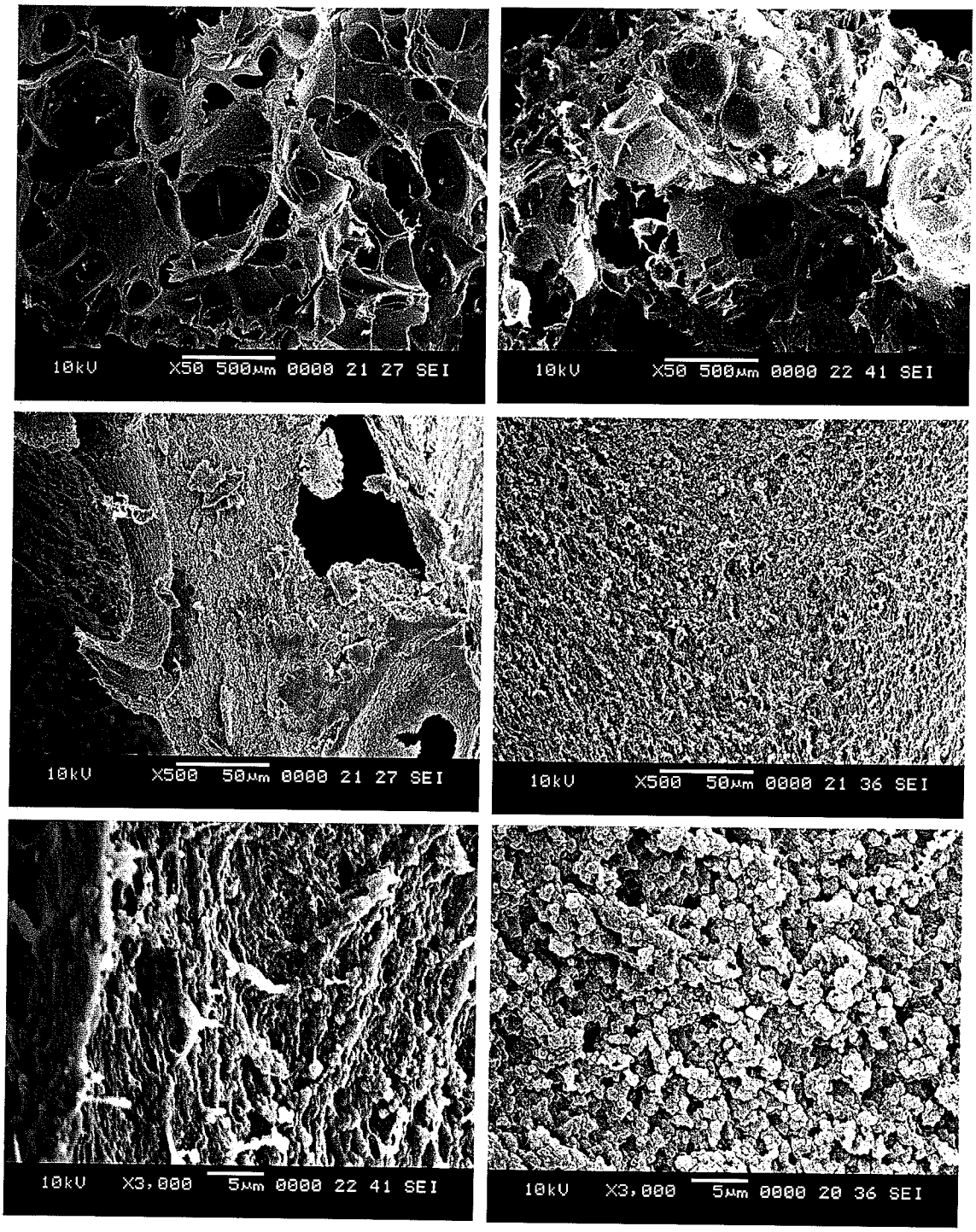


Figure 35. SEM of canola protein isolate-κ-carrageenan gels. A: high G′, low tan δ (pH 6, 3% (w/v) κ-CAR, 15% (w/v) CPI, 0.05 M NaCl); B: low G′, high tan δ (pH 10, 3% (w/v) κ-CAR, 20% (w/v) CPI, 0.25 M NaCl). (X50, X500, X3000 micrographs).

This demonstrates a very strong and elastic network character. Comparing the CPI alone and κ-CAR gel micrographs (Figs. 33A and 34) showed that the structures of the individual gels were greatly improved when the two macromolecules (CPI and κ-CAR) were combined to form a gel system. The results obtained when the two biopolymers were mixed showed the complementary character of each biopolymer. The rheological and microstructural properties of the mixed gels were enhanced as there was increased crosslinking in a mixed state. This improved network structure (shown as properly formed network) suggests a synergistic behaviour between CPI and κ-CAR as was reported in the gelation analysis of CPI and κ-CAR (Chapter 3).

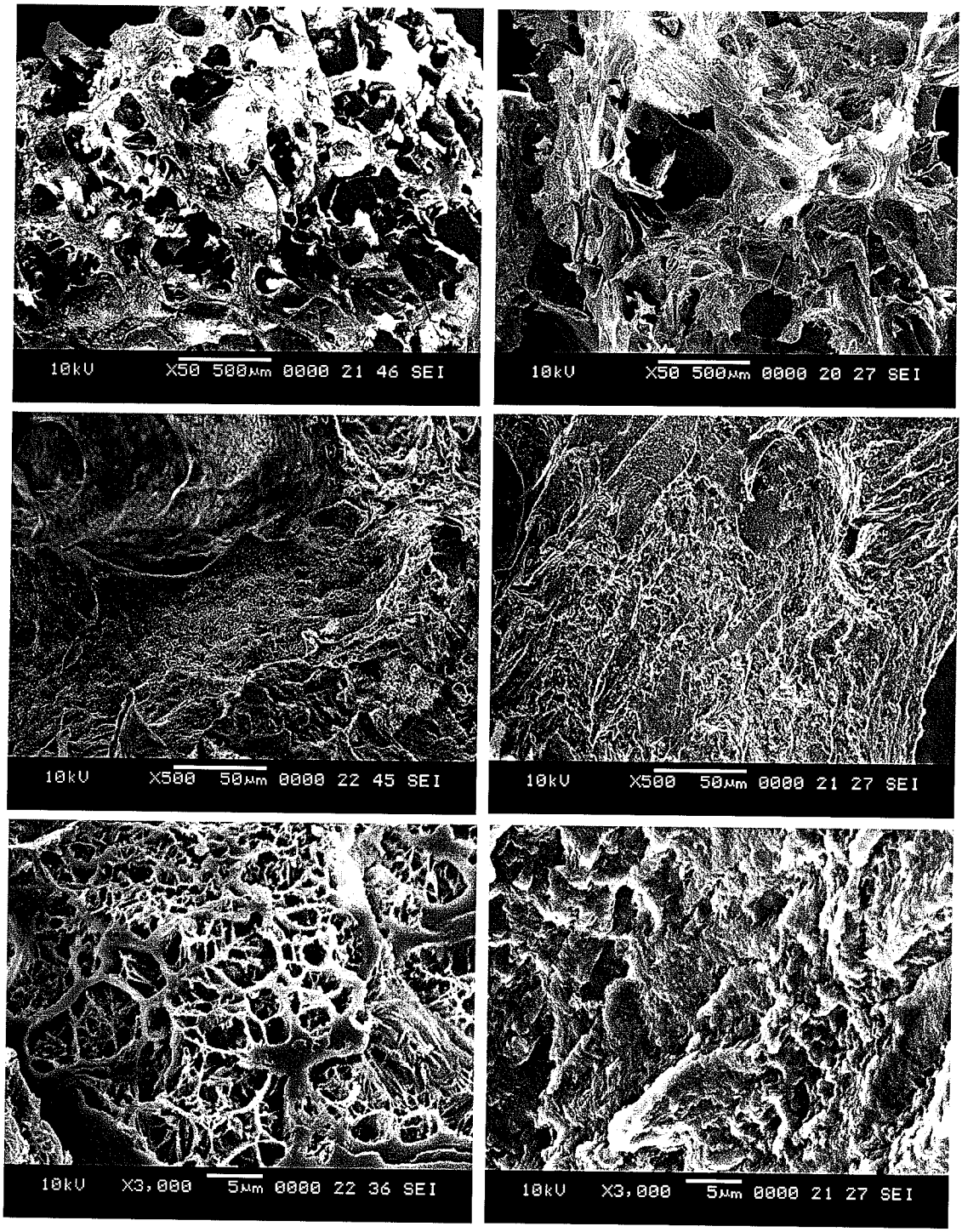
Other rheological investigations confirmed the synergistic effects in denatured protein/κ-CAR systems involving work with κ-CAR and β-lactoglobulin (Tziboula and Horne, 1999; Ould-Eleya et al., 2000a, 2000b; Neiser et al., 2000). The appearance of the gel structures for CPI-κ-CAR mixture (prepared at non-optimal conditions) shown in Figure 33B was quite different (at higher magnifications) from that obtained for the control (Fig. 35A). The image shown in X500 micrograph appeared as small strands of networks. At X3000, it was apparent that the network was composed of tight clumps of aggregated strands. The structure at X3000 was highly aggregated though the evidence of alignment into strands may have been responsible for the somewhat high rheological characteristic (G′=12944 Pa). In some systems, clumps of aggregates have been shown to yield high G′ values due to the relative closeness of the neighbouring molecules (Ismond and Welsh, 1992). The rheological data (stated above) for gel sample shown in Figure 35B was characterized by higher tan δ value (0.19) than that for the control sample (tan δ=0.15) shown in Figure 35A. The tan δ values indicated significant

structural differences for the two gel samples and this was clearly reflected in the microstructural data. Canola protein gels with high tan δ values have been characterized to have aggregated structures (Léger and Arntfield, 1993).

Influence of denaturants on canola protein-κ-carrageenan gel structure

The inclusion of denaturants (urea, DTT) in canola protein-carrageenan dispersions prior to heat treatment was specifically intended to examine the interactions involved in CPI-κ-CAR gelation. The SEM images shown in Figure 36 illustrate the effect of denaturants (urea, DTT), on the structures of CPI-κ-CAR gels formed under optimal conditions. Micrographs in Figure 36A show the systems treated with urea while the DTT-treated systems are shown in Figure 36B. The structural alignments of the CPI-κ-CAR networks due to the effects of the two denaturants are quite different.

Effect of urea: The urea-treated CPI-κ-CAR mixture formed a gel at room temperature prior to heat treatment, suggesting a change in native structure. The gel system treated with urea showed some structural ordering with excessively-crosslinked networks (with large pores), demonstrating a significant disorganization of the gel structure. This is quite visible at high magnification. The x3000 micrograph reveals a markedly decreased organization of the cellular structures, with some portions of the network completely disintegrated with a smeared appearance. Comparing the micro-structural data shown in Figure 35A (properly crosslinked elastic network) to that shown in Figure 36A (excessively crosslinked gel structure) further clarifies the significant difference in structural arrangement of the gel systems due to treatment with urea. The



A B

Figure 36. SEM of canola protein-κ-carrageenan gels (pH 6, 3% (w/v) κ-CAR, 15% (w/v) canola protein, 6 M urea or 0.15 M DTT). A: Urea-treated gels; B: DTT-treated gels. (X50, X500, X3000 micrographs).

addition of 6 M urea, a hydrogen bond blocker (Phillips et al., 1994) into a gelling system is designed to completely unfold the protein molecule and prevent the formation of noncovalent intermolecular interactions. This implies that any network formed under this condition would be entirely due to disulfide bonds. The excessive disorganization of the properly-crosslinked network shown in Figure 35A was very observable in Figure 36A; this implicates noncovalent forces (e.g. hydrophobic interactions and hydrogen bonds) as a factor in the network formation of CPI-κ-CAR systems. However, the fact that the SEM images for urea-treated CPI-κ-CAR system showed some network formation demonstrates that canola protein has sufficient or appropriately located cysteine/cystine residues to support a gel structure. This result supports the involvement of disulfide bonds in the network formation of CPI-κ-CAR systems. The rheological data for the urea-treated CPI-κ-CAR (G'=19340 Pa; tan δ=0.07) and CPI-κ-CAR without urea (G'=95465 Pa; tan δ=0.15) systems further corroborates the microstructural data and gives insight on the contribution of noncovalent and disulfide interactions to the gel network. The following explanation can give more insight on why CPI-κ-CAR system can form a network when treated with urea: In 6 M urea, the inaccessible thiol groups become exposed and can be activated by alteration in solvent conditions or by unfolding of the protein during heating. Such newly exposed activated reactive thiol groups may then form covalent intermolecular disulfide bonds via thiol-disulfide interchange and oxidation reactions (Matsudomi et al., 1991; Xiong and Kinsella, 1990a). The thiol-disulfide interchange reaction can result in increased intermolecular disulfide crosslinked network required for gelation.

Influence of DTT: The marked differences in the structural alignment of gel networks shown in Figure 36 confirm the involvement of covalent and noncovalent forces in the stabilization of gel systems. The system treated with DTT (Fig. 36B) had an amorphous structure which was quite evident at X3000. The distinct properly crosslinked network seen in Figure 35A (X3000; G′=95465 Pa; tan δ=0.15) was not evident in Figure 36B (X3000). Figure 36B (X3000) shows less network formation, which may be due to disruption of disulfide bonds by DTT. As a reducing agent, DTT is capable of disrupting existing disulfide linkages, thus causing destabilization and modification of protein's native conformation (Cheftel et al., 1985). The amorphous structure seen in DTT-treated CPI-κ-CAR gel (Fig. 36B) may be due to disruption and modification of protein's native structure by DTT. However, the rheological data for the DTT-treated CPI-κ-CAR (Fig. 36B) indicates a network with enhanced gel strength (G′=105500 Pa; tan δ=0.14). Reports on the effect of DTT on protein gelation have been variable. Léger and Arntfield (1993) reported high G′ values for DTT-treated canola protein gels; confirming the rheological data of the present study. It seems that the cleavage of disulfide bonds prior to heating, along with thermal degradation of the protein-hydroclolloid complex, exposes binding sites that otherwise would have remained buried within the molecule. As a result, enhanced crosslinking (G′=105500 Pa) was obtained for DTT-treated CPI-κ-CAR gel. This may explain the higher G′ value obtained for the DTT-treated CPI-κ-CAR gel. In contrast, high concentrations of sulfhydryl reagent may decrease the number of thiol groups available for the thiol-disulfide reaction, thus weakening the structure of heat-induced gels (Xiong and Kinsella, 1990b; Zirbel and Kinsella 1988b). The tan δ values for the DTT-treated CPI-

κ-CAR (tan δ=0.14) gel and that without DTT (tan δ=0.15) did not differ much as did their structural arrangement observed by SEM. The addition of sulfhydryl reagents (i.e. DTT, N-ethylmaleimide, cysteine hydrochloride) interferes with interactions (e.g. intermolecular hydrogen and ionic bonds, hydrophobic interactions and disulfide bonds) that affect gel network formation and thus properties of gels (Clark et al., 1981; Schmidt et al., 1979). Our finding on DTT-treated CPI-κ-CAR gel supports the involvement of covalent and noncovalent forces in the gel formation of this system.

Structure of canola protein isolate-guar gum networks

The detailed structural arrangement of CPI-guar gum gels prepared at the established optimum conditions (20% canola protein, 1.5% guar gum, 0.05 M NaCl, pH 10) and non-optimal conditions (20% canola protein, 1% guar gum, 0.05 M NaCl, pH 6) are shown in Figures 37A and B, respectively. The two gel samples shown in Figures 37A and 37B did not differ from each other at X50 as the overall structure looked similar at this low magnification. However, there were differences in structure at higher magnifications. For the gels prepared at optimum conditions which serves as the control (Fig. 37A), the X3000 micrograph shows a properly crosslinked, elastic network with long fibers. Since individual guar gum dispersion prepared at the established optimum conditions did not form a gel, only a slushy mess was obtained, it is apparent that the network shown in Figure 37A probably represents the network mainly formed by CPI prepared at pH 10. Canola protein gel prepared at pH 10 was characterized by enhanced rheological properties (G′=60320 Pa, tan δ=0.15) and the observed microstructure was shown earlier in Figure 33A; which was also described as properly crosslinked. The

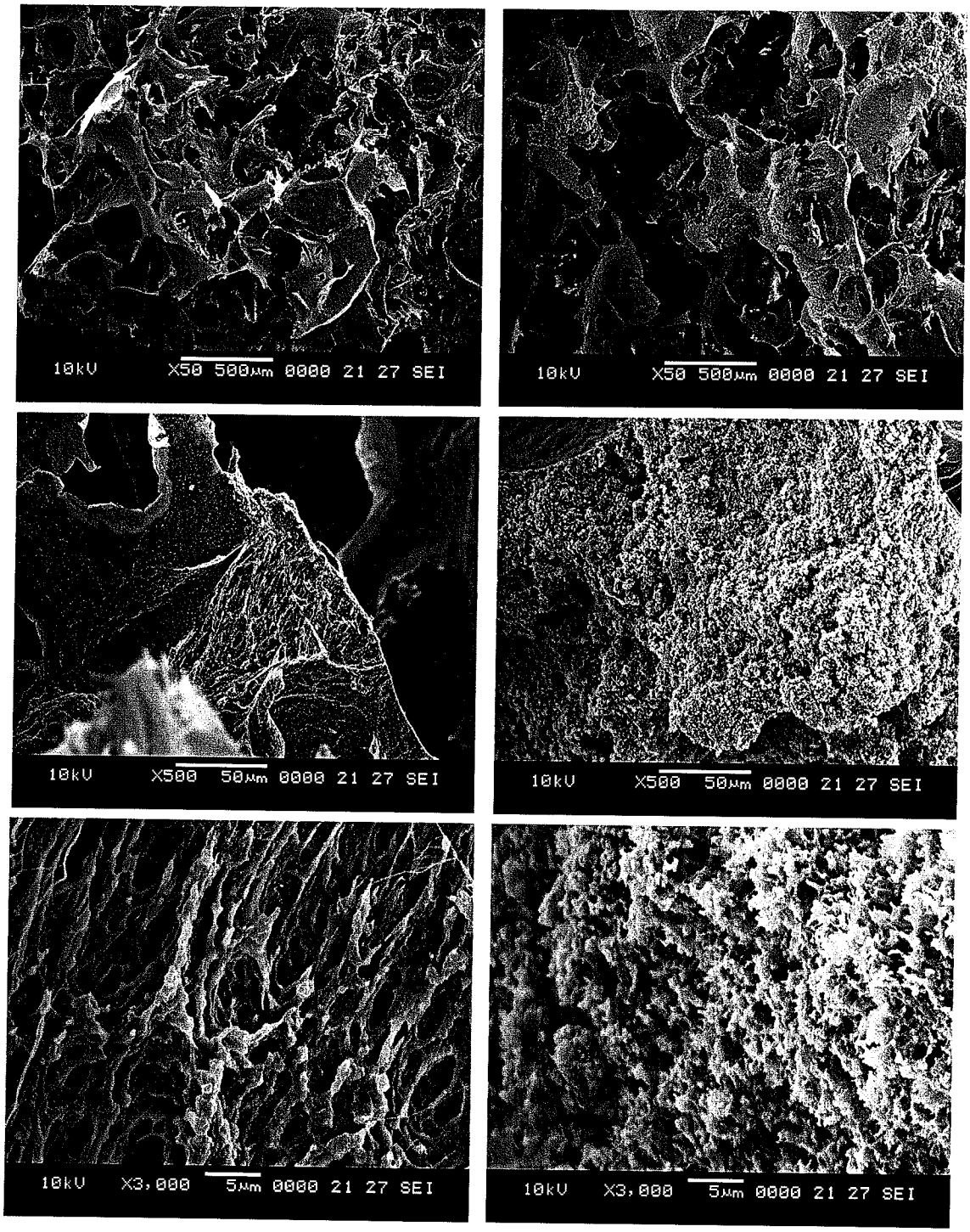


Figure 37. SEM of canola protein-guar gum gels. A: high G′, low tan δ (pH 10, 1.5% (w/v) guar gum, 20% (w/v) CPI, 0.05 M NaCl); B: low G′, high tan δ (pH 6, 1% (w/v) guar gum, 20% (w/v) canola protein, 0.05 M NaCl). (X50, X500, X3000 micrographs).

improved gel properties of CPI at high pH was also confirmed by the findings of Léger and Arntfield (1993). In contrast, the gel system prepared under non-optimal conditions is an amorphous mass seen at X500 with small aggregates (Fig. 37B). The amorphous structure appeared fluffy and spongy at X3000. The rheological data for CPI-guar gum gel prepared at the established optimum conditions (Fig. 37A) showed a strong and elastic network (G'=56440 Pa, tan δ=0.18), whereas the rheological data for CPI-guar gum gel prepared at non-optimal conditions (Fig. 37B) were less elastic and not as strong (G'=18570 Pa, tan δ=0.26). This trend was supported by the SEM images (Figs. 37A and B).

Effect of urea and DTT on the structure of CPI-guar gum networks

In an effort to assess the molecular forces that contribute to network formation of CPI-guar gum mixtures, denaturants such as urea and DTT were added to the protein-hydrocolloid dispersions prior to thermal treatment. The SEM images shown in Figure 38 illustrate the effect of denaturants (urea, DTT) on the structure of CPI-guar gum gels. The gel systems treated with urea are shown in Figure 38A whereas those treated with DTT are shown in Figure 38B. The network structures shown in Figures 38A and B which reflects the effects of the two denaturants are quite different.

Influence of urea: The gel system treated with urea (Fig. 38A) displays some structural crosslink which looks somewhat disrupted and squashed at X500 and X3000, indicating a disorganization of the gel structure. The X3000 micrograph reveals a markedly distortion of the cellular structures with threadlike strands forming the network. Comparing the microstructural data shown in Figure 37A (properly

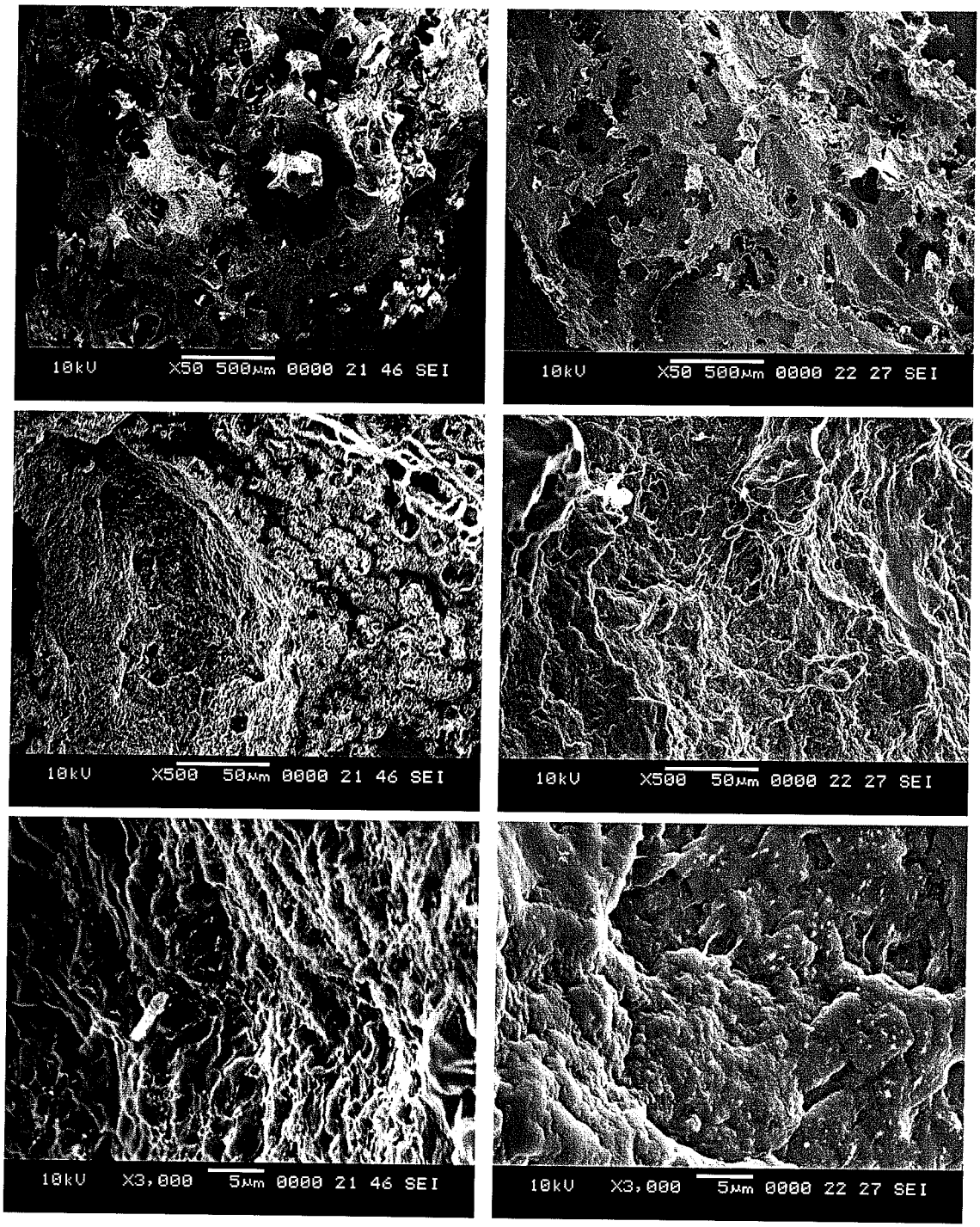


Figure 38. SEM of canola protein-guar gum gels (pH 10, 1.5% (w/v) guar gum, 20% (w/v) canola protein, 6 M urea or 0.15 M DTT). A: Urea-treated gels; B: DTT-treated gels. (X50, X500, X3000 micrographs).

crosslinked elastic network) to that shown in Figure 38A (somewhat distorted and squashed structure) further clarifies the difference in structural arrangement of the gel systems due to treatment with urea. It was observed that CPI-guar gum dispersions treated with urea formed a gel at room temperature (23-25°C) prior to heat treatment. The immediate formation of a gel on the addition of 6 M urea indicates significant changes in molecular structure and conformation. As noted by (Xiong and Kinsella, 1990c), the spontaneous formation of gels formed from whey protein isolate in 6 M urea results from protein-protein crosslinkages via oxidation of thiol groups and thiol-disulfide interchange reactions. The observable disruption of the properly crosslinked network shown in Figure 38A was very apparent in Figure 38A; this implicates noncovalent forces (e.g. hydrophobic interactions and hydrogen bonds) as factors in the network formation of CPI-guar gum systems. Further, the fact that there were some evidence of alignment of strands in the SEM images for urea-treated CPI-guar gum mixture shows that disulfide bonds contributes to the network formation of this system. The rheological data for the urea-treated CPI-guar gum (G'=1396 Pa; tan δ=0.19) and CPI-guar gum without urea (G'=56440 Pa; tan δ=0.18) systems further confirms the observations displayed in the micrographs and supports the involvement of noncovalent and disulfide interactions in network formation of the mixed gels.

Effects of DTT: The CPI-guar gum system treated with DTT (Fig. 38B) had an amorphous and rubbery structure which was quite evident at x3000. Figure 38B shows less network formation, suggesting the destruction of disulfide linkges. The structure seen in Figure 38B is similar to that seen in Figure 36B (which reveals the network arrangement of DTT-treated CPI-κ-CAR gels). The rheological data for the

DTT-treated CPI-guar gum (G′=8213 Pa; tan δ=0.16) and CPI-guar gum without DTT (G′=56440 Pa; tan δ=0.18) differ significantly, showing a much higher value for the gel without DTT. Reports on the effect of DTT on protein gelation have been variable with some findings indicating enhanced gel network (Léger and Arntfield, 1993; Schmidt et al., 1979) while some indicated poor network formation (Xiong and Kinsella, 1990b; Schmidt, 1981). The result of this study indicates the formation of fewer crosslinks (G′=8213 Pa) by the DTT-treated CPI-guar gum gel. Our result on DTT-treated CPI-guar gum gel network suggests that covalent and noncovalent forces are involved in the gel formation of this system.

CHAPTER 6

Molecular Interactions Involved in Network Formation of Commercial Canola Protein-Hydrocolloid Mixtures

Abstract

Biopolymer mixtures contribute to the formation of gel networks in food systems. Effective utilization of these networks depends on the understanding of the type and role of interactions involved in network formation. There is paucity of information on how molecular interactions are related to the network formation of food systems that contain commercial canola proteins. This study examined the gelling behaviour of canola protein isolate-hydrocolloid mixtures and the molecular forces that stabilize and strengthen network structure. The effects of sodium salts (sulfate, acetate, chloride, thiocyanate), denaturants (urea and dithiothreitol) and pH on network formation of canola protein isolate (CPI), κ-carrageenan (κ-CAR), guar gum and CPI-hydrocolloid mixtures were examined by dynamic rheological testing. The polypeptide composition of CPI was assessed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The data from the SDS-electrophoresis showed that CPI has disulfide bonds in its native structure. Treating CPI-κ-CAR mixture with sodium salts affected the rheological (G′, tan δ) properties of the gel system. Enhanced CPI-κ-CAR gel properties followed the lyotropic series; for increased G′ values: $SO_4^{2-} > Cl^- > C_2H_3O_2^- > SCN^-$, whereas for decreased tan δ values: $SCN^- > Cl^- > C_2H_3O_2^- > SO_4^{2-}$. Urea-treated CPI-κ-CAR gels had firm and elastic networks, indicating that CPI has sufficient disulfide linkages to support a gel network. The DTT-treated CPI-κ-CAR gel had improved elastic character (low tan δ values), suggesting that the gel structure was properly

formed. Results support the involvement of disulfide bonds and noncovalent interactions (e.g. hydrogen bonds, hydrophobic interactions) in the network formation of CPI-κ-CAR gels. The rheological data of CPI-guar gum gels showed that the gel properties were not improved in the presence of guar gum, indicating that guar gum interfered with CPI gelation. Thus, the interactions involved in CPI-guar gum gelation may be comparable to those involved in CPI alone. An understanding of the factors that enhance the physical and textural attributes of CPI in food systems will add value to industrial canola meal.

Introduction

Protein-polysaccharide interactions give a realistic indication of the behaviour of proteins in multicomponent food systems and can provide useful information for improving protein functionality. Canola meal, predominantly used as an animal feed ingredient, is a potential source of protein for use in food systems. The ability of canola protein to improve food quality (e.g. textural property) will determine its successful utilization in food systems. An understanding of the molecular basis for macromolecular interactions that contribute to food quality is important for successful development of functional canola protein products that can be incorporated into foods.

Léger and Arntfield (1993) reported that hydrophobic and electrostatics interactions were responsible for the establishment of canola protein gel networks, whereas gel stabilization and strengthening were attributed to disulfide linkages, electrostatic interactions and hydrogen bonding. The report by Gill and Tung (1978) indicated that gel formation in the rapeseed protein systems is a complex phenomenon which may involve covalent, ionic, disulfide, hydrophobic and hydrogen bonding. In an

effort to determine the interactions responsible for canola protein-hydrocolloid (κ-CAR, guar gum) network formation, the environment in which the gelation took place was selectively altered to identify the role of individual molecular forces. Since no single environment can affect a specific molecular force, a composite of data was used to understand the gelling mechanism.

The addition of sodium salts to canola protein-hydrocolloid dispersions can be an effective probe for evaluating the contribution of hydrophobic interactions to the network formation process. Neutral salts such as sodium sulfate (Na_2SO_4), sodium acetate ($NaC_2H_3O_2$), sodium chloride (NaCl), and sodium thiocyanate (NaSCN) affect protein-protein and protein-polysaccharides interactions; either by ionic strength effects, binding to the protein charged groups, or at high concentrations by altering water structure with subsequent changes in hydrophobic effects (Damodaran and Kinsella, 1982). Further, a conventional method for studying disulfide bonds interactions is the inclusion of reagents that modify or prevent the formation of disulfide bonds prior to heat treatment; these reagents include dithiothreitol (DTT) cysteine hydrochloride (CysHCl) and 2-mercaptoethanol (ME). In this study, the effects of denaturing agents (e.g. urea, DTT) have been used to determine forces that impact gel formation in canola and canola-hydrocolloid systems.

Materials and Methods

Source of materials

Food grade κ-carrageenan (κ-CAR; No. C-1013) that contains predominantly κ- and lesser amounts of λ-carrageenan, was purchased from Sigma Chemical Co. (St. Louis,

MO, USA). The composition of κ-CAR powder as indicated by the manufacturer showed it contained a mixture of the following cations: K^+ (10.4%), Ca^{2+} (2.3%) and Na^+ (0.9%). Commercial (BMW Canola, Winnipeg, Canada) canola protein isolate (CPI) was purchased and used without further purification. Proximate analysis (AOAC, 1990) 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), with a viscosity of 6000-10000 mPas (Whistler and BeMiller, 1997), urea (U-15; Lot No. 863571) and DTT (D-0632; Lot No. 61K16571) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). $NaC_2H_3O_2$ (Lot No. 7364 KCLZ) was procured from Mallinckrodt Inc. (Paris, Kentucky, USA). All other chemicals such as NaCl (BP358-212; Lot No. 028091), HCl (A144-225; Lot No. 296220), NaOH (BP359-212; Lot No. 974661), NaSCN (S441-500; Lot No. 987676) and Na_2SO_4 (S421-500; Lot No. 985711) were certified reagent grade (Fisher Scientific Co., NJ, USA).

Experimental design

All experimental parameter measurements were done in duplicate. Design-Expert® Software (Stat-Ease Inc., MN) was used to generate the model experimental design for factorial and response surface optimization. Appropriate combinations of factors (CPI, κ-CAR, guar gum, NaCl concentrations as well as pH) and optimum conditions for gelation of CPI-κ-CAR mixtures (pH 6, 0.05 M NaCl, 15% (w/v) CPI, 3% (w/v) κ-CAR) were established as previously described (Chapter 3). The procedure described in Chapter 4 was used to generate the optimum gelling conditions for CPI-guar gum

mixtures: pH 10, 0.05 M NaCl, 20% (w/v) CPI, 1.5% (w/v) guar gum. For interaction studies, neutral salts such as sodium sulfate (Na_2SO_4), sodium acetate ($NaC_2H_3O_2$), sodium chloride (NaCl) and sodium thiocyanate (NaSCN) at varied concentrations (0.05, 0.5 M) and denaturants (0.15 M DTT, 6 M urea) were used (at the established optimum conditions stated above) for the individual CPI, guar gum and κ-CAR dispersions. Control runs with dispersions of individual CPI (20%, w/v), κ-CAR (3%, w/v), guar gum (1.5%, w/v), CPI-κ-CAR and CPI-guar gum without sodium salts or denaturants at optimum conditions (stated above) were carried out.

Sample preparation

Dispersions of individual CPI (15%, w/v protein-basis; 0.05 M NaCl; pH 6 and 10), κ-CAR (3%, w/v; pH 6; 0.05 M NaCl) and guar gum (1.5%, w/v; 0.05 M NaCl; pH 10) were prepared. Similarly, dispersions of CPI-κ-CAR (15%, w/v; pH 6; 0.05 M NaCl; 3%, w/v κ-CAR) and CPI-guar gum (20%, w/v; pH 10; 0.05 M NaCl; 1.5%, w/v guar gum) mixtures were prepared. The mixture was stirred for approximately 1 h at room temperature or until a complete dispersion of the mixture was achieved. Dispersions of individual CPI, κ-CAR and guar gum samples in appropriate solutions were prepared in similar manner to serve as controls. The use of dispersions allows monitoring of network formation from the onset of conformational changes. As reported by Arntfield (1989), it was more advantageous to heat-set the protein in the rheometer and follow structure development during heating and cooling. In this way, possible compression problems associated with loading preset gels as observed by Gill and Tung (1978) were avoided. The CPI, κ-CAR, guar gum, pH and NaCl combinations were the optimum conditions

generated using Design-Expert® Software; and the method of establishing optimum conditions have been reported (Chapter 1). For interaction studies, dispersions of CPI-κ-CAR (15% w/v, 3% κ-CAR, pH 6) and CPI-guar gum (20% w/v, 1.5% w/v guar gum, pH 10) mixtures were treated with sodium salts (Na_2SO_4, $NaC_2H_3O_2$, NaCl, NaSCN) at varied concentrations (0.05 M, 0.5 M) and denaturants (0.15 M DTT or 6 M urea) prior to heat treatment. Samples were adjusted to appropriate pH values with 1 M NaOH or 1 M HCl. To ensure that pH was maintained, samples were allowed to equilibrate for 30 min at room temperature and the pH was rechecked prior to further testing. The 30 min time limit was adequate since it was observed that no further change in pH occurred after 15 min. Samples treated with urea formed gels at room temperature prior to heat treatment.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out as described by Aluko and McIntosh (2001) with some modifications. Gel electrophoresis of reduced and non-reduced canola protein isolate (CPI) were run on 3% stacking and 12% separating gels using the Hoefer Separation and Control Unit (Hoefer Scientific Instruments-SE600, California) according to the manufacturer's instructions. Samples were prepared for non-reduced SDS-PAGE by mixing 10 mg CPI, 1 mL deionized water and 0.4 mL stock solution (20 mL glycerol, 12.5 mL stacking gel buffer solution (1 M Tris-HCl buffer solution at pH 6.8), 24.1 mL deionized water, 4g SDS and 20 mg pyronin Y). Samples were heated in microcentrifuge tube at 90°C for 3 min, cooled at room temperature for approximately 2 h (or until sample dissolved), then centrifuged at 15,000 rpm for 3 min, and aliquots (4,

8, 12 μL) of the supernatant were loaded onto the gel. Reduced samples were prepared by adding 5% (v/v) 2-mercaptoethanol (ME) to an aliquot of the supernatant from the 4g SDS extraction, and aliquots (4, 8, 12 μL) were loaded onto the gel. Standard proteins (SigmaMarker, M-4038, Lot 122K9283; Sigma Chemical Co., St. Louis) sample was prepared according to manufacturer's instructions and 12 μL was loaded onto the gel. Separation of samples was done on a 16×18 cm gel for 4 h at 40 mA.

Rheology: Assessment of gel properties

Dynamic rheological testing on an advanced rheometer 2000 (AR2000, TA Instruments, New Castle, DE, USA) was used in stages one and two to monitor CPI-hydrocolloid network formation during heating and cooling as well as to characterize the resulting networks. The AR2000 was equipped with 40mm parallel plate geometry and a built-in automated sensitivity. Input strain amplitude for dynamic analysis was 0.02, a value found to be in the linear viscoelastic region in an experiment with canola protein (Arntfied et al., 1990a). This strain was used for all rheological measurements.

Approximately 1 mL canola protein-hydrocolloid dispersion was placed between parallel plates in the rheometer and the gap between the plates was adjusted to 1 mm when the upper plate was lowered. To prevent sample drying during heating, paraffin oil (Mallinckrodt, Paris, Kentucky, USA; Lot No. 6358 KJPC) was placed in the shallow well on top of the upper plate and the edges of the solvent trap were also covered with oil. Samples were heated and cooled over a temperature range of 25° to 95°C at 2°C/min with a 2-min hold time at the final temperature for both the heating and cooling phases. A frequency of 1 Hz was used for the thermal scans. Sample temperature was controlled

by a circulating tap water. Rheological data were collected at 2 min intervals with a thermal equilibrium time of 10 sec. Frequency sweeps of the final product were measured over a range of 0.01-10 Hz at 25°C by using the same strain amplitude and sensitivity as in the thermal scans. The rheological properties, G′ (storage modulus), G″ (loss modulus) and loss tangent or tan delta (tan δ=G″/G′) for representative heating, cooling and frequency sweep curves were recorded. The parameters, G′ and G″ represent the elastic and the viscous components of the gel, respectively; while tan δ reflects the relative energy from the viscous and the elastic components. However, the key gel properties, G′ and tan δ have been reported. Values at 1 Hz were recorded for comparison and statistical analysis. The use of a single frequency (1 Hz) for data comparison has been reported (Arntfield et al., 1989, 1990c).

Statistical analysis

All analyses were conducted in duplicates. Data used in tables and figures were average values. Data were analyzed by analysis of variance (ANOVA) procedure using SAS® 1999-2001 Proprietary Software Release 8.2 (TS20; SAS Institute Inc., Cary, NC, USA); using Duncan's multiple range test to determine statistical differences ($P \leq 0.05$) between treatment means (Steel et al., 1997).

Results and Discussions

Visual assessment

On visual examination, only a single phase was observed at the CPI, κ-CAR and guar gum concentrations used for rheological evaluations. There was no evidence of separate

liquid phases. A single-phase mixture can exist in a system of incompatible polymers and this occurs when the protein-polysaccharide concentration is below the phase separation threshold (Tolstoguzov, 1991). The mixtures were well mixed or agitated prior to rheological measurements. For systems treated with sodium salts: CPI, CPI-κ-CAR and CPI-guar gum dispersions formed light brown, opaque networks; whereas κ-CAR dispersion formed a white, translucent gel. The opaqueness of these gels is due to light scattering caused by the unordered network of insoluble protein aggregates (via hydrophobic interactions), while the slow rate of association (via hydrogen bonding) of the soluble complexes facilitates formation of an ordered translucent gel network (Damodaran, 1996). There was no visual evidence of gel formation when CPI-hydrocolloid, κ-CAR, guar gum and CPI dispersions were treated with sodium salts at room temperature; suggesting that there was no significant conformational change in the presence of these salts at room temperature. For systems treated with denaturants: CPI-hydrocolloid dispersions treated with urea formed a gel at room temperature; indicating significant exposure of functional groups, denaturation, and structural modification of biopolymer molecules. The spontaneous formation of whey protein isolate gels in 6 M urea (disrupts hydrogen bonds) results from protein-protein cross-linkages via sulfhydryl-disulfide linkages (Xiong and Kinsella, 1990a; Phillips et al., 1994). Systems treated with DTT formed opaque, yellow gels. Guar gum (1.5%, w/v) dispersion prepared with 0.05 M NaCl at pH 10 did not form a gel after heat treatment, probably due to complete structural breakdown at this pH.

SDS-PAGE: Polypeptide composition of canola protein isolate

The results of the SDS-PAGE assessment of canola protein isolate in the presence and absence of 2-mercaptoethanol (ME) are shown in Figure 39. CPI samples without ME had polypeptides with molecular weights (MW) ranging from 17 to 116 kDa. There were four major polypeptides in the non-reduced samples with MW of 17 (E), 21 (D), 27 (C) and 45 kDa (B). Similar results were reported by Schwenke et al. (1983) who showed that the 12S globulin from rapeseed contains the following four polypeptide chains differing by their MW in the SDS-electrophoresis: 18500 ± 800, 21100 ± 500, 26800 ± 900, and 31200 ± 1600. Thus the 'C, D and E' bands are tentatively identified as components of the 12S globulin fraction of the canola protein isolate (Fig. 39). Furthermore, the protein profile of canola meals analyzed by non-reducing SDS-PAGE showed that four major polypeptides bands (16, 18, 30 and 53 kDa) were prominent in all samples (Aluko and McIntosh, 2001).

A 52 kDa polypeptide has been shown to be present in the purified 12S preparation from *Brassica napus* seeds (Dalgalarrondo et al., 1986). The presence of 52 kDa polypeptide ('A' band in Fig. 39) in the CPI sample examined further suggests that 12S globulin constitutes part of the proteins extracted by SDS-electrophoresis in the present study. In CPI sample treated with ME, polypeptide bands with MW of 17 kDa (band E), 52 kDa (band A) and above disappeared, indicating the presence of disulfide bonds in the native protein molecules (Fig. 39). A similar finding by Dalgalarrondo et al. (1986) showed that the 52 kDa protein band of rapeseed 12S globulin disappeared when SDS-PAGE was run in the presence of ME. Similar results were reported by Aluko and McIntosh (2001). The protein composition of the samples treated with ME also showed

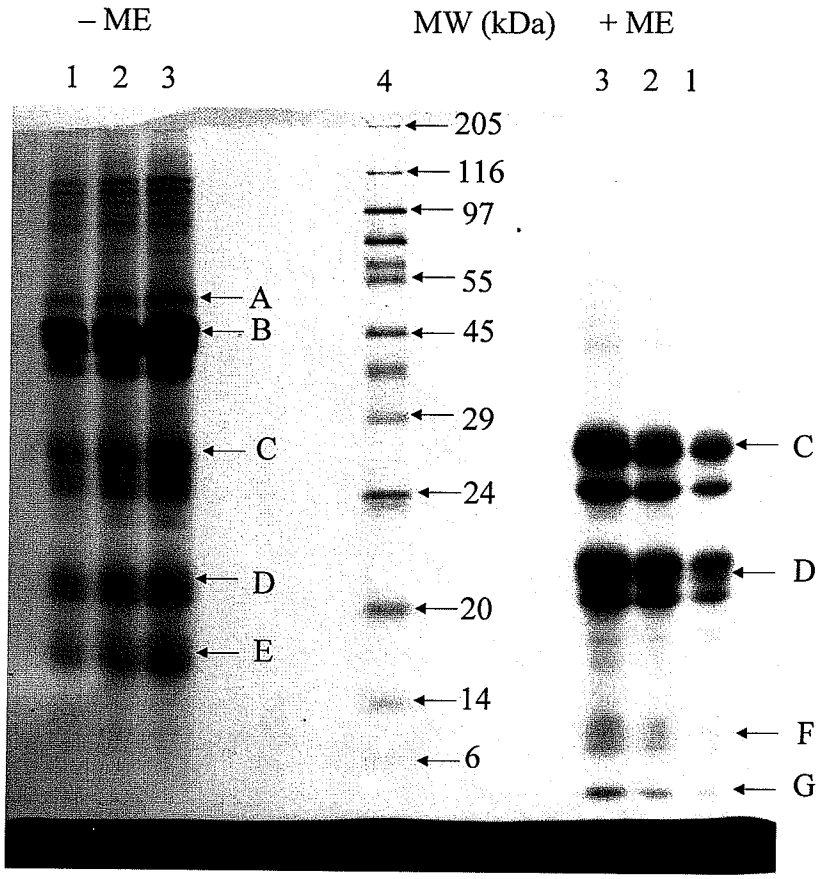


Figure 39. SDS-PAGE compositions of canola protein isolate (CPI) under non-reducing (–ME) and reducing (+ME) conditions. Lanes 1: 4 μl sample each; Lanes 2: 8 μl sample each; Lanes 3: 12 μl sample each; Lane 4: standard proteins. Estimated relative molecular weights (kDa): A ≈52; B ≈45; C ≈27; D ≈21; E ≈17; F ≈12; G ≈3. The gel was stained with Coomassie Brilliant Blue.

two additional polypeptide bands with estimated MW of 3 and 12 kDa (bands G and F, respectively) that were not present under non-reducing conditions (Fig. 39). The cleavage of disulfide bonds (by ME) responsible for subunit association may have resulted in dissociation of the major polypeptides into smaller components. This may account for the two additional polypeptide bands (G and F) obtained in the presence of ME. It is possible that the two additional bands may be associated with any or all the four major polypeptide chains in CPI through disulfide bonding. The presence of other polypeptide bands indicates that commercial CPI is not pure 12S globulin but a mixture of globulins.

Canola protein isolate-κ-carrageenan gels

Influence of sodium salts on gel properties

In an effort to determine the interactions responsible for canola protein-hydrocolloid gel formation, the environment in which the gelation took place was selectively altered to identify the role of individual molecular forces. Since no single environment can affect a specific molecular force, a composite of data was used to understand the gelling mechanism. The addition of sodium salts to canola protein-hydrocolloid dispersions can be an effective probe for evaluating the contribution of hydrophobic interactions to the network formation process. Neutral salts such as sodium sulfate (Na_2SO_4), sodium acetate ($NaC_2H_3O_2$), sodium chloride (NaCl), and sodium thiocyanate (NaSCN) affect the physicochemical properties and interactions between proteins, and proteins and polysaccharides; either by ionic strength effects, binding to the protein charged groups, or at high concentrations by altering water structure with

subsequent changes in hydrophobic effects (Damodaran and Kinsella, 1982).

Heating phase: During the heating regime, the control (no salt) gels produced the greatest increase in G′ values and this trend continued during the cooling cycle. For the salt-treated gels, the SCN^- ion produced the greatest increase in G′ values, followed by Cl^-, SO_4^{-2}, and $C_2H_3O_2^-$ ions, respectively (Fig. 40). The destabilizing effect of SCN^- may be responsible for the increase in G′ values for gels treated with NaSCN; binding of this chaotropic salt exposes the hydrophobic residues, thereby promotes protein-protein interactions that lead to gel formation. Among the salt-treated gels, increased G′ values occurred at high temperature (above 80°C). Given that hydrophobic interactions are favoured at high temperatures, the increase in G′ values during the initial stage of gelation (the latter heating stage) is attributed to hydrophobic interactions. This observation was similar to the findings of Léger and Arntfield (1993).

Cooling cycle: The pattern was different during the cooling phase; the SO_4^{-2} anion had the greatest increase in G′ values, followed by Cl^-, $C_2H_3O_2^-$ and SCN^-, respectively (Fig. 41A). The shapes of the heating and cooling curves were similar, suggesting that all gels underwent the same gelling mechanism. The stabilizing effect of SO_4^{-2} anion may explain the increased gel strength (high G′ values) observed for the gels treated with Na_2SO_4, due to protein-protein and protein-hydrocolloid interactions. The SCN^- ion showed the most reduction in tan δ whereas the SO_4^{-2} anion exhibited the least reduction. Lower tan δ values indicate a more elastic network structure. The reduction in tan δ values followed the lyotropic series: $SCN^- > Cl^- > C_2H_3O_2^- > SO_4^{-2}$. The favourable elastic property (low tan δ values) produced by NaSCN may be due to the binding effect of SCN^- anion, whereas the stabilizing effect of Na_2SO_4 may be responsible for the high

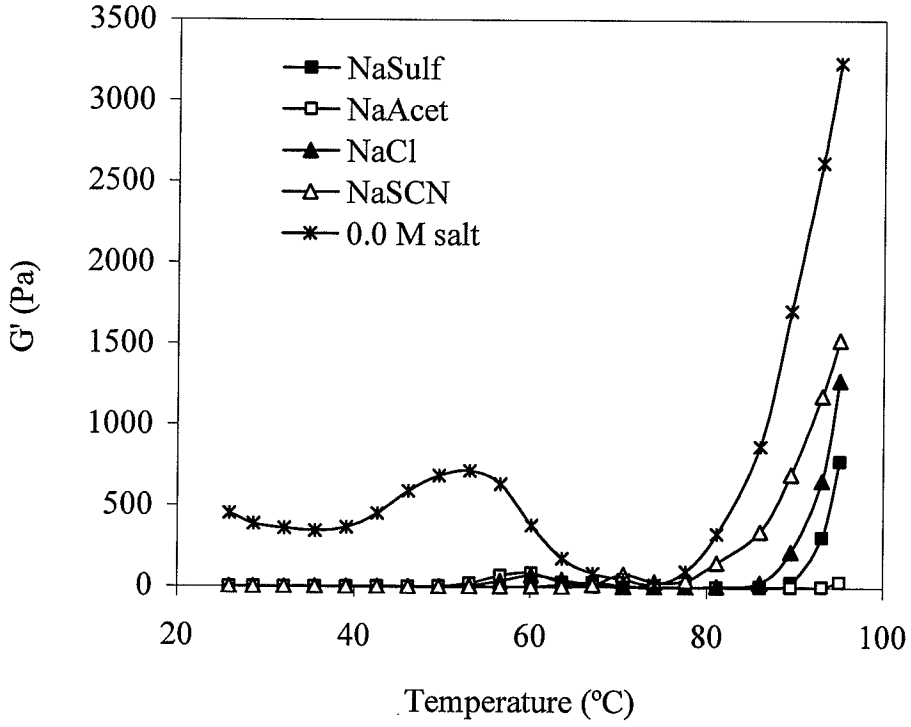


Figure 40. Influence of 0.5 M sodium salts on the storage modulus (G′) of canola protein-κ-carrageenan gels (15% w/v protein, 3% w/v κ-carrageenan, pH 6) as a function of temperature. Curves show changes in G′ during the heating phase.

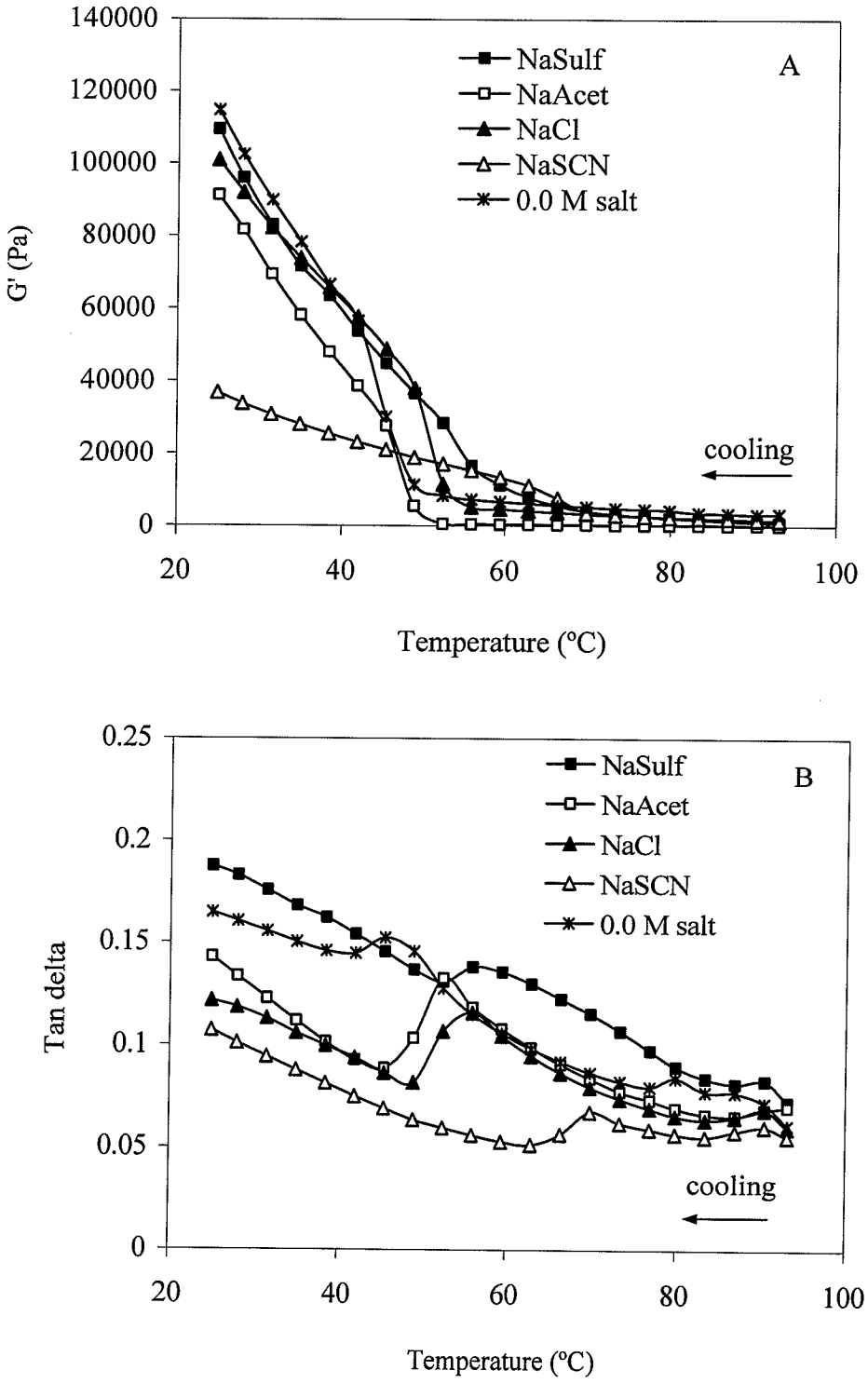


Figure 41. Effect of 0.5 M sodium salts on G′ (A) and tan δ (B) as a function of temperature during cooling of canola protein-κ-carrageenan gels (15% w/v protein, 3% w/v κ-carrageenan, pH 6). Curves show changes in gel properties during the cooling cycle.

tan δ values (less elastic structure) shown by gels treated with SO_4^{2-} anion (due to increased nonpolar aggregation at 0.5 M salt level). The shapes of the cooling curves were similar for all treatments, suggesting that the actual crosslinking pattern is the same among the treatments. In Figure 41B, the tan δ values of all treatments displayed unique cooling patterns. At the onset of the cooling regime the curves showed an upward trend, which reversed downwards and later upwards. Overall, between 50 and 60°C (mid-cooling phase), the cooling curves showed a downward trend, resulting in improved network structure in all treatments. Given that hydrogen bonding is favoured at low temperatures, it is evident that hydrogen bonds are responsible for this stabilization and/or strengthening. The contribution of hydrogen bonds (amongst other molecular forces) to the stabilization and strengthening of canola protein gels has been noted previously (Léger and Arntfield, 1993). However, below 50°C, the gels appear to lose their integrity. The gradual increase in tan δ values during the later cooling phase showed the disintegration of the gel structures, yielding 'gels' with less elastic character. These results suggest that the forces responsible for stabilizing and/or strengthening the gel matrix in the final cooling stage appeared to have been disrupted. Based on the results, it is evident that hydrophobic forces, electrostatic interactions, and hydrogen bonding are involved in CPI-κ-CAR gel formation.

After cooling – effect of salt types: Rheological properties of the final CPI-κ-CAR gels are shown in Table 14. Significant differences ($P \leq 0.01$) in gel characteristics were observed in the salt (Na_2SO_4, $NaC_2H_3O_2$, NaCl, NaSCN) treatments. At 0.05 M salt concentration, the SCN^- anion exhibited the greatest increase in G′ value (G′=182500 Pa) whereas the Cl^- anion showed the smallest increase (G′=95465 Pa). However, this

Table 14. Influence of salt type and concentration on the rheological properties (G′, tan δ) of canola protein-κ-carrageenan (κ-CAR) gels prepared with 15% (w/v) protein and 3% (w/v) κ-CAR at pH 6. Measurements were taken during frequency sweep (after cooling) at 1 Hz.

Salt type	**G′ (Pa)***	
Control (0.0)	123000 ± 4243^{bc1}	123000 ± 4243^{a1}
	0.05 M	**0.5 M**
Na_2SO_4	154000 ± 11314^{ab1}	96980 ± 5685^{ab1}
$NaC_2H_3O_2$	136500 ± 19092^{b1}	87180 ± 4978^{b1}
NaCl	95465 ± 2454^{c1}	107500 ± 3536^{ab1}
NaSCN	182500 ± 19092^{a1}	37685 ± 163^{c2}

Salt type	**Tan δ***	
Control (0.0)	0.16 ± 0.001^{a1}	0.16 ± 0.001^{a1}
	0.05 M	**0.5 M**
Na_2SO_4	0.14 ± 0.005^{b1}	0.18 ± 0.001^{b2}
$NaC_2H_3O_2$	0.16 ± 0.002^{a1}	0.14 ± 0.002^{c2}
NaCl	0.15 ± 0.005^{a1}	0.11 ± 0.006^{d2}
NaSCN	0.16 ± 0.001^{a1}	0.10 ± 0.004^{d2}

*Mean ± SD. For a given parameter (G′ or tan δ), column values with the same letter and row values with the same number are not significantly different ($P<0.02$)

pattern was different in tan δ values of the salt-treated gels at 0.05 M salt level (Table 14) with SO_4^{2-} showing the greatest decrease (improved network structure) in tan δ value. Despite the fact that SCN^- is regarded as a destabilizing anion, it produced high G′ values in this study. Similar result was reported for canola protein isolate where in the presence of 0.1 M NaSCN, high G′ values were observed (Léger and Arntfield, 1993). At low concentrations, ions interact with proteins via non-specific electrostatic interactions. This electrostatic neutralization of protein charges usually stabilizes protein structure (Shih et al., 1992). Although electrostatic interactions are thought to be the main noncovalent force at work at low salt concentration (von Hippel and Schleich, 1969), the rheological data support the possibility of lyotropic effects on protein structure development at the 0.05 M salt level. This may explain the improved network strength (high G′ value) observed for salt-treated gels in our study. For example, the binding of SCN^- to the protein changes the charge profile of the protein (by creating an excess negative charge) and helps it to unfold, exposing formerly buried functional groups and making them available for network crosslinking. This observation indicates that hydrophobic interactions and electrostatic interactions are involved in the network formation of CPI-κ-CAR gels. Similar finding was noted by Léger and Arntfield (1993) in their work with the 12S canola protein.

However, among the salt-treated gels at 0.5 M, the G′ value for gels prepared with NaSCN were significantly different ($P<0.05$) when compared to those treated with Na_2SO_4, $NaC_2H_3O_2$ and NaCl (Table 14). NaSCN-treated gels had the lowest G′ value (G′=37685 Pa). A chaotropic salt such as NaSCN destabilizes the structure of protein. It encourages the transfer of polar residues to water by binding to a protein's surface at this

concentration. This can increase the electrostatic repulsion between protein molecules which does not favour interaction. As a result, the protein destabilizes and becomes more soluble. This electrostatic repulsion may have caused the decreased G′ value (G′=37685 Pa) of the CPI-κ-CAR gels treated with NaSCN. Similarly at 0.5 M salt level, Na_2SO_4-treated gels had the highest tan δ value of 0.18; suggesting increased hydrophobic interactions and gel aggregation.

Influence of salt concentration: Three salt-treated gels (NaSCN; NaCl; $NaC_2H_3O_2$) had lower tan δ values at 0.5 M, indicating a more elastic structure. On the other hand, Na_2SO_4-treated gel had higher tan δ value at 0.5 M. As a nonchaotropic salt, Na_2SO_4 influences the order of water such that exposure of nonpolar protein residues is thermodynamically unfavourable. With increased concentration, there is increased nonpolar aggregation and protein precipitation. The increased protein-protein aggregation may be responsible for the less elastic gel structure. In our study, the degree to which tan δ value was lowered by the anions (at 0.5 M) followed the lyotropic series: $SCN^- = Cl^- > C_2H_3O_2^- > SO_4^{2-}$ (Table 14).

Influence of urea on network properties

Urea is one of the standard denaturing agents employed to study the transition between native and unfolded states of proteins. This denaturing agent induces unfolding of proteins by reducing protein-protein and enhancing protein-solvent interactions (Phillips et al., 1994).

Heating regime: Figure 42 shows the G′ values for CPI-κ-CAR and individual CPI gels treated with 6 M urea, as well all that for the control (CPI-κ-CAR gel without

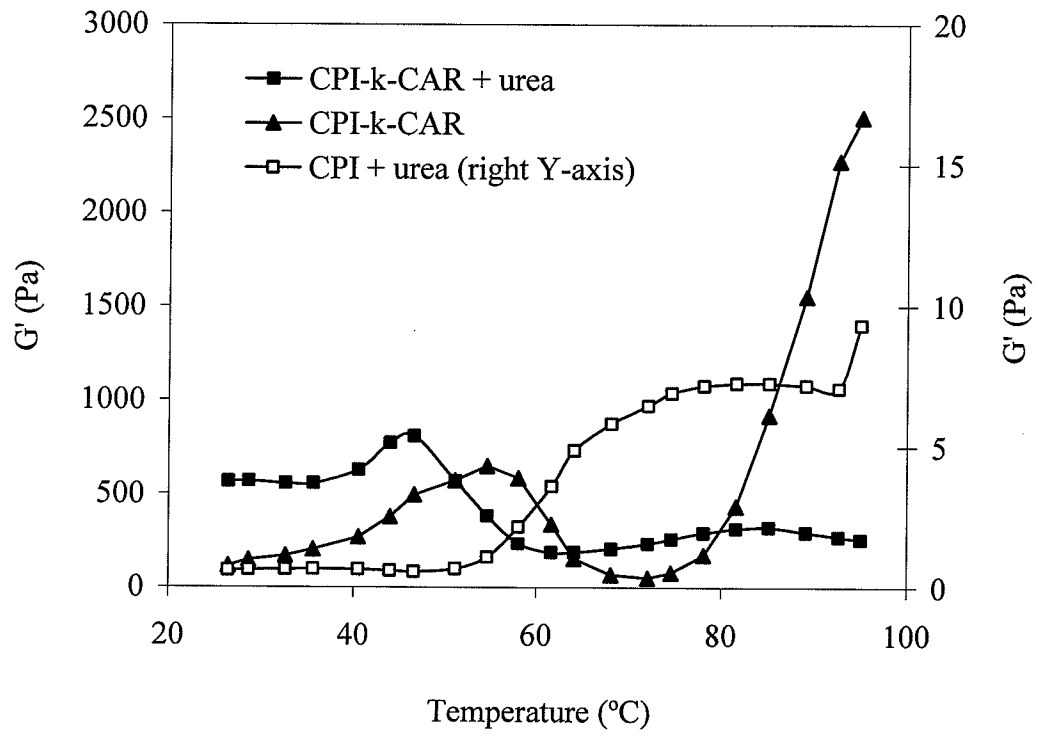


Figure 42. Influence of urea on G′ as a function of temperature for individual canola protein isolate (CPI), κ-carrageenan (κ-CAR) and mixed CPI-κ-CAR gels (15% CPI, 6 M urea, 3% κ-CAR, pH 6). Curves show changes in G′ during the heating stage.

urea). The urea-treated CPI-κ-CAR gel developed structure prior to thermal gelation (a gel was formed at room temperature). Increased G′ value was observed at approximately 48°C. After this temperature, the G′ values decreased markedly; an evidence of conformational changes (Fig. 42). Xiong and Kinsella (1990a, 1990b) reported a spontaneous formation of gels from whey protein isolate in 6 M urea due to protein-protein crosslinkages via oxidation of thiol groups and thiol-disulfide interchange reactions. On the other hand, the individual CPI treated with urea did not form a gel; only a slushy mess was produced. The structure of CPI was completely disrupted (G′=9 Pa, maximum; Fig. 42). In contrast, the control (CPI-κ-CAR without urea) sample

developed structure at about 50-60°C and structure formation increased significantly after 80°C.

Cooling phase: During cooling, the control samples continued to show an increase in network formation and attained a G′ value of approximately 81000 Pa at 25°C (Fig. 43A). The increase in G′ values was pronounced during the final cooling phase (i.e. at 25-45°C). The CPI only (G′=1074 Pa at 25°C) and CPI-κ-CAR (G′=19250 Pa at 25°C) gels showed an increase in network formation during the final cooling stage, however the extent to which structure developed among the urea-treated samples was far much less than that of sample prepared without urea. Comparing the urea-treated and control samples, it could be seen that the G′ values of the control gel were far greater than those of the urea-treated gels. The denaturing effect of urea was excessive, indicating destabilization and complete unfolding of protein (and polysaccharide) molecules. The urea-treated samples appear to plateau by the end of the cooling period, whereas that of the control did not. Further, Figure 43B shows the tan δ values for the urea-treated and control samples (shapes of the curves are different). While the tan δ values for the urea-treated samples were decreasing, that for the control are increasing. The decrease in tan δ values was greatest for the urea-treated CPI-κ-CAR gels (tan δ=0.08 at 25°C), an evidence of improved network structure (very low tan δ implies more elastic structure).

Standard denaturants such as urea and guanidine hydrochloride are hydrogen bond formers (Phillips et al., 1994). As a competitive hydrogen bond former, urea is capable of strong interactions with water, which alter the structure of the aqueous phase around the protein (and polysaccharide) molecule and increase the solubility of

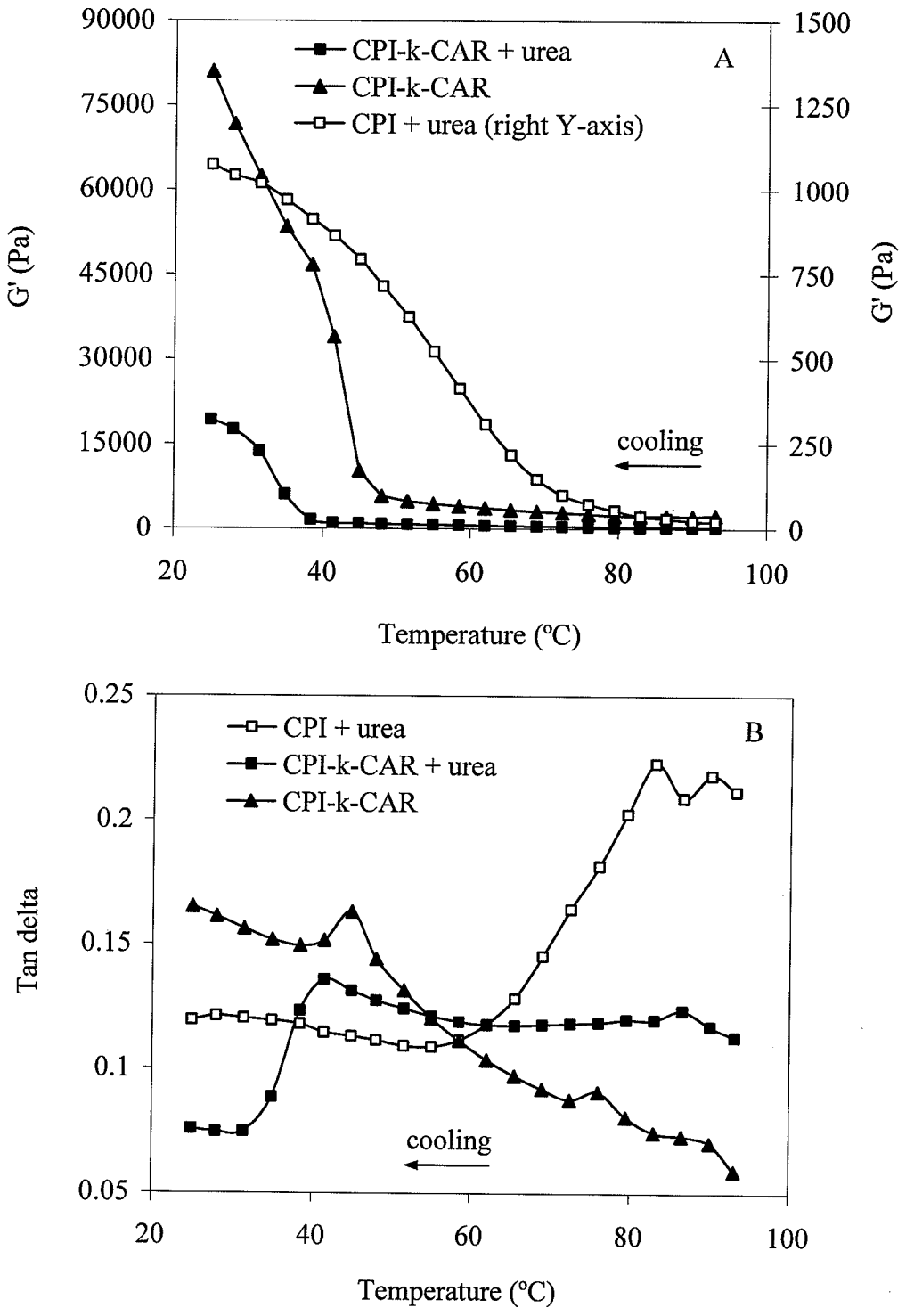


Figure 43. Influence of urea on G′ (A) and tan δ (B) as a function of temperature for individual canola protein isolate (CPI), κ-carrageenan (κ-CAR) and mixed CPI-κ-CAR gels (15% w/v CPI, 6 M urea, 3% w/v κ-CAR, pH 6) during the cooling regime.

hydrophobic amino acids. As a result, urea can disrupt both hydrogen bonds and hydrophobic interactions involved in the maintenance of protein structure. Further, it is possible that disulfide bond formation contributed to the improved network structure (shown by low tan δ values) seen in urea-treated gels (Fig. 43B). This factor can be explained further by the plots shown in Figure 44. The addition of urea into a gelling system is designed to completely unfold the protein and prevent the formation of noncovalent intermolecular interactions (hydrogen bonds, hydrophobic interactions).

This means that any networks formed in the presence of urea would be entirely due to disulfide bonds. As shown in Figure 44, when CPI and CPI-κ-CAR gels are treated with urea, straight lines are obtained for plots of storage modulus (G′) and loss modulus (G″; plot not shown) as a function of oscillatory frequency. The straight line plots demonstrate that the gels are very firm (almost ideal elastic networks); an indication that CPI has sufficient or appropriately located disulfide bonds to support a gel network. These results support the involvement of noncovalent interactions (e.g. hydrogen bonds, hydrophobic interactions) and disulfide bonds in canola protein-κ-CAR network formation.

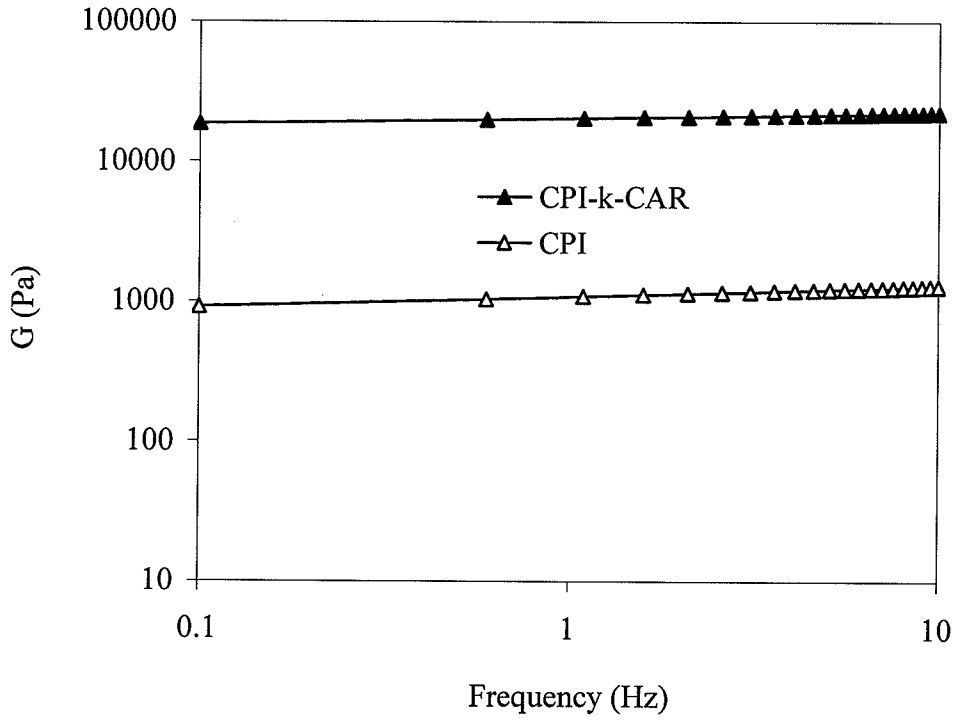


Figure 44. Influence of urea on storage modulus (G′) as a function of temperature for canola protein isolate (CPI) and CPI-κ-carrageenan gels (15% w/v CPI, 6 M urea, pH 6, 3% w/v κ-CAR) during frequency sweep (after cooling).

Influence of DTT on network properties

A common technique for studying disulfide interactions is the inclusion of reagents that modify or prevent the formation of disulfide bonds prior to heating.

Heating phase: The G′ values of DTT-treated and control (CPI-κ-CAR without DTT) gels are shown in Figure 45. The G′ values of the DTT-treated CPI-κ-CAR gel reached a maximum at approximately 85°C and declined at the end of the heating regime; whereas the DTT-treated CPI gel appear to plateau at the end of the heating cycle. This finding corroborates the report of Léger and Arntfield (1993) in their work with 12S canola globulin. As a reducing agent, DTT is capable of disrupting existing

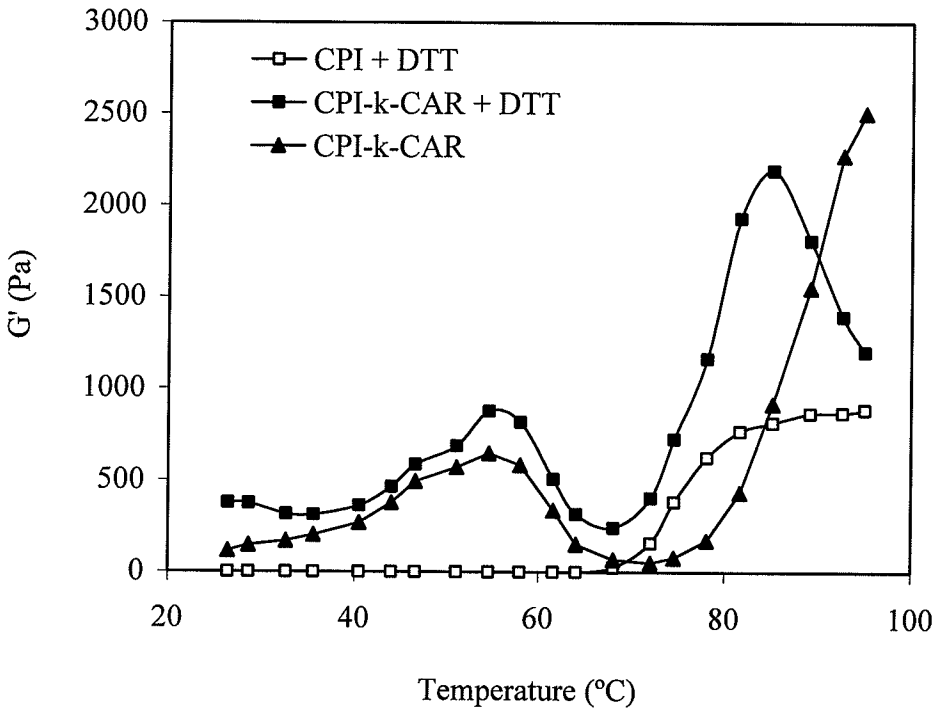


Figure 45. Influence of dithiothreitol (DTT) on storage modulus (G′) as a function of temperature for individual canola protein isolate (CPI), κ-carrageenan (κ-CAR) and CPI-κ-CAR gels (pH 6, 15% w/v CPI, 3% w/v κ-CAR, 0.15 M DTT) during heating.

disulfide crosslinks; causing destabilization and modification of a protein's native conformation (Cheftel et al., 1985). The control and the DTT-treated CPI-κ-CAR gels had similar shape of curve within the temperature range of 25-85°C, suggesting similar gelling mechanism for these two samples within the stated temperature range. Structure development in the DTT-treated individual CPI gel started at about 70°C and increased gradually during the final heating cycle.

Cooling regime: As was observed during the heating phase, the control and DTT-treated CPI-κ-CAR gels had similar shape of curve (Fig. 46A). The G′ values of the DTT-treated CPI-κ-CAR gel are greater than those of the control. It appears that the

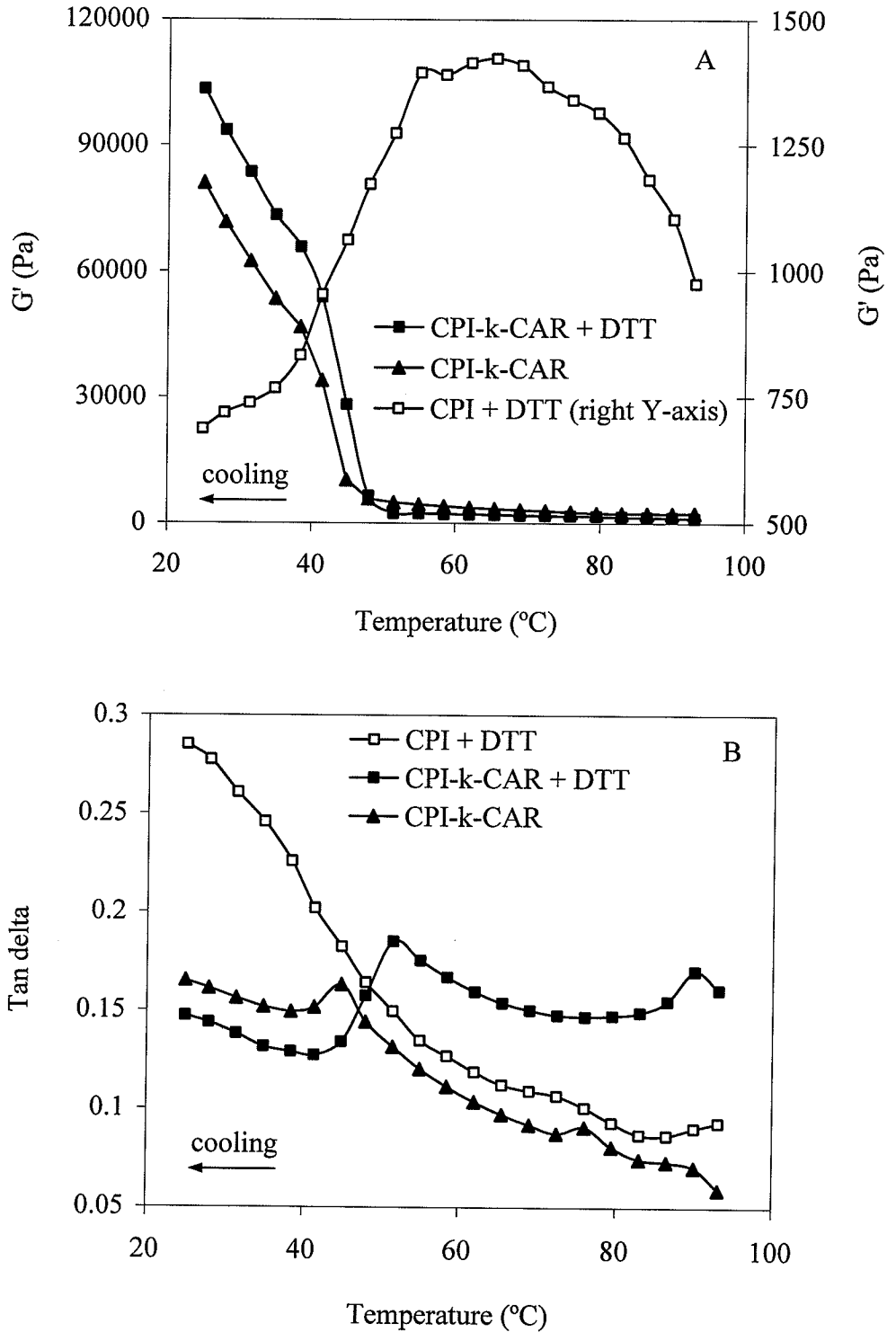


Figure 46. Influence of dithiothreitol (DTT) on G′ (A) and tan δ (B) as a function of temperature for individual canola protein isolate (CPI), κ-carrageenan (κ-CAR) and CPI-κ-CAR gels (pH 6, 15% CPI, 3% κ-CAR, 0.15 M DTT) during cooling.

cleavage of disulfide bonds (by DTT) prior to heating and the resultant thermal disintegration of the protein molecule exposed binding sites that would have remained buried within the molecule (Léger and Arntfield, 1993). As a result, extensive crosslinking and subsequent structure formation are produced by DTT-treated mixed gel. The CPI alone gel treated with DTT had high G′ values during the initial cooling cycle which decreased during the final cooling phase. Figure 46B shows the changes in tan δ values for the three gels. The tan δ values of the DTT-treated CPI-κ-CAR gel decreased during the final cooling phase, suggesting that the gel structure was well established; and the gel had elastic character. It is possible that DTT modified the protein conformation such that the structural integrity of the gel was maintained. Furthermore, the tan δ values of the CPI alone gel increased as cooling progressed. This could be attributed to the destabilization of the protein's native structure and the disruption of the covalent forces (i.e. disulfide bonds) that are involved in stabilizing network structure. Considering the strength of disulfide bonds, it was not surprising that CPI alone gel was weakened by the reduction of these bonds. DTT has been shown to decrease G′ while increasing tan δ value for rapeseed gels, this implicated disulfide bond interactions as a factor in the elasticity of the network (Gill and Tung, 1978).

The formation of networks on treating CPI-κ-CAR mixtures with DTT or urea (Fig. 47) demonstrates that noncovalent forces (e.g. hydrogen bonds, hydrophobic interactions) and disulfide bonds are involved in the gelation of CPI-κ-CAR systems, with hydrophobic interactions and hydrogen bonds initiating the network formation while disulfide bonds contribute to network stabilization. The result obtained from SDS-PAGE analysis (Fig. 39) showed that two of the four major protein bands (17 and 52

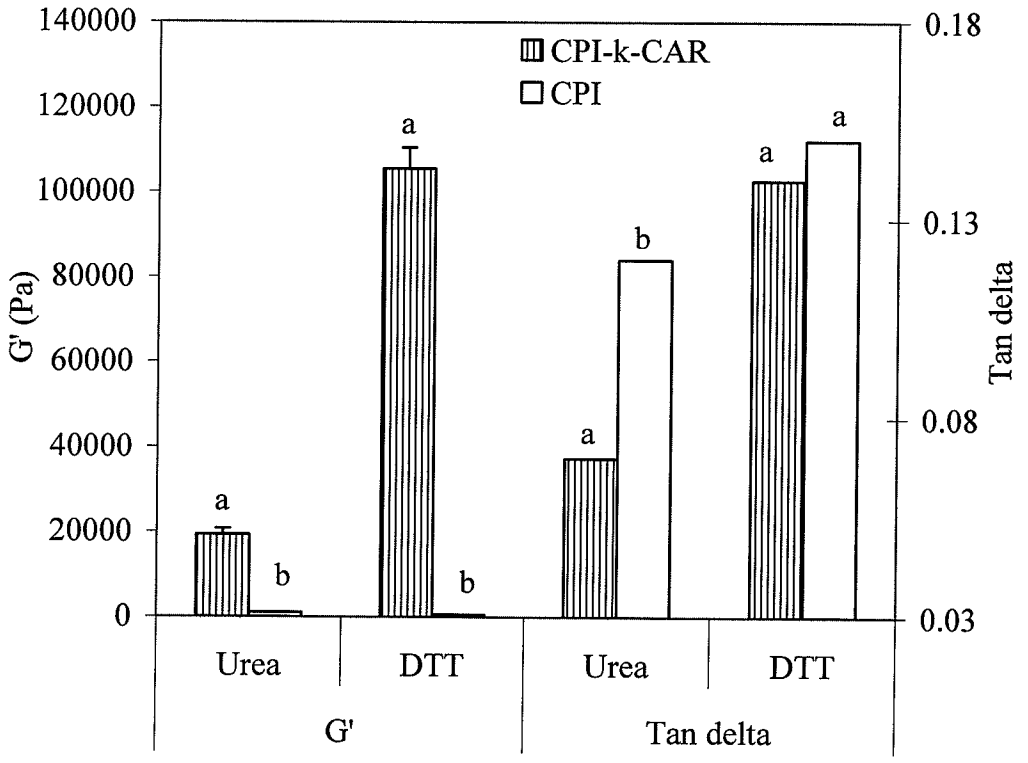


Figure 47. Effect of urea and dithiothreitol (DTT) on the gel properties of individual canola protein isolate (CPI) and CPI-κ-carrageenan (κ-CAR) mixtures (15% (w/v) CPI, 3% (w/v) κ-CAR, 0.15 M DTT or 6 M urea, pH 6). For G′ or tan δ and within treatments, bars with same letter are not significantly different ($P<0.01$). Measurements were taken during frequency sweep (after cooling) at 1 Hz.

kDa) disappeared in the presence of ME, confirming that CPI has sufficient disulfide bonds which can contribute to the gel strength of canola protein in foods of mixed composition.

Canola protein isolate-guar gum gels

The rheological data of CPI-guar gum mixtures showed that the gel properties (G′, tan δ) were not improved in the presence of guar gum, suggesting that guar gum interfered

with the gelation of canola protein isolate (see details in Chapter 4). Based on the results shown in Chapter 4, it is apparent that the interactions involved in network formation of CPI-guar gum systems will not differ from those responsible for the gelling behaviour of CPI system. These interactions have been discussed above.

CHAPTER 7

Emulsifying Characteristics of Commercial Canola Protein-Hydrocolloid Systems

Abstract

The ability to stabilize emulsions is one of the most important functions of food proteins. This study examined the type of interactions between commercial canola protein isolate (CPI) and κ-carrageenan (κ-CAR) or guar gum under varied conditions (salt, pH) in order to establish the optimum conditions for emulsion formation and stability. The effects of hydrocolloids and salts on the emulsifying activity index (EAI) and emulsion stability (ES) of CPI-hydrocolloid-stabilized emulsion were examined by turbidimetric testing at 500 nm, while optimization software was used to identify the optimum conditions (pH, CPI, salt and hydrocolloid concentrations) for emulsification. Emulsions stabilized by CPI-hydrocolloid mixtures were prepared by homogenizing an aqueous dispersion of CPI mixed with κ-CAR or guar gum and canola oil. Results showed that the optimum conditions for CPI-κ-CAR-stabilized emulsions were pH 6, 0.25 M NaCl, 1% (w/v) κ-CAR, and 10% (w/v) CPI; whereas those for CPI-guar gum systems were pH 10, 0.25 M NaCl, 3% (w/v) guar gum, and 10% (w/v) CPI. Salt concentration significantly ($P<0.05$) affected emulsifying properties; higher EAI values resulted at intermediate salt level (up to 0.25 M) but not at 0.5 M level. At a level of 0.25 M salt, increases in EAI of CPI-κ-CAR emulsions followed the series: chloride = sulfate > acetate > thiocyanate. The emulsifying properties of CPI-κ-CAR mixtures were improved by electrostatic complexing; while improvements in CPI-guar gum systems were probably due to the incompatibility of the biopolymers. CPI-hydrocolloid complexes can serve as surface-active ingredient in multicomponent food systems.

Introduction

Proteins constitute an important group of emulsifying agents used in foods; they stabilize the oil droplets against coalescence as well as reduce the oil-water interfacial tension, which facilitates formation of emulsions. The requirements for a protein to exhibit satisfactory emulsifying properties include ability of the molecules to adsorb rapidly at the newly created oil-water interfaces during the process of emulsification, to undergo structural change and rearrangement at the interface, followed by the formation of a cohesive film with viscoelastic properties due to intermolecular interactions (Damodaran, 1989; Kinsella, 1982). Thus, the unique appearance and textural characteristics of several food products such as mayonnaise, salad cream, milk and dairy cream is due to oil incorporation in the form of small droplets with the aid of food emulsifiers (Dickinson and Stainsby, 1982).

However, proteins are normally used in emulsions in combination with polysaccharides in order to improve the stability of oil droplets against creaming. Studies have shown that emulsion capacity and stability increased on transition from a protein to its complex with polysaccharide (Gurov et al., 1983; Larichev et al., 1983). The consistency of an emulsion stabilized by protein-anionic polysaccharide can be controlled from a liquid to a solid state. For example, a thixotropic protein-fat emulsion, containing about 20% casein, has been used to produce cooked sausages. The emulsion is produced using conventional sausage-making equipment and allows replacement of 30% of the meat in the production of cooked sausages (Tolstoguzov, 1986). As shown by Tolstoguzov (1991), the addition of an equal weight of pectin to a solution of legumin increases the emulsion stability and also decreases the emulsifying threshold of legumin by a factor of 3, under conditions of incompatibility (pH 7.6) and complexing

(pH 4.2). According to this author, the use of a polysaccharide (dextran, pectin) incompatible with the protein (legumin) intensifies the protein adsorption and decreases the requirements for protein content sufficient for a multilayer adsorption. This is attributed to an altered conformation of the protein at the interface resulting in larger contact between protein and polysaccharide molecules and thereby providing strong layers at low adsorption.

Diftis and Kiosseoglou (2003) reported an improvement in emulsifying properties (oil droplet size reduction, emulsion stabilization against creaming) of soybean protein isolate mixed with sodium carboxymethyl cellulose and heated at 60° C for a period of up to 5 weeks. The authors attributed the improvement to the formation (upon heating) of a covalent protein-polysaccharide conjugate which may enhance repulsive steric forces between the oil droplets as a result of polysaccharide chain overlapping. The use of canola meal (mainly used as an animal feed ingredient) as a protein source in foods will depend on the functional properties of its protein. This study evaluated the ability of canola protein isolate to form and stabilize emulsions when mixed with hydrocolloids.

Materials and Methods

Source of materials

Food grade κ-carrageenan (κ-CAR; No. C-1013) that contains predominantly κ- and lesser amounts of λ-carrageenan, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The composition of κ-CAR powder as indicated by the manufacturer showed it contained a mixture of the following cations: K^+ (10.4%), Ca^{2+} (2.3%) and

Na^+ (0.9%). Commercial (BMW Canola, Winnipeg, Canada) canola protein isolate (CPI) was purchased and used without further purification. Proximate analysis (AOAC, 1990) of the CPI 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), with a viscosity of 6000-10000 mPas (Whistler and BeMiller, 1997), urea (U-15; Lot No. 863571) and DTT (D-0632; Lot No. 61K16571) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). $NaC_2H_3O_2$ (Lot No. 7364 KCLZ) was procured from Mallinckrodt Inc. (Paris, Kentucky, USA). All other chemicals such as NaCl (BP358-212; Lot No. 028091), HCl (A144-225; Lot No. 296220), NaOH (BP359-212; Lot No. 974661), NaSCN (S441-500; Lot No. 987676) and Na_2SO_4 (S421-500; Lot No. 985711) were certified reagent grade (Fisher Scientific Co., NJ, USA).

Experimental design

All experimental parameter measurements were done in duplicate. Design-Expert® Software (Stat-Ease Inc., MN) was used to generate the experimental model for a factorial design and response surface optimization. The experiment was carried out in two phases. In phase one, a series of experiments of different combinations of CPI and hydrocolloids (κ-CAR, guar gum) following a full factorial model were conducted. The design was a 2-level factorial of 4 factors in 20 experiments including four replicates of the center point. The factors included pH (6, 10), CPI (10, 20% w/v), NaCl (0.05, 0.25 M) and hydrocolloid (1, 3% w/v) concentrations. The emulsifying activity (EA) and emulsion stability (ES) were measured. Using these data, model fitting was performed

using the numerical, graphical and point prediction models (Design-Expert® Software) to determine the optimum conditions for maximizing EA and ES. Two-dimensional contour plots were generated from the fitted model. In phase two, experiments were carried out to verify the optimum conditions obtained in phase one. The model generated conditions for CPI-κ-CAR mixtures were pH 6, 0.25 M NaCl, 10% (w/v) CPI, 1% (w/v) κ-CAR; while those for CPI-guar gum systems were pH 10, 3% (w/v) guar gum, 10% (w/v) CPI, 0.25 M NaCl (i.e. verification tests were carried out at these conditions). The effect of sodium salts (Na_2SO_4, $NaC_2H_3O_2$, NaCl, NaSCN; at 0.25 and 0.5 M) and denaturants (0.15 M dithiothreitol, DTT; 6 M urea) on CPI and CPI-hydrocolloid systems were examined (at the established optimum conditions stated above). Control tests of CPI-stabilized emulsions with or without hydrocolloids, sodium salts and denaturants were also carried out.

Statistical analysis

Data used in tables and figures were average values. Data from confirmatory and control tests (phase two) were analyzed by analysis of variance (ANOVA) procedure using SAS® 1999-2001 Proprietary Software Release 8.2 (TS20; SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was used to determine statistical differences ($P \leq 0.05$) between treatment means (Steel et al., 1997).

Sample preparation

For phase one, two concentrations of CPI (10, 20% w/v; protein-basis), κ-CAR (1, 3% w/v) and guar gum (1, 3% w/v) were tested. Dispersions of CPI-κ-CAR and CPI-guar

gum mixtures were prepared by mixing appropriate amounts of CPI and κ-CAR or guar gum powders in appropriate NaCl solutions (0.05, 0.25 M) and at appropriate pH (6, 10). The CPI, κ-CAR, guar gum, pH and NaCl combinations were generated using Design-Expert® Software. Each mixture was stirred for approximately 1 hr at room temperature or until a complete dispersion was achieved. For confirmatory tests (phase two), dispersions of CPI (10% w/v protein-basis, 0.25 M NaCl, pH 6 and 10), CPI-κ-CAR systems (10% w/v, pH 6, 0.25 M NaCl, 1% w/v κ-CAR) and CPI-guar gum mixtures (10% w/v, pH 10, 0.25 M NaCl, 3% w/v guar gum) were prepared. Further, dispersions of CPI with or without salt, hydrocolloid and denaturants in appropriate solutions were prepared in similar manner to serve as control. The impact of neutral salts on CPI-κ-CAR (10% (w/v) CPI, 1% (w/v) κ-CAR, pH 6) and CPI-guar gum (10% (w/v) CPI, 3% (w/v) guar gum, pH 10) emulsions were assessed by treating the mixtures with sodium salts (Na_2SO_4, $NaC_2H_3O_2$, NaCl, NaSCN; at 0.25 and 0.5 M) and denaturants (0.15 M DTT, 6 M urea). Samples were adjusted to appropriate pH values with 1 M NaOH or 1 M HCl. To ensure that pH was maintained, samples were allowed to equilibrate for 30 min at room temperature and the pH was rechecked prior to further testing.

Turbidimetric testing: Measurement of emulsifying properties

Emusifying activity index (EAI) was determined according to a modification of the spectroturbidimetric procedure of Pearce and Kinsella (1978) reported by Dyer-Hurdon and Nnanna (1993). CPI alone and CPI-hydrocolloid dispersions were homogenized at setting #5 for 1.5 min using the Sorvall® Omni-Mixer (Ivan Sorvall Inc., Norwalk) at room temperature (24 ± 1° C). The mixture was prepared by pipetting 16

mL of sample dispersion into a 150 mL glass beaker, followed by addition of 24 mL pure commercial canola oil. This oil-containing mixture was homogenized at setting #5.5 using the Sorvall® Omni-Mixer for 1 min at room temperature to produce an emulsion. A 30 μL aliquot of the emulsion was removed and diluted with 20 mL of 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance (*A*) at 500 nm was measured on a spectrophotometer (Ultraspec 2000; Pharmacia Biotech) using the 0.1% SDS solution as a blank. EAI was calculated as follows:

$$EAI = 2T/\phi C$$

where T = turbidity (2.303*A*) of sample, ϕ = volume fraction of dispersed phase, C = weight of protein per unit volume of aqueous phase before the emulsion is formed.

Emulsion stability (ES) was determined according to the procedure described by Chung and Ferrier (1992) with the following modification. Emulsions prepared as stated above were allowed to stand for 24 h at room temperature. The total height, along with heights of the emulsion layer and the bottom aqueous layer were recorded to the nearest 1 mm. The ES was calculated as the percentage (v/v) emulsion layer remaining. ES was calculated as follows:

$$ES = \text{Height of emulsion layer/Total height of emulsion} \times 100\%$$

Results and Discussions

Table 15 shows the emulsification responses (EAI, ES) and the model equations from the full factorial design for canola protein-κ-carrageenan mixtures at varied conditions.

Table 15 – Interfacial stabilizing properties (EAI, ES) of canola protein-κ-carrageenan mixtures under varied conditions.

pH	NaCl (M)	κ-Carrageenan (%)	Protein (%)	EAI (m^2/g)	ES (%)
6	0.05	1	10	131.4	88
6	0.05	1	20	133.8	75
6	0.05	3	10	89.4	72
6	0.05	3	20	99.4	76
6	0.25	1	10	197.4	95
6	0.25	1	20	200.9	79
6	0.25	3	10	245.5	72
6	0.25	3	20	186.7	82
8	0.15	2	15	156.7	80
8	0.15	2	15	117.5	81
8	0.15	2	15	136.1	81
8	0.15	2	15	114.6	82
10	0.05	1	10	120.6	83
10	0.05	1	20	165.3	91
10	0.05	3	10	99.3	65
10	0.05	3	20	136.5	76
10	0.25	1	10	134.8	100
10	0.25	1	20	125.7	100
10	0.25	3	10	134.4	77
10	0.25	3	20	194.2	76

EAI = 27.11 + 10.6 pH + 950 NaCl + 47.74 κ-CAR + 5.64 CPI – 96.59 pH*NaCl – 8.99 pH*κ-CAR – 0.35 pH*CPI + 142.81 NaCl*κ-CAR – 12.36 NaCl*CPI – 5.72 κ-CAR*CPI + 0.73 pH*κ-CAR*CPI (P=0.008).

ES = 152.56 – 7.06 pH + 108.75 NaCl – 31.94 κ-CAR – 6.33 CPI + 2.44 pH*κ-CAR + 0.72 pH*CPI – 21.25 NaCl*κ-CAR – 3.38 NaCl*CPI + 2.52 κ-CAR*CPI – 0.26 pH *κ-CAR*CPI + 0.63 NaCl*κ-CAR*CPI (P=0.001).

Canola protein isolate-κ-carrageenan systems

Effect of NaCl-κ-carrageenan interaction on emulsifying activity (EAI)

Significant factors and interactions were identified using the ANOVA model (Design-Expert® software). NaCl concentration, interaction between pH and NaCl concentration and interaction between NaCl and κ-CAR concentrations were significant ($P<0.05$, Appendix 12). Thus, contour plots of EAI as a function of NaCl and κ-CAR concentrations at pH 6 and 10 are shown in Figure 48. Higher EAI values resulted at higher NaCl concentrations at pH 6 and 10. However, EAI values were higher at pH 6 (Fig. 48A) than at pH 10 (Fig. 48B). According to Phillips et al. (1994), changing the pH affects the net charge of the adsorbed layer and thus the conformation of protein molecules. This in turn affects protein load and electrostatic interactions at the oil-water interface which controls the film cohesiveness and interfacial rheology.

In the present study, the lower EAI values obtained at pH 10 may be attributed to a progressive decrease in association of CPI molecules because of increased electrostatic repulsion between CPI molecules, Cl^- and κ-CAR (an anionic polysaccharide). It has been reported that protein-protein interactions promote the formation of a thick viscoelastic film at the interface, which contribute to emulsion stability (Damodaran, 1996). At pH values below the protein isoelectric point (IEP), the protein and anionic polysaccharide carry opposite net charges (Tolstoguzov, 1986). The IEP of canola protein isolate is at ≈ pH 7 (Schwenke et al., 1987). Thus CPI carries a net positive charge at pH 6 and κ-CAR has a negative charge. Electrostatic complexing results at pH values below the protein IEP, where the biopolymers carry net opposite charges (Tolstoguzov, 1991). The electrostatic complex formation and enhanced molecular

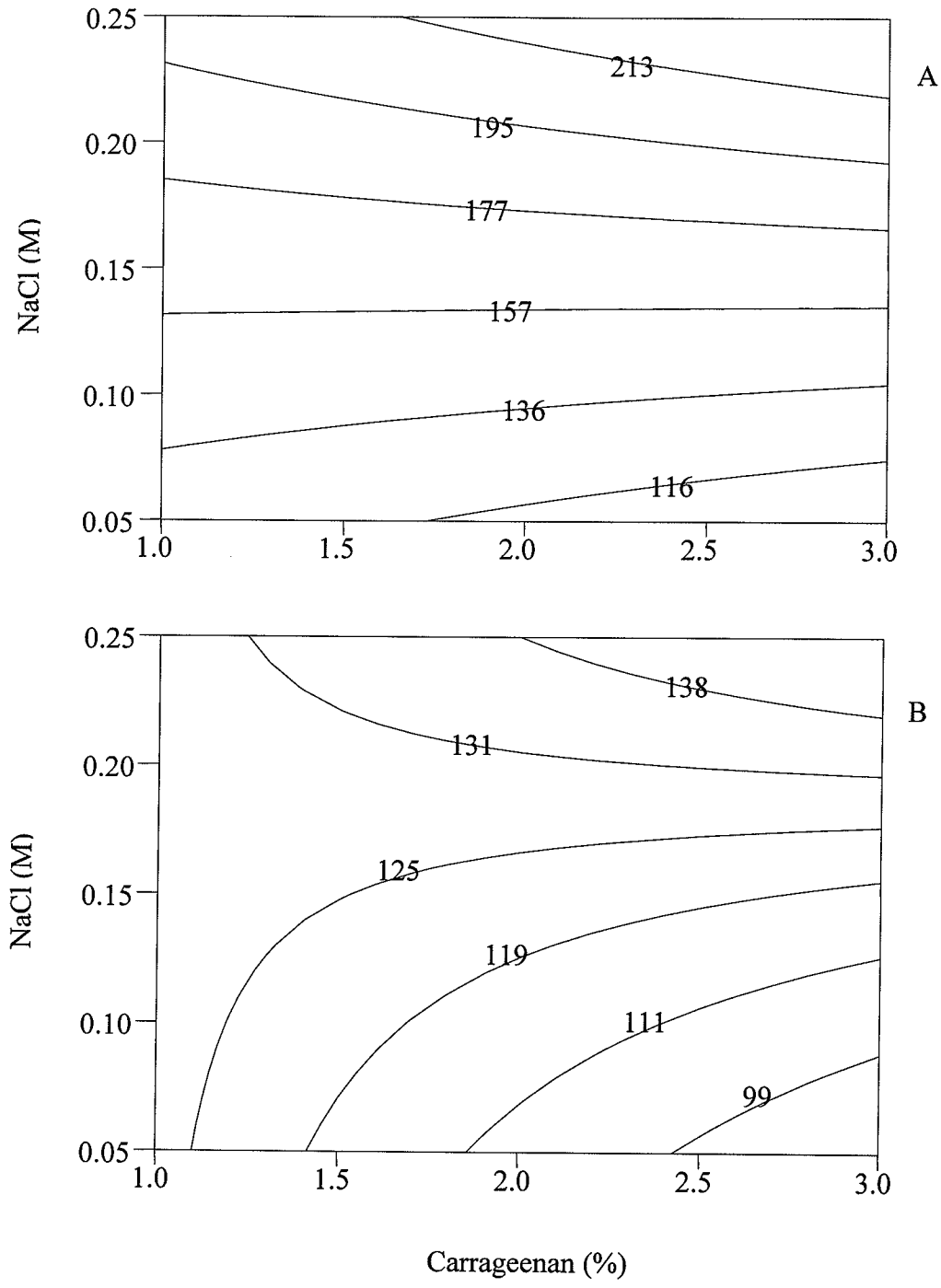


Figure 48. Emulsifying activity index (EAI) contour plot showing the effects of κ-carrageenan (κ-CAR) and salt concentrations on the EAI of CPI-κ-CAR emulsions at 10% (w/v) CPI and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given EAI value. For example, in Figure 48A; at 2.5% (w/v) κ-CAR and 0.1 M NaCl, EAI=136 m^2/g.

interaction at pH 6 may be responsible for the improved emulsifying activity observed in CPI-κ-CAR systems. Scientists studied the effect of κ-CAR on the surface properties of bovine serum albumin (BSA)-stabilized emulsions and demonstrated that κ-CAR forms electrostatic complex with BSA at neutral pH which becomes much stronger at pH 6 (Dickinson and Pawlowsky, 1998). The authors interpreted the system behaviour at pH 6 as bridging flocculation, which formed an emulsion droplet gel network over a certain limited polysaccharide concentration range. In the present study, the higher EAI values observed in CPI-κ-CAR emulsions at pH 6 may be due to bridging flocculation.

Effects of CPI-κ-carrageenan interaction on emulsion stability (ES)

Based on the ANOVA model (Design-Expert® software), the significant ($P<0.05$) factors were NaCl concentration, κ-CAR concentration, interaction between κ-CAR and CPI, and interaction between pH and the biopolymers (Appendix 11). There was also a significant interaction between pH, CPI and κ-CAR and the contour plots of ES as a function of κ-CAR and CPI concentrations at pH 6 and 10 are shown in Figure 49. At pH 6, stable emulsion was achieved at low κ-CAR and low CPI concentrations and the ES values decreased as the concentration of the biopolymers increased. At this pH which is below CPI's IEP, the two macromolecules carry a net opposite charge; thus an attractive electrostatic complex results at pH below the protein IEP (Tolstoguzov, 1991). This interaction may have encouraged aggregation of the biopolymer molecules in the present study, which decreased as biopolymer concentration increased. This may explain the low ES values obtained at high biopolymer concentration.

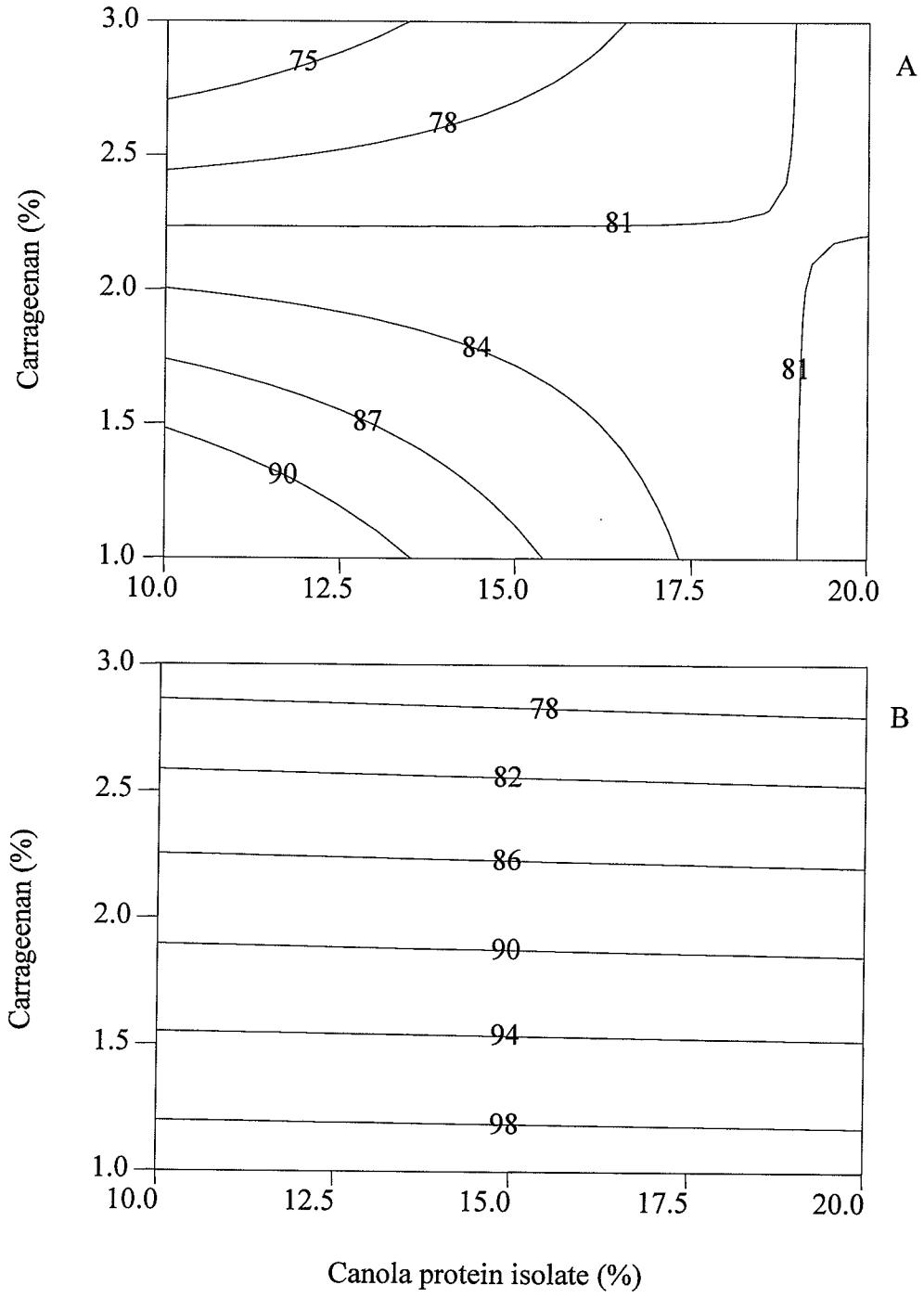


Figure 49. Emulsion stability (ES) contour plot showing the effects of canola protein isolate (CPI) and κ-carrageenan (κ-CAR) concentration on the ES of CPI-κ-CAR emulsions at 0.25 M NaCl and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given ES value. For example, in Figure 49B; at 1.2% (w/v) κ-CAR and any CPI concentration, ES=98%.

As stated by Damodaran (1996), if attractive interactions are too strong, this can lead to interfacial aggregation and coagulation, which will be detrimental to emulsion stability. Also, at pH 10, ES values decreased as κ-CAR level increased and CPI concentration appear not to affect the ES (Fig. 49B). Kappa-CAR and CPI carry a net negative charge at pH 10. This leads to an electrostatic repulsion between the biopolymer molecules, and the repulsion increases as κ-CAR concentration increases. This may explain the low ES values at high κ-CAR level. As reported by Damodaran (1996), if repulsive electrostatic forces are much stronger than attractive interactions, this may prevent formation of a thick, cohesive film. Therefore, a proper balance of attractive, repulsive, and hydration forces is required to form a stable emulsion.

Canola protein isolate-guar gum systems

The emulsification responses (EAI, ES) and the model equations from the full factorial design for canola protein-guar gum mixtures at varied conditions are shown in Table 16.

Table 16 – Surface stabilizing characteristics (EAI, ES) of canola protein-guar gum mixtures under varied conditions.

pH	NaCl (M)	Guar gum (%)	Protein (%)	EAI (m^2/g)	ES (%)
6	0.05	1	10	172.9	86
6	0.05	1	20	171.6	86
6	0.05	3	10	131.1	86
6	0.05	3	20	191.7	83
6	0.25	1	10	174.6	100
6	0.25	1	20	87.3	100
6	0.25	3	10	218.1	100
6	0.25	3	20	107.3	100
8	0.15	2	15	109.6	100
8	0.15	2	15	107.9	100
8	0.15	2	15	110.4	100
8	0.15	2	15	108.6	100
10	0.05	1	10	172.6	83
10	0.05	1	20	331.8	84
10	0.05	3	10	249.0	87
10	0.05	3	20	162.3	85
10	0.25	1	10	176.1	100
10	0.25	1	20	152.3	100
10	0.25	3	10	293.5	100
10	0.25	3	20	187.6	100

EAI = + 423.74 – 46.47 pH + 1200.12 NaCl – 189.42 Ggum – 27.31 CPI – 108.56 pH*NaCl + 27.38 pH*Ggum + 5.09 pH*CPI – 194.62 NaCl*Ggum – 57.45 NaCl*CPI + 14.70 Ggum*CPI + 50.19 pH*NaCl*Ggum – 2.29 pH*Ggum*CPI (P=0.02).

ES = 92.86 – 1.68 pH + 25.88 NaCl – 4.12 Ggum – 0.043 CPI + 5.75 pH*NaCl + 0.70 pH*Ggum + 0.016 pH*CPI + 21.75 NaCl*Ggum + 0.35 NaCl*CPI – 0.087 Ggum* CPI – 2.81 pH*NaCl*Ggum (P<0.0001).

Effect of CPI-guar gum interaction on emulsifying activity index (EAI)

Based on the ANOVA model, the significant ($P<0.05$) factors and interactions were pH, interaction between NaCl and guar gum concentrations, interaction between NaCl and CPI concentrations, interaction between guar gum and CPI concentrations, and a 3-way interaction between pH, guar gum and CPI concentrations (Appendix 13). Thus, the contour plots of EAI as a function of guar gum and CPI concentrations at pH 6 and 10 are shown in Figure 50.

At pH 6, high EAI values resulted at high guar gum concentration and decreased as CPI concentration increased. At this pH value, CPI carries a net positive charge while guar gum is nonionic. As CPI level increases, the net positive charge probably increases and may lead to electrostatic repulsion between the CPI molecules. The repulsion between CPI molecules may be responsible for the lower EAI values observed as CPI concentration increased. Arntfield and Cai (1998) observed an incompatible interaction between canola protein isolate and guar gum. The incompatibility between these two biopolymers may account for the high EAI values obtained as guar gum level increased.

At pH 10, the increase in EAI values with increase in guar gum concentration was more evident. Overall, EAI values were higher at pH 10. At this pH, CPI carries a net negative charge. This probably increased the incompatibility between CPI and guar gum. It is possible that the increased incompatibility at this pH produced emulsions with high EAI values (Fig. 50). Incompatibility has been shown to stabilize legumin-pectin emulsions (Tolstoguzov, 1991).

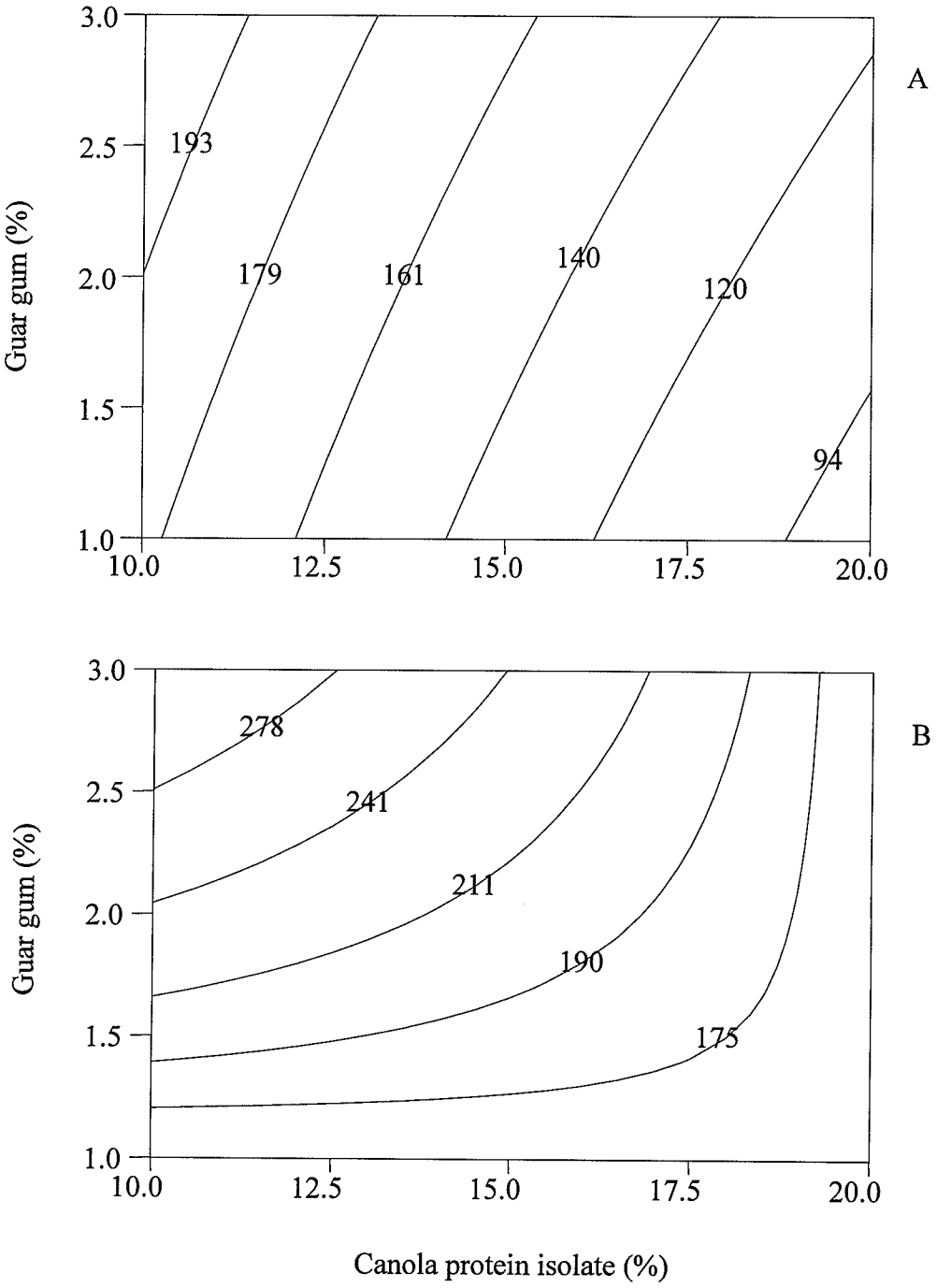


Figure 50. Emulsifying activity index (EAI) contour plot showing the effects of canola protein isolate (CPI) and guar gum concentrations on the EAI of CPI-guar gum emulsions at 0.25 M NaCl and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given EA value. For example, in Figure 50A; at 15% (w/v) CPI and 1.5% (w/v) guar gum, EAI=140 m^2/g.

Influence of NaCl-guar gum interaction on emulsion stability (ES)

Significant factors and interactions were identified using the ANOVA model (Design-Expert® software). NaCl concentration, interaction between pH and guar gum concentration, and a 3-way interaction between pH, NaCl and guar gum concentrations was significant ($P<0.05$, Appendix 14), thus the contour plots of ES as a function of NaCl and guar gum concentrations at pH 6 and 10 are shown in Figure 51. Stable CPI-guar gum emulsions were obtained as NaCl concentration increased, and this was evident at both pH 6 and 10 (Fig. 51). According to Tolstoguzov (1991), higher salt concentrations screen the electrostatic interaction between biopolymer molecules and encourage the self-association of protein molecules. This attractive electrostatic interaction probably enhanced the formation of a stable viscoelastic film which apparently improved emulsion stability. Tolstoguzov (1991) noted that incompatibility is observed when the salt (e.g. NaCl) level is 0.2 M and higher. In the present study, it is possible that incompatibility between the biopolymers may account for the increase in ES values with increasing NaCl concentration (Fig. 51).

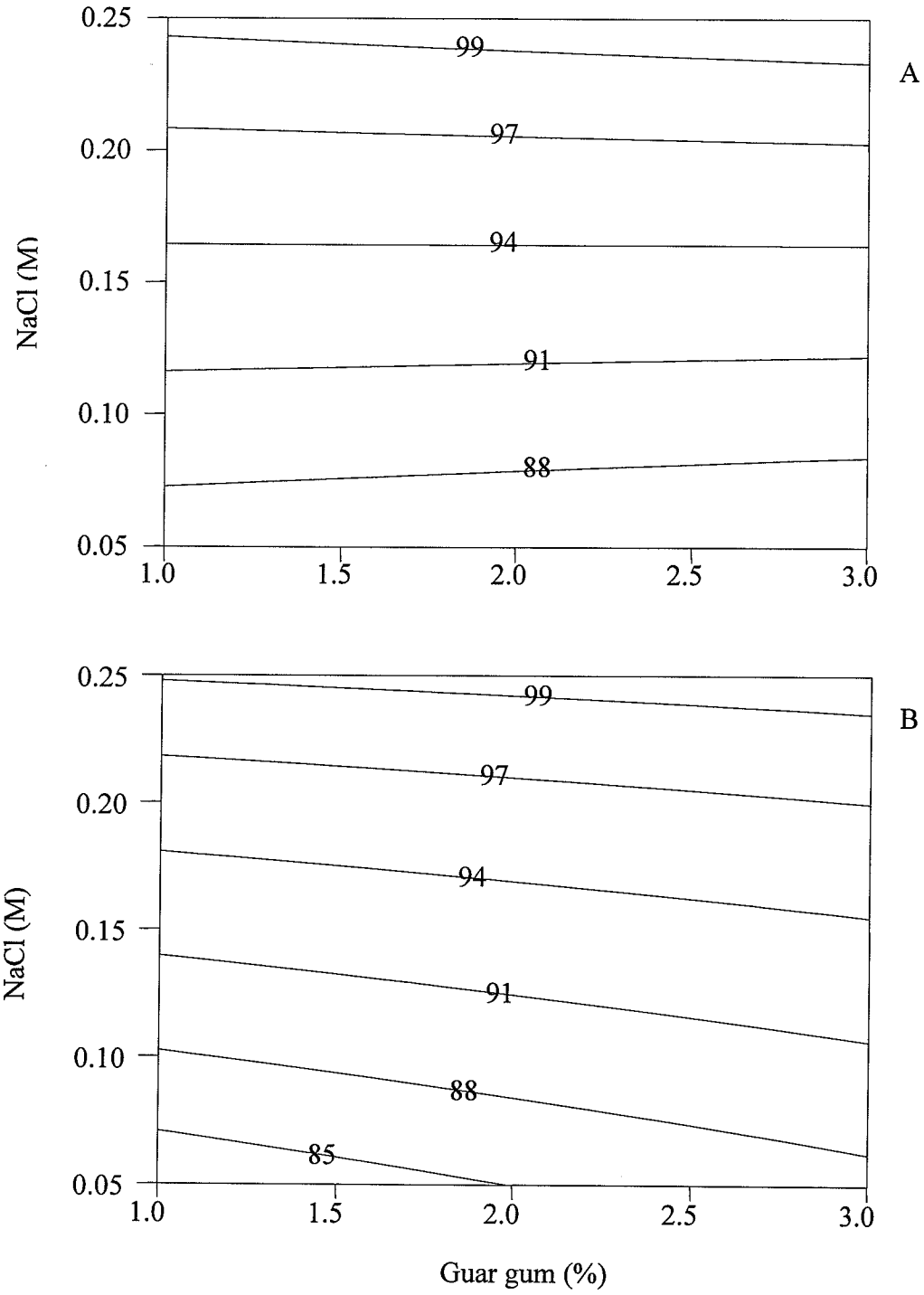


Figure 51. Emulsifying activity index (ES) contour plot showing the effects of NaCl and guar gum concentrations on the ES of CPI-guar gum emulsions at 10% (w/v) CPI and varied pH (A: pH 6; B: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given EA value. For example, in Figure 51B; at 1.5% (w/v) guar gum and 0.1 M NaCl, ES=88%.

Prediction of optimum conditions for CPI-hydrocolloid emulsions

Numerical optimization for CPI-κ-CAR systems: Given that colloids (e.g. food emulsions) are inherently unstable systems, the goal was to obtain CPI-hydrocolloid emulsions with improved interfacial stabilizing properties such as emulsifying activity index (EAI) and emulsion stability (ES). The numerical and graphical features of response surface optimization model (Design-Expert® Software) were used to identify the optimum conditions that would result in emulsions with high EAI and ES values. Numerical and graphical optimizations were completed using the combinations from the full factorial design for CPI-κ-CAR systems. Optimization was based on the criteria set for both factors (CPI concentration, pH, NaCl concentration and κ-CAR concentration) and responses (EAI and ES). EAI and ES are the parameters used to characterize emulsions and these values were used in optimization procedure. Table 17 shows the parameters and criteria set for factors and responses; and summarizes the optimum predicted solutions characterizing improved emulsion formation and stability according to desirability. Based on the solutions outlined in Table 17, the model numerical predictions for CPI-κ-CAR-stabilized emulsions were:

10% (w/v) CPI, 1% (w/v) κ-CAR, 0.25 M NaCl and pH 6

At these conditions, the emulsion was predicted to have EAI and ES values of 203 m^2/g and 97%, respectively (i.e. solution No. 1, Table 17). Using the set parameters at pH 10, the desired criteria could not be met (graph not shown).

Table 17. Predicted optimum combination of factors and responses characterizing improved CPI-κ-CAR emulsions based on set criteria within numerical optimization model.

Constraints

Parameters	Goal	Lower limit	Upper limit
pH	within range	6	10
NaCl concentration (M)	within range	0.05	0.25
κ-CAR concentration (%)	within range	1	3
CPI concentration (%)	within range	10	20
EAI (m^2/g)	maximize	89.4	245.5
ES (%)	maximize	65	100

Solutions

No.	pH	NaCl (M)	κ-CAR (%)	CPI (%)	EAI (m^2/g)	ES (%)	Desirability
1	6	0.25	1.0	10.0	202.8	97	0.80
2	6	0.25	1.0	10.0	201.8	97	0.80
3	6	0.24	1.0	10.0	199.1	97	0.79
4	6	0.24	1.0	10.0	196.4	96	0.78
5	6	0.25	1.0	10.0	203.8	95	0.78

Graphical optimization for CPI-κ-carrageenan systems: To maximize the emulsifying properties (EAI, ES), the lower limits for EAI and ES values for CPI-κ-carrageenan mixtures were set at 160 m^2/g and 80%, respectively (Fig. 52). These criteria limits were sufficient to give a rough idea of which combinations of factors would be used in the confirmatory experiments to produce stable emulsions. Guided by these range of parameters and using the graphical feature of the optimization model (Design-Expert® Software), corresponding response surface overlay contour plot as a function of κ-carrageenan and NaCl concentrations were generated at pH 6.

Figure 52 shows the overlay plot of the response (EAI and ES) surface graphs at pH 6.0 with the regions of optimum conditions shaded. Within the shaded area of the CPI-κ-CAR systems (Fig. 52), any combination of κ-CAR and NaCl concentrations is predicted to give an emulsion with EAI and ES values not less than 160 m^2/g and 80%, respectively. Figure 52 illustrates that an adequate emulsion will be obtained at high NaCl and low κ-CAR concentrations. These criteria were selected to achieve the desired goal which was to maximize the interfacial stabilizing properties, EAI and ES.

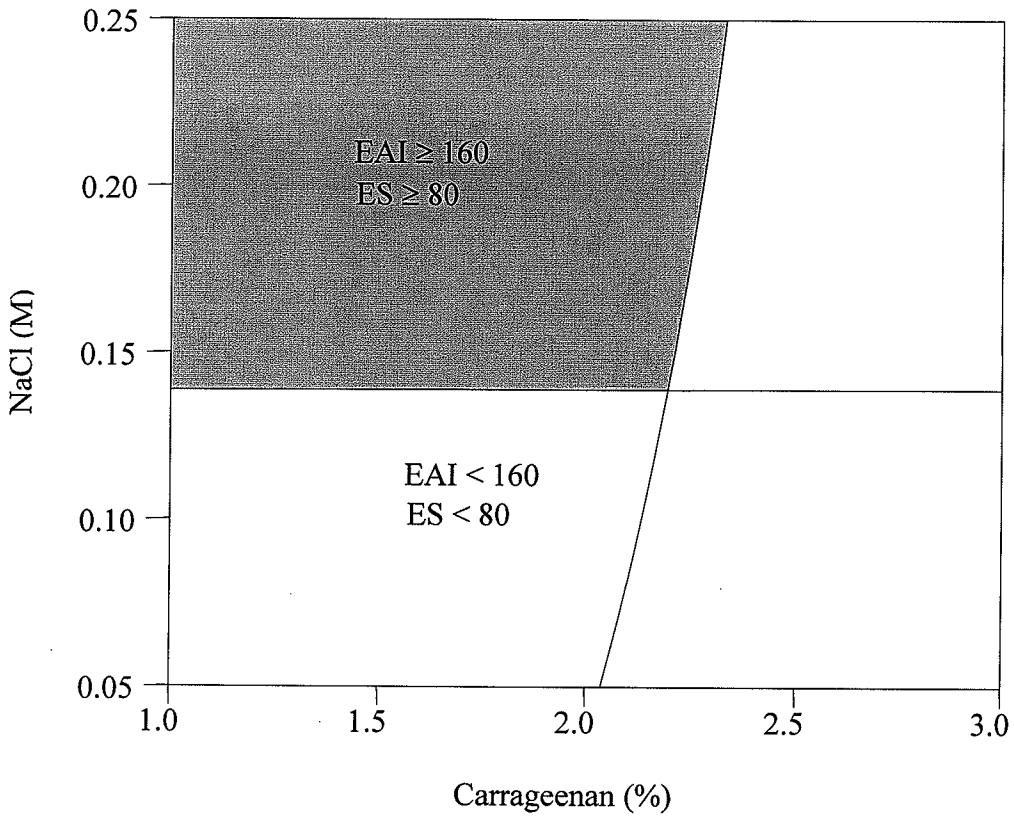


Figure 52. Graphical optimization display of EAI and ES as a function of κ-carrageenan (κ-CAR) and NaCl concentrations at 10% (w/v) CPI and pH 6. The clear areas on the plot do not meet the selected criteria. The lines mark the limits on the responses. Shaded areas indicate where factors can be set to satisfy the requirements on both responses. For example, any point in the shaded region shows the κ-CAR and NaCl combination that will give an emulsion with EAI and ES values not less than 160 m^2/g and 75%, respectively.

Numerical optimization for CPI-guar gum systems: As shown in the CPI-κ-CAR emulsions, Table 18 shows the parameters and criteria set for factors and responses; and summarizes the optimum predicted solutions characterizing improved emulsion formation and stability according to desirability. Based on the solutions outlined in Table 18, the model numerical predictions for CPI-guar gum-stabilized emulsions were:

10% (w/v) CPI, 3% (w/v) guar gum, 0.25 M NaCl and pH 10

Under these conditions, the emulsion was predicted to have EAI and ES values of 317 m^2/g and 100%, respectively (i.e. solution No. 1, Table 18). Within the parameters used for evaluation in this study, the desired criteria could not be met at pH 6 for CPI-guar gum mixtures. Given that alkaline pH values (above pH 8) are rarely encountered in food systems, the model used indicated pH 10 as the optimum pH for CPI-guar gum mixtures. The desired criteria could not be met at pH 6 for CPI-guar gum mixtures.

Table 18. Predicted optimum combination of factors and responses characterizing improved CPI-guar gum emulsions based on set criteria within numerical optimization model.

Constraints

Parameters	Goal	Lower limit	Upper limit
pH	within range	6	10
NaCl concentration (M)	within range	0.05	0.25
Ggum concentration (%)	within range	1	3
CPI concentration (%)	within range	10	20
EAI (m^2/g)	maximize	87.3	331.8
ES (%)	maximize	82.4	100

*Ggum = Guar gum

Solutions

No.	pH	NaCl (M)	Guar gum (%)	CPI (%)	EAI (m^2/g)	ES (%)	Desirability
1	10	0.25	3.0	10.0	317.2	100	0.97
2	10	0.25	3.0	10.0	312.2	100	0.96
3	10	0.25	3.0	10.0	312.8	100	0.96
4	10	0.25	2.9	10.0	311.0	100	0.96
5	10	0.25	3.0	10.6	308.3	100	0.95

Graphical optimization for CPI-guar gum systems: As previously described in CPI-κ-carrageenan mixtures, the EAI and ES values for CPI-guar gum mixtures were set at 215 m^2/g and 85%, respectively (Fig. 53). Guided by these range of parameters and using the graphical feature of the optimization model (Design-Expert® Software), corresponding response surface overlay contour plot as a function of CPI and guar gum concentration were generated at pH 10.

Figure 53 show the overlay plot of the response (EAI and ES) surface graphs at pH 10.0 with the regions of optimum conditions shaded. Within the shaded area of the CPI-guar gum systems (Fig. 53), any combination of CPI and guar gum concentrations is predicted to give an emulsion with EAI and ES values not less than 215 m^2/g and 85%, respectively. Figure 53 illustrates that an adequate emulsion will be obtained at high guar gum level and any CPI concentration. These criteria were selected to achieve the desired goal which was to maximize the emulsifying characteristics, EAI and ES.

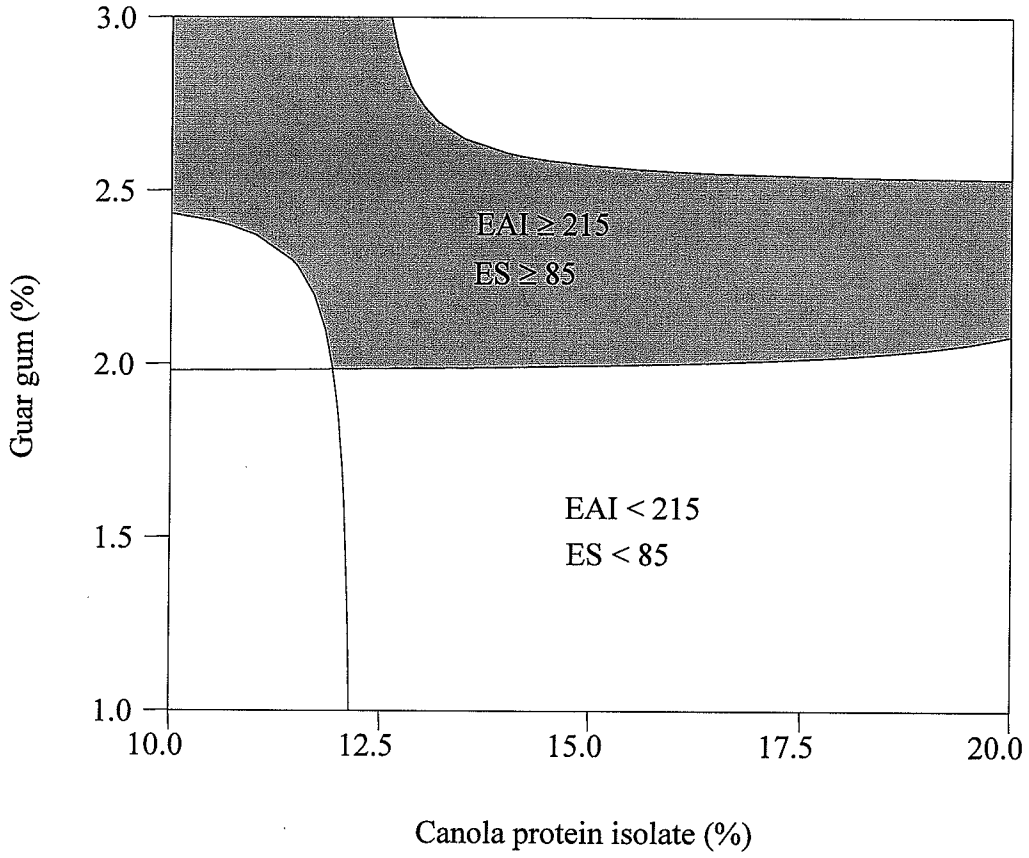


Figure 53. Graphical optimization display of EAI and ES as a function of canola protein isolate (CPI) and guar gum concentrations at pH 10. The clear areas on the plot do not meet the selected criteria. The lines mark the limits on the responses. Shaded areas indicate where factors can be set to satisfy the requirements on both responses. For example, any point in the shaded region shows the CPI and guar gum combination that will give an emulsion with EAI and ES values not less than 215 m^2/g and 75%, respectively. The desired criteria could not be achieved at pH 6 with guar gum.

Verification of model predictions for CPI-hydrocolloid mixtures

The model numerical predictions (Design-Expert® Software) for CPI-hydrocolloid-stabilized emulsions were verified by conducting confirmatory tests at the established optimum conditions for CPI-κ-CAR mixtures (pH 6; 10%, w/v CPI; 1%, w/v κ-CAR; 0.25 M NaCl) and CPI-guar gum mixtures (pH 10; 10%, w/v CPI; 3%, w/v guar gum; 0.25 M NaCl). Predicted EAI and ES values for CPI-κ-CAR-stabilized emulsions and CPI-guar gum-stabilized emulsions were established using the point prediction model (Design-Expert® Software).

Figures 54 and 55 show the predicted and experimental EAI and ES values for CPI-κ-CAR-stabilized emulsions and CPI-guar gum-stabilized emulsions, respectively. The experimental and predicted EAI and ES values for CPI-κ-CAR mixtures showed strong positive correlations as follows: for EAI; R^2=0.9999 and for ES; R^2=0.9985 (Fig. 54). Also, the experimental EAI and ES values for CPI-guar gum systems correlated positively to the predicted values as follows: for EAI; R^2=0.9939 and for ES; R^2=1 (Fig. 55). These results indicate statistical similarity ($P>0.05$) in the model predictions and experimental data.

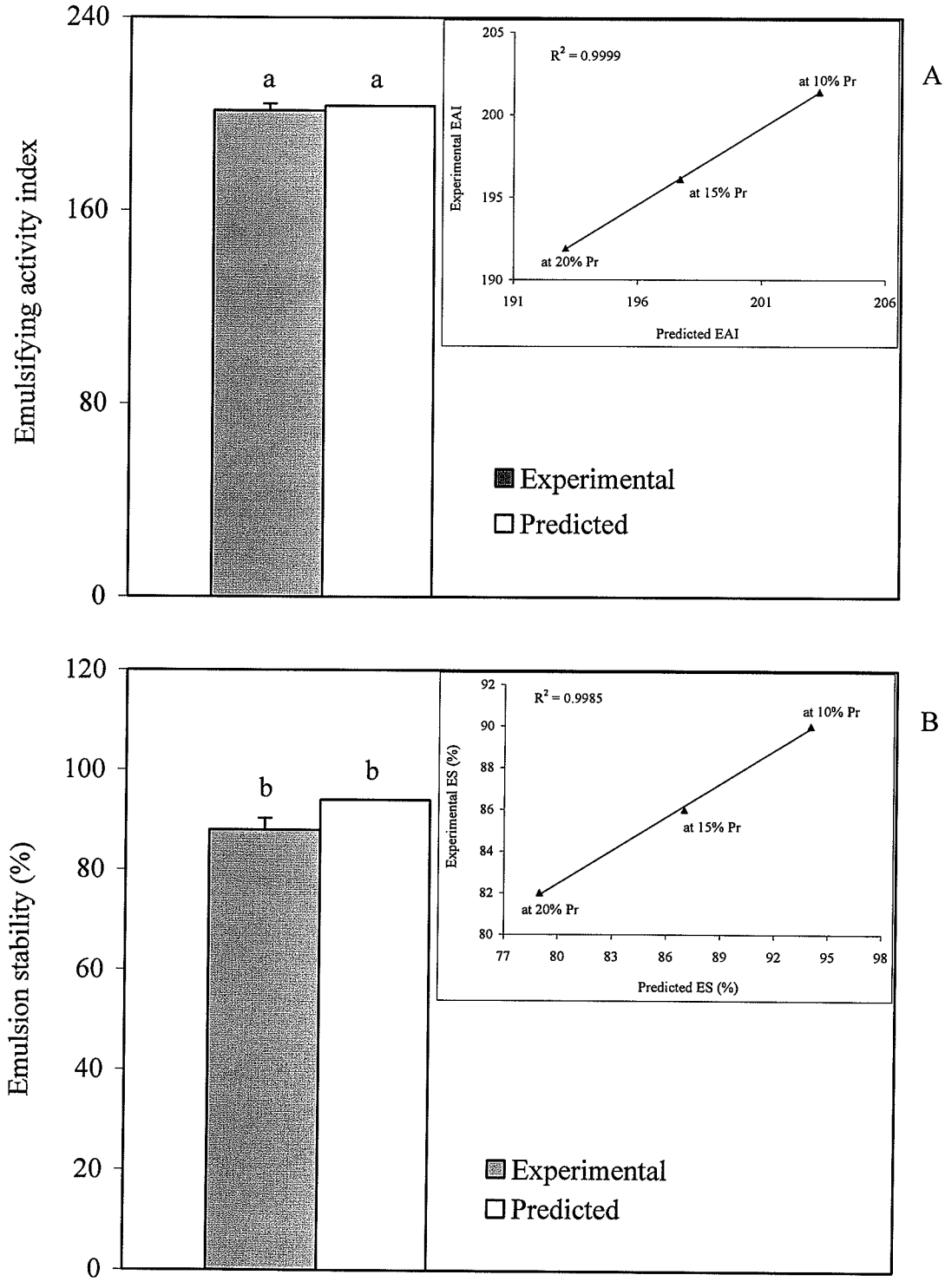


Figure 54. Experimental and predicted interfacial properties, EAI (A) and ES (B), of CPI-κ-carrageenan mixtures at 10% (w/v) CPI, pH 6, 0.25 M NaCl and 1% κ-carrageenan. *Insets: Pr = Protein. For each parameter (EAI or ES), bars with same letters are not significantly different (P>0.05).

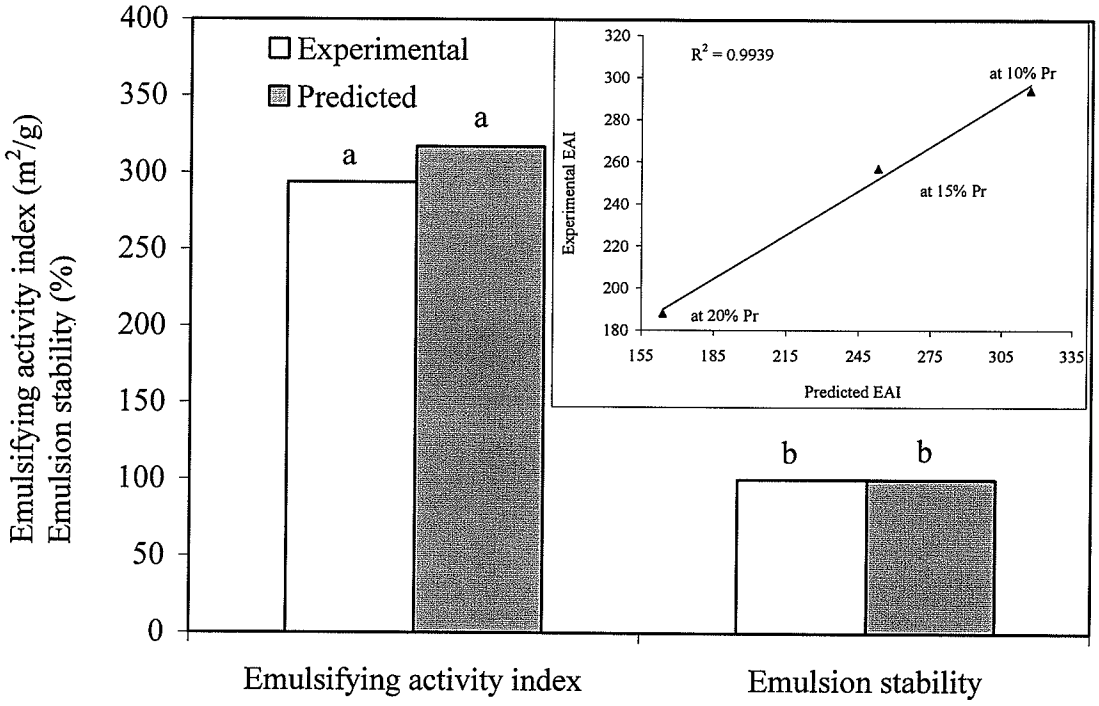


Figure 55. Experimental and predicted interfacial properties, EAI and ES, of CPI-guar gum mixtures at 10% (w/v) CPI, pH 10, 0.25 M NaCl and 3% guar gum. *Insets: Pr = Protein. For emulsion stability (ES), R^2=1. For each parameter (EAI or ES), bars with same letters are not significantly different (P>0.05).

Effect of CPI-hydrocolloid interactions on emulsifying properties

The interfacial stabilizing characteristics of canola protein-stabilized emulsions alone and in the presence of hydrocolloids, κ-carrageenan (κ-CAR) and guar gum, are shown in Figure 56. At pH 6 (optimum pH for CPI-κ-CAR emulsions), the EAI and ES values for CPI alone were 162 m^2/g and 68%, respectively; whereas at pH 10 (optimum pH for CPI-guar gum emulsions), the EAI and ES values for CPI alone were 68 m^2/g and 66%, respectively. On the addition of hydrocolloids (1% and 3% (w/v) κ-CAR or guar gum), the emulsifying properties were greatly improved (Fig. 56). Given that at pH 6, CPI carries a net positive and κ-CAR is negatively charged, the interaction between the two biopolymers will lead to the formation of electrostatic CPI-κ-CAR complexes. Further, guar gum is non-ionic and its interaction with CPI at pH 10 (where CPI carries a net negative charge) may lead to the formation of an incompatible CPI-guar gum system.

A study on protein polysaccharide interactions involving canola protein isolates showed that neutral polysaccharides such as guar gum and canola protein tended to be incompatible, which led to improved gelation properties (Arntfield and Cai, 1998). The results of the present study show that under the complexing conditions (at pH 6), the addition of 1% (w/v) κ-CAR increased the EAI of CPI-stabilized emulsions from 162 to 201 m^2/g and its ES increased from 68 to 95% (Fig. 56A). Also, under the incompatibility conditions (at pH 10), the use of 1% (w/v) guar gum increased the EAI of CPI-stabilized emulsions from 68 to 177 m^2/g and its ES increased from 66 to 100% (Fig. 56B). These findings are supported by previous reports which showed that the emulsifying capacity and emulsion stability increased on transition from a protein to its

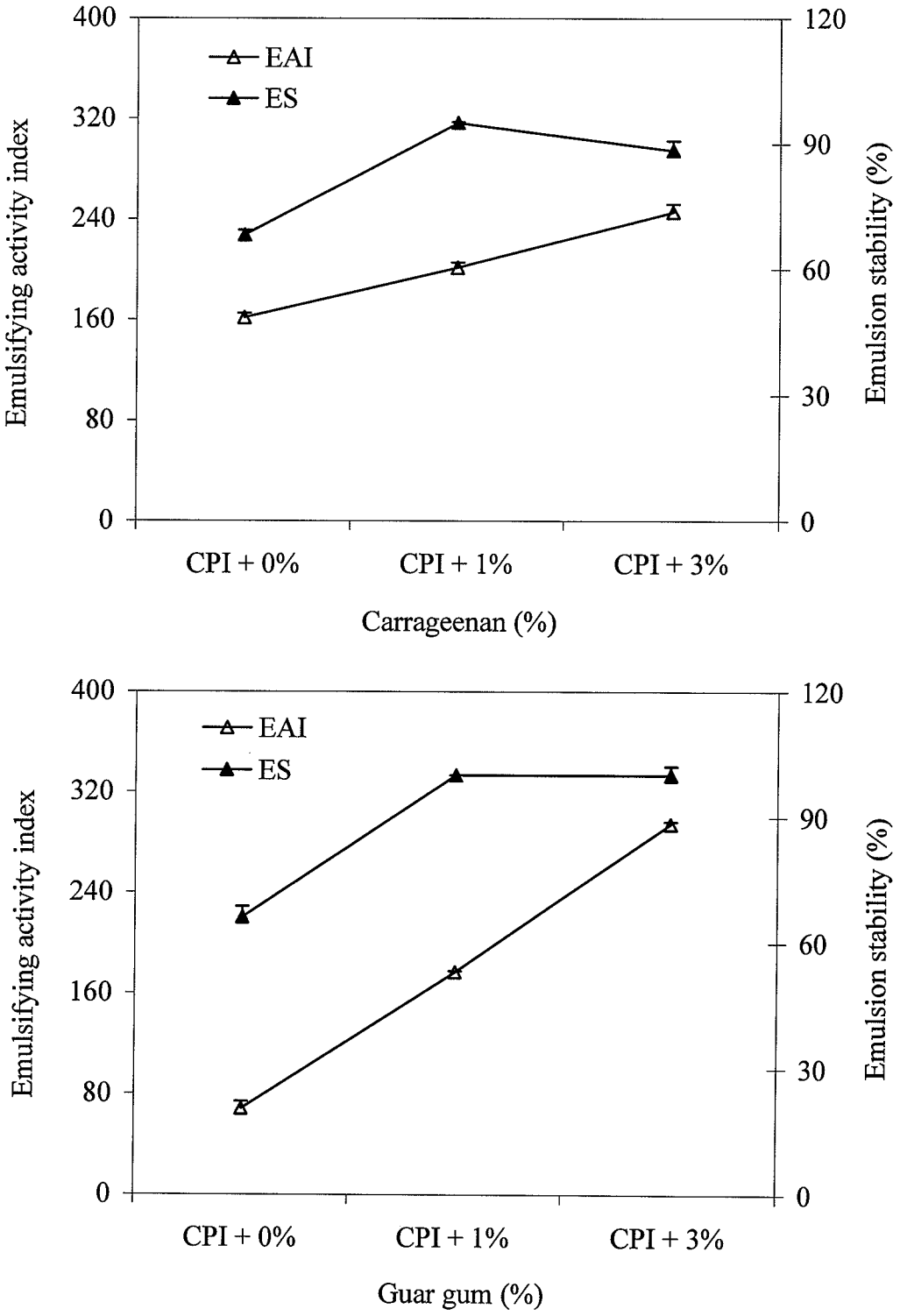


Figure 56. Influence of protein-hydrocolloid interactions on the emulsifying properties (EAI, ES) of canola protein-stabilized emulsions. A: CPI-κ-CAR systems (10% w/v CPI, 0.25 M NaCl, pH 6). B: CPI-guar gum systems (10% w/v CPI, 0.25 M NaCl, pH 10).

complex with polysaccharides (Gurov et al., 1983; Larichev et al., 1983). As shown by Tolstoguzov (1991), on addition of equal weight of pectin to a solution of legumin, the emulsion stability increases, while the emulsifying threshold for legumin decreases by a factor of 3. This is the case under conditions of complexing (stability increased from 67 to 96% at pH 4.2) and incompatibility (stability increased from 40 to 84% at pH 7.6).

The use of a polysaccharide (dextran), which is incompatible with the protein (legumin), intensifies the protein adsorption and decreases the requirements for protein content sufficient for a multilayer adsorption (Tolstoguzov, 1991). This could be attributed to an altered conformation of the protein at the interface resulting in larger contact between protein and polysaccharide molecule, and thereby providing strong layers at low adsorption (Samant et al., 1993). Soy proteins can be conjugated with dextran, resulting in a hybrid which possesses improved emulsifying properties compared to commercial emulsifiers (Kobayashi et al., 1990). In a recent study, soy protein isolate was mixed with sodium carboxymethyl cellulose and dry-heated at 60°C for up to 5 weeks. An improvement in emulsifying properties of the mixture, especially on oil droplet size reduction and emulsion stabilization against creaming was observed (Diftis and Kiosseoglou, 2003). Dispersed systems such as CPI-hydrocolloid-stabilized emulsions could be made more stable by improving the surface film via incompatibility and complexing of the surface active protein with polysaccharides.

Effect of sodium salts on emulsifying properties of CPI-hydrocolloid systems

Canola protein-κ-carrageenan systems: The addition of sodium salts to canola protein-hydrocolloid dispersions can be an effective probe for evaluating the contribution of hydrophobic interactions to the network formation process. Salts affect protein (or protein-polysaccharide mixtures) stability in two ways. At low concentrations (below 0.2 ionic strength), ions interact with proteins via nonspecific electrostatic interactions (Damodaran, 1996). However, at higher concentrations (>1 M), salts have ion specific effects that influence the structural stability of proteins (or protein-polysaccharide systems). The effect of sodium salts on the emulsifying properties (EAI, ES) of CPI-κ-CAR mixtures are shown in Table 19. As the salt concentration increased from 0.25 M to 0.5 M, the EAI and ES values decreased (Table 19). An exception to this trend was NaSCN which showed improved emulsifying activity at 0.5 M (EAI=136.5 m^2/g) than at 0.25 M (EAI=115.9 m^2/g). At 0.25 M salt concentration, increase in EAI of CPI-κ-CAR mixtures followed the series: chloride = sulfate > acetate > thiocyanate; whereas at 0.5 M salt level, the increase followed the order: acetate = chloride > sulfate > thiocyanate.

The series observed in the present study is different from the series reported by Damodaran (1996). According to this author, the relative ability of anions to influence the structural stability of proteins in general follows the lyotropic series: $F^- < SO_4^{2-} < Cl^- < Br^- < I^- < ClO_4^- < SCN^- < Cl_3CCOO^-$. The difference in the order of anions to affect protein conformation observed in the present study compared to that reported by Damodaran (1996) may be due to the presence of κ-CAR. It is possible that the κ-CAR modified the lyotropic effect probably via electrostatic complex formation with CPI. The

Table 19. Influence of salt type and concentration on the interfacial properties (EAI, ES) of canola protein-κ-carrageenan emulsions prepared with 10% (w/v) canola protein isolate and 1% (w/v) κ-carrageenan at pH 6.

Salt type	**EAI (m^2/g)***	
Control (no salt)	63.6 ± 0.72^{d1}	63.6 ± 0.72^{d1}
	0.25 M	**0.5 M**
Na_2SO_4	240.1 ± 3.44^{a1}	153.4 ± 2.17^{b2}
$NaC_2H_3O_2$	200.9 ± 3.17^{b1}	162.5 ± 3.80^{a2}
NaCl	241.2 ± 2.19^{a1}	161.1 ± 1.99^{a2}
NaSCN	115.9 ± 3.44^{c1}	136.5 ± 3.80^{c2}

Salt type	**ES (%)***	
Control (no salt)	81.9 ± 2.08^{b1}	81.9 ± 2.08^{ab1}
	0.25 M	**0.5 M**
Na_2SO_4	84.0 ± 2.84^{ab1}	81.1 ± 4.73^{ab1}
$NaC_2H_3O_2$	72.0 ± 2.88^{c1}	61.4 ± 0.26^{c2}
NaCl	88.4 ± 2.31^{ab1}	78.3 ± 3.19^{b1}
NaSCN	90.8 ± 2.75^{a1}	89.5 ± 4.52^{a1}

*Mean ± SD. For a given parameter (EAI or ES), column values with different letters and row values with different numbers are significantly different ($P<0.05$)

decrease in the thickness of electrostatic double layer may explain the lower EAI and ES values noted at 0.5 M salt level for CPI-κ-CAR emulsions (Table 19). It has been reported that divalent salts (e.g. Na_2SO_4) reduce the double layer thickness at lower concentrations (Phillips et al., 1994); thereby promoting coagulation of emulsion droplets at lower concentrations. However, data from present study showed that Na_2SO_4 and NaCl (at iosionic concentration) had a comparable effect on the emulsifying properties of CPI-κ-CAR systems.

Canola protein-guar gum systems: Increasing salt level from 0.25 M to 0.5 M decreased the emulsifying activity (EAI) of these systems in the presence of NaCl, Na_2SO_4, and NaSCN but not when treated with $NaC_2H_3O_2$ (Table 20). Phillips et al. (1994) noted a decrease in the thickness of electrical double layer with increasing salt concentration. This may explain the low EAI values observed in CPI-guar gum emulsions at 0.5 M salt level. At 0.25 M salt level, increase in EAI of CPI-guar gum mixtures followed the sequence: chloride > sulfate > thiocyanate > acetate; whereas at 0.5 M salt level, the increase followed the order: sulfate = acetate = chloride > thiocyanate (Table 20). At 0.25 M salt level, the EAI values of CPI-κ-CAR emulsions (optimum pH 6; Table 19) were higher than those of the CPI-guar gum emulsions (optimum pH 10; Table 20). Tolstoguzov (1991) reported that emulsions stabilized by legumin-pectin complexes in an acid (pH 4.2) medium had higher emulsifying capacity and emulsion stability than those under incompatible conditions at pH 7.6. This author attributed the increase to stronger adsorption layers formed by the complexes as a result of intensified intermolecular interactions of macromolecules at the interface.

Table 20. Influence of salt type and concentration on the emulsifying properties (EAI, ES) of canola protein isolate-guar gum systems prepared with 10% (w/v) canola protein isolate and 3% (w/v) guar gum at pH 10.

Salt type	**EAI (m^2/g)***	
Control (no salt)	137.3 ± 3.82^{c1}	137.3 ± 3.82^{a1}
	0.25 M	**0.5 M**
Na_2SO_4	151.9 ± 3.48^{b1}	135.5 ± 2.44^{a2}
$NaC_2H_3O_2$	123.4 ± 3.14^{d1}	140.2 ± 4.67^{a1}
NaCl	293.6 ± 0.18^{a1}	132.7 ± 4.45^{a2}
NaSCN	131.8 ± 3.32^{c1}	112.1 ± 2.99^{b2}

Salt type	**ES (%)***	
Control (no salt)	88.1 ± 3.44^{b1}	88.1 ± 3.44^{a1}
	0.25 M	**0.5 M**
Na_2SO_4	78.7 ± 1.82^{c1}	91.0 ± 2.64^{a2}
$NaC_2H_3O_2$	69.2 ± 0.28^{d1}	77.9 ± 1.90^{b2}
NaCl	100.0 ± 0.0^{a1}	75.5 ± 4.14^{b2}
NaSCN	97.5 ± 3.61^{a1}	95.4 ± 3.61^{a1}

*Mean ± SD. For a given parameter (EAI or ES), column values with different letters and row values with different numbers are significantly different ($P<0.05$)

Effect of urea and DTT on surface properties of CPI-hydrocolloid systems

The influence of urea and dithiothreitol (DTT) on the emulsifying properties of canola protein-hydrocolloid mixtures are shown in Figure 57. Figure 57A shows the emulsifying activity index (EAI) and the emulsion stability (ES) of CPI-κ-CAR systems without denaturants (control) and those treated with urea and DTT. The DTT-treated (EAI=118 m^2/g) and urea-treated (EAI=84 m^2/g) emulsions had higher EAI values than the control (EAI=64 m^2/g). In terms of the stability of the systems, the trend was different with the control (ES=82 m^2/g) being more stable than the DTT-treated (ES=70 m^2/g) and urea-treated (ES=62 m^2/g) emulsions (Fig. 57A). Urea (a hydrogen bond former) interacts very strongly with water and alters the structure of the aqueous phase around the protein molecule. It induces unfolding of proteins by reducing protein-protein and enhancing protein-solvent interactions (Phillips et al., 1994). Given that urea can disrupt hydrogen bonds and hydrophobic interactions, it is possible that the disruption of the hydrophobic interactions exposes the hydrophobic amino acid residues, causing them to interact at the interface and leading to improved emulsion formation.

This may account for the higher EAI values observed in urea-treated samples compared to the control. Further, significant differences ($P<0.05$) in EAI values of DTT-treated emulsions compared to the urea-treated and control emulsions indicates the contribution of disulfide bonds in the emulsification of mixed food systems containing canola protein. It has been shown that canola protein isolate has disulfide bonds in its native structure (Chapter 6). As a reducing agent, DTT can disrupt existing disulfide bonds, causing destabilization and modification of the native structure of a protein (Cheftel et al., 1985). The higher EAI value of DTT-treated CPI-κ-CAR emulsions

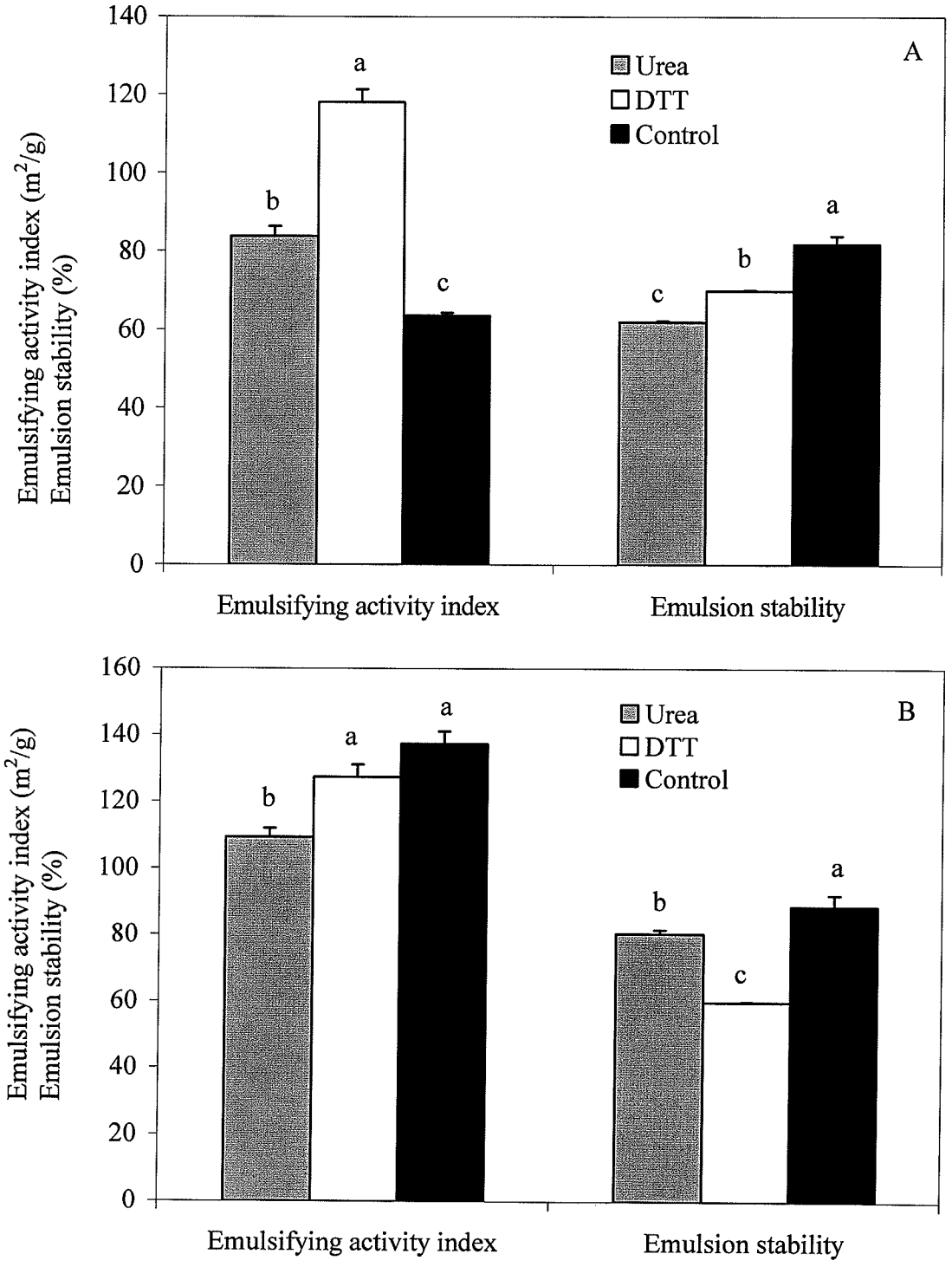


Figure 57. Effect of urea and DTT on the emulsifying properties (EAI, ES) of canola protein-hydrocolloid systems with or without denaturants. A: CPI-κ-CAR systems (10% w/v CPI, 1% w/v κ-CAR, 0.15 M DTT or 6 M urea, pH 6). B: CPI-guar gum systems (10% w/v CPI, 3% w/v guar gum, 0.15 M DTT or 6 M urea, pH 10). For EAI or ES and within treatments, bars with different letter are significantly different ($P<0.05$).

suggests that the cleavage of disulfide bonds exposes hydrophobic groups that would have remained buried within the molecules. As a result, increased interfacial interaction and enhanced emulsification were observed for these systems. In contrast, lower ES values were observed in emulsions treated with urea and DTT compared to that of the control (Fig. 57A). Lower ES values implicated noncovalent interactions and disulfide linkages as factors in emulsion stability.

The ES values of CPI-guar gum emulsions treated with urea and DTT were lower than that of the control (Fig. 57B), indicating that noncovalent interactions and disulfide bonds are contributing factors in emulsion stability. A similar trend was observed in CPI-κ-CAR emulsions (Fig. 57A). The EAI values of DTT-treated (127 m^2/g) and control (137 m^2/g) emulsions are comparable; however, they are higher than that of urea-treated (109 m^2/g) emulsions. In terms of emulsion stability, the DTT-treated CPI-guar gum emulsions had the lowest ES (60 m^2/g) value compared to the control (EAI=81 m^2/g) and the urea-treated (EAI=89 m^2/g) emulsions.

Overall, EAI and ES values were lower in the emulsions treated with denaturants compared to those of the control. An exception to this trend was the higher EAI values observed in CPI-κ-CAR emulsions treated with denaturants compared to that of the control. Lower EAI and ES values of CPI-κ-CAR and CPI-guar gum emulsions treated with urea and DTT indicate that noncovalent interactions and disulfide bonds are contributing factors in the formation and stability of these emulsions (Fig. 57). The effects of denaturants (e.g. urea, sulfhydryl reagents) on protein systems have been variable. In 6 M urea, the secondary and tertiary structures of most whey proteins were disrupted; this promotes interactions between free thiol groups and catalyzes thiol-

disulfide interchanges (Phillips et al., 1994). The addition of DTT (0-32 mM) to whey protein isolate systems inhibited the formation of intermolecular disulfide bonds (Zirbel and Kinsella, 1988).

GENERAL DISCUSSIONS

The role of protein-polysaccharide interaction in canola protein gelation

Gelling biopolymer dispersion is one of reactive macromolecules becoming progressively more and more crosslinked and aggregated as physical and/or chemical interactions occur. Globular protein gelation is the conversion of a viscous solution of individual globular molecules into a soft viscoelastic solid through the process of branched aggregation (Clark and Lee-Tuffnell, 1986). A denaturation temperature (T_d) of 86°C was determined for commercial CPI (Chapter 1). The T_d was determined to ensure that the heating temperature range used in rheological assessment was sufficient to denature the protein, a necessary step in the gelation process. The formation of CPI-κ-CAR complexes resulted in changes in the CPI structure, as reflected in the decrease in ΔH values as κ-CAR concentration increased at pH 6. Incompatibility between CPI and guar gum probably stabilized the structure of CPI. This was observed in the increase in ΔH values as CPI and guar gum concentration increased at pH 6.

Proteins and polysaccharides interact non-specifically and can create junctions which are mainly due to noncovalent interactions. Ionic interaction (under suitable conditions of pH and ionic strength) is one form of such interactions. At pH values below the protein isoelectric point (IEP), the protein and anionic polysaccharide carry net opposite charges. In this pH region, insoluble complexes can be formed provided the ionic strength is below 0.1-0.2 (Doublier et al., 2000; Tolstoguzov, 1986). In the present study, when canola protein isolate (CPI) was mixed with κ-carrageenan at pH 6, an insoluble electrostatic complex was formed. However, mixing CPI and guar gum at pH 10 resulted in interference from guar gum. We compared the effect of mixing CPI with

κ-carrageenan or guar gum on the gelation properties of the protein. On the addition of κ-carrageenan (κ-CAR) to a CPI dispersion, a strong and elastic gel was produced (Table 21). In contrast, the addition of guar gum produced a gel whose G′ value was lower than that of CPI alone, suggesting that guar gum interfered with the gelling ability of CPI. The gel strength was greatly increased under complex formation (pH 6) but not under interference (pH 10). The lower G′ value obtained for CPI-guar gum gel may be due to electrostatic repulsion between CPI and guar gum at pH 10. The study on CPI-polysaccharide interactions during network formation indicated that guar gum and CPI tended to be incompatible which led to improved gelation properties (Léger and Arntfield, 1993). Incompatibility is believed to promote network formation but that was not evident in CPI-guar gum gels in the present study. This needs further investigation.

Modern food processing uses an increasing amount of isolated food macromolecular components to provide specific functional properties to a wide range of formulated foods. These food additives can contain denatured, aggregated, or hydrolyzed biopolymers. For this reason compatibility and phase behaviour of biopolymer mixtures is of practical importance. Incompatibility of biopolymers, self-association and interbiopolymer complexing contribute to synergistic and antagonistic effects of food formulation (Tolstoguzov, 2003). The rheological data of the present study showed a synergistic interaction between CPI and κ-CAR which resulted in gels with strong and elastic structure (Table 21). This was ascribed to the complexing phenomenon.

Table 21. Gelation properties of canola protein isolate (CPI) in the presence and absence of κ-carrageenan or guar gum (0.05 M NaCl, 3% w/v κ-carrageenan or 1.5% w/v guar gum).

Properties	CPI (pH 6)	CPI (pH 10)	CPI-κ-CAR	CPI-guar gum
			Complexing (pH 6)	Interference (pH 10)
G'* (Pa)	27755 ± 757	68795 ± 1082	97465 ± 2454	56440 ± 2051
Tan δ*	0.19 ± 0.002	0.14 ± 0.002	0.15 ± 0.005	0.18 ± 0.004

*Mean ± SD.

Microstructure in relation to gelation of CPI-hydrocolloid gels

The functional attribute of CPI-hydrocolloid mixtures as a gelling agent may not be totally determined by its rheological properties, thus, their microstructural properties must always be of major importance. Structure-function relationships of CPI-hydrocolloid mixtures provide some information on how structures affect the behaviour of CPI in mixed foods. The scanning electron microscopy (SEM) images (Chapter 5) showed that CPI gels prepared at pH 6 had loosely crosslinked network structure with large and small pores, whereas properly crosslinked network structure with very small pores was observed at pH 10. This improved structural arrangement at pH 10 was reflected in the rheological data (Table 21). Given that tan δ reflects the actual arrangement of structures formed in a gel system, the lower tan δ (0.14) value at pH 10 compared to that at pH 6 (tan δ=0.19) reflected the properly connected structure of CPI gels at pH 10; indicating that elastic gels were formed. The SEM analysis on 12S rapeseed glycoprotein revealed a decrease in pore size as pH was increased from 6 to 10 (Gill and Tung, 1978).

The structure of CPI-κ-CAR gels prepared at pH 6 had short, tightly packed strands, whereas the structure of CPI-guar gum gels prepared at pH 10 consisted of long, properly crosslinked strands (Chapter 5). However, the tan δ values of the CPI-κ-CAR and CPI-guar gum systems (Table 21) were not completely reflected in the microstructural results. The SEM images of CPI in the presence of κ-CAR showed the complementary character of each macromolecule. The rheological and microstructural characteristics of the mixed gels were improved since properly crosslinked structure was observed in the mixed state. This improved network structure (properly formed structure) suggests a synergistic behaviour between CPI and κ-CAR as was reported in the gelation analysis of CPI and κ-CAR (Chapter 3). Properly crosslinked network structures are indicative of strong and firm gels.

The structures of CPI-hydrocolloid gel systems treated with urea had excessively crosslinked and disrupted strands, whereas DTT-treated gel systems had amorphous structures. Given that urea and DTT disrupt noncovalent interactions and disulfide bonds, respectively, the microstructural and rheological data support the involvement of these forces in network formation of CPI-hydrocolloid systems.

Increase in tan δ values produced CPI-κ-CAR gels with highly aggregated structures (Chapter 5, Fig. 35B). High tan δ values indicate weaker structures. It was evident from the SEM images that the network strands were not fully aligned. This gives gels a fluid character because the liquid phase is not completely entrapped in a continuous three-dimensional network. Microstructural data complemented the rheological properties that characterized the final gels.

Molecular interactions involved in structure formation

Food formulation aims to control interactions between proteins, polysaccharides and their interactions with other food components. In order to identify the molecular forces that contribute to the network formation of CPI-hydrocolloid (κ-CAR, guar gum) systems, the environment in which the mixed gels were formed was selectively altered. To achieve this goal, varied data was used to provide insight on the gelling mechanism of CPI-hydrocolloid systems.

Increases in G′ values of CPI-κ-CAR gelling systems treated with sodium salts followed a lyotropic series. At 0.5 M salt concentration, mixed systems treated with Na_2SO_4 and NaCl had the highest G′ values, whereas NaSCN-treated gels had the lowest G′ value. High G′ values indicate that the network structure CPI-κ-CAR mixtures was stabilized by Na_2SO_4 and NaCl. On the other hand, NaSCN (a chaotropic salt) destabilized the protein structure. Based on these results, it is evident that hydrophobic interactions contribute to the gelation of CPI-κ-CAR systems. Protein (protein-polysaccharide) stabilization or destabilization by salts is related to their effect on bulk water structure. Salts that stabilize protein structure enhance the hydrogen-bonded structure of water, whereas salts that denature proteins break down bulk water structure and make it a better solvent for nonpolar molecules (Damodaran, 1996). In other words, the denaturing effect of chaotropic salts might be related to destabilization of hydrophobic interactions in proteins or protein-polysaccharide systems.

More evidence from the present study that demonstrate the involvement of hydrophobic interactions in the development of structure of CPI-κ-CAR gels was shown in the heating and cooling curves obtained during the rheological evaluation. Given that

hydrophobic interactions are favoured at high temperatures, rheological data showed that network formation in most of the mixed systems started at high temperatures attained during the heating cycle and the initial cooling phase. Léger and Arntfield (1993) made a similar observation in a study on the types of interactions involved in the formation of CPI gels.

The contribution of hydrogen bonding in network strengthening and/or stabilization was seen during the final cooling cycle. Since hydrogen bonding is enhanced at low temperatures, the gradual increase in G′ values and the decrease in tan δ values during the final cooling phase and frequency sweep (further cooling) of gelation is ascribed to this molecular force. Additional data that support the role of hydrophobic interactions and hydrogen bonds in the formation of CPI-κ-CAR networks is shown in the urea and DTT influences. As a hydrogen bond former, urea can disrupt hydrogen bonds and hydrophobic interactions that contribute to the stability of biopolymer structure (Phillips et al., 1994). The poor gel strength (low G′ values) observed in urea-treated systems during the heating and cooling cycles could be attributed to the disruption of hydrophobic interactions and hydrogen bonds. Given that DTT is a disulfide reducing agent, its addition to CPI-κ-CAR dispersions prior to heating appears to improve the gel strength (high G′ values) of the mixture especially during the cooling stage. It is possible that the cleavage of the disulfide bonds by DTT greatly altered the biopolymer structure, which promoted interactions among hydrophobic groups. Thus, enhanced structure formation in DTT-treated CPI-κ-CAR systems was observed.

Studies have shown that a proper balance between attractive and repulsive forces was essential for a good gel formation to occur (Ferry, 1948; Tolstoguzov, 1997, 2003).

The disruption of this balance may explain the poor gel strength (low G′ values) obtained with urea-treated CPI-κ-CAR systems and improved network formation (during cooling) by DTT treatment. Changes in pH also affect electrostatic interactions and hydrophobic interactions. At pH below the IEP of CPI (i.e. at pH 6), electrostatic attraction between CPI and κ-CAR promoted protein-protein interaction among hydrophobic groups, thereby strong and elastic gels were produced (Table 21). At pH 10, electrostatic repulsion between biopolymer molecules may encourage unfolding of buried hydrophobic residues. Subsequently, this promotes intermolecular interaction among exposed hydrophobic groups. This may explain the strong gels obtained for CPI and CPI-guar gum systems at pH 10. Although guar gum interfered with the gelation of CPI at pH 10, a very strong gel was still produced (G′=56440 Pa; Table 21).

Gelation of CPI-hydrocolloid mixtures is a complex phenomenon that involves various molecular interactions. The rheological and microstructural data demonstrated that hydrophobic interactions, a balance between attractive and repulsive forces as well as electrostatic interactions are the primary forces involved in the gel formation of CPI-κ-CAR systems. Disulfide linkages and hydrogen bonding contribute to network strengthening and stabilization of these systems. These observations were similar to previous findings, in terms of interactions involved in network formation, made for CPI gelling systems such as 12S canola globulin (Léger and Arntfield, 1993), 12S rapeseed glycoproteins (Gill and Tung, 1978), and CPI (Paulson and Tung, 1989).

Surface hydrophobicity in relation to emulsification of CPI-based systems

Surface hydrophobicity (S_o) is that portion of the nonpolar surface of the protein that makes contact with the surrounding bulk water. It was observed that the S_o values of CPI-guar gum mixtures were higher than those of CPI-κ-CAR systems (Chapter 2). The different physical properties of κ-CAR and guar gum may explain the difference in S_o values. The formation of an electrostatic CPI-κ-CAR complex at pH 6 may have encouraged protein-protein or protein-hydrocolloid interactions, thereby making the hydrophobic groups unavailable as exposed residues for interaction at the oil-water interface. This may explain the lower S_o values noted for CPI-κ-CAR systems. Guar gum has been reported to be incompatible with CPI resulting in improved gelation properties (Arntfield and Cai, 1998); it is possible that the incompatibility increased at pH 10 used in the present study, and resulted in high S_o value (indicative of a good emulsifier).

The emulsifying activity index (EAI) of CPI-stabilized emulsions at pH 6 and 10 were 162 and 68 m^2/g, respectively (Chapter 7). The EAI of CPI-stabilized emulsion in the presence of κ-CAR was 201 m^2/g, whereas that in the presence of guar gum was 177 m^2/g. The EAI of emulsions stabilized by CPI-κ-CAR complexes at pH 6 was greater than that stabilized by CPI-guar gum mixture at pH 10. However, the use of hydrocolloids increased the emulsifying activity index (EAI) under incompatibility conditions to a more considerable extent (from 68 to 177 m^2/g at pH 10) than under complexing conditions (from 162 to 201 m^2/g at pH 6). A similar trend was observed in the emulsion stability (ES) of the two CPI-hydrocolloid systems (Table 22). This observation supported the findings of Tolstoguzov (1991) in terms of the stability of

emulsions stabilized by legumin in the presence of pectin. The ability of various proteins to decrease interfacial tension at the oil-water interface and to increase the EAI is related to their S_o values. However, this relationship is by no means perfect (Damodaran 1996). The emulsifying properties of several proteins (soy proteins, β-lactoglobulin, α-lactalbumin) do not show a strong correlation with surface hydrophobicity (Damodaran 1996).

Our results (Chapters 2 and 7) showed that the surface hydrophobicity of CPI-hydrocolloid systems strongly correlated with the emulsifying properties of these systems. If a protein system, alone or in a mixed state, has a high number of nonpolar amino acid groups on the surface, greater interaction with the oil surface is possible and this results in a finer emulsion. As hydrophobic residues tend to be buried during folding, the presence of hydrophobic residues on the surface of the protein (protein-hydrocolloid) can give insight on how proteins unfold and interact in food systems.

Table 22. Stability of emulsions (%) stabilized by canola protein isolate (CPI) in the presence of κ-carrageenan or guar gum (1% w/v, hydrocolloids; 10%, w/v CPI; 0.25 M NaCl).

System	Complexing region (pH 6)	Incomaptibility region (pH 10)
CPI	68 ± 1.20	66 ± 2.55
CPI-κ-CAR	95 ± 0.11	–
CPI-guar gum	–	100 ± 0.00

*Mean ± SD.

CONCLUSIONS

The results of this study give new insights into the thermal and structural stability, rheology, microstructure, molecular interactions and emulsification of commercial canola protein isolate (CPI) in multicomponent food systems. Many challenges still remain.

Hydrophobic interactions improved the structural stability of CPI-hydrocolloid mixtures, as evidenced in the increase in denaturation temperature (T_d) values with increasing NaCl concentration. The formation of CPI-κ-CAR complexes resulted in changes in the CPI structure. This was reflected in the decreases in enthalpy of denaturation (ΔH) values as κ-CAR concentration increased at pH 6. Incompatibility between CPI and guar gum probably stabilized the structure of CPI. This was observed in the increase in ΔH values as CPI and guar gum concentration increased at pH 6.

CPI interacting with κ-CAR formed strong and elastic gels via electrostatic complexing at pH 6; and a synergistic behaviour between the two biopolymers was noted. Under conditions which improved protein-polysaccharide gelation (incompatibility), guar gum interfered with the gelation of canola protein isolate.

Microstructural assessment using scanning electron microscopy (SEM) gave more realistic information on network structures of CPI-hydrocolloid gelling systems. CPI-hydrocolloid gels prepared at optimum conditions were characterized by properly crosslinked networks, whereas gels prepared at non-optimal conditions had aggregated networks. Treating CPI-hydrocolloid mixtures with denaturants caused excessive structural changes to gels, implicating disulfide bonds and noncovalent forces as factors in network formation. The SEM data complemented the rheological results and provided

further insights on the type of structures (e.g. aggregated strands) associated with gels that have high tan δ values, which could not be distinguished from rheological data alone.

The major molecular interactions that contribute to the initiation of network formation of CPI-hydrocolloid systems are hydrophobic and electrostatic interactions as well as proper balance between repulsive and attractive forces. Disulfide linkages and hydrogen bonding are involved in the gel stabilization and strengthening. These were reflected in the rheological, structural, thermal, microstructural and emulsification data.

CPI and CPI-hydrocolloid mixtures each had higher S_o at pH 10 than at pH 6. The increase in surface hydrophobicity (S_o) of CPI was greater in the presence of guar gum when compared to κ-CAR. The results indicate that the addition of hydrocolloids resulted in increased exposure of hydrophobic amino acid residues on the protein surface, with the S_o greatly enhanced in the presence of guar gum and at pH 10. Higher S_o values suggests increase in hydrophobic patches on the protein surface. As the number of these patches increases, greater and rapid adsorption at an oil-water interface (which is required for emulsion formation) becomes more probable.

Hydrophobic interactions, hydrogen bonds and disulfide linkages are contributing factors in emulsion formation and stability. This was reflected in lower EAI and ES values observed in CPI-hydrocolloid-stabilized emulsions treated with sodium salts, urea or DTT. The addition of κ-CAR or guar gum to CPI-stabilize emulsions resulted in improved interfacial properties of these systems. This improvement was attributed to the formation of CPI-κ-CAR complexes at pH 6. The enhanced emulsifying characteristics observed in CPI-guar gum systems may be due to the incompatibility

between CPI and guar gum. CPI-hydrocolloid complexes can serve as surface-active ingredients.

PRACTICAL AND FUTURE RESEARCH CONSIDERATIONS

Practical applications

A better understanding of the interactions between biopolymers will enhance the manipulation of food quality (e.g. sensory properties) by adjusting the interaction in a desirable way. For example, food products with novel structures can be produced by allowing a mixed food system to partially phase separate.

Protein-polysaccharide interactions such as complexing can be used to lower processing costs and the overall product cost. For instance, given that proteins are more expensive than polysaccharides, using proteins in combination with polysaccharides will lower the amount of proteins required in a food system. Thus, a reduced cost of protein can be achieved by the addition of right amount of polysaccharide. In turn, this will lower the overall processing cost. In this study, CPI-κ-carrageenan complexing produced gels and emulsions with improved properties. Using CPI in combination with hydrocolloids will reduce the costs of food products containing mixed biopolymers.

Further research considerations

The interaction between canola protein isolate (CPI) and guar gum needs further assessment since guar gum interfered with CPI gel formation. The incompatibility previously reported to produce improved gelation between the two macromolecules (Arntfield and Cai, 1998) did not result in improved gel properties in this study

(especially at pH 6). Further examination of the phase separation phenomenon will provide more insight on the incompatibility between CPI and neutral polysaccharides (e.g. guar gum).

Superior gel properties were obtained for CPI-κ-carrageenan mixtures (at pH 6) and this was attributed to the electrostatic complex formation between the two macromolecules. The possibility of a chain segment binding occurring between CPI and κ-carrageenan requires further consideration.

Some of the observations of this study were limited to the experimental design used. Examining different set of points (absent in the experimental design) could provide additional information on the interactions and mechanisms that enhance gelation and emulsification of CPI in multicomponent systems.

Another consideration will be to compare the functionality of CPI to that of soy protein ingredients, which are best known as emulsifying and gelling agents (Utsumi et al., 1997). The gelation and emulsification data of the present research showed that CPI can serve as a gelling and emulsifying ingredient, especially when used in combination with hydrocolloids. Since soy protein products are the leading plant protein commercially available and are widely used, comparing the gelation and emulsifying properties of commercial CPI to that of soy protein ingredient would be useful.

The gelation of CPI-hydrocolloid mixtures were carried out at temperatures ranging from 25° to 95°C, it is possible that improved network properties may be produced at temperature above 100°C. Investigation into the network properties of CPI-hydrocolloid systems at temperatures higher than 95°C is recommended.

The functional foods and nutraceuticals industry is an emerging and rapidly

growing sector. Investigation into the potential of CPI as a source of bioactive compounds (e.g. bioactive peptides) should be explored. This could provide opportunity that will enhance fabrication of new food products with functional, nutritious and organoleptically acceptable characteristics.

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APPENDICES

Appendix 1. Selected factorial model – ANOVA of denaturation temperature (T_d) of canola protein isolate-κ-carrageenan systems.

Response: T_d

Factor	Name	Units	Type	-1 Level	+1 level
A	pH		Numeric	6.00	10.00
B	Salt conc	M	Numeric	0.05	0.25
C	κ-CAR	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00
Source	**Sum/Squares**	**DF**	**Mean Square**	**F-Value**	**Prob >F**
Model	35.80	15	2.39	69.25	0.0025
Curvature	4.46	1	4.46	129.41	0.0015
Residual	0.10	3	0.034		
Pure Error	0.10	3	0.034		
Cor Total	40.37		19		
Root MSE	0.19		R-Squared	0.9971	
Dep Mean	90.09		Adj R-Squared	0.9827	
C.V.	0.21				
PRESS	N/A		Adeq Precision	28.102	Desire > 4
Factor	**Coefficient Estimate**	**DF**	**Standard Error**	**T for Ho Coeff=0**	**Prob > |t|**
Intercept	89.85	1	0.046		
A-pH	0.89	1	0.046	19.16	0.0003
B-Salt	0.97	1	0.046	20.99	0.0002
C-κ-CAR	0.19	1	0.046	4.19	0.0248
D-Prot conc	0.57	1	0.046	12.24	0.0012
AB	-0.20	1	0.046	-4.21	0.0244
AC	-0.051	1	0.046	-1.09	0.3552
AD	-0.21	1	0.046	-4.56	0.0197
BC	0.057	1	0.046	1.23	0.3079
BD	8.125×10^{-03}	1	0.046	0.18	0.8722
CD	0.016	1	0.046	0.34	0.7586
ABC	6.875×10^{-03}	1	0.046	0.15	0.8916
ABD	-0.16	1	0.046	-3.38	0.0431
ACD	-0.089	1	0.046	-1.93	0.1498
BCD	-0.057	1	0.046	-1.23	0.3079
ABCD	0.11	1	0.046	2.33	0.1022
C/Point	1.18	1	0.10	11.38	0.0015

Appendix 2. Selected factorial model – ANOVA of enthalpy of denaturation (ΔH) of canola protein isolate-κ-carrageenan systems.

Response: ΔH

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	κ-CAR	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	45.35	11	4.12	5.04	0.0208
Curvature	1.03	1	1.03	1.26	0.2984
Residual	5.73	7	0.82		
Lack of Fit	4.51	4	1.13	2.76	0.2154
Pure Error	1.22	3	0.41		
Cor Total	52.12	19			

Root MSE	0.90	R-Squared	0.8878	
Dep Mean	16.97	Adj R-Squared	0.7115	
C.V.	5.33	Pred R-Squared	-0.4251	
PRESS	74.27	Adeq Precision	7.115	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	16.86	1	0.23		
A-pH	-0.14	1	0.23	-0.62	0.5539
B-Salt	0.64	1	0.23	2.82	0.0257
C-κ-CAR	-0.54	1	0.23	-2.39	0.0482
D-Prot.	-0.98	1	0.23	-4.32	0.0035
AB	-0.23	1	0.23	-1.01	0.3443
AC	0.55	1	0.23	2.42	0.0463
AD	-0.52	1	0.23	-2.28	0.0567
BC	0.22	1	0.23	0.96	0.3670
CD	0.15	1	0.23	0.67	0.5235
ABC	-0.48	1	0.23	-2.13	0.0706
ACD	-0.49	1	0.23	-2.16	0.0678
C/ Point	0.57	1	0.51	1.12	0.2984

Appendix 3. Selected factorial model – ANOVA of denaturation temperature (T_d) of canola protein isolate-guar gum mixtures.

Response: T_d

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	Ggum	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	41.48	14	2.96	26.41	0.0031
Curvature	8.06	1	8.06	71.81	0.0011
Residual	0.45	4	0.11		
Lack of Fit	0.045	1	0.045	0.34	0.6030
Pure Error	0.40	3	0.13		
Cor Total	49.99	19			

Root MSE	0.33	R-Squared	0.9893
Dep Mean	88.73	Adj R-Squared	0.9518
C.V.	0.38	Pred R-Squared	0.7544
PRESS	12.28	Adeq Precision	20.915

Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	88.4	11	0.084		
A-pH	0.45	1	0.084	5.37	0.0058
B-Salt	1.07	1	0.084	12.77	0.0002
C-Ggum	0.15	1	0.084	1.80	0.1465
D-Protein	0.74	1	0.084	8.84	0.0009
AB	-0.50	1	0.084	-5.93	0.0040
AC	-0.11	1	0.084	-1.35	0.2481
AD	-0.63	1	0.084	-7.53	0.0017
BC	-0.028	1	0.084	-0.34	0.7539
BD	4.375E-03	1	0.084	0.052	0.9608
CD	-9.375E-03	1	0.084	-0.11	0.9163
ABC	-0.087	1	0.084	-1.04	0.3581
ABD	-0.072	1	0.084	-0.86	0.4391
ACD	0.017	1	0.084	0.20	0.8501
BCD	-0.068	1	0.084	-0.81	0.4616
C/ Point	1.59	1	0.19	8.47	0.0011

Appendix 4. Selected factorial model – ANOVA of enthalpy of denaturation (ΔH) of canola protein isolate-guar gum mixtures.

Response: ΔH

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	Ggum	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	122.15	12	10.18	4.21	0.0445
Curvature	76.73	1	76.73	31.73	0.0013
Residual	14.51	6	2.42		
Lack of Fit	8.70	3	2.90	1.50	0.3736
Pure Error	5.81	3	1.94		
Cor Total	213.39	19			

Root MSE	1.56	R-Squared	0.8938	
Dep Mean	13.97	Adj R-Squared	0.6815	
C.V.	11.13	Pred R-Squared	-0.2087	
PRESS	257.92	Adeq Precision	6.839	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	12.99	1	0.39		
A-pH	-1.67	1	0.39	-4.30	0.0051
B-Salt	0.62	1	0.39	1.59	0.1637
C-Ggum	0.98	1	0.39	2.52	0.0453
D-Protein	-0.19	1	0.39	-0.48	0.6456
AB	0.14	1	0.39	0.36	0.7345
AC	-0.60	1	0.39	-1.55	0.1726
AD	-0.48	1	0.39	-1.23	0.2659
BC	0.84	1	0.39	2.16	0.0738
BD	-0.25	1	0.39	-0.64	0.5449
CD	-1.03	1	0.39	-2.65	0.0381
ABD	-0.56	1	0.39	-1.44	0.2011
BCD	-0.85	1	0.39	-2.18	0.0719
C/ Point	4.90	1	0.87	5.63	0.0013

Appendix 5. Selected factorial model – ANOVA of surface hydrophobicity (S_o) of canola protein isolate-κ-carrageenan systems.

Response: S_o

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	κ-CAR	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	1349.01	12	112.42	6.99	0.0129
Curvature	354.90	1	354.90	22.06	0.0033
Residual	96.52	6	16.09		
Lack of Fit	96.49	3	32.16	3216.40	<0.0001
Pure Error	0.03	3	0.010		
Cor Total	1800.44	19			
Root MSE	4.01		R-Squared	0.9332	
Dep Mean	132.32		Adj R-Squared	0.7997	
C.V.	3.03		Pred R-Squared	-0.5245	
PRESS	2744.71		Adeq Precision	8.810	Desire>4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	130.22	1	1.00		
A-pH	-3.36	1	1.00	-3.35	0.0155
B-Salt	-0.67	1	1.00	-0.67	0.5296
C-κ-CAR	-2.57	1	1.00	-2.56	0.0428
D-Prot conc	-0.79	1	1.00	-0.79	0.4587
AB	-0.42	1	1.00	-0.42	0.6908
AC	2.38	1	1.00	2.37	0.0552
AD	0.13	1	1.00	0.13	0.9001
BC	-0.68	1	1.00	-0.68	0.5222
BD	-4.33	1	1.00	-4.23	0.0050
CD	2.02	1	1.00	2.01	0.0907
ABC	-6.01	1	1.00	-5.99	0.0010
ACD	0.37	1	1.00	0.37	0.0257
C/Point	10.53	1	2.24	4.70	0.0033

Appendix 6. Selected factorial model – ANOVA of surface hydrophobicity (S_o) of canola protein isolate-guar gum systems.

Response: So

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	Ggum	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	28687.94	15	1912.53	11908.65	< 0.0001
Curvature	24459.42	1	24459.42	1.523E+05	< 0.0001
Residual	0.48	3	0.16		
Pure Error	0.48	3	0.16		
Cor Total	53147.84	19			

Root MSE	0.40	R-Squared	1.0000	
Dep Mean	481.99	Adj R-Squared	0.9999	
C.V.	0.083			
PRESS	N/A	Adeq Precision	500.876	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	464.50	1	0.10		
A-pH	1.77	1	0.10	17.68	0.0004
B-Salt	-3.83	1	0.10	-38.28	0.0201
C-Ggum	-0.64	1	0.10	-6.39	0.0678
D-Prot conc	-15.75	1	0.10	-157.22	0.0311
AB	6.57	1	0.10	65.56	0.0921
AC	3.09	1	0.10	30.88	0.0101
AD	-12.97	1	0.10	-129.43	0.0563
BC	-14.26	1	0.10	-142.33	0.0621
BD	-2.39	1	0.10	-23.89	0.0732
CD	11.03	1	0.10	110.06	0.0321
ABC	20.41	1	0.10	203.76	0.0761
ABD	-3.82	1	0.10	-38.13	0.0401
ACD	-7.58	1	0.10	-75.71	0.0091
BCD	10.30	1	0.10	102.77	0.0621
ABCD	-19.50	1	0.10	-194.64	0.5301
C/Point	87.43	1	0.22	390.26	0.0031

Appendix 7. Selected factorial model – ANOVA of storage modulus (G′) of canola protein isolate-κ-carrageenan systems.

Response: G′

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	κ-CAR	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob >F
Model	1.297E+10	14	9.266E+08	16.79	0.0073
Curvature	1.580E+09	1	1.580E+09	28.63	0.0059
Residual	2.208E+08	4	5.519E+07		
Lack of Fit	2.186E+08	1	2.186E+08	301.17	0.0004
Pure Error	2.177E+06	3	7.258E+05		
Cor Total	1.477E+10	19			

Root MSE	7429.23	R-Squared	0.9833	
Dep Mean	25133.50	Adj R-Squared	0.9247	
C.V.	29.56	Pred R-Squared	-2.7883	
PRESS	5.596E+10	Adeq Precision	13.025	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	29577.50	1	1857.31		
A-pH	-15748.75	1	1857.31	-8.48	0.0011
B-Salt	-6433.75	1	1857.31	-3.46	0.0257
C-κ-CAR	-4160.00	1	1857.31	-2.24	0.0886
D-Prot conc	-4536.25	1	1857.31	-2.44	0.0710
AB	7140.00	1	1857.31	3.84	0.0184
AC	-5411.25	1	1857.31	-2.91	0.0435
AD	4715.00	1	1857.31	2.54	0.0641
BC	-13521.25	1	1857.31	-7.28	0.0091
BD	-585.00	1	1857.31	-0.31	0.7685
CD	4076.25	1	1857.31	2.19	0.0932
ABC	12227.50	1	1857.31	6.58	0.0028
ABD	816.25	1	1857.31	0.44	0.6830
ACD	-4797.50	1	1857.31	-2.58	0.0611
BCD	2827.50	1	1857.31	1.52	0.2026
C/Point	-22220.00	1	4153.06	-5.35	0.0059

Appendix 8. Selected factorial model – ANOVA of loss tangent (tan δ) of canola protein isolate-κ-carrageenan systems.

Response: Tan δ

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	κ-CAR	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	0.094	9	0.010	8.99	0.0016
Curvature	1.786E-03	1	1.786E-03	1.54	0.2459
Residual	0.010	9	1.159E-03		
Lack of Fit	4.088E-03	6	6.813E-04	0.32	0.8896
Pure Error	6.347E-03	3	2.116E-03		
Cor Total	0.11	19			

Root MSE	0.034	R-Squared	0.8999	
Dep Mean	0.11	Adj R-Squared	0.7997	
C.V.	30.51	Pred R-Squared	0.6193	
PRESS	0.040	Adeq Precision	9.445	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	0.11	1	8.513E-03		
A-pH	-5.750E-03	1	8.513E-03	-0.68	0.5164
B-Salt	4.000E-03	1	8.513E-03	0.47	0.6496
C-κ-CAR	0.048	1	8.513E-03	5.65	0.0003
D-Prot.	-0.015	1	8.513E-03	-1.70	0.1227
AB	-4.125E-03	1	8.513E-03	-0.48	0.6396
AC	0.039	1	8.513E-03	4.58	0.0013
BC	0.025	1	8.513E-03	2.97	0.0158
CD	-0.027	1	8.513E-03	-3.14	0.0119
ABC	-0.020	1	8.513E-03	-2.33	0.0444
C/ Point	0.024	1	0.019	1.24	0.2459

Appendix 9. Selected factorial model – ANOVA of storage modulus (G') of canola protein isolate-guar gum systems.

Response: G'

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	Ggum	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob >F
Model	6.444E+09	13	4.957E+08	8.35	0.0144
Curvature	1.491E+08	1	1.491E+08	2.51	0.1739
Residual	2.970E+08	5	5.939E+07		
Lack of Fit	1.620E+08	2	8.098E+07	1.80	0.3066
Pure Error	1.350E+08	3	4.501E+07		
Cor Total	6.890E+09	19			

Root MSE	7706.78	R-Squared	0.9559	
Dep Mean	16613.40	Adj R-Squared	0.8414	
C.V.	46.39	Pred R-Squared	-0.5392	
PRESS	1.060E+10	Adeq Precision	9.069	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	15248.00	1	1926.70		
A-pH	5479.38	1	1926.70	2.84	0.0361
B-Salt	-2502.12	1	1926.70	-1.30	0.2507
C-Ggum	-6023.75	1	1926.70	-3.13	0.0261
D-Protein	12494.38	1	1926.70	6.48	0.0013
AB	-5462.75	1	1926.70	-2.84	0.0364
AC	1883.13	1	1926.70	0.98	0.3733
AD	5203.50	1	1926.70	2.70	0.0427
BC	-4603.87	1	1926.70	-2.39	0.0624
BD	-3427.75	1	1926.70	-1.78	0.1354
ACD	-5202.63	1	1926.70	-2.70	0.0428
ABC	-2312.00	1	1926.70	-1.20	0.2839
ABD	-5367.12	1	1926.70	-2.79	0.0386
BCD	-4407.25	1	1926.70	-2.29	0.0709
C/ Point	6827.00	1	4308.22	1.58	0.1739

Appendix 10. Selected factorial model – ANOVA of loss tangent (tan δ) of canola protein isolate-guar gum systems.

Response: Tan δ

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	Ggum	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	0.13	15	8.840E-03	368.32	0.0002
Curvature	2.245E-04	1	2.245E-04	9.35	0.0551
Residual	7.200E-05	3	2.400E-05		
Pure Error	7.200E-05	3	2.400E-05		
Cor Total	0.13	19			

Root MSE	4.899E-03	R-Squared	0.9995	
Dep Mean	0.20	Adj R-Squared	0.9967	
C.V.	2.42			
PRESS	N/A	Adeq Precision	88.561	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	0.20	1	1.225E-03		
A-pH	-0.034	1	1.225E-03	-28.07	< 0.0001
B-Salt	0.022	1	1.225E-03	17.86	0.0004
C-Ggum	0.032	1	1.225E-03	26.03	0.0001
D-Protein	-0.024	1	1.225E-03	-19.90	0.0003
AB	-0.017	1	1.225E-03	-13.78	0.0008
AC	-0.022	1	1.225E-03	-17.86	0.0004
AD	0.017	1	1.225E-03	13.78	0.0008
BC	0.019	1	1.225E-03	15.82	0.0005
BD	-0.022	1	1.225E-03	-17.86	0.0004
CD	-0.024	1	1.225E-03	-19.90	0.0003
ABC	-0.019	1	1.225E-03	-15.82	0.0005
ABD	0.024	1	1.225E-03	19.90	0.0003
ACD	0.022	1	1.225E-03	17.86	0.0004
BCD	-0.022	1	1.225E-03	-17.86	0.0004
ABCD	0.024	1	1.225E-03	19.90	0.0003
C/ Point	-8.375E-03	1	2.739E-03	-3.06	0.0551

Appendix 11. Selected factorial model – ANOVA of emulsion stability (ES) of canola protein isolate-κ-carrageenan mixtures.

Response: ES

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	κ-CAR	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.0	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob >F
Model	1537.19	11	139.74	12.50	0.0014
Curvature	1.51	1	1.51	0.14	0.7239
Residual	78.25	7	11.18		
Lack of Fit	76.25	4	19.06	28.59	0.0101
Pure Error	2.00	3	0.67		
Cor Total	1616.95	19			

Root MSE	3.34	R-Squared	0.9516
Dep Mean	81.55	Adj R-Squared	0.8754
C.V.	4.10	Pred R-Squared	0.2433
PRESS	1223.56	Adeq Precision	11.408 Desire >4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob >|t|
Intercept	81.69	1	0.84		
A-pH	1.81	1	0.84	2.17	0.0668
B-Salt	3.44	1	0.84	4.11	0.0045
C-κ-CAR	-7.19	1	0.84	-8.60	< 0.0001
D-Protein	0.19	1	0.84	0.22	0.8289
AC	-2.81	1	0.84	-3.36	0.0120
AD	2.06	1	0.84	2.47	0.0430
BC	-1.19	1	0.84	-1.42	0.1984
BD	-1.06	1	0.84	-1.27	0.2443
CD	2.81	1	0.84	3.36	0.0120
ACD	-2.56	1	0.84	-3.07	0.0182
BCD	0.31	1	0.84	0.37	0.7196
C/ Point	-0.69	1	1.87	-0.37	0.7239

Appendix 12. Selected factorial model – ANOVA of emulsifying activity index (EAI) of canola protein isolate-κ-carrageenan mixtures.

Response: EAI

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.05	0.25
C	κ-CAR	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob >F
Model	27586.63	11	2507.88	6.84	0.0088
Curvature	1092.98	1	1092.98	2.98	0.1278
Residual	2565.04	7	366.43		
Lack of Fit	1427.54	4	356.88	0.94	0.5418
Pure Error	1137.51	3	379.17		
Cor Total	31244.66	19			

Root MSE	19.14	R-Squared	0.9149
Dep Mean	146.01	Adj R-Squared	0.7812
C.V.	13.11	Pred R-Squared	0.2043
PRESS	24862.84	Adeq Precision	9.531 Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob >|t|
Intercept	149.71	1	4.79		
A-pH	-10.86	1	4.79	-2.27	0.0576
B-Salt	27.74	1	4.79	5.80	0.0007
C-κ-CAR	-1.53	1	4.79	-0.32	0.7583
D-Protein	5.61	1	4.79	1.17	0.2797
AB	-19.32	1	4.79	-4.04	0.0050
AC	3.78	1	4.79	0.79	0.4554
AD	10.97	1	4.79	2.29	0.0556
BC	14.28	1	4.79	2.98	0.0204
BD	-6.18	1	4.79	-1.29	0.2375
CD	0.42	1	4.79	0.088	0.9327
ACD	7.26	1	4.79	1.52	0.1732
C/ Point	-18.48	1	10.70	-1.73	0.1278

Appendix 13. Selected factorial model – ANOVA of emulsifying activity index (EAI) of canola protein isolate-guar gum mixtures.

Response: EAI

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.050	0.25
C	Ggum	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob >F
Model	54824.83	12	4568.74	6.15	0.0179
Curvature	19028.28	1	19028.28	25.60	0.0023
Residual	4459.52	6	743.25		
Lack of Fit	4455.89	3	1485.30	1228.36	<0.0001
Pure Error	3.63	3	1.21		
Cor Total	78312.63	19			

Root MSE	27.26	R-Squared	0.9248
Dep Mean	170.81	Adj R-Squared	0.7743
C.V.	15.96	Pred R-Squared	-0.6185
PRESS	1.268E+05	Adeq Precision	10.406 Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	186.24	1	6.82		
A-pH	29.41	1	6.82	4.32	0.0050
B-Salt	-11.64	1	6.82	-1.71	0.1386
C-Ggum	6.34	1	6.82	0.93	0.3883
D-Protein	-12.25	1	6.82	-1.80	0.1224
AB	-1.64	1	6.82	-0.24	0.8181
AC	1.11	1	6.82	0.16	0.8757
AD	5.10	1	6.82	0.75	0.4826
BC	20.69	1	6.82	3.04	0.0229
BD	-28.72	1	6.82	-4.21	0.0056
CD	-18.10	1	6.82	-2.66	0.0377
ABC	10.04	1	6.82	1.47	0.1913
ACD	-22.90	1	6.82	-3.36	0.0152
C/ Point	-77.11	1	15.24	-5.06	0.0023

Appendix 14. Selected factorial model – ANOVA of emulsion stability (ES) of canola protein isolate-guar gum mixtures.

Response: ES

Factor	Name	Units	Type	-1 Level	+1 Level
A	pH		Numeric	6.00	10.00
B	Salt Conc	M	Numeric	0.050	0.25
C	Ggum	%	Numeric	1.00	3.00
D	Protein conc	%	Numeric	10.00	20.00

Source	Sum of Squares	DF	Mean Square	F Value	Prob >F
Model	929.85	12	77.49	133.41	< 0.0001
Curvature	183.01	1	183.01	315.09	< 0.0001
Residual	3.48	6	0.58		
Lack of Fit	3.48	3	1.16	6.366E+07	< 0.0001
Pure Error	0.000	3	0.000		
Cor Total	1116.35	19			

Root MSE	0.76	R-Squared	0.9963	
Dep Mean	93.95	Adj R-Squared	0.9888	
C.V.	0.81	Pred R-Squared	0.9112	
PRESS	99.13	Adeq Precision	27.602	Desire > 4

Factor	Coefficient Estimate	DF	Standard Error	t for H_0 Coeff=0	Prob > |t|
Intercept	92.44	1	0.19		
A-pH	-0.025	1	0.19	-0.13	0.8999
B-Salt	7.56	1	0.19	39.69	< 0.0001
C-Ggum	0.075	1	0.19	0.39	0.7075
D-Protein	-0.18	1	0.19	-0.92	0.3938
AB	0.025	1	0.19	0.13	0.8999
AC	0.56	1	0.19	2.95	0.0255
AD	0.16	1	0.19	0.85	0.4265
BC	-0.075	1	0.19	-0.39	0.7075
BD	0.18	1	0.19	0.92	0.3938
CD	-0.44	1	0.19	-2.30	0.0614
ABC	-0.56	1	0.19	-2.95	0.0255
ACD	0.000	1	0.19	0.000	1.0000
C/ Point	7.56	1	0.43	17.75	< 0.0001