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Food Chemistry 95 (2006) 255–263
Food Chemistry
www.elsevier.com/locate/foodchem

Surface hydrophobicity of commercial canola proteins mixed with κ-carrageenan or guar gum

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Received 13 September 2004; received in revised form 4 January 2005; accepted 4 January 2005

Abstract

The presence of hydrophobic patches on the protein surface can aid in its ability to adsorb at an oil–water or air–water interface. The surface hydrophobicity (S_0) of canola protein isolate (CPI)-hydrocolloid (κ-carrageenan, guar gum) systems was evaluated by fluorometric testing under varied conditions (NaCl, CPI and hydrocolloid concentrations; pH) using 8-Anilino-1-NaphthaleneSulphonate as a fluorescent probe. The S_0 values of CPI-hydrocolloid mixtures increased as hydrocolloid concentrations increased. High S_0 values reflect increased exposure of nonpolar amino acid residues due to changes in protein structure. This effect was greater at high pH and when guar gum was used.

Keywords: Fluorescence probe; Hydrophobic patches; Hydrocolloids; Functional ingredients

1. Introduction

In food systems, proteins interact with food components such as polysaccharides and this modifies protein and/or polysaccharide functionality (Damodaran, 1996). The overall structure–function relationships of food systems containing both biopolymers depends on the individual biopolymers as well as the strength and interaction of the biopolymers (Galazka, Smith, Ledward, & Dickinson, 1999). 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-carrageenan provide stability to milk products by interacting directly with the milk proteins (Drohan, Tziboula, McNulty, & Horne, 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, Aminot, Somard, Brisson, & Jones, 1984).

An understanding of the functional characteristics of canola proteins and the changes they undergo during processing are essential to compete with traditional food proteins. Surface and emulsifying properties of proteins are strongly correlated to their structure (Damodaran, 1996; Dickinson & Stainsby, 1988). 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 & Ma, 1989; Koning & Visser, 1992), and thus protein hydrophobicity (Alizadeh-Pasdar & Li-Chan, 2001). Galazka et al. (1999) studied the effect of high pressure treatment on bovine serum albumin (BSA)-sulphated polysaccharide complexes, and noted that the addition of κ-carrageenan or ι-carrageenan to BSA (at pH 7)

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doi:10.1016/j.foodchem.2005.01.030

decreased the surface hydrophobicity (S_0) of the protein. They suggested that the decrease in S_0 was mainly due to electrostatic repulsion between the two negatively charged molecules (κ-carrageenan, 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 (Royer, 1995; Slavic, 1994) and a number of fluorescence probes have been used for this purpose. 8-Anilino-1-NaphthaleneSulfonate (ANS), an anionic hydrophobic probe (Alizadeh-Pasdar & Li-Chan, 2001) is the most popular marker (Clarke & Nakai, 1972; Horiuchi, Fukushima, Sugimoto, & Hattori, 1978). *cis*-Parinaric acid ($C_{18}H_{28}O_2$; C18:4), a natural polyene fatty acid was used to assess the S_0 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_0 (Voutsinas, Nakai, & Harwalker, 1983). As shown by Alizadeh-Pasdar and Li-Chan (2001), an uncharged fluorescent probe, 6-Propionly-2-*N*-*N*-Dimethylamino-Naphthalene (PRODAN), has also been used to determine the hydrophobicity of proteins (whey protein isolate, β-lactoglobulin, BSA) mixed with κ-carrageenan. 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 examined the surface activity of CPI in multicomponent food systems.

2. Materials and methods

2.1. Source of materials

Commercial 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). As indicated by the manufacturer, the κ-CAR powder consists of the following cations: K^+ (10.4%), Ca^{2+} (2.3%) and Na^+ (0.9%). Commercial grade guar gum (G-4129; Lot 95H0653) and 8-Anilino-1-NapthaleneSulphonate (ANS; Lot 109C-0640; A-5144) were purchased from Sigma Chemical Co. (St. Louis, MO). 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% ($N \times 5.7$), 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 028091), HCl (A144-225, Lot 296220), and NaOH (BP359-212, Lot 974661) were certified reagent grade (Fisher Scientific Co., New Jersey).

2.2. 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 (Stat-Ease Inc., Minneapolis, MN). The mixture was stirred for approximately 1 h at room temperature or until a complete dispersion of the mixture was achieved. Dispersions of CPI alone in appropriate pH and NaCl solutions were prepared in similar manner to serve as 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.

2.3. Experimental design

All experimental measurements were performed in duplicate. Design-Expert® Software 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 solvent conditions (pH, NaCl concentration). In stage two, series of experiments were carried out to characterize different combinations of CPI and hydrocolloids (i.e., 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-CAR (1, 3% w/v) concentrations. The surface hydrophobicity (S_0) of the samples was measured. Two-dimensional contour plots were generated from the fitted model using Design-Expert® Software.

2.4. Fluorometry: measurements of hydrophobic region on molecular surface

Surface hydrophobicity (S_0) 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_0 of CPI-κ-CAR and CPI-guar gum systems were studied at varied pH (6, 10) and salt concentration (0.05, 0.25 M). Each sample was serially diluted with 0.1 M 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) 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_0) 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. Control tests of CPI alone at varied concentration (10, 20%, w/v), pH (6, 10) and salt concentration (0.05, 0.25 M) were also carried out. All determinations were done in duplicate. Several conditions (e.g., pH, salt and protein concentration) encountered in conventional food systems affect the functional properties and interactions of proteins. As cited by Phillips, Whitehead, and Kinsella (1994), the addition of salts (e.g., Na_2SO_4, NaCl or NaSCN at $\leqslant$0.1 M) improved the foaming properties of β-lactoglobulin via reduced charge repulsion (an electrostatic effect). Positive correlations between the exposed hydrophobic patches on protein surfaces and the adsorption capacity of some proteins (e.g., β-casein) have been reported (Horiuchi et al., 1978). In the present study, the conditions stated above were varied to identify some characteristics of CPI in multicomponent food systems.

2.5. Statistical analysis

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). Duncan's multiple range test was used to determine statistical differences ($P \leqslant 0.05$) between treatment means (Steel, Torrie, & Dickie, 1997).

3. Results and discussions

3.1. Canola protein isolate-κ-carrageenan systems

The model equation from the full factorial design for surface hydrophobicity (S_0) values of canola protein-κ-carrageenan mixtures at varied conditions is as follows:

$$S_0 = 224.08 - 11.85\text{pH} - 326.88\text{NaCl} - 48.74\kappa\text{-CAR} + 0.82\text{CPI} + 57.97\text{pH} * \text{NaCl} + 5.14\text{pH} * \kappa\text{-CAR} - 0.061\text{pH} * \text{CPI} + 233.44\text{NaCl} * \kappa\text{-CAR} - 8.66\text{NaCl} * \text{CPI} + 0.11\kappa\text{-CAR} * \text{CPI} - 30.03\text{pH} * \text{NaCl} * \kappa\text{-CAR} + 0.037\text{pH} * \kappa\text{-CAR} * \text{CPI} \ (P = 0.013).$$

Significant factors and interactions were identified using the ANOVA model (Design-Expert® software). κ-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 ($P < 0.05$). To best demonstrate the nature of these interactions, contour plots have been used (Figs. 1–4).

3.1.1. Effects of pH-NaCl-κ-carrageenan interaction on surface hydrophobicity

The contour plots of S_0 as a function of NaCl and κ-CAR concentrations at pH 6 and 10 are shown in Fig. 1. S_0 is indicative of the number of hydrophobic amino acid residues on the protein surface. At pH 6 (Fig. 1(a)), the interaction between NaCl and κ-CAR affected the protein or protein-κ-CAR conformation. It was evident from the curves that S_0 values decreased with increasing levels of κ-CAR. At κ-CAR levels below 2% (w/v), higher S_0 values were associated with low NaCl levels. It is possible that low S_0 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_0 values.

At pH 10 (Fig. 1(b)), the interaction between CPI and the other system components (e.g., NaCl and κ-CAR) became more complex. S_0 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_0 values observed at higher NaCl level in the present study.

3.1.2. Impact of canola protein-pH-κ-carrageenan interaction on surface property

The contour plots of S_0 as a function of κ-CAR and CPI concentrations at pH 6 and 10 are shown in Fig. 2. At pH 6, S_0 decreased as κ-CAR concentration increased (Fig. 2(a)). κ-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

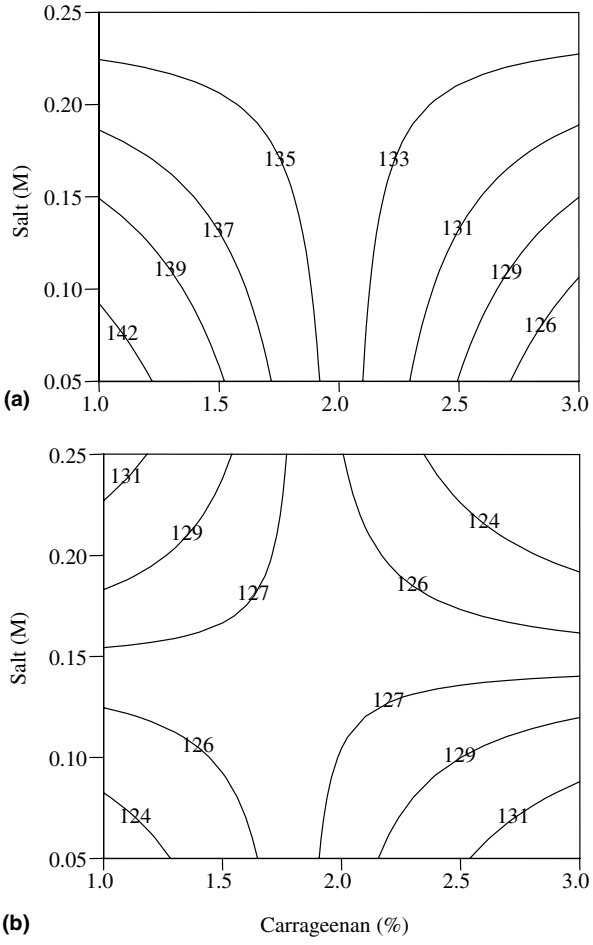

Fig. 1. Contour plots showing the influence of κ-carrageenan (κ-CAR) and NaCl concentrations on the surface hydrophobicity (S_0) of CPI-κ-CAR systems at 15% (w/v) CPI and varied pH (a: pH 6; b: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_0 value. For example, in (b); at 2.5% (w/v) κ-CAR and 0.1 M NaCl, $S_0 = 129$.

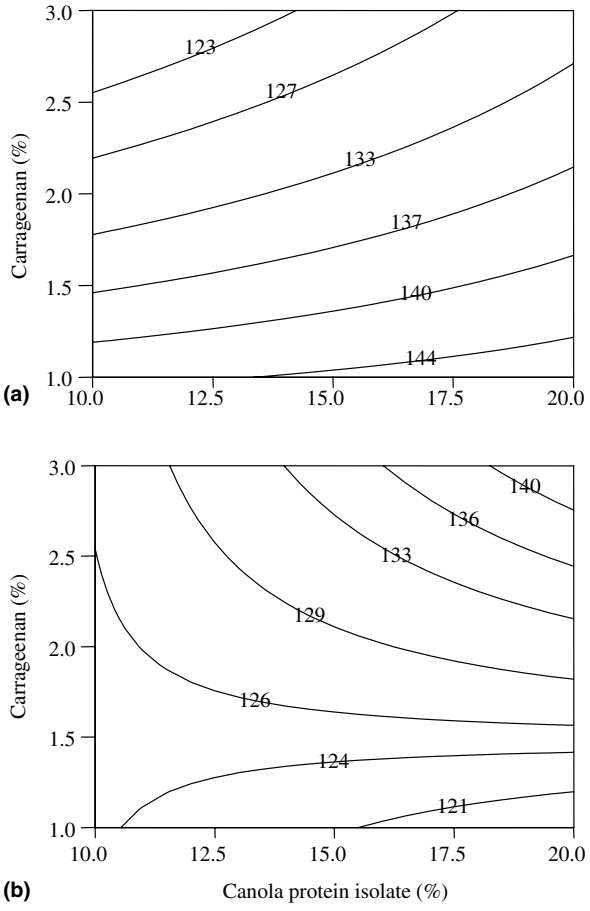

Fig. 2. Contour plots showing the effects of canola protein isolate (CPI) and κ-carrageenan (κ-CAR) concentrations on the surface hydrophobicity (S_0) of CPI-κ-CAR systems at 0.05 M NaCl and varied pH (a: pH 6; b: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_0 value. For example, in (a); at 15% (w/v) CPI and 2% (w/v) κ-CAR, $S_0 = 133$.

an electrostatic interaction results between the two biopolymers (Dalgleish & Hollocou, 1996). This interaction increased as κ-CAR concentration increased, thus making sites on the protein molecule inaccessible for binding with ANS. This may explain the low S_0 values observed at high κ-CAR levels. In contrast, at pH 10, increased S_0 resulted as κ-CAR and CPI levels increased (Fig. 2(b)). 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_0 values as CPI and κ-CAR levels increased. The increase in S_0 could also be due to quantitative effects (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. 2(b)). This binding of ANS to hydrophobic sites on the canola protein surface indicates that these hydrophobic areas are available for other possible reactions. With the effect of increasing κ-CAR level at pH 10, the high pH possibly exposed the buried hydrophobic groups in the protein molecule. 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_0 of whey protein isolate at high pH (e.g., pH 9) in the presence of κ-CAR, and attributed the increase in S_0 to increased exposure of hydrophobic sites resulting from the unfolding of protein molecules in the presence of κ-CAR.

3.2. Canola protein isolate-guar gum systems

The model equation from the full factorial design for surface hydrophobicity (S_0) values of canola pro-

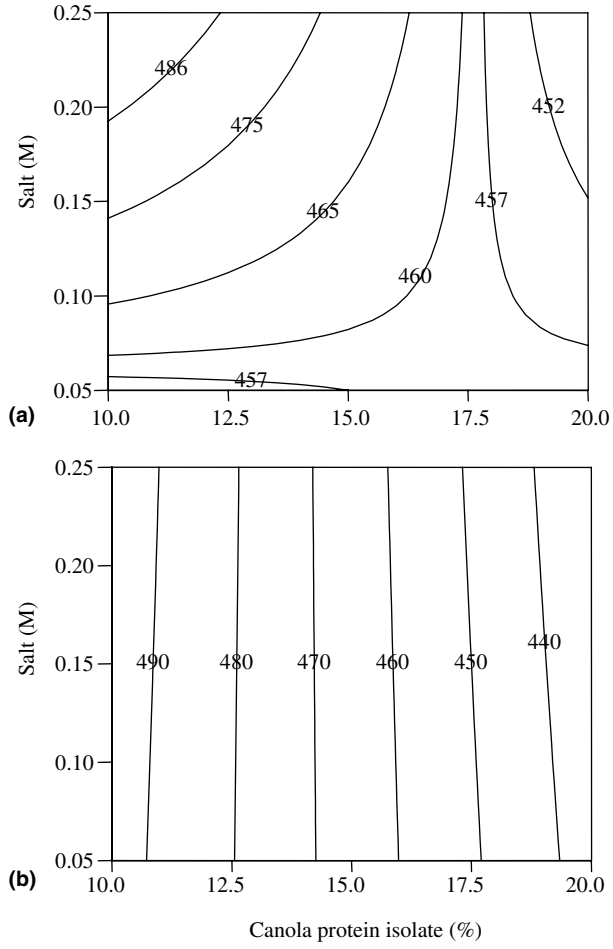

Fig. 3. Contour plots showing the influence of canola protein isolate (CPI) and NaCl concentrations on the surface hydrophobicity (S_0) of CPI-guar gum systems at 1.5% (w/v) guar gum and varied pH (a: pH 6; b: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_0 value. For example, in (a); at 12.5% (w/v) CPI and 0.1 M NaCl, $S_0 = 465$.

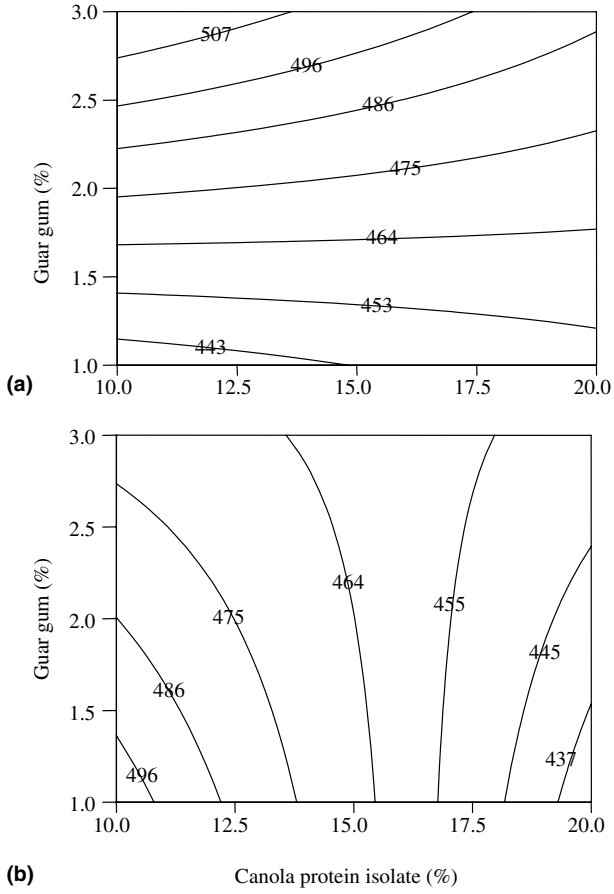

Fig. 4. Contour plots showing the influence of canola protein isolate (CPI) and guar gum concentrations on the surface hydrophobicity (S_0) of CPI-guar gum systems at 0.05 M NaCl and varied pH (a: pH 6; b: pH 10). The significant interaction identified by the ANOVA model was used to produce the contour plots. Each contour line corresponds to a given S_0 value. For example, in (b); at 12.5% (w/v) CPI and 2% (w/v) guar gum, $S_0 = 475$.

tein-guar gum mixtures at varied conditions is as follows:

$$\begin{aligned} S_0 = {} & -355.97 + 99.34\text{pH} + 6528.39\text{NaCl} + 404.09\text{Ggum} \\ & + 39.79\text{CPI} - 698.99\text{pH} * \text{NaCl} - 46.26\text{pH} * \text{Ggum} \\ & - 5.06\text{pH} * \text{CPI} - 3608.04\text{NaCl} * \text{Ggum} \\ & - 327.41\text{NaCl} * \text{CPI} - 18.22\text{Ggum} * \text{CPI} \\ & + 394.57\text{pH} * \text{NaCl} * \text{Ggum} + 35.18\text{pH} * \text{NaCl} \\ & * \text{CPI} + 2.17\text{pH} * \text{Ggum} * \text{CPI} + 176.59\text{NaCl} \\ & * \text{Ggum} * \text{CPI} - 19.50\text{pH} * \text{NaCl} * \text{Ggum} \\ & * \text{CPI} \ (P < 0.05). \end{aligned}$$

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 ($P < 0.05$).

3.2.1. Effect of CPI-pH-NaCl interaction on molecular conformation

The contour plots of S_0 as a function of NaCl and CPI concentrations at pH 6 and 10 are shown in Fig. 3. At pH 6 (Fig. 3(a)), the S_0 values increased as NaCl levels increased and CPI concentration decreased. Reports on the effect of NaCl on protein structure have been varied. NaCl has been described as a stabilizing salt by its ability to increase the thermal stability of plant proteins (Arntfield, Murray, & Ismond, 1986; Ismond, Murray, & Arntfield, 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 prior protein denaturation (Arntfield, Murray, & Ismond, 1990). It is possible that NaCl (at high molar concentration) acted to unfold

or partially denature the protein in the present study. This may explain the high S_0 values observed for CPI-guar gum mixtures at high NaCl concentration (Fig. 3(a)).

In contrast, at pH 10, S_0 values decreased as CPI concentration increased (Figs. 3(b) and 4(b)). 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 the protein–protein association that occurred at the lower protein level. The decrease in S_0 at increasing CPI level may be due to hydrophobic interactions between neighbouring non-polar residues (Samant, Singhal, Kulkarni, & Rege, 1993) following unfolding of polypeptide chains as stated by Paulson and Tung (1989).

3.2.2. Effects of canola protein-guar gum interaction on surface hydrophobicity

The 3-way interaction between CPI concentration, guar gum concentration and pH was significant ($P < 0.05$). The contour plots of S_0 as a function of NaCl and CPI concentrations at pH 6 and 10 are shown in Fig. 4. According to Petkowicz, Reicher, and Mazeau (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. 4(a)), S_0 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_0 values are higher. This change in protein conformation was more noticeable at high guar gum concentrations. 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_0 values as guar gum concentration increased.

3.3. Surface properties of canola protein as affected by hydrocolloids

Changes in the conformation of canola protein isolate (CPI) in mixed systems with hydrocolloids (κ-CAR, guar gum) and 0.5 M NaCl are further illustrated in Fig. 5. The S_0 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. 5(a)). Voutsinas et al. (1983) reported a S_0 value of 65 for a laboratory prepared CPI using *cis*-parinaric acid (measures aliphatic amino acid residues) as a fluorescence probe. In this

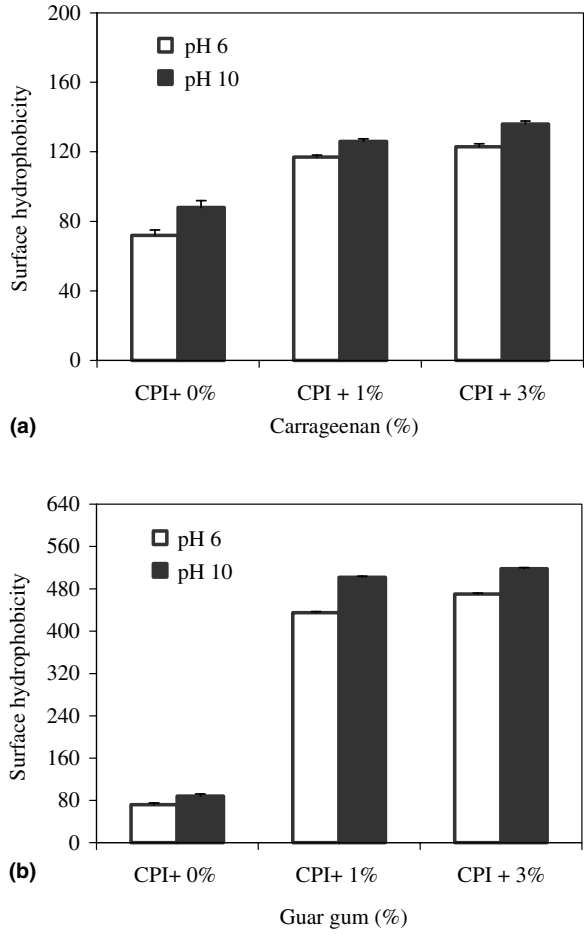

Fig. 5. Effects of κ-carrageenan (κ-CAR) and guar gum concentrations on the surface hydrophobicity of canola protein isolate (CPI)-hydrocolloid systems (10% w/v CPI, 0.05 M NaCl). (a) CPI-κ-CAR mixtures; (b) CPI-guar gum mixtures. Error bars represent standard deviation.

study (Fig. 5), CPI-κ-CAR mixtures treated with 0.05 M NaCl and 1% κ-CAR had S_0 values of 117 (at pH 6) and 126 (at pH 10).

These data indicate that the S_0 values increased in the presence of κ-CAR. Kato, Tsutsui, Matsudomi, Kobayashi, and Nakai (1981) revealed that most proteins probably increase their S_0 as denaturation proceeds, because the hydrophobic residues buried in the interior of proteins were exposed at the molecular surface. For example, the emulsifying properties of ovalbumin and lysozyme increased with denaturation, and correlated linearly with S_0 (Kato et al., 1981). 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 increasing S_0 values in the presence of κ-CAR. Our results showed that the S_0 values were higher at pH 10 than at pH 6. This finding corroborates a report cited by Phillips et al. (1994), which showed a compact structure of β-lactoglobulin at low pH with enhanced

susceptibility to surface denaturation and a more 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_0 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 the WPI molecules.

For CPI-guar gum systems, S_0 values increased in the presence of guar gum with higher S_0 values observed at pH 10 than at pH 6 (Fig. 5(b)) as was seen with CPI and κ-CAR. However, the S_0 values for CPI-guar gum mixtures were significantly ($P < 0.05$) higher than those for CPI-κ-CAR systems.

3.4. Comparison of surface hydrophobicity in CPI-κ-CAR and CPI-guar gum systems

It was noted that the S_0 values for all CPI-guar gum mixtures (Figs. 3–5(b); Table 1) were higher than those of CPI-κ-CAR systems (Figs. 1, 2 and 5(a); Table 1). One explanation could be due to the chemical properties of the two hydrocolloids used in this study. Guar gum is nonionic (Whistler & 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 interaction with ANS, while disrupting the protein structure.

Galazka et al. (1999) investigated bovine serum albumin (BSA)-sulphated polysaccharide complexes and showed that adding κ-CAR or ι-CAR to BSA (at pH 7) decreased S_0 of the protein. They assumed that the decrease in S_0 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 (Dickinson, 1998; Tolstoguzov, 1986). As stated by Tolstoguzov (1986), at pH values below the protein IEP, protein and anionic polysaccharides 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_0 values observed for CPI-κ-CAR systems when compared to CPI-guar gum mixtures (Fig. 5).

As reported by Alizadeh-Pasdar and Li-Chan (2001), decreases in the S_0 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 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_0 of CPI mixed with κ-CAR and NaCl at pH 6 and 10. The lower S_0 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 (such as the formation of hydrogen bonds; electrostatic interactions between CPI and NaCl; incompatibility between CPI and guar gum) occurred in CPI-guar gum systems which resulted in higher S_0 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 1. The S_0 values for CPI-κ-CAR and CPI-guar gum systems at 0.25 M NaCl (Table 1) were higher than those at 0.05 M NaCl (Fig. 5). It is possible that at 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 & Li-Chan, 2001).

Table 1
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_0[a] at pH 6	S_0[a] 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]

Within column, S_0 values with different letters are significantly different ($P < 0.05$).
[a] Means ± SD.

4. Conclusions

The surface hydrophobicity (S_0) of canola protein isolate (CPI) was affected by NaCl and hydrocolloid

concentrations as well as pH and hydrocolloid type. CPI and CPI-hydrocolloid mixtures each had higher S_0 at pH 10 than at pH 6. The increase in S_0 of CPI was greater in the presence of guar gum than in 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_0 greatly enhanced in the presence of guar gum and at pH 10. Higher S_0 values suggests increase in hydrophobic patches on the protein surface. As the number of these patches is increased, the potential for greater and rapid adsorption at an oil–water interface (which is required for emulsion formation) becomes more probable.

Acknowledgement

The financial support from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

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