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Network formation of canola protein–κ-carrageenan mixtures as affected by salts, urea and dithiothreitol

Florence O. Uruakpa*, S.D. Arntfield

Department of Food Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

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Abstract

Rheological characterization of plant proteins can be improved by inclusion of low levels of polysaccharides. The gelling behavior of canola protein isolate (CPI)–κ-carrageenan (κ-CAR) mixtures were evaluated to determine the effects of sodium salts (sulfate, acetate, chloride, and thiocyanate), urea, and dithiothreitol (DTT) on network formation and structure. The G' values for gels prepared from the CPI–κ-CAR mixtures in the presence of various 0.5 mol/l salts in the lyotropic series were ranked as follows: Cl^-–SO_4^{2-}–$C_2H_3O_2^-$ > SCN^-. $\tan\delta$ values ranked as follows: SO_4^{2-} > $C_2H_3O_2^-$ > SCN^-–Cl^-. A CPI–κ-CAR gel prepared in the presence of 360 g/l urea had a weak elastic network, indicating that CPI has sufficient linkages to support a gel network; this was not the case when κ-CAR was not part of the system. DTT-treated CPI–κ-CAR gels had G' values comparable to the untreated sample, suggesting that non-covalent forces play a major role in gel formation. Only weak interactions were seen for the CPI in DTT. Unlike gels formed with CPI alone, the gels from the CPI–κ-CAR mixtures provided enough covalent linkage to form a gel when non-covalent interactions are inhibited, and at the same time, did not require disulfide bonding to form strong elastic gel. This demonstrates that CPI–κ-CAR not only produces better networks than CPI alone, but these networks are less sensitive to environmental factors.

Keywords: Protein–hydrocolloid networks; Gel properties; Dynamic rheology

1. Introduction

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 properties) will determine its successful utilization. Léger and Arntfield (1993) reported that hydrophobic and electrostatic 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. Gill and Tung (1978) indicated that gel formation in the rapeseed protein system is a complex phenomenon which may involve covalent, ionic, disulfide, hydrophobic and hydrogen bonding. The strength of these networks, however, was not as good as those from egg white (Léger & Arntfield, 1993).

The addition of low levels of polysaccharides such as guar gum (Cai & Arntfield, 1997), and κ-carrageenan (κ-CAR) (Uruakpa & Arntfield, 2004) has been shown to improve gel properties in comparison to canola protein alone. The impact of protein polysaccharide mixtures on network formation has been attributed to a coacervation of the polymers (Grinberg & Tolstoguzov, 1997; Tolstoguzov, 1991) or an incompatibility between the polymers (Tolstoguzov, 1991). These reactions vary depending on the nature of the polysaccharide and the pH of the system (Imeson, Ledward, & Mitchell, 1977).

The anionic sulfated polysaccharide κ-CAR has been used to improve gelation in a number of mixed systems. An improvement in the gelation process has been

*Corresponding author. Tel.: +1 204 474 9621; fax: +1 204 474 7630.
E-mail address: Ojiugou@yahoo.com (F.O. Uruakpa).

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demonstrated for β-lactoglobulin (Capron, Nicolai, & Durand, 1999; Capron, Nicolai, & Smith, 1999; Ould Eleya & Turgeon, 2000a, b) and bovine serum albumin (Oakenfull, Nishinari, & Miyoshi, 2000). These networks were believed to result from two co-continuous separate phases (Ould Eleya & Turgeon, 2000a, b). In a recent investigation, the optimum conditions for the formation of a gel containing canola protein and κ-CAR was shown to be at pH 6, with 0.05 mol/l NaCl concentration, 3 g/100 g κ-CAR and 15 g/100 g canola protein isolate (CPI) (Uruakpa & Arntfield, 2004).

This research has been undertaken to determine the interactions responsible for canola protein–κ-CAR network formation by altering the environment in which gelation takes place. The addition of sodium salts to CPI–κ-CAR dispersions can be an effective probe for evaluating the contribution of hydrophobic interactions to the network formation process. 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 & Kinsella, 1982). A conventional method for studying disulfide bonds interactions is the inclusion of a reagent that modifies or prevents the formation of disulfide bonds prior to heat treatment. These reagents include dithiothreitol (DTT) cysteine hydrochloride (CysHCl) and 2-mercaptoethanol (ME); DTT will be used in this study. The contribution of hydrogen bonds can be evaluated by including urea (Léger & Arntfield, 1993). Through the inclusion of various salts, urea and DTT, we hope to determine how the interactions responsible for the formation of canola protein–κ-CAR gels differ from those for canola protein alone.

2. Materials and methods

2.1. Source of materials

Commercial grade κ-CAR (κ-CAR; C-1013) that contains predominantly κ- and lesser amounts of λ-carrageenan, was purchased from Sigma Chemical Co. (St. Louis, MO). The exact κ-CAR concentration was not available; however, the manufacturer indicated that it was a mixture of the following cations: K^+ (10.4 g/100 g), Ca^{2+} (2.3 g/100 g) and Na^+ (0.9 g/100 g). Commercial CPI was purchased from BMW Canola, Winnipeg, and used without further purification. The ratio of 2S to12S/7S proteins of the CPI was 3:97. Proximate analysis (AOAC, 1990) of the CPI sample indicated a protein content of 87 g/100 g ($N \times 5.7$), 0.7 g/100 g oil, 2 g/100 g ash, 5.9 g/100 g moisture and 4.4 g/100 g total carbohydrate (determined by difference). Urea (U-15; Lot 863571) and DTT (D-0632; Lot 61K16571) were purchased from Sigma Chemical Co. (St. Louis, MO). $NaC_2H_3O_2$ (Lot 7364 KCLZ) was procured from Mallinckrodt Inc. (Paris, Kentucky). All other chemicals such as NaCl (BP358-212; Lot 028091), HCl (A144-225; Lot 296220), NaOH (BP359-212; Lot 974661), NaSCN (S441-500; Lot 987676) and Na_2SO_4 (S421-500; Lot 985711) were certified reagent grade (Fisher Scientific Co., Fair Lawn, NJ).

2.2. Experimental design

Optimum conditions for gelation of CPI–κ-CAR mixtures (pH 6, 0.05 mol/l NaCl, 15 g/100 g CPI, 3 g/100 g κ-CAR) that were established previously (Uruakpa & Arntfield, 2004) are being used as the basis for this investigation. To examine the effects of neutral salts, sodium sulfate (Na_2SO_4), sodium acetate ($NaC_2H_3O_2$), sodium chloride (NaCl) and sodium thiocyanate (NaSCN) at concentrations of 0.05 and 0.5 mol/l were used in place of the 0.05 mol/l NaCl. The urea (360 g/l) and DTT (23.1 g/l) were added to the mixture containing no salt. Samples containing 15 g/100 g CPI alone were prepared in either urea or DTT.

2.3. Sample preparation

Dispersions of CPI and CPI–κ-CAR in the appropriate mixture were prepared by stirring for approximately 1 h at room temperature or until a complete dispersion of the mixture was achieved. Samples were adjusted to pH 6 with 1 mol/l NaOH or 1 mol/l 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.

2.4. Rheology: assessment of gel properties

Dynamic rheological testing on an Advanced Rheometer 2000 (AR2000, TA Instruments, New Castle, DE) was used to monitor CPI–κ-CAR network formation during heating and cooling as well as to characterize the resulting networks. The AR2000 was equipped with 40 mm 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 visco-elastic region in an experiment with laboratory-prepared CPI (Arntfield, Murray, & Ismond, 1990a). This strain was used for all rheological measurements.

Approximately 1 ml CPI–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 sample drying during heating, paraffin oil (Mallinckrodt, Paris, Kentucky; Lot 6358 KJPC) was placed in the shallow well