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Dispersion of food proteins in water-alcohol mixed dispersants

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Abstract

Dispersions of commercial casein and whey protein and laboratory-prepared soybean protein were studied in mixed dispersants of water with various aliphatic alcohols, methanol, ethanol, *n*-propanol and 2-propanol. Supernatant and protein sediments were separated by centrifugation in two steps: 1800 rpm 10 min, followed by centrifugation of the supernatant at 50 000 rpm for 60 min (125 000×***g***). A gel-like protein sediment obtained at low alcohol concentration by high-***g*** centrifugation increased in amounts as a function of the alcohol concentration until it progressively transformed, with higher alcohol concentrations, into an opaque flock (precipitate), sedimenting at 1800 rpm. It was concluded that the sediment obtained by ultracentrifugation was a protein of increased density which was produced by partial and progressive dehydration and alcohol binding. The conversion of the sediment into a flock or precipitate is discussed in terms of the hydrophilic-lipophilic balance of the protein and of the polar-nonpolar character of the dispersant.

Keywords: Casein; Soybean; Whey protein; Methanol; Ethanol; Propanol; Dispersion; Precipitation

1. Introduction

Protein is an important food component. Apart from its contribution to the nutritional value, it plays a role in texture and flavour through complex interactions with other food ingredients. Thickening, aggregation, gelation, emulsion and foam formation are among the functional properties associated with proteins (Damodaran, 1994). Most of these properties depend on the balance between protein-protein and protein-dispersant interactions. In food processes, many factors can influence protein structure and its ability to interact with other constituents. Heating, shearing or the use of high pressure were shown to alter protein structure and properties (Spiegel, 1999; Tedford, Kelly, Price & Schaschke, 1998). Proteolytic enzymes can increase protein solubility and interfacial activity, but usually decrease gelation and emulsion stability (Nakai & Li-Chan, 1988). Changes in protein environment (pH, ionic strength, polarity) can also affect its behaviour in food systems. In order to optimize the processed foods properties, all factors affecting protein structure need to be controlled.

Changes in pH and ionic strength alter the ionization of protein functional groups and the thickness of the electrical double layer, affecting protein–protein interactions (Walstra & Jenness, 1984). The influence of these factors on protein voluminosity and aggregation has been previously studied (Boulet, Britten & Lamarche, 1998, 2000). Organic solvents added to protein aqueous dispersions were also shown to affect protein structure (Griebenow & Klibanov, 1996). Alcohol-induced precipitation was proposed to produce protein concentrates from cheese whey (Morr & Lin, 1970). Addition of alcohol to milk was studied in relation to the colloidal stability of casein micelle dispersions (Horne & Parker, 1980). Alcohols modify protein structure and interaction properties through specific binding, the binding being accompanied by dehydration (Bull & Breese, 1978). As a rule, protein denaturation increases with increasing alcohol chain length (Herskovits, Gadegbeku & Jaillet, 1970) and decreasing hydroxyl content (Bull & Breese, 1978). Branching of the hydrocarbon chain was shown to decrease the effectiveness of denaturation (Herskovits et al., 1970). Protein–protein interactions are also

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influenced by the non specific effect of alcohols on the dielectric constant of the dispersant (Horne & Parker, 1981a). The study of protein dispersion in the presence of alcohol is useful to better-understand the factors responsible for protein–protein interactions. It is the purpose of this paper to determine the effect of increasing alcohol concentration on the colloidal properties of some food proteins.

2. Materials and methods

Casein (Anachemia, Sigma Chemicals, USA) soybean protein and commercial whey protein (Bipro from Le Sueur Isolates, Le Sueur, USA) were dipersed in water and the pH was adjusted to predetermined values with a solution of NaOH. The dispersions were then freeze-dried and the resulting preparations were used as the experimental material. Dispersions were prepared by agitating, together, 15 ml of water or water-alcohol mixed dispersant and 0.600 g of dry protein preparation 16 h at $23 \pm 1^{\circ}C$. The dispersant contained various alcohols — methanol, ethanol, *n*-propanol and 2-propanol, in various proportions to water to give various mole fractions (M/M). The presence of the alcohol in the dispersion affects its pH but no attempt was made to control it. Replicate preparations of the dispersions were centrifuged 10 min at 1800 rpm to remove sedimentable protein or precipitate. The supernatant was then centrifuged at 50 000 rpm, or $125\,000 \times g$, for 60 min and the residue was considered the sedimentable colloidal fraction. Protein concentration in the supernatant was determined by subtracting the dry weight of the residue from the initial protein dry weight and then converting to %w/v using the partial specific volume, 0.73 ml/g. The effect of the alcohol concentration on the partial specific volume was not considered. Standard deviation of the protein concentration in the centrifuged extracts varied randomly with replicates between 0.1 and 3.7% for casein and between 0.0 and 4.4% for whey protein.

3. Results

The distribution of casein in the centrifuged extracts as a function of the ethanol concentration is illustrated in Fig. 1. The precipitate, obtained with 1800 rpm centrifugation, appeared as an opaque coagulum or flock while the sediment obtained at 50 000 rpm was a translucent gel or colloidal sediment. This result indicates that the destabilized protein was not uniform in its properties but probably consisted of particles of different solvation, size (degree of aggregation) and density (voluminosity). Colloidal casein particles, sedimenting in the form of a gel, increased with the ethanol concentration to a maximum and then decreased, suggesting the conversion of the gel into precipitate. It appears that, as the alcohol concentration was increased in the dispersant, the dispersed particles were progressively transformed into sedimentable particles with the 50 000 rpm centrifugation and finally the latter were converted into a flock or precipitate. This was a general observation since it was present with the three proteins and with the different pH values and alcohols used.

One to 5% casein was extracted with water and with the water-*n*-propanol mixed dispersants and the protein concentration in the high-***g*** supernatants was measured (Fig. 2). The curves show that the protein was totally dispersed, irrespective of the amount present and the alcohol concentration, until about 0.21–0.25 M/M alcohol. The concentration decreased at higher alcohol concentrations in two steps. The transition at 0.30–0.34 M/M corresponded to the appearance of the precipitate. The initiation of sedimentation, as well as the transition

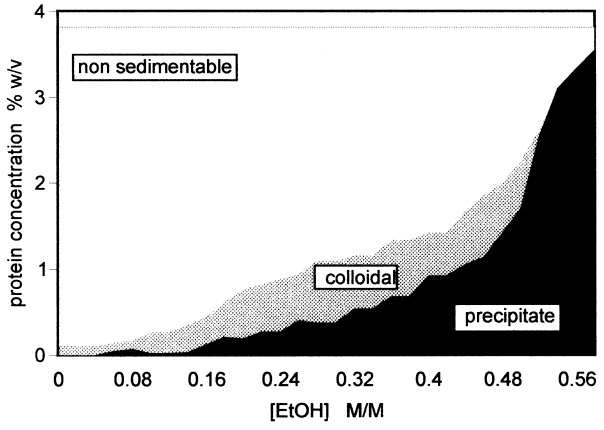

Fig. 1. Effect of ethanol molar fraction of dispersant on the distribution of casein following centrifugation at 1800 rpm 10 min (precipitate) and 50 000 rpm 60 min (colloidal). Total casein concentration was 3.88% w/v. The casein was prepared at pH 6.95.

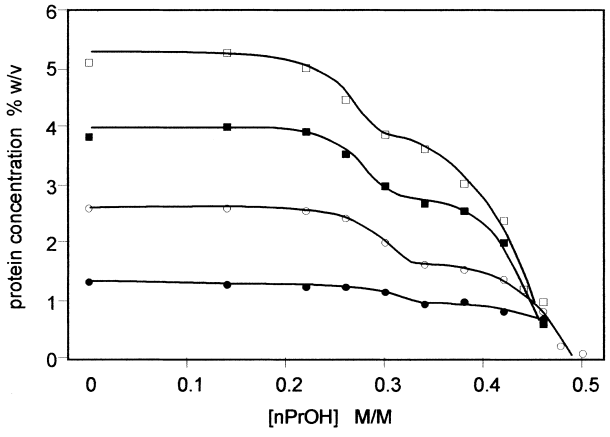

Fig. 2. Effect of *n*-propanol molar fraction of dispersant on casein concentration in the high-***g*** supernatant. Initial casein concentration was: ●, 1.34%; ○, 2.60%; ■, 3.84%; and □, 5.12%. The casein was prepared at pH 7.8.

in the curve, appeared to depend on the dispersant alcohol concentration only. This result excludes the possibility that solubility is the phenomenon controlling dispersibility, but suggests that colloidal properties of the protein are responsible for the dispersion stability. The proportion of the alcohol to water in the dispersant, required to initiate both high-***g*** sedimentation and precipitation, appeared to be increasing by about 0.04 M/M as the total protein concentration decreased over the range studied (Fig. 2). This indicates that the mixed dispersant mole fraction affects the stability of the protein but the latter may also be affected by the initial protein concentration or the ionic strength of the dispersant since they are both linked together in this experiment.

Comparing the dispersion curves of the various proteins at pH near neutrality (Fig. 3), it can be seen that dispersibility in the presence of ethanol varied greatly between proteins, casein being the most dispersable and soybean protein the least. The typical protein dispersion-alcohol concentration relationship described above is present to various degrees with the three proteins but is clearly shown by the whey protein dispersions.

The effect of the nature of the alcohol on the dispersibility of casein has been examined with casein at a fixed pH of 7.8 (Fig. 4). The concentration of protein in the high-***g*** supernatants started to decrease at about the same alcohol concentration (0.2 M/M) with the different alcohols but the rate of change diverged markedly as the alcohol concentration increased. The rate of decrease of dispersibility of the protein appeared to be related to the size of the nonpolar residue of the alcohol: the larger the residue, the greater the rate.

The effect of pH on the dispersibility of casein has been examined in water-ethanol and in water-2-propanol mixed dispersants (Fig. 5a, b). A gradual decrease of the supernatant concentration was obtained with casein at pH 5.95, corresponding to the sedimentation of colloidal particles. Abrupt changes of the slope of the concentration curves corresponding to the transition and precipitation, occurred with this protein and the two alcohols. Increases of the casein pH extended the initiation of sedimentation and the transition to higher alcohol concentrations, indicating that increasing the protein pH counteracted the effect of the alcohol on dispersibility. A concentration curve of casein at pH 1.83 in water-ethanol mixed dispersant showed initiation of sedimentation at the alcohol concentration of 0.36 M/M and a transition at 0.63 M/M (curve not shown). The initiation of sedimentation and the transition occurred at high ethanol concentrations in the extreme acid as well as extreme alkaline pH, indicating that the dispersibility of the positively as well as the negatively charged caseins are similarly affected by the alcohol concentration.

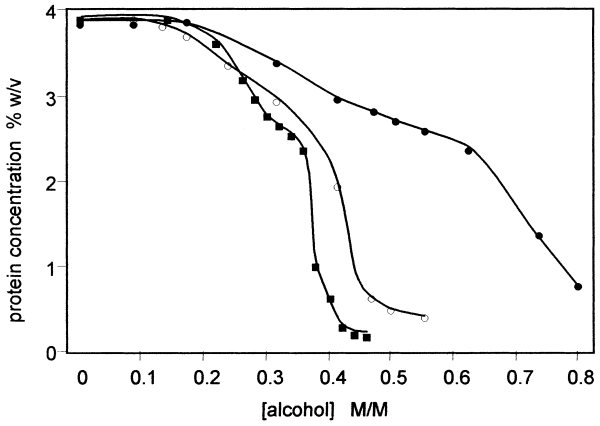

Fig. 4. Effect of alcohol molar fraction of dispersant on casein concentration in high-***g*** supernatants. Dispersant alcohols were: ●, methanol; ○, ethanol; and ■, 2-propanol. Casein was prepared at pH 7.8.

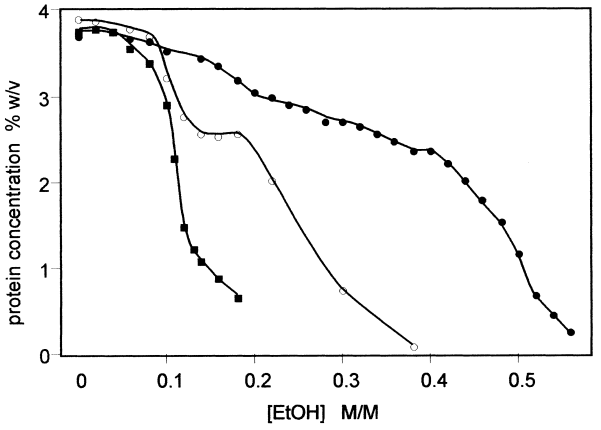

Fig. 3. Effect of ethanol molar fraction of dispersant on protein concentration in the high-***g*** supernatants: ●, casein prepared at pH 6.95; ○, whey protein prepared at pH 6.90; and ■, soybean protein prepared at pH 7.50.

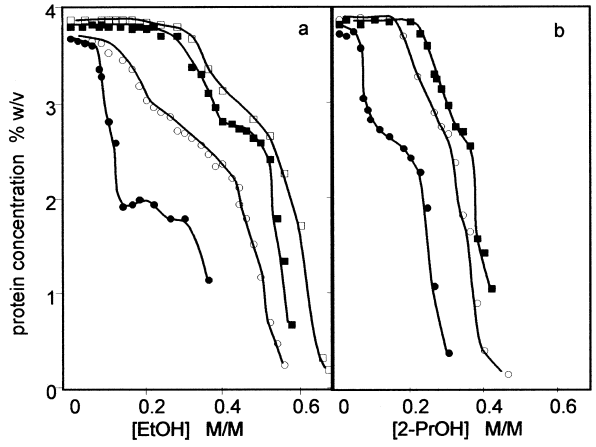

Fig. 5. Effect of alcohol molar fraction of dispersant on casein concentration in the high-***g*** supernatants. Casein was dispersed in (a) water/ethanol and (b) water/2-propanol mixed dispersants. Casein was prepared at the following pH: ●, 5.95; ○, 6.95; ■, 9.40; and □, 10.0.

Table 1
Effect of pH on the stability of various proteins dispersed in mixed water-alcohol dispersants

Protein/dispersant	pH	$\Phi_{5\%}$[a]	Φ_{ppt}[b]
Casein/ethanol	1.83	0.36	0.63
	5.95	0.07	0.14
	6.95	0.12	0.2
	7.88	0.17	0.26
	9.4	0.28	0.4
	10	0.3	0.4
Casein/2-propanol	5.95	0.06	0.07
	6.95	0.18	0.22
	7.88	0.21	0.3
	9.4	0.24	0.26
Casein/*n*-propanol	7.88	0.24	0.3
Casein/methanol	7.8	0.27	0.42
Whey protein/ethanol	6.9	0.07	0.13
Soybean protein/ethanol	7.5	0.07	0.08

[a] Alcohol molar fraction of the dispersant required to reduce protein concentration in high-***g*** supernatant by 5%.
[b] Alcohol molar fraction of the dispersant required to initiate precipitation.

Results from Fig. 5 allow a comparison of the effect of the nature of the alcohol at constant pH on the casein dispersibility. It is clear that the alcohol with the longer aliphatic chain has a greater destabilizing effect on the casein dispersion. A similar conclusion was already drawn from data reported with a different pH and different alcohols (Fig. 4). The protein dispersibilities in both the high and the low alcohol concentration zones were affected by the nature of the alcohol but to a greater extent in the high concentration zone.

Horne and Parker (1980, 1981b) defined stability as the ethanol concentration at which the first protein flocks were apparent and, for Schmidt and Koops (1977), it was the maximum ethanol concentration at which no flocculation occurred. This condition corresponds to the transition or the beginning of precipitation in the high alcohol concentration zone in our work. Horne and Parker (1980, 1981b) reported that the stability of sodium caseinate at the concentration of 2.5%, increased with pH in the range 6.0–7.1 where the protein coagulated at EtOH concentrations of approximately 0.18–0.26 M/M, respectively. The stability of casein dispersions in water-ethanol, as reported by Schmidt and Koops (1977), was 78% ethanol which is equivalent to a final concentration of approximately 0.16 M/M. Our results with casein, at pH5.95 and 6.95 in water-ethanol dispersants, show that the first appearance of the flock occurred at a similar ethanol concentration (Fig. 5a). However, comparison between different casein preparations must be regarded with caution because the residual calcium content of the casein preparation, which has a large effect on its stability toward alcohol (data not shown), is often not known and is probably different between preparations. This is the reason for carrying out this work with one and the same protein preparation.

4. Discussion

We considered the data in two groups, those obtained in the low zone of alcohol concentration, below the transition concentration where the high-***g*** sediment was obtained, and those obtained in the high zone, where both the sediment and flock were obtained. The initiation of sedimentation was taken as 5% reduction of protein concentration in the high-***g*** supernatant and the initiation of precipitation was taken as the transition concentration. These values were determined from polynomial regression curves and the results are presented in Table 1.

The colloidal sediment (Fig. 1) was probably deposited because the sedimentation rate of the protein colloidal particles was so high that they reached the bottom of the centrifuge tube in the time allowed, where they accumulated as a translucent gel-like layer. The rate of sedimentation of colloidal particles at constant centrifugal force depends on the balance between the frictional force and the effective mass. The latter depend on the specific volume of the protein and the density of the dispersant while the frictional force depends on the friction coefficient which is given by the Stokes' relation: $f = 18.85a\eta$ where "a" is a factor dependent on the radius and shape of the particle and "η" is the viscosity of the dispersant.

The presence of alcohol decreases the density of the dispersant but simultaneously increases its viscosity so that the net contribution of the dispersant to the rate of the sedimentation may be relatively small and the factors related to protein properties may be predominant. The changes in the protein which may increase the rate of sedimentation are: decrease of the protein partial specific volume, and decrease of the particle radius or asymmetry or both. Particle radius and asymmetry probably increase rather than decrease in the presence of alcohol due to the reduction of hydrophobic forces. The most likely change to be produced by the presence of alcohol is the decrease of the protein specific volume or increase of the protein molecular density. It was concluded in a previous work on the viscosity and voluminosity of the same three proteins (Boulet et al., 1998, 2000) that the aqueous dispersions were made of large and voluminous particles built by the aggregation of subparticles. Under viscosity and diffusion transport, these particles behave as hydrodynamic particles but, because of their high voluminosity, one may assume that they are permeated by the dispersant and move through it under the centrifugal force as hydrated subparticles and not as hydrodynamic particles.

The increase of the protein density in the low alcohol concentration zone appears to be progressive, allowing

sedimentation to take place at different alcohol concentrations, depending on the type of alcohol, the type and pH of the protein (Table 1), and the initial protein concentration (Fig. 2).

To understand the effect of alcohol on protein dispersions, one must consider its effect on the dispersant as well as the protein. Van Oss (1989) proposed a hydration-dehydration mechanism for the destabilization of the proteins by alcohols. Bull and Breese (1978) have shown that monohydric alcohols are strongly bound to proteins and that the binding is accompanied by partial dehydration. Chung and Villota (1989) concluded, from diffusion experiments, that the absorption mechanism of *n*-butanol and *n*-hexanol, to native soybean protein, involved hydrogen-bonding in addition to hydrophobic interactions. This suggests competition between the alcohol and the water molecules for binding to the protein. This type of interaction may explain sedimentation of the colloidal protein particles at low alcohol concentrations because binding of alcohol would increase the protein density. The stabilizing effect of pH (Table 1) may be explained by this competition between the alcohol and water if it is assumed that the affinity of the protein for water increases with pH. This is expected since the protein electrostatic charges are involved in hydration and their number increases with pH. Similarly, increases in the strength of the protein electrostatic charges, as it occurs with decreasing ionic strength of the dispersant, are also expected to favour hydration against ethanol binding. This is apparent in the effect of initial protein concentration on the initiation of sedimentation (Fig. 2). The increase of the concentration of ethanol required to initiate sedimentation may be attributed to the decreases of the ionic strength, which accompany the decrease of the initial protein concentration. It may be concluded that sedimentation of the protein at low alcohol concentrations by the high-***g*** centrifugation is due to an increase of the protein density which may be explained by assuming that the alcohol establishes bonds with the protein in competition with water.

Protein precipitation or flocculation started at the alcohol concentrations corresponding to the transition and appeared to be due to interactions between the protein particles of increased density and the dispersant.

Horne and Parker (1980, 1981b) suggested that a relationship exists between the stability and the ionization of the ester-phosphate residues of casein. Factors that are known to repress ionization of the charged residues, such as the presence of calcium ions, were also shown to decrease the protein stability. They proposed that the role of the ethanol is merely to reduce the dielectric constant of the medium, thereby reducing the energy barrier preventing coagulation. Addition of ethanol is known to reduce the dielectric constant of the dispersing agent and compression of charges was proposed as an explanation of the alcohol instability of colloidal milk particles (Horne and Parker, 1981b) and of calcium caseinate (Dalgleish, 1982).

Decrease of charge repulsion in protein-water dispersions by increase of ionic strength was found to decrease the voluminosity and aggregation of protein supramolecular particles (Boulet et al., 1998, 2000). Similar changes of supramolecular properties were obtained by reducing the protein charges upon addition of ethanol to the dispersion (Boulet et al., in preparation). The decrease of charge repulsion was not accompanied in these cases by precipitation, suggesting that other factors, in addition to charge repulsion, must be considered to explain the stability of protein dispersions.

Van Oss (1989) concluded that ethanol was bound to protein at the hydrophobic areas through the $-CH_2CH_3$ residue. Binding of alcohol may explain flocculation of the protein by changing its surface characteristics. This is supported by the observation that the flock formation appeared at lower alcohol concentration with the large size alcohol hydrophobic residue than with the small size (Table 1). However, the pH-dependence of precipitation with both the long- and short-chain alcohols suggests that the dehydration capability of the alcohol may coexist with hydrophobic binding at the high alcohol concentration zone and that dehydration may be the most important factor for precipitation of the protein.

Solvation is dependent on favourable energy of transfer with the dispersant, which is the sum of the enthalpy and entropy changes. Free energy of transfer is affected by compositional and structural characteristics of the solvated molecule and of the dispersant composition. In water and in alcohol concentrations up to the precipitation transition, energy of transfer was favourable to dispersion. Favourable energy of transfer and solvation in a given dispersant depends on the balance between the hydrophilic-lipophilic character of the protein surface and that of the dispersant. The influence of the concentration of alcohol, in water-mixed dispersants, on attraction between colloidal particles was examined by Sonntag and Strenge (1987). With hydrophobic (methylated) silica particles, increasing the ethanol content resulted in progressive reduction of the attraction between the particles. However, with hydrophilic silicate particles, the reverse was obtained and increasing alcohol concentration increased the particle–particle attraction, probably through lipophobic interaction. The lipophilic character of the protein increases with increasing concentration of alcohol because of alcohol binding but, at the same time, the dispersant becomes less hydrophilic. The change in protein character induced by alcohol binding seems more important than the change in dispersant properties, which lead to protein precipitation at a critical alcohol concentration where the energy balance promotes protein–protein interactions. This effect is expected to be larger with

larger size of the alcohol nonpolar residue. Protein precipitation would then occur at lower alcohol concentration. The effect of alcohol nonpolar residues was demonstrated by Cecil (1967) by dispersing methylated glass beads (0.2 mm diameter) in water-alcohol mixed dispersants of increasing alcohol concentration. The changeover point from an aggregating to a non-aggregating dispersant occurred at decreasing alcohol concentrations as the nonpolar residue of the alcohol increases in size, from methanol to *n*-butanol. In protein dispersion, the changing polar character of the dispersant and the shift of the hydrophilic-lipophilic balance of the protein may take place progressively and the transformation of colloidal gel-like particles into a flock may take place at the changeover of the balance between the two.

In the high alcohol concentration zone (Figs. 3–5), flock formation corresponds to a mechanism of destabilization in which the changing protein, resulting from the binding of ethanol, interacts with the changing dispersant. As the concentration of the alcohol increases, the dispersant becomes less and less polar and the affinity of protein for dispersant decreases in favour of lipophilic association between protein molecules. It is expected that this transition will shift to higher alcohol concentrations as the pH increases and the ionic strength decreases.

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