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Influence of pH and sodium chloride on the high pressure-induced gel formation of a whey protein concentrate

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Whey protein concentrate (WPC) gels induced by high pressure (4000 bar/30 min) at a protein concentration of 132 g/liter in the presence and absence of NaCl, 0.5 mol/liter, and at pH-values ranging from 3 to 9, were characterized by rheology, electron microscopy, and gel dissolution tests performed in the presence of bond breaking agents (salts, urea, SDS, DTT). A weak coagulum was formed around the IEP (pH 4-5). Below the IEP, gel formation did only occur in the presence of NaCl. At pH 3, gels showed a high force decay during relaxation, and were stabilized by electrostatic and hydrogen bonds. Gel networks produced above the IEP showed a high gel strength and G'-value, and a low force decay during relaxation. NaCl acts negatively on their rheological properties, while disulfide bonds contribute significantly to the stabilization of these gels. © 1997 Elsevier Science Ltd

INTRODUCTION

Recently, more detailed information on the use of high pressure (1000-8000 bar) to induce denaturation, aggregation and gel formation of whey proteins has become available. Pressurization of β-lactoglobulin (β-Lg) at pH 7.0 and at a protein concentration of 25 g/liter, results in unfolding and aggregation which is partially reversible with storage time after pressurization (Dumay *et al.*, 1994). At higher protein concentrations (100-180 g/liter), gel formation of β-Lg (Zasypkin *et al.*, 1996) and whey protein concentrate (Van Camp & Huyghebaert, 1995*a,b*; Van Camp *et al.*, 1996) has been demonstrated. The resulting gel networks are unstable in function of storage time, and differ from thermally-induced gels, both in terms of rheological behaviour and gel microstructure.

The gel formation of whey protein concentrate (WPC) as induced by high pressure is significantly influenced by pH (Van Camp & Huyghebaert, 1995*b*). However, to date, no detailed study on the rheological properties and the microstructure of high pressure-induced WPC gels in function of pH has been performed. Also, a number of authors (Shimada & Cheftel, 1988; Lupano *et al.*, 1992) have demonstrated for thermally-induced WPC gels that additional information on the type of bonds stabilizing the networks can be obtained by performing dissolution tests in the presence of bond breaking agents like urea and sodium dodecyl sulphate (SDS) for hydrogen bonds and hydrophobic interactions, salts for electrostatic interactions, and β-mercaptoethanol or dithiothreitol (DTT) for disulfide bonds. A thorough characterization of protein gels can thus be obtained by combining the data from rheology, microscopy, and dissolution tests.

In this paper, high pressure-induced WPC gels obtained at various pH-values in the presence and absence of sodium chloride, have been characterized by rheological measurements at both small (oscillation and creep tests) and large (gel strength and relaxation tests) sample deformations, by scanning electron microscopy, and by dissolution tests in the presence of salts, urea, SDS and DTT. The influence of storage time after pressurization was derived from rheological measurements and from electron microscopy.

MATERIALS AND METHODS

Sample preparation — high pressure-treatments

Whey protein concentrate (WPC; Lacprodan 80) was obtained from Denmark Protein (DK) and contained per kg powder 733±10 g of protein, 27±2 g of ash

from which 1.85 g sodium and 0.25 g chloride, 74 ± 4 g of fat, and app 47 g of lactose. The dry matter content of the powder was 915 ± 6 g/kg (Van Camp & Huyghebaert, 1995*b*). Also, Lacprodan 80 contains 10-20% thermal denatured whey proteins and 3-4% Non-Protein Nitrogen (NPN), because of it's processing history (De Wit *et al.*, 1988).

The WPC was solubilized in demineralized water by shaking for 1-2 h in an Erlenmeyer flask at room temperature. Solutions were adjusted to pH 3, 4, 5, 6, 7, 8 or 9 with HCl or NaOH, 1 mol/liter, and diluted to a final protein concentration of 132 g/liter. No buffers were applied in view of possible interferences by buffer salts on aggregation and gelation of proteins under high pressure (Funtenberger *et al.*, 1995; Van Camp & Huyghebaert, 1995*b*). If applicable, 0.5 mol/liter sodium chloride (analytical grade) was added. Final protein solutions were poured into high density polyethylene (HDPE) bottles ($5\times5\times10$ cm^3) and placed for 12 h at 4°C for deaeration and complete hydration. Care was taken not to leave any head space between the closing screwcap and the liquid solution.

Prior to pressurization, samples were equilibrated at 25°C. High pressure treatments were performed for 30 min at 25°C at 4000 bar operating pressure without additional supply of heat to the pressure vessel, as described previously (Van Camp & Huyghebaert, 1995*b*). After the high pressure treatment, samples were immediately placed at 4°C for a period of 24 h prior to analysis, unless indicated differently.

Rheological measurements — scanning electron microscopy

Rheological measurements at large sample deformations were performed in six-fold by means of an INSTRON Universal Testing Machine. The gel strength was defined as the amount of work (in mJ) necessary to compress a gel of 2 cm thickness for 8 s using a flat circular plunger (3.6 cm diameter) at a vertical displacement speed of 50 mm/min (Van Camp & Huyghebaert, 1995*b*). Relaxation tests were performed for a total relaxation time of 10 min, as described previously (Van Camp & Huyghebaert, 1995*a*). The residual force at various relaxation times was expressed relative to the initial force obtained immediately after compression (in %).

Rheological measurements at small sample deformations were performed in six-fold at 25°C on a Bohlin controlled stress (CVO) rheometer, using the plate-plate geometry with a diameter of 4 cm and a gap setting of 3 mm. During creep measurements, an instantaneous stress of 4 Pa was applied to the sample, after which the sample deformation was followed for a total creep time of 10 min. Results are expressed in compliance units, ie the proportion of the measured strain to the applied stress (in 1/Pa). The storage modulus (G′-value) and loss modulus (G″-value) obtained from oscillation were derived exactly as described previously (Van Camp & Huyghebaert, 1995*a*).

Gel slides ($6\times2\times1$ cm^3) of WPC gels produced by high pressure were prepared for scanning electron microscopy by fixation overnight at 4°C in 40 g/liter glutardialdehyde, followed by dehydration in a graded series of water/ethanol mixes, and critical point drying through carbon dioxide. Dried samples were coated with gold and examined with a Philips SEM 505 at a magnification of 2000 × and 8000 ×, respectively, and at a voltage of 30 kV.

Dissolution experiments

After pressurization, WPC gels were carefully removed from the recipients and separated from the non-incorporated liquid (NIL), which was transferred for volumetric measurement to a graduated cilinder. NIL-values were expressed relative to the initial sample volume in the recipients prior to pressurization (Van Camp & Huyghebaert, 1995*b*).

Dissolution experiments were performed on gels obtained at pH 3 in the presence of sodium chloride, and at pH 5, 6, 7 and 9 in the absence of sodium chloride. The protein content of the gels was determined by Kjeldahl, using a conversion factor of 6.38. An amount of gel containing 0.03 g of protein was transferred to a centrifuge tube of 50 ml, after which 20 ml of demineralized water (solution 1), a standard buffer containing 0.086 mol/liter Tris, 0.09 mol/liter glycine, 4×10^{-3} mol/liter ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), pH 8.0 (solution 2), solution 2 additionally containing 8 mol/liter urea and 17.3×10^{-3} mol/liter SDS (solution 3), or solution 3 additionally containing 10×10^{-3} mol/liter DTT (solution 4) was added. After homogenization with an Ultra Turrax at room temperature for 3 min, protein solutions were adjusted with the appropriate solubilizing medium (solution 1, 2, 3 or 4) to a protein concentration of 1 g/liter (0.03 g of protein/30 ml of buffer), and centrifuged at 20000 × ***g*** for 15 min(Shimada & Cheftel, 1988). Each gel was independently extracted six times with each of the four solutions.

The protein content in the supernatant of each protein solution was determined by the colorimetric method of Bensadoum and Weinstein (1976), with a few modifications. After a 25-fold dilution with demineralized water, 3 ml of solution was transferred to a centrifuge tube of 10 ml and 0.025 ml of sodium deoxycholate, 20 g/liter, was added. After an equilibration period of 15 min, 1 ml of trichloroacetic acid solution, 240 g/liter, was added and insoluble protein was removed from solution by centrifugation at 3800 × ***g*** for 30 min. The pellet was resolubilized in 1 ml of demineralized water and the protein content of the solution was determined by the modified Lowry method of Schacterle and Pollack (1973). Conversion of absorbance to protein was achieved by simultaneously analysing a

series of WPC-solutions with a known (Kjeldahl) protein content (correlation coefficient $r^2 = 0.99$ for calibration curves in the concentration range of 4.7 to 117 mg protein/liter, conversion factor 6.38). The protein determination step was performed on each of the supernatant fractions obtained from the six individual dissolution tests. Results are expressed in *g* of protein solubilized/100 *g* of protein in the gel.

RESULTS AND DISCUSSION

Influence of pH and NaCl on the rheological properties of WPC gels

In view of the strong influence of pH on the high pressure-induced gel formation of WPC (Van Camp & Huyghebaert, 1995*b*), a more detailed rheological study was performed, both in the presence and absence of sodium chloride, 0.5 mol/liter, which, as suggested by a number of authors (Harwalkar & Kalab, 1985*a*,*b*; Mulvihill & Kinsella, 1988; Mulvihill *et al.*, 1991), acts primarily on electrostatic and hydrophobic interactions during the course of protein gelation. In Fig. 1, the results of the rheological tests performed at large sample deformations (gel strength, part a; relaxation tests, part b) and at small sample deformations (oscillation tests, part c; creep tests, part d) are given in function of pH with and without added sodium chloride for high pressure-induced WPC gels.

The iso-electric point (IEP) of the whey proteins is situated near pH 5.0 (Kinsella & Whitehead, 1989). At this pH, a weak and white coagulum was formed which was characterized by a low gel strength (Fig. 1(a)), a large force decay during relaxation (Fig. 1(b)), a low storage modulus G′ (Fig. 1(c)), and high compliance values during creep (Fig. 1(d)). In the presence of sodium chloride, no significant influence on the rheological properties of the coagulum could be detected. According to the Ferry-theory, which has been used by several authors to explain the process of protein gelation (Poole & Fry, 1982; Gosset *et al.*, 1984; Ziegler & Acton, 1984; Cheftel *et al.*, 1985; Harwalkar & Kalab, 1985*b*), gel formation of proteins is the result of a two-step process involving the partial denaturation of individual proteins to allow more access to reactive side groups within the protein molecules, and the aggregation of these proteins by means of reactive side groups into a three-dimensional network structure capable of retaining significant amounts of water. Near the IEP,

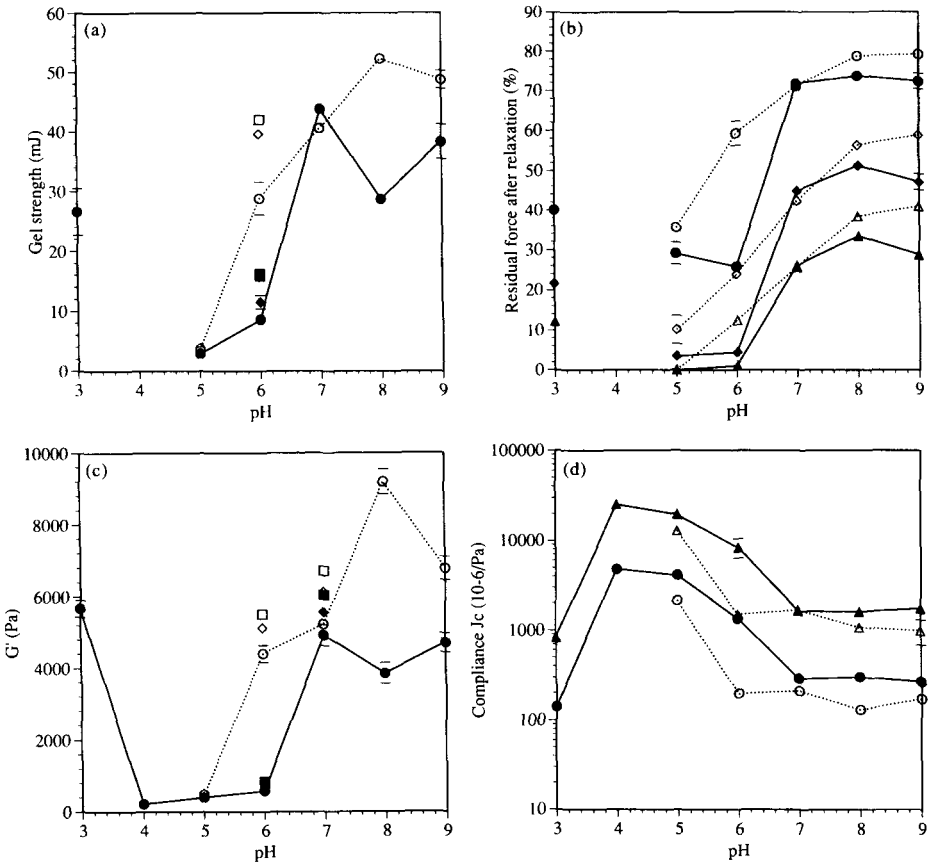

Fig. 1. Gel strength (○ or ●) (part a), residual force after relaxation at 0.25 (○ or ●), 2.50 (◇ or ◆) and 10.00 (△ or ▲) min relaxation time (part b), storage modulus (○ or ●) (part c) and compliance values at 0.5 (○ or ●) and 500 (△ or ▲)s creep time (part d) for high pressure-induced WPC gels at a protein concentration of 132 g/liter as a function of pH with (filled symbols) and without (open symbols) addition of 0.5 mol/liter NaCl. Results of gel strength and storage modulus at pH 6 and 7 are given after 1 day (○ or ●), 3 days (◇ or ◆), and 6 days (□ or ■) of storage at 4°C. Other measurements relate to 1 day of storage at 4°C.

electrostatic attraction forces can dominate over electrostatic repulsion forces, which reduces the effect of salt, promotes aggregation instead of denaturation, and results in the formation of a coagulum where random protein-protein interactions predominate (Poole & Fry, 1982; Gosset *et al.*, 1984). In view of the rheological properties of the gels, it may be suggested that, for the latter, primarily non-covalent, short term intermolecular interactions are involved (Van Camp & Huyghebaert, 1995*a*).

At pH 3 and 4 in the absence of sodium chloride, no gel formation occurred after pressurization. Addition of 0.5 mol/liter sodium chloride induced, at pH 3 and after 12 h of storage at 4°C, a viscous paste matrix which after pressurization resulted in a gel network with a fairly high gel strength and storage modulus (Figs 1(a)–(c), and low compliance values during creep (Fig. 1(d)). The force decay of the gel during relaxation was pronounced but remained low at each relaxation time compared to those of the gels formed around the IEP (Fig. 1(b)). On visual examination, the gel had a white appearance and contained small granular aggregates embedded in a viscous paste matrix. The occurrence of this type of network structure may be explained by a combined effect of pH and increased ionic strength. At pH 3 and 4, repulsive forces among positively charged protein molecules prevent gel formation in the absence of salt (Mulvihill & Kinsella, 1988; Mulvihill *et al.*, 1991). At pH 3, the viscosity effect observed prior to pressurization may be related to a partial denaturation of some of the whey proteins (e.g. α-lactalbumin and serum albumin; Whitney, 1987). In view of the soft coagulum formed at pH 4 in the presence of salt, it is postulated that under these process conditions the shielding effect of sodium chloride strongly reduces the repulsive forces acting among proteins, creating an identical situation as for the IEP of the whey proteins (see above). At pH 3, part of the repulsive forces are maintained, which delays the aggregation step during gelation and as a result leads to the formation of a stronger gel network (Harwalkar & Kalab, 1985*a*,*b*). The relaxation behaviour of the gel at pH 3 suggests that an important part of the network bonds are composed of non-covalent, short term intermolecular interactions (Peleg, 1987; Shimada & Cheftel, 1988; Van Camp & Huyghebaert, 1995*a*).

Above the IEP, the gel networks produced in the absence of NaCl are characterized by a high gel strength (Fig. 1(a)), a rather small force decay during relaxation (Fig. 1(b)), a high storage modulus G′ (Fig. 1(c)), and a low compliance value during creep (Fig. 1(d)). In the presence of salt, significant reductions in gel strength and G′-values are recorded, accompanied by higher values for compliance during creep and a higher force decay during relaxation. The gels are homogeneous and appear to be more ordered and more elastic compared to the coagulums and gels formed at lower pH. Taking the Ferry-theory into account, an increase in pH will generate negative repulsive forces among proteins which delay the aggregation step and allow the formation of an ordered network structure by interaction of proteins at specific reactive sides. Increasing the ionic strength by adding an extra amount of NaCl influences the gel forming process negatively, possibly by decreasing protein unfolding (Boye *et al.*, 1995) and/or by acceleration of the aggregation step (Cheftel *et al.*, 1985; Mulvihill & Kinsella, 1988). In addition, the lower force decay of the alkaline gels during relaxation compared to acid pH (Fig. 1b) and the decreasing G″/G′-value for increasing pH (i.e. from 0.25 at pH 3 + NaCl down to 0.12 at pH 9 with no influence from the salt at pH 5 or higher) suggests that more long term intermolecular interactions are involved in the stabilization of these gels (Van Camp & Huyghebaert, 1995*a*).

Non-incorporated liquid

High pressure-induced WPC gels are characterized by an amount of liquid surrounding the gel in the recipient after pressurization, i.e. the non-incorporated liquid (NIL). In Fig. 2, the NIL of high pressure-set gels is given in function of pH, both in the presence and absence of sodium chloride, 0.5 mol/liter.

In accordance with results given in a previous publication (Van Camp & Huyghebaert, 1995*b*), the highest NIL-values are found at pH 6 and 7. At extreme alkaline pH (=9) and at the IEP, low NIL-values are registered. A similar profile is obtained when salt is present, although the NIL at pH 6 and 8 is significantly reduced when compared to the NIL without salt added. At pH 3 and 4 in the presence of salt, NIL is low or completely absent.

The occurrence of NIL is related to a low water holding capacity (WHC) of the WPC gels. It has been demonstrated for whey protein aggregation and gelation induced by heat (Shimada & Cheftel, 1988; Mulvihill *et al.*, 1991; Lupano *et al.*, 1992) as well as by high pressure (Funtenberger *et al.*, 1995; Van Camp & Huy-

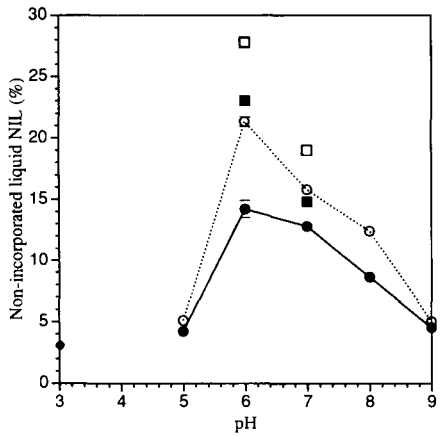

Fig. 2. Percentage of non-incorporated liquid (NIL) for high pressure-induced (4000 bar/30 min) WPC gels at a protein concentration of 132 g/liter as a function of pH with (●) and without (○) addition of 0.5 mol/liter NaCl. Results are given after 1 day (○ or ●) and for pH 6 and 7 also after 6 days (□ or ■) of storage at 4°C.

ghebaert, 1995*b*), that intermolecular disulfide bonds become important at and above neutrality as a result of an increased reactivity of SH-groups. By increasing the pH from 6 to 9, protein unfolding may thus be stimulated, which improves protein-water interactions (Chou & Morr, 1979). In addition, the formation of more stable intermolecular contacts can improve the retention of physically entrapped water. Since pressurization at 4000 bar results in a 12% volume reduction of the recipients (Crum, 1991), decompression may aid in the formation of NIL by forcing free or loosely bound water between the gel and the recipient wall during pressure release. At pH 5, the weak coagulum formed is more able to adapt to this volume increase compared to the gels formed at pH 6, 7 and 8, which may explain its low NIL. A similar explanation may be given for the significant reduction in NIL at pH 6 and 8 in the presence of salt, since weaker gels (cf. Fig. 1) with a larger cross-section area compared to those formed in the absence of salt (results not shown) are produced.

Influence of storage time after pressurization

Previous publications on pressure-treated whey proteins (Dumay *et al.*, 1994; Funtenberger *et al.*, 1995; Van Camp & Huyghebaert, 1995*a*) have demonstrated that time-dependent alterations in molecular structure and gel properties may occur during storage after pressurization. Further evidence on this matter was obtained by comparing the rheological properties (i.e. gel strength, G′ and G″) and the NIL of high pressure-set WPC gels after 1, 3 and 6 days of storage at 4°C. Measurements were performed at pH 6 and 7, with and without addition of NaCl, 0.5 mol/liter. For each measurement, a new recipient was taken from the refrigerator.

Up to 6 days' storage after pressurization at pH 6, a significant increase in gel strength and G′-modulus was found, which was most pronounced during the first 3 days of storage. At pH 7, the relative increments of G′ were comparable to those obtained at pH 6, although no significant changes in gel strength occurred (Figs 1(a)–(c)). Although not significant, the G″/G′-values tend to decrease during storage at both pH 6 and 7. The effect of salt on these alterations was limited (Table 1). These results suggest that, during storage, the amount of stable long-term intermolecular bonds is increased relative to the more labile, short-term intermolecular interactions (Whorlow, 1992; Van Camp & Huyghebaert, 1995*a*). According to Funtenberger *et al.* (1995), this may reflect the continuous formation of intermolecular disulfide bonds by SH/SS interchange or by SH-oxidation reactions after pressure release.

Table 1. G″/G′-values for high pressure-induced (4000 bar/30 min) WPC gels (protein concentration 132 g/liter), at pH 6 and 7 with or without addition of NaCl, 0.5 mol/liter, and after 1, 3 and 6 days of storage at 4°C

pH	NaCl	Storage time		
		1 day	3 days	6 days
6	–	0.176(0.015)	0.161(0.003)	0.152(0.008)
6	+	0.180(0.006)	0.163(0.004)	0.149(0.019)
7	–	0.154(0.005)	0.135(0.011)	0.133(0.018)
7	+	0.150(0.006)	0.134(0.014)	0.131(0.006)

Oscillation experiments were performed in six-fold; the standard deviation of each mean is given between brackets. – = without salt addition, + = with addition of 0.5 mol/liter NaCl prior to pressurization.

The NIL increased during storage, particularly at pH 6 (Fig. 2). The formation of more stable intermolecular bonds tends to decrease the pore dimensions of the gels (Johnston *et al.*, 1993) and, as a consequence, the void volume available for the liquid diminishes (Van Marle & Zoon, 1995). This in turn may stimulate syneresis of the gels.

Gel microstructure

The microstructure of the pressure-induced gels obtained at pH 3, 5, 6, 7 and 9 in the presence and absence of NaCl, was analysed by SEM at a magnification of 2000 × and 8000 ×, respectively.

The porous network structure obtained for high pressure-set WPC gels at pH 7 (Fig. 3(a)) has been described previously (Van Camp & Huyghebaert, 1995*a*). At pH 6, a similar structure is found (Fig. 3(b)), although the larger porosity suggests that fewer intermolecular contacts have been formed. At pH 9, the porosity of the gel is diminished (results not shown), possibly illustrating a larger reactivity of protein side-chains with more intermolecular bond formation as a result. The appearance of a porous network structure at pH 6 to 9 may also be related to the expansion effect mentioned previously whereby the liquid is forced out of the gel during pressure release. In the presence of salt, and for pH 6 and 7 after 6 days instead of 1 day of storage, no significant influence on the gel microstructure was found.

At pH 5, the coagulum is composed of large aggregates (Fig. 3(c)). At pH 3 in the presence of salt, the gel contains a large number of individual aggregates which are comparable to the network bonds described for the gel at pH 7 (Fig. 3(d)).

Dissolution Experiments

To identify the type of bonds stabilizing the networks of high pressure-induced WPC gels, a series of dissolution experiments in the presence of bond breaking agents (salt, SDS, urea, DTT) was performed. Analyses were done on gels obtained at pH 3 in the presence of NaCl, and at pH 5, 6, 7 and 9 without salt added. The solubilized protein was expressed relative to the amount of protein in the gel (Fig. 4).

All gels were readily dissolved in the standard buffer containing urea, SDS, and DTT. When DTT was absent, the solubility was significantly reduced at neutral and

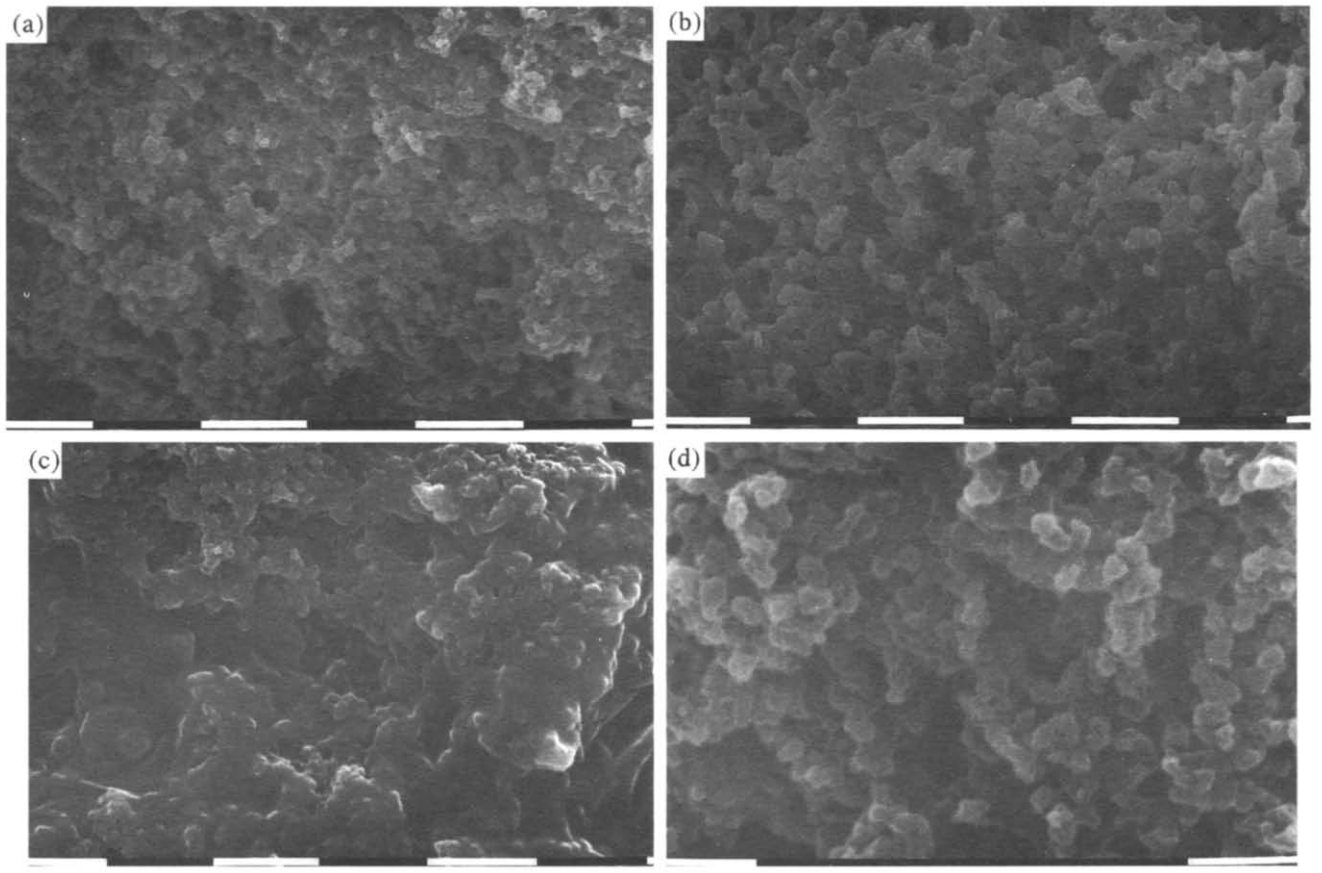

Fig. 3. Electron microscopic analysis of high pressure-induced (4000 bar/30 min) WPC gels at a protein concentration of 132 g/liter in the absence of NaCl at pH 7 (part a, left, top), pH 6 (part b, right, top) and pH 5 (part c, left, bottom), and in the presence of NaCl, 0.5 mol/liter, at pH 3 (part d, right, bottom). Analysis was performed at a magnification of 2000 × (part a, b and c) and 8000 (part d). The bars in the figures correspond to 10 μm.

alkaline pH-values, but not at acid pH. At pH 6, an intermediate behaviour was found. These results are in accordance with previous publications on heat-set whey protein gels (Shimada & Cheftel, 1988; Wang & Damodaran, 1990; Mulvihill *et al.*, 1991; Lupano *et al.*, 1992; Mangino, 1992), and with suggestions made earlier on disulfide bonds in high pressure-set gels. The reactivity of SH-groups increases at neutral and alkaline pH, allowing more SS-groups to participate in the stabilization of these gels. At acid pH, the contribution of intermolecular SS-bonds to the stabilization of the gels remains limited.

Independent from pH, the removal of urea and SDS from the solubilizing buffer did not result in extra reduction of the gel solubility. On the contrary, at acid pH and partly at pH 6, gel solubility is markedly enhanced by dilute salt solutions, suggesting that electrostatic interactions (Mulvihill & Kinsella, 1988; Shimada & Cheftel, 1988), and possibly also hydrogen bonds (Lupano *et al.*, 1992), are dominant in the stabilization of these gels. The fact that hydrophobic interactions do not contribute significantly to high pressure-set WPC gels may indicate that only a limited protein unfolding occurred during gelation. This in turn may explain the results obtained in previous studies, where a more limited number of intermolecular interactions were found in high pressure compared to heat-set gels (Van Camp & Huyghebaert, 1995*a*).

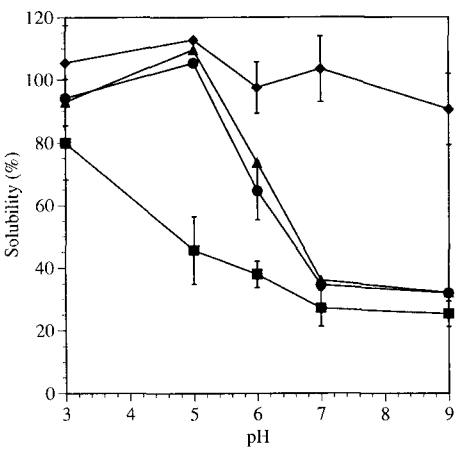

Fig. 4. Protein solubility (in g/100 g of protein in the gel) of high pressure-induced (4000 bar/30 min) WPC gels (protein concentration 132 g/liter) at pH 3 in the presence of NaCl, 0.5 mol/liter, and at pH 5, 6, 7 and 9 in the absence of NaCl, using demineralized water (■), standard buffer (●), standard buffer with urea and SDS (▲), and standard buffer with urea, SDS and DTT (◆) as solubilizing medium.

The solubility of high pressure-induced gels in demineralized water is most pronounced at acid pH, but remains significant at pH 6 to 9. This result may reflect that part of the protein in the gels is entrapped physically in the form of liquid, or is only loosely bound to the gel network structure. It cannot be excluded that the NIL produced at pH 6 to 9 is equivalent to, or derived from, this liquid present in the porous network structure.

CONCLUSIONS

By combining the results from rheological measurements, scanning electron microscopy, and dissolution tests, new data were obtained on the influence of pH and NaCl on the high pressure-induced gel formation of whey proteins. Below the IEP, gelation only occurs in the presence of salt. Above the IEP, salt impairs the formation of a gel network. Similar to heat, pressure-set gels at neutral and at alkaline pH are stabilized by disulfide bonds. At acid pH, WPC gels contain more electrostatic interactions and possibly also hydrogen bonds. More information concerning the role of hydrogen bonds in pressure-set gelation of WPC may be obtained by the use of Fourier transformation infra-red spectroscopy (FTIR), as demonstrated in a recent publication by Heremans *et al.* (1997). The apparently limited contribution of hydrophobic interactions to pressure-induced WPC gels should receive more attention in future research.

Non-incorporated liquid (NIL) has to be considered as a characteristic of high pressure-induced WPC gels. The impact of decompression on its formation may be studied by performing pressure experiments at different decompression rates. Moreover, analysis of the protein in the NIL might give more information on the amount and the type of protein actively participating in the gel-forming process. The instability of WPC gels in function of storage time has been confirmed. Addition of polysaccharides like xanthan may be a possible means to reduce the loss of NIL during storage (Zasypkin *et al.*, 1996).

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