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A comparative rheological study of heat and high pressure induced whey protein gels

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The rheological properties of whey protein concentrate (WPC) gels induced by high pressure (4000 bar/30 min), were compared to those induced by heat (80°C/30 min) at protein concentrations ranging from 110 up to 183 g/liter. Oscillation measurements at 1 Hz and 0.001 strain showed the highest storage and loss moduli for heat set gels, while creep experiments at a stress level of 40 Pa gave larger sample deformations for high pressure induced gels. Relaxation experiments performed at 17 and 33% deformation were characterized by a higher force decay as a function of time for the high pressure gels, while during compression the compression modulus was always higher in the case of heat set gels. Electron microscopy showed a higher level of cross links in the heat induced gels; high pressure generated a more porous network with a lower amount of intermolecular cross links.

INTRODUCTION

High hydrostatic pressure processing (1000 – 8000 bar) is investigated with growing interest by various researchers in view of its potential applications in the manufacture and stabilization of food products. Recent publications in this area report on the inactivation of micro-organisms (Carlez & Cheftel, 1994) and spores (Sojka & Ludwig, 1994), the denaturation of proteins (Dumay *et al.*, 1994), and the effects of pressure on nutritional compounds like vitamins, flavors and colorants (Hayashi *et al.*, 1989; Carlez *et al.*, 1993). Up to now, information on the high pressure induced gel formation of food proteins remains limited.

Whey protein concentrates (WPC) have been used by various authors to elucidate the mechanism of heat induced whey protein gelation (Kohnhorst & Mangino, 1985; Kinsella & Whitehead, 1989; Mangino, 1992). As a consequence, the substrate seems well suited to study the gel formation of whey proteins under high pressure. In a previous publication (Van Camp & Huyghebaert, 1995), it has been shown that WPC gels produced by high pressure (4000 bar/30 min) at a protein concentration of 110 up to 183 g/liter differ significantly from heat induced protein gels (80°C/30 min) with respect to gel strength and appearance: high pressure induced WPC gels were characterized by a lower gel strength and were surrounded by non-incorporated liquid after pressurization; heat induced WPC gels, on the contrary, were stronger and appeared dry and firm without significant loss of liquid.

In this paper, more information is given on the rheological properties of high pressure induced WPC gels compared to those obtained by heat. The analysis of gel networks at large sample deformations (relaxation experiments, stress–strain relationships), as well as the network properties obtained at small sample deformations (oscillation and creep experiments), are being used to obtain information on the structure of both types of gel networks. Additional information on the gel microstructures in relation to their rheological properties is obtained by electron microscopy.

MATERIALS AND METHODS

Sample preparation — heat and high pressure treatments

The whey protein concentrate (WPC; Lacprodan 80) used throughout the experiments was derived from Danmark Protein (DK), and contained 733.5 g protein/kg powder. A more detailed description of the protein concentrate has been reported previously (Van Camp & Huyghebaert, 1995).

The WPC was solubilized in 0.05 mol/liter phosphate buffer solution by shaking for 1–2 h in an Erlenmeyer

flask at room temperature. Solutions were diluted to the desired protein concentration, and (if necessary) adjusted to pH 7.0 with NaOH, 1 mol/liter. Final protein solutions were poured in high density polyethylene (HDPE) bottles (5 × 5 × 10 cm) and placed for 12 h at +4°C for deaeration and complete hydration. With the exception of the experiment where the influence of the recipient filling grade was evaluated, care was taken not to leave any head space between the closing screwcap and the liquid solution.

Heat treatments were performed at 80°C for 30 min using a circulating water bath. High pressure treatments were performed for 30 min at 4000 bar operating pressure without additional supply of heat to the pressure vessel as described previously (Van Camp & Huyghebaert, 1995). After the heat or high pressure treatment, samples were immediately placed at +4°C for a period of 24 h prior to analysis, unless indicated differently.

Measurements of the gel strength and deduction of the amount of non-incorporated liquid (NIL) were as described previously (Van Camp & Huyghebaert, 1995). 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.

Oscillation and creep experiments

All oscillation and creep experiments (Whorlow, 1992) were performed at 25°C on a Bohlin controlled stress (CS-50) rheometer, using the plate–plate geometry with a diameter of 4 cm and a gap setting between the upper and lower plate of 3 mm. WPC gels were carefully removed from the HDPE recipient and cut into circular slices of 4 cm diameter and 3 mm thickness prior to analysis.

Oscillation experiments were performed in the linear region at 1 Hz and at a strain level of 0.001. In contradiction to the heat induced WPC gels, it was found that the storage modulus (G' value) of the high pressure induced WPC gels increased slightly as a function of time after the gap setting. The loss modulus (G'' value) for both heat and high pressure induced gels remained constant after an initial and rather rapid decrease during the first 100 s of the measurement. For the high pressure induced gels, the effects on G' and G'' may be caused by the loss of liquid during the measurement (as noticed experimentally). In comparison to the variation obtained between the individual sample slices of the same gel, the effect described remained rather limited. All G' and G'' values presented are for both type of protein gels the mean of six individual determinations, whereby the values used from each determination were obtained after a delay period of 100 s.

During the creep experiments, an instantaneous stress of 40 Pa was applied to the sample, after which the resulting sample deformation was followed for an additional 10 min. Each analysis was performed in six-fold, and results are presented in compliance units, i.e. the proportion of the measured strain to the applied stress (in 1/Pa).

Relaxation experiments and stress–strain relationships

The rheological characterization of heat and high pressure induced WPC gels at large deformations was investigated by means of an INSTRON Universal Testing Machine. The protein gels were carefully removed from the HDPE recipients, cut into slices of 2 cm thick, and analysed to produce a true stress–true strain curve (Calzada & Peleg, 1978; Rosenau *et al.*, 1978; Torres *et al.*, 1978; Johnson *et al.*, 1980; Peleg, 1987) and a relaxation curve (Peleg, 1987; Ziegler & Rizvi, 1989; Foegeding, 1992; Gamero *et al.*, 1993; Hsieh & Regenstein, 1993).

The relaxation experiments were performed by placing a Petri dish (diameter 9.6 cm) on top of the gel slide and following the force decay for 10 min after deformation of the protein gel with a flat circular plunger (diameter 3.6 cm) at a vertical displacement speed of 50 mm/min. The deformation time was set to 4 and 8 s (i.e. a vertical displacement of 3.3 and 6.7 mm or a relative deformation of 17 and 33%), respectively.

The *true stress* σ_T generated in the gel slide is calculated by dividing the measured force (in N) during penetration by the actual cross-sectional area S (in cm^2) of the deformed specimen. The *true strain* ϵ_T for compression of the gel slide is given by the logarithmic dimensionless expression:

$$\epsilon_T = \ln\left(\frac{H}{H_0 - \Delta H}\right) \quad (1)$$

where: H_0 = initial thickness of the gel slide (= 2 cm); ΔH = length decrease in the direction of the applied force (in cm).

The compression force F as a function of the compression depth was recorded for 8 s using the experimental set up already described for the relaxation experiment. The vertical displacement speed of the plunger was maintained at 50 mm/min. The corresponding sample deformation during compression was measured by the use of a Petri dish equipped with a mm dimension scale in two perpendicular directions, which permitted to follow the sample top area S as a function of the compression depth.

For both heat and high pressure induced WPC gels, the true stress–true strain and relaxation curves were constructed as an average of four individual repetitions of each experiment.

Scanning electron microscopy

Gel slides (6 × 2 × 1 cm) of 180 g/liter WPC gels produced by high pressure and by heat were prepared for scanning electron microscopy by fixation overnight at 4°C in 40 g/liter glutardialdehyde, followed by dehydration in a graded ethanol 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 500 × and 2000 ×, respectively, and at a voltage of 30 kV.

RESULTS AND DISCUSSION

Influence of container filling grade during pressurization and sample storage time after pressurization

In view of the high compressibility of gasses compared to liquids under high pressure (Cheftel, 1992), it was investigated how partially filled sealed containers would react to the gel forming behavior of liquid protein solutions under pressure. A series of WPC solutions with a final protein concentration of 132 g/liter were pressurized in sealed (250 ml) HDPE-recipients at 4000 bar for 30 min with a sample filling grade in the recipient of 30 –50 –80 and 100%, respectively. The gel strength and the NIL values for the gels formed are summarized in Table 1. After the pressure treatment at low filling grades (30 and 50%), the gel networks formed had a rather flat structure along the length axis of the container and collapsed under their own weight. This result suggests that during pressurization the recipient wall deforms at its weakest parts (i.e. sideways) whereby the liquid inside the recipient is redistributed along the length axis. After decompression, the resulting gel networks are not able to maintain their original shape when the recipient walls reinstall their original configuration. From 80% filling degree onwards, self supporting gel network structures are obtained with a similar gel strength compared to these obtained at 100% filling grade. The amount of non-incorporated liquid (NIL) is maximized at a filling grade of 30 –50%, and progressively decreases at higher filling grades. The latter result indicates that gel networks with a high water holding capacity are predominantly formed beyond 80% filling degree.

The influence of storage time at + 4°C was investigated by following the storage (G') and loss (G'') modulus of WPC gels with a protein concentration of 132 g/liter up to 14 days after pressurization. For each measuring point gathered, a new container was taken from the refrigerator. As indicated in Table 2, the gel networks show a significant increase in sample storage modulus up to 3 days (72 h) after pressurization; longer storage times up to 14 days after pressurization do not change the modulus significantly. The loss modulus did not change significantly throughout the storage, which is further indicated by a significantly decreasing G''/G' value during the first three days of storage. An increase in G' coupled with a decrease in G''/G' can be associated with a decrease in energy dissipation caused by the relaxation of network bondings (Whorlow, 1992). Apparently some structural reorientation and/or additional formation of bondings takes place between the protein molecules following the decompression and cooling step. The adulteration effects found above could not be visualized when large sample deformations were applied. Gel strength and NIL values derived with an INSTRON device were not significantly different up to 5 days after pressurization (Table 2).

Table 1. Influence of recipient filling grade on the strength and on the amount of NIL of high pressure induced (4 kbar; 30 min) WPC gels. Protein conc. = 132 g/liter in 0.05 mol/liter phosphate buffer pH 7.0

Recipient filling grade	Gel strength (mJ)	Non-incorporated liquid (%)
30 %	—	34.6 (2.3)
50 %	—	38.0 (3.0)
80 %	36.1 (1.7)	28.2 (0.6)
100 %	32.0 (0.5)	18.0 (0.8)

NIL determinations were performed in three-fold; measurements of the gel strength in eight-fold. The standard deviation for each mean is given between brackets.

Rheological characterization of WPC gels at small deformations: oscillation and creep measurements

A holding time of 30 min at 4000 bar induces gel formation of WPC at a minimum protein concentration of 110 g/liter (Van Camp & Huyghebaert, 1995). To investigate the effect of protein concentration on the rheological properties of both heat and high pressure induced WPC gels beyond this limiting protein concentration, the corresponding G' and G'' values were deduced at a concentration of 110 –132 –147 –161 and 183 g/liter ,respectively. Both moduli are indicated in Fig. 1 (top), while the corresponding G''/G' values are presented in Fig. 1 (bottom). Increasing the protein concentration definitely increases the G' values of both heat and high pressure induced protein gels, whereby higher values are noticed in the case of heat compared to high pressure induced WPC gels. A similar line of

Table 2. Influence of storage time on different characteristics (G', G'', GS, NIL) of high pressure induced (4 kbar; 30 min) WPC gels. Protein conc = 132 g/liter in 0.05 mol/liter phosphate buffer pH 7.0

Storage time	5 h	24 h	72 h	144 h	192 h	312 h
G' (kPa)	6.92 (0.28)	7.67 (0.32)	9.93 (0.31)	9.37 (0.18)	9.06 (0.30)	9.19 (0.34)
G'' (kPa)	1.19 (0.04)	1.25 (0.07)	1.40 (0.05)	1.28 (0.02)	1.28 (0.03)	1.27 (0.05)
G''/G'	0.17	0.16	0.14	0.13	0.14	0.14
GS (mJ)	32.5 (0.8)	36.1 (1.4)	N.D.	34.7 (1.0)	N.D.	N.D.
NIL (%)	17.2 (1.9)	16.6 (3.2)	N.D.	17.6 (3.2)	N.D.	N.D.

Oscillation experiments were performed in six-fold; values of the gel strength and NIL are the mean of 12 and three repeated determinations, respectively. The standard deviation for each mean is given between parentheses. N.D. = not determined.

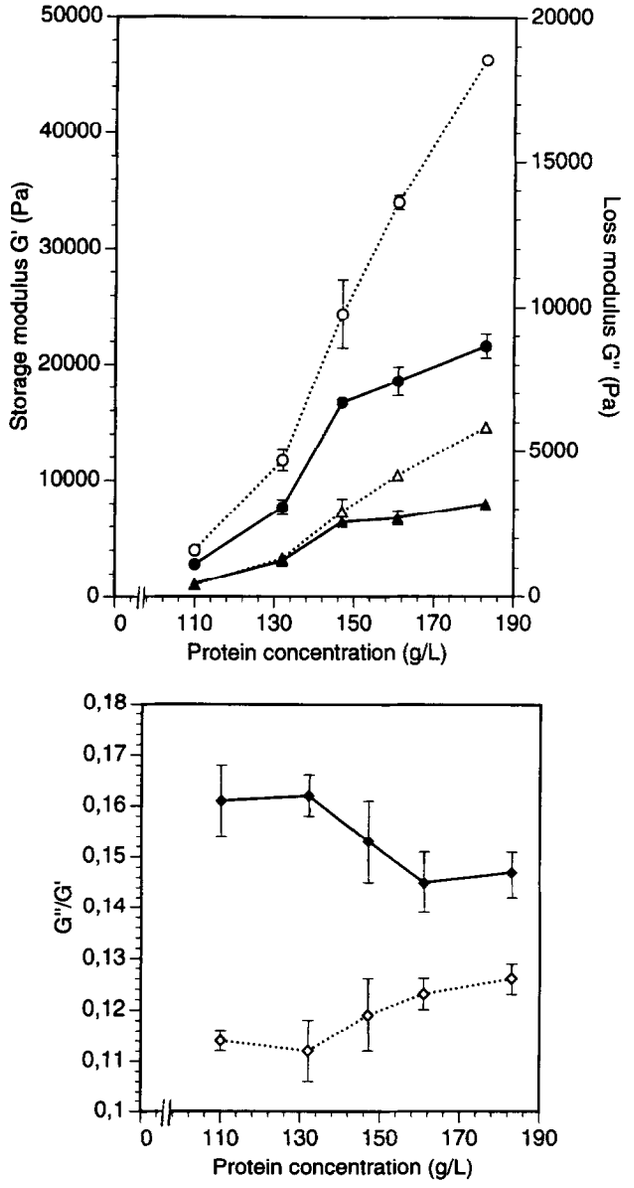

Fig. 1. Storage (● or ○) and loss (▲ or △) modulus (top) and G''/G' proportion (◆ or ◇) (bottom) of high pressure (4 kbar/30 min; filled symbols) and heat (80°C/30 min; open symbols) induced WPC gels as a function of the protein concentration.

reasoning is possible for the G'' values. Increasing the protein concentration thus markedly increases the formation of both long and short term bondings, due to the availability of more contact points between neighboring polypeptide side chains (Cheftel *et al.*, 1985). The number of bondings is presumably higher in the case of heat induced protein gels, which might form the basis of a stronger gel network with higher water holding capacity compared to high pressure induced gels (Van Camp & Huyghebaert, 1995). The G''/G' proportion is significantly higher in the case of high pressure induced gels, and shows a tendency to decrease with increasing protein concentration. This implies that high pressure induced WPC gels show an increasing elastic behavior at higher protein concentrations, but remain under all conditions less elastic compared to heat induced WPC gels (Hung & Smith, 1993).

The behavior of a solid gel network under constant stress can be followed by a creep experiment (Kamata *et al.*, 1988; Kamata & Kinsella, 1989; Katsuta *et al.*, 1990). The creep–compliance curves for three of the protein concentrations applied —110, 132 and 161 g/liter — are presented for both high pressure and heat induced WPC gels in Fig. 2 (top). The resulting

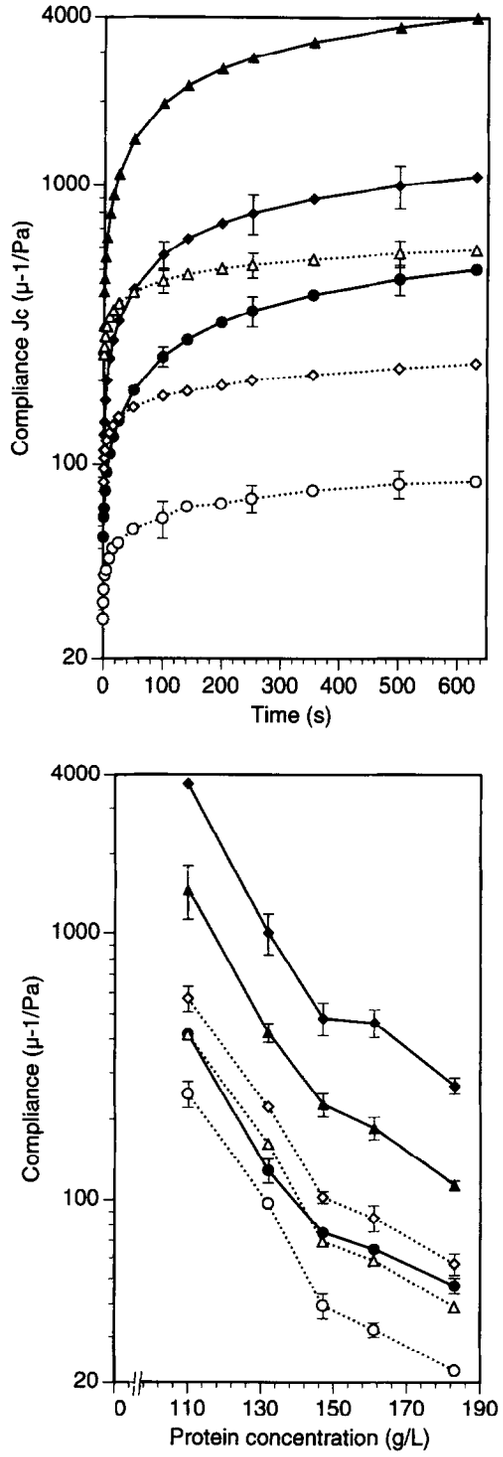

Fig. 2. Creep/compliance curves as a function of time at a protein concentration of 110 (▲ or △)–132 (◆ or ◇) and 161 (● or ○) g/liter (top), and as a function of protein concentration at 0.5 (● or ○)–50 (▲ or △) and 500 (◆ or ◇) s creep time (bottom) for high pressure (4 kbar/30 min; filled symbols) and heat (80°C/30 min; open symbols) induced WPC gels.

compliance values as a function of the protein concentration at various time intervals after application of the stress, are outlined in Fig. 2 (bottom). The first stable reading after application of the stress was obtained after 0.5 s, which is taken as a measure for the instantaneous compliance of the sample. The resulting values decrease with increasing protein concentration for both heat and high pressure induced protein gels, and are for a given protein concentration the lowest in the case of heat induced gels. From these results it might be deduced that the polypeptide strands in the high pressure induced gel networks have a higher degree of mobility to rearrange between the crosslinks in comparison to heat induced gels (Katsuta *et al.*, 1990). Increasing the protein concentration increases the chance of forming intermolecular bondings (Cheftel *et al.*, 1985), which decreases the extent of rearrangement.

After an instantaneous deformation, a further more slowly increasing strain occurs, whereby the more solid-like nature of the heat induced gels is stressed by the smaller slope of the creep/compliance curve (Peleg, 1987), and as a consequence also by the more closely approaching compliance curves as a function of the time in Fig. 2 (bottom). For the high pressure induced WPC gels a higher slope is noticed, which in this case might be attributed to weaker interactions achieved between neighboring and denatured protein molecules. Also, during the creep measurement a liquid layer became visible around the protein gel slide which illustrates the rather low water holding capacity of these gel networks in comparison to the heat induced protein gels, and which might have contributed to the more viscous nature of the high pressure induced protein gels.

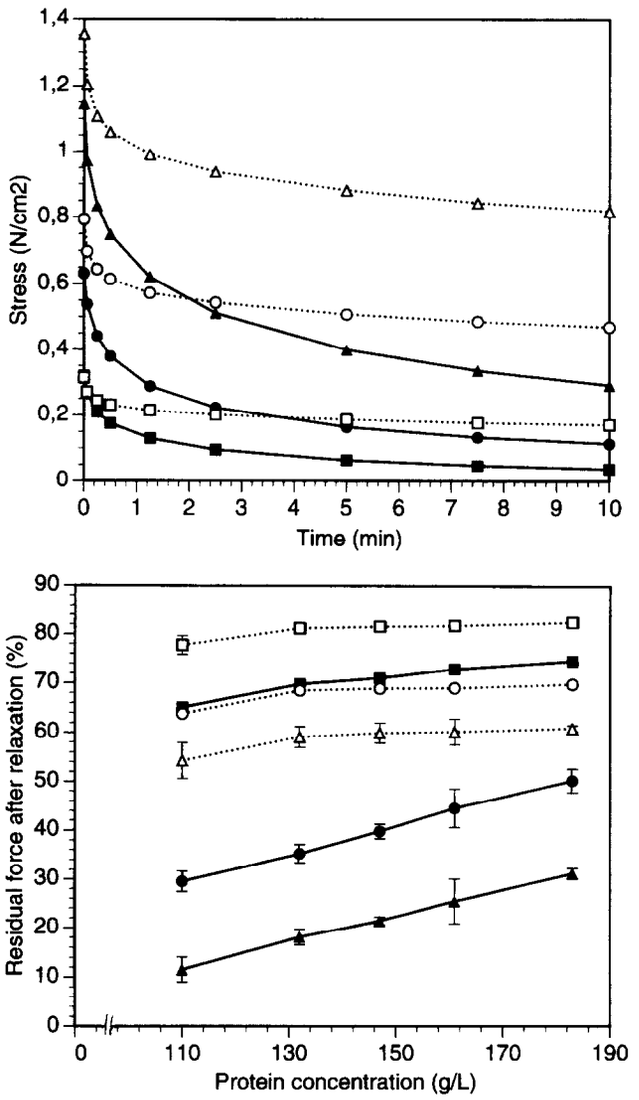

Fig. 3. Relaxation curves as a function of time at a protein concentration of 110 (■ or □)–132 (● or ○) and 161 (▲ or △) g/liter (top), and as a function of protein concentration at 0.25 (■ or □)–2.50 (● or ○) and 10.00 (▲ or △) min relaxation time (bottom) for high pressure (4 kbar/30 min; filled symbols) and heat (80°C/30 min; open symbols) induced WPC gels.

Rheological characterization of WPC gels at large deformations: relaxation experiments and stress–strain-relationships

By the use of a relaxation experiment at large sample deformations, the resulting stress relaxation in the gel network can be followed as a function of time (Peleg, 1987). In Fig. 3 (top) the stress decay during relaxation at a deformation level of 33% is given for various concentrations of high pressure and heat induced WPC gels. The residual force at various relaxation times, expressed in relative terms to the initial force obtained immediately after compression, is presented for both types of WPC gels in Fig. 3 (bottom). Deformation of both heat and high pressure induced gel networks to a level of 17 instead of 33% reduced the absolute values of the registered stress with 50 to 60%, but did not significantly affect the relative force decay as a function of time. There was no need to convert the relative force decay curves to their stress equivalents since for both heat and high pressure induced gel networks at all protein concentrations and at both deformation levels studied, no change in sample top area during relaxation occurred.

WPC gels generated by heat are characterized by a rather small force decay as a function of time, which is only slightly affected by the protein concentration below 130 g/liter (Fig. 3 bottom). The behavior can clearly be catalogued as being viscoelastic, with a predominant influence from the elastic component. WPC gels generated by high pressure maintain viscoelastic properties, but differentiate from heat induced gels by a more pronounced force decay as a function of time. Also, the effect of protein concentration is clearly more accentuated. During the course of the relaxation experiment, the total force generated in the gel network as a result of compression is reduced by the rupture of high energetic bondings, followed by the concomitant formation of bondings with lower energy content (Peleg, 1987). This stress relaxation process is clearly more pronounced in high pressure induced WPC gels, presumably caused by the presence of weaker intermolecular bondings. Increasing the protein concentration increases the amount and proportion of stable intermolecular

contacts between adjacent polypeptide side chains, and as a consequence also reduces the relaxation phenomenon.

The contours of true stress–true strain relationships allow the deduction of the behavior of gel networks during the process of compression to large sample deformations (Peleg, 1987; Hsieh *et al.*, 1993). The true stress–true strain relationships for high pressure and heat induced WPC gels up to a deformation level of 33% are presented for various protein concentrations in Fig. 4. Apart from a concave upward curve during the initial stages of compression, a linear relationship was found for all gel networks studied. A concave upward true stress–true strain relationship is characteristic for a predominantly compressive material (Johnson *et al.*, 1980). Compaction of the gel network produces a denser structure which tends to increase the overall stress measured (Calzada & Peleg, 1978). For all strain levels evaluated, no collapse point for WPC gels could be found. As a consequence, it can be assumed that for both type of protein gels the linear part of the stress–strain relationship is associated with a rather elastic or rubbery gel network where no fragmentation or excessive compaction occurs under the strain levels applied. Increasing the protein concentration for WPC produces a higher true stress for a given strain level, and as a consequence increases the slope of the stress–strain relationship which is also indicated as 'compression modulus' and used to quantify the stiffness of a material (Peleg, 1987). For all protein concentrations evaluated, the compression modulus for the high pressure induced WPC gels is always lower compared to these of the corresponding heat induced WPC gels, although its increase with protein concentration is lower for the latter type of gels: increasing the protein concentration from 110 up to 183 g/liter increases the modulus from 0.4 to 3.1 in the case of high pressure induced gels, and from 1.1 up to 5.4 in the case of heat induced gels.

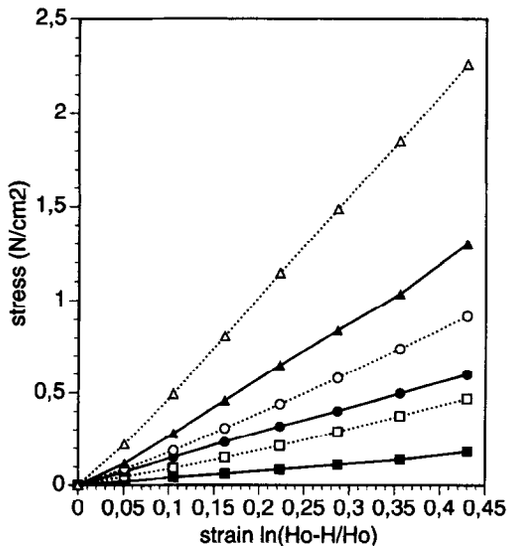

Fig. 4. Stress–strain relationships of high pressure (4 kbar/30 min; filled symbols) and heat (80°C/30 min; open symbols) induced WPC gels at a protein concentration of 110 (■ or □)–132 (● or ○) and 183 (▲ or △) g/liter.

Gel microstructure

To investigate the microstructure of the heat and high pressure induced WPC gels, an electron microscopic analysis at both 500 × and 2000 × magnification has been performed at a final protein concentration of 132 g/liter (Fig. 5).

High pressure induced WPC gels (Fig. 5, left) are characterized by a porous, finely stranded netwerk structure in which the holes around the polypeptide chains are presumably filled with non-incorporated liquid (NIL). Deformation of the sample specimen, or application of a stress in order to induce deformation, can promote the loss of liquid from the gel network, which indeed has been confirmed during the rheological measurements.

In the case of heat induced WPC gels (Fig. 5, right), a less porous, more compact network structure is obtained in which clearly more intermolecular network bondings between the adjacent polypeptide side chains have been formed. The narrow hollow spacings (diameter 1.5–4.5 μm) form separate entities capable of maintaining the liquid enclosed during deformation. The more continuous network structure with a larger number of contact points found for heat induced gels in comparison to high pressure induced gels, is positively correlated with their higher gel strength (Van Camp & Huyghebaert, 1995), their increased storage modulus during oscillation (Fig. 1), their smaller sample deformation during creep (Fig. 2), their reduced force decay during relaxation (Fig. 3), and their higher compression modulus during compression (Fig. 4).

CONCLUSIONS

The rheological properties of WPC gels obtained after a pressure treatment at 4000 bar for 30 min without additional supply of heat to the pressure vessel, differ significantly from those obtained after a heat treatment of 80°C for 30 min at atmospheric pressure. Both from the experiments performed at small and at large sample deformations it is suggested that more long term bondings have been formed between the polypeptide side chains in the case of heat induced WPC gels. High pressure gels on the contrary presumably contain a higher amount of weaker, short term bondings which more easily disrupt upon deformation of the gel network. Furthermore, the use of electron microscopy has highlighted that a more porous structure with a lower number of contact points is valid for the high pressure gel networks.

Based on these results it cannot be excluded that deviations occur in the mechanisms of protein denaturation and aggregation during gelation induced by heat and by high pressure. In order to obtain information on the nature of the interactions formed (hydrogen bonds, hydrophobic interactions, electrostatic interactions, SS-bonds), and on their relative contribution to the formation of the network structure, it might be of interest to characterize the high pressure induced WPC

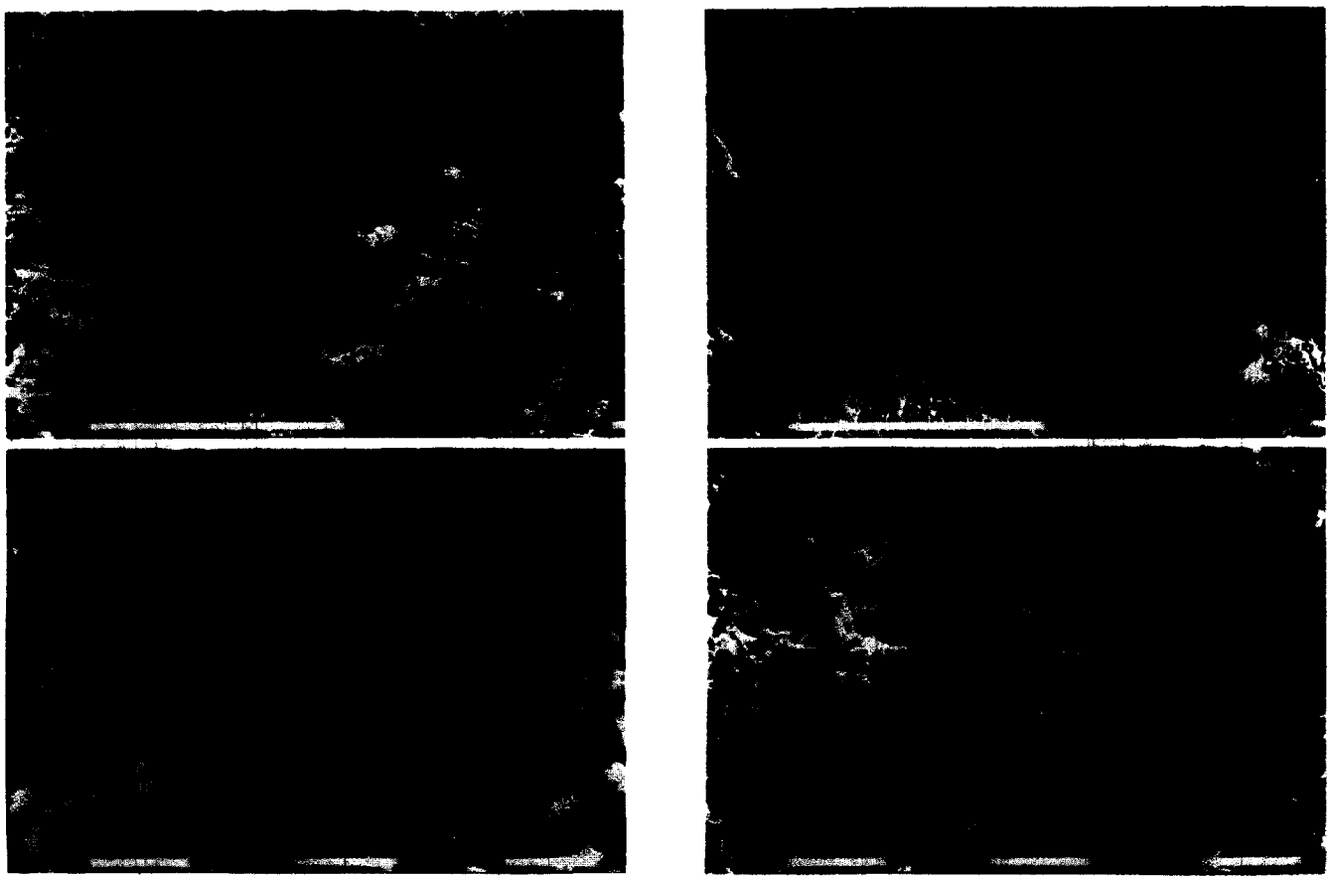

Fig. 5. Electron microscopic analysis of high pressure (4 kbar/30 min; left) and heat (80°C/30 min; right) induced WPC gels at a protein concentration of 132 g/liter. Solubilization was achieved in 0.05 mol/liter phosphate buffer, pH 7.0. Analysis was performed at a magnification of 500 × (top) and 2000 × (bottom). The bars in the top figures and the bottom figures correspond to 100 and 10 μm, respectively.

gels in the presence of food additives (e.g. salts, sugars), and denaturing agents (e.g. β-mercapto-ethanol, urea, sodium dodecyl sulphate) (Shimada & Cheftel, 1988; Dumay *et al.*, 1994). Also, the use of rheological measurements at various operating temperatures (Kamata *et al.*, 1988; Kamata & Kinsella, 1989; Katsuta & Kinsella, 1990;Katsuta *et al.*, 1990) might give some additional information.

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