

Gelation of faba bean proteins - Effect of extraction method, pH and NaCl

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ABSTRACT

The effect of extraction method, pH and NaCl addition on rheological properties and microstructure of heat-induced faba bean protein gels was evaluated. Gels formed at pH 7 (no NaCl) of alkaline-extracted protein had the densest and finest network structure and highest stress and strain at fracture. The high density of nodes and small pores in the protein network could contribute to those mechanical properties. In contrast, storage modulus (G') and Young's modulus were lowest for protein gels at pH 7. The gels formed at pH 5 had high G' and Young's modulus, whereas stress and strain at fracture were lower, especially for gels formed from alkaline-extracted protein. Gels formed at pH 5 with 2% NaCl had two types of internal gel network, caused by a change in solubility of 7S globulins. When the protein powder was dissolved in water, particle size was dependent on the extraction method, with alkaline extraction giving much larger protein particles than soaked extraction.

1. Introduction

The challenges of climate change and population growth are generating demand for innovative, locally produced vegetable-based protein-rich foods. The key to success is knowledge of designing multi-scale protein structures. Most texturised vegetable-based protein-rich foods are currently made from either soybean, little of which is grown in the Scandinavian countries, or wheat gluten, which may be undesirable due to the growing incidence and awareness of coeliac disease. Faba bean grows well throughout Sweden and is extensively produced, but this protein and energy-rich crop is primarily used as animal feed (<https://www.ja.se/artikel/51581/kerbnoor-ska-bli-mat.html>).

Gelation of protein is the crucial first step in the manufacture of various foods, but there is a knowledge gap regarding the molecular mechanisms that result in high quality gel structures based on legumes and how these can be achieved with commercial manufacturing techniques. Fundamental data regarding the conditions and mechanisms of gelation of legume products can provide new possibilities in the field of sustainable food science. Furthermore, extraction of protein from faba beans has been demonstrated to almost completely remove the favism-causing glucosides vicine and convicine (Vioque, Alaiz, & Girón-Calle, 2012). This is desirable when faba beans are to be used for food applications.

There are many products based on legumes available on the market (e.g. tofu, tempeh, alternatives to dairy milks, pasta). Legumes have a

high protein content and high protein quality, so are ideal for inclusion in a daily diet. The vast majority of processed vegetarian protein foods are based on soybean, which is a leading source of vegetable proteins worldwide. Faba bean has somewhat similar physical and functional properties to soybean (Zee, Boudreau, Bourgeois, & Breton, 1988), with around 27% protein (dry weight). It has a lower lipid content (1%) than soybean (18%) and less flatulence factors (raffinose and stachyose) than cowpea (Abdel-Gawad, 1993). The major proteins are globulins, which consist of two high-molecular-weight proteins called legumin and vicilin (11S and 7S) (Danielsson, 1950; Kimura et al., 2008).

Pre-treatment of flour and extraction parameters, such as choice of solvent, pH and temperature, determine the protein fractions and other compounds found in the protein isolate and their characteristics. Choice of extraction conditions can affect the gel characteristics of the extracted protein.

A range of different extraction methods have been developed for different protein sources. Here we compared what we refer to as soaked extraction and alkaline extraction. Soaked extraction is similar to the method commonly used for tofu production and has been used for extraction of soybean and faba bean (Zee et al., 1988). Protein extraction by alkaline NaOH solution has been used for a range of legumes, including pea and faba bean (Arogundade, Tshay, Shumey, & Manazie, 2006; McCurdy & Knifel, 1990; Munialo, Martin, Van Der Linden, & De Jongh, 2014).

The properties of protein gels depend not only on their composition,

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but also on the conditions used to induce gelation. Globular proteins, such as those found in faba bean protein isolates, usually form two types of gels, depending on how much charge the native protein carries. For example, in whey protein fine-stranded gels are formed when repulsion is large, while a network of colloidal particles is formed when the isoelectric point is approached (Langton & Hermansson, 1992). It has been shown that soy protein gels are more homogeneous when formed at pH 6.4 than when formed at lower pH (Chen, Zhao, Niepcron, Nicolai, & Chassenieux, 2017a). Increasing heterogeneity has also been observed in gels made of soy protein isolate on increasing the NaCl content to 0.4 M (Chen, Zhao, Chassenieux, and Nicolai, 2017b). Faba bean proteins have an isoelectric point of 5.0–5.5 (Danielsson, 1950). Many food applications have pH in the range of 5–7, often with NaCl addition, and understanding of how protein gels behave within this range is therefore crucial.

Microstructure affects the texture of products. The texture can be evaluated by determining the mechanical properties in small, non-destructive or large, destructive, deformation tests (Langton, Åström, & Hermansson, 1996). The two methods reflect different properties of the product. Langton et al. (1996) found that, in the case of particulate whey protein gels, the overall network dimensions (particle size and pore size) are revealed in the destructive tests, while the non-destructive tests are sensitive to strand characteristics. Thus, it is important to evaluate texture using different methods.

The objectives of this study were to determine the effects of protein extraction method for faba bean protein, pH and NaCl addition on gel texture and microstructure. The texture was analysed using both small and large deformation tests. The microstructure of the set hydrogels was analysed using light microscopy. The correlation between rheology and structure was investigated.

2. Materials and methods

All chemicals used were of reagent grade quality. Faba bean (*Vicia faba minor*) of the variety *Gloria* was kindly provided by RISE (Research Institutes of Sweden). *Gloria* is a white flowering species that is usually lower in tannins than varieties with more highly pigmented flowers (Sverigeförsöken, 2012).

2.1. Design

To evaluate the effect of pH and NaCl concentration on the textural and structural properties of gels made from different faba bean protein extracts, a 2² full factorial design with duplicates at each point was used (Table 1).

2.2. Protein extraction

Two different methods were used for extraction of proteins from faba beans, soaked extraction and alkaline extraction. All beans were dehulled prior to extraction. Protein content of the flour and the extracts was determined using the Kjeldahl method with a conversion factor of 5.4. Protein content was found to be 32.3%, 67.0% and 81.7% (dry

Table 1

Sample design, SPE = soaked protein extract, APE = alkaline protein extract.

Protein	pH	Added NaCl (%)
SPE	5	0
SPE	5	2
SPE	7	0
SPE	7	2
APE	5	0
APE	5	2
APE	7	0
APE	7	2

weight basis) for the flour, soaked extract and alkaline extract, respectively. The crude yield and protein yield was 21% and 43.4% for soaked extract, and 24.4% and 61.7% for alkaline extract. For dry content analysis, samples were dried at 105 °C overnight.

2.2.1. Soaked extraction

Dehulled faba beans were soaked in water overnight at a ratio of 1:2 and then water was added to obtain a bean to water ratio of 1:3. The soaked beans were processed in a high-speed blender (2 × 2 min) and the pH of the slurry obtained was adjusted to 8 with 5 M NaOH. The slurry was then stirred for 1 h with a magnetic stirrer, before centrifugation at 3700g (20 °C, 15 min). The supernatant was collected, frozen at −21 °C and lyophilised in an Edwards Modulyo freeze dryer (Edwards, UK).

2.2.2. Alkaline extraction

For alkaline extraction, faba beans were first cryo-milled using an Ultra-Centrifugal Mill (ZM-1, Retsch GmbH, Germany) to yield a fine flour. The flour was then dispersed in distilled water at a ratio of 1:10 and the pH was adjusted to 8 using 5M NaOH. The slurry obtained was stirred at room temperature (20 ± 2 °C) for 2 h with a magnetic stirrer and then centrifuged at 3700 g for 30 min. The supernatant was collected and the pellet discarded. The pH of the supernatant was adjusted to 4.0 using 1 M HCl to precipitate the protein and stirred for 30 min before centrifugation at 3700g for 30 min. The supernatant was discarded and the pellet was re-dispersed in distilled water at a ratio of 1:6. The pH was adjusted to 4.0 and the dispersion was centrifuged again at 3700 g for 30 min. Finally, the pellet was re-dispersed in distilled water and the pH adjusted to 8. The sample was then frozen and lyophilised as described for the soaked extract.

2.3. SDS-PAGE and size exclusion chromatography

In order to investigate whether there were any differences in the content of proteins extracted using the two different methods, i.e. soaked extraction and alkaline extraction, one-dimensional SDS-PAGE was performed on Mini-PROTEAN TGX, 4–20%. For this, 0.05 g of dried protein from each method was mixed with 2.5 mL of 30 mM bicine buffer pH 9.0 containing 0.5 M NaCl. After complete solubilisation, the protein solutions were run through two different PD-10 columns. The absorbance of the solutions was then measured and an appropriate dilution was made. A few microlitres of each solution were used for the gel tests (Fig. 1). To further assess the nature of the proteins obtained from the two methods, size exclusion chromatography (SEC) was performed by loading 500 µL of protein solution obtained after PD10 onto a Superdex-200. Two major peaks were obtained, corresponding to molecular weights of 353 and 150 KD.

2.4. Gel formation

Protein isolates were dispersed in distilled water and stirred for 10 min with a magnetic stirrer before adjustment of pH to 5 or 7 with 1 M HCl. After pH adjustment the mixture was stirred for an additional 30 min, followed by pH adjustment if required. The volume was then adjusted to give a final protein concentration of 150 mg/mL. For the samples with 2% NaCl (0.342 mol/L), the salt was added after volume adjustment and the mixture was stirred for an additional 30 min. Gels were then produced by adding 30 mL of sample to 50 mL Falcon tubes and heating the tubes for 30 min in a water bath at 95 °C. This was followed by rapid cooling in tap water. All gels were stored in a fridge overnight before compression testing and preparation of samples for microscopy.

2.5. Compression testing

Gels prepared as described in section 2.3 were cut into cylindrical

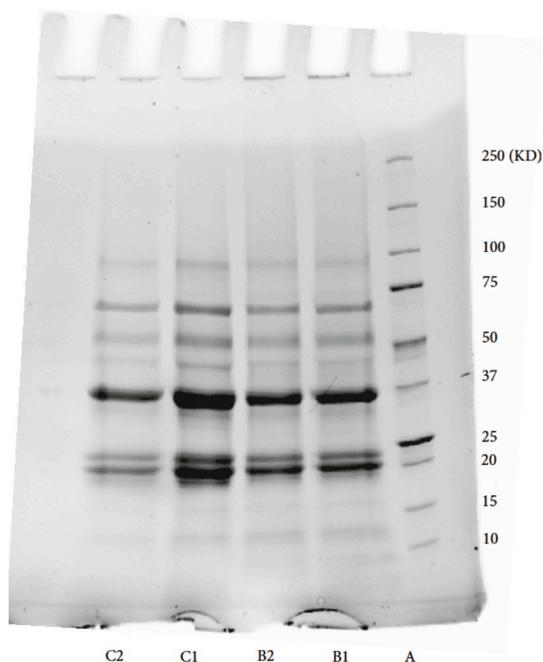


Fig. 1. SDS-PAGE of faba bean proteins extracted by soaked and alkaline extraction methods. A, molecular weight standard, B1 and B2 soaked extract, C1 and C2 alkaline extract.

pieces with diameter 21 mm and height 15 mm, using a cutting die. Compression tests were performed in a Texture Analyzer HDi (Stable Micro Systems, UK) equipped with a 500 N load cell and a 50 mm cylindrical aluminium probe. The samples were compressed to 80% at a rate of 1 mm/s. Young's modulus, compressive stress at 40% strain, compressive stress at fracture and strain at fracture were determined. Fracture was defined either as the first peak in the stress strain curve or, when a clear peak could not be detected, as the onset of the first plateau.

2.6. Rheology

A Bohlin C-VOR rheometer (Malvern Instruments Nordic AB, Sweden) equipped with a 25 mm concentric cylinder geometry was used to study the gelation process as a function of temperature of the protein mixtures, prepared as described above. Small angle oscillatory measurements at 1 Hz and 1% strain were performed with a temperature profile going from 25 to 95 °C at 1.5 °C/min, 30 min hold at 95 °C followed by a decrease to 25 °C at a rate of 1.5 °C/min, which was held for 10 min. The storage modulus (G') was then measured.

2.7. Microscopy

Gels prepared as described in section 2.3 were cut into pieces of approximately 2 mm × 2 mm and fixated overnight in a mixture of 2% formaldehyde, 2.5% glutaraldehyde solution and 0.075% ruthenium red. The samples were then fixated with 1% osmium tetroxide. After fixation, the samples were dehydrated using ethanol of increasing concentration, followed by infiltration with LR white plastic resin. Finally, the resin was hardened at 65 °C. Embedded samples were cut into 1 μm thick sections using a Leica Ultramicrotome and stained with Light Green, which colours protein green/turquoise.

Protein mixtures were diluted in water and examined directly using differential interference contrast microscopy.

Protein mixtures and stained sections were examined using a Nikon Eclipse Ni-U microscope and images were captured with a Nikon Digital Sight DS-Fi2 camera equipped with a 40x (0.95 NA) and 60x (1.49 NA) apochromatic objective. The images were processed with the software

NIS-Elements BR (Nikon Instruments Inc., New York, USA).

2.8. Statistical analysis

SAS Proc GLM (SAS 9.4, SAS Institute Inc. Cary, NC, USA) was used to determine significant effects ($P < 0.05$) in the design. The effect of pH, NaCl and pH × NaCl interactions was investigated. Coefficients with $P < 0.2$ were excluded from the model. When a significant interaction was found, pairwise comparisons with adjustment for multiple comparisons according to Tukey were made between all samples. If no interaction was found, comparisons were only made between sample groups at pH 5 and 7 and between sample groups with 0% and 2% added NaCl. Analyses of samples with soaked protein extract and alkaline protein extract were performed separately.

3. Results and discussion

In a pre-study, the critical gel concentration was investigated for soaked protein extract and alkaline protein extract at pH 5 and pH 7, with and without NaCl. Not all samples formed proper gels at 13% and soaked extract had a somewhat lower critical concentration than alkaline extract. Thus 15% protein concentration was chosen for the hydrogels analysed in this study. This is similar to that used in a previous study for preparation of faba beans gels (15.4% w/w) (Makri, Papalamprou, & Doxastakis, 2006).

3.1. Rheology

The texture was first analysed by small deformation tests using a rheometer and recording the storage modulus, G' . Fig. 2 shows the gel formation of the two extracts, at pH 5 and 7, with and without NaCl. As can be seen from the diagram, the onset of G' increase occurred earlier, at lower temperature, for pH 5 gels compared with pH 7 gels. This is in agreement with results found for β-lactoglobulin and soy protein gels. For β-lactoglobulin an early stage of gelation was observed at pH 5.3, close to isoelectric point, but not at other pH (Stading & Hermansson, 1990). For soy protein an earlier onset of gelation was observed when decreasing pH from 6.8 to 5.8 (Chen et al., 2017a). The early increase in G' was most obvious for soaked protein extract (upper diagram in Fig. 2) at pH 5 with and without NaCl, and for alkaline extract at pH 5 without NaCl. It is possible that a similar early increase exists for the alkaline extract at pH 5 with NaCl but that the rheometer is not sensitive enough to detect it.

For soaked protein extract, the effect of both pH and NaCl on the final G' value was significant, with an increase in either pH or NaCl leading to a decrease in the final G' value, as seen in Fig. 2 and Table 2. For alkaline protein extract, the situation was more complex; the effect of both pH and NaCl was significant and there was a significant interaction effect between pH and NaCl. In pairwise comparison of samples with and without added NaCl, significantly higher G' was found for the pH 5 samples, but not for the pH 7 samples (see Table 2). The storage modulus, G' , of pH 5 samples with and without added NaCl was also higher than for the corresponding pH 7 samples. A similar increase in G' when lowering pH from around 7 to closer to the isoelectric point has previously been observed for soy and pea protein (Renkema, Gruppen & van Klijet, 2002; Sun & Arntfield, 2011). Both Renkema et al. and Sun & Arntfield also observed a pH dependent effect on G' of increasing the ionic strength.

3.2. Compression testing

The mechanical properties were also investigated using large deformation tests. The results from these compression tests are shown in Table 4 and Fig. 3, where the data are grouped to show the significant effects. For soaked protein extract gels, the results demonstrated significant interaction effects (Table 3) for compressive strain at fracture

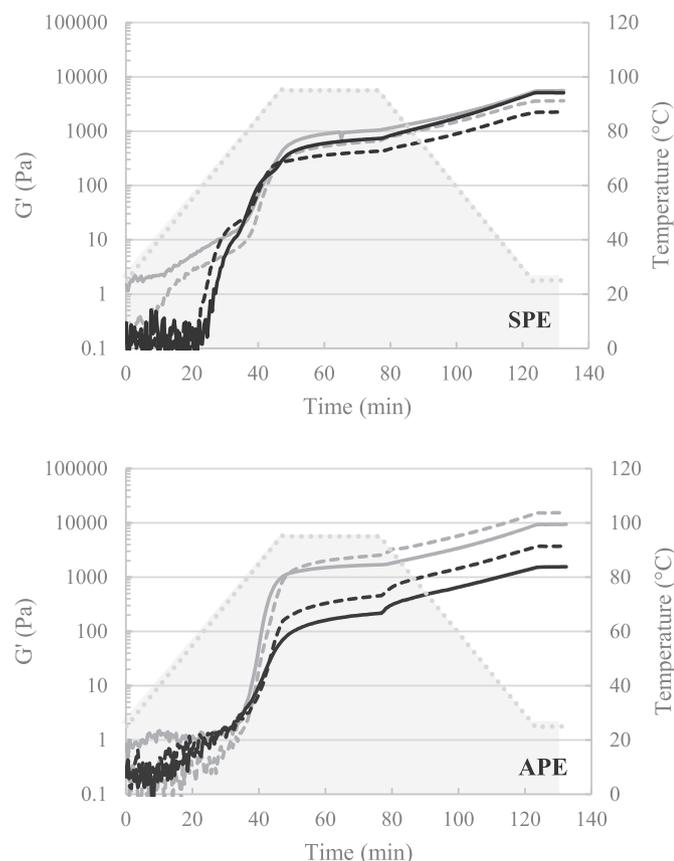


Fig. 2. Change in storage modulus (G') of protein isolates during temperature-induced gelation in a rheometer. Top: Soaked protein extract (SPE). Bottom: Alkaline protein extract (APE). Grey full lines: pH 5. Grey dashed lines: pH 5, 2% NaCl. Black full lines: pH 7. Black dashed lines: pH 7, 2% NaCl. Shaded area: Temperature profile.

Table 2

P-values obtained for different design factors in small deformation tests. SPE = soaked protein extract, APE = alkaline protein extract.

	SPE	APE
Storage modulus, G' (final)		
pH	<.0001	<.0001
NaCl	<.0001	0.0017
pH x NaCl	-	0.0238

and Young's modulus. Only NaCl had a significant effect on compressive stress at 40% strain, while both pH and NaCl had significant main effects on compressive stress at fracture (Table 3) (see Table 4).

For the gels made from alkaline protein extract, significant interactions were found for compressive stress and strain at fracture, and higher values were achieved at pH 7 without NaCl (Fig. 3, Table 3). For compressive stress at 40% strain, only NaCl had a significant effect, with NaCl yielding lower strain values. At pH 7, the stress and strain at fracture for alkaline extract decreased with addition of NaCl. This is in agreement with Makri et al. (2006), who found a significant reduction in stress at fracture for faba bean gels at pH 6.5 when adding 0.25 mol/L NaCl (Makri et al., 2006). A significant reduction in stress at fracture has also been found for soy protein gels at pH 3.8, 5.2 and 7.6 when adding NaCl (Renkema, 2004).

Overall, our results show that pH by itself had an effect on Young's modulus and storage modulus (G' value), with higher values being achieved at pH 5 compared with pH 7 (see Fig. 3).

3.3. Microstructure

Fig. 4 shows micrographs of embedded and sectioned gels made with soaked protein extract at pH 5 and 7, with and without added NaCl. Gels formed at pH 5 had a particulate structure, while gels formed at pH 7 had a denser and finer microstructure. At pH 5 with added NaCl, a secondary fine-grained protein network structure formed between the protein particles and in the cavity (c) in the gel. Addition of NaCl at pH 7 appeared to yield a more open structure (more heterogeneous) than in the gel at pH 7 without added NaCl.

Fig. 5 shows micrographs of embedded and sectioned gels made with alkaline protein extract at pH 5 and 7, with and without added NaCl. Gels at pH 5 had a particulate microstructure (coarser than for the soaked extract at pH 5), while gels at pH 7 had a denser, more homogeneous structure. At pH 5 with added NaCl, a secondary fine-grained protein network structure developed between the coarse protein particles. At pH 7, addition of NaCl appeared to yield a more heterogeneous structure than in the gel without NaCl. The gel formed from alkaline extract at pH 7 and no NaCl had the densest and finest microstructure.

As expected, all pH 5 gels had a coarser, more particulate structure and all pH 7 gels had a finer structure with smaller pores. This is in agreement with results found for soy protein at pH 6.4–pH 5.8 (Chen et al., 2017a) and for whey protein gels/beta-lactoglobulin gels (Langton & Hermansson, 1992), all of which formed coarser gels around their isoelectric point. However, the influence of NaCl is not always predictable.

Faba bean protein is composed of two major proteins, 7S and 11S globulins (Kimura et al., 2008). To check for presence of the two globulins in the protein extracts, size exclusion chromatography was performed. The chromatograms obtained for faba proteins extracted by the two methods showed a similar pattern, with two major peaks. Using the elution volume at the two separate peaks, the molecular weight of the proteins was determined to be 353 and 150 KD which corresponds to the 11S and 7S globulins, respectively (Derbyshire, Wright, & Boulter, 1976).

The solubility of 7S and 11S from faba bean is dependent on the pH and ionic strength. Kimura et al. (2008) found that at low ionic strength, 7S and 11S globulins from faba bean had low to intermediate solubility at both pH 5 and 7. At a concentration of 0.5M NaCl the solubility of both 7S and 11S increased to around 90% at both pH 5 and 7. Notably, at high ionic strength there was a sharp decrease in solubility of 11S, but not 7S, when going from pH 5 to 4.5. Considering this, it is possible that slightly lower ionic strength would result in lower solubility of 11S compared with 7S at pH 5. Furthermore, Arogundade et al. (2006) detected a peak around 0.4 M NaCl in protein solubility both at pH 4 and 7. Although, the results did not include data for 0.3 or 0.5 M, it does indicate that a shift in ionic strength in that range will alter the solubility.

If the solubility of the two fractions is different under the conditions used in the current study, the two different protein network structures observed in the gels at pH 5 with added NaCl could have different protein composition. It then seems reasonable to expect that 7S would be the more soluble fraction and consequently be the main constituent of the secondary protein network structure surrounding the larger protein particles. This gel, at pH 5 with NaCl, had the highest G' value for the alkaline protein extract, indicating that the two internal protein networks could influence the rheological properties. This would then indicate that the ratio of 7S and 11S could have an impact when using faba bean protein extract in food applications, where pH 5 and NaCl addition is common. This needs to be further explored.

There was no obvious difference in the SDS-PAGE profiles between the different extracts (Fig. 1), whereas there was a difference between the solubility of the two extracts (Fig. 6). The soaked protein extract dissolved more fully in water, whereas the alkaline freeze-dried extract remained in large lumps when redispersed. When comparing the micrographs of the dispersed protein before and after freeze drying it is

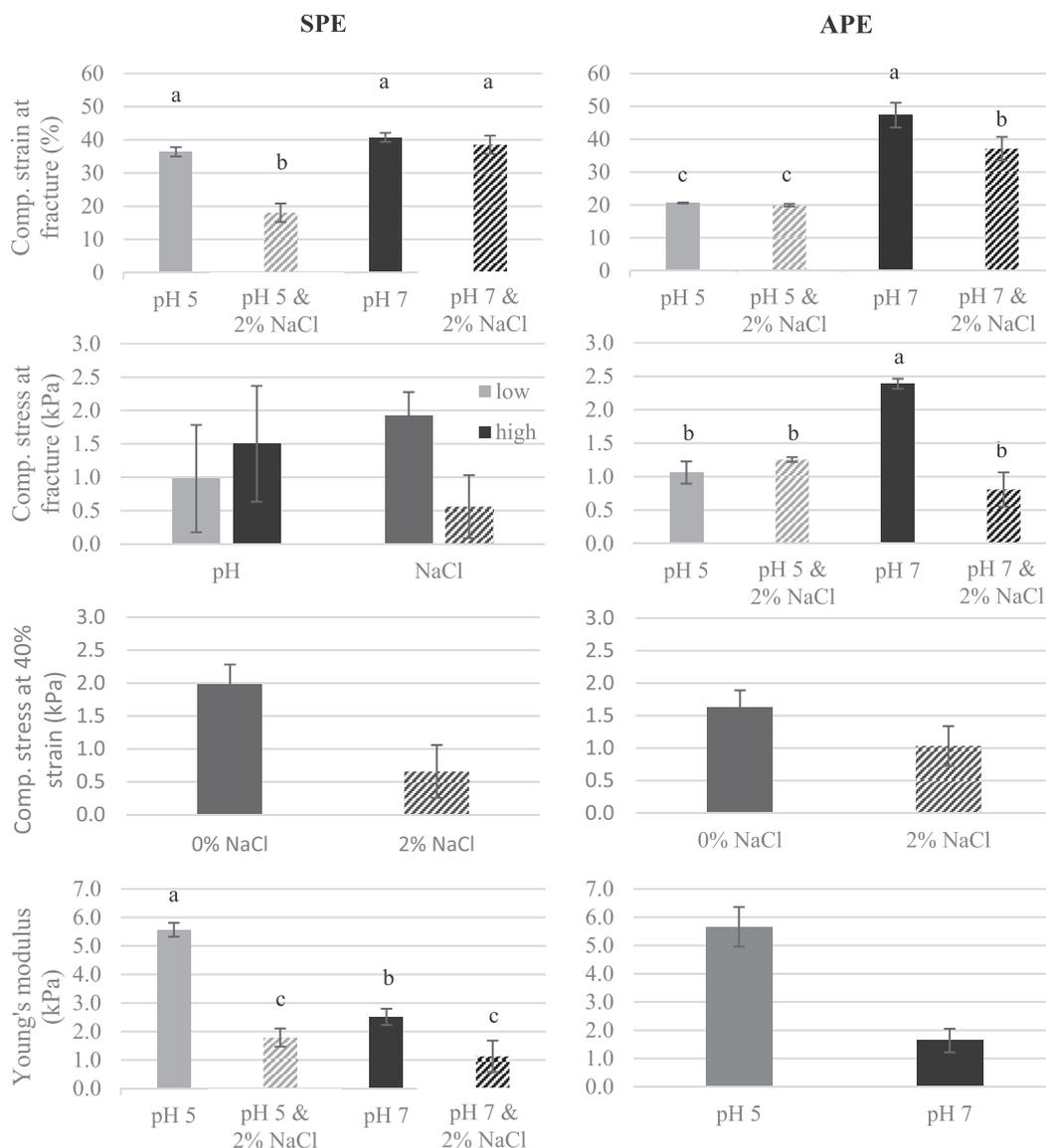


Fig. 3. Results from compression testing of protein gels. When no significant interaction effect between pH and NaCl was found, data are grouped to show the significant main effects. When significant interactions were found, significant differences between samples are indicated with letters. Left column: Soaked protein extract (SPE). Right column: Alkaline protein extract (APE).

Table 3

P-values obtained for different factors in large deformation tests. SPE = soaked protein extract, APE = alkaline protein extract.

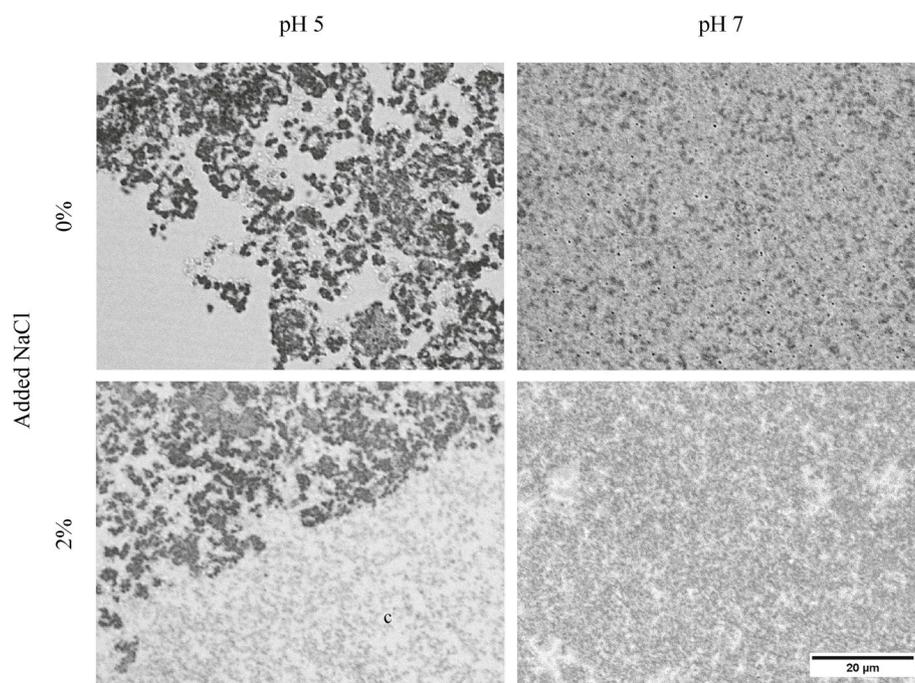
	SPE	APE
Compression strain at fracture		
pH	0.0002	0.0006
NaCl	0.0004	0.0338
pH x NaCl	0.0007	0.0468
Compression stress at fracture		
pH	0.0370	0.0423
NaCl	0.0013	0.0127
pH x NaCl	-	0.0063
Compression stress at 40% strain		
pH	-	0.8087
NaCl	0.0038	0.0341
pH x NaCl	-	0.0913
Young's modulus		
pH	0.0003	0.0003
NaCl	0.0001	-
pH x NaCl	0.0012	-

clear that the formation of large aggregates occurred during the freeze drying process and that it was most prominent for the alkaline extract (Fig. 6). This difference in particle size/solubility in water could be explained by differences in the extraction process; during alkaline extraction the pH is adjusted to make the protein precipitate, whereas no precipitation occurs during soaked extraction. This could yield larger particles in the alkaline extract prior to freeze drying which then more easily forms larger extracts during the drying process. Furthermore, the absence of a precipitation step for the soaked extract also means that water soluble non protein components, e.g. oligosaccharides, could remain in the extract. This could account for the lower protein content in the soaked extract. The presence of these compounds might stabilize the protein during freeze drying, preventing aggregation and easing the redispersal. The protein aggregation during lyophilisation likely explains the much larger particles in the alkaline extract gels at pH 5. After adjustment of the pH to 7 and heating both extracts dissolved and, judging from the appearance of the gel microstructure, the alkaline process extracted more protein on average than the soaked extraction process. As a result, the alkaline extract gel had the finest network

Table 4

Results from compression tests. Average values of two replicates, standard deviation in brackets. SPE = soaked protein extract, APE = alkaline protein extract.

Protein isolate	Sample	Compression strain at fracture (%)	Compression stress at fracture (kPa)	Compression stress at 40% strain (kPa)	Young's modulus (kPa)
SPE	pH 5	36.4 (1.4)	1.7 (0.01)	1.8 (0.3)	5.6 (0.2)
	pH 5 & 2% NaCl	18.0 (2.8)	0.3 (0.1)	0.4 (0.01)	1.8 (0.3)
	pH 7	40.7 (1.4)	2.2 (0.4)	2.1 (0.3)	2.5 (0.3)
	pH 7 & 2% NaCl	38.5 (2.7)	0.8 (0.6)	0.9 (0.5)	1.1 (0.6)
APE	pH 5	20.6 (0.2)	1.1 (0.2)	1.5 (0.2)	5.2 (0.7)
	pH 5 & 2% NaCl	19.9 (0.4)	1.3 (0.04)	1.3 (0.1)	6.2 (0.1)
	pH 7	47.3 (3.8)	2.4 (0.1)	1.8 (0.2)	1.9 (0.1)
	pH 7 & 2% NaCl	37.1 (3.6)	0.8 (0.3)	0.8 (0.3)	1.4 (0.5)

**Fig. 4.** Micrographs of embedded and sectioned gels made with soaked protein extract (SPE) at pH 5 and 7, with and without added NaCl. Proteins are coloured green-blue.

structure of all gels investigated (compare Figs. 5 and 4).

3.4. Structure and texture

The alkaline protein extract gel formed at pH 7, no NaCl, had the densest and finest network structure, as well as the highest stress and strain at fracture. In contrast, the G' and Young's modulus values were lowest for protein gels at pH 7. The gels formed at pH 5 had high G' and Young's modulus values and lower stress and strain at fracture, especially for the alkaline extract gels. High Young's modulus and G' in combination with low fracture stress or strain has previously been observed for soy and whey protein gels (Langton et al., 1996; Renkema, 2004; Stading & Hermansson, 1991). It has been suggested that the number nodes, curvature and thickness of protein strands to a large degree determine the modulus and fracture properties of protein gels. The high strain/stress at fracture of the pH 7 gels could be due to a high amount of nodes in a more fine-stranded network possibly formed of flexible curved strands; which is the case for β -lactoglobulin gels. However, we have not conducted structural investigations at a magnification sufficient to determine the curvature or number of nodes of the protein strands in the pH 7 gels. The high G' /Young modulus of the pH 5 gels could be due to that we are measuring on the particles visible in the micrographs (Figs. 4 and 5), which forms strands, like strings of beads, with low flexibility. This again shows the importance of measuring

texture using different methods.

4. Conclusions

- Coarse particulate gels formed at pH 5 and finer gels formed at pH 7, independent of extraction method and NaCl addition.
- At pH 5, addition of 2% NaCl led to the microstructure separating into a coarser and a finer network within the same gel.
- Different responses were obtained when using large and small deformation tests, relating to pore size (or amount of nodes in the network) and large particles forming the gels, respectively.
- There were very small differences between the two extraction methods used.

CRediT authorship contribution statement

Maud Langton: Conceptualization, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **Sohail Ehsanzamir:** Investigation. **Saeid Karkehabadi:** Investigation. **Xinmei Feng:** Investigation, Resources, Funding acquisition. **Monika Johansson:** Investigation, Funding acquisition. **Daniel P. Johansson:** Conceptualization, Writing - review & editing, Investigation, Formal analysis, Validation, Visualization, Methodology.

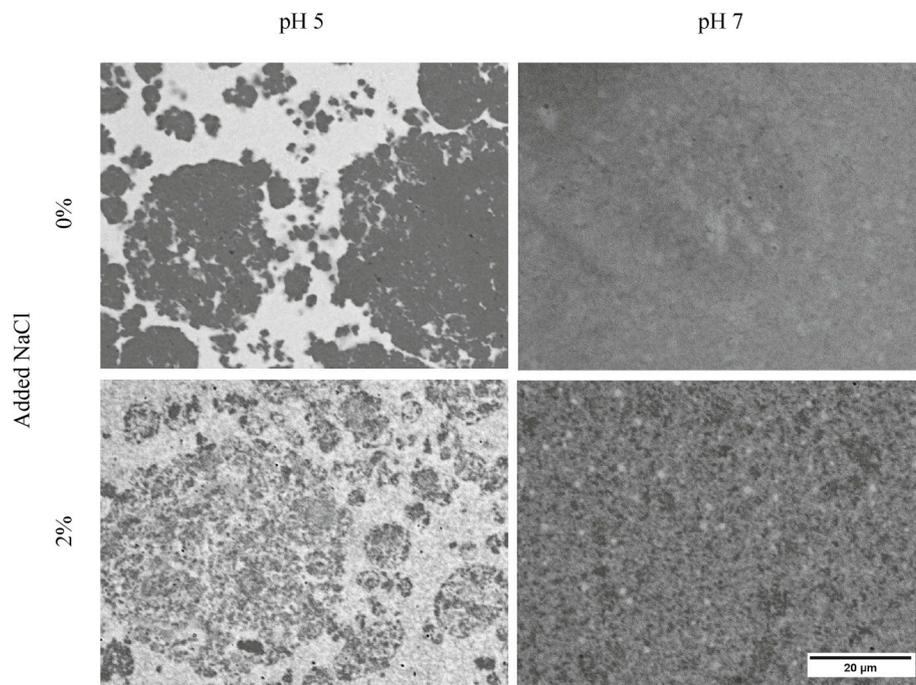


Fig. 5. Micrographs of embedded and sectioned gels made with alkaline protein extract (APE) at pH 5 and 7, with and without added NaCl. Proteins are coloured green-blue. Gels at pH 5 have a particulate structure (coarser than in soaked protein extract at pH 5), while gels at pH 7 have a denser, more homogeneous structure.

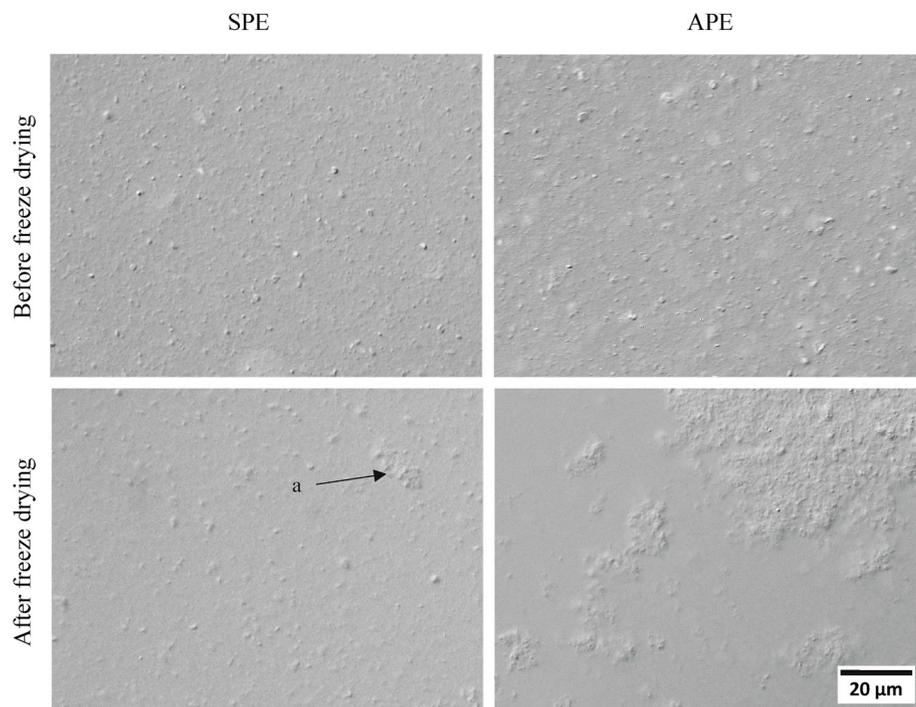


Fig. 6. Micrographs showing protein particles in samples before pH (~8) adjustment and heating. Unstained samples visualised using differential interference contrast microscopy. Left: SPE = soaked protein extract, Right: APE = alkaline protein extract.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2019.105622>.

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