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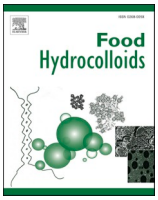

Gelation behaviour and gel properties of the 7S and 11S globulin protein fractions from faba bean (*Vicia faba* var. minor) at different NaCl concentrations

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ABSTRACT

The 7S and 11S globulins, the main protein fractions found in legumes, may differ in functionality. This study evaluated gel formation and rheological and microstructural properties of gels formed from the 7S and 11S protein fractions of faba bean. The effect of adding sodium chloride (NaCl) was also investigated. In terms of rheological and mechanical properties, NaCl addition appeared to have an opposing effect on 7S and 11S gels. Gels formed from 7S showed increases in storage modulus and peak stress when NaCl was added, whereas gels formed from 11S showed decreases. Microstructural changes were observed only for 7S gels, for which addition of NaCl resulted in transition from a fine-stranded to a coarse-stranded gel network. The 11S gels showed a fine-stranded gel network at all NaCl concentrations investigated. Gels formed from a mixture of 7S and 11S (7S:11S ratio 3:7) showed similar rheological properties and microstructure as the 11S gels.

1. Introduction

Legumes are an important source of proteins and could be included as a novel and more sustainable alternative to animal-based foods in the human diet. However, development of novel foods based on non-soy legumes is hampered by lack of understanding of the mechanisms governing formation of gels and other structures. Legumes, such as faba bean (*Vicia faba*), contain a large amount of proteins. The two major globular proteins in faba bean are vicilin and legumin, known as 7S and 11S, respectively (Warsame, Michael, O'Sullivan, & Tosi, 2020). Around 20–34% of the total protein in faba bean consists of vicilin/convicilin and 35–60% consists of legumin (Multari, Stewart, & Russell, 2015; Warsame et al., 2020). The 7S:11S ratio differs between faba bean varieties, ranging from 1:1 to 1:3.6 (Warsame et al., 2020). Both 7S and 11S have similar amino acid composition (Multari et al., 2015), but the 7S globulins are trimeric proteins with molecular weight of around 158 kDa, whereas 11S globulins form hexameric proteins with molecular weight of around 340 kDa (Multari et al., 2015). 7S and 11S globulins are also the main fractions obtained with alkaline extraction.

The solubility of protein is crucial for physicochemical properties such as gelation, foaming and emulsification and is dependent on factors such as presence of salts and pH (Multari et al., 2015; Nicolai & Chassenieux, 2019). The 11S protein fraction of faba bean has been shown to have low solubility between pH 3 and 4.5 at ionic strength (μ) = 0.5 and between pH 4 and 7 at μ = 0.08 (Kimura et al., 2008). Thus solubility can be altered by pH and salt concentration.

Different protein fractions are likely to behave differently in terms of gelling. For soybean, the corresponding protein fractions conglycinin (7S) and glycinin (11S) have been demonstrated to differ in how gelation is driven and to yield gels with different textural characteristics (Nakamura, Utsumi, & Mori, 1986; Utsumi & Kinsella, 1985). Hydrogen bonding and hydrophobic interactions are reported to be strongly involved in gel formation of glycinin, while electrostatic interactions and disulphide bonds are more important for gel formation of conglycinin (Utsumi & Kinsella, 1985).

Proteins from other legumes behave differently. For example, the pea protein vicilin, but not legumin, has been demonstrated to form heat-set gels (Bora, Brekke, & Powers, 1994). Information on the gelation of specific protein fractions of faba bean is limited as only a few studies have been performed, the majority focusing on the 11S fraction (Burova, Grinberg, Grinberg, Leontiev, & Tolstoguzov, 1992; Grinberg, Grinberg, Bikbov, Bronich, & Mashkevich, 1992; Kimura et al., 2008; Zheng, Matsumura, & Mori, 1993a, 1993b). However, protein isolates from faba beans have been demonstrated to form gels at lower concentrations than

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pea protein isolates (Fernández-Quintela, Macarulla, del Barrio, & Martínez, 1997). The functionality of faba bean proteins is therefore likely to differ from that of pea proteins.

The properties of protein gels depend not only on their composition, but also on the conditions used to induce gelation. Opaque, particulate heat-set gels are usually formed near the isoelectric point, whereas transparent, fine-stranded gels are formed further away from the isoelectric point (at higher/lower pH) (Langton & Hermansson, 1992). This is also the case for whole faba bean protein extracts, independent of extraction method (soaking or alkaline extraction), with the hydrogels formed having a coarser protein network structure at pH 5 compared with pH 7 (Langton et al., 2020).

This study aimed to evaluate the gelation process and rheological and microstructural properties of gels formed from the two major faba bean protein fractions, 7S and 11S, at different NaCl concentrations. The strength of the gels (storage modulus, G') was measured by oscillatory rheology. Further characterisation of material properties was performed by applying large deformation stress growth measurements. The microstructure of the set gels was analysed by electron microscopy under similar conditions to those in the rheological analyses, in order to obtain comparable results. To the best of our knowledge, it is the first study to investigate the gel formation of both the 7S and 11S protein fractions from faba bean var. minor and the influence of NaCl on their gel formation. With knowledge of the gel formation of specific protein fractions, the properties of gels can be tailored for specific applications by choosing appropriate varieties and extraction processes to yield a protein isolate with the desired composition. We have previously reported differences in the gel formation of whole faba bean protein isolates extracted by soaking and alkaline extraction (Langton et al., 2020). Differences that could potentially be related to differences in the 7S to 11S ratios of the extracted protein isolates.

2. Materials and methods

All chemicals used were of reagent grade quality. Faba bean (Vicia faba var. minor) of the variety Gloria was kindly provided by RISE (Research Institutes of Sweden). Gloria is a white-flowering species that usually contains a lower amount of tannins than varieties with more highly pigmented flowers (Sverigeförsöken, 2012). The beans were dehulled and cryo-milled using an Ultra-Centrifugal Mill (ZM-1, Retsch GmbH, Germany) to yield a fine flour.

2.1. Extraction of protein isolates

The 7S and 11S protein fractions of faba bean were isolated based on differential solubility in sodium chloride (NaCl), using a protocol similar to that described in a previous study (Suchkov, Popello, Grinberg, & Tolstoguzov, 1990), with some modifications. In brief, 100 g of faba flour were mixed with 900 ml water for 1 h. The pH was adjusted to 8.0 using 0.5 M NaOH and mixing was continued at 45 °C for another hour. The suspension obtained was centrifuged at 5000×*g* for 30 min. The pellet was discarded and NaCl was added to the supernatant to a concentration of 0.6 M. The solution was stirred for 30 min and the pH was adjusted to 5.1 by adding 0.2 M HCl. The suspension obtained was centrifuged at 5000×*g* for 20 min, water was added to the supernatant to bring the NaCl concentration to 0.3 M and the solution was centrifuged at 1000×*g* for 15 min. The supernatant was placed in a cold room, while the pellet was dissolved in 500 ml of 0.6 M NaCl. The suspension obtained was centrifuged at 5000×*g* for 30 min and the supernatant was diluted to 0.3 M NaCl and further centrifuged at 1000×*g* for 10 min, after which the sediment (representing the 11S fraction) was collected. The solution previously saved in the cold room was centrifuged at 1000×*g* and 4 °C for 10 min and the supernatant was diluted to twice the volume and further centrifuged at 1000×*g* and 4 °C for 10 min. The pellet (representing the 7S fraction) was collected and saved. Both the 11S and 7S fractions were freeze-dried (Scanvac CoolSafe 110–4, Labogene, Lynge, Denmark).

2.2. Validation of purity of protein fractions

The molecular weight and purity of the two globulin fractions isolated were assessed by size exclusion chromatography (SEC), using a HiLoad 16/600 Superdex-200 (Cytiva, Marlborough, MA, USA) on the choromatography system Äkta explorer (GE Healthcare, Chicago, IL, USA). For this, a small amount of 11S or 7S was dissolved in 25 mM bicine (pH 8.8, 0.3 M NaCl). The protein solution was run through a PD-10 column and 0.5 ml of the eluted protein was loaded onto a Superdex-200 HiLoad 16/600 size exclusion column using the same buffer.

2.3. Solubility measurements on 7S and 11S globulins

One gram of 7S or 11S was solubilised in 500 ml of 25 mM bicine (pH 8.8, with 0.1, 0.3 or 0.5 M NaCl). The protein solution was aliquoted to 15 Falcon tubes and kept at room temperature and adjusted to cover a range of pH between 1.5 and 8.8. The Falcon tubes were then centrifuged at 11000×*g* for 45 min. The absorbance of the supernatant was read at 280 nm and plotted against the pH, and percentage solubility was calculated by assuming the solubility of the solution at pH 8.8 to be 100%.

2.4. Sample preparation

For gel preparation, 0.5 g of protein isolate of 7S, 11S, or a mixture of 7S and 11S in a ratio of 3:7 (based on the ratio of extracted 7S and 11S fractions obtained) was dispersed in deionized water or in a NaCl solution of 0.1 M or 0.3 M. The pH was adjusted to 7 with 1 M NaOH and the dispersion was agitated using a magnetic stirrer for 30 min, before a final adjustment of pH to 7 if needed. This was followed by another 30 min of agitation. The procedure yielded 4 g of dispersion with 12% w/w (dry basis, db) of protein isolate. A new dispersion was prepared for each rheology measurement. It should be noted that, due to the NaCl remaining in the protein isolate after extraction, the actual NaCl concentration will be slightly higher than the one stated for each gel. Based on the NaCl concentration at the final centrifugation step and the dry matter of the protein pellet, the actual NaCl concentration would be approximately 0.05 M or 0.09 M higher for the 7S and 11S gels respectively.

2.5. Rheological properties

A DHR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with 40 mm cross-hatched plate-plate geometry was used to study the gelation and mechanical properties of the gels formed from protein mixtures prepared as described in section 2.4. Small-angle oscillatory measurements at 1 Hz and 1% strain were performed with a temperature profile increasing from 25 to 95 °C at 1.5 °C/min, held at 95 °C for 30 min, decreasing to 25 °C at a rate of 1.5 °C/min, and held at 25 °C for 10 min. The chosen strain value was confirmed by preliminary experiments to lie within the linear viscoelastic region. To prevent evaporation, the edge was covered with a layer of low-viscosity paraffin oil. The apparent gelation temperature (hereafter referred to simply as gelation temperature) was estimated based on the cross-over point for storage modulus (G′) and loss modulus (G″). If the cross-over was below the instrument torque detection limit, the slope of G′ and G″ was used to extrapolate to an estimated cross-over point.

The temperature sweep was followed by a stress growth measurement where a constant shear rate of 0.01 s^{-1} was applied and the stress was continuously measured. This yielded a stress-strain curve, from which shear modulus was calculated and the peak stress and shear strain required for shear fracture were identified. Shear modulus was calculated as the slope of the linear region of the stress-strain curve. The fracture point was determined by calculating the derivate ($d\sigma/d\gamma$) for

each point at the stress-strain curve and calculating the intersection of a line fitted to the constant region and a line fitted to the slope where the value of the derivate started to decline (Fig. 1). All rheological measurements were performed at least in triplicate except for the gels prepared in deionized water, where only duplicates were run.

2.6. Microscopy

2.6.1. Fixation

For microscopy, gels were produced by heating 1 ml of protein dispersion at 95 °C for 30 min. After heating, the gels were rapidly cooled in tap water and cut into cubes with sides of approximately 2 mm. The samples were fixated overnight in 2.5% glutaraldehyde with 0.1% ruthenium red in 0.1 M phosphate buffer (pH 7), after which they were then washed and further fixed in 1% OsO_4. After these two fixation steps, the samples were dehydrated in a series of aqueous ethanol of increasing concentration.

2.6.2. SEM

For visualisation using scanning electron microscopy (SEM), fixated and dehydrated samples were dried in a critical-point drier, fractured and mounted on a specimen stub using carbon tape. The mounted samples were then sputter-coated with gold and imaged in a FlexSEM 1000 (Hitachi, Tokyo, Japan) using high vacuum. Micrographs were collected at magnification of × 30 000 (3.3 nm/pixel). Contrast and brightness of images were adjusted using an open-source image processing program (ImageJ).

2.7. Statistical analysis

Analysis of variance (ANOVA) was performed using a general linear model in Minitab (Minitab 19.2020.1, Minitab LCC, State College, PE, USA) to determine significant effects ($p < 0.05$). The ratio of 7S to 11S, NaCl concentration and the interaction between these were included as factors in the model. When a significant interaction or single effect was found, pairwise comparison with adjustment for multiple comparisons according to Tukey was made between samples.

3. Results and discussion

The gelation properties of proteins are generally highly dependent on the purity and the solubility of the protein of interest. In this study, different methods for extraction of the two main protein components of faba bean (7S and 11S) were applied and differences in solubility, gelation and aggregation properties at different NaCl concentrations were examined.

The purity of the two isolated globulin fractions were assessed by size exclusion chromatography (SEC). The SEC chromatograms obtained for the two globulin fractions extracted based on differential solubility in NaCl solution showed nearly single peaks, at elution volumes of 67 ml and 58 ml for the 7S and 11S fraction, respectively (Fig. 2). These elution volumes correspond to molecular weight of 150 kD for 7S and 350 kD for 11S. The SEC results also indicated purity of above 85% for both fractions, based on the calculated area of peaks (Fig. 2). The small peaks observed at an elution volume of 42 ml correspond to the void volume and are most likely due colour pigments. The minor peak observed around the elution volume of 50 ml corresponds to a molecular weight greater than 550 KD and could potentially be due to aggregation of protein molecules.

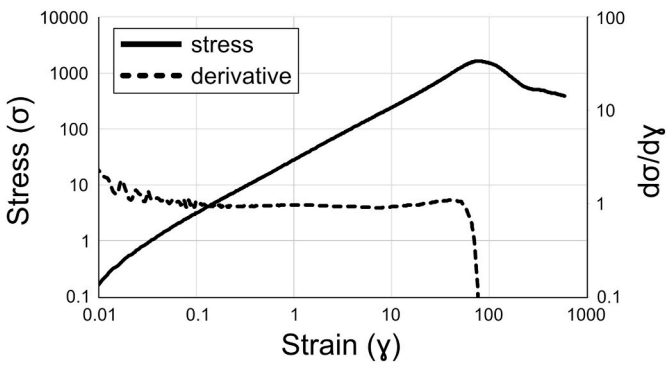

Fig. 1. Example of a stress growth curve (solid line) and its derivate (dashed line) for one of the 7S gels at 0.1 M NaCl.

The solubility of the 7S and 11S globulins, evaluated at pH 1.5–8.8 and at 0.1 M, 0.3 M and 0.5 M NaCl, is shown in Fig. 3. Both 7S and 11S exhibited low solubility around pH 5 (isoelectric point), although 11S was less soluble than 7S, and both exhibited higher solubility at pH above 7 and at pH below around 2, regardless of NaCl concentration. At the lowest NaCl concentration tested (0.1 M), both 7S and 11S showed low solubility and hence isoelectric precipitation. As the NaCl concentration increased, 7S became almost completely soluble at pH above 5, whereas 11S showed a lower degree of solubility, especially at pH 3–5. These results are in agreement with findings reported previously (Kimura et al., 2008). Furthermore, it should be noted that solubility, as measured by centrifugation, depends on a variety of factors such as centrifugal force and time (Silva, Cochereau, Schmitt, Chassenieux, & Nicolai, 2019).

3.1. Texture and gel formation

Gel formation by 12% w/w (db) protein dispersions was monitored by measuring storage modulus (G') using small deformation tests. Only pH 7 was evaluated, as solubility was an issue at pH 5, especially for 11S. Fig. 4 shows gel formation for 7S, a mixture of 7S and 11S, and 11S prepared in different NaCl solutions (diH_2O, 0.1 M, 0.3 M NaCl). For the mixture, a 7S to 11S ratio of 3:7 was chosen, based on the amounts of 7S and 11S obtained from extraction. This ratio is within the range reported for other faba bean varieties (Warsame et al., 2020). Previous research on gelation of specific protein fractions from faba bean has focused mainly on the 11S fraction from *Vicia faba* var. major (e.g. broad bean) (Burova et al., 1992; Grinberg et al., 1992; B.-A. Zheng et al., 1993a, 1993b; B. Zheng, Matsumura, & Mori, 1991), which is a different subspecies from the *V. faba* var. minor used in this study (Burova et al., 1992; Grinberg et al., 1992; Kimura et al., 2008; B.-A. Zheng et al., 1993a, 1993b; B. Zheng et al., 1991). Furthermore, none of those studies investigated the effect of NaCl on the 7S and 11S protein fractions.

The rheological test results showed a difference in behaviour between the 7S and 11S fractions on adding NaCl. For 7S, NaCl addition resulted in a faster increase in G′ during heating and a higher final G′ value (Fig. 4A). In contrast, for the 11S fraction NaCl addition resulted in a delayed and reduced increase in G' (Fig. 4C), especially at higher concentrations (0.3 M NaCl). For most samples the increase in G′ was very steep. However, 7S without NaCl and both high-NaCl samples (0.3 M NaCl) of the 7S + 11S mixture and 11S showed a slower increase in G'. The overall appearance of gelation curves and dependence on NaCl concentration for the 7S + 11S mixture were more similar to those of 11S than 7S. With a higher proportion of 11S compared to 7S in the studied mixed sample, the behaviour of the mixed systems might be a result of a simple cancellation of the two trends observed for the 7S and 11S individually.

The final G′ values of the different gels are summarised in Fig. 5A (data from individual replicates can be found in Table S1). With NaCl added, final G′ for 7S was higher than for 11S, whereas in water G′ was lower for 7S than for 11S. For 7S, preparation in 0.3 M NaCl increased final G' and stress at fracture by 7- to 9-fold more than for gels prepared in deionized water. On the other hand, gels of 11S prepared in 0.3 M NaCl showed 9- to 12-fold lower final G' and stress at fracture than 11S gels formed in deionized water. The mixed 7S + 11S gels had a final G' value that lay between those observed for the 7S and 11S gels.

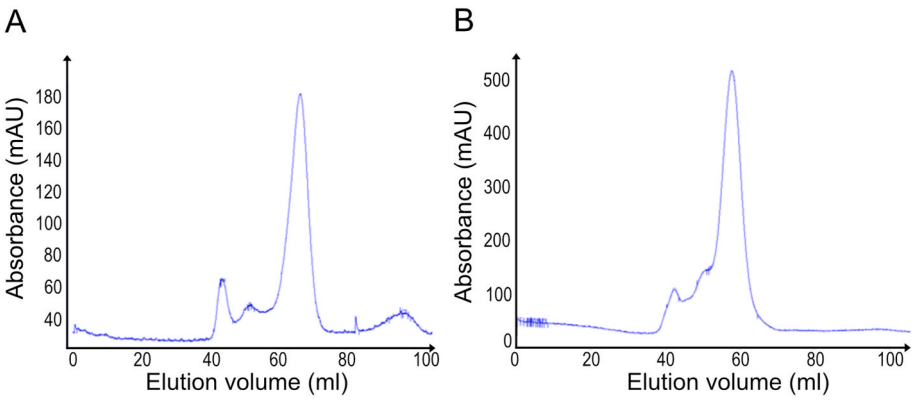

Fig. 2. Size exclusion chromatographs (Superdex-200 device, running buffer 30 mM Tris-HCl, pH 8.5, 0.5 M NaCl) obtained for the two major protein components of faba bean. (A) 7S and (B) 11S (B). Running buffer: 30 mM Tris-HCl pH 8.5, 0.5 M NaCl. Elution volume of 7S (67 ml) and 11S (58 ml) corresponds to molecular weight of approximately 150 KD and 350 KD, respectively.

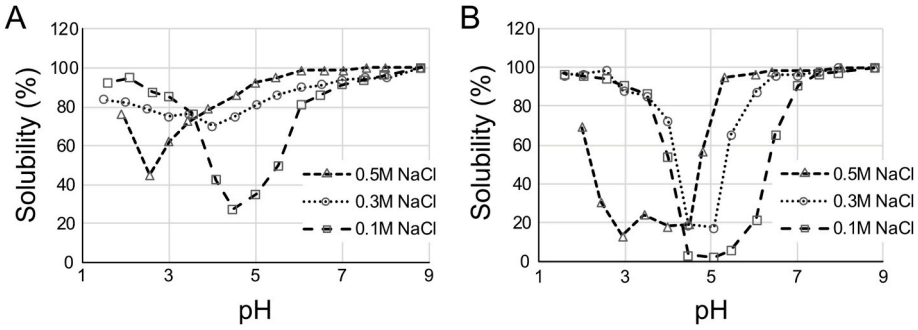

Fig. 3. Solubility curves for the 7S (A) and 11S (B) globulin fractions at 0.1 M, 0.3 M and 0.5 M NaCl.

For both 7S, 11S and the mixture, one of the NaCl concentrations resulted in a comparably slower and/or delayed increase of G' as well as a continued increase throughout the holding time of 30 min at 95 °C (Fig. 4). The heating time is a known factor to influence the gelation and final gel properties of different protein gels (Clark, Kavanagh, & Ross-Murphy, 2001; Renkema & van Vliet, 2002). Hence, the differences observed in the final G' (Fig. 5A) would potentially be less pronounced using a longer holding time.

A similar effect of NaCl addition on the 7S and 11S protein fractions in soybean has been observed by others (Shimada & Matsushita, 1980). In that study, NaCl addition to the 7S protein fraction resulted in increased hardness of the gels and lower minimum gelling concentration, while NaCl addition to the 11S protein fraction suppressed gel formation. Those authors suggested that during gel formation, the 11S protein fraction can form more hydrogen bonds and ionic interactions and fewer hydrophobic interactions than the 7S fraction (Shimada & Matsushita, 1980).

On comparing the results in this study with those in our previous study on whole faba bean protein extracts (Langton et al., 2020), we found that the alkaline-extracted protein in our previous study showed higher G′ at pH 7 with added NaCl, which was similar to the trend observed for 7S in this study. However, the soaked protein extract in our previous study showed lower final G' with added NaCl, which was similar to the trend observed for 11S in this study. This may indicate that alkaline extraction results in a higher content of 7S than when using a soaking protein extraction method.

The gelation temperature was estimated based on the cross-over point for storage modulus (G′) and loss modulus (G″). The gelation temperature depends on the heating rate and is controlled by an activation energy (Chen, Zhao, Chassenieux, & Nicolai, 2016; Sun & Arntfield, 2011). Hence, the gelation temperature reported here is referred to as the apparent gelation temperature. The 7S fraction showed a decrease in gelation temperature when prepared in 0.1 M NaCl compared with 7S in deionized water or in 0.3 M NaCl (Fig. 5B). In contrast, the 11S fraction showed an increase in gelation temperature with increasing NaCl concentration. When prepared in 0.3 M NaCl, 11S did not form a gel until after a temperature of 95 °C had been reached, and hence no gelation temperature is reported. The observed increase in gelation temperature for 11S with addition of NaCl is in line with the increase in denaturation point for faba bean 11S reported by others at comparable NaCl concentrations (Zheng et al., 1993a). The higher observed gelation temperature could potentially also be related to kinetics and a slower gelation rate with increasing NaCl concentration. Reduced gelation rate has previously been observed for the 7S fraction of soy protein with addition of NaCl (Nagano, Mori, & Nishinari, 1994). Similarly to the 11S gels, gels formed from the 7S + 11S mixture showed an increase in gelation temperature with increasing NaCl concentration, but with lower gelation temperatures compared with 11S at each respective NaCl concentrations. The overall higher gelation temperature observed for the 11S fraction compared with the 7S fraction is in line with the higher denaturation temperature reported for faba bean 11S compared with 7S when measured by differential scanning calorimetry (DSC) (Kimura et al., 2008).

Large deformations were observed by shear stress growth measurements and the peak stress and strain at fracture were successfully identified (Fig. 5C–D, data from individual replicates can be found in Table S1). Peak stress and strain at fracture both showed similar trends to final G'. Shear modulus from the stress growth experiment was not included due to it having a very strong correlation ($R^2 = 0.997$) with the final G' from the temperature sweep. Peak stress and strain from large deformations are typically determined by compression rather than shear. However, due to small sample volumes, large deformation tests

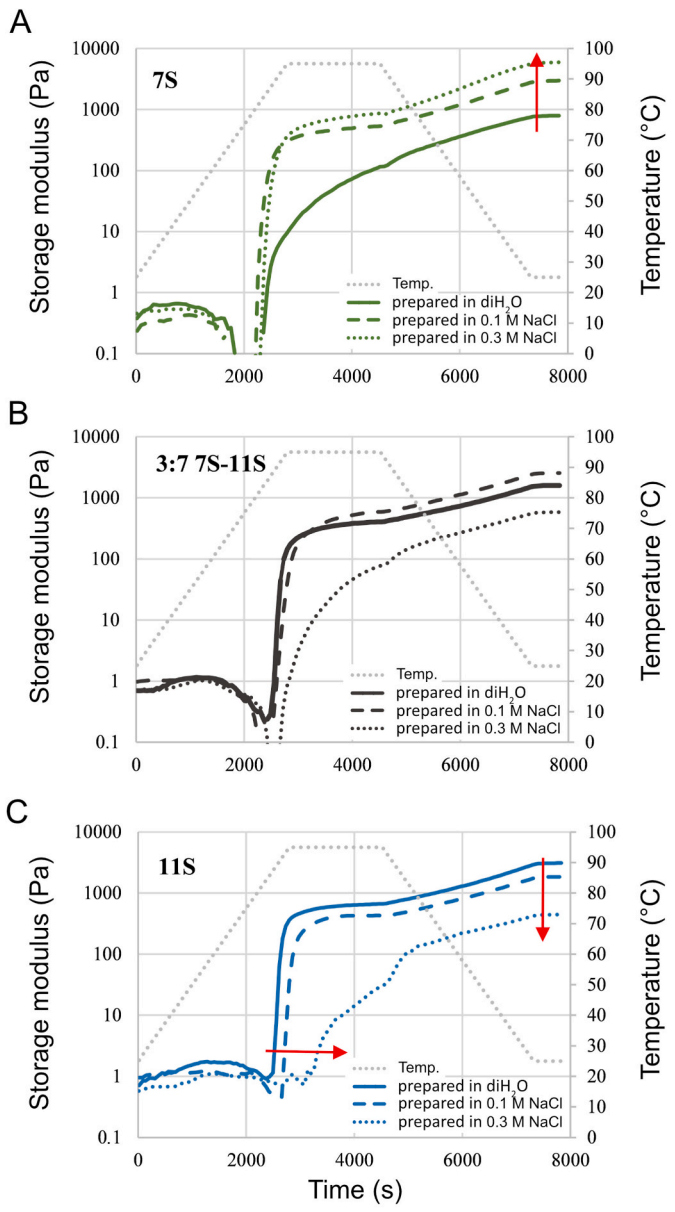

Fig. 4. Plots showing gelation (storage modulus, G') over time as a function of temperature for the protein isolates 7S (A), a 3:7 mixture of 7S and 11S (B) and 11S (C) at different NaCl concentrations. Red arrows indicate shifts in G' occurring with increasing NaCl concentrations. Note: The stated NaCl concentration is the concentration of the solution in which the protein was dispersed during sample preparation. The actual salt concentration will be slightly higher due to salt present in the protein isolate.

were performed by shear stress growth measurements on gels directly after the temperature ramp and oscillatory measurements. Deformation by shear differs from compression, e.g. by not causing volume changes in the sample and the fracture of a material might occur differently depending on the type of force applied.

Peak stress showed similar differences between the samples, as observed for G'. For 7S, the peak stress increased with increasing NaCl concentration, while for 11S the peak stress appeared to have an inverse dependence on the NaCl concentration. However, there was no significant difference between gels prepared in deionized water and 0.1 M NaCl for 11S. A similar decrease in gel hardness with increasing NaCl concentration has been observed previously by compression of gels from 11S from faba bean prepared at a heating temperature of 95 °C (Zheng et al., 1993a). The behaviour of the 7S + 11S mixture was again somewhat more similar to that of 11S.

The 7S gels tended to show increases in fracture strain with increasing NaCl concentration, while the opposite trend was seen for 11S. However, there was large variation in the data and the results should be interpreted with caution.

The differences in behaviour upon addition of NaCl observed for 7S and 11S in terms of viscoelastic properties and peak stress could potentially be explained by differences and changes in their denaturation temperature. A heating temperature during gel formation of just above the protein denaturation point has been shown previously to give the strongest gels for faba bean 11S protein (Zheng et al., 1993a). That study found that a heating temperature between the peak temperature and endset temperature (as obtained from DSC measurements) resulted in the highest hardness and viscoelastic properties for gels from faba bean 11S. Consequently, heating to temperatures further away from this region, either below the peak temperature or above the endset temperature, resulted in weaker gels (Zheng et al., 1993a). At the same time, addition of NaCl is known to alter denaturation temperature (Kimura et al., 2008; Zheng et al., 1993a). Increased NaCl concentration has been shown to lead to an increase in denaturation temperature for both 7S and 11S from faba bean (Kimura et al., 2008; Zheng et al., 1993a). With an increase in ionic strength from 0.08 to 0.5, the denaturation temperature has been reported to increase from 76.5 °C to 83.8 °C for 7S and from 85.0 °C to 95.4 °C for 11S (Kimura et al., 2008). Slightly higher denaturation point, around 98 °C, has also been reported for 11S at 0.4 M NaCl (Zheng et al., 1993a).

Hence, the increase and decrease in G' and peak stress for 7S and 11S, respectively, could potentially be explained by different shifts in their denaturation point. With increasing NaCl concentration, the denaturation point for 7S would be expected to increase towards (but not above) 95 °C and an increase in gel hardness and viscoelastic properties could be expected. On the other hand, the decrease observed for 11S with increasing NaCl concentration could be explained by an increase in the denaturation point to above 95 °C. However, as the heating protocol was fixed and different heating times were not investigated, also differences in kinetics and gelation rate might have influenced the results and comparison between the samples (Nicolai & Chassenieux, 2019). Further studies would be needed to elucidate whether the observed differences are also related to differences in gelation rate.

3.2. Microstructure

The SEM images of gel microstructure of fractured and sputter-coated samples after fixation, dehydration and drying revealed that all gels had a fine-stranded structure when prepared in deionized water (Fig. 6). Similar fine-stranded structure was observed with addition of NaCl for the gels formed from 11S and the 7S + 11S mixture. However, the structure of the 7S gels changed from a fine-stranded type into a coarser structure at both NaCl concentrations tested. This was unexpected based on the solubility results (see Fig. 3), where higher NaCl concentration for 7S gave higher solubility at pH 7. Comparable, but slightly denser, fine-stranded microstructure has been observed previously for faba bean protein gels at a higher protein concentration (20% w/w) (Johansson et al., 2022).

Gel protein network formation is affected by factors such as pH and salt concentration. Typically, a fine-stranded protein network is formed when repulsion between proteins is great, while a more coarse-stranded or particulate network is formed at lower repulsion, such as at high salt concentration or pH close to the isoelectric point (Langton & Hermansson, 1992; Mulvihill, Rector, & Kinsella, 1990). As seen from the solubility measurements (Fig. 3), pH 7 is far from the isoelectric point of both 7S and 11S. Hence, a more fine-stranded microstructure observed for gels prepared in deionized water compared to the higher NaCl concentrations could be expected. The change towards a more coarse or particulate structure for 7S at higher NaCl concentrations is in line with

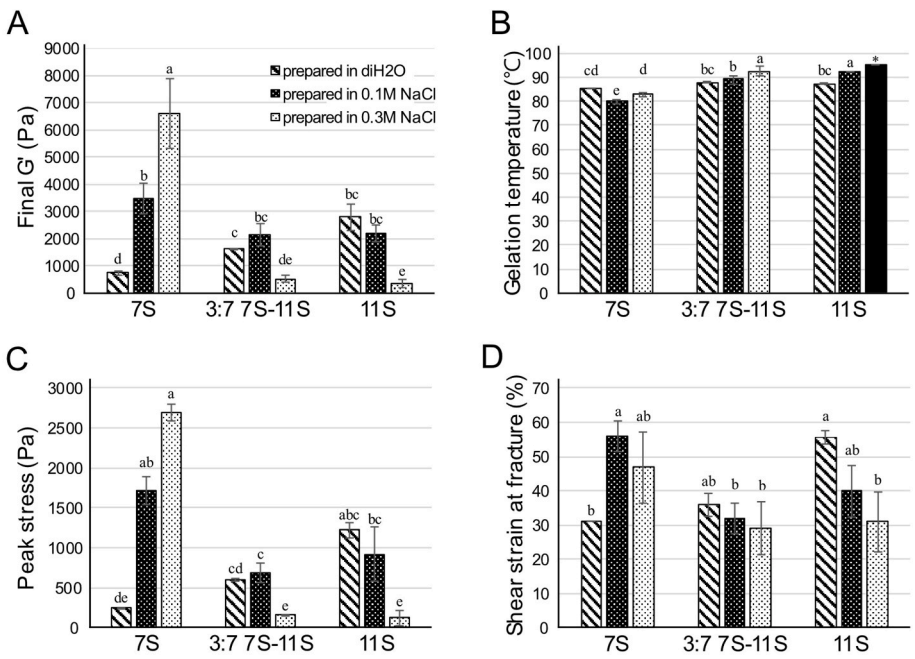

Fig. 5. Mechanical and rheological properties of gels formed from the 7S and 11S fractions and a 3:7 mixture of both, all at pH 7. Different letters on bars indicate significant differences between samples. Data on the gelation temperature for pure 11S in 0.3 M NaCl are excluded since gelling occurred after 95 °C was reached. Different letters indicate significant differences. Note: The stated NaCl concentration is the concentration of the solution in which the protein was dispersed during sample preparation. The actual salt concentration will be slightly higher due to salt present in the protein isolate.

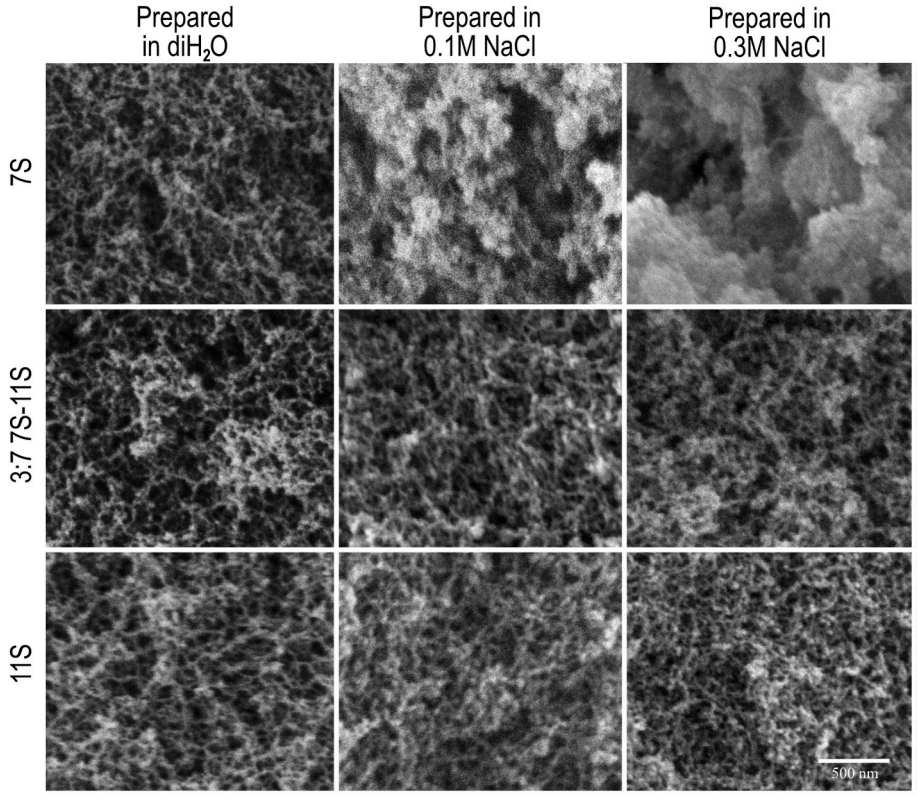

Fig. 6. Scanning electron micrographs (594 × 508 pixels) of gels made from faba bean protein fractions 7S, 11S and a 3:7 mixture of 7S and 11S at different NaCl concentrations, all at pH 7. Note: The stated NaCl concentration is the concentration of the solution in which the protein was dispersed during sample preparation. The actual salt concentration will be slightly higher due to salt present in the protein isolate.

the decrease in repulsion, as discussed earlier, but is perhaps slightly surprising considering the simultaneous observed increase in solubility (Fig. 3). No change towards a more coarse-stranded structure was observed for 11S and 7S + 11S gels as the NaCl concentration increased. It is possible that a change towards a more coarse-stranded network will occur also for 11S and 11S+7S at NaCl concentrations higher than those used in this study. Similar network structure has been observed by others for the 11S protein fraction from faba bean at 0.2 M NaCl (Zheng et al., 1993a).

Gels were also investigated by light microscopy (See supplementary information for method and sample preparation). The micrographs from light microscopy (LM) revealed a change in microstructure for 7S from a

dense and homogenous to a more particulate microstructure as the NaCl concentration was increased (Fig. S1). For gels from 11S and the 7S + 11S mixture, a dense and homogenous microstructure was observed at all NaCl concentrations investigated. These observations were in line with the observations made from the SEM micrographs. However, as the same fixation method was used for both SEM and LM it is possible that artefacts occurring due to sample preparation could be present in both cases. The extensive sample preparation needed due to the high water content and the need for chemical fixation may change the microstructure and cause artefacts (Gordon & Barbut, 1990; Liu & Lanier, 2015).

There were also visual macroscopic differences observed between the gel samples for 7S prepared in deionized water and 0.1 M NaCl. A change in appearance from beige and transparent to white and opaque was observed for the 7S gels as the NaCl concentration was increased (Fig. 7). This is in line with the change towards a more coarse-stranded network structure observed by SEM and further support that the observed change in microstructure for 7S was not a result of artefacts introduced during sample preparation.

3.3. Relationship between texture and microstructure

In terms of mechanical properties, the dependence on NaCl concentration for 7S gels appeared to be the inverse of that for 11S (Figs. 4 and 5). However, changes in microstructure depending on NaCl concentration were only detected for 7S (Fig. 6). It is possible that the mechanical properties of the 7S gels can be explained by structural/aggregational changes, while the changes in mechanical properties of 11S gels are related to changes in kinetics. However, further research is needed to confirm this.

For the 7S gels, storage modulus increased and peak stress decreased with increasing NaCl concentration, while the opposite was seen for 11S (Fig. 5). This is in line with the shift towards a more coarse network structure in 7S gels as NaCl was added (Fig. 6), as coarser gel structure has been correlated to an increase in G' and a decrease in fracture stress in previous studies (Munialo, van der Linden, Ako, & de Jongh, 2015; Renkema, 2004; Stading & Hermansson, 1990; Stading, Langton, & Hermansson, 1993; Van Vliet, 2013). However, it should be noted that other factors, such as the bond strength, curvature of the protein strands, will also influence the textural and rheological properties (Renkema, 2004). This could potentially explain why differences in textural and rheological properties were observed also between the 11S and mixed 7S + 11S samples, despite these showing similar fine-stranded structure at all NaCl concentrations.

In terms of fracture strain, a tendency for an increase was observed with increasing NaCl concentration for 7S, while the opposite trend was seen for 11S. There is no unequivocal relationship between fracture strain and gel coarseness (Renkema, 2004). However, a similar increase in fracture strain with an increase in coarseness has been observed for pea protein gels (Munialo et al., 2015). Again, the changes in mechanical properties for 11S and 7S + 11S could not be explained by any observed changes in microstructure. However, there was large variation in the fracture strain data and the results should be interpreted with caution.

4. Conclusions

This study analysed gel formation at different NaCl concentrations and the rheological and microstructural properties of gels from the 7S and 11S protein fractions of faba bean. The solubility of the protein fractions was also investigated. The results revealed differences in gel formation between the 7S and 11S fractions. In terms of rheological and mechanical properties, the effect of NaCl concentration showed opposing trends for 7S and11S. Addition of NaCl to 7S resulted in an increase in storage modulus and peak stress of the gels, whereas decreases in these two parameters were observed for 11S gels upon addition of NaCl. Changes in microstructure were observed only for 7S gels, where addition of NaCl resulted in transition from a fine-stranded to a coarse-stranded gel network. The 11S gels showed a fine-stranded gel network at all NaCl concentrations investigated. A mixture of 7S and 11S (7S–11S ratio of 3:7) showed similar rheological properties and microstructure as the 11S sample.

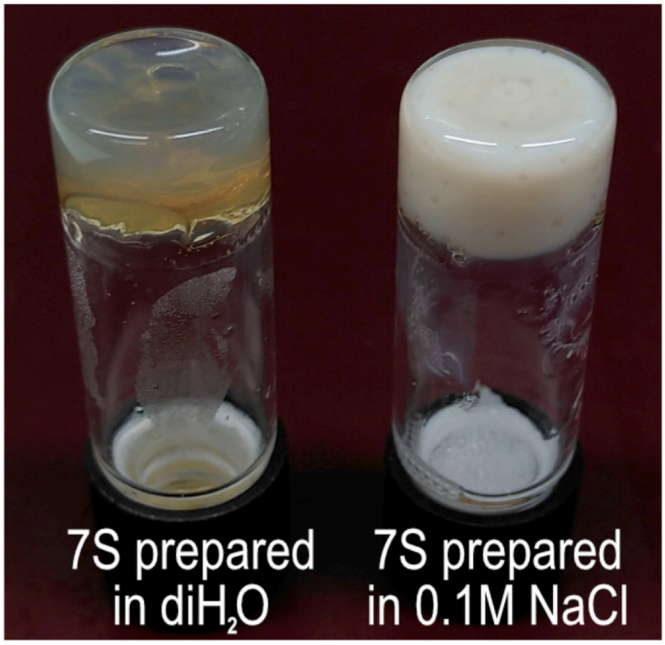

Fig. 7. Gels prepared from 7S at different NaCl concentrations, both at pH 7. Note: The stated NaCl concentration is the concentration of the solution in which the protein was dispersed during sample preparation. The actual salt concentration will be slightly higher due to salt present in the protein isolate.

Author contributions

Mathias Johansson: Visualisation; writing - original draft; writing - review & editing. **Saeid Karkehabadi:** Conceptualization; Investigation; methodology; writing - review & editing. **Daniel P. Johansson:** Conceptualization; formal analysis (statistics, data analysis); investigation; methodology; validation; visualisation; writing – original draft. **Maud Langton:** Conceptualization; funding acquisition; methodology; supervision; writing - review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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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.2023.108789.

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