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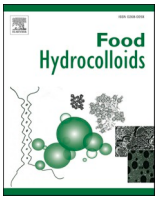

Effect of cellulose-rich fibres on faba bean protein gels is determined by the gel microstructure

Mathias Johansson [a,*], Jakob Karlsson [b], Frans W.J. van den Berg [c], Anna Ström [b], Lilia Ahrne [c], Corine Sandström [a], Maud Langton [a]

[a] Department of Molecular Sciences, Swedish University of Agricultural Sciences, Box 7015, SE-750 07, Uppsala, Sweden
[b] Department of Chemistry and Chemical Engineering, Chalmers University of Technology, SE-412 96, Gothenburg, Sweden
[c] Department of Food Science, Univesity of Copenhagen, DK-1958, Frederiksberg C, Denmark

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ABSTRACT

Increased consumption of plant-based foods and better utilisation of side-streams can reduce the environmental impact of food consumption. A promising crop for production of protein-rich plant-based foods is faba bean, which can serve as a local alternative to soy in cold-climate regions. This study investigated faba bean protein gelation at multiple pH values and the effect of adding a fibre-rich side-stream from protein extraction. Two different sources were used to extract the fibre (cotyledon and hull). The gels were characterised in terms of textural properties, microstructure and water mobility. Gels produced at pH 4 and 5 showed reduced fracture stress, fracture strain and water-holding capacity, but higher Young's modulus, than gels produced at pH 7. The effect of adding fibre (at fixed solids content) varied with pH. Differences observed were attributed to the gel microstructure, as light and scanning electron micrographs showed coarse, aggregated microstructure at pH 4 and 5 and a fine-stranded protein network at pH 7. Irrespective of fibre source (cotyledon/hull), addition of fibre had comparable effects on textural properties. Low-field NMR revealed differences in water mobility between gels at pH 4–5 versus pH 7, and between gels with/without added fibre, likely related to contrasting microstructures and the water-binding properties of the fibre fractions.

1. Introduction

Reducing consumption of animal-based proteins by shifting to plant-based alternatives can significantly reduce of the environmental impact of food consumption (Detzel et al., 2022; Willett et al., 2019). Furthermore, utilisation of side-streams originating from extraction and purification of plant proteins can reduce waste in the value chain of food production. Proteins can be extracted by dry or wet fractionation, where wet fractionation yields a higher protein content but is also more energy-intense (Schutyser, Pelgrom, van der Goot, & Boom, 2015; Vogelsang-O'Dwyer et al., 2020). In both cases, a fibre-rich side-stream can be obtained and potentially used as an addition of fibre to different food products.

Faba beans are rich in protein and can be used as a protein source for human consumption. They can be grown in northern Europe (Jensen, Peoples, & Hauggaard-Nielsen, 2010) and thus potentially serve as an alternative to soy. The main proteins in faba beans are globulins, and the major globular proteins are vicilin and legumin (Warsame, Michael, O'Sullivan, & Tosi, 2020).

Heat-induced gelation is an important process in production of many foods. When present at sufficiently high concentrations, globular proteins can form gels upon heating (Nicolai & Chassenieux, 2019; Zha, Rao, & Chen, 2021). Depending on environmental conditions such as pH and salt, different types of gels are formed (Langton et al., 2020; Nicolai & Chassenieux, 2019). At high ionic strength or pH close to the isoelectric point (pI), the repulsion between proteins is low and coarse-stranded or particulate protein gel networks are formed (Langton & Hermansson, 1992). At low ionic strength or at pH further from pI, repulsion is stronger and more fine-stranded gels are formed (Langton & Hermansson, 1992). The type of network formed influences the textural properties of the gel (Langton, Aström, & Hermansson, 1996). For example, studies on the effect of pH, salt and extraction method on gel formation by faba bean protein have demonstrated differences in microstructure depending on pH and NaCl concentration (Langton et al., 2020), while studies on gel formation by the two major globular protein fractions in faba bean (7S and 11S) have revealed differences in gel

* Corresponding author.
E-mail address: mathias.johansson@slu.se (M. Johansson).

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formation between the two fractions (Johansson, Karkehabadi, Johansson, & Langton, 2023).

Most foods also contain other macromolecules, *e.g.* fibre, which can affect protein gel formation. Effects of adding okara, a fibre-rich side-stream from soy milk and tofu production, on soy protein gelation have been examined in several studies (Arai, Nishinari, & Nagano, 2021, 2022; Lan et al., 2020; Lv et al., 2022; Ullah et al., 2019). Okara addition has been found to both increase and decrease the fracture stress of different soy protein gel systems, depending on the particle size and pre-treatment, *e.g.* by ultra-sound (Fan et al., 2021; Lv et al., 2022; Ullah et al., 2019).

In a previous study by our research group examining the effect of adding a fibre-rich side-stream from protein extraction on the texture and microstructure of faba bean protein gels at pH 7, replacing part of the protein with fibre was found to result in increased storage modulus (G′) and decreased fracture stress (Johansson, Johansson, et al., 2022). The decrease in fracture stress was hypothesised to derive from introduction of inhomogeneities into the protein matrix via larger particles present in the fibre fraction and the increase in G′ to water absorption by the fibre, leading to an increase in effective protein concentration in the surrounding matrix (Johansson, Johansson, et al., 2022). However, further research is needed to confirm these hypotheses, including investigating the effect of adding fibre to gels with different protein microstructures and determining the water mobility within the gels.

The present study builds on our previous work on mixed faba bean gels (Johansson, Johansson, et al., 2022), with the objective to evaluate the effect of addition of two fibre fractions on gel formation, texture and microstructure of faba bean protein gels at different pH values. The fibre fractions were extracted from side-streams of the protein extraction process. Textural properties were characterised by both small and large deformations and the microstructure was examined at different length scales using light and electron microscopy. Water-holding capacity (WHC) was measured and water mobility was assessed in the gels by low-field nuclear magnetic resonance (LF-NMR) and related to gel textural properties.

2. Materials and methods

2.1. Materials

Dehulled and milled faba beans (*Vicia faba*, variety *Tiffany*) were purchased from the company *Svensk fava* (http://svensk-fava.se/). The hull fraction obtained from dehulling the faba beans was kindly provided by the same company. The hull fraction was milled using a Retsch ZM 200 mill (Retsch, Germany) at 18000 rpm and an insert with mesh size 0.5 mm.

Analytical grade NaOH and HCl were purchased from Merck KGaA (Darmstadt, Germany), Technovit 7100 from KULZER (Hanau, Germany) and osmium tetrahydroxide from Ted Pella (Redding, CA, USA). Glutaraldehyde, ruthenium red and chemicals and sugar standards used for analysis of monosaccharides and uronic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extraction

Extraction of protein and fibre was based on a previously described procedure, with some modifications (Johansson, Johansson, et al., 2022; Johansson et al., 2022). Faba bean flour obtained from cotyledon or hull was dispersed in deionised water at a ratio of 1:10 and pH was adjusted to 8. The mixture was stirred for 1 h at 40 °C before centrifugation (5000×*g*, 30 min) to separate the protein and starch/fibre-rich pellet. The supernatant was adjusted to pH 4.8 to precipitate the protein. After centrifuging (5000×*g*, 30 min), the protein was collected, washed once by redispersing in deionised water (1:10) and adjusting to pH 4.8 if needed. After centrifuging again (5000×*g*, 30 min), the protein was redispersed a final time and pH adjusted to 7, followed by freeze-drying (Scanvac CoolSafe 110-4, Labogene, Lynge, Denmark). Only protein extracted from faba bean cotyledon was used in this study.

Fibre was extracted from the starch/fibre-rich pellet resulting from the first centrifugation step during protein extraction. The pellet was dispersed in 0.1% NaOH at a ratio of 1:5 and stirred for 24 h at room temperature (recorded pH of approximately 9.5 after the 24-h stirring). The mixture was then left without agitation for an additional 24 h. Next, the dispersion was washed by centrifugation (3700×*g*, 5 min) and redispersed in deionised water until pH 7 was reached. After the final centrifuging, the pellet was redispersed and filtered multiple times through a nylon fibre cloth until the filtrate only contained minor amounts of starch. The fibre-rich filter cake was collected and freeze-dried to obtain the fibre fraction. Freeze-dried materials were milled into flours and sieved through a 150 μm (protein) or 250 μm (fibre) mesh to remove larger particles.

2.3. Characterisation

2.3.1. Compositional analysis

Total starch content was analysed using a Total Starch Assay Kit (Total starch assay kit (AA/AMG), Megazyme Ltd, Wicklow, Ireland). Protein content was determined by the Kjeldahl method with a conversion factor of 5.4 (Mosse, 1990), using a DT 220 Digestor system followed by a Kjeltec 8200 analyser unit (Foss Analytical A/S, Hillerød, Denmark). Ash content was determined by incineration at 550 °C for 24 h in a muffle furnace and moisture content by oven-drying at 105 °C for 24 h (Nielson, 1994). Starch analysis was performed in duplicate, while protein, ash and moisture measurements were performed in triplicate.

Analysis of crude fibre was performed by the Analytical Laboratory, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Ultuna, following the method of Jennische & Larsson, 1990. The analysis was performed in duplicate.

The composition of the raw materials and extracted fractions is summarised in Table 1. The protein content of the faba bean flour (cotyledon, 33.1%) was within the general range reported for faba bean, but higher than reported for the variety Tiffany (23.3%) (Griffiths & Lawes, 1978; Mayer Labba, Frøkiær, & Sandberg, 2021). The protein content of the hull fraction (15.2%) and the extracted protein and fibre fractions (85.6% and 7.9%, respectively) were within the ranges reported for similarly extracted fractions from the faba bean variety Gloria (Johansson, Johansson, et al., 2022). The relatively high starch content in the hull fraction (12.2%) was a result of contamination from the cotyledon during dehulling.

The total solid contents for the different raw materials and extracted fractions did not add up to 100% (Table 1). This is especially true for the two extracted fibre fractions. All samples are expected to contain only minor amounts of fat (<3%) (Johansson, Johansson, et al., 2022). A

Table 1
Composition (percentage of dry weight) of faba bean cotyledon/hull and fractions extracted by wet fractionation. Error intervals represent ±1 standard deviation.

	Extracted fractions			Faba bean	
	Protein	Cotyledon fibre	Hull fibre	Cotyledon	Hull
Crude protein	85.6 ± 0.9	7.9 ± 0.5	4.3 ± 0.3	33.1 ± 1.3	15.2 ± 0.2
Crude fibre	0.9 ± 0.0	22.6 ± 0.1	47.4 ± 0.3	5.1 ± 0.0	28.8 ± 0 0.1
Starch	<0.2	7.4 ± 1.1	0.6 ± 0.0	32.6 ± 0.1	12.2 ± 0.3
Ash	4.1 ± 0.0	5.3 ± 0.0	3.8 ± 0.3	4.6 ± 0.0	3.2 ± 0.1
Moisture[a]	5.3 ± 0.1	5.0 ± 0.4	3.1 ± 0.1	9.9 ± 0.1	10.5 ± 0.1

[a] Expressed as g/100g sample.

small part could also be sugars and low molecular weight carbohydrates which has been estimated to around 7% in mature whole seeds (Landry, Fuchs, & Hu, 2016). However, the main reason for the total solids not adding up to 100% is likely an underestimation of the total fibre content as estimated by the crude fibre content. Crude fibre content has in multiple studies been reported to be significantly lower than the fibre content measured as *e.g.* neutral detergent fibre or total dietary fibre (Gdala & Buraczewska, 1997; Sobota & Rzedzicki, 2009; Sosulski & Cadden, 1982).

2.3.2. Monosaccharides

Monosaccharide content was analysed using a modified version of an existing method (Sluiter et al., 2008) as described in our previous study (Johansson, Johansson, et al., 2022). In short, 200 mg sample followed by 3 mL 72% H_2SO_4 were added to a 150 mL beaker and placed under vacuum for 15 min. The samples were kept at 30 °C for 1 h with stirring every 20 min, followed by the addition of 84 g deionised water and autoclaving at 125 °C for 1h. They were then vacuum-filtered and diluted to a total volume of 100 mL. Further dilutions (1:10 and 1:50) with deionised water were performed, when necessary, to remain within the calibration range. Fucose, at a concentration of 400 mg/L, was used as an internal standard. The samples were filtered through 0.45 μm filters into HPLC vials. Monosaccharide composition was analysed using high-performance anion exchange chromatography (HPAEC) with a pulsed amperometry detector (PAD) (ICS 3000 Dionex, Sunnyvale, USA) equipped with an AEC column (CarboPac PA 1 analytical 4 × 250 nm). Milli-Q water was used as solvent and 60% v/v 200 mM NaOH with 40% v/v 200 mM NaOH +170 mM NaOAc was run as cleaning between the injections. Standards used were D (+) glucose, D (+) xylose, D (+) galactose, L (+) arabinose, L (+) rhamnose and D (+) mannose. All samples were analysed in duplicate.

2.3.3. Uronic acids

Uronic acid content was quantified using the Blumenkrantz-Asboe-Hansen method (Blumenkrantz & Asboe-Hansen, 1973) with some modifications as described previously (Johansson, Johansson, et al., 2022). In brief, 10.0 ± 0.5 mg sample was weighed in a 10 mL glass tube. While kept on ice, 0.5 mL 96% H_2SO_4 was added twice, with each addition followed by 5 min of vortexing. In the same way, 0.25 mL deionised water was added twice with intermittent vortexing. The samples were then diluted to a total volume of 10 mL. After dilution, 160 μL sample was added to 1.5 mL Eppendorf tubes together with 40 μL of 4 M sulphamic acid-potassium sulfamate (pH 1.6) followed by 800 μL borax in 96% H_2SO_4. The tubes were incubated at 95 °C for 20 min. After cooling to room temperature, absorbance was pre-read at 525 nm using a UV–Vis spectrophotometer (Cary 60 UV–Vis Spectrophotometer, Agilent Technologies), followed by addition of 40 μL 0.15% (w/v) 3-phenylphenol in 0.5% (w/v) NaOH and reading again at 525 nm overtime, recording the peak value. A standard curve was created using D (+) galacturonic acid (0, 40, 80, 120, 160 and 200 μg/mL). All samples were analysed in duplicate.

2.3.4. Zeta potential

Zeta potential was measured using a Zetaziser device (Zetasizer Nano ZS90, Malvern Panalytical, Malvern, UK). Samples were prepared at a concentration of 5 mg/mL and pH 3–10, adjusted using 1 M HCl or 1 M NaOH. To improve dispersibility of the materials, each sample was treated with ultrasound before analysis (Vibra-Cell, Sonics & Materials Inc, Newton, MA, USA). Samples were analysed in triplicate.

2.3.5. Water-binding capacity

Water-binding capacity (WBC) of the raw materials and extracted fibre fractions was determined in triplicate following existing methodology (Bravo-Núñez & Gómez, 2019), with modifications. The fibre fractions were dispersed in deionised water to make a 5% (w/w) dispersion and rotated for 1 h at 30 rpm using a tube revolver (Thermo Fischer Scientific, Waltham, MA, USA). The samples were then centrifuged at 580×*g* for 10 min and excess water was removed by decantation. WBC was calculated as grams of retained water per grams of dry sample.

2.4. Preparation of protein dispersions and gels

Gels and protein dispersions used for the different analyses were prepared as follows (Table 2). Protein and fibre were mixed, dispersed in deionised water and stirred for 30 min. The pH was adjusted using 1 M HCl or 1 M NaOH and the dispersion was diluted to a concentration of 15% (g flour/g sample). After an additional 30 min of stirring, the dispersion was degassed by applying moderate vacuum to a desiccator using a water aspirator. Samples were then either used directly for rheological analysis or transferred to glass cylinders and heat-treated. The glass cylinders were sealed at the bottom using a rubber lid and the top was sealed with thread tape, punctuated with a small needle to prevent pressure build-up. Heat treatment involved heating the samples in a water bath to 95 °C at 1.5 °C/min, and holding at 95 °C for 30 min and then lowering the temperature to 25 °C at a rate of 1.5 °C/min. Gels were stored overnight at 4 °C for compression testing or preparation for microscopy.

2.5. Rheology

A DHR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with 40 mm plate-plate geometry was used to study the gelation and rheological properties of the gels formed from the protein-fibre mixtures prepared as described in section 2.4. Small-angle oscillatory measurements at a 1 mm gap, 1 Hz and 0.2% 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 30 min. Preliminary experiments confirmed that the chosen strain was within the linear viscoelastic region. A custom-made solvent trap combined with a layer of low viscous paraffin oil covering the sample edges was used to prevent evaporation. All measurements were performed in triplicate.

2.6. Compression tests

Large deformation properties were analysed by compression tests using a texture analyser (Stable Micro Systems, TA-HDi, Surrey, UK) equipped with a 500 N load cell and a 36 mm cylindrical aluminium probe. Gels were prepared as described in section 2.4 (cut into pieces with diameter 12 mm and height 14 mm) and allowed to equilibrate at room temperature for approximately 1 h before measurements. Samples were compressed at a speed of 1 mm/s to 70% of their initial height using a trigger force of 0.02 N to initiate data collection. True stress and true strain were calculated as described previously (Munialo, van der Linden, & de Jongh, 2014) and used to calculate true fracture stress, true fracture strain (hereafter referred to as fracture stress and fracture strain) and Young's modulus. Fracture stress and fracture strain were defined as the maximum stress and corresponding strain at the first distinct peak before fracture and Young's modulus as the slope of the initial 1–5% of the stress-strain curve (chosen to be within the linear region without including the potentially noisy data at the first few measurement points). Measurements were performed in three batches,

Table 2
Composition of the faba bean samples, expressed as weight percentage of the different raw materials added.

Sample	Protein (%)	Cotyledon fibre (%)	Hull fibre (%)	Water (%)
Protein	15	0	0	85
Cotyledon	12.75	2.25	0	85
Hull	12.75	0	2.25	85

with 3–5 gels per batch, for each sample. Statistical analysis was performed on the mean value obtained from each batch.

2.7. Water-holding capacity of gels

WHC was measured on gels prepared in glass tubes with 6 mm diameter as described in section 2.4. Gels were cut into pieces with height 10 mm and carefully placed in a Spin-X Centrifuge Tube Filter (Corning Inc. Life Sciences, Corning, NY, USA) with 0.22 μm pore size. Samples were centrifuged with increasing speed (50, 500 and 5000×*g*), with the liquid released weighed between each run. WHC was calculated (Li et al., 2022):

$$WHC\ (\%) = \left(1 - \frac{m_{serum}}{m_{gel}}\right) \times 100 \quad \text{(Eq. 1)}$$

where m_{serum} is weight of serum expelled during centrifugation and m_{gel} is original weight of the gel before centrifugation. For each sample, measurements were performed on a total of eight gels split over two batches.

2.8. LF-NMR

Gels for LF-NMR measurements were prepared as described in section 2.4 with some modifications. Degassing was performed using a degassing station (TA instruments, New Castle, DE, USA) and gels were produced directly in the LF-NMR tubes (13 mm diameter) by heating in a water bath for 30 min at 95 °C. The gels were allowed to cool at room temperature and stored overnight at 4 °C. Samples were allowed to equilibrate at room temperature for approximately 1 h before measurements.

Measurements were performed at 25 °C with an operating frequency of 20.75 MHz for protons using a MQR Spectro-P spectrometer (Oxford Instruments, Oxfordshire, UK) controlled by the Oxford Instruments NMR application Developer Software. Transverse relaxation times (T_2) were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with the parameters set as follows: relaxation delay 0.8 s, τ-delay 200 μs, 10000 echoes, eight scans and receiver gain 5.

In-house MATLAB (R2019a, MathWorks, MA, USA) scripts for exponential fitting according to Eq. (2) were used to determine transverse relaxation time ($T_{2,n}$) and relative abundance ($M_{2,n}$) of the different proton populations:

$$I(t) = \sum_{n=1}^{N} M_{2,n} \bullet e^{-t/T_{2,n}} \quad \text{(Eq. 2)}$$

where *I(t)* is intensity as a function of time and *N* is number of relaxation components. The number of components was chosen based on visual inspection of inverse Laplace distributions obtained using Tikhonov regularisation from the CPMG time profiles (Engelsen & van den Berg, 2017). Each sample was analysed in three separate batches, with 3–5 gels per batch.

2.9. Microscopy

Gel samples were obtained as specified in section 2.4 and prepared for microscopy as described previously (Johansson, Johansson, et al., 2022). In short, samples were cut into approximately 2 mm × 2 mm × 2 mm cubes and fixed overnight in 0.1 M NaCl solution with 2.5% glutaraldehyde and 0.1% ruthenium red, followed by a second fixation step with 1% osmium tetroxide for 2 h. The samples were dehydrated using a series of solutions with increasing ethanol concentration before being either dried using a critical point drier (Quorum Technologies Ltd, K850 Critical Point Dryer, East Sussex, UK) for scanning electron microscopy (SEM) or embedded for light microscopy (LM) using Technovit 7100. Samples for LM were sectioned (Leica Microsystems GmbH, Leica EM UC6, Wetzlar, Germany) into 2 μm thick sections and investigated using a Nikon Eclipse Ni–U microscope (Nikon, Tokyo, Japan) equipped with a 60 × (1.4 N.A.) plan apochromatic objective. Micrographs were captured using a Nikon Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan) with 0.08 μm/pixel (data size: 2560 × 1920 pixels). Samples prepared for SEM were fractured, sputter-coated with gold (Cressington Scientific Instruments, Sputtercoater-108 auto, Watford, UK) and examined at 5 kV (Hitachi, FlexSEM 1000II, Tokyo, Japan). Micrographs were digitally recorded (19.84 nm/pixel, data size: 1280 × 960 pixels).

2.10. Statistics

Analysis of variance (ANOVA) and pairwise comparison (Tukey) with significance level 95% ($p < 0.05$) were performed using R studio (Version February 1, 5033, RStudio Inc., MA, USA). Young's modulus values were log-transformed to obtain normally distributed residuals before statistical analysis (both ANOVA and pairwise comparison).

3. Results and discussion

3.1. Characterisation

Relative monosaccharide composition was determined for the raw materials and extracted fibre fractions (Table 3). The glucose originated mainly from cellulose, but in the case of cotyledon fibre also from starch and possibly from small proportions of other glucose-containing polysaccharides, *e.g.* xyloglucan. The fibre obtained from faba bean hull had a similar monosaccharide composition to that reported previously for hull from yellow pea (Karlsson et al., 2024a).

Increases in arabinose and xylose content were observed for both the cotyledon and hull fibre compared with their respective raw material. This is in agreement with previous findings for a similarly extracted faba bean fibre fraction from a different variety (*Gloria*) (Johansson, Johansson, et al., 2022). The higher arabinose content indicates the presence of arabinan, arabinoxylan or other arabinose-containing polysaccharides (Bhatty, 1990). The xylose content indicates the presence of *e.g.* xylan and xyloglucans. Xyloglucans are generally found in relatively high levels in the cell walls of pulses (Jackman & Stanley, 1995).

Relatively high levels of uronic acids were detected for both fibre fractions, indicating presence of pectins such as homogalacturonan and rhamnogalacturonan (Jackman & Stanley, 1995). The levels observed were higher than reported previously for the cotyledon raw material and the extracted fibre fraction (Johansson, Johansson, et al., 2022).

Zeta potential was measured for the extracted protein and fibre materials (Fig. 1). As expected, the faba bean protein had a positive charge at low pH and negative charge at pH above pI (zero charge). The zero charge occurred between pH 4 and 5 and is within the range observed previously (Sosulski & McCurdy, 1987).

The fibre fractions had a similar charge to the protein fraction at pH above 7. The fibre fractions showed an increase in charge as the pH decreased, but remained negatively charged until at least pH 4. The

Table 3
Monosaccharide composition (wt % of total monosaccharides) of the faba bean raw materials and extracted fractions. Mean of duplicate measurements. N.D.: not detected.

	Extracted fractions		Raw material	
	Cotyledon fibre	Hull fibre	Cotyledon	Hull
Arabinose	21.4	4.1	5.6	2.2
Rhamnose	N.D.	1.0	0.6	0.8
Galactose	3.9	1.9	6.0	2.9
Glucose	31.4	53.6	74.5	69.3
Xylose	4.3	8.9	1.7	6.2
Mannose	0.3	N.D.	0.3	N.D.
Uronic acids	38.7	30.5	11.4	18.7

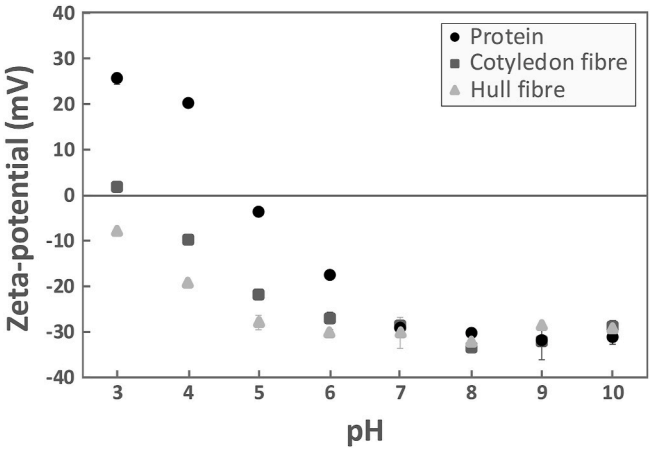

Fig. 1. Zeta potential of the different faba bean raw materials over the pH range 3–10. Error bars represent ±1 standard deviation.

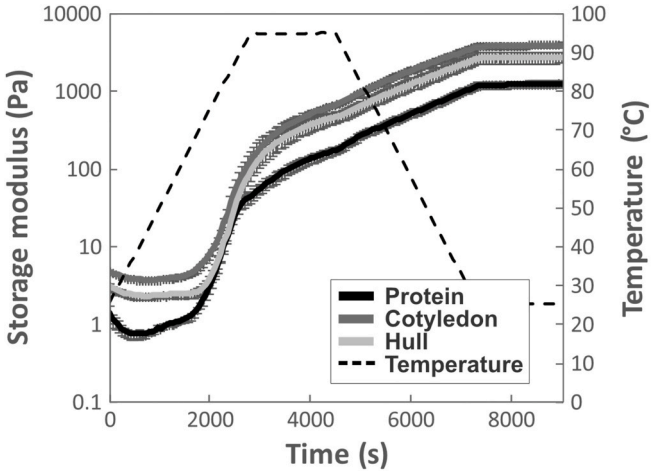

Fig. 2. Storage modulus (G′) during the heating ramp of faba bean protein gels at pH 7, with and without fibre extracted from either the cotyledon or hull of the bean. Error bars indicate ±1 standard deviation.

decrease in charge at pH < 5 is in agreement with the pK_a of pectin, which is typically in the range 3.5–4.5 (Dumitriu, 2004).

However, it should be noted that due to the low solubility and large particle size of the fibre fractions, a proportion of the original sample was removed during the centrifugation step before determination of zeta potential. Hence, the measured charge of the fibre fractions is not representative for the fraction as a whole. In addition, it is noticed that the fibre extracted from the cotyledon has positive charge at low pH, which possible is related to the protein content of this fraction.

WBC (g retained water/g dry sample) was determined for the two raw materials (cotyledon flour and hull flour) and the fibre fractions extracted from these (cotyledon fibre and hull fibre, respectively) (Table 4). The WBC of the raw materials was lower than that of the extracted fibre fractions. Among the two raw materials, WBC was higher for hull flour than for cotyledon flour, probably due to the higher fibre content of the hull flour (Table 1). The higher protein content in the cotyledon flour may also have influenced the results, as part of the protein was solubilised and lost during decanting, reducing the weight of the measured pellet. The WBC of cotyledon fibre was higher than that of hull fibre, as previously observed for pea cotyledon fibre and hull fibre (Dalgetty & Baik, 2003).

3.2. Rheology

Gel formation and rheological properties were determined by oscillatory rheology (Fig. 2). Results from rheological measurements are presented only for the samples prepared at pH 7 as the variation in G′ between replicates was very large at pH 4 and 5. This was possibly related to the larger protein particles, resulting in sedimentation/phase separation.

Gels prepared at pH 7 showed an increase in G′ upon addition of fibre (Fig. 2). Gel formation and gel properties of the protein and protein fibre (cotyledon) gels were similar to those observed previously for mixed faba bean gels (Johansson, Johansson, et al., 2022). A similar increase in G′ has been observed on substituting part of the protein for fibre in pea protein-pea fibre gels (Karlsson et al., 2024b). At pH 7, G′ of the mixed gels with cotyledon fibre was higher than for those with hull fibre, possibly due to the higher WBC of fibre extracted from the cotyledon. A large water-holding capacity of insoluble sugarcane fibre has been suggested to explain the increase in G′ of myofibrillar protein gels upon addition of sugarcane fibre, as the water absorption by the fibre results in less available water for the protein and thus increase in the effective protein concentration of the surrounding matrix (Zhuang et al., 2020).

Table 4
Water-binding capacity (WBC, g water retained/g flour) of the faba bean raw materials and extracted fractions. Error intervals represent ±1 standard deviation.

	Sample	Water binding capacity
Raw materials	Cotyledon flour	2.9 ± 0.1
	Hull flour	4.0 ± 0.2
Extracted fractions	Cotyledon fibre	10.2 ± 0.4
	Hull fibre	6.4 ± 0.1

3.3. Compression tests

Textural properties of the gels were further investigated by large deformations in the form of compression tests, where both fracture stress and fracture strain increased with increasing pH (Fig. 3). Young's modulus showed a different trend, with the highest values observed at pH 5. Similar trends in fracture properties have been observed previously for gels prepared at pH 5 and 7 from alkaline-extracted faba bean protein (Langton et al., 2020). For the pure faba bean protein sample, a more distinct peak and clearer fracture were observed at pH 7 compared with pH 4 and 5 (Fig. S1 in Supplementary Information).

After gel formation by heating, the protein and protein-hull fibre samples at pH 5 showed signs of syneresis and a liquid layer had formed on the top of the gels (Fig. S2). This might have affected the results, due to the higher solids content in the gels compared with the other samples, on which no water layer was observed. The absence of a liquid layer for the samples with cotyledon fibre added might be related to its higher WBC and starch content compared with samples with hull fibre. Due to the syneresis of protein gels and mixed gels with hull fibre added, the results (highlighted in red in Fig. 3) for these gels should be interpreted with caution.

Overall, the effect of the fibre fraction compared with the pure protein gels was similar irrespective of the fibre source used (cotyledon or hull). At pH 4 and 5, addition of fibre increased fracture stress while at pH 7 it decreased fracture stress (Fig. 3). No significant effect of fibre on fracture strain was detected at lower pH, but a clear decrease was noted at pH 7. Young's modulus increased at all pH values upon addition of fibre fraction from either source (cotyledon/hull), with the most drastic increase observed at pH 7. The trends at pH 7 (increasing fracture stress/strain, decreasing Young's modulus) were similar to those observed previously at a higher solids content (20%) upon addition of a similarly extracted fibre fraction (Johansson, Johansson, et al., 2022).

In general, higher fracture stress, fracture strain and Young's modulus were observed for the mixed gels with addition of fibre extracted from faba bean cotyledon compared with fibre extracted from

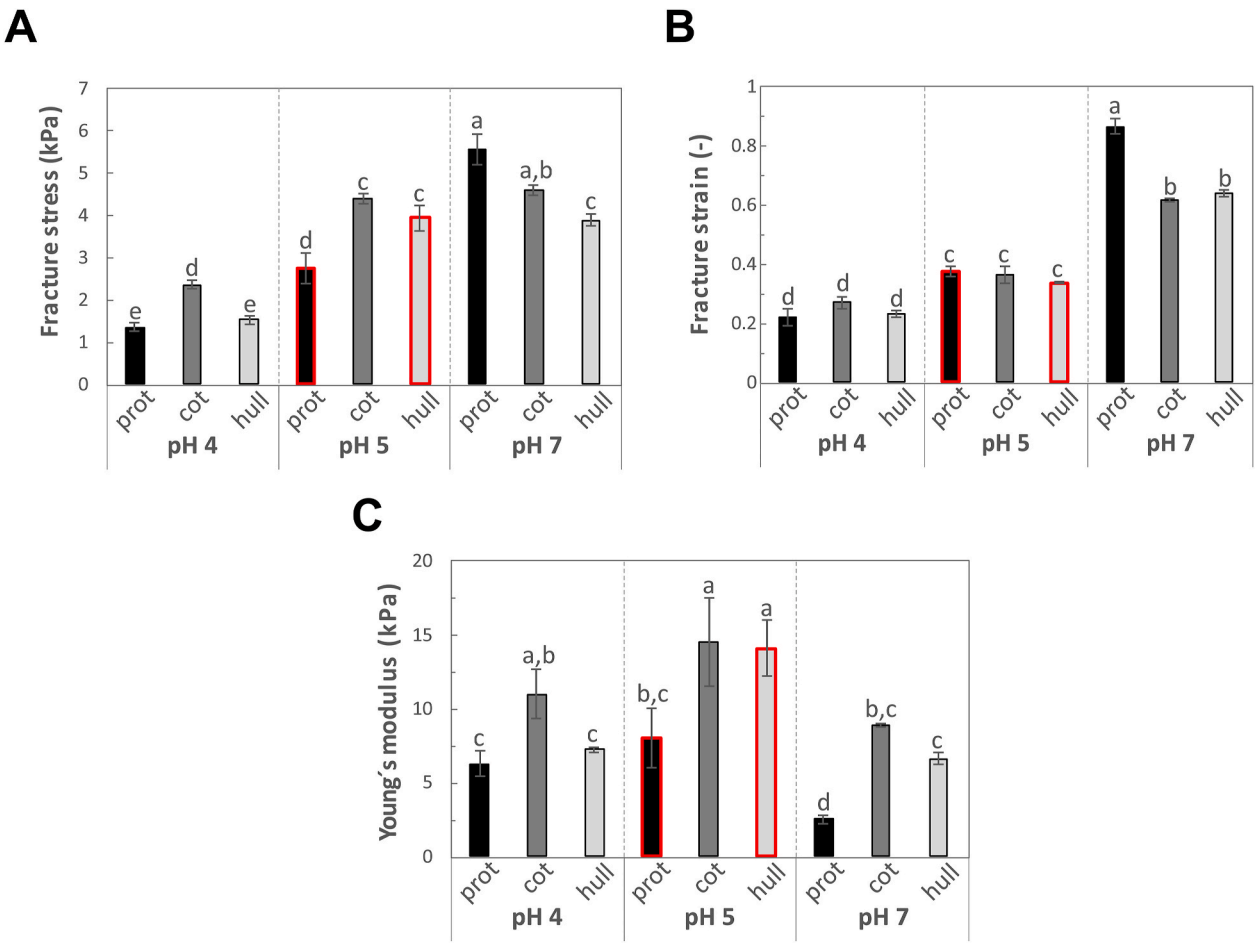

Fig. 3. (A) Fracture stress, (B) fracture strain and (C) Young's modulus from compression tests of faba bean protein gels with or without added cotyledon or hull fibre, produced at pH 4, 5 and 7. Samples highlighted in red showed signs of syneresis with a water layer forming on top of the gels. Different letters (a–d) within panels indicate significant differences ($p < 0.05$). Error bars represent ±1 standard deviation.

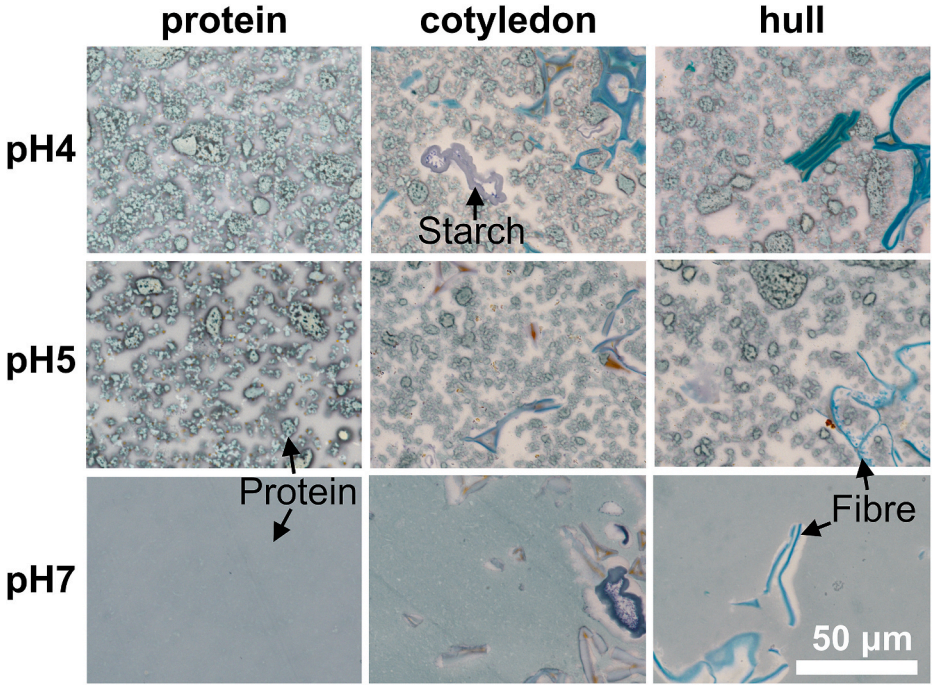

Fig. 4. Light microscopy micrographs of faba bean protein gels prepared at pH 4, 5 and 7, with and without added fibre (cotyledon/hull).

hull. As with the increase in G′, this difference may be related to the higher WBC of cotyledon fibre.

3.4. Microstructure

Light microscopy (LM) revealed clear differences between the protein gel structures formed at the different pH levels (Fig. 4). At pH 7, the protein formed a dense, homogenous network visible at the applied magnification, while at pH 4 and 5, a particulate and aggregated structure was observed. The changes in microstructure as the pH approaches the pI is in agreement with what could be expected at low repulsion between protein molecules (Langton & Hermansson, 1992; Nicolai & Durand, 2013). Similar microstructures have been observed previously for faba bean protein gels at pH 5 and 7 (Langton et al., 2020).

Larger protein aggregates/particles (>10 μm) were observed at pH 4 and 5 and were possibly the result of the low repulsion between protein molecules close to pI. The larger particles might also be partly due to aggregation during the extraction process or during the sample preparation as the pH was adjusted through pI. Further LM investigation of the protein dispersion during preparation revealed that some particles were present before pH-adjustment, while others formed during adjustment. Less harsh pH adjustment with a lower HCl concentration or using glucono-δ-lactone, giving a slower decrease in pH over time, could potentially have reduced the formation of these particles.

For samples with added fibre, the fibre fraction was visible as cell wall fragments distributed throughout the gel (Fig. 4). Minor amounts of starch were also observed in gels with cotyledon fibre added. Addition of fibre had no clear effect on the protein network microstructure as observed by LM.

The SEM micrographs revealed a more coarse-stranded protein network structure composed of larger aggregates at pH 4 and 5 and a more fine-stranded structure at pH 7 (Fig. 5). This correlates well with the LM observations and is in line with previous findings on protein gels that a more coarse-stranded protein network develops close to pI (Langton et al., 2020; Langton & Hermansson, 1992; Nicolai & Durand, 2013). The addition of fibre-fraction had no clear effect on the protein network structure.

The contrasting microstructures at the different pH values and the introduction of larger cell wall fragments throughout the structure are likely to have influenced the textural properties of the gels. A clear difference in fracture behaviour was observed between gels prepared at pH 7 and gels prepared at pH 4 and 5 (Fig. S1). More ductile deformation and less pronounced fracture were observed at the two lower pH values and might be related to gel microstructure with larger (>10 μm) protein particles and aggregates. Weak interaction between particles and continuous rearrangement of particles during compression might explain the less pronounced drop in force during fracture.

Fracture strain decreased upon addition of fibre at pH 7 or with decreasing pH, possibly also because of the difference in microstructure and the presence of larger particles in the form of fibre or protein aggregates. Similarly, whey protein gels containing dense protein particles are reported to have lower fracture strain (Sağlam, Venema, de Vries, van den Berg, & van der Linden, 2014).

Addition of fibre had a different effect on gel fracture properties (fracture stress and fracture strain) for gels prepared at pH 4 and 5 compared with pH 7 (Fig. 3). Adding fibre decreased fracture stress and strain at pH 7, while an increase in fracture stress and no change in fracture strain were observed at pH 4 and 5. This difference might be partly explained by the contrasting microstructures observed at pH 7 compared to the lower pH values. Assuming that the effect of adding the fibre fractions at pH 7 was related to introduction of larger inhomogeneities in the structure, the effect of adding fibre is likely to be smaller at pH 4 and 5, at which the protein structure already contains protein particles with similar sizes as the cell wall fragments in the added fibre fractions. Hence, addition of the fibre fractions will not introduce inhomogeneities to the gel network at pH 4 and 5 in the same way as for the more fine-stranded gel network at pH 7.

The differences observed in textural properties at pH 4 and 5 is not explained by the hypothesis suggested in the previous paragraph or by any clear differences in microstructure. Instead, it might be related to differences in *e.g.* bond strength, protein charge, protein-protein interactions or more subtle differences in microstructure which are not captured by visual evaluation of the micrographs. Further investigation and quantitative analysis of the microstructure could have yielded further insight into the differences between these samples.

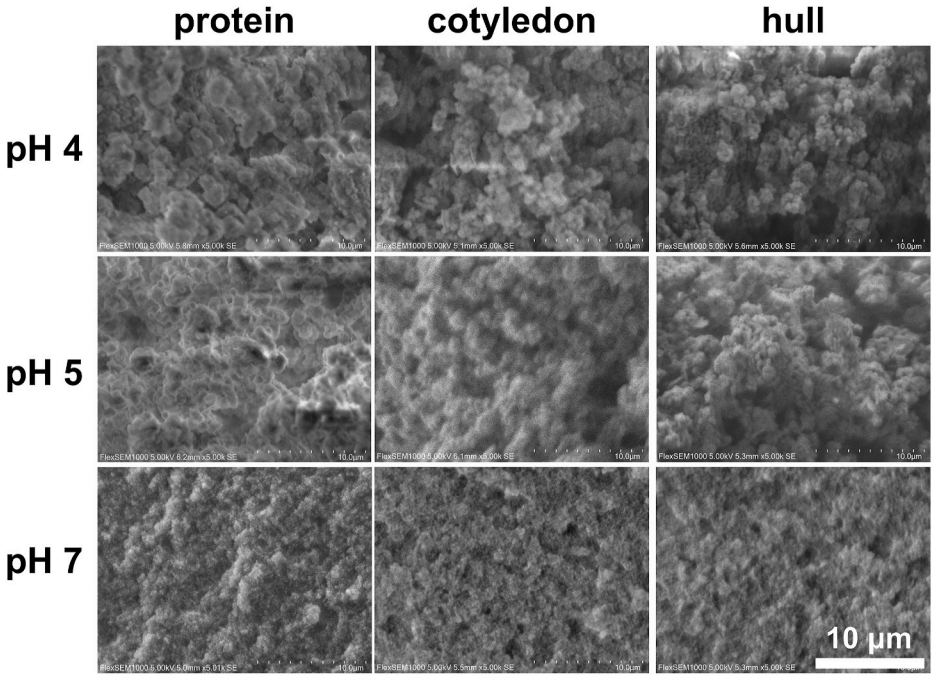

Fig. 5. Scanning electron microscopy micrographs of faba bean protein gels prepared at pH 4, 5 and 7, with and without added fibre (cotyledon/hull).

3.5. Water-holding capacity

The WHC of the gels was determined by centrifugation at multiple centrifugal speeds, as this has previously been shown to influence the results (Kocher & Foegeding, 1993; Urbonaite et al., 2016). Similarly to the gels produced for compression tests, syneresis occurred for protein gels and mixed gels with hull fibre added at pH 5, and the WHC of these gels should be interpreted with caution. A clear effect of pH was observed (Fig. 6). Up to centrifugal force of 500×*g*, almost no liquid was released from the gels at pH 7. The gels at pH 4 and 5 showed lower WHC already at 50×*g* than seen for pH 7 gels at the highest centrifugal force used (5000×*g*). This difference likely relates to the contrasting microstructures, with the coarser protein network and large protein particles (>10 μm) present in gels at pH 4 and 5. Reductions in WHC of protein gels have been observed previously for gels with increasing coarseness or aggregate size, with *e.g.* chemically cross-linked soy protein gels formed from larger aggregates showing reduced WHC (Wu et al., 2019). Similarly, both soy and whey protein gels are reported to show reduced WHC as the coarseness of their protein network structure increases (Puppo & Añón, 1998; Urbonaite et al., 2016), and increased porosity of whey protein/polysaccharide mixed gels is correlated with increased serum release upon compression (van den Berg, van Vliet, van der Linden, van Boekel, & van de Velde, 2007).

For most samples, no significant difference was observed between the protein gels and the mixed protein/fibre gels (Fig. 6). Furthermore, despite the difference in WBC between the cotyledon and hull fibre, in most cases no significant difference was observed in WHC between the mixed gels with cotyledon or hull fibre added. Others have studied different protein-fibre systems and the effect of fibre on WHC. One such study investigated the effect of adding increasing amounts of okara fibre to soy tofu and found no statistically significant difference in WHC between most samples, but generally higher WHC for the mixed systems than for the system without okara (Ullah et al., 2019).

3.6. LF-NMR

LF-NMR was used to further investigate water distribution and mobility within the gels. Because of the differences in sample preparation compared to gels used in the other analyses (*e.g.* WHC), the LF-NMR data was primarily used for comparisons between samples rather than with data from the other analyses. Water protons in *e.g.* soy and milk protein gel systems can be divided into various populations based on their transverse relaxation times (T_2), where a shorter relaxation time indicates lower mobility (Hansen et al., 2010; Laursen, Rovers, van den Berg, & Ahrné, 2023; Ullah et al., 2019).

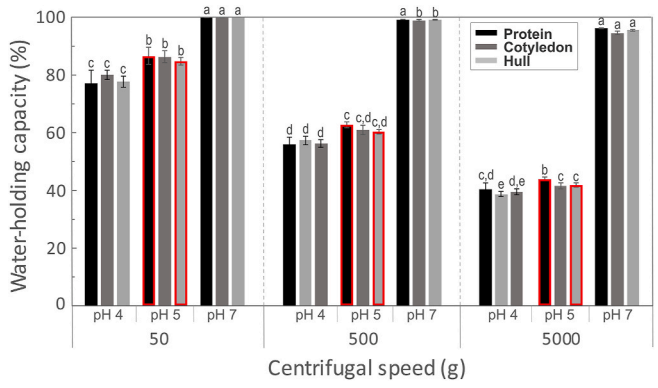

Fig. 6. Water-holding capacity of faba bean protein and protein-fibre gels prepared at pH 4, pH 5 and pH 7. Samples highlighted in red showed signs of syneresis with a water layer forming on top of the gels. Different letters (within centrifugal speeds) indicate significant differences. Error bars represent ±1 standard deviation.

For gels prepared at pH 4 and 5, three major peaks were identified (Fig. 7). The signal with the lowest relaxation time, *i.e.* least mobile water ($T_{2,1}$), was observed in the range 5–25 ms. The second population ($T_{2,2}$) was the largest for all samples and occurred around 40–55 ms. The third population ($T_{2,3}$) with the most mobile water was observed around 300–400 ms. Others have reported similar distributions with three major peaks and comparable relaxation times for soy tofu with added okara fibre (Ullah et al., 2019).

For samples prepared at pH 7, no third peak was observed at 300–400 ms. Relaxation times between 100 and 1000 ms are typically interpreted as serum or bulk water (Gianferri, D'Aiuto, Curini, Delfini, & Brosio, 2007; Gilbert, Rioux, St-Gelais, & Turgeon, 2020). Absence of a third water population with the highest mobility could potentially be related to the difference in microstructure. Gels at pH 7 had a more fine-stranded protein network and a more homogenous structure also at larger length scales. On the other hand, gels prepared at pH 4 and 5 had a more coarse stranded protein network (Fig. 5) and a more aggregated structure in which larger cavities were visible by LM (see Fig. 4). Assuming that the peak around 300–400 ms ($T_{2,3}$) is related to bulk water or more freely moving water, a smaller population contribution in this range could be expected at pH 7 based on the gel microstructure. Likewise, the population corresponding to the most mobile water has been observed to almost completely disappear for egg protein gels as the pH increases from 5 to 9, changes suggested to relate to potential changes in microstructure and a more well-developed protein network

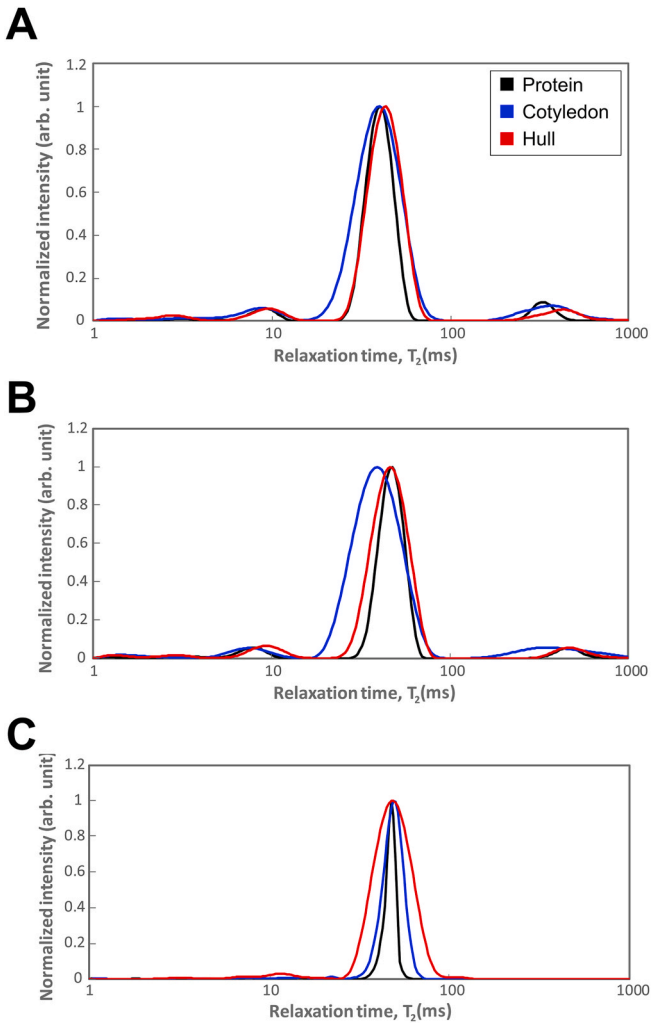

Fig. 7. Inverse Laplace distributions of the different faba bean protein and protein-fibre systems at (A) pH 4, (B) pH 5 and (C) pH 7.

at pH 9, further away from pI (Li et al., 2018).

Similarly to the peak around 300–400 ms, the first peak observed around 5–25 ms appeared to be absent or very small at pH 7 (Fig. 7). However, this was related to the large variation in relaxation times for $T_{2,1}$ at pH 7, averaging out close to zero in the mean curves plotted in Fig. 7. Individual replicates showed a peak occurring around 5–25 ms.

Transverse relaxation times and population sizes for the different samples are shown in Fig. 8. In general, a shift towards longer relaxation time was observed as the pH increased. With the exception of relaxation times $T_{2,2}$ and $T_{2,3}$ at pH 5, an increase in relaxation times was also observed upon addition of fibre.

The population sizes were estimated using discrete exponential fitting (Eq. (1)). The second relaxation time ($T_{2,2}$) was the largest population for all samples (75–97%). An increase in the population size of $T_{2,1}$ was observed upon addition of hull fibre or cotyledon fibre compared with gels with only protein. This increase could be related to WHC of the fibre fractions, increasing the proportion of less mobile water. A study investigating soy protein-corn starch gels observed comparable transverse relaxation time curves with three major peaks and a similar increase in the population related to $T_{2,1}$ on increasing the proportion of starch in the system (He et al., 2024). This was attributed to the ability of starch molecules to bind a larger proportion of water by hydrogen bonds (He et al., 2024).

4. Conclusions

This study investigated the effect of adding two different fibre-rich side-streams (cotyledon and hull fibre) from protein extraction on faba

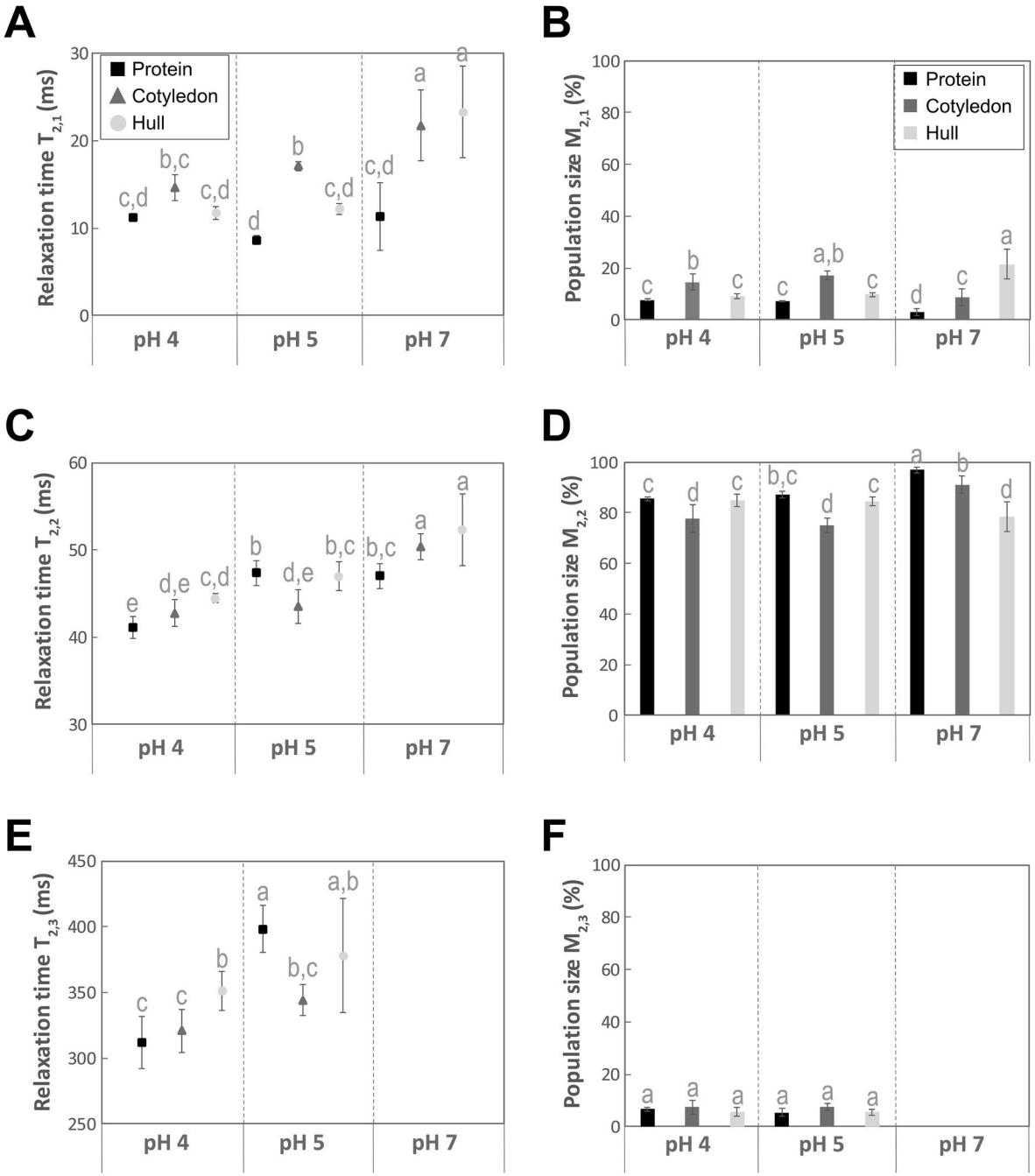

Fig. 8. Relaxation times $T_{2,n}$ of faba bean protein and protein-fibre systems (A, C, E) and relative proportion $M_{2,n}$ (B, D, F) of corresponding water populations. Error bars represent ±1 standard deviation. Different letters (a–e) within panels indicate statistical difference.

bean protein gelation in terms of textural properties, water mobility and microstructure at pH 4, 5 and 7. Gel properties were clearly affected by changes in pH, with lower fracture stress, fracture strain and WHC observed for samples at pH close to pI of the faba bean protein. These changes may relate to observed differences in microstructure, with a more coarse protein network and larger protein particles (>10 μm) present at pH 4 and 5. The effect of adding fibre differed depending on the initial microstructure of the gel. An increase in fracture stress was observed when fibre was added to gels at pH 4 and 5, while a decrease was observed at pH 7. The decrease at pH 7 was hypothesised to relate to introduction of inhomogeneities by the addition of fibre particles to the otherwise homogenous and fine-stranded protein gel network. At pH 4 and 5, the gel microstructure already contained protein particles in a similar size range as the fibre and addition of fibre did not introduce new inhomogeneities in the same way as at pH 7. Some differences in water mobility were observed by LF-NMR between gels formed at different pH values and between protein and protein-fibre gels, again, possibly related to observed changes in microstructure at the different pH and water absorption by the fibre.

Overall, these results indicate that fibre-rich side-streams from protein extraction can be used to modify the textural properties of faba bean protein gels, with the effect on textural properties of adding such fibre-fractions depending on the microstructure of the pure protein gels. This finding possibly also applies to other protein-fibre gel systems, but must be validated in further research.

CRediT authorship contribution statement

Mathias Johansson: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Jakob Karlsson:** Writing – review & editing, Investigation. **Frans W.J. van den Berg:** Writing – review & editing, Methodology, Formal analysis. **Anna Ström:** Writing – review & editing, Supervision. **Lilia Ahrne:** Writing – review & editing, Supervision. **Corine Sandström:** Writing – review & editing, Supervision. **Maud Langton:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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.2024.110295.

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