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Mixed legume systems of pea protein and unrefined lentil fraction: Textural properties and microstructure

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ABSTRACT

Within the context of circular economy, there is an increasing interest to utilise agrifood by-products. However, extensive extraction and purification steps make the valorisation of side streams not always cost effective. Therefore, an increased knowledge of the functionality of unrefined side streams could increase their utilisation in food products. We investigated the thermal gelation of mixed legume systems containing a commercial pea protein isolate (*Pisum sativum*) and the unrefined fraction remaining after protein extraction from lentils (*Lens culinaris*). The unrefined lentil fraction contained mainly starch (~45 g/100 g) and insoluble cell wall polysaccharides (~50 g/100 g) with minor amounts of soluble protein (4 g/100 g) and polyphenols (<1 mg GAE/g). The addition of the unrefined lentil fraction increased the strength and Young's modulus of pea protein gels in the pH range 3–4.2, and also increased the gels' elastic modulus G'. The microstructure could be described as a mixed network of swollen protein particles of different sizes (5–50 μm), gelatinised starch and cell wall fragments. The results demonstrate that unrefined side streams from lentils could be used for textural modification of plant protein gels, with implications for the design of novel plant-based foods.

1. Introduction

Strategies to reduce the environmental impact of agrifood industry include replacing the consumption of animal proteins by plant alternatives and to valorise agrifood by-products (Ripple et al., 2017, Poore & Nemecek, 2018; Willett et al., 2019). Legumes are rich in protein and carbohydrates and they are used in a range of different foods such as extruded meat substitutes, snacks and beverages. Peas (*Pisum sativum*) have a high protein content (23–31 wt %) (Lam, Karaca, Tyler, & Nickerson, 2018) and can supply a well-balanced amino acid profile when combined with proteins from grains (J. Boye, Zare, & Pletch, 2010). The main proteins in peas and other legumes are globulins (Sharif et al., 2018), with legumin (11 S) and vicilin (7 S) being the main globulin protein groups in peas (Derbyshire, Wright, & Boulter, 1976; Lam et al., 2018). The general process of heat-induced gelation of globular proteins includes partial denaturation, aggregation due to newly exposed residues and agglomeration of the aggregates to form a spatial network (Clark, Kavanagh, & Ross-Murphy, 2001). Studies on heat-induced pea protein gelation have mainly focused on the effect of salt, pH, concentration and extraction methods in single-component systems (Munialo, van der Linden, Ako, & de Jongh, 2015; Munialo, van der Linden, & de Jongh, 2014; Sun & Arntfield, 2011). The pH during gel formation has been found to largely affect the structure of the formed pea protein network (Ako, Nicolai, Durand, & Brotons, 2009; Munialo et al., 2015) as well as the viscoelastic properties (Munialo et al., 2015).

Extraction and purification of side streams for further use as food ingredients is not always sustainable nor cost effective, as large amounts of energy, chemicals and water are required. The main sustainability issues are related to the traditional wet extraction methods, as well as the subsequent energy-demanding drying of the extracts (Berghout, Pelgrom, Schutyser, Boom, & Van Der Goot, 2015). Therefore, the utilisation of side streams as unrefined, or mildly fractionated, materials deserves greater attention (Y. Peng, Kersten, Kyriakopoulou, & van der

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Goot, 2020).

Many studies have been conducted on mixed systems of starch and proteins from cereals; however, few studies have been performed on gelation of pea protein in more complex systems containing starch. Pressure-induced gelation of pea protein/starch mixtures was investigated showing that non-gelatinised starch could act as a filler material in the protein gels (Sim & Moraru, 2020). Multicomponent systems with pea protein and κ-carrageenan, maize starch and sucrose (Nunes, Raymundo, & Sousa, 2006), showed that lower cooling rates promote phase separation between pea protein and κ-carrageenan. In general structuring of pea protein remains challenging because it has lower solubility and gelling capacity than other plant proteins such as those ones from soy (Bildstein, Lohmann, Hennigs, Krause, & Hilz, 2008).

This study aims to determine the effect of the addition of an unrefined side stream from lentils on the mechanical, rheological and microstructural properties of pea protein gels as a function of pH and unrefined fraction concentration. A better understanding of the technological applications of unrefined side streams in complex systems containing other food ingredients, such as plant proteins, could aid reutilisation of agrifood by-products reducing the need for costly and extensive purification processes.

2. Materials and methods

2.1. Materials

A commercial pea protein isolate (PPI) (NUTRALYS® S85F, Roquette, France) was used as pea protein source. This PPI had a minimum protein content of 84 g/100 g and initial pH 7.4, as per manufacturer specification. Green lentils, *Lens culinaris*, (Gröna Linser, Saltå kvarn, Sweden) were purchased at a local supermarket in Gothenburg (Sweden). HCl was purchased from Fisher Scientific (NH, USA) and Merck (NJ, USA). Glutaraldehyde was from Ted Pella (CA, USA) and Technovit 7100 was from Kulzer (Hanau, Germany). Iodine, light green, rhodamine B, Trifluoroacetic acid (TFA), sulphuric acid, L-Fucose (Fuc), L-Arabinose (Ara), D-Galactose (Gal), L-Rhamnose (Rha), D-Glucose (Glc), D-Xylose (Xyl), D-Mannose (Man), D-Galacturonic acid (GalA), D-Glucuronic acid (GlcA), Folin-Ciocalteu's reagent, α-amylase, amyloglucosidase and oxidase/peroxidase reagent (GOPOD) were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of the unrefined fraction from lentils

An unrefined fraction (UF) was obtained from green lentils after separation of soluble proteins as follows: 100 g of lentils were ground using a knife mill (RETSCH Knife Mill Grindomix GM200, Retsch, Haan, Germany) resulting in a coarse flour. Following grinding, 35 g of the lentil flour was slowly added to 350 mL distilled water while continuously adjusting the pH to 2.5 using 0.5 M HCl and stirred for an additional 60 min. The resulting suspension was centrifuged at 3600 rpm (2434×*g*) for 20 min (Heraeus Megafuge 16 R centrifuge equipped with a TX400 rotor, Thermo Scientific, MA, USA). The supernatant containing soluble protein was discarded, and the pellet collected and frozen (−80 °C) before freeze-drying (Alpha 1–2 LDplus freeze dryer, Martin Christ, Osterode, Germany). The preparation of the unrefined fraction was done twice to evaluate the reproducibility of the extraction method.

The obtained unrefined fraction was used for mechanical compression tests and microscopy. Due to the presence of few large particles (>0.5 mm), which could interfere with the rheological measurements, part of the unrefined fraction was sieved (Vibratory Sieve Shaker Analysette 3, Fritsch, Idar-Oberstein, Germany) through a 125 μm sieve, and used for rheology experiments and differential scanning calorimetry (DSC).

2.3. Chemical analysis of unrefined fraction (UF)

The chemical composition of the unrefined fraction obtained from two extractions was analysed to ensure that the extraction procedure itself was reproducible.

Analysis of total starch content was done using Megazyme kits according to AACC Method 76-13.01. Samples were measured in triplicate.

The content of soluble protein was determined using Bradford protein assay kit from Bio-rad. The dye solution was added to diluted samples and absorbance was measured at 595 nm. Bovine globuline standard calibration was used to determine protein content. Samples were measured in triplicate.

The determination of total phenolic content (TPC) was carried out using Folin-Ciocalteu's reagent (Folin & Ciocalteu, 1927). The absorbance was measured at 725 nm and results were expressed as mg gallic acid equivalents (GAE) per 100 g dry weight of freeze-dried sample using a gallic acid standard calibration. Samples were measured in triplicate.

Monosaccharide composition analysis using ion chromatography (IC-3000 system Dionex) on an HPAEC-PAD system was performed after trifluoroacetic acid (TFA) and sulphuric acid hydrolysis of samples (Saeman, Moore, Mitchell, & Millett, 1954). The monosaccharide identification and quantification were carried out according to a previously described method (McKee et al., 2016). Cellulose content was estimated based on the glucose content of the samples after hydrolysis with sulphuric acid and minus the starch and glucose content from TFA hydrolysis. Samples were measured in triplicates.

2.4. Protein solubility

The solubility of the pea protein isolate at different pH was evaluated by preparing 1 mg/100 mL PPI dispersions. Samples were prepared by dispersing the PPI in distilled water for 1.5 h. The pH was adjusted with 1 M HCl, pH measurements were taken again after 30 min stirring to ensure that the pH was stable. After centrifuging for 10 min at 10.000×*g* (Sorvall ST 8 Small Benchtop Centrifuge equipped with a HIGHConic III Fixed Angle Rotor, Thermo Scientific, MA, USA) the protein concentration in the supernatant was quantified using Bradford's method (Bradford, 1976) measuring absorbance at 595 nm using a NanoVue Plus spectrophotometer (GE Healthcare, IL, USA). Bovine serum albumin (BSA) was used as standard. For each formulation 2 samples were measured.

2.5. Preparation of pea protein and mixed gels

Gels for mechanical testing were prepared by dispersing the pea protein isolate in deionised (MilliQ) water and stirring for 1.5 h before adjusting the pH (3, 3.6 and 4.2) using 0.5 M HCl. After pH-adjustment, water was added to reach a final concentration of 13 g/100 g PPI. For mixed gels, the unrefined fraction was first added to the water and stirred for 15 min before the addition of PPI. The unrefined fraction was added in different ratios to the PPI, 0.1:1, 0.2:1 and 0.3:1 (UF/PPI), leading to gels with constant PPI concentration (13 g/100 g) and increasing total solids. Solutions were poured into glass cylinders with an inner diameter of 16 mm. A small hole in the rubber lid prevented pressure build-up during heating. Samples were heated in a water bath at 95 °C for 30 min. After heating, samples were left to cool down at 20 °C overnight. For each formulation 10 gels were prepared.

A set of gels were prepared for confocal laser scanning microscopy (CLSM) by adding Rhodamine B (0.2 wt %) to the solutions, prior to thermal treatment, and gels were prepared as described above for mechanical tests. For each formulation 3 gels were prepared.

For light microscopy, PPI and UF were dispersed in water and stirred for 1.5 h. The pH (3, 3.6 and 4.2) was adjusted using 1 M HCl and water was added to the reach the final concentration and ratios (0.1:1, 0.2:1 and 0.3:1 UF/PPI). Solutions were poured into 15 mL falcon tubes and

heated in a water bath at 95 °C for 30 min. After heating, samples were left to cool down at 20 °C overnight prior to microscopy observations. For each formulation 3 gels were prepared.

2.6. Microstructural characterisation

For light microscopy, gels were cut into approximately 2 mm × 2 mm × 2 mm pieces and fixated overnight in 2.5 wt % glutaraldehyde. The samples were then dehydrated using ethanol in increasing concentrations followed by infiltration by plastic resin (Technovit 7100) and hardening at room temperature. Sample sections with a thickness of 2 μm were obtained using a Leica Ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Double staining was performed using light green to stain proteins and iodine for staining starch. Stained sections were examined using a Nikon Eclipse Ni–U microscope (Nikon, Tokyo, Japan) equipped with a 40 × (0.75 NA) apochromatic objective and images captured with a Nikon Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan).

For confocal laser scanning microscopy, a piece of the gel was cut with a sharp razor blade and introduced in a small metal cup placed onto a glass slide. A cover slide was used to cover the surface of the gel. Micrographs were obtained using a Leica TCS SP2 (Leica Microsystems GmbH, Wetzlar, Germany) confocal laser scanning microscope configured with an upright microscope using a HeNe laser for excitation at 543 nm. Emission was recorded between 550 and 650 nm. A 20x (0.7 NA) glycerol/water objective was used.

2.7. Differential scanning calorimetry (DSC)

Thermal properties of PPI and UF were evaluated by differential scanning calorimetry (1 Star system, Mettler Toledo, OH, USA) which allows determination of the onset and peak denaturation temperatures. An aliquot of 9 ± 2 μg of the solution (pH 3), prepared as described in section 2.5 excluding the heating step, was placed in the centre of a 40 μL aluminium crucible which was hermetically sealed. Measurements were run from 20 to 120 °C with a heating rate of 10 °C/min. A sealed empty crucible was used as a reference. All measurements were performed in duplicates.

2.8. Uniaxial compression

Compression tests were performed using a material testing instrument (INSTRON 5542 Universal Testing Instrument, Instron Corporation, MA, USA) with a 500 N load cell and a cylindrical probe with a diameter of 30 mm. Sandpaper with a grit size of P180 was used on both the probe and bottom plate to prevent slip. Gels with a height of 12 mm and diameter of 16 mm were compressed at a rate of 0.1 mm/s up to ~70% deformation. From the resulting true stress-true strain curves, the fracture stress i.e. maximum true stress, and the corresponding true strain (fracture strain) were selected. The Young's modulus was obtained from the slope of the linear part of the true stress-true strain curve. For each formulation 6 gels were measured, and average and standard deviation of the different parameters were calculated for comparison.

2.9. Dynamic rheological measurements

Dynamic rheological measurements were performed using a strain-controlled rheometer (ARES-G2, TA Instruments, DE, USA). Concentric cylinders were used as the measuring system, with a 30 mm diameter cup and a 27.7 mm diameter bob. The solution (pH 3) was carefully poured into the cup, which was preconditioned at 20 °C, a constant volume of 20 mL was used for all the samples. Thereafter, a resting time of 1 min was applied before starting the temperature cycle. The temperature cycle began with heating to 95 °C followed by a holding time of 30 min before cooling to 20 °C and an additional holding time of 30 min. A rate of 5 °C/min was used for the heating and 1 °C/min for cooling. Experiments were run at a strain of 0.5% and an angular frequency of 6.28 rad/s, selected from independent measurements to determine the linear viscoelastic region. Measurements were performed in duplicates. The elastic modulus was normalised by using equation (1) (Munialo et al., 2015)

$$G'^{*} = \frac{G'(t)}{G'(t = t^{*})} \quad \text{(equation 1)}$$

where t* is the time corresponding to the end of the cooling phase and start of the holding time at 20 °C.

2.10. Statistical data analysis

Statistical analysis of protein solubility data and rheological measurements was performed using XLStats (version 2015, Excel Microsoft, Redmond, WA, USA), a post-hoc Tukey's test ($p < 0.05$) was used to determine significant differences between mean values of independent replicates. The Origin (Pro) (version 2017 9.40.00, OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis of the mechanical results by two-way ANOVA. The analysis was performed on mean values of independent replicates and the significance level was assessed using the Tukey's test ($P < 0.05$).

3. Results

3.1. Chemical composition, microstructure and thermal stability (DSC) of the unrefined lentil fraction

The starch content represented 42–48 g/100 g of the unrefined fraction (Table 1), which is in agreement with previous reports on starch content in lentils (Brummer, Kaviani, & Tosh, 2015; Dalgetty & Baik, 2003; García-Alonso, Goni, & Saura-Calixto, 1998). The cellulose and hemicellulose, which represent the cell wall components, were a major part of the unrefined fraction. Other minor components were remaining soluble proteins ca. 4 wt % and phenolic compounds (Table 1). Pulse proteins are highly soluble under alkaline and acidic conditions therefore most of the protein was separated during the acid extraction (J. I. Boye, Zare, & Pletch, 2010).

The monosaccharide analysis by 2-step sulphuric hydrolysis revealed that the dominant sugar was glucose 91 g/100 g, followed by 5 g/100 g arabinose, 2 g/100 g xylose and 2 g/100 g galactose. Minor amounts of uronic acids (GlcA and GalA) were detected after trifluoroacetic acid (TFA) hydrolysis, but they were not quantified as their content was below <1% (results not presented). Sugars were present in similar concentration ranges to those previously found in the insoluble fraction

Table 1
Composition of the green lentil unrefined fraction remaining after soluble protein extraction. The results represent the average and standard deviation of 2 independently extracted batches. Fuc, Rha, Man were not detected. GalA and GlcA were not quantified (<1%).

Component	mg/g flour
Total carbohydrate	943 ± 78
Starch	454 ± 55
Cellulose	405 ± 21
Other polysaccharides	84 ± 9
Soluble protein	3.9 ± 0.5
	mg GAE/g
Total phenolics	0.73 ± 0.06
Monosaccharide content	**(mg/g flour)**
Glc	859 ± 70
Ara	48 ± 5
Xyl	19 ± 3
Gal	17 ± 2

of green lentils (Brummer et al., 2015). Based on the monosaccharides content the polysaccharides present in lentils' cell walls have been suggested to be mainly cellulose, arabinan or arabinogalactan types with xylose side chains (Bhatty, 1990). Soluble polysaccharides such as pectins could be removed during protein extraction as reflected by the low content of galacturonic acid (Brummer et al., 2015). The chemical composition of both batches was very similar represented by the standard deviation in Table 1, indicating that extraction method was reproducible.

As observed in Fig. 1a, the microstructure of the unrefined fraction agreed well with the chemical analysis showing the presence of starch granules (in black) and insoluble materials i.e. cell wall fragments, of different sizes. The integrity of the cell structures seemed to be unaffected by the thermal treatment (95 °C, 30 min) (Fig. 1b), whereas the starch granules were disintegrated and gelatinised.

Regarding the thermal analysis of the unrefined fraction, a peak with a maximum at 69.9 °C (onset temperature: 62.4 °C) was detected, which corresponds to the gelatinisation of starch (Fig. 2).

3.2. Particle size, solubility and thermal stability (DSC) of the commercial pea protein isolate

Light microscopy (Fig. 1c) showed that the commercial pea protein isolate (original pH 7.4) was characterised by particles of different sizes ranging from few μm to ~200 μm, resembling typical spray dried protein particles. The lowest protein solubility was measured at pH 4.2, closer to the pea protein isoelectric point, IEP at 4.5, and the highest solubility at pH 7.4 (Table 2). The concentrations corresponded to 4.7 g/100 g, 3.5 g/100 g, 2.3 g/100 g and 7.1 g/100 g of the total protein for pH 3, pH 3.6, pH 4,2 and pH 7.4 respectively.

The thermal stability was evaluated by differential scanning calorimetry. Thermal properties of globular proteins are related to their heat-induced aggregation and gelation behaviour. No obvious denaturation peak was observed for the native pea protein in solution (Fig. 2), which could be due to the low protein concentration used, but more likely due to the fact that the protein is denatured, supported by solubility data (Table 2). It has been previously reported that commercial pea protein isolate could be denatured as a result of the harsh conditions used during extraction (Shand, Ya, Pietrasik, & Wanasundara, 2007).

3.3. Gel strength

The concentration of pea protein isolate required to obtain self-standing gels was set at 13 g/100 g based on previous experiments. Fracture properties of heat-induced pea protein gels with a PPI concentration of 13 g/100 g were measured at three different pH (3.0, 3.6 and 4.2), selected below the protein isoelectric point, IEP 4.5

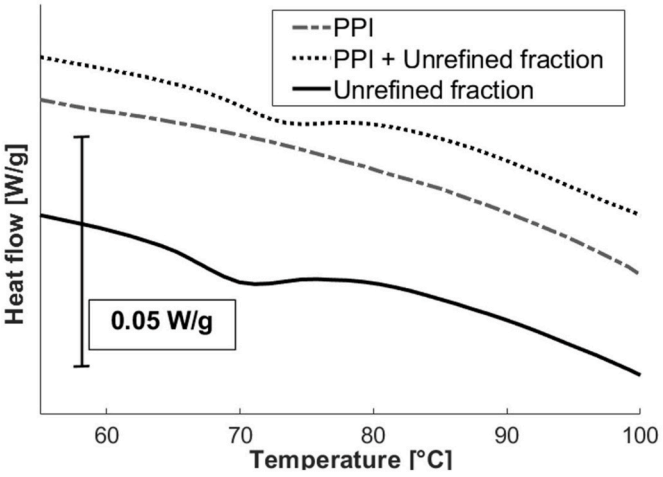

Fig. 2. Differential scanning calorimetry (DSC) measurements of lentil unrefined fraction, pea protein isolate PPI (13 wt %) and 0.3:1 unrefined fraction/PPI dispersions. All samples were measured at pH 3 in duplicates.

Table 2
Amount of soluble protein at different pH in a 1% (w/v) PPI dispersion. The results represent the average and standard deviation of three experiments. Different letters indicate significant differences.

pH	Protein (μg/mL)
3.0	395 ± 58[a]
3.6	293 ± 47[a]
4.2	194 ± 27[b]
7.4	595 ± 23[c]

(Withana-Gamage, Wanasundara, Pietrasik, & Shand, 2011). The effect of pH and addition of unrefined fraction on fracture stress, fracture strain and Young's modulus is represented in Fig. 3.

Decreased pH resulted in an increase in fracture stress and Young's modulus. PPI gels at pH 4.2 were too weak to be handled and measured. The effect of pH on fracture strain was less clear, however lower average values were observed at pH 3.6 and 4.2 compared to pH 3. Addition of unrefined fraction resulted in an increase of the fracture stress and Young's modulus independently of the pH. No significant differences were observed in fracture strain as a function of the unrefined fraction. Statistical analysis showed that both pH and addition of unrefined fraction had a significant effect on gel strength and Young's modulus, whereas only pH had a significant effect on the fracture strain as

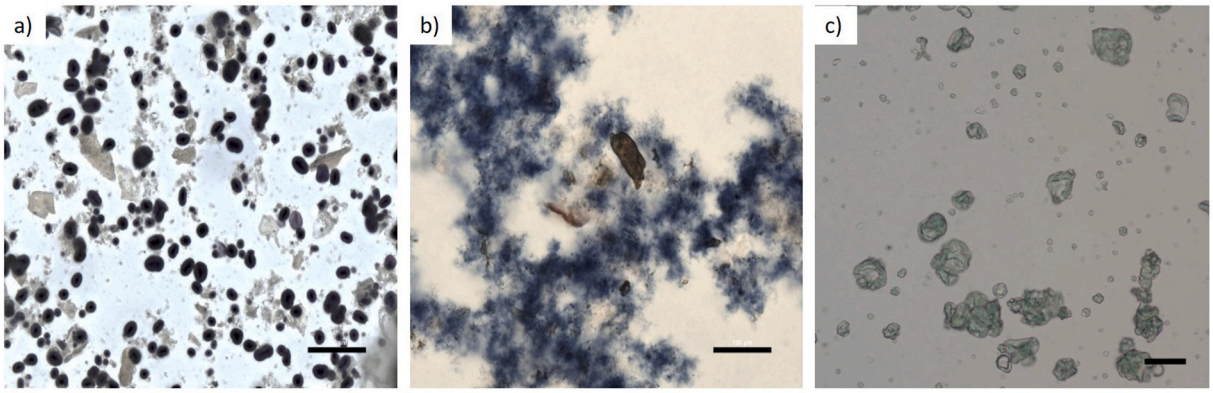

Fig. 1. Unrefined fraction obtained after lentil protein extraction, before (a) and after thermal treatment (95 °C, 30 min) and cooled down to 20 °C (b). Pea protein isolate in water 1% (w/v) pH 7.4 (c). Scale bars correspond to 100 μm. Staining: iodine for starch (purple) and light green for proteins (green/turquoise).

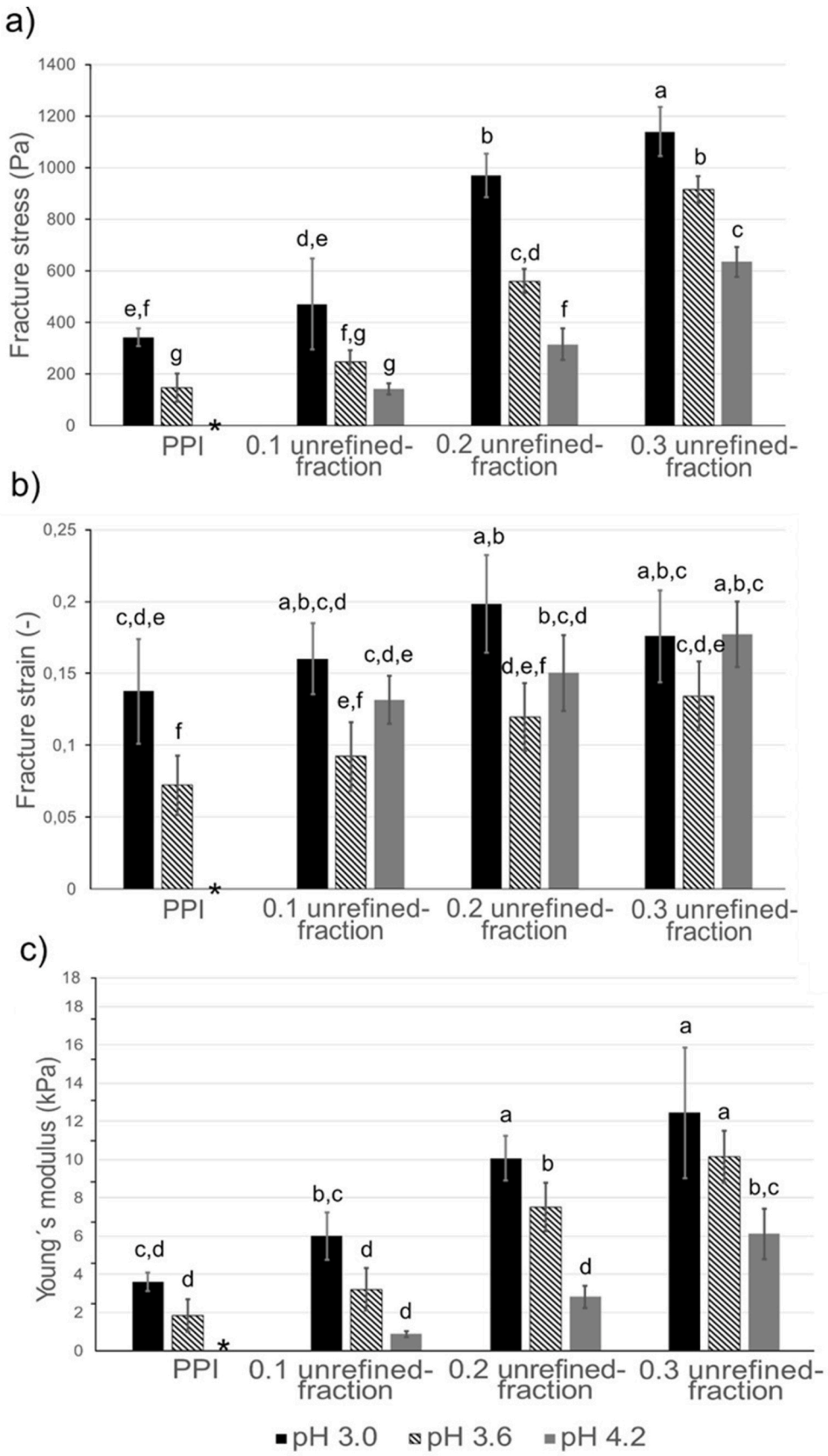

Fig. 3. Fracture stress (a), fracture strain (b) and Young's modulus (c) of 13 wt % pea protein gels (PPI) at different pH 3, 3.6 and 4.2, and with different lentil unrefined fraction (UF) contents (0:1, 0.1:1, 0.2:1 and 0.3:1, UF: PPI). Error bars represent the average and standard deviation of at least 6 replicates. Different letters indicate significant differences. *indicates that the gels were not self-standing and was not possible to measure them.

reflected by p-values lower than 0.05.

3.4. Gel microstructure

Light microscopy revealed differences in the microstructure as a function of pH as well as with the addition of the unrefined fraction (Fig. 4 a-f). The micrographs showed that the PPI gels could be described as a network of swollen particles clustered together. Two main protein particle populations could be identified with sizes of approximately 10–100 μm, and smaller particles surrounding the larger particles. There was an increase in overall gel density with decreasing pH, which was attributed to the changes in pea protein solubility with pH (Table 2). As the solubility increases, soluble protein released into the continuous phase is gelled and leads to a more homogenous network at pH 3. Addition of the unrefined fraction was visible by the presence of starch (purple) in the mixed gels. The starch granules were swollen and deformed, part of the amylose leaked out from the starch granules and was present across the gel surrounding the protein particles. Confocal scanning laser micrographs of gels stained for protein (Fig. 4 g,h), confirmed the nature of the protein gels i.e. network of swollen particles of different sizes (in green). As the ratio of UF to PPI increases larger black areas (no fluorescence signal) were present in the gels, suggesting that the unrefined fraction containing starch and cell fragments was localised in those regions throughout the gels.

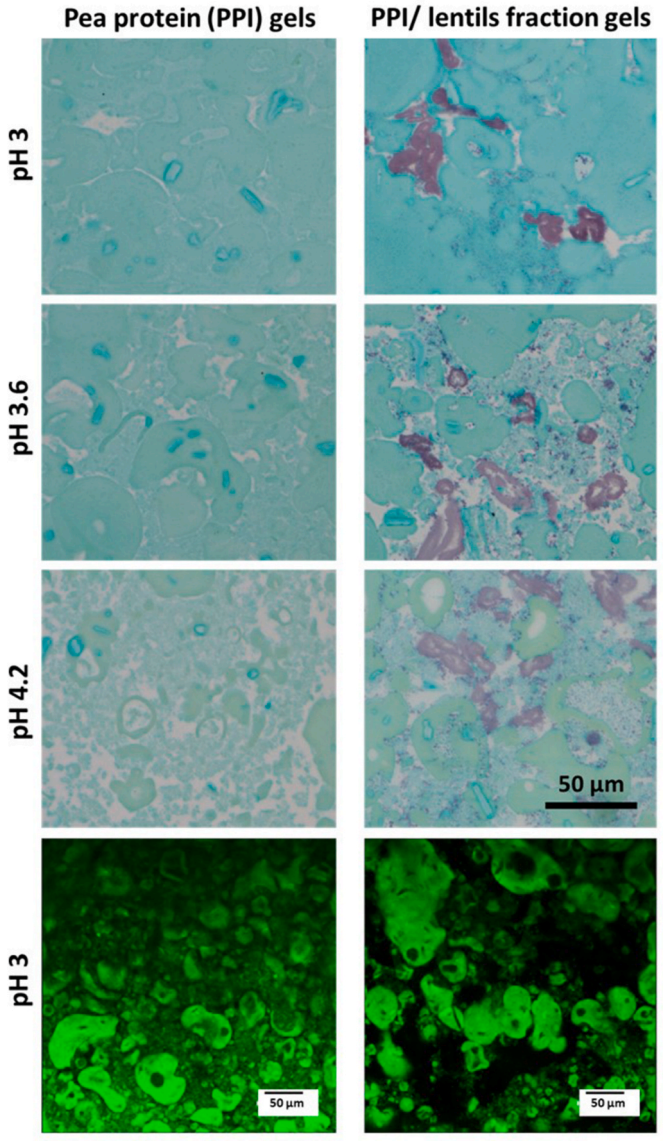

Fig. 4. Light micrographs of heat-induced pea protein gels (13 % wt) at different pH, and with added lentil unrefined fraction (0.3:1 UF: PPI). Scale bar corresponds to 50 μm in all images. Staining: light green for protein and iodine for starch (in purple). Confocal laser scanning micrographs of heat-induced pea protein gels (13 wt %) at pH 3 without added lentil unrefined fraction and with lentil unrefined fraction added at a ratio 0.3:1 UF/PPI. Samples were stained for protein with Rhodamine B.

3.5. Viscoelastic properties

To study the impact that the addition of the unrefined fraction had on the gelation mechanism, small amplitude oscillatory shear (SAOS) measurements were performed. The storage G' and loss moduli G'' were recorded in situ during the thermal gelation of PPI and UF/PPI solutions at pH 3, for the lowest and the highest UF content (Fig. 5).

The final storage modulus G' was larger than the loss modulus G'' for all types of gels (Table 3), with tan δ values between 0.18 and 0.22, indicative of the elastic nature of the gels. The addition of the unrefined fraction increased the storage and loss modulus of the gels, twofold for the 0.1:1 and more than threefold for the 0.3:1 gels. The development of G' started with an increase during heating and continuing during the holding step at 95 °C. At ~60 °C the G' of the samples containing unrefined fraction increased faster than for the only protein samples (indicated by an arrow in Fig. 5a), and ~70 °C the maximum rate of increase in G' was reached. These two temperatures correspond to the reported start and maximum gelatinisation temperature of lentil starch.

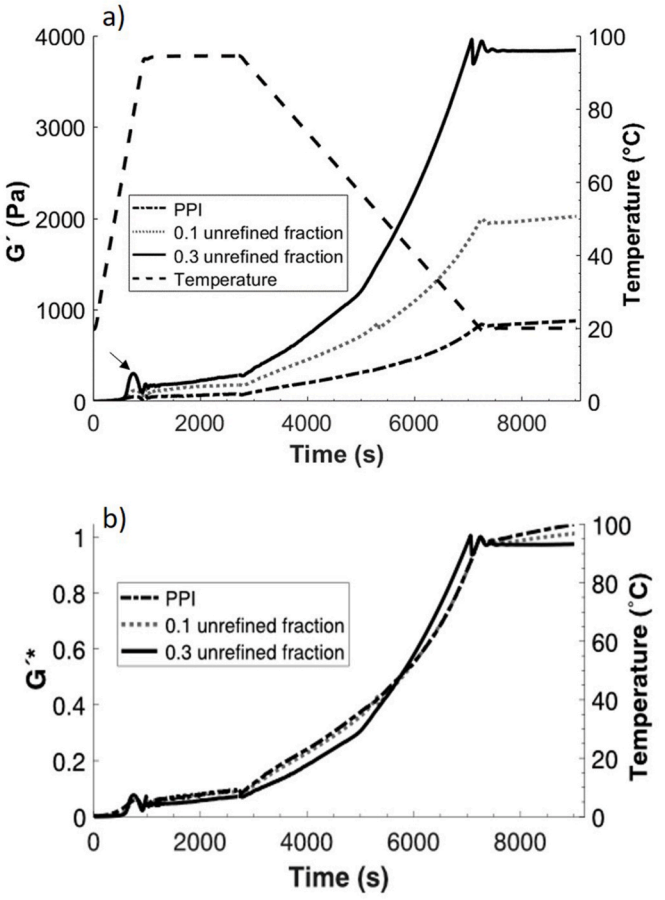

Fig. 5. Storage modulus (G′) evolution of 13 wt % pea protein isolate (PPI)) with and without the addition of lentil unrefined fraction (UF) at ratios 0.1:1 and 0.3:1 UF/PPI (a) and normalised G′ obtained using equation (1) (b). Samples were measured in duplicated.

Table 3
Storage modulus G ′ and loss modulus G'' selected at the final point of the thermal cycle. Pea protein (PPI) and lentil unrefined fraction (UF) at different ratios 0:1, 0.1:1 and 0.3:1 (UF/PPI). Values represent the average and standard deviation calculated from 2 measurements. Different letters represent significant differences.

Gel composition	G' (Pa)	G'' (Pa)
0:1 UF/PPI	1038 ± 0.4^{a}	201 ± 5.8^{a}
0.1:1 UF/PPI	1970 ± 51^{b}	392 ± 22^{b}
0.3:1 UF/PPI	3624 ± 217^{c}	723 ± 61^{c}

A substantial increase in the storage modulus was observed during cooling down to 20 °C, but no remarkable changes were observed when holding the temperature at 20 °C.

As can be seen in Fig. 5b. The gelation curves of the 0.1:1 UF/PPI and PPI superimposed when the G' was normalised using equation (1), indicating that the mechanism of gel formation was similar however, the shape of the master curve varied for the 0.3:1 UF/PPI suggesting that with an increase unrefined fraction content the gelation differed.

4. Discussion

Proteins and starch are affected by processing parameters such as water availability, temperature, pH, and the presence of other food components, leading to different microstructures and texture related properties. Regarding the commercial pea protein (13 g/100 g at pH 3) the results showed similar gelation pattern and G' values (~1000 Pa) to those reported in the literature (Munialo et al., 2015). However, the overall gel strength (0.2–0.4 kPa) and Young's modulus (4 kPa) was lower than in that published study (fracture stress 1–5 kPa and Young's modulus 40 kPa). The fracture point in the true stress/true strain curves for all gels was not sharp, indicating a ductile nature. The functional properties of commercial soy protein isolates (Hermansson, 1983), and pea protein isolates (Lan et al., 2019) have been reported to differ from those of mildly prepared isolates. In addition, less than 10 g/100 g of the PPI was soluble at the pH investigated here, in agreement with reported solubility of this commercial PPI showing a solubility of 5 wt % and 15 g/100 g for pH 4.2 and pH 3 respectively (Wei et al., 2020). Our results showed that PPI particles tend to swell and aggregate leading to particulate gels, rather than a continuous protein strand network, as reflected in microscopy images. As the particles get closer together, they might stick to each other as result of protein crosslinking by hydrogen bonds, van der Waals and hydrophobic interactions.

The increase in fracture stress and Young's modulus (Fig. 3) as the concentration of UF increased was mainly attributed to the increase total solids and, in particular to the presence of starch. Starch gelatinisation is a complex process including structural changes leading to swelling of the granules, leaching and loss of granular integrity. The micrographs (Fig. 4) revealed that starch granules were disintegrated in the mixed gels, as a result of the thermal treatment. Previous studies reported that starch affected the textural properties of heat-induced protein gels, both increasing the firmness of lentil protein gels (Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014) and decreasing the hardness of soy protein gels (Yu, Ren, Zhao, Cui, & Liu, 2020). The development of viscoelastic properties during the thermal cycle (Fig. 5a), combined with DSC results (Fig. 2), indicated that the increase in viscoelastic moduli of PPI/UF gels was related to the presence of starch. Research on mixed systems of starch and proteins has concentrated on dairy and soy proteins (Aguilera & Baffico, 1997; Bertolini, Creamer, Eppink, & Boland, 2005; Dang, Loisel, Desrumaux, & Doublier, 2009; J.-Y. Li, Yeh, & Fan, 2007; Nayak et al., 2004; Yu et al., 2020). Those studies showed that the effect of the starch on gelation and thermal stability are related to the water uptake by starch granules and proteins, and that the starch concentration determined which of the components, i.e. protein or starch, formed a continuous phase (J.-Y. Li et al., 2007). Absorption of water and swelling of starch granules could increase the local protein concentration in the surrounding solution (Aguilera & Rojas, 1997), leading to an increase in the viscoelastic moduli, in strength and in the Young's modulus of pea protein gels (Munialo et al., 2015). The temperature at which an increase in G' was observed during heating of the mixed gels corresponded to the starch gelatinisation temperature measured by DSC (Fig. 2), as well as previously reported gelatinisation temperature of lentil starch (Joshi et al., 2013). DSC showed a slight increase in the transition temperature for the mixed systems as compared to only unrefined fraction, for the PPI/UF fraction the onset was 67.0 °C and the peak maximum at 73.3 °C. A similar increase has been observed for gelatinisation of corn starch in the presence of soy protein and is hypothesised to be related to the reduced water availability for the starch (S. Li, Wei, Fang, Zhang, & Zhang, 2014). The sharp increase in G' during cooling of the mixed gels (Fig. 5a) is believed to be the result of reassociation and network formation of amylose molecules, which leaked from the starch granules surrounding the protein particles, as seen in Fig. 4. The gels became weaker with increasing pH (Fig. 3a), a reduction in gel strength has been attributed to decreased connectivity between protein aggregates (Munialoet al., 2015; Weijers, van de Velde, Stijnman, van de Pijpekamp, & Visschers, 2006). As the pH decreased, the network became denser (Fig. 4), this has been previously shown for pea protein gels an attributed to a change from a coarse network with heterogeneous pores size to a finer pea protein network (Munialo et al., 2014). In our study, due to the limited amount of protein soluble and available for the formation of a protein-strand network, is also possible that as the pH increases to values close to the IEP, and the solubility of the protein decreases, the network is more open and heterogenous introducing weaker points that can easier yield under stress.

The change in microstructure with pH could also explain the higher fracture strain at pH 3. It has previously been shown that for pea protein gels (Munialo et al., 2015) and soy protein gels (Renkema, Gruppen, & Van Vliet, 2002) a change in porosity with pH led to an increased brittleness. In addition to the effect due to the starch content, the presence of insoluble cell wall polysaccharides (Table 1 and Fig. 1), mainly cellulose, in the unrefined fraction could be playing a role as structural reinforcements leading to increase gel strength. As compared to starch, less is known of the impact of insoluble fibres on gelation of plant proteins. In recent years, several studies have shown that insoluble cell wall polysaccharides i.e dietary fibre, are effective in improving the gelling properties of protein due to the formation of a stable network structure (J. Peng et al., 2019; Ullah et al., 2019). The effect and mechanism of adding wheat bran cellulose to soy protein gels have been recently investigated (Xiao et al., 2020) showing an increased in gel strength. In our study insoluble cell wall polysaccharides are present as cell structures of different size (Fig. 1), and although the starch and the insoluble cell wall polysaccharides were not separated, the results showed that presence of the latter is not detrimental for the functionality and reinforcement of the gels, and it could even have a nutritional benefit as they are considered dietary fibre which are related to health benefits.

5. Conclusion

We have shown that an unrefined fraction obtained as a by-product of protein extraction from green lentils was rich in starch and insoluble cell wall polysaccharides. This unrefined fraction could be added to pea protein gels to induce changes in their rheological and textural properties. Heat-induced gels were characterised by a network of swollen particles of approximately 10–100 μm in size. The addition of the unrefined fraction led to a mixed network in which starch was gelatinised and distributed throughout the gels. We propose that i) starch is the main responsible for changes in gels viscoelasticity, as suggested by DSC and rheological measurements, and ii) the insoluble cell wall fragments in the unrefined fraction act as a filler in the gels, reinforcing the structure and leading to an increase in gel strength. The ductile and weak nature of the gels suggests that these ingredients could be further

functionalised during food manufacturing. Our results indicate that extraction and purification steps of side streams from legumes might not always be required to utilise them as food ingredients. Indeed, unrefined fractions containing starch and cell wall components tuned the functional properties of protein-based gels, opening the possibility to develop new textures for plant-based foods.

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CRediT authorship contribution statement

Mathias Johansson: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Epameinondas Xanthakis:** Conceptualization, Methodology, Writing - review & editing. **Maud Langton:** Funding acquisition, Methodology, Resources, Writing - review & editing. **Carolin Menzel:** Investigation, Methodology, Writing - review & editing. **Francisco Vilaplana:** Investigation, Methodology, Writing - review & editing. **Daniel P. Johansson:** Methodology, Writing - review & editing. **Patricia Lopez-Sanchez:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Validation, Writing - review & editing.

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.

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