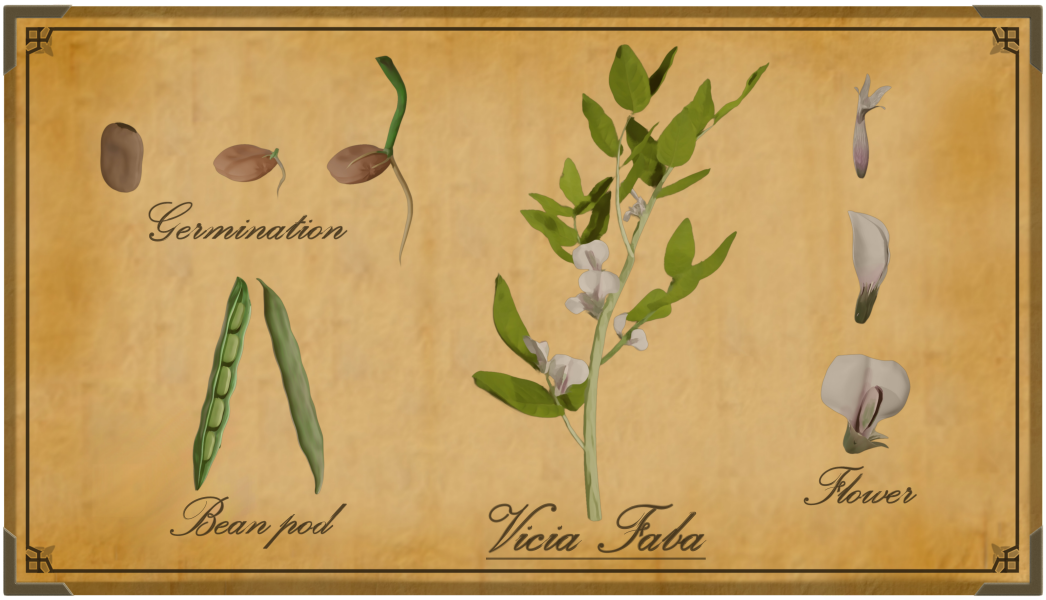
Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2024:21
Faculty of Natural Resources and Agricultural Sciences

Texture and microstructure of legume-based mixed gels

Mathias Johansson



Texture and microstructure of legume-based mixed gels

Mathias Johansson
Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences
Uppsala

SLU
SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS
Uppsala 2024

Acta Universitatis Agriculturae Sueciae
2024:21

Cover: Vintage botanical poster of faba bean (*Vicia faba*)
(Illustration: Mathias Johansson, 2024)

ISSN 1652-6880
ISBN (print version) 978-91-8046-306-5
ISBN (electronic version) 978-91-8046-307-2
https://doi.org/10.54612/a.6v1pth6al3

Swedish University of Agricultural Sciences, Department of Molecular Sciences, Uppsala, Sweden

Print: SLU Grafisk service, Uppsala 2024

Texture and microstructure of legume-based mixed gels

Abstract

Reducing consumption of animal-based proteins by shifting to plant-based alternatives can significantly reduce of the environmental impact of food consumption. Faba bean and pea are protein-rich crops which could act as local plant protein sources in cool climate regions such as Sweden.

Focusing on texture and microstructure, this thesis explored creation of mixed gels mainly from laboratory-extracted faba bean protein, starch and fibre but also from a commercial pea protein isolate. Analysis revealed that gel microstructures dominated by larger protein aggregates resulted in weak gels with low fracture stress. Addition of NaCl was found to have different effects on the gel formation by the two major protein fractions (7S and 11S) in faba bean. Gel texture was also modified by adding starch- and fibre-rich side-streams obtained from protein extraction. At pH 7, substituting part of the protein (constant solids content) with starch and/or fibre increased gel storage modulus and decreased fracture stress and fracture strain. The decrease in fracture stress was attributed to introduction of inhomogeneities into the protein matrix. At pH 4 and 5, fibre addition increased fracture stress, an effect attributed to the existing microstructure already containing larger protein particles in the same size range as the added fibre.

The results presented in this thesis extend knowledge of gel formation by plant-based proteins, and specifically of texture formation by protein from non-soy legumes. This can facilitate development of plant-based foods from a broader range of protein sources.

Keywords: faba bean, pea, protein gelation, starch, fibre, rheology

Textur och mikrostruktur av blandgeler från baljväxter

Sammanfattning

En övergång till en mer växtbaserad kost kan bidra till att minska miljöpåverkan från vår matkonsumtion. Men, detta kräver att hälsosamma och välsmakande alternativ finns tillgängliga. Två grödor som kan fungera som lokala proteinkällor i denna omställning är åkerböna och ärta, vilka båda kan odlas i kalltempererade regioner som Sverige.

Med fokus på textur och mikrostruktur rapporteras i denna avhandling resultaten från karaktärisering av blandgeler från framförallt egen-extraherat protein, stärkelse och fibrer från åkerböna men även från ett kommersiellt ärtproteinisolat. Resultaten visar att geler med en mikrostruktur som domineras av större proteinaggregat resulterar i svaga geler med låg gelstyrka. Vidare undersöktes gelbildningen av de två största proteinfraktionerna i åkerböna, 7S och 11S, där en tillsats av salt (NaCl) hade olika effekt på lagringsmodulen hos geler från de två fraktionerna. Effekten av stärkelse- och fiber-rika sidoströmmar från proteinextraktionen undersöktes genom att byta ut en del av proteinet mot stärkelse och/eller fibrer (konstant mängd fast material). Vid pH 7 ökade lagringsmodulen samtidigt som gelstyrkan minskade när stärkelse och/eller fiber tillsattes. Minskningen i gelstyrkan antogs relatera till den mer heterogena mikrostrukturen hos blandgeler jämfört med motsvarande proteingeler. Vid pH 4 och 5 ökade istället gelstyrkan när fiber tillsattes, något som kopplades till att den befintliga mikrostrukturen från proteinet redan innehöll proteinpartiklar med likande storlek som partiklarna i den tillsatta fiberfraktionen.

Resultaten som presenteras i denna avhandling bidrar till att öka kunskapen om gelbildning av växtbaserade proteiner från åkerböna och ärta. Detta kan i sin tur underlätta utvecklingen av växtbaserade livsmedel från ett bredare utbud av proteinkällor.

Nyckelord: åkerböna, ärta, protein gelbildning, stärkelse, fiber, rheologi

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Mathias Johansson, Saeid Karkehabadi, Daniel P. Johansson, Maud Langton (2023). Gelation behaviour and gel properties of the 7S and 11S globulin protein fractions from faba bean (*Vicia faba* var. *minor*) at different NaCl concentrations. *Food Hydrocolloids* 142 (108789).

II. Klara Nilsson, Mathias Johansson, Corine Sandström, Hanna Eriksson Röhnisch, Mikael S. Hedenqvist, Maud Langton (2023). Pasting and gelation of faba bean starch-protein mixtures. *Food Hydrocolloids* 138 (108494).

III. Mathias Johansson, Jakob Karlsson, Frans W.J. van den Berg, Anna Ström, Lilia Ahrne, Corine Sandström, Maud Langton. Effect of cellulose-rich fibres on faba bean protein gels is determined by the gel microstructure (submitted).

IV. Mathias Johansson, Daniel Johansson, Anna Ström, Jesper Rydén, Klara Nilsson, Jakob Karlsson, Rosana Moriana, Maud Langton (2022). Effect of starch and fibre on faba bean protein gel characteristics. *Food Hydrocolloids* 131 (107741).

V. Mathias Johansson, Epameinondas Xanthakis, Maud Langton, Carolin Menzel, Francisco Vilaplana, Daniel P. Johansson, Patricia Lopez-Sanchez (2021). Mixed legume systems of pea protein and unrefined lentil fraction: Textural properties and microstructure. *LWT* 144 (111212).

The contribution of Mathias Johansson to the papers included in this thesis was as follows:

I. Evaluated the results and wrote the manuscript together with the co-authors.

II. Contributed equally with Klara Nilsson. Designed the study together with the co-authors, performed the experiments, evaluated the results and wrote the manuscript.

III. Designed the study together with the co-authors, performed the majority of the experiments, evaluated the results and wrote the manuscript.

IV. Designed the study together with the co-authors, performed the majority of the experiments, evaluated the results and wrote the manuscript.

V. Performed the majority of the experiments, evaluated the results and wrote the manuscript.

The following papers were published during the timeframe of the doctoral project, but are not part of this thesis.

VI. Mathias Johansson, Klara Nilsson, Fanny Knab, Maud Langton (2022). Faba Bean Fractions for 3D Printing of Protein-, Starch- and Fibre-Rich Foods. *Processes* 10 (466).

VII. Tina Arndt, Kristaps Jaudzems, Olga Shilkova, Juanita Francis, Mathias Johansson, Peter R. Laity, Cagla Sahin, Urmimala Chatterjee, Nina Kronqvist, Edgar Barajas-Ledesma, Rakesh Kumar, Gefei Chen, Roger Strömberg, Axel Abelein, Maud Langton, Michael Landreh, Andreas Barth, Chris Holland, Jan Johansson, Anna Rising (2021). Spidroin N-terminal domain forms amyloid-like fibril based hydrogels and provides a protein immobilization platform. *Nature Communications* 13 (4695).

VIII. Jaqueline Auer, Johanna Östlund, Klara Nilsson, Mathias Johansson, Anja Herneke, Maud Langton (2023). Nordic Crops as Alternatives to Soy—An Overview of Nutritional, Sensory, and Functional Properties. *Foods*, 12 (2607).

IX. Jenny Johannesson, Malhar Manik Pathare, Mathias Johansson, Christel A.S. Bergström, Alexandra Teleki (2023). Synergistic stabilization of emulsion gel by nanoparticles and surfactant enables 3D printing of lipid-rich solid oral dosage forms. *Journal of Colloid and Interface Science* 650, 1253-1264.

X. Jenny Johannesson, Mingjun Wu, Mathias Johansson, Christel A.S. Bergström (2023). Quality attributes for printable emulsion gels and 3D-printed tablets: Towards production of personalized dosage forms. *International Journal of Pharmaceutics* 646 (123413).

XI. Jaqueline Auer, Marie Alminger, Marina Marinea, Mathias Johansson, Galia Zamaratskaia, Anders Högberg, Maud Langton (2024). Assessing the digestibility and estimated bioavailability/ bioaccessibility of plant-based proteins and minerals from soy, pea, and faba bean ingredients. *LWT* (115893).

Abbreviations

G'	Storage modulus
G"	Loss modulus
IEP	Isoelectric point
LF-NMR	Low-field nuclear magnetic resonance
LM	Light microscopy
LVR	Linear viscoelastic region
PCA	Principal component analysis
SEM	Scanning electron microscopy
WHC	Water-holding capacity

1. Introduction

The food sector accounts for a large part of global environmental impact and emissions of greenhouse gases, significantly contributing to climate change (Crippa *et al.* 2021). One way to reduce the environmental impact of the food sector is to shift towards eating more plant-based foods (Willett *et al.* 2019; Scarborough *et al.* 2023). Demand for plant-based foods has increased significantly in recent decades (Tziva *et al.* 2020; Aschemann-Witzel *et al.* 2021; Tso *et al.* 2021), possibly due to increasing awareness of issues related to animal welfare and the environmental unsustainability of the current food production system (Siegrist & Hartmann 2019; Giacalone *et al.* 2022).

The current market for plant-based foods, particularly meat substitutes, is dominated by a handful of crops. Prominent among these are soy, with limited cultivation feasibility in Scandinavian countries, and wheat, which may be undesirable due to the high frequency of coeliac disease and gluten intolerance among consumers. Therefore, it is desirable to use other protein-rich crops that can be extensively cultivated in Scandinavia. However, development of new foods based on protein-rich crops other than soy is hampered by lack of understanding of the mechanisms that govern formation of gels and other structures, such as extrudates, from these protein sources. To address this knowledge gap and facilitate development of appealing foods from alternative protein sources, more research is needed.

This thesis examined heat-induced gel formation by legume proteins (faba bean and pea), alone and in mixed systems including starch and fibre obtained as side-streams from protein extraction (Figure 1). The overarching aim was to correlate the structure and properties of mixed gels prepared from protein, starch and fibres extracted from legumes, mainly focusing on Swedish faba bean.

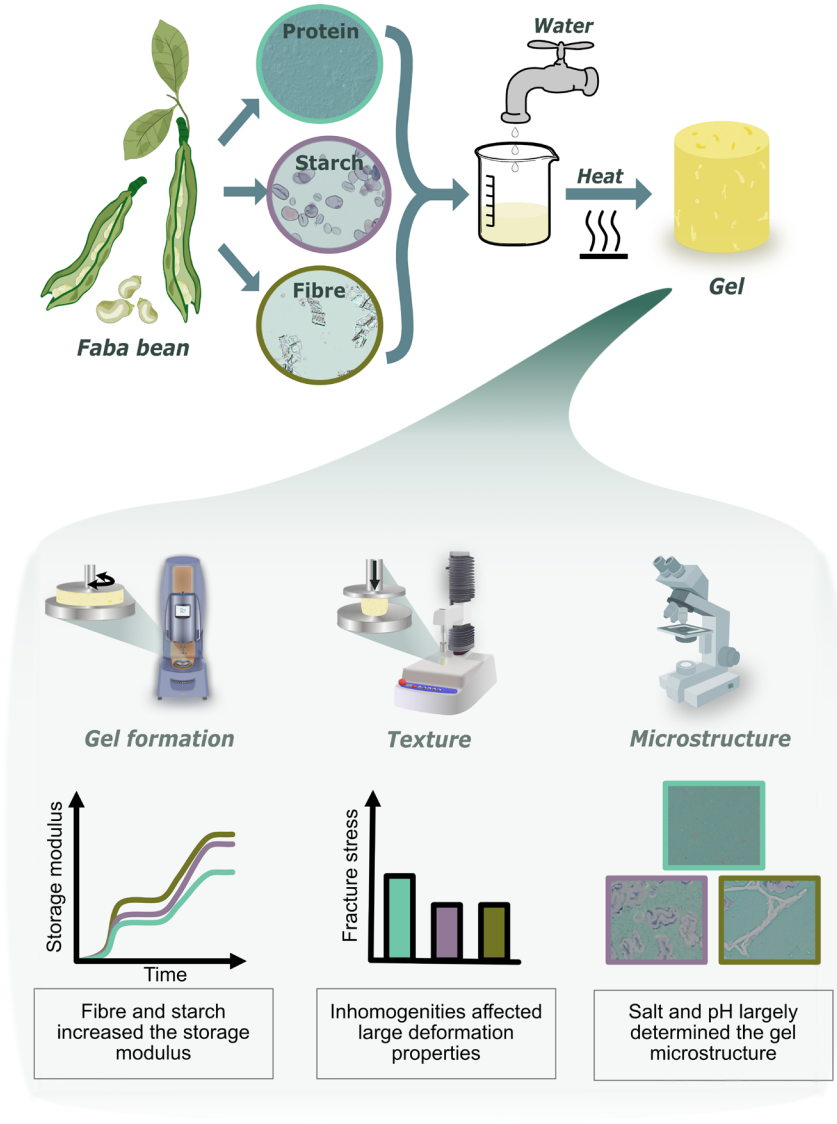


Figure 1. Graphical abstract outlining the main components and findings of this thesis work.

2. Background

Protein gel formation is a commonly used process in the food industry, giving texture to many different types of foods such as yoghurts and sausages. The majority of research performed on protein gel formation has focused on milk proteins, but since the early 2000s interest in gel formation by plant proteins has increased significantly (Figure 2). However, most research on plant protein gel formation has concentrated on only a few crops, namely soy and wheat (although with peas also increasing in popularity in recent years). Thus, there is a lack of knowledge on gel formation by proteins from legumes other than soy, such as faba bean, a shortcoming that needs to be addressed to meet increasing demand for alternative plant protein sources.

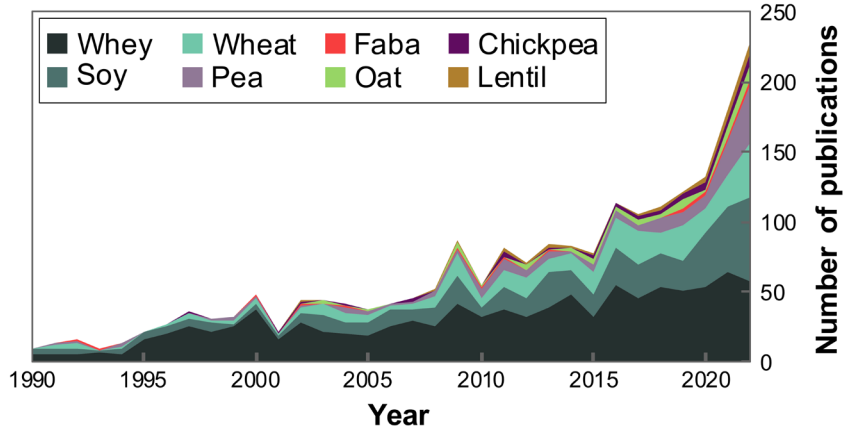


Figure 2. Number of articles published per year in the period 1990-2022 on gel formation by proteins from different sources. Data obtained in November 2023 from the Scopus database using the search term "protein gelation" combined with the different protein sources.

Legumes are commonly used plant-based protein sources and often contain high amounts of starch that act with proteins as the main components of many food matrices, along with water. Most pulses are also rich in fibres and minerals, providing nutrition and health benefits (Didinger & Thompson 2022). However, during protein extraction from starch-rich pulses such as faba beans and lentils, most starch and fibre end up as waste or side-streams that are currently used only in a limited number of food, feed and industrial applications (Ratnayake & Naguleswaran 2022). Valorisation of these side-streams would reduce waste and increase the sustainability of production. Hence, incorporating these side-streams back into the food supply chain is important when aiming for increased consumption of plant proteins.

Faba beans (*Vicia faba*) and peas (*Pisum sativum*) are protein-rich legumes cultivated worldwide, including in temperate climate regions such as Sweden, for both feed and food. Legumes are nitrogen-fixing crops, meaning that they can reduce the need for fertiliser when grown in a rotational crop system (Jensen *et al.* 2010). The chemical composition of faba beans varies depending on cultivar, but roughly consists of 33-53% starch, 19-33% protein, 11-22% dietary fibre and 0.5-2.8% lipids on a dry matter basis (Griffiths & Lawes 1978; Duc *et al.* 2015; Mayer Labba *et al.* 2021). Peas have a similar chemical composition, containing 19-55% starch, 19-34% protein, 11-26% dietary fibre and 0.5-4.7% lipids on a dry matter basis (Bastianelli *et al.* 1998; Chen *et al.* 2023). Within-crop variation in composition occurs due to factors such as genetic variation between cultivars, environmental conditions and harvesting time (Ntatsi *et al.* 2018). Legumes also typically contain high levels of certain vitamins and micronutrients, such as folate, iron and zinc (Mayer Labba *et al.* 2021; Rahate *et al.* 2021). However, they also contain antinutrients, *e.g.* phytate, which can lower the bioavailability of the minerals present (Mayer Labba *et al.* 2021). In addition, faba beans contain vicine and convicine, which can cause favism (acute haemolysis) in individuals with glucose-6-phosphate dehydrogenase deficiency (Arese & De Flora 1990).

The composition and the levels of antinutrients present vary between cultivars and thus, depending on the end-need, different cultivars can be grown. For example, the faba bean cv. *Tiffany* is low in vicine and convicine, while cv. *Gloria* has higher levels of vicine and convicine, but also a high protein content and low levels of tannins (Vilariño *et al.* 2009; Mayer Labba *et al.* 2021).

2.1 Protein gel formation

A gel can be described as a soft, solid or solid-like system with two or more components, one of which is a liquid present in substantial quantity (Almdal *et al.* 1993). If the liquid is water, the gel is defined as a hydrogel. In protein gels, the second main component is protein, forming a spatial network filling the entire volume of the system.

Protein gelation, or protein gel formation, is an important process in the food industry, giving texture to many foods. Understanding gel formation by the relevant protein can help predict the physicochemical properties of these foods, and how changes in ingredients and processing might affect their textural properties. Protein gelation can be induced by different means, such as changes in temperature, pressure or pH.

Multiple factors, such as protein source, pH and addition of salt, are known to affect gel formation by plant proteins (Nicolai & Chassenieux 2019; Langton *et al.* 2020). Individual protein fractions from the same source may also gel differently, as has been observed *e.g.* for soy, faba bean and pea proteins (Utsumi & Kinsella 1985; Bora *et al.* 1994; Johansson *et al.* 2023). Differences in gel formation between protein fractions from the same plant can be caused by differences in *e.g.* molecular weight and amino acid composition.

The main protein fractions in most legume seeds (including faba bean, pea and soy) are 7S and 11S globulins (Fukushima 1991; Lu *et al.* 2020; Warsame *et al.* 2020). The ratio of these individual protein fractions differs between cultivars (Multari *et al.* 2015; Warsame *et al.* 2020), meaning that choice of cultivar is likely to influence gel formation by the extracted protein.

The protein extraction method used also affects gel formation, with different extraction methods resulting in differences in gelling properties that can often be as large or larger than those deriving from using different plant sources (Nicolai & Chassenieux 2019). Harsh processing conditions, such as high pH and temperatures during extraction, can cause proteins to denature (Nash *et al.* 1971; Arntfield & Murray 1981; Hansen *et al.* 2022). This can lead to low solubility and poor techno-functional properties of the extracted protein. Inherently low solubility and a tendency for the proteins to aggregate during extraction are some of the major difficulties when using plant globulins for different food applications (Nicolai & Chassenieux 2019). Faba bean and pea protein both have a solubility profile similar to that of most other plant proteins, such as soy, typically showing the lowest solubility and

isoelectric point (IEP) around pH 4-5 (Sosulski & McCurdy 1987; Otegui *et al.* 1997).

2.1.1 Gel formation by globular proteins

Most plant proteins are globular proteins, including the majority of proteins found in faba beans and peas. Heat-induced gelation of globular proteins typically occurs as the protein, in sufficiently high concentration, is heated to above its denaturation temperature (Clark 1998; Nicolai & Chassenieux 2019). Upon heating, the protein denatures, exposing hydrophobic (and other) interaction sites (Hermansson 1979; Nakai 1983; Delahaije *et al.* 2016). This leads to aggregation of the protein and eventually the aggregates grow and connect to form a continuous network if the concentration is sufficiently high (Nicolai & Chassenieux 2019). Intermolecular interactions between proteins within the gel can be covalent (disulphide bonds) and/or non-covalent (hydrogen bonds, electrostatic and hydrophobic interactions) (Foegeding & Davis 2011; Nicolai & Chassenieux 2019). During subsequent cooling of the system, the gel network is strengthened further by formation of additional hydrogen bonds and other non-covalent short-range interactions (Beveridge *et al.* 1984).

The denaturation temperature of plant proteins such as faba bean and pea is typically reported to lie in the range 75-95 °C (Arntfield & Murray 1981; Sun & Arntfield 2011a; Mession *et al.* 2013; Nivala *et al.* 2017; Bühler *et al.* 2020). The denaturation temperature depends on factors such as extraction method and ionic strength, and differs between different protein fractions isolated from the same source (Arntfield *et al.* 1986; Kimura *et al.* 2008). At higher salt concentrations, denaturation temperature above 100 °C has been reported for both faba bean and pea protein (Arntfield *et al.* 1986; Sun & Arntfield 2011b). This could reduce the applicability of these proteins in processes relying on gel formation at high salt concentrations.

2.1.2 Gel microstructure

Protein gels are typically classified by their microstructure as either fine-stranded or particulate (Clark 1998; Nicolai & Durand 2013) (Figure 3). Fine-stranded networks consist of strands with diameter most often less than 10 nm and length ranging from tens of nanometres to tens of microns (Nicolai & Durand 2013). Fine-stranded networks tend to form when the repulsion between proteins is high, *e.g.* at pH far away from IEP or at low

ionic strength (Langton & Hermansson 1992; Nicolai & Durand 2013). Particulate or coarse-stranded gels often consist of spherical particles with diameter ranging from 50 nm to a few microns and tend to form when the repulsion between proteins is low, *e.g.* at pH close to IEP or at high ionic strength. Faba bean and pea proteins have been shown to follow similar trends, *i.e.* they form coarse-stranded gels when the repulsion between proteins is low and fine-stranded gels when the repulsion is high (Munialo *et al.* 2014; Langton *et al.* 2020).

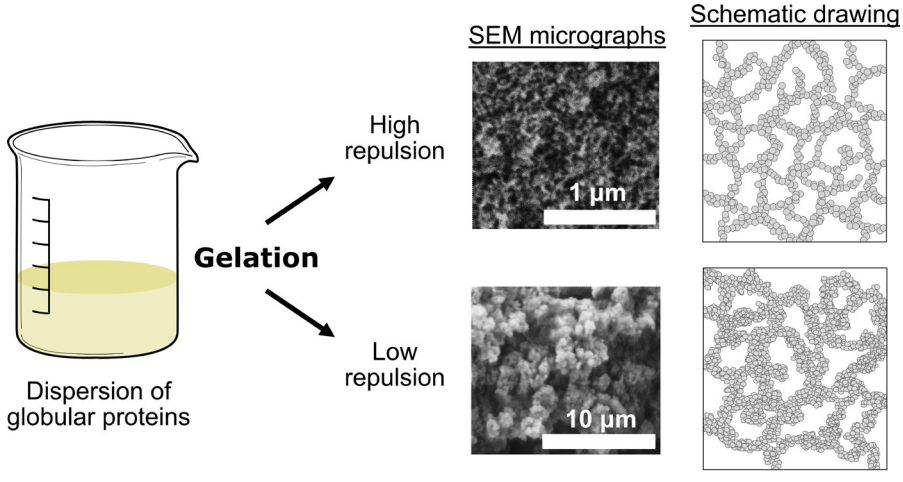


Figure 3. Schematic representation of (upper panel) fine-stranded and (lower panel) coarse-stranded protein gels formed at high and low repulsion, respectively, and scanning electron microscopy (SEM) images of the corresponding gels.

2.1.3 Gel texture

The textural properties of protein gels can be characterised by using small and/or large deformations. Small deformation properties can be measured by oscillatory rheology, which involves subjecting the material to periodic deformations by applying oscillatory stress or strain, typically between two parallel plates, and recording the resulting dynamic mechanical properties. The deformation applied is set to be small enough not to damage the material structure (*i.e.* measurements are performed within the linear viscoelastic region (LVR) of the sample). The rheological properties are typically reported as storage modulus (G') and loss modulus (G") and can be measured on the gels produced or during gel formation. Storage modulus represents the elastic proportion of the sample and the energy that is stored within the

material upon deformation, while loss modulus represents the viscous part and corresponds to the energy dissipated and lost as heat.

Large deformation properties can be measured *e.g.* by compression tests, where the sample is compressed between two surfaces until fracture while recording the force and deformation. Fracture stress, which relates to gel strength, is typically defined as the maximum stress recorded just before fracture occurs. Fracture strain, the corresponding strain value at fracture, describes the brittleness of the material. Young's modulus, defined as the slope of the initial linear region of the stress-strain curve, describes the stiffness of the gel.

Large and small deformation tests can provide information on different properties of a food. Langton *et al.* (1996) showed that for whey protein gels, textural properties measured with small non-destructive deformations are more sensitive to protein strand characteristics, while textural properties measured with large destructive deformations are more sensitive to overall network dimensions such as pore size and particle size (Langton *et al.* 1996). Hence, a combination of the two can provide complementary information on gel properties. Furthermore, large deformation and fracture properties tend to be more affected by inhomogeneities than small deformation properties (Vliet 1995).

2.2 Starch gelatinisation and pasting

Similarly to proteins, starch can form gels upon heating. When starch is heated in the presence of water, water begins to enter the granules as they start to swell and increase in size. Upon further increase in temperature, low-molecular-weight components (mainly amylose) of the starch start to leach out (Ambigaipalan *et al.* 2011). Swelling of the granules and leaching of amylose increase the viscosity of the mixture to a peak viscosity that is reached as the starch granules start to rupture and collapse. Upon cooling, amylose and amylopectin reassociate, a process known as setback, increasing the viscosity and forming a gel (Singh *et al.* 2003). The pasting properties of different starches depend on factors such as the starch granule morphology, amylose and amylopectin contents and structure, heating rate/temperature, solvent type and presence of other components, *e.g.* emulsifiers and proteins (Evans & Haisman 1982; Jane *et al.* 1999; Richardson *et al.* 2004; Nilsson *et al.* 2022).

In general, legume starches are characterised by high pasting temperature, absence of peak viscosity and high setback (Hoover *et al.* 2010). Faba bean starch is reported to have relatively low temperature of gelatinisation (within the range 64-75 °C) compared with other bean starches (Ambigaipalan *et al.* 2011; Li *et al.* 2019; Zhang *et al.* 2019; Nilsson *et al.* 2022). The gelatinisation temperature of lentil starch is within a similar range (65-69 °C) (Joshi *et al.* 2013; Li *et al.* 2019). For faba bean starch, no peak in viscosity is observed if it is heated to 95 °C, while if it is heated to 140 °C, peak viscosity is observed at around 119 °C (Nilsson *et al.* 2022). On the other hand, lentil starch shows peak viscosity already at around 72 °C (Joshi *et al.* 2013). For faba bean starch, amylose leakage has been found to start at a temperature of ca. 75 °C (Ambigaipalan *et al.* 2011).

2.3 Mixed gel systems

Analysis of single-component systems of protein and starch is suitable for obtaining a general understanding of how these components behave and interact during texture formation. However, most food matrices are not single-component systems, but rather a mixture of components that interact and interfere with each other to create a more complex system. For a better understanding of how different components such as protein, starch and fibre interact during texture formation, mixed gel systems can be studied.

2.3.1 Protein-starch gels

Multiple studies have investigated mixed starch-protein gels from different sources, such as lentil starch-lentil protein, corn starch-soy protein and rice starch-soy protein systems (Li *et al.* 2007; Joshi *et al.* 2014; Pang *et al.* 2022). The textural and microstructural properties of mixed starch-protein gels depend on the physicochemical properties, and hence the source, of the starch and protein used (Muhrbeck & Eliasson 1991). The microstructure of mixed starch-protein gels depends on whether the starch or the protein gels first. A complex system with two continuous networks has been shown to form if the starch gels before the protein, while diffusion and aggregation of amylose are hindered and the starch does not form a continuous phase if the protein gels first (Muhrbeck & Eliasson 1991).

During pasting, the viscosity of different starch-protein mixtures tends to decrease and the pasting temperature increases if the proportion of protein is

increased (Joshi *et al.* 2014; Pang *et al.* 2022). The gel strength of protein-starch gels has been found to both decrease (Joshi *et al.* 2014; Bravo-Núñez *et al.* 2019; Min *et al.* 2022) and increase (Ribotta *et al.* 2007; Yu *et al.* 2020; Zhang *et al.* 2021) with increasing protein content, indicating differences depending on starch-protein ratio, source and treatment.

2.3.2 Protein-fibre gels

Dietary fibre plays a significant role in a healthy diet, with consumption of dietary fibre linked to multiple health benefits (Stephen *et al.* 2017). Despite this, average intake of dietary fibre tends to be below the recommended level (Stephen *et al.* 2017). Hence, it is important to devise new ways of including fibre in foods, while determining the effect this might have on texture and sensory properties.

The effect of adding fibre to protein gels will differ widely depending on the properties and structure of the fibres/fibre source used, *e.g.* if present as individual fibre molecules or bound within a larger cell wall fragment. Furthermore, predicting the properties of mixed gel systems remains challenging even if the behaviour of the individual components are well characterized, necessitating the need to investigate each system of interest individually (Chassenieux & Nicolai 2024).

Many studies examining the effects on gels of adding fibre-rich materials including larger particles, such as cell wall fragments, have investigated the effect of okara, a fibre-rich side-stream from tofu production, on soy protein gels (Ullah *et al.* 2019; Arai *et al.* 2021; Arai *et al.* 2022; Lv *et al.* 2022b). The effect of dietary fibre from soybean on soy protein gelation has been shown to depend on the particle size of the added fibre (volume mean particle diameter of 80 or 139 μm) (Lv *et al.* 2022a). Addition of insoluble dietary fibre from sugarcane has been found to improve the gel strength of myofibrillar protein gels, probably as a result of increased moisture stability and water-holding capacity (Zhuang *et al.* 2020).

Other studies have examined protein-fibre systems based on purified fibre sources, *e.g.* multiple studies have investigated the role of pectin in gels from different protein sources such as whey, soy and pea (Beaulieu *et al.* 2001; Munialo *et al.* 2016; Zhang *et al.* 2022). Addition of pectin tends to increase the storage modulus and fracture stress of mixed gels (Munialo *et al.* 2016; Zhang *et al.* 2022).

2.4 Texture, microstructure and sensory relationships in protein gels

Textural properties of a food and the sensory perception when it is consumed are often the result of, or affected by, the structure of that food on macro and micro scale (Figure 4). The gold standard in sensory analysis and studies of food texture is to use a trained sensory panel, but training and maintaining such a panel is both expensive and time-consuming (Joyner 2018). Hence, finding instrument-based methods producing values that relate to the sensory perception of texture is of great interest to the food industry. One of the main obstacles to identify such relations is coincidental correlations without a plausible mechanistic link, so efforts have to be made to eliminate such links between instrument measurements and sensory attributes (Foegeding & Drake 2007).

For protein gels, different gel microstructures typically give rise to different textural properties (van den Berg *et al.* 2007). A coarse gel structure has been correlated to an increase in G' and a decrease in fracture stress (Stading & Hermansson 1990; Stading *et al.* 1993; Renkema 2004; van Vliet 2013; Munialo *et al.* 2015). The relationship between fracture strain and coarseness of the protein network is less clear, and no unequivocal relationship between fracture strain and gel coarseness has been identified (Renkema 2004). Other factors, such as the number and strength of protein-protein bonds and the flexibility and curvature of protein strands, also affect the textural and rheological properties of gels, influencing the correlation between microstructure and texture (Renkema 2004; Nicolai & Chassenieux 2019).

Different gel systems have been investigated for correlations between textural and microstructural properties with sensory perception (Langton *et al.* 1997; Gwartney *et al.* 2004; Foegeding 2007; van den Berg *et al.* 2007). Van den Berg *et al.* (2007) used principal component analysis (PCA) to find correlations between data from sensory analysis and textural properties of protein/polysaccharide gels and found that microstructure played a significant role in perceptions of these gels by influencing serum release and fracture mechanism. They also found that the sensory attribute “firm” correlated well with Young’s modulus, energy for fracture and true fracture stress (van den Berg *et al.* 2007). High fracture stress of whey protein emulsion gels has been shown to be correlated with high scores for sensory attributes such as tough, firm and crumbly (Sala *et al.* 2007). Studies on whey

protein gels with different microstructures have also found correlations between microstructure and textural and sensory properties (Langton *et al.* 1997; Gwartney *et al.* 2004). Hence, knowledge of how to obtain a certain microstructure or textural properties can be crucial in food product development.

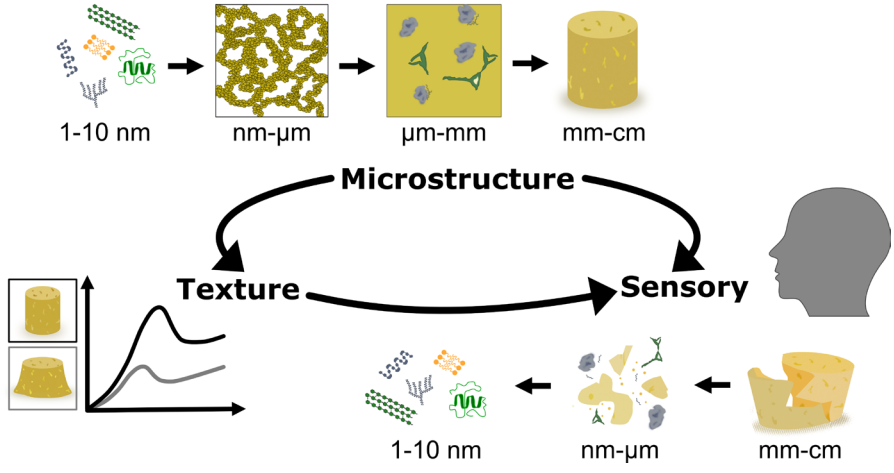


Figure 4. Schematic diagram of the relationship and interplay between microstructure, texture and sensory perception of foods.

3. Aims and objectives

The overall aim of this thesis was to determine the relationship between the microstructure and textural properties of mixed gels based on protein, starch and fibres extracted from legumes. Specific objectives of the work were to:

- Evaluate gel formation and gels produced from commercial and laboratory-extracted plant proteins, testing the hypothesis that presence of stable insoluble protein particles/aggregates strongly influences textural properties (Papers I-V).

- Induce changes in gel microstructure by altering pH or salt concentration and/or by addition of other components (starch/fibre) to correlate with changes in textural properties, testing the hypothesis that changes in protein gel textural properties are related to changes in microstructure (Papers I-V).

- Investigate the effect on gel textural properties of adding starch and/or fibre to protein gels with different types of microstructure (homogeneous and fine-stranded or particulate and/or coarse-stranded), testing the hypothesis that the effect on textural properties of introducing starch/fibre differs depending on gel microstructure of the corresponding protein gels (Papers II-V).

4. Materials and methods

This chapter describes how the different protein, starch and fibre fractions used were extracted and how the gels were formed and analysed. The methods used are described only in brief (see Papers I-V for more detailed descriptions).

4.1 Extraction of protein, starch and fibre

Extraction of protein was based on isoelectric precipitation, either alone (Papers II-IV) or in combination with alterations in solubility caused by addition of salt (NaCl) (Paper I). Starch- and fibre-rich fractions were obtained by centrifugation and filtration of side-streams from protein extraction.

In brief, dehulled and milled faba beans (cv. *Gloria* (Papers I, II, IV) or cv. *Tiffany* (Paper III)) were dispersed in deionised water. Gloria was used due to its high protein content, while Tiffany was used due to its low content of vicine and convicine. The pH was adjusted to 8 (Papers I, III) or 9 (Papers II, IV) and insoluble material was removed by centrifugation. The protein was then precipitated by lowering the pH to 4 (Papers II, IV), 4.8 (Paper III) or 5.1 (Paper I). In Paper I, additional changes in temperature and NaCl concentration were used to extract the major faba bean protein fractions 7S and 11S. The protein pellets obtained were washed and freeze-dried as-is (Papers I, IV), or pH adjusted to 7 before drying (Papers II, III).

The starch and fibre fractions used in Papers II-IV were extracted from the insoluble material obtained from the first centrifugation step during protein extraction. The pellet was redispersed in alkaline solution, washed and filtered through a 70 µm nylon filter to separate the starch (filtrate) from the fibre-rich fraction (filter cake). The starch was oven-dried at 40 °C, while

the fibre fraction was freeze-dried. The fibre fraction extracted from the faba bean hull was obtained using the same procedure as described above, discarding the extracted protein and starch. The starch- and fibre-rich lentil fraction used in Paper V was the pellet obtained after removal of the protein by solubilisation and centrifugation at pH 2.5.

4.2 Compositional analysis

Protein content was determined using the Kjeldahl method and a conversion factor of 5.4. Total starch content was determined using a Total Starch HK (Hexokinase) Assay Kit (Megazyme Ltd, Wicklow, Ireland) based on enzymatic degradation of the starch.

4.3 Preparation of protein dispersions and gels

Gels and dispersions were prepared by mixing the dry flours (protein/starch/fibre) before dispersing in deionised water or NaCl solution. Samples in Papers I-IV were stirred for 30 min using a magnetic stirrer, followed by pH adjustment, final adjustment of the concentration and an additional 30 min of stirring. Samples prepared for Paper V were stirred for 1.5 h before pH adjustment and induction of gelation. Dispersions prepared for Paper IV were degassed by applying a moderate vacuum using a water aspirator connected to a desiccator with the sample placed inside. Samples were then either preheated, to avoid sedimentation of the starch, or loaded directly into glass tubes for heating and gel formation. Gels produced for compression tests or microscopy in Papers II and III were heated to 95 °C and cooled back to room temperature at a controlled rate (1.5 °C/min). Gels in Papers I, IV and V were heated directly in a water bath at 95 °C for 30 min. All samples were stored at 4 °C overnight before compression testing or preparation for microscopy. The composition of the different mixed systems analysed in Papers I-V is shown in Table 1.

Table 1. *Composition of gels produced in Papers I-V. Solids content, expressed as weight percentage (g flour/100g sample, wet basis), and proportions of solids added from the different fractions*

Paper	Source	Solids content (%)	Protein (%)	Starch (%)	Fibre (%)
I	Faba bean (cv. Gloria)	12[a]	100	0	0
II	Faba bean (cv. Gloria)	12	0-100	0-100	0
III	Faba bean (cv. Tiffany)	15	85-100	0	0-15
IV	Faba bean (cv. Gloria)	20	65-100	0-35	0-10
V	Pea	13-16[b]	77-100	0-23[c]	

[a]Dry matter basis, considering moisture content of added raw materials. [b]Increasing with increasing amounts of starch/fibre added. [c]Starch/fibre not separated.

4.4 Rheology

Samples for rheological measurements were prepared as described in section 4.3, but without the final heating step to 95 °C. Rheological measurements in Papers I-IV were performed using a DHR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with 40 mm aluminium plate-plate (Papers II-IV) or 40 mm cross-hatched plate-plate (Paper I) geometry. An ARES-G2 rheometer (TA Instruments, DE, USA) equipped with a concentric cylinder set-up (30 mm diameter cup, 27.7 mm diameter bob) was used in Paper V. Gel formation was monitored over a temperature profile by small amplitude oscillatory shear measurements at a gap of 1 mm, frequency 1 Hz and strain within the linear viscoelastic region. The temperature profile consisted of heating from 25 °C to 95 °C at 1.5 °C/min, 30 min holding time and cooling to 25 °C at 1.5 °C/min. A slightly different temperature ramp was used in Paper V, with 5 °C/min heating and 1 °C/min cooling to 20 °C. Evaporation was limited by using a solvent trap and covering the sample edges with low-viscosity paraffin oil.

In Paper II, the pasting behaviour of the starch-protein mixtures was investigated using the DHR-3 rheometer equipped with a Peltier pressure cell and steel starch paddle. Pasting measurements were performed by heating to either 95 °C or 150 °C, the latter to ensure that peak viscosity was achieved for all samples.

4.5 Texture analysis (compression tests)

Compression tests were performed by compressing cylindrical gel samples, prepared as described in section 4.3, at a rate of 1 mm/s until fracture (Texture analyser, Stable Micro Systems, TA-HDi, Surrey, UK or INSTRON 5542 Universal Testing Instrument, Instron Corporation, MA, USA). True stress and true strain were calculated from the force and displacement values as described previously (Munialo *et al.* 2014). True fracture stress and true fracture strain were defined as the maximum true stress and corresponding true strain at the first clear peak before fracture (hereafter referred to simply as fracture stress and fracture strain). Young's modulus was calculated as the slope of the true stress-true strain curve during the initial linear region.

4.6 Microscopy (LM and SEM)

Gels prepared as described in section 4.3 were cut into small pieces (approximately 2 mm × 2 mm × 2 mm) and fixed overnight in 2.5% glutaraldehyde. In Papers I-IV, 0.1% ruthenium red was used together with glutaraldehyde and a second fixation step was performed in 1% osmium tetraoxide for 2 h. Thereafter, samples were washed and dehydrated in a series of ethanol solutions of increasing concentration. Samples prepared for light microscopy (LM) were embedded in plastic and sectioned using an ultramicrotome before staining and examination (Nikon, Eclipse Ni–U microscope, Tokyo, Japan). Samples prepared for scanning electron microscopy (SEM) were critical point dried, fractured and sputter-coated with gold before examination (Hitachi, FlexSEM 1000II, Tokyo, Japan).

4.7 Water-holding capacity of gels

Gels for measurement of water-holding capacity (WHC) (Paper III) were prepared as described in section 4.3. The gels were cut into cylinders 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. The samples were then centrifuged with increasing speed (50, 500 and 5000 × g). The expelled liquid was removed and the WHC was determined as the weight ratio (W/W_0), where W is the gel weight after centrifugation and W_0 is the initial gel weight before centrifugation.

4.8 Low-field NMR

Samples for low-field nuclear magnetic resonance (LF-NMR) (Paper III) were obtained by preparing dispersions as described in section 4.3, but degassed using a degassing station (TA Instruments, New Castle, DE, USA). Samples were gelled directly in the LF-NMR tubes by heating in a water bath at 95 °C for 30 min, cooled down at room temperature and stored at 4 °C overnight. LF-NMR measurements were performed using a MQR Spectro-P spectrometer (Oxford Instruments, Oxfordshire, UK).

5. Results and discussion

The texture and sensory properties of protein gels, and of foods in general, are greatly influenced by their microstructure. Hence, being able to predict and control gel microstructure enables better control of texture and sensory properties. The microstructure of protein gels can be altered *e.g.* by changing the pH and salt concentration or by incorporation of other ingredients.

Many studies have been performed on milk and soy protein, but fewer studies have focused on gels from other plant protein sources, such as faba bean. In this thesis, gel formation by faba bean protein (whole isolate and specific protein fractions) and pea protein (commercial isolate) at different pH and NaCl concentrations was studied. Mixed systems with added starch- and/or fibre-rich side-streams from protein extraction, to systematically increase the complexity of the gel matrices, were also studied.

The starch and protein content of the protein isolates and extracted starch and fibre fractions used in this thesis were characterised to obtain an overview of their composition. The protein isolates contained 77-86% protein (dry matter basis), with minor amounts of other components. Thus protein content was within the range found in most commercial plant protein isolates. The extracted starch contained approximately 86% starch. The composition of the extracted fibre fractions in Papers III-V differed due to use of different extraction protocols. The starch- and fibre-rich side-stream, obtained during protein extraction from green lentils, used in Paper V contained the highest amount of starch (45%), as the starch was not separated from the fibre. The faba bean fibre fraction used in Paper IV contained around 22% starch, while the two fibre fractions used in Paper III contained 7% and 1% starch, for the fractions extracted from the cotyledon and hull, respectively. Examination of the fibre fractions by LM revealed the presence of larger cell and cell wall fragments in all fibre fractions and higher starch

content in the cotyledon fibre fraction in Paper IV than corresponding fraction in Paper III (Figure 5). More detailed information on the composition of the materials used in the different studies is provided in Papers I-V. Further purification steps could have been added to obtain purer fractions, but this would increase the cost and potentially reduce the relevance and applicability for the industry.

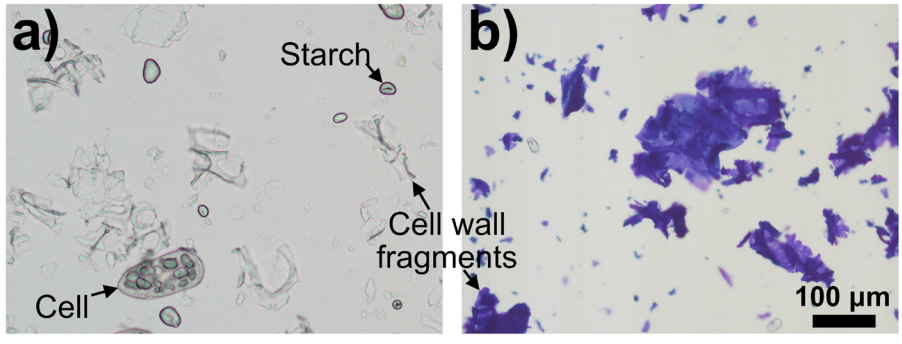


Figure 5. Light microscopy images of the fibre fraction extracted from faba bean showing the presence of cells, cell wall fragments and starch granules. (a) Unstained fibre fraction from Paper IV and (b) cotyledon fibre fraction from Paper III stained with methylene blue.

5.1 Effects of extraction method and solubility on protein gel formation

Protein solubility is a crucial factor in many applications and processes, including protein gelation. However, plant proteins typically show relatively poor solubility. In addition to inherent low solubility, the solubility of some commercially produced protein isolates can be further reduced during extraction and drying. This includes some isolates where particles formed during spray-drying are sufficiently stable to remain intact throughout the gelation process (Paper V). This can limit the ability of the protein to participate in formation of a fine- or coarse-stranded protein network, where proteins or smaller protein aggregates form a network consisting of strands with thickness of less than a few microns. There are significant differences in terms of both microstructure and textural properties between such poorly soluble protein isolates and isolates produced for higher solubility. Some of these differences are described in the following subsections using the example of gel formation by a laboratory-extracted faba bean protein under

high solubility conditions at pH 7 and a commercial pea protein with poor solubility under the used conditions (pH 3-4.2).

5.1.1 Gels from a soluble faba bean protein

Poor solubility and the presence of non-dissolved large protein particles or aggregates can hamper gel formation and impair the textural properties of protein gels. Most plant proteins show high solubility at pH 7 and minimum solubility around their IEP (typically around pH 4-5). In Papers II-IV, protein gels were prepared from a laboratory-extracted faba bean protein isolate at pH 7 and investigated in terms of microstructure and textural properties.

Microstructure

All protein gels prepared at pH 7 in Papers II-IV showed a homogeneous structure in LM analysis, with a fine-stranded protein network observed by SEM (Figure 6). This is in line with previous findings on faba bean protein gels (Langton *et al.* 2020) and on plant protein gels from other sources, such as pea and soy (Munialo *et al.* 2015; Chen *et al.* 2017).

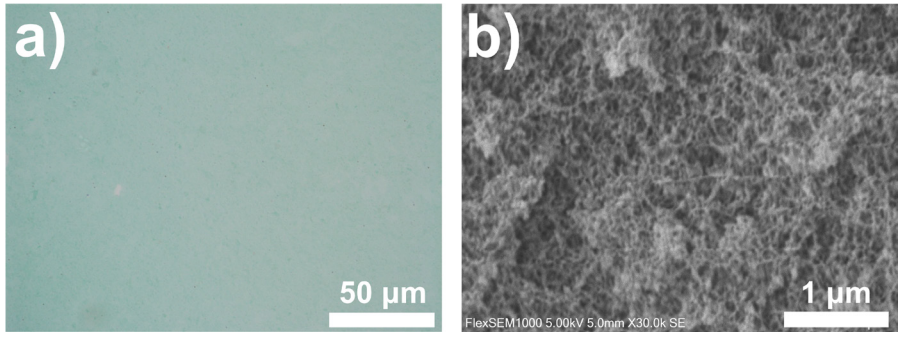


Figure 6. (a) Light microscopy (with protein stained blue/green) and (b) scanning electron images of faba bean protein gel prepared at pH 7.

Gel formation and texture

Gel formation at pH 7 occurred as expected for most plant proteins (Papers II-IV), with an initial increase in G' during heating and a further increase during the cooling phase (Figure 7). An initial increase in G' occurs due to protein denaturation and initial aggregation and network formation. This is followed by further strengthening of the network, and an associated increase in G', during the subsequent cooling phase. The textural properties in terms

of fracture stress, fracture strain and Young's modulus were within the ranges reported previously for faba bean protein gels (Langton *et al.* 2020).

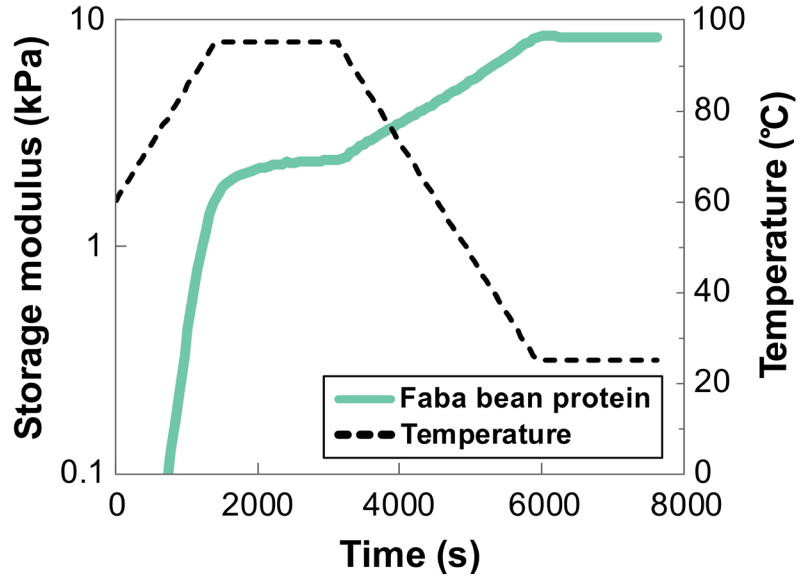


Figure 7. Typical change over time in storage modulus (G') during gel formation by faba bean protein at pH 7.

5.1.2 Gels from a poorly soluble pea protein isolate

In Paper V, pea protein gels were produced at pH 3, 3.6 and 4.2 using a commercial pea protein isolate. These pH levels are relatively close to the IEP of pea protein, which is typically at around pH 4.7 (Stone *et al.* 2015). In contrast to the faba bean protein gels described above, the gel microstructure and texture of the pea protein gels were highly influenced by poor solubility of the protein and presence of large insoluble protein particles (approximately 5-50 μm).

Microstructure

Light microscopy revealed that the protein particles formed during extraction and drying of the pea protein isolate were still present after the gelation process. Rather than a homogeneous microstructure with a coarse- or fine-stranded protein network, the gels mainly consisted of closely packed large swollen protein particles similar to those observed in the protein raw material (Figure 8). Only a small proportion of the protein was present as smaller

aggregates, forming a more typical protein network in between the larger protein particles. This was further confirmed by solubility measurements, where poor solubility (<10%) was observed at all pH values investigated (see Paper V for more details).

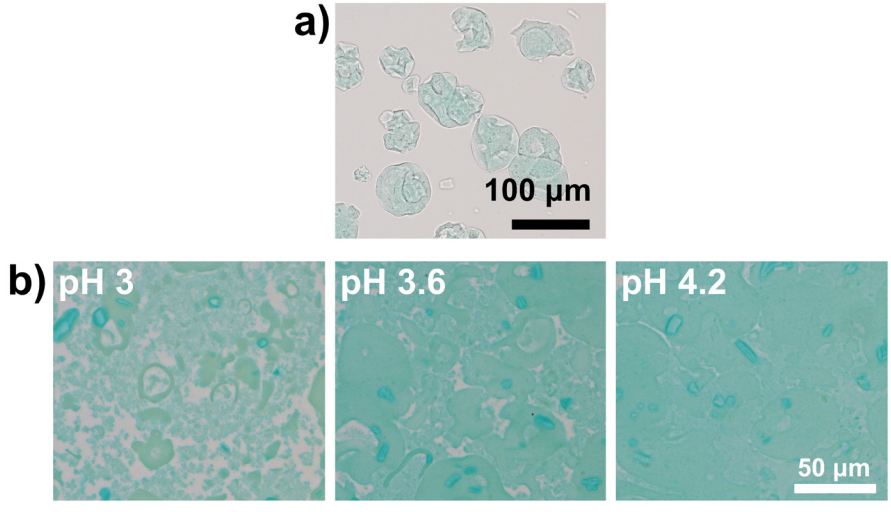


Figure 8. Light microscopy images of a dispersion of (a) a commercial pea protein isolate and (b) pea protein (blue/green) gels prepared at pH 3, 3.6 and 4.2 from the same commercial isolate. Images from Paper V, with permission.

Gel formation and texture

Despite the poor solubility, gel formation as observed by monitoring G' during a temperature ramp occurred as expected for a typical globular plant protein and the final G' was similar to that reported by others for pea protein gels (Munialo *et al.* 2015). However, textural properties in terms of fracture stress and fracture strain were significantly lower than reported in the literature for pea protein gels at similar concentrations and pH (Munialo *et al.* 2015). The lower fracture stress and fracture strain are likely related to the poor solubility of the pea protein and presence of large insoluble protein particles, creating a structure of closely packed insoluble protein particles rather than the coarse/fine-stranded network typically seen for protein gels.

In summary, the results highlighted the importance of extraction and drying method and solubility for protein functionality and gel formation. At pH 7, laboratory-extracted faba bean protein formed homogeneous, fine-stranded gels and the gelation process occurred in a similar way to that of

most globular plant proteins. In contrast, gels produced using a commercial pea protein isolate were weak and brittle, due to the poor solubility and a microstructure consisting of swollen protein particles.

5.2 Microstructure tuning using salt and pH

The microstructure of heat-induced gels from globular proteins is strongly influenced by pH and ionic strength. Changes in these parameters influence the charge of proteins, altering protein-protein interactions and aggregation behaviour during gel formation. Globular proteins are known to form fine-stranded protein gel networks at low ionic strength and pH far from IEP, *i.e.* when repulsion between proteins is high (Langton & Hermansson 1992; Nicolai & Durand 2013). In contrast, they form coarse-stranded networks at high ionic strength and pH close to IEP, where the repulsion between proteins is lower (Langton & Hermansson 1992; Nicolai & Durand 2013).

The effect of NaCl on gel formation by the two major faba bean protein fractions, 7S and 11S, is described below, followed by a description of the effect of pH on gel formation by faba bean protein with regard to texture and microstructure. Gel properties at the different pH levels were also investigated in terms of water-holding capacity and water mobility, properties which were then linked back to gel microstructure.

5.2.1 Effect of NaCl

In order to evaluate the protein fraction in faba bean that dominates gel formation properties, the two major protein fractions (7S and 11S) were analysed at different NaCl concentrations (Paper I). Gels were produced by heating protein dispersions of these fractions prepared in deionised water or 0.1M or 0.3M NaCl and analysed in terms of texture and microstructure.

Microstructure

A homogeneous microstructure with a fine-stranded protein network was observed for gels from both 7S and 11S when prepared in deionised water. As the NaCl concentration increased, microstructural changes were observed only for the 7S gels (Figure 10), with both LM and SEM micrographs showing a coarser structure as the NaCl concentration was increased. A clear difference was also visible to the naked eye, where the 7S gels at high NaCl were white and more opaque, while beige, transparent gels formed when no

additional NaCl was added (Figure 9). These differences are likely related to differences in the size of protein aggregates and protein gel network characteristics, resulting in different scattering of light. They were in line with the change towards a coarser network structure observed by SEM (Figure 10) and were in agreement with typical changes as the pH approaches IEP or electrostatic interactions decrease (Langton & Hermansson 1992; Nicolai & Durand 2013).

The lack of microstructural changes for the 11S gels upon increasing NaCl concentration was somewhat surprising (Figure 10), as coarser structures is generally observed for globular protein gels upon increased ionic strength (Puppo & Añón 1998; Nicolai & Durand 2013). It is possible that a change towards a coarser structure would occur at NaCl concentrations higher than those investigated in this thesis.

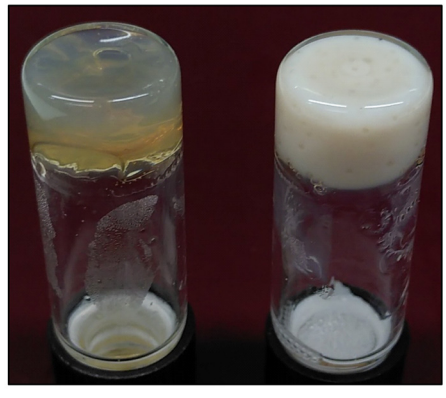


Figure 9. Gels formed from the 7S protein fraction from faba bean prepared in (left) deionised water and (right) 0.1M NaCl. Image from Paper I, with permission.

Gel formation and texture

The effect of NaCl on gel formation by the two major protein fractions (7S and 11S) was monitored during a temperature ramp. Gel formation differed between the protein fractions (Figure 10). With no additional NaCl added, 7S gelled later and formed weaker gels (lower G') than 11S. As additional NaCl was added and the NaCl concentration increased, the final G' of 7S gels increased and the gelation temperature decreased. In contrast, the 11S gels showed a decrease in final G' and an increase in gelation temperature as the NaCl concentration increased.

The hampered gel formation by the 11S fraction at 0.3M NaCl might be related to an increase in denaturation temperature to above 95 °C as the NaCl concentration increased. Addition of NaCl is known to alter the denaturation point of proteins and has previously been shown to increase the denaturation temperature of faba bean proteins (Zheng *et al.* 1993; Kimura *et al.* 2008).

The observed increase in G' for 7S gels at increasing NaCl concentration is in line with the shift towards coarser network structure, which has been shown to be correlated with increased G' values (Stading & Hermansson 1990; Stading *et al.* 1993; Renkema 2004; van Vliet 2013; Munialo *et al.* 2015). The differences in rheological properties observed for the 11S gels, despite the lack of differences in microstructure, could be due to changes in protein-protein interactions and protein strand characteristics as the NaCl concentration changed.

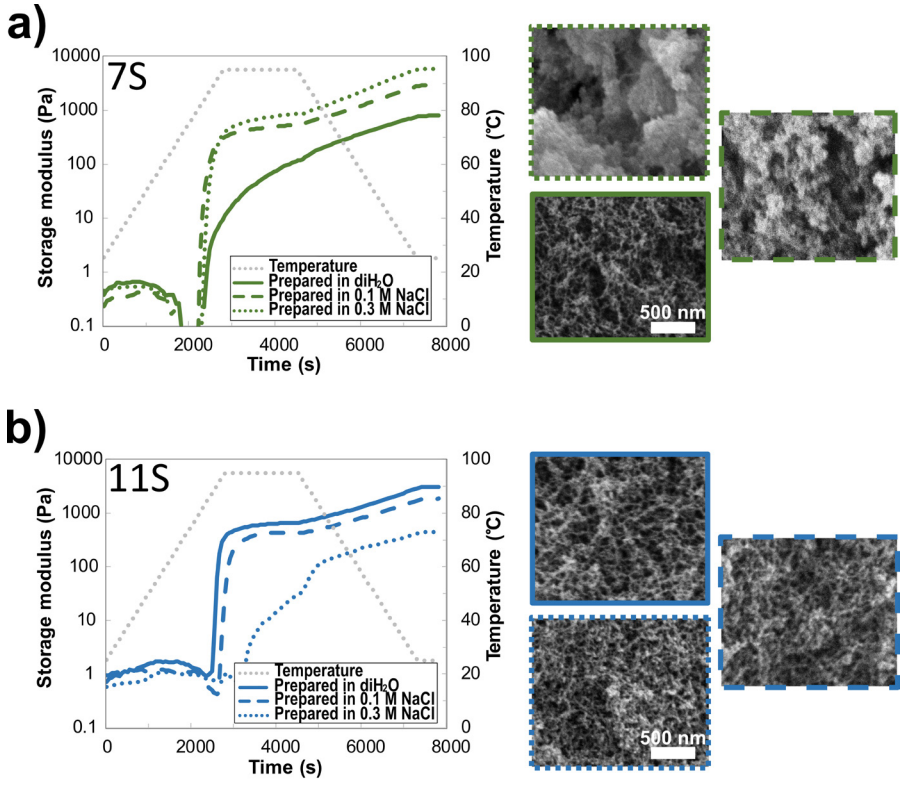


Figure 10. Gel formation and microstructure of gels prepared from (a) the 7S protein fraction and (b) the 11S protein fraction from faba bean at different NaCl concentrations. Images adapted from Paper I, with permission.

5.2.2 Effect of pH

Similarly to changes in NaCl, adjustments in pH are known to affect gel properties. In Paper III, gel formation, texture and microstructure of faba bean protein gels formed at pH 4, 5 and 7 were analysed. The pH level was found to significantly affect both the microstructure and textural properties of the gels.

Microstructure

Both LM and SEM micrographs showed clear differences in the microstructure of faba bean protein gels formed at pH 4 and 5 compared with pH 7 (Figure 11). At the two lower pH-values, LM revealed a coarse, particulate structure where protein particles/aggregates were clearly visible. In contrast, a more dense, homogeneous protein network was formed at pH 7. The larger protein particles (approximately 5-50 μm) observed at pH 4 and 5 were not observed in gels produced at pH 7.

Further investigation of the protein dispersions at pH 4 and 5 before heat treatment and gel formation revealed the presence of similarly sized particles (Figure 11). Consequently, some of the larger particles observed in the gels at the two lower pH levels are likely to have formed either during extraction and drying of the protein or during preparation of the dispersions, and remained intact throughout the gelation process. Observations of microstructure at different stages during preparation of dispersions revealed that some particles never dissolved fully during the dispersion step, while others formed during the pH adjustment step (as the pH was adjusted through IEP).

The SEM micrographs revealed similar differences in the protein network formed at the different pH values. A fine-stranded network was observed at pH 7, while a coarser network structure with larger aggregates was observed at pH 4 and 5. The changes in microstructure identified by both LM and SEM as the pH approached IEP have been observed previously by others and can be expected for globular plant proteins (Langton *et al.* 2020).

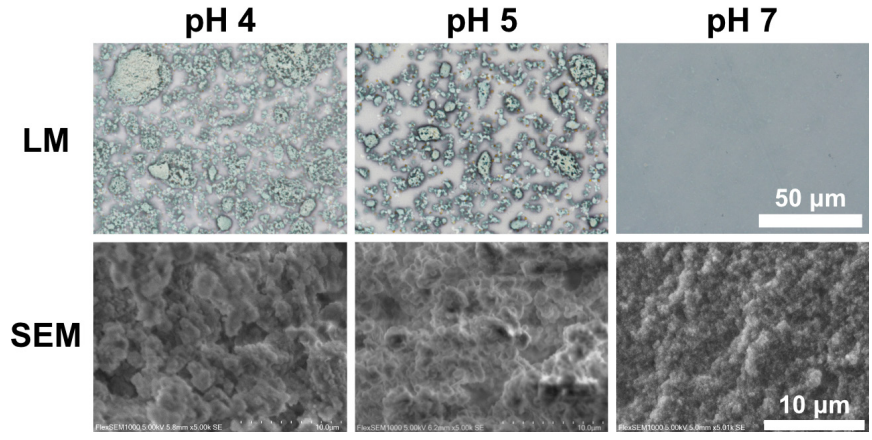


Figure 11. (Upper panels) Light microscopy (LM) images with protein stained blue and (lower panels) scanning electron microscopy (SEM) images of faba bean protein gels prepared at pH 4, 5 and 7.

Gel texture

Compression tests showed a decrease in fracture stress and fracture strain as the pH was reduced from pH 7 (Figure 12). On the other hand, Young's modulus increased as IEP was approached and was highest at pH 5 and lowest at pH 7. A similar decrease in fracture stress and fracture strain, with a simultaneous increase in Young's modulus, has been reported for gels from alkaline-extracted faba bean protein prepared at pH 5 compared with pH 7 (Langton *et al.* 2020). The higher Young's modulus and lower fracture stress of gels at pH 4 and 5 compared with pH 7 are likely related to increasing coarseness of the protein gel network, as a coarse protein network has previously been correlated with an increase in G' and a decrease in fracture stress (Stading & Hermansson 1990; Stading *et al.* 1993; Renkema 2004; van Vliet 2013; Munialo *et al.* 2015).

In addition to the coarser protein network, the reduction in fracture strain was probably related to the larger protein particles observed at pH 4 and 5, but not at pH 7. These protein particles create a more inhomogeneous microstructure which is typically related to a decrease in both fracture stress and fracture strain (Munialo *et al.* 2014; Dille *et al.* 2015).

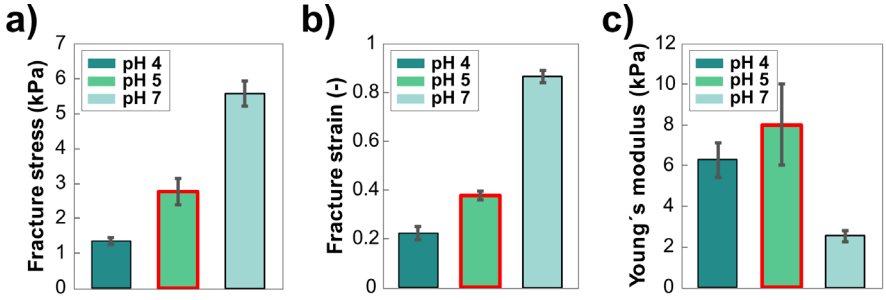


Figure 12. (a) Fracture stress, (b) fracture strain and (c) Young's modulus of faba bean protein gels prepared at pH 4, 5 and 7. Samples highlighted in red showed partial sedimentation or syneresis and a thin water layer formed on top of the gels upon gelation. Error bars represent ±1 standard deviation.

Despite increasing attention in recent years, there are still relatively few studies on heat-induced gelation of faba bean protein compared with many other protein sources (Zheng *et al.* 1993; Makri *et al.* 2006; Langton *et al.* 2020; Vogelsang-O'Dwyer *et al.* 2020; Nivala *et al.* 2021). The results in this thesis and in previous research indicate that the overall behaviour and characteristics in terms of microstructure and textural properties of faba bean protein gels are similar to those of most other globular plant proteins, such as soy and pea protein.

Water-holding capacity and water mobility

The faba bean protein gels prepared at pH 4, 5 and 7 were further investigated in terms of water-holding capacity (WHC). Large differences were observed between the gels formed at the different pH values (Figure 13). At pH 7, most water was retained within the gel (WHC > 95%), even at the highest centrifugal speed applied (5000 × g). In comparison, water retention in gels formed at pH 4 and 5 was much lower, with WHC below 90% already at 50 × g and below 50% at 5000 × g. These large differences are likely related to differences in microstructure, as increased coarseness of microstructure in whey protein gels is reported to be correlated with reduced WHC (Urbonaite *et al.* 2016). Similarly, chemically cross-linked soy protein gels formed from aggregates have lower WHC as aggregate size increases (Wu *et al.* 2019). Besides increased coarseness of the protein network structure, the large protein particles (approximately 5-50 µm) present in the gels formed at pH 4 and 5 are likely to have further limited the ability of these gels to hold water compared with the gels formed at pH 7.

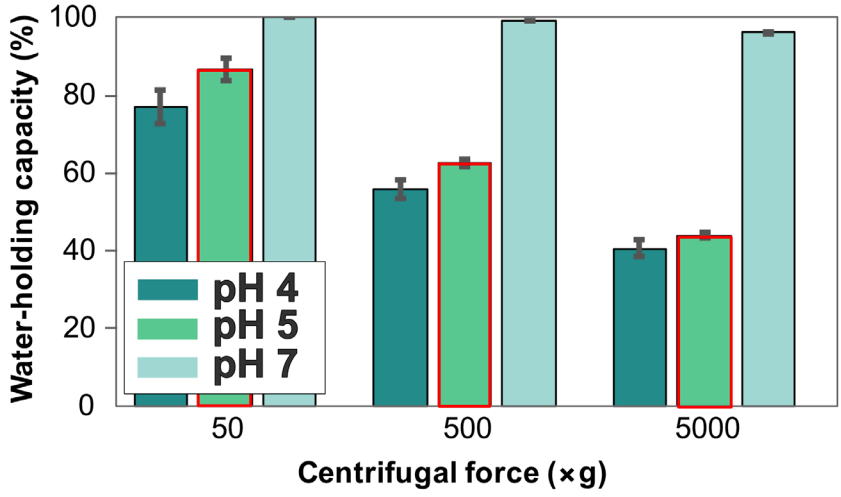


Figure 13. Water-holding capacity (WHC) of faba bean protein gels prepared at pH 4, pH 5 and pH 7 and increased centrifugal force. Samples highlighted in red showed partial sedimentation or syneresis and a thin water layer formed on top of the gels upon gelation. Error bars represent ±1 standard deviation.

In additional analyses, LF-NMR was used to investigate the water bound in the gel structure at different pH levels. LF-NMR is a non-destructive technique that can give information about the mobility of water molecules within a food matrix. Based on transversal relaxation times (T_2) of the water protons, different water populations can be identified and their relative proportions can be determined (Ullah *et al.* 2019; Laursen *et al.* 2023).

For gel samples at pH 4 and 5, three different water populations were identified based on their T_2 proton relaxation times (Table 2). The first population, corresponding to the most strongly bound water, was observed in the range 8-12 ms. This water is typically considered to be strongly bound to the protein polymer chain (Ullah *et al.* 2019; Zheng *et al.* 2021). The second population was the largest and occurred at 40-50 ms. This population is often assumed to relate to immobilised water entrapped within the protein network structure and smaller pores within the structure (Ullah *et al.* 2019; Zheng *et al.* 2021). The third population, corresponding to the most mobile water, was observed around 300-400 ms. Relaxation times between 100-1000 ms are typically interpreted as bulk or serum water (Gianferri *et al.* 2007; Gilbert *et al.* 2020). At pH 7, only the first two water populations were identified and the third population (T_2 > 100ms) seemed to be lacking. This

could be explained by the denser protein network observed at pH 7 and the lack of larger cavities or pores as observed in the more particulate structure at pH 4 and 5 (see Figure 11). A similar observation has been made for egg protein gels, where the water population with relaxation time between 100-1000 ms declines significantly as the pH increases from pH 5 to pH 9 (Li *et al.* 2018). As in the case of the faba bean gels examined in this thesis, these changes coincided with a simultaneous increase in protein solubility and potential change in gel microstructure (Li *et al.* 2018).

The proportions of the different water populations ($M_{2,n}$) were estimated by discrete exponential fitting. The second population ($M_{2,2}$), with a relaxation time of around 40-50 ms, was the largest for all samples, corresponding to 85-97% of total water. The largest amount of water in the second population ($M_{2,2}$) was observed at pH 7, while a lower proportion was observed at pH 4 and 5. The higher $M_{2,2}$ at pH 7 could be related to the lack of a third peak, but also to a decrease in the population size of the most strongly bound water ($M_{2,1}$).

Table 2. *Relaxation time and population size of faba bean protein gels prepared at pH 4, 5 and 7. Mean values ±1 standard deviation.*

	$T_{2,1}$ (ms)	$M_{2,1}$ (%)	$T_{2,2}$ (ms)	$M_{2,2}$ (%)	$T_{2,3}$ (%)	$M_{2,3}$ (%)
pH 4	11.2 ± 0.5	7.7 ± 0.3	41.1 ± 1.2	85.6 ± 0.7	311.8 ± 19.7	6.6 ± 0.8
pH 5	8.7 ± 0.5	7.3 ± 0.3	47.4 ± 1.4	87.3 ± 1.3	398.3 ± 18.2	5.4 ± 1.5
pH 7	11.4 ± 3.9	3.0 ± 1.3	47.0 ± 1.4	97.0 ± 1.3	-	-

Overall, differences in T_2 relaxation times between gels prepared at the different pH levels were relatively small. This indicates that the water in the different gels was present within populations of similar mobility, except for the highly mobile water populations ($T_{2,3}$), which was absent for gels prepared at pH 7. Similar relaxation times, with the major peak located in the range 10-100 ms and corresponding to 80-95% of total water, have been reported for many other gel systems, such as tofu, egg protein gels and soy protein-corn starch gels (Liu *et al.* 2013; Zheng *et al.* 2021; He *et al.* 2024).

In summary, increasing NaCl concentration resulted in differences in gel formation by the 7S and 11S protein fractions from faba bean, where an increase in G' was observed for 7S, while a decrease was observed for 11S. Simultaneously, microstructural changes were observed only for the 7S gels.

Homogeneous gels with a fine-stranded protein network were formed at pH 7, whereas a more coarse-stranded protein network was formed at pH 4 and 5 and the gels contained larger protein aggregates (approximately 5-50 μm) that formed during extraction and pH-adjustment. Gels prepared at pH 7 had higher fracture stress and fracture strain, but lower Young's modulus than gels prepared at pH 4 and 5. Gels at pH 7 also showed a higher capacity for retaining water upon exposure to external centrifugal forces. LF-NMR revealed differences in water populations with different mobility, where the water population typically corresponding to bulk or serum water was present only at the two lower pH-levels. The differences in textural properties, WHC and water mobility upon changes in pH were suggested to relate to dissimilarities in microstructure.

5.3 Starch and fibre as inhomogeneities in mixed gel systems with different microstructures

The microstructure of protein gels can also be modified by introducing other macromolecules, such as starch and fibre, which are commonly found in many foods. Hence, determining the influence of these components on protein gel formation is important for a better understanding of foods with a more complex food matrix. The effects of introducing starch- and/or fibre-rich fractions to protein gels are discussed below. The starch and fibre fractions contained relatively large particles in the form of starch granules and cell wall fragments (approximately 5-100 μm). Section 5.3.1 describes the effect of starch and fibre when added to faba bean and pea protein gels with different microstructures, while section 5.3.2 describes the effects for mixed protein-starch gels where starch is the major component.

5.3.1 Effects of adding starch/fibre to gels with fine-stranded or particulate microstructure

In Papers II-IV, the effect on faba bean protein gel formation of adding starch- and fibre-rich side-streams from the protein extraction step was investigated. The protein isolate used had high solubility at pH 7 and low solubility at pH 4 and 5, and was extracted by isoelectric precipitation followed by freeze-drying.

The effect of adding a starch- and fibre-rich lentil fraction to gels produced using a commercial pea protein isolate was also studied (Paper V),

where the effects on fracture stress and Young's modulus were similar to those observed at pH 4 and 5 for the faba bean gels. However, it should be noted that the starch/fibre fraction was added in addition to the protein in Paper V, rather than replacing part of the protein as in Papers II-IV, *i.e.* increasing the solids content of the gels.

Microstructure

In gels with added fibre fraction (Papers III-IV), cells and cell wall fragments (approximately 5-100 μm) were observed by LM to be distributed throughout the gel structure (Figure 14). Gels at pH 7 showed a dense, homogeneous protein network surrounding the starch and fibre. Similar microstructures were observed at both 15% (Paper III) and 20% solids content (Paper IV). For gels prepared at pH 4 and 5, the gel microstructure contained larger protein aggregates (as discussed in section 5.2.2). No clear effect on protein network structure was observed by SEM after addition of fibre at any of the pH values investigated (Figure 14).

For the protein-starch gels prepared at pH 7 (Paper IV), with minimum 65% of the solids added as protein, all gels had a well-defined continuous protein phase with starch granules distributed throughout the gel structure (Figure 14). Leaked-out amylose was observed to form aggregates within the protein network. Smaller regions with amylose networks and aggregates were also observed on the surfaces of starch granules. Again, no clear changes in the continuous protein network were observed by SEM upon inclusion of starch in the mixed gel systems (Figure 14).

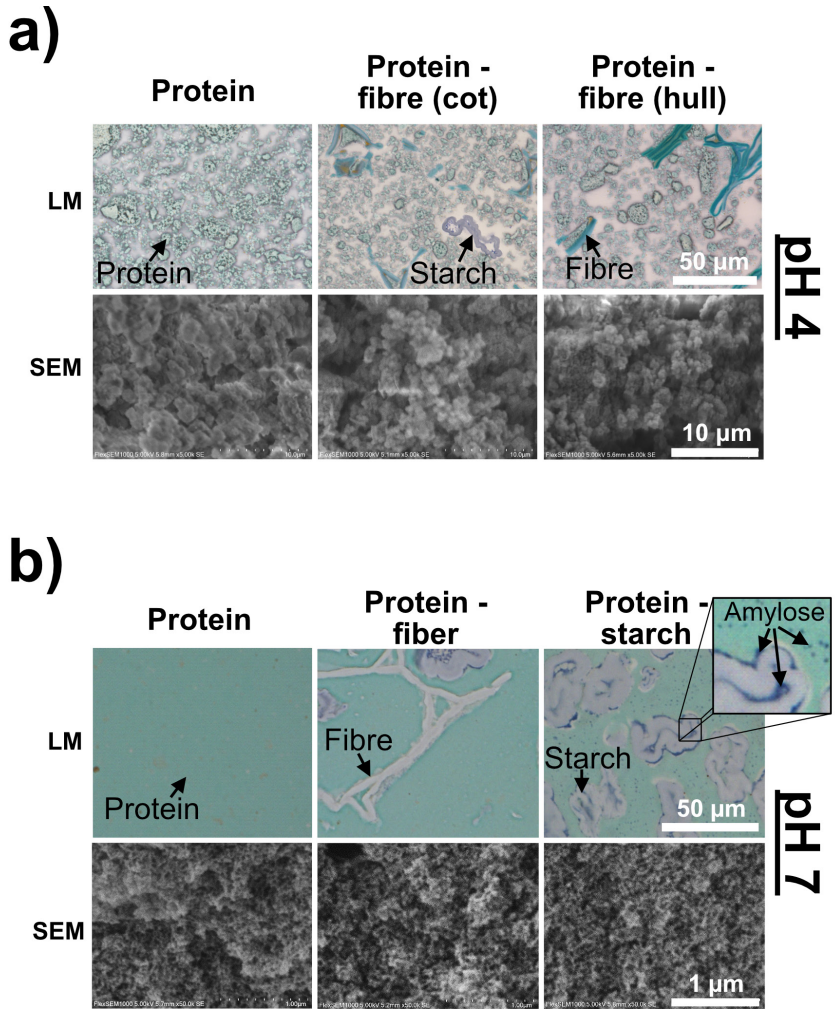


Figure 14. Light microscopy and scanning electron microscopy images of mixed gels from faba bean protein with (a) added fibre prepared at pH 4 with 15% solids content and (b) added fibre or starch prepared at pH 7 with 20% solids content. Images from Paper IV, with permission.

In contrast, the gels formed using the commercial pea protein isolate consisted mainly of swollen protein particles (see section 5.1.2). The microstructure of the pea protein gels was somewhat similar to that of the faba bean gels produced at pH 4 and 5, but with larger protein particles. The added starch- and fibre-rich fraction did not influence the microstructure and was evident mainly as swollen and deformed starch granules in between the protein particles (Figure 15).

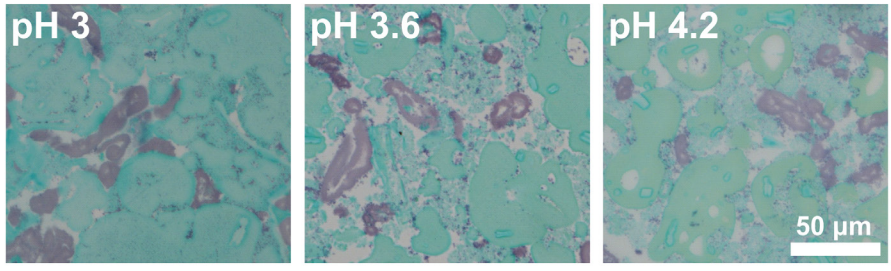


Figure 15. Light microscopy images of mixed gels prepared at pH 3, 3.6 and 4.2 formed with commercial pea protein isolate and a starch/fibre-rich side-stream from lentil protein extraction. Protein stained blue/green, starch stained purple. Images from Paper V, with permission.

Gel formation and texture

In Paper IV, gels were prepared at pH 7 and partial substitution of protein with starch or fibre resulted in an increase in final G' and a decrease in fracture stress and fracture strain (Figure 16). The increase in G' potentially related to water absorption by the starch/fibre, increasing the effective protein concentration in the surroundings. On the other hand, large deformation properties tend to be more affected by inhomogeneities (Vliet 1995). Hence, the reduction in fracture stress and fracture strain might relate to inhomogeneities in the protein matrix introduced by the fibre, starch granules and leaked amylose overriding any potential increase arising due to an increase in effective protein concentration in the surrounding protein matrix.

A similar trend with increasing G' and decreasing fracture stress was observed for gels prepared at pH 7 in Paper III using fibre extracted from both the cotyledon and hull of faba bean (Figure 17). These changes were again attributed to the introduction of inhomogeneities and water absorption by the fibre.

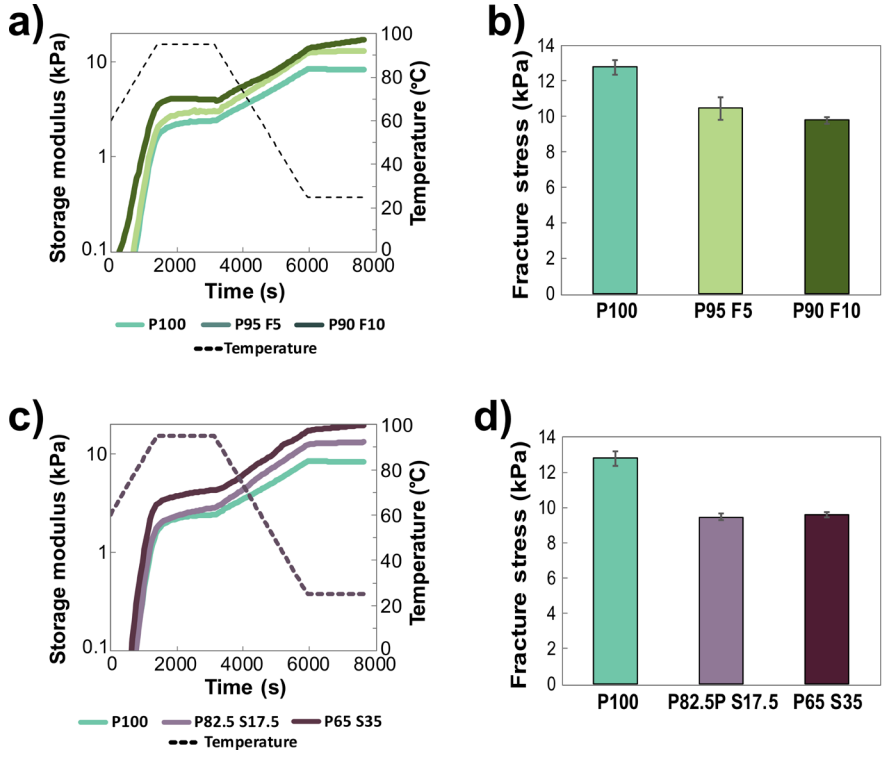


Figure 16. Change in storage modulus (G') over time during gel formation and fracture stress of (a, b) mixed protein-fibre gels and (c, d) protein-starch gels prepared at pH 7. Sample labels indicate percentage of solids added as protein (P) and as starch (S) or fibre (F) (P% S/F%). Error bars represent ±1 standard deviation.

In Paper III, the effect of fibre extracted from cotyledon and hull was investigated at pH 4 and 5, in addition to pH 7 (Figure 17). In compression tests, the effect of including fibre in the gel structure seemed to differ depending on pH, as a different trend was observed at pH 4 and 5 compared with pH 7. Instead of a decrease in fracture stress and fracture strain as observed at pH 7, an increase was observed upon addition of fibre at the two lower pH levels. This difference could be explained by differences in microstructure. Assuming that the reduction in fracture stress at pH 7 was due to the introduction of inhomogeneities by adding fibre, this effect would be smaller at pH 4 and 5 as protein particles of similar size were already present in the gel matrix. The increase in fracture stress and fracture strain that occurred instead was possibly due to water absorption by the fibre.

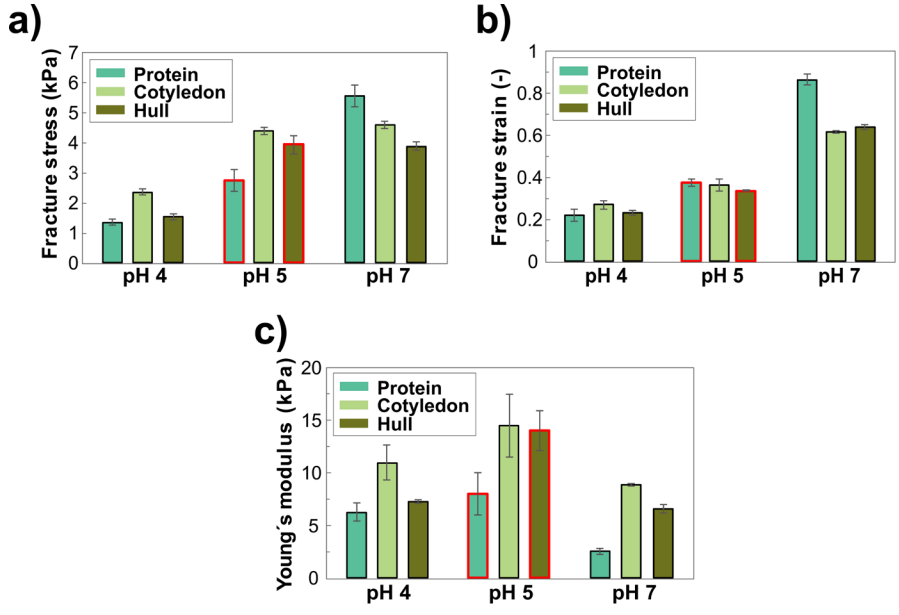


Figure 17. (a) Fracture stress, (b) fracture strain and (c) Young's modulus of protein-fibre mixed gels prepared at pH 4, 5 and 7. Samples highlighted in red showed partial sedimentation or syneresis and a thin water layer formed on top of the gels upon gelation. Error bars represent ±1 standard deviation.

In Paper V, a starch- and fibre-rich lentil fraction was added to gels formed from a commercial pea protein isolate. As seen for the faba bean gels, addition of starch and fibre resulted in an increase in G', likely because of gelatinisation of starch present in the added fraction, combined with the overall higher solids content of the system. It also resulted in an increase in both fracture stress and fracture strain similar to that observed for the faba bean gels at pH 4 and 5. The increase could be attributable to the overall increase in solids content for the pea protein gels. It could also be attributable to water absorption by the starch and the fact that the pea protein gels already contained particles in the same size range as the added starch and fibre (as discussed above for the faba bean gels produced at pH 4 and 5).

Water-holding capacity and water mobility

Paper III investigated the effect of the fibre fraction on gel WHC and water mobility (Figure 18-19). As discussed in section 5.2.2, pH strongly influenced the WHC of the protein gels. A similar effect of pH was observed for the mixed protein-fibre gels. However, addition of fibre had little or no

effect on WHC of the gels. The limited effect of adding fibre was somewhat surprising as a similarly extracted faba bean fibre fraction has previously been observed to increase the water binding capacity and significantly improve the structure stability of faba bean protein, starch and fibre mixtures used for food 3D-printing (Johansson *et al.* 2022).

In general, the effect of fibre on the WHC of protein gels tend to depend not only on the type of fibre, but also on the amount of fibre added. Previous studies on mixed protein-fibre systems have reported an increase in WHC as insoluble fibre is added, but only up to a certain concentration, after which the WHC stabilises or starts to decrease (Choi *et al.* 2011; Zhuang *et al.* 2016; Ullah *et al.* 2019). However, in most of these previous studies, the increase in WHC after addition of fibre was relatively small, increasing only a few percentage points.

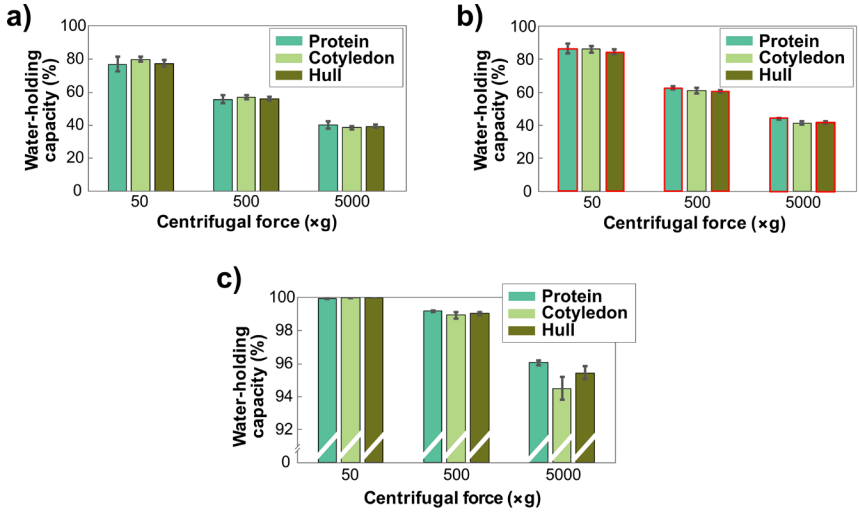


Figure 18. Water-holding capacity at increasing centrifugal force of faba bean protein gels with and without fibre (cotyledon/hull) prepared at (a) pH 4, (b) pH 5 and (c) pH 7. Samples highlighted in red showed partial sedimentation or syneresis and a thin water layer formed on top of the gels upon gelation. Error bars represent ± 1 standard deviation.

To further investigate potential correlations between textural changes and water absorption by the fibre, LF-NMR measurements were performed in Paper III to study water mobility in the protein/fibre gels (Figure 19). In general, the transverse relaxation time of the water protons seemed to increase upon addition of fibre (except for $T_{2,2}$ and $T_{2,3}$ at pH 5), indicating more mobile water. This may have been due to the fibre fraction interfering

with the protein matrix. However, an increase in the population size of the least mobile water ($M_{2,1}$) was also observed upon addition of fibre. The increase in the proportion of this water population could be related to the high WHC of the fibre fractions. A similar increase in the population related to $T_{2,1}$ has been observed previously for mixed soy protein-corn starch gels upon increasing the starch concentration (He *et al.* 2024). The increase was attributed to the ability of starch molecules to interact with and reduce the mobility of large amounts of water.

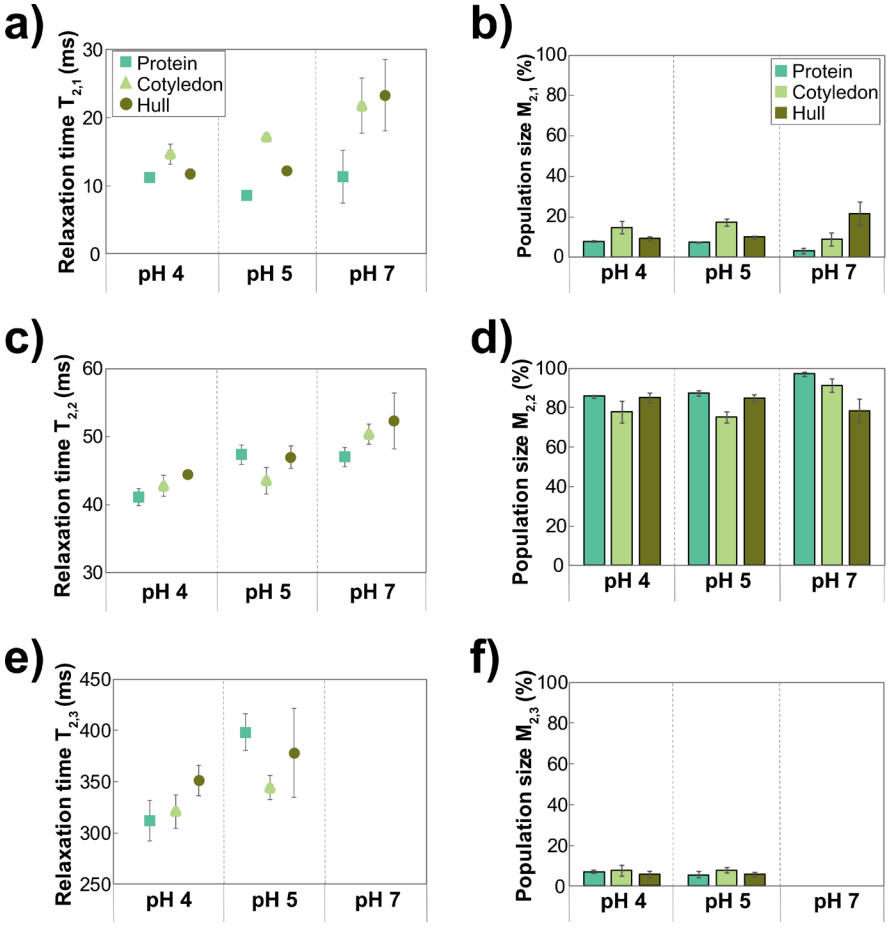


Figure 19. (a, c, e) Relaxation times ($T_{2,n}$) and (b, d, f) corresponding population sizes ($M_{2,n}$) identified in LF-NMR analyses of mixed protein-fibre gels prepared at pH 4, 5 and 7. Error bars represent ±1 standard deviation.

In summary, the addition of starch and/or fibre to faba bean protein gels formed at pH 7 created microstructural inhomogeneities. However, the protein network itself was not clearly affected, as observed by SEM. Differences in the effect of adding fibre were observed depending on the pH, and hence the protein gel structure. Addition of fibre to homogeneous protein gels formed at pH 7 decreased fracture stress and fracture strain, while Young's modulus and G' increased. On the other hand, when fibre was added to particulate gels formed at pH 4 or 5, fracture stress increased rather than decreased and fracture strain did not change significantly. A similar effect was observed when a starch- and fibre-rich lentil fraction was added to gels produced from a commercial pea protein isolate with a microstructure consisting mainly of swollen protein particles. The differences in effect on textural properties of addition of starch and/or fibre to the different gel systems were attributed to differences in microstructure. There was no clear effect of adding fibre on WHC of the gels at any of the pH values investigated.

5.3.2 Starch-rich mixed gels

Gels where starch, rather than protein, was the main component were studied in Paper II.

Microstructure

In the starch-rich gels studied in Paper II, starch was the continuous phase for gels with a starch content of 70% or more (of total solids added) (Figure 20). At 60% starch, the continuous network was more difficult to define, and either a potential bicontinuous network was formed or protein seemed to form the continuous phase. As the proportion of protein increased from around 10%, the amylose aggregated within the protein network, rather than forming a network on its own. Subsequent SEM analysis revealed a more porous structure with thicker strands for the starch network compared with the protein network (Figure 20).

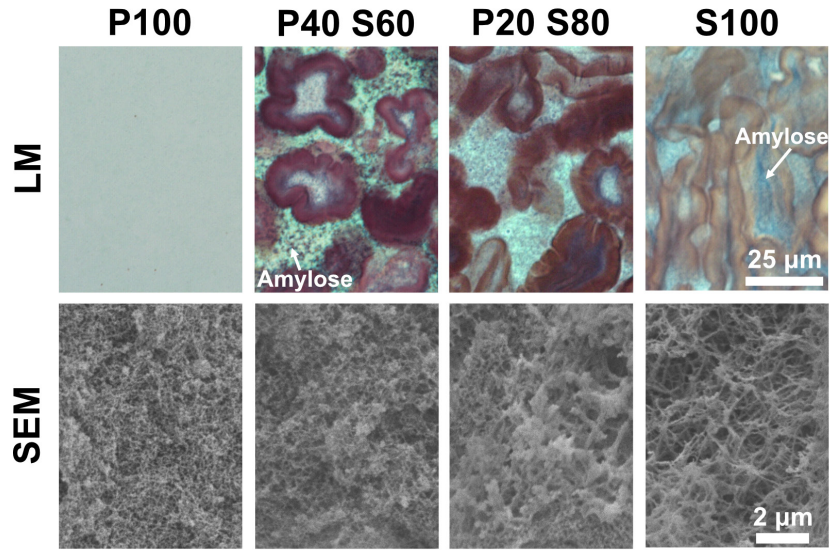


Figure 20. (Upper panels) Light microscopy (LM) and (lower panels) scanning electron microscopy (SEM) images of mixed faba bean starch-protein gels prepared at pH 7. Sample labels indicate percentage of solids added as protein (P%) or starch (S%). Protein stained blue/green, starch stained purple. Images from Paper II, with permission.

Pasting, gel formation and texture

The pasting properties of the starch-protein mixtures were investigated during heating to both 95 °C and 150 °C. In both cases, the final viscosity increased with increasing proportion of starch. The higher temperature was used to ensure peak viscosity was reached (Nilsson *et al.* 2022), and was recorded at 121 °C for the pure starch sample. The high temperature needed to reach peak viscosity could be related to the high amylose content in faba bean starch helping to maintain granular integrity (Hoover *et al.* 2010; Nilsson *et al.* 2022). The temperature required to reach peak viscosity increased with increasing protein content, with the highest temperature (145 °C) observed for the sample containing 60% starch and 40% protein (of total solids added).

Final G' of the starch-protein gels also increased with increasing starch content, and the initial increase in G' during heating seemed to occur earlier (Figure 21). At a starch content of 80% or more (of total solids added), a peak in G' was observed during the initial heating of the sample and the change in G' during cooling was similar for all samples. At lower starch content, the overall change in G' was more similar to that of the pure protein

system. The shift in behaviour during gel formation could have been caused by a shift in the component (starch/protein) dominating the continuous gel network.

Amplitude sweeps were performed on the gels obtained after the temperature ramp in Paper II. Except for the pure protein system, the linear viscoelastic region (LVR) extended to encompass greater strains as the starch content increased. The systems containing a higher proportion of starch also showed a more clear and abrupt drop in G' as the breakdown in structure occurred. This could be related to changes in composition and structure, *e.g.* with a more inhomogeneous system or a system with a wider particle size distribution typically resulting in a drop in G' occurring over a larger range of strains. The wider LVR observed for the pure protein gels could be because the pure one-component system had fewer inhomogeneities, which typically lead to a reduction in fracture stress. It could also be that the definition of LVR applied did not capture potential nonlinearities occurring at lower strains for the pure protein system.

The textural properties of the starch-protein gels showed similar trends as the viscosity and G'. Fracture stress, fracture strain and Young's modulus all gradually decreased as the proportion of starch decreased (Figure 21). The pure protein gels were not self-standing and could not be analysed by compression tests. Overall, the higher G' and fracture stress observed for samples with higher starch content correlated well with the simultaneous increase in water-holding capacity of the systems.

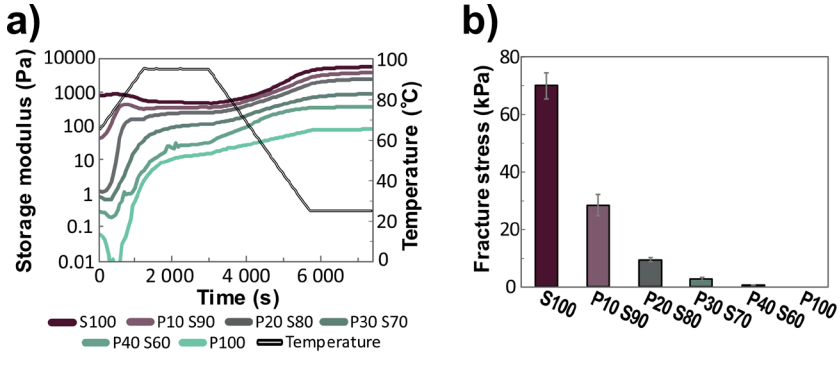


Figure 21. (a) Change in storage modulus (G') over time during gel formation and (b) fracture stress of gels prepared at pH 7 with different protein-starch mixtures. Sample labels indicate percentage of solids added as protein (P%) or starch (S%). Error bars represent ±1 standard deviation.

Differences in protein- and starch-rich gels

A reduction in textural properties was observed when part of the starch in faba bean starch gels was replaced with protein (Paper II) or when part of the protein in faba bean protein gels was replaced by starch (Paper IV). The different changes in fracture stress of the starch- and protein-rich systems upon increasing the proportion of starch could be due to multiple factors.

First, in both cases addition of the minor component probably interfered with gel and network formation by the major component. Protein and polysaccharides are typically seen as incompatible (Grinberg & Tolstoguzov 1997), and if no strong interactions/bonds are formed between the two phases, this is likely to lead to reduced textural properties.

Second, total solids content differ substantially between the two types of gels (starch-rich gels: 12% solids, protein-rich gels: 20% solids). The different water availability in the two systems might have affected gelatinisation of the starch and the water distribution between the protein and starch, *e.g.* the ability of starch to absorb water may have had a different effect at concentrations closer to the lowest gelling concentration of the protein than at higher concentrations. Higher solids content and viscosity of the protein-rich system might also have affected amylose leakage from the starch granules and aggregation and network formation during starch gelatinisation.

In summary, mixed starch-protein gels had a continuous protein phase when the protein content was 40% or higher, while a continuous starch phase was observed at starch concentrations of 70% or above. In the mixed gels, leaked-out amylose aggregated into more amylose-dense regions within the protein phase. In terms of textural properties, both fracture stress and G' increased with increasing starch content.

6. Conclusions

This thesis examined the microstructure and textural properties of legume-based mixed gels based on protein, starch and fibre. The main conclusions from the work were that:

- Increased NaCl concentration induced changes in the microstructure of gels from faba bean protein fraction 7S, but not protein fraction 11S, while changes in rheological properties occurred for both fractions but with opposing trends (increasing storage modulus for 7S gels, decreasing for 11S gels).
- Gels with microstructure dominated by larger protein particles (approximately 5-50 μm) had lower fracture stress and fracture strain than gels with a homogeneous, fine-stranded protein network.
- The water-holding capacity and water mobility of gels were significantly affected by changes in pH, and the effect was attributed to differences in microstructure.
- Addition of starch/fibre to protein gels formed at pH 7, with a homogeneous, fine-stranded protein network, decreased fracture stress and fracture strain, while fracture stress increased and fracture strain remained constant on addition of starch/fibre to gels with a particulate structure of larger protein particles (pH 4 or 5).
- Addition of starch/fibre increased storage modulus of homogeneous, fine-stranded faba bean protein gels (pH 7) and of pea protein gels with a microstructure dominated by larger protein particles.

In a wider context, the findings presented in this thesis extend current knowledge on microstructure and texture formation by faba bean and pea proteins. Furthermore, it improves understanding of how inclusion of starch

and fibre affects the textural properties of gels with different microstructures (*e.g.* homogeneous and fine-stranded *vs.* particulate) and reduce the knowledge gap on texture formation of protein from non-soy legumes. Something that can facilitate development of plant-based foods from a wider range of protein sources.

7. Future work

This thesis investigated gel formation by faba bean protein alone, or with addition of starch and/or fibre. It also investigated the impact of incorporating a lentil side-stream rich in starch and fibre on gelation of commercial pea protein. Future research should address additional aspects in order to deepen understanding of these and similar gel systems.

The influence of starch and fibre addition may vary depending on the solids content of the system, *e.g*. below, near or far above the lowest gelling concentration of the protein. Investigating these scenarios could provide valuable insights into water competition dynamics at different solids contents. In addition, studies on water mobility and water-holding capacity of protein-starch gels, as done in this thesis for protein-fibre gels, would yield valuable information. Moreover, comparing gels formed from dry fractionated faba bean protein and gels produced from a mixture of extracted protein, starch and fibre of similar composition could provide valuable knowledge on the effect of the extraction process and the less harsh processing conditions during dry fractionation.

The fibre fractions employed in this thesis were left unprocessed post-extraction. Future investigations could explore the effects of functionalisation/post-processing techniques such as heat treatment, ultrasound or milling in altering the functionality of the fibre fraction and its subsequent impact when added to protein gel systems.

Fermentation and enzymatic treatments are two other process that could be used both to induce gel formation or as pre-treatments to change the functionality of the raw materials. The fermentation process will also influence the flavour profile of the gels and have the potential to create beneficial nutritional effects, *e.g*. by reducing the phytate content.

In this thesis, mixed legume-based gels containing protein, starch and fibre were investigated. Fat is another component present in many foods, and further studies could examine the effect of oil in both protein and mixed protein-starch/fibre gels, to evaluate whether the added oil affects the systems differently depending on their composition. The effect of oil when added to protein gels with different microstructures, such as homogeneous, fine-stranded gels or particulate gels with large protein particles, could also be evaluated.

This thesis examined gel formation by the 7S and 11S protein fractions in faba bean. In further assessments of gel formation by individual protein fractions, it would be interesting to evaluate gel formation by protein isolates extracted from different faba bean varieties with varying 7S/11S ratio. This would allow evaluation of whether information on the individual protein fractions could be extended to isolates extracted from these.

Microstructure was a consistent theme throughout the work in this thesis. The microstructure and properties of the gel network, *e.g.* pore size, affect the release of water and water-soluble compounds, which is important for flavour release and sensory experience. The pore size distribution in the different systems could be further quantified and correlated to the release of liquid and water-soluble compounds.

Future work could also include sensory evaluations of protein gels with added starch or fibre, to determine whether differences in microstructure and textural properties are correlated to perceived sensory differences and whether addition of starch or fibre affects sensory properties such as graininess, mouth-coating and adhesiveness.

References

Almdal, K., Dyre, J., Hvidt, S. & Kramer, O. (1993). Towards a phenomenological definition of the term 'gel'. *Polymer Gels and Networks,* 1, 5-17. https://doi.org/10.1016/0966-7822(93)90020-I

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., Nantanga, K.K.M. & Seetharaman, K. (2011). Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. *Food Research International,* 44, 2962-2974. https://doi.org/10.1016/j.foodres.2011.07.006

Arai, Y., Nishinari, K. & Nagano, T. (2021). Developing soybean protein gel-based foods from okara using the wet-type grinder method. *Foods,* 10, 348. https://doi.org/10.3390/foods10020348

Arai, Y., Nishinari, K. & Nagano, T. (2022). Wet grinder-treated okara improved both mechanical properties and intermolecular forces of soybean protein isolate gels. *Gels,* 8, 616. https://doi.org/10.3390/gels8100616

Arese, P. & De Flora, A. (1990). Pathophysiology of hemolysis in glucose-6-phosphate dehydrogenase deficiency. *Seminars in Hematology*, 1-40.

Arntfield, S.D. & Murray, E.D. (1981). The influence of processing parameters on food protein functionality I. Differential scanning calorimetry as an indicator of protein denaturation. *Canadian Institute of Food Science and Technology Journal,* 14, 289-294. https://doi.org/10.1016/S0315-5463(81)72929-8

Arntfield, S.D., Murray, E.D. & Ismond, M.A.H. (1986). Effect of salt on the thermal stability of storage proteins from faba bean (Vicia Faba). *Journal of Food Science,* 51, 371-377. https://doi.org/10.1111/j.1365-2621.1986.tb11133.x

Aschemann-Witzel, J., Gantriis, R.F. & Fraga, P. (2021). Plant-based food and protein trend from a business perspective : markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition,* 61, 3119-3128. https://doi.org/10.1080/10408398.2020.1793730

Bastianelli, D., Duparque, M., Grosjean, F., Peyronnet, C. & Régnier, J.M. (1998). Feeding value of pea (*Pisum sativum*, L.) 1. Chemical

composition of different categories of pea. *Animal Science,* 67, 609-619. https://doi.org/10.1017/S1357729800033051

Beaulieu, M., Turgeon, S.L. & Doublier, J.-L. (2001). Rheology, texture and microstructure of whey proteins/low methoxyl pectins mixed gels with added calcium. *International Dairy Journal,* 11, 961-967. https://doi.org/10.1016/S0958-6946(01)00127-3

Beveridge, T., Jones, L. & Tung, M.A. (1984). Progel and gel formation and reversibility of gelation of whey, soybean, and albumen protein gels. *Journal of Agricultural and Food Chemistry,* 32, 307-313. https://doi.org/10.1021/jf00122a033

Bora, P.S., Brekke, C.J. & Powers, J.R. (1994). Heat induced gelation of pea (*Pisum sativum*) mixed globulins, vicilin and legumin. *Journal of Food Science,* 59, 594-596. https://doi.org/10.1111/j.1365-2621.1994.tb05570.x

Bravo-Núñez, Á., Garzón, R., Rosell, C.M. & Gómez, M. (2019). Evaluation of starch-protein interactions as a function of pH. *Foods,* 8, 2-11. https://doi.org/10.3390/foods8050155

Bühler, J.M., Dekkers, B.L., Bruins, M.E. & van der Goot, A.J. (2020). Modifying faba bean protein concentrate using dry heat to increase water holding capacity. *Foods,* 9, 1077. https://doi.org/10.3390/foods9081077

Chassenieux, C. & Nicolai, T. (2024). Mechanical properties and microstructure of (emul)gels formed by mixtures of proteins and polysaccharides. *Current Opinion in Colloid & Interface Science,* 70, 101781. https://doi.org/10.1016/j.cocis.2023.101781

Chen, N., Zhao, M., Niepceron, F., Nicolai, T. & Chassenieux, C. (2017). The effect of the pH on thermal aggregation and gelation of soy proteins. *Food Hydrocolloids,* 66, 27-36. https://doi.org/10.1016/j.foodhyd.2016.12.006

Chen, S.-K., Lin, H.-F., Wang, X., Yuan, Y., Yin, J.-Y. & Song, X.-X. (2023). Comprehensive analysis in the nutritional composition, phenolic species and in vitro antioxidant activities of different pea cultivars. *Food Chemistry: X,* 17, 100599. https://doi.org/10.1016/j.fochx.2023.100599

Choi, Y.-S., Choi, J.-H., Han, D.-J., Kim, H.-Y., Lee, M.-A., Kim, H.-W., Jeong, J.-Y. & Kim, C.-J. (2011). Effects of rice bran fiber on heat-induced gel prepared with pork salt-soluble meat proteins in model system. *Meat Science,* 88, 59-66. https://doi.org/10.1016/j.meatsci.2010.12.003

Clark, A.H. (1998). Gelation of globular proteins. In: Hill, S.E., Ledward, D.A. & Mitchell J.R. (eds) *Functional properties of food*

macromolecules. (2) Aspen Publishers, Inc., Gaithersburg, USA. 77-142.

Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F.N. & Leip, A. (2021). Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*, 2, 198-209. https://doi.org/10.1038/s43016-021-00225-9

Delahaije, R.J.B.M., Gruppen, H., van Eijk−van Boxtel, E.L., Cornacchia, L. & Wierenga, P.A. (2016). Controlling the ratio between native-like, non-native-like, and aggregated β-lactoglobulin after heat treatment. *Journal of Agricultural and Food Chemistry,* 64, 4362-4370. https://doi.org/10.1021/acs.jafc.6b00816

Didinger, C. & Thompson, H.J. (2022). The role of pulses in improving human health: A review. *Legume Science,* 4, e147. https://doi.org/10.1002/leg3.147

Dille, M.J., Draget, K.I. & Hattrem, M.N. (2015). 9 - The effect of filler particles on the texture of food gels. In: Chen, J. & Rosenthal, A. (eds) *Modifying Food Texture*. Woodhead Publishing. 183-200. https://doi.org/10.1016/B978-1-78242-333-1.00009-7

Duc, G., Aleksić, J.M., Marget, P., Mikic, A., Paull, J., Redden, R.J., Sass, O., Stoddard, F.L., Vandenberg, A., Vishnyakova, M. & Torres, A.M. (2015). Faba Bean In: De Ron, A.M. (ed.) *Grain Legumes*. Springer New York. 141-178. https://doi.org/10.1007/978-1-4939-2797-5_5

Evans, I.D. & Haisman, D.R. (1982). The effect of solutes on the gelatinization temperature range of potato starch. *Starch - Stärke,* 34(7), 224-231. https://doi.org/10.1002/star.19820340704

Foegeding, E.A. (2007). Rheology and sensory texture of biopolymer gels. *Current Opinion in Colloid & Interface Science,* 12, 242-250. https://doi.org/10.1016/j.cocis.2007.07.001

Foegeding, E.A. & Davis, J.P. (2011). Food protein functionality: A comprehensive approach. *Food Hydrocolloids,* 25, 1853-1864. https://doi.org/10.1016/j.foodhyd.2011.05.008

Foegeding, E.A. & Drake, M.A. (2007). Invited review: Sensory and mechanical properties of cheese texture. *Journal of Dairy Science,* 90, 1611-1624. https://doi.org/10.3168/jds.2006-703

Fukushima, D. (1991). Recent progress of soybean protein foods: Chemistry, technology, and nutrition. *Food Reviews International,* 7, 323-351. https://doi.org/10.1080/87559129109540915

Giacalone, D., Clausen, M.P. & Jaeger, S.R. (2022). Understanding barriers to consumption of plant-based foods and beverages: insights from

sensory and consumer science. *Current Opinion in Food Science,* 48, 100919. https://doi.org/10.1016/j.cofs.2022.100919

Gianferri, R., D'Aiuto, V., Curini, R., Delfini, M. & Brosio, E. (2007). Proton NMR transverse relaxation measurements to study water dynamic states and age-related changes in Mozzarella di Bufala Campana cheese. *Food Chemistry,* 105, 720-726. https://doi.org/10.1016/j.foodchem.2007.01.005

Gilbert, A., Rioux, L.-E., St-Gelais, D. & Turgeon, S.L. (2020). Characterization of syneresis phenomena in stirred acid milk gel using low frequency nuclear magnetic resonance on hydrogen and image analyses. *Food Hydrocolloids,* 106, 105907. https://doi.org/10.1016/j.foodhyd.2020.105907

Griffiths, D.W. & Lawes, D.A. (1978). Variation in the crude protein content of field beans (*Vicia faba* L.) in relation to the possible improvement of the protein content of the crop. *Euphytica,* 27, 487-495. https://doi.org/10.1007/BF00043174

Grinberg, V.Y. & Tolstoguzov, V.B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids,* 11, 145-158. https://doi.org/10.1016/S0268-005X(97)80022-7

Gwartney, E.A., Larick, D.K. & Foegeding, E.A. (2004). Sensory texture and mechanical properties of stranded and particulate whey protein emulsion gels. *Journal of Food Science,* 69, S333-S339. https://doi.org/10.1111/j.1365-2621.2004.tb09945.x

Hansen, L., Bu, F. & Ismail, B.P. (2022). Structure-function guided extraction and scale-up of pea protein isolate production. *Foods,* 11, 3773. https://doi.org/10.3390/foods11233773

He, M., Zhang, M., Gao, T., Chen, L., Liu, Y., Huang, Y., Teng, F. & Li, Y. (2024). Assembly of soy protein-corn starch composite gels by thermal induction: Structure, and properties. *Food Chemistry,* 434, 137433. https://doi.org/10.1016/j.foodchem.2023.137433

Hermansson, A.-M. (1979). Aggregation and denaturation involved in gel formation. In: Gould, R.F. (ed) *Functionality and Protein Structure*. American Chemical Society. 81-103. https://doi.org/10.1021/bk-1979-0092.ch005

Hoover, R., Hughes, T., Chung, H.J. & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International,* 43, 399-413. https://doi.org/10.1016/j.foodres.2009.09.001

Jane, J., Chen, Y.Y., Lee, L.F., McPherson, A.E., Wong, K.S., Radosavljevic, M. & Kasemsuwan, T. (1999). Effects of

amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry,* 76, 629-637. https://doi.org/10.1094/CCHEM.1999.76.5.629

Jensen, E.S., Peoples, M.B. & Hauggaard-Nielsen, H. (2010). Faba bean in cropping systems. *Field Crops Research,* 115, 203-216. https://doi.org/10.1016/j.fcr.2009.10.008

Johansson, M., Karkehabadi, S., Johansson, D.P. & Langton, M. (2023). Gelation behaviour and gel properties of the 7S and 11S globulin protein fractions from faba bean (*Vicia faba* var. minor) at different NaCl concentrations. *Food Hydrocolloids,* 142, 108789. https://doi.org/10.1016/j.foodhyd.2023.108789

Johansson, M., Nilsson, K., Knab, F. & Langton, M. (2022). Faba bean fractions for 3D printing of protein-, starch- and fibre-rich foods. *Processes,* 10, 466. https://doi.org/10.3390/pr10030466

Joshi, M., Aldred, P., McKnight, S., Panozzo, J.F., Kasapis, S., Adhikari, R. & Adhikari, B. (2013). Physicochemical and functional characteristics of lentil starch. *Carbohydrate Polymers,* 92, 1484-1496. https://doi.org/10.1016/j.carbpol.2012.10.035

Joshi, M., Aldred, P., Panozzo, J.F., Kasapis, S. & Adhikari, B. (2014). Rheological and microstructural characteristics of lentil starch–lentil protein composite pastes and gels. *Food Hydrocolloids,* 35, 226-237. https://doi.org/10.1016/j.foodhyd.2013.05.016

Joyner, H.S. (2018). Explaining food texture through rheology. *Current Opinion in Food Science,* 21, 7-14. https://doi.org/10.1016/j.cofs.2018.04.003

Kimura, A., Fukuda, T., Zhang, M., Motoyama, S., Maruyama, N. & Utsumi, S. (2008). Comparison of physicochemical properties of 7S and 11S globulins from pea, fava bean, cowpea, and french bean with those of soybean—french bean 7S globulin exhibits excellent properties. *Journal of Agricultural and Food Chemistry,* 56, 10273-10279. https://doi.org/10.1021/jf801721b

Langton, M., Aström, A. & Hermansson, A.-M. (1996). Texture as a reflection of microstructure. *Food Quality and Preference,* 7, 185-191. https://doi.org/10.1016/S0950-3293(96)00030-4

Langton, M., Ehsanzamir, S., Karkehabadi, S., Feng, X., Johansson, M. & Johansson, D.P. (2020). Gelation of faba bean proteins - Effect of extraction method, pH and NaCl. *Food Hydrocolloids,* 103, 105622. https://doi.org/10.1016/j.foodhyd.2019.105622

Langton, M. & Hermansson, A.-M. (1992). Fine-stranded and particulate gels of β-lactoglobulin and whey protein at varying pH. *Food*

Hydrocolloids, 5, 523-539. https://doi.org/10.1016/S0268-005X(09)80122-7

Langton, M., Åström, A. & Hermansson, A.-M. (1997). Influence of the microstructure on the sensory quality of whey protein gels. *Food Hydrocolloids,* 11, 217-230. https://doi.org/10.1016/S0268-005X(97)80028-8

Laursen, A.K., Rovers, T.A.M., van den Berg, F.W.J. & Ahrné, L. (2023). Formulation of heat- and acid-induced milk gels – Effect on texture, microstructure, and water distribution. *Food Structure,* 37, 100341. https://doi.org/10.1016/j.foostr.2023.100341

Li, J.-Y., Yeh, A.-I. & Fan, K.-L. (2007). Gelation characteristics and morphology of corn starch/soy protein concentrate composites during heating. *Journal of Food Engineering,* 78, 1240-1247. https://doi.org/10.1016/j.jfoodeng.2005.12.043

Li, J., Zhang, M., Chang, C., Gu, L., Peng, N., Su, Y. & Yang, Y. (2018). Molecular forces and gelling properties of heat-set whole chicken egg protein gel as affected by NaCl or pH. *Food Chemistry,* 261, 36-41. https://doi.org/10.1016/j.foodchem.2018.03.079

Li, L., Yuan, T.Z., Setia, R., Raja, R.B., Zhang, B. & Ai, Y. (2019). Characteristics of pea, lentil and faba bean starches isolated from air-classified flours in comparison with commercial starches. *Food Chemistry,* 276, 599-607. https://doi.org/10.1016/j.foodchem.2018.10.064

Liu, J., Zhu, K., Ye, T., Wan, S., Wang, Y., Wang, D., Li, B. & Wang, C. (2013). Influence of konjac glucomannan on gelling properties and water state in egg white protein gel. *Food Research International,* 51, 437-443. https://doi.org/10.1016/j.foodres.2013.01.002

Lu, Z.X., He, J.F., Zhang, Y.C. & Bing, D.J. (2020). Composition, physicochemical properties of pea protein and its application in functional foods. *Critical Reviews in Food Science and Nutrition,* 60, 2593-2605. https://doi.org/10.1080/10408398.2019.1651248

Lv, Y., Tang, T., Xu, L., Wang, J., Su, Y., Li, J., Gu, L., Zhang, M., Yang, Y. & Chang, C. (2022a). Influence of soybean dietary fiber with varying particle sizes and transglutaminase on soy protein isolate gel. *Food Research International,* 161, 111876. https://doi.org/10.1016/j.foodres.2022.111876

Lv, Y., Wang, J., Xu, L., Tang, T., Su, Y., Gu, L., Chang, C., Zhang, M., Yang, Y. & Li, J. (2022b). Gel properties of okara dietary fiber-fortified soy protein isolate gel with/without NaCl. *Journal of the Science of Food and Agriculture,* 103, 411-419. https://doi.org/10.1002/jsfa.12155

Makri, E.A., Papalamprou, E.M. & Doxastakis, G.I. (2006). Textural properties of legume protein isolate and polysaccharide gels. *Journal of the Science of Food and Agriculture,* 86, 1855-1862. https://doi.org/10.1002/jsfa.2531

Mayer Labba, I.-C., Frøkiær, H. & Sandberg, A.-S. (2021). Nutritional and antinutritional composition of fava bean (*Vicia faba* L., var. minor) cultivars. *Food Research International,* 140, 110038. https://doi.org/10.1016/j.foodres.2020.110038

Mession, J.-L., Blanchard, C., Mint-Dah, F.-V., Lafarge, C., Assifaoui, A. & Saurel, R. (2013). The effects of sodium alginate and calcium levels on pea proteins cold-set gelation. *Food Hydrocolloids,* 31, 446-457. https://doi.org/10.1016/j.foodhyd.2012.11.004

Min, C., Ma, W., Kuang, J., Huang, J. & Xiong, Y.L. (2022). Textural properties, microstructure and digestibility of mungbean starch–flaxseed protein composite gels. *Food Hydrocolloids,* 126, 107482. https://doi.org/10.1016/j.foodhyd.2022.107482

Muhrbeck, P. & Eliasson, A.C. (1991). Rheological properties of protein/starch mixed gels. *Journal of texture studies,* 22, 317-332. https://doi.org/10.1111/j.1745-4603.1991.tb00023.x

Multari, S., Stewart, D. & Russell, W.R. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. *Comprehensive Reviews in Food Science and Food Safety,* 14, 511-522. https://doi.org/10.1111/1541-4337.12146

Munialo, C.D., van der Linden, E., Ako, K. & de Jongh, H.H.J. (2015). Quantitative analysis of the network structure that underlines the transitioning in mechanical responses of pea protein gels. *Food Hydrocolloids,* 49, 104-117. https://doi.org/10.1016/j.foodhyd.2015.03.018

Munialo, C.D., van der Linden, E., Ako, K., Nieuwland, M., Van As, H. & de Jongh, H.H.J. (2016). The effect of polysaccharides on the ability of whey protein gels to either store or dissipate energy upon mechanical deformation. *Food Hydrocolloids,* 52, 707-720. https://doi.org/10.1016/j.foodhyd.2015.08.013

Munialo, C.D., van der Linden, E. & de Jongh, H.H.J. (2014). The ability to store energy in pea protein gels is set by network dimensions smaller than 50nm. *Food Research International,* 64, 482-491. https://doi.org/10.1016/j.foodres.2014.07.038

Nakai, S. (1983). Structure-function relationships of food proteins: with an emphasis on the importance of protein hydrophobicity. *Journal of agricultural and food chemistry,* 31, 676-683. https://doi.org/10.1021/jf00118a001

Nash, A.M., Kwolek, W.F. & Wolf, W.J. (1971). Denaturation of soybean proteins by isoelectric precipitation. *Cereal Chemistry,* 48, 360-368.

Nicolai, T. & Chassenieux, C. (2019). Heat-induced gelation of plant globulins. *Current Opinion in Food Science,* 27, 18-22. https://doi.org/10.1016/j.cofs.2019.04.005

Nicolai, T. & Durand, D. (2013). Controlled food protein aggregation for new functionality. *Current Opinion in Colloid & Interface Science,* 18, 249-256. https://doi.org/10.1016/j.cocis.2013.03.001

Nilsson, K., Sandström, C., Özeren, H.D., Vilaplana, F., Hedenqvist, M. & Langton, M. (2022). Physiochemical and thermal characterisation of faba bean starch. *Journal of Food Measurement and Characterization,* 16, 4470-4485. https://doi.org/10.1007/s11694-022-01543-7

Nivala, O., Mäkinen, O.E., Kruus, K., Nordlund, E. & Ercili-Cura, D. (2017). Structuring colloidal oat and faba bean protein particles via enzymatic modification. *Food Chemistry,* 231, 87-95. https://doi.org/10.1016/j.foodchem.2017.03.114

Nivala, O., Nordlund, E., Kruus, K. & Ercili-Cura, D. (2021). The effect of heat and transglutaminase treatment on emulsifying and gelling properties of faba bean protein isolate. *LWT,* 139, 110517. https://doi.org/10.1016/j.lwt.2020.110517

Ntatsi, G., Gutiérrez-Cortines, M.E., Karapanos, I., Barros, A., Weiss, J., Balliu, A., Rosa, E.A.d.S. & Savvas, D. (2018). The quality of leguminous vegetables as influenced by preharvest factors. *Scientia Horticulturae,* 232, 191-205. https://doi.org/10.1016/j.scienta.2017.12.058

Otegui, I., Fernández-Quintela, A., Diego, A.D., Cid, C., Macarulla, M.T. & Partearroyo, M.A. (1997). Properties of spray-dried and freeze-dried faba bean protein concentrates. *International Journal of Food Science & Technology,* 32, 439-443. https://doi.org/10.1111/j.1365-2621.1997.tb02118.x

Pang, Z., Bourouis, I., Sun, M., Cao, J., Liu, P., Sun, R., Chen, C., Li, H. & Liu, X. (2022). Physicochemical properties and microstructural behaviors of rice starch/soy proteins mixtures at different proportions. *International Journal of Biological Macromolecules,* 209, 2061-2069. https://doi.org/10.1016/j.ijbiomac.2022.04.187

Puppo, M.C. & Añón, M.C. (1998). Structural properties of heat-induced soy protein gels as affected by ionic strength and pH. *Journal of Agricultural and Food Chemistry,* 46, 3583-3589. https://doi.org/10.1021/jf980006w

Rahate, K.A., Madhumita, M. & Prabhakar, P.K. (2021). Nutritional composition, anti-nutritional factors, pretreatments-cum-processing impact and food formulation potential of faba bean (Vicia faba L.): A comprehensive review. *LWT,* 138, 110796. https://doi.org/10.1016/j.lwt.2020.110796

Ratnayake, W.S. & Naguleswaran, S. (2022). Utilizing side streams of pulse protein processing: A review. *Legume Science,* 4, e120. https://doi.org/10.1002/leg3.120

Renkema, J.M.S. (2004). Relations between rheological properties and network structure of soy protein gels. *Food Hydrocolloids,* 18, 39-47. https://doi.org/10.1016/S0268-005X(03)00040-7

Ribotta, P.D., Colombo, A., León, A.E. & Añón, M.C. (2007). Effects of soy protein on physical and rheological properties of wheat starch. *Starch - Stärke,* 59, 614-623. https://doi.org/10.1002/star.200700650

Richardson, G., Kidman, S., Langton, M. & Hermansson, A.M. (2004). Differences in amylose aggregation and starch gel formation with emulsifiers. *Carbohydrate Polymers,* 58, 7-13. https://doi.org/10.1016/j.carbpol.2004.06.013

Sala, G., van de Velde, F., Cohen Stuart, M.A. & van Aken, G.A. (2007). Oil droplet release from emulsion-filled gels in relation to sensory perception. *Food Hydrocolloids,* 21, 977-985. https://doi.org/10.1016/j.foodhyd.2006.08.009

Scarborough, P., Clark, M., Cobiac, L., Papier, K., Knuppel, A., Lynch, J., Harrington, R., Key, T. & Springmann, M. (2023). Vegans, vegetarians, fish-eaters and meat-eaters in the UK show discrepant environmental impacts. *Nature Food,* 4, 565-574. https://doi.org/10.1038/s43016-023-00795-w

Siegrist, M. & Hartmann, C. (2019). Impact of sustainability perception on consumption of organic meat and meat substitutes. *Appetite,* 132, 196-202. https://doi.org/10.1016/j.appet.2018.09.016

Singh, N., Singh, J., Kaur, L., Singh Sodhi, N. & Singh Gill, B. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry,* 81, 219-231. https://doi.org/10.1016/S0308-8146(02)00416-8

Sosulski, F.W. & McCurdy, A.R. (1987). Functionality of flours, protein fractions and isolates from field peas and faba bean. *Journal of Food Science,* 52, 1010-1014. https://doi.org/10.1111/j.1365-2621.1987.tb14263.x

Stading, M. & Hermansson, A.-M. (1990). Viscoelastic behaviour of β-lactoglobulin gel structures. *Food Hydrocolloids,* 4, 121-135. https://doi.org/10.1016/S0268-005X(09)80013-1

Stading, M., Langton, M. & Hermansson, A.-M. (1993). Microstructure and rheological behaviour of particulate β-lactoglobulin gels. *Food Hydrocolloids,* 7, 195-212. https://doi.org/10.1016/S0268-005X(09)80172-0

Stephen, A.M., Champ, M.M.-J., Cloran, S.J., Fleith, M., van Lieshout, L., Mejborn, H. & Burley, V.J. (2017). Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. *Nutrition Research Reviews,* 30, 149-190. https://doi.org/10.1017/S095442241700004X

Stone, A.K., Avarmenko, N.A., Warkentin, T.D. & Nickerson, M.T. (2015). Functional properties of protein isolates from different pea cultivars. *Food Science and Biotechnology,* 24, 827-833. https://doi.org/10.1007/s10068-015-0107-y

Sun, X.D. & Arntfield, S.D. (2011a). Gelation properties of salt-extracted pea protein isolate induced by heat treatment: Effect of heating and cooling rate. *Food Chemistry*, 124, 1011-1016. https://doi.org/10.1016/j.foodchem.2010.07.063

Sun, X.D. & Arntfield, S.D. (2011b). Dynamic oscillatory rheological measurement and thermal properties of pea protein extracted by salt method: Effect of pH and NaCl. *Journal of Food Engineering,* 105, 577-582. https://doi.org/10.1016/j.jfoodeng.2011.03.008

Tso, R., Lim, A.J. & Forde, C.G. (2021). A critical appraisal of the evidence supporting consumer motivations for alternative proteins. *Foods,* 10, 24. https://doi.org/10.3390/foods10010024

Tziva, M., Negro, S.O., Kalfagianni, A. & Hekkert, M.P. (2020). Understanding the protein transition: The rise of plant-based meat substitutes. *Environmental Innovation and Societal Transitions,* 35, 217-231. https://doi.org/10.1016/j.eist.2019.09.004

Ullah, I., Hu, Y., You, J., Yin, T., Xiong, S., Din, Z.-u., Huang, Q. & Liu, R. (2019). Influence of okara dietary fiber with varying particle sizes on gelling properties, water state and microstructure of tofu gel. *Food Hydrocolloids,* 89, 512-522. https://doi.org/10.1016/j.foodhyd.2018.11.006

Urbonaite, V., van der Kaaij, S., de Jongh, H.H.J., Scholten, E., Ako, K., van der Linden, E. & Pouvreau, L. (2016). Relation between gel stiffness and water holding for coarse and fine-stranded protein gels. *Food Hydrocolloids,* 56, 334-343. https://doi.org/10.1016/j.foodhyd.2015.12.011

Utsumi, S. & Kinsella, J.E. (1985). Structure-function relationships in food proteins: subunit interactions in heat-induced gelation of 7S, 11S, and soy isolate proteins. *Journal of Agricultural and Food Chemistry,* 33, 297-303. https://doi.org/10.1021/jf00062a035

van den Berg, L., van Vliet, T., van der Linden, E., van Boekel, M.A.J.S. & van de Velde, F. (2007). Breakdown properties and sensory perception of whey proteins/polysaccharide mixed gels as a function of microstructure. *Food Hydrocolloids,* 21, 961-976. https://doi.org/10.1016/j.foodhyd.2006.08.017

van Vliet, T. (2013). *Rheology and fracture mechanics of foods*. CRC Press.

Vilariño, M., Métayer, J.P., Crépon, K. & Duc, G. (2009). Effects of varying vicine, convicine and tannin contents of faba bean seeds (*Vicia faba* L.) on nutritional values for broiler chicken. *Animal Feed Science and Technology,* 150, 114-121. https://doi.org/10.1016/j.anifeedsci.2008.08.001

Vliet, T. (1995). Large deformation and fracture behaviour of gels. *Faraday Discussions,* 101, 359-370. https://doi.org/10.1039/FD9950100359

Vogelsang-O'Dwyer, M., Petersen, I.L., Joehnke, M.S., Sørensen, J.C., Bez, J., Detzel, A., Busch, M., Krueger, M., O'Mahony, J.A., Arendt, E.K. & Zannini, E. (2020). Comparison of faba bean protein ingredients produced using dry fractionation and isoelectric precipitation: Techno-functional, nutritional and environmental performance. *Foods,* 9, 322. https://doi.org/10.3390/foods9030322

Warsame, A.O., Michael, N., O'Sullivan, D.M. & Tosi, P. (2020). Identification and quantification of major faba bean seed proteins. *Journal of Agricultural and Food Chemistry,* 68, 8535-8544. https://doi.org/10.1021/acs.jafc.0c02927

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L.J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J.A., De Vries, W., Majele Sibanda, L., Afshin, A., Chaudhary, A., Herrero, M., Agustina, R., Branca, F., Lartey, A., Fan, S., Crona, B., Fox, E., Bignet, V., Troell, M., Lindahl, T., Singh, S., Cornell, S.E., Srinath Reddy, K., Narain, S., Nishtar, S. & Murray, C.J.L. (2019). Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet,* 393, 447-492. https://doi.org/10.1016/S0140-6736(18)31788-4

Wu, C., Ma, W., Chen, Y., Navicha, W.B., Wu, D. & Du, M. (2019). The water holding capacity and storage modulus of chemical cross-

linked soy protein gels directly related to aggregates size. *LWT,* 103, 125-130. https://doi.org/10.1016/j.lwt.2018.12.064

Yu, B., Ren, F., Zhao, H., Cui, B. & Liu, P. (2020). Effects of native starch and modified starches on the textural, rheological and microstructural characteristics of soybean protein gel. *International Journal of Biological Macromolecules,* 142, 237-243. https://doi.org/10.1016/j.ijbiomac.2019.09.095

Zhang, B., Qiao, D., Zhao, S., Lin, Q., Wang, J. & Xie, F. (2021). Starch-based food matrices containing protein: Recent understanding of morphology, structure, and properties. *Trends in Food Science and Technology,* 114, 212-231. https://doi.org/10.1016/j.tifs.2021.05.033

Zhang, D., Chen, D., Patel, B. & Campanella, O.H. (2022). Pectin as a natural agent for reinforcement of pea protein gel. *Carbohydrate Polymers,* 298, 120038. https://doi.org/10.1016/j.carbpol.2022.120038

Zhang, Z., Tian, X., Wang, P., Jiang, H. & Li, W. (2019). Compositional, morphological, and physicochemical properties of starches from red adzuki bean, chickpea, faba bean, and baiyue bean grown in China. *Food Science & Nutrition,* 7, 2485-2494. https://doi.org/10.1002/fsn3.865

Zheng, B.-A., Matsumura, Y. & Mori, T. (1993). Relationships of molecular forces to rheological and structural properties of legumin gels from broad beans. *Bioscience, Biotechnology, and Biochemistry,* 57, 1257-1260. https://doi.org/10.1271/bbb.57.1257

Zheng, L., Wang, Z., Kong, Y., Ma, Z., Wu, C., Regenstein, J.M., Teng, F. & Li, Y. (2021). Different commercial soy protein isolates and the characteristics of Chiba tofu. *Food Hydrocolloids,* 110, 106115. https://doi.org/10.1016/j.foodhyd.2020.106115

Zhuang, X., Jiang, X., Han, M., Kang, Z.-l., Zhao, L., Xu, X.-l. & Zhou, G.-h. (2016). Influence of sugarcane dietary fiber on water states and microstructure of myofibrillar protein gels. *Food Hydrocolloids,* 57, 253-261. https://doi.org/10.1016/j.foodhyd.2016.01.029

Zhuang, X., Jiang, X., Zhou, H., Chen, Y., Zhao, Y., Yang, H. & Zhou, G. (2020). Insight into the mechanism of physicochemical influence by three polysaccharides on myofibrillar protein gelation. *Carbohydrate Polymers,* 229, 115449. https://doi.org/10.1016/j.carbpol.2019.115449

Popular science summary

It is becoming increasingly urgent to address challenges related to climate change and environmental impact. The current food sector contributes significantly to global environmental impact, but its impact could be lowered by shifting to a more plant-based diet. However, for this to be achieved, healthy and tasty plant-based alternative foods need to be available.

A few crops dominate the current market in plant-based foods, especially meat substitutes. Soy occupies a prominent position on the market, but cultivation of soy faces limitations in Sweden and other Scandinavian countries. Wheat, another significant crop in production of plant-based foods, can be grown in Sweden, but there are concerns due to the prevalence of coeliac disease and gluten intolerance. There is thus a need to explore alternative protein sources from other crops. This thesis examined the possibility of creation of textured foods using protein from non-soy legumes, with particular emphasis on gel formation by faba bean protein since this crop can be grown in Sweden and other countries in northern Europe.

The process of gel formation plays a crucial role in producing various foods such as yoghurts, sausages and gelled desserts. Understanding the intricate process of protein gel formation is crucial for developing appealing plant-based foods. Soy and milk proteins have been extensively studied, but there is a lack of knowledge about the mechanisms governing gel formation by many alternative proteins, a knowledge gap addressed in this thesis by investigating the relationship between microstructure and textural properties of legume-based mixed gels.

Different factors influencing protein gel formation, such as salt, pH and protein source, were analysed as they tend to vary also between foods. There were great differences in gel properties and microstructure for faba bean protein gels formed at different pH values. Weaker gels with particulate

microstructure were formed at pH 4 and 5, while gels with a stronger and more homogeneous structure were formed at pH 7. The overall behaviour was similar to that typically observed for many other plant proteins, including soy.

The protein extraction process used affected protein functionality, with drastic differences in gel strength between laboratory-extracted and commercial proteins. These differences were mainly due to differences in microstructure, *e.g.* the commercial protein contained insoluble protein particles, limiting the availability of proteins for gel formation and resulting in a structure consisting of densely packed, swollen protein particles.

By adjusting the extraction process, the two major protein fractions in faba bean, 7S and 11S, were extracted. These 7S and 11S fractions exhibited distinct gelling properties and responded differently to changes in salt (NaCl) content. This opens up the possibility of breeding and growing faba bean varieties with specific 7S/11S ratios tailored for specific applications or functionality.

In terms of broader nutritional composition, legumes (and most foods) include fibre and starch, in addition to protein, so the impact of these components on protein gel formation was explored. In an attempt to create gels with desired properties and simultaneously reduce waste from process side-streams, mixed gels containing protein together with starch and fibre fractions obtained during protein extraction were studied. The findings revealed nuanced effects based on the microstructure of the surrounding protein matrix. For instance, addition of starch and fibre influenced fracture stress differently depending on gel microstructure, causing an increase in gels with large protein particles and a decrease in fine-stranded, homogeneous protein gels.

In conclusion, the work presented in this thesis provided valuable insights to the broader context of developing sustainable and appealing plant-based foods. Addressing the knowledge gap about gel formation by proteins from alternative plant-based sources paves the way for creation of innovative, environmentally-friendly foods that can promote a shift towards a more sustainable and plant-based future.

Populärvetenskaplig sammanfattning

Livsmedelsindustrin har en betydande inverkan på miljön och står för en stor del av de globala utsläppen av växthusgaser. En ökad konsumtion av växtbaserad kost kan reducera utsläppen av växthusgaser från livsmedelsindustrin. Men, för att detta ska vara gångbart måste hälsosamma och välsmakande alternativ finnas tillgängliga. Idag domineras marknaden för växtbaserade livsmedel, framförallt köttsubstitut, av ett fåtal grödor. Två av dessa grödor är soja och vete. Soja vars odling är begränsad i Sverige, samt vete som kan odlas i Sverige men har begränsningar på grund av förekomsten av celiaki och glutenintolerans. Forskningen som presenteras i avhandling adresserar behovet av att utforska alternativa proteinkällor och fokuserar på skapandet av konsistens och tuggmotstånd med hjälp av protein från baljväxter, med särskilt fokus på gelbildning av protein från åkerböna.

Gelbildning är en viktig process i produktionen av en rad olika livsmedel så som yoghurt, korv och gelade desserter. Att förstå processen när proteingeler bildas är avgörande för att utveckla tilltalande växtbaserade livsmedel. Majoriteten av forskningen på gelbildning av proteiner har än så länge fokuserat främst på soja- och mjölkproteiner samtidigt som det saknas kunskap om gelbildning av många alternativa proteiner.

Arbetet som presenteras i denna avhandling syftar till att etablera ett samband mellan mikrostruktur och konsistens hos blandgeler från baljväxter och undersöker hur vanliga faktorer inom livsmedelsindustrin så som salt, pH och proteinkälla kan påverka gelbildningen av proteiner. Stora skillnader observerades i både konsistens och mikrostruktur för geler från åkerbönprotein bildade vid olika pH. Svagare geler med en partikelformig mikrostruktur observerades vid pH 4 och 5, jämfört med en mer homogen struktur och starkare geler vid pH 7. Det övergripande beteendet liknade det som vanligtvis observeras för andra växtproteiner, *t.ex.* soja.

Ytterligare en faktor som påverkar proteinets funktionalitet är extraktionsprocessen. Stora skillnader i gelstyrka observerades mellan laboratorieextraherade och kommersiella proteiner. Skillnaderna förklarades huvudsakligen av olikheter i gelernas mikrostruktur, där det kommersiella proteinet innehöll olösliga proteinpartiklar som begränsade proteinernas tillgänglighet för gelbildning.

Extraktionsprocessen kan också justeras för att erhålla specifika proteinfraktioner. I denna avhandling extraherades de två huvudsakliga proteinfraktionerna i åkerböna, 7S och 11S. Proteinfraktionerna uppvisade distinkta gelningsegenskaper och svarade olika på förändringar i salthalt (NaCl). Denna kunskap öppnar för förädling eller odling av sorter med specifika 7S/11S-förhållanden skräddarsydda för specifika användningsområden.

De flesta livsmedel innehåller fler komponenter än endast protein och vatten, *t. ex.* stärkelse och fibrer. För att bättre efterlikna ett livsmedel och samtidigt reducera avfall från sidoströmmar under proteinextraktionen studerades blandgeler innehållande protein, stärkelse och fibrer. Tillsats av stärkelse eller fiber hade olika effekt på proteingelerna på grund av deras mikrostruktur. Till exempel observerades en ökning i gelstyrka när stärkelse eller fibrer tillsattes till geler med stora proteinpartiklar samtidigt som en minskning observerades när tillsatsen gjordes till homogena proteingeler.

Sammanfattningsvis bidrar denna forskning med insikter till utvecklandet av hållbara och tilltalande växtbaserade livsmedel. Genom att minska kunskapsklyftan om gelbildning av alternativa proteinkällor möjliggör resultaten som redovisas i denna avhandling för skapandet av innovativa och miljövänliga livsmedel och främjar därigenom en förändring mot en mer hållbar och växtbaserad framtid.

Acknowledgements

The journey towards completing this PhD thesis has been a remarkable and transformative experience, marked by both challenges and triumphs. In the midst of a global pandemic, I embarked on this academic pursuit, leaving behind my home in Gothenburg and the comfort of proximity to family and loved ones. Despite the distance, this afforded me the opportunity to connect with numerous exceptional individuals who made significant contributions in shaping my academic and personal growth. As I reflect on this journey, I am filled with gratitude for the support and encouragement I received along the way, and there are many people I would like to thank for making this thesis possible.

First and foremost, I would like to extend my heartfelt gratitude to my main supervisor **Maud**. Your confidence in my capabilities and your continuous support throughout this project were crucial for my success. I am deeply grateful for the freedom you granted me to explore my research interests and the possibilities for collaboration that you facilitated. I also want to extend my sincerest thanks to my co-supervisors, each of whom contributed uniquely to my progress. I am truly fortunate to have had the opportunity to work alongside you. Thank you **Corine** for all your support, kind words and help with NMR. Thank you **Anna** for all the valuable input during my project and for always being so warm and welcoming during my visits at Chalmers. I also want to say a big thank you to **Daniel** for the invaluable support during the initial part of my PhD. Your knowledge and introduction to instruments and techniques made the start-up phase so much easier than it could have been.

There are multiple people at SLU to whom I wish to express my gratitude, beginning with the dedicated members of our research group STEG. To my office mates during these four years: **Klara**, it was a pleasure to work with you and my thesis would not have been the same without our collaborations. **Anja** and **Solja**, thank you for your warm and welcoming attitude in the office and for making sure that vegan options were always available. To my fellow PhD students in the group: Thanks to **Jaqueline** for all the valuable discussions and for bringing me back to the football pitch; **Johanna** for your kindness and taking over my position as PhD representative in the leading group; and **Alejandra** for reviewing my thesis and letting us taste the most delicious tempeh. To the more senior group members: Thank you **Hanna** for all the kind words and understanding; **Jing** for your microscopy skills and help during lab teaching; and **Saeid** for sharing your knowledge on proteins and protein extraction. Thank you **Henrik** for your support on the Dionex and introducing us to brewing, and **Galia** for increasing the visibility of our work and teaching us all about mushrooms.

To all present and previous recurring visitors to the fikaroom outside the D2-corridor: thank you **Ani**, **Björn**, **Christina**, **Elin**, **Filip**, **Fredric**, **Johnny**, **Luying**, **Marijana**, **Nazila**, **Oksana**, **Sara**, **Tahereh**, **Tarja**, **Troy**, **Yan** and others for all the interesting and entertaining conversations.

I would also like to thank **Mathilde** and **Yashaswini** for being such good lab-teaching partners. Thank you **Monika** for taking such good care of me and all the other PhD students at the department, I hope you find a good replacement for all your Ladok requests. Thank you **Gulaim** for your introduction and help with the SEM; **Peter** for your support with the NMR; **Gunilla** for all the help and support with MEKÖL and the student labs; and **Anders** for the booking and supplying us with a new digester.

Further, I want to express my appreciation for **Nils** and **Mikolaj**, without your technical support very few at the department would have time for anything but troubleshooting. **Anna**, **Erica**, **Eva-Marie** and **Jaana**, thank you for always answering questions and providing support related to employment, receipts and other administrative questions. Thanks to **Vadim** for all the administrative support and signing of documents.

To all my fellow PhD students and other members of the department, thank you for making BioCentrum such a welcoming workplace during these four years.

This journey also involved many wonderful people from outside SLU, starting with **Frans** and **Wenbo** for introducing the LF-NMR instrument and showing me the ways in the lab in Copenhagen. Special thanks to **Lilia** for your support, kindness and taking such good care of me during and after my unintentional encounter with the Danish health care system.

I would also like to thank all my co-authors and collaborators. Special thanks to **Alexandra**, **Anna**, **Carlos**, **Jenny**, **Malhar** and **Tina** for the collaborations and opportunities to look beyond protein gelation; **Epameinondas** for the help with extraction of lentil starch/fibre; **Fanny** for your work on the 3D printing; **Jesper** for your help with statistics; and **Mikael** for your valuable comments and input. Additionally, thank you **Mary** for your outstanding and remarkably swift language revisions of this thesis and other manuscripts, leaving little time for rest after sending text for correction.

My thanks also to **Annelie** and the LiFT mentorship programme for providing valuable guidance and mentorship, and to all the LiFT students and organisers for making courses and study visits such a nice experience. Moreover, thanks to everyone involved in PAN Sweden for all the stimulating discussions. Special thanks to the PAN PhD students, **Ansung** and **Annalena** for being such nice company during meetings and seminars and **Jakob** for the warm welcome and generous assistance during my visits to Chalmers.

Special mention also to my supervisor during my Master's thesis, **Patricia**. Thank you for your supervision and support, and for setting me on this path of discovery.

I am also grateful to my family and friends for their unwavering encouragement and understanding throughout this demanding journey. Tack **Pappa** för att du tog dig ända till Köpenhamn när jag behövde det och **Mamma** för att du alltid stöttar och tror på mig. Tack till **Cecilia** för alla vetenskapliga diskussioner och läxhjälp som påmint mig om hur mycket jag lärt mig, och ibland glömt, under mina år på Chalmers. Tack **Sofie** för den tidiga examenspresent som skänkt både glädje och frustration i vintermörkret men tillslut resulterade i framsidan till denna avhandling.

Tack **Nimbus**, **Otto** och **Barney** för all den glädje ni skänker mig och **Viktor** för att du får mig att tänka på något annat än jobb ibland. Och ett varmt tack till alla ni som fått Varberg att kännas som ett andra hem: Tack

Dan för alla frallor och crawl-kurser; **Birgitta** för tips på tv-serier och jordgubbsplock; **Leif** och **Viveca** för alla kalas; **Dan** för att jag fick födelsedagssång på min 30-års dag; **Malin** och **Flora** ert alltid lika glada välkomnande; och till **Tilde** och **Signe** för att jag får följa med på allt från långpromenader till skridskoutflykter.

Till tjejen som större delen av de senaste åren befunnit sig 435 km bort men trots avståndet alltid funnits där för mig. Älskade **Tea**, tack för allt ditt stöd, din omtanke och den glädjen du skänkt under resans gång, den hade inte varit detsamma utan dig. Jag längtar och ser fram emot både vardagen och alla de äventyr som väntar oss framöver. Tack för att du så tålmodigt väntat på västkusten - äntligen är jag färdig!

I

Food Hydrocolloids 142 (2023) 108789

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

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

Mathias Johansson *, Saeid Karkehabadi, Daniel P. Johansson, Maud Langton

Department of Molecular Sciences, Swedish University of Agricultural Sciences, Box 7015, SE-750 07, Uppsala, Sweden

ARTICLE INFO

Keywords:
Faba bean
Protein
Gelation
Rheology
Microstructure
Texture

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

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

https://doi.org/10.1016/j.foodhyd.2023.108789
Received 1 February 2023; Received in revised form 31 March 2023; Accepted 17 April 2023
Available online 17 April 2023

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.

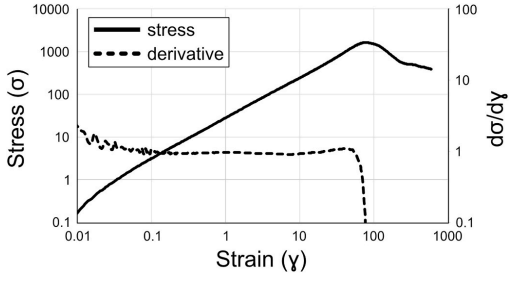


Fig. 1. Example of a stress growth curve (solid line) and its derivate (dashed line) for one of the 7S gels at 0.1 M NaCl.

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.

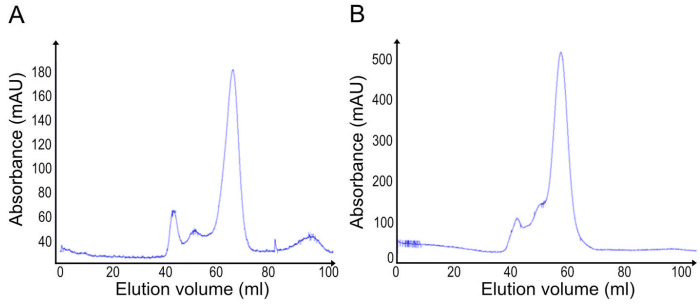


Fig. 2. Size exclusion chromatographs (Superdex-200 device, running buffer 30 mM Tris-HCl, pH 8.5, 0.5 M NaCl) obtained for the two major protein components of faba bean. (A) 7S and (B) 11S (B). Running buffer: 30 mM Tris-HCl pH 8.5, 0.5 M NaCl. Elution volume of 7S (67 ml) and 11S (58 ml) corresponds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

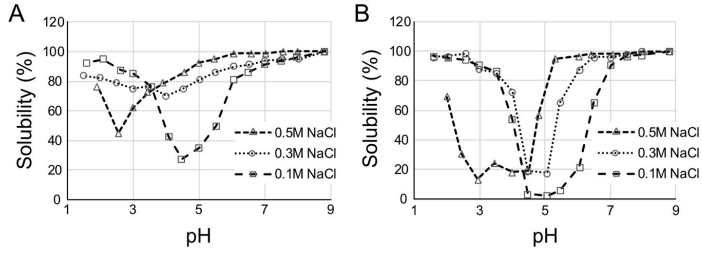


Fig. 3. Solubility curves for the 7S (A) and 11S (B) globulin fractions at 0.1 M, 0.3 M and 0.5 M NaCl.

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

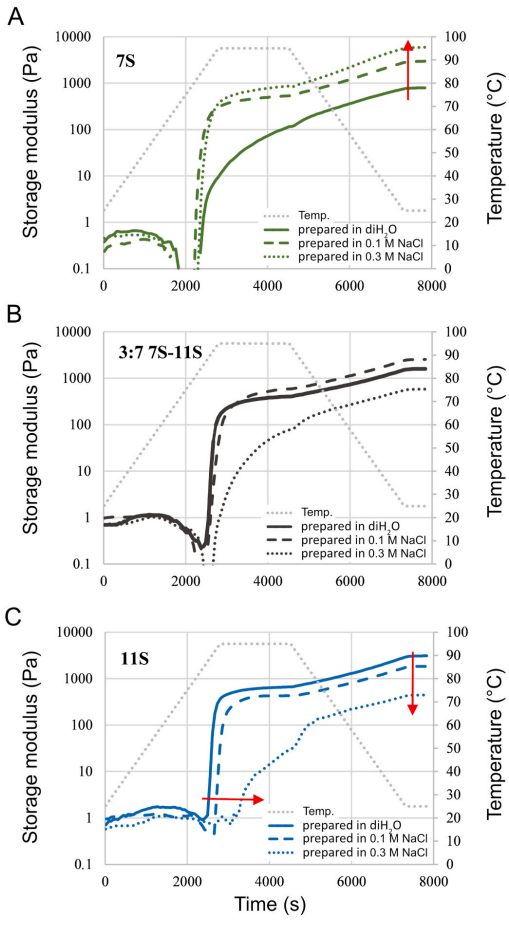


Fig. 4. Plots showing gelation (storage modulus, G') over time as a function of temperature for the protein isolates 7S (A), a 3:7 mixture of 7S and 11S (B) and 11S (C) at different NaCl concentrations. Red arrows indicate shifts in G' occurring with increasing NaCl concentrations. Note: The stated NaCl 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

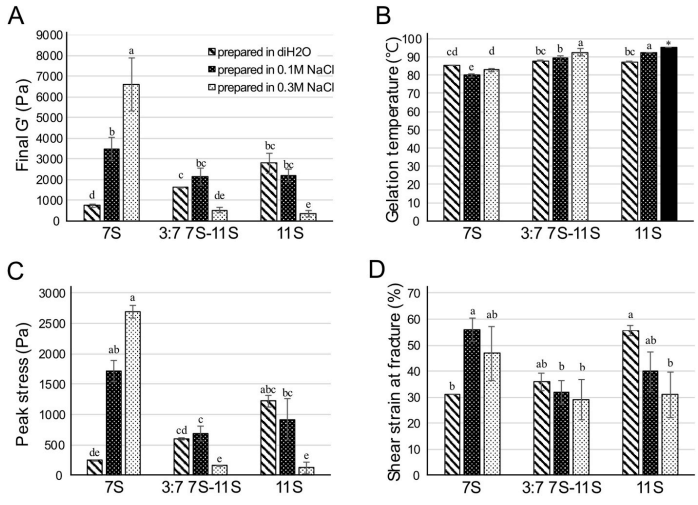


Fig. 5. Mechanical and rheological properties of gels formed from the 7S and 11S fractions and a 3:7 mixture of both, all at pH 7. Different letters on bars indicate significant differences between samples. Data on the gelation temperature for pure 11S in 0.3 M NaCl are excluded since gelling occurred 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.

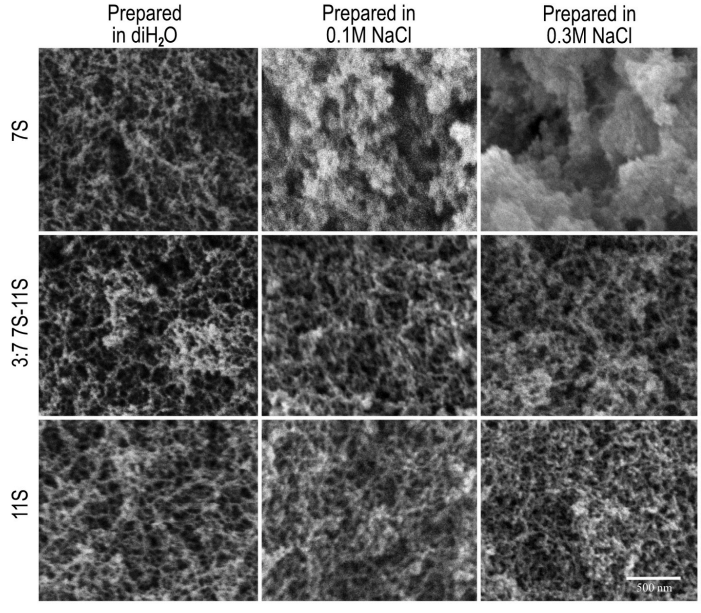


Fig. 6. Scanning electron micrographs (594 × 508 pixels) of gels made from faba bean protein fractions 7S, 11S and a 3:7 mixture of 7S and 11S at different NaCl concentrations, all at pH 7. Note: The stated NaCl concentration is the concentration of the solution in which the protein was dispersed during 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.

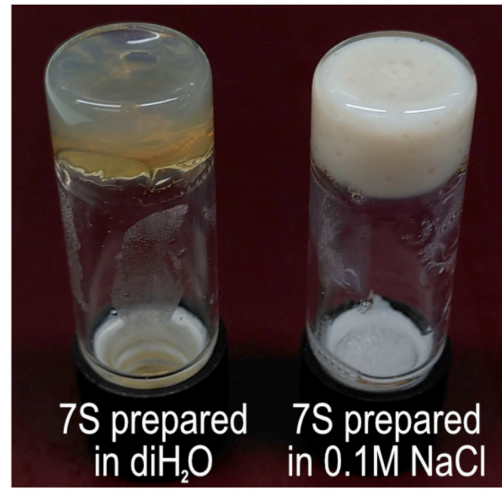


Fig. 7. Gels prepared from 7S at different NaCl concentrations, both at pH 7. Note: The stated NaCl concentration is the concentration of the solution in which the protein was dispersed during sample preparation. The actual salt concentration will be slightly higher due to salt present in the protein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

Acknowledgements

This work was supported by FORMAS – a Swedish Research Council for Sustainable Development [grant numbers 2018–01869, 2017–00426]; and Trees and Crops for the Future (TC4F), a Strategic Research Area at SLU, supported by the Swedish Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2023.108789.

References

Bora, P. S., Brekke, C. J., & Powers, J. R. (1994). Heat induced gelation of pea (Pisum sativum) mixed globulins, vicilin and legumin. *Journal of Food Science, 59*(3), 594–596. https://doi.org/10.1111/j.1365-2621.1994.tb05570.x

Burova, T. V., Grinberg, N. V., Grinberg, V. Y., Leontiev, A. L., & Tolstoguzov, V. B. (1992). Effects of polysaccharides upon the functional properties of 11 S globulin of broad beans. *Carbohydrate Polymers, 18*(2), 101–108. https://doi.org/10.1016/0144-8617(92)90131-9

Chen, N., Zhao, M., Chassenieux, C., & Nicolai, T. (2016). Thermal aggregation and gelation of soy globulin at neutral pH. *Food Hydrocolloids, 61*, 740–746. https://doi.org/10.1016/j.foodhyd.2016.06.028

Clark, A. H., Kavanagh, G. M., & Ross-Murphy, S. B. (2001). Globular protein gelation—theory and experiment. *Food Hydrocolloids, 15*(4), 383–400. https://doi.org/10.1016/S0268-005X(01)00042-X

Fernández-Quintela, A., Macarulla, M. T., del Barrio, A. S., & Martínez, J. A. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition, 51*(4), 331–341. https://doi.org/10.1023/A:1007936930354

Gordon, A., & Barbut, S. (1990). Cold stage scanning electron microscopy study of meat batters. *Journal of Food Science, 55*(4), 1196–1198. https://doi.org/10.1111/j.1365-2621.1990.tb01640.x

Grinberg, V. Y., Grinberg, N. V., Bikbov, T. M., Bronich, T. K., & Mashkevich, A. Y. (1992). Thermotropic gelation of food proteins. *Food Hydrocolloids, 6*(1), 69–96. https://doi.org/10.1016/S0268-005X(09)80058-1

Johansson, M., Johansson, D., Ström, A., Rydén, J., Nilsson, K., Karlsson, J., et al. (2022). Effect of starch and fibre on faba bean protein gel characteristics. *Food Hydrocolloids, 131*, Article 107741. https://doi.org/10.1016/j.foodhyd.2022.107741

Kimura, A., Fukuda, T., Zhang, M., Motoyama, S., Maruyama, N., & Utsumi, S. (2008). Comparison of physicochemical properties of 7S and 11S globulins from pea, fava bean, cowpea, and French bean with those of soybean—French bean 7S globulin exhibits excellent properties. *Journal of Agricultural and Food Chemistry, 56*(21), 10273–10279. https://doi.org/10.1021/jf801721b

Langton, M., Ehsanzamir, S., Karkehabadi, S., Feng, X., Johansson, M., & Johansson, D. P. (2020). Gelation of faba bean proteins - effect of extraction method, pH and NaCl. *Food Hydrocolloids, 103*, Article 105622. https://doi.org/10.1016/j.foodhyd.2019.105622

Langton, M., & Hermansson, A.-M. (1992). Fine-stranded and particulate gels of β-lactoglobulin and whey protein at varying pH. *Food Hydrocolloids, 5*(6), 523–539. https://doi.org/10.1016/S0268-005X(09)80122-7

Liu, W., & Lanier, T. C. (2015). Combined use of variable pressure scanning electron microscopy and confocal laser scanning microscopy best reveal microstructure of comminuted meat gels. *LWT - Food Science and Technology, 62*(2), 1027–1033. https://doi.org/10.1016/j.lwt.2015.02.001

Multari, S., Stewart, D., & Russell, W. R. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. *Comprehensive Reviews in Food Science and Food Safety, 14*(5), 511–522. https://doi.org/10.1111/1541-4337.12146

Mulvihill, D. M., Rector, D., & Kinsella, J. E. (1990). Effects of structuring and destructuring anionic ions on the rheological properties of thermally induced β-lactoglobulin gels. *Food Hydrocolloids, 4*(4), 267–276. https://doi.org/10.1016/S0268-005X(09)80203-8

Munialo, C. D., van der Linden, E., Ako, K., & de Jongh, H. H. J. (2015). Quantitative analysis of the network structure that underlines the transitioning in mechanical responses of pea protein gels. *Food Hydrocolloids, 49*, 104–117. https://doi.org/10.1016/j.foodhyd.2015.03.018

Nagano, T., Mori, H., & Nishinari, K. (1994). Effect of heating and cooling on the gelation kinetics of 7S globulin from soybeans. *Journal of Agricultural and Food Chemistry, 42*(7), 1415–1419.

Nakamura, T., Utsumi, S., & Mori, T. (1986). Mechanism of heat-induced gelation and gel properties of soybean 7S globulin. *Agricultural and Biological Chemistry, 50*(5), 1287–1293.

Nicolai, T., & Chassenieux, C. (2019). Heat-induced gelation of plant globulins. *Current Opinion in Food Science, 27*, 18–22. https://doi.org/10.1016/j.cofs.2019.04.005

Renkema, J. M. S. (2004). Relations between rheological properties and network structure of soy protein gels. *Food Hydrocolloids, 18*(1), 39–47. https://doi.org/10.1016/S0268-005X(03)00040-7

Renkema, J. M. S., & van Vliet, T. (2002). Heat-induced gel formation by soy proteins at neutral pH. *Journal of Agricultural and Food Chemistry, 50*(6), 1569–1573. https://doi.org/10.1021/jf010763l

Shimada, K., & Matsushita, S. (1980). Gel formation of soybean 7S and 11S proteins. *Agricultural and Biological Chemistry, 44*(3), 637–641. https://doi.org/10.1080/00021369.1980.10863995

Silva, J. V. C., Cochereau, R., Schmitt, C., Chassenieux, C., & Nicolai, T. (2019). Heat-induced gelation of mixtures of micellar caseins and plant proteins in aqueous solution. *Food Research International, 116*, 1135–1143. https://doi.org/10.1016/j.foodres.2018.09.058

Stading, M., & Hermansson, A.-M. (1990). Viscoelastic behaviour of β-lactoglobulin gel structures. *Food Hydrocolloids, 4*(2), 121–135. https://doi.org/10.1016/S0268-005X(09)80013-1

Stading, M., Langton, M., & Hermansson, A.-M. (1993). Microstructure and rheological behaviour of particulate β-lactoglobulin gels. *Food Hydrocolloids, 7*(3), 195–212. https://doi.org/10.1016/S0268-005X(09)80172-0

Suchkov, V. V., Popello, I. A., Grinberg, V. Y., & Tolstoguzov, V. B. (1990). Isolation and purification of 7S and 11S globulins from broad beans and peas. *Journal of Agricultural and Food Chemistry, 38*(1), 92–95.

Sun, X. D., & Arntfield, S. D. (2011). Gelation properties of salt-extracted pea protein isolate induced by heat treatment: Effect of heating and cooling rate. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2010.07.063

Utsumi, S., & Kinsella, J. E. (1985). Structure-function relationships in food proteins: Subunit interactions in heat-induced gelation of 7S, 11S, and soy isolate proteins. *Journal of Agricultural and Food Chemistry, 33*(2), 297–303.

Van Vliet, T. (2013). *Rheology and fracture mechanics of foods*. CRC Press.

Warsame, A. O., Michael, N., O'Sullivan, D. M., & Tosi, P. (2020). Identification and quantification of major faba bean seed proteins. *Journal of Agricultural and Food Chemistry, 68*(32), 8535–8544. https://doi.org/10.1021/acs.jafc.0c02927

Zheng, B., Matsumura, Y., & Mori, T. (1991). Thermal gelation mechanism of legumin from broad beans. *Journal of Food Science, 56*(3), 722–725.

Zheng, B.-A., Matsumura, Y., & Mori, T. (1993a). Relationship between the thermal denaturation and gelling properties of legumin from broad beans. *Bioscience, Biotechnology, and Biochemistry, 57*(7), 1087–1090.

Zheng, B.-A., Matsumura, Y., & Mori, T. (1993b). Relationships of molecular forces to rheological and structural properties of legumin gels from broad beans. *Bioscience, Biotechnology, and Biochemistry, 57*(8), 1257–1260. https://doi.org/10.1271/bbb.57.1257

Web references

Sverigeförsöken. (2012). Retrieved from http://www.forsoken.se/Mellansvenska/rapport2012.htm. (Accessed 23 November 2022).

II

Food Hydrocolloids 138 (2023) 108494

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Pasting and gelation of faba bean starch-protein mixtures

Klara Nilsson [a,**,1], Mathias Johansson [a,*,1], Corine Sandström [a], Hanna Eriksson Röhnisch [a], Mikael S. Hedenqvist [b], Maud Langton [a]

[a] Department of Molecular Sciences, Swedish University of Agricultural Sciences, Box 7015, SE-750 07, Uppsala, Sweden
[b] Division of Polymeric Materials, Department of Fibre and Polymer Technology, KTH-Royal Institute of Technology, 10044, Stockholm, Sweden

ARTICLE INFO

Keywords:
Faba bean
Protein
Starch
Texture
Microstructure
Gelation

ABSTRACT

Starch and protein are major components in many foods, contributing to nutritional and textural properties. Understanding how the behaviour and interactions of these components contribute to different textures is important. In this study, mixed gel systems were created with different ratios of starch to protein (constant solid content 12%) extracted from faba bean, a promising crop for locally produced plant-based foods in cold climate regions. The mixed starch-protein gels were characterised in terms of pasting, texture and microstructure. Starch-rich mixtures showed higher water binding and water absorption than samples with higher protein content. A tendency for more efficient hydration in starch-rich samples was confirmed by NMR. Iodine affinity appeared to be lower for high-protein samples, particularly at higher temperatures. Mixtures with high starch content also showed higher viscosity during pasting, higher storage modulus throughout gelation, lower tan δ and lower frequency dependence of the final gel. Characterisation by compression tests showed stronger and more elastic gels with increasing starch content. Light microscopy revealed that starch granules were tightly packed, especially at higher starch content, with protein filling the spaces between starch granules. SEM micrographs revealed a network structure with larger pores and thicker strands in samples with higher starch content. Overall, increasing protein content reduced viscosity during pasting and caused softer gels, likely owing to different gelation and hydration properties of starch and protein.

1. Introduction

Transitioning to more plant-based diets is suggested to have nutritional and environmental benefits (Röös et al., 2020; Willett et al., 2019). Across Europe, a trend for greater interest in plant-based foods is emerging (Aschemann-Witzel, Gantriis, & Fraga, 2021). There are many reasons why consumers opt for plant-based foods, including health, sustainability and animal welfare (Aschemann-Witzel et al., 2021). Consumers are now demanding a greater array of plant-based products using simple ingredients with good organoleptic properties. Faba bean already has an established market for animal feed and an emerging market for human consumption, and is a high-protein crop that can be grown in Northern Europe. With increased research and technological innovation, faba bean has the potential to be used in locally produced and sustainable plant-based foods (Multari, Stewart, & Russell, 2015).

Under appropriate processing conditions, faba bean starch and faba bean protein can both act as a gelling agent, but with different gelling abilities and properties. In real food matrices, starch and protein are likely to be blended and interactions between the biopolymers will influence the formation and texture of the final gel. Depending on the final food product, different gel textures and structures will be acceptable. The gelling properties and mixability of starch and protein are among the most important features in food formulation (Onwulata, Tunick, & Thomas-Gahring, 2014; Schorsch, Wilkins, Jones, & Norton, 2001).

Gelatinisation is a process whereby starch granules swell and leach out amylose during heating in excess water. According to Walstra (2002), as the system cools a gel can develop through the formation of microcrystallites between the amylose chains and/or as a closely packed system of granules, with the amylose layer acting as glue between the granules. Faba bean starch gels are reported to be strong and firm (Li et al., 2019). Faba bean also has high final viscosity during pasting, which has been attributed to the relatively high amylose content

* Corresponding author.
** Corresponding author.
E-mail addresses: klara.nilsson@slu.se (K. Nilsson), mathias.johansson@slu.se (M. Johansson).
[1] These authors contributed equally.

https://doi.org/10.1016/j.foodhyd.2023.108494
Received 2 November 2022; Received in revised form 30 December 2022; Accepted 18 January 2023
Available online 19 January 2023

(Ambigaipalan et al., 2011; Li et al., 2019; Punia, Dhull, Sandhu, & Kaur, 2019; Zhang, Tian, Wang, Jiang, & Li, 2019) that aggregates to form a gel network upon cooling. High amylose content has also been related to retarded swelling of starch granules (Nilsson et al., 2022; Sasaki & Matsuki, 1998; Tester & Morrison, 1990; Vamadevan & Bertoft, 2020). Faba bean starch has been found to leach out more amylose than other bean varieties with similar amylose content, contributing to a rise in viscosity (Ambigaipalan et al., 2011; Ambigaipalan, Hoover, Donner, & Liu, 2013). Two other factors that result in faba bean starch gels having higher viscosity than e.g. wheat starch gels are larger size of the swollen granules and higher prevalence of larger polymer chains (Nilsson et al., 2022). Amylose and amylopectin molecular weight in faba bean starch is reported to be 10–20 MDa and 60–100 MDa, respectively, and heating the starch at a higher rate (12 °C/min vs 1.5 °C/min) to temperatures below peak viscosity (119 °C for faba bean) has been found to produce the most viscous gels (Nilsson et al., 2022). The temperature range for gelatinisation of faba bean starch has been shown to be 59–75 °C (Ambigaipalan et al., 2011; Li et al., 2019; Nilsson et al., 2022; Punia et al., 2019; Zhang et al., 2019), with pasting commencing at around 77 °C.

Similarly to starch gelatinisation, protein gelation is an essential process in the production of many foods. Most legumes, including faba bean, contain mainly globular proteins (Kimura et al., 2008; Nicolai & Chassenieux, 2019). The globular proteins in faba bean can be divided into two major fractions, hexameric legumin-type (11S) and trimeric vicilin-type (7S) globulins, with an approximate size of 330 and 150 kDa, respectively (Sharan et al., 2021; Warsame, O'Sullivan, & Tosi, 2018). Gelation of globular proteins occurs by complete or partial denaturation of the protein, exposing hydrophobic residues and other interaction sites (Zha, Rao, & Chen, 2021). The newly exposed parts of the protein interact and aggregate to form a network if the protein concentration is sufficiently high (Zha et al., 2021). The gelation process and gel properties of proteins are affected by multiple factors, such as pH, presence of salt, extraction method and protein source (Langton et al., 2020; Ma et al., 2022; Nicolai & Chassenieux, 2019). A denaturation point within the range 75–95 °C, depending on the ionic strength of the environment, has been reported for the 7S and 11S fractions in faba bean protein (Kimura et al., 2008). Gelation of faba bean protein and the effects of salt, pH and extraction method have been studied previously (Langton et al., 2020). The effect of partly replacing faba bean protein with starch and/or fibre has been investigated previously, resulting in an increased storage modulus of gels whilst the fracture stress was simultaneously reduced (Johansson, Johansson, et al., 2022).

In brief, concentrated starch gels can be described as a composite system consisting of swollen granules embedded in a three-dimensional network of aggregated amylose chains (Yang, Irudayaraj, Otgonchimeg, & Walsh, 2004), while globular proteins form fine-stranded gel networks at high repulsion or a coarse-stranded network of colloidal particles as the isoelectric point is approached (Langton et al., 2020; Langton & Hermansson, 1992). Depending on the ratio of starch to protein, gel formation and properties may differ, with e.g. higher protein content in starch-protein composite gels being associated with augmented pasting temperature and reduced gel firmness (Bravo-Núñez, Garzón, Rosell, & Gómez, 2019; Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014; Núñez-Santiago, Bello-Pérez, & Tecante, 2004; Oñate Narciso & Brennan, 2018; Onwulata et al., 2014; Ribotta, Colombo, León, & Añón, 2007; Yang et al., 2004). According to Eliasson (1983), higher protein content alters the water retention capacity of the system because the proteins compete with starch for available water, thereby causing an increase in pasting temperature. Protein adsorption to granule surfaces during pasting could explain both the reduced pasting viscosity and increased pasting temperature, as the adsorbed proteins would restrict water diffusion and thus reduce and delay granule swelling (Bravo-Núñez et al., 2019; Bravo-Núñez & Gómez, 2019; Oñate Narciso & Brennan, 2018). Joshi et al. (2014) found that the pasting temperature increased from 73.5 °C in 100% starch systems to 82.4 °C in 50:50 lentil starch/lentil protein systems, and attributed this to the characteristically high denaturation temperature (118 °C) of lentil protein isolate. The decrease in pasting viscosity with increasing protein content may also be partly due to an overall reduction in the starch fraction, as starch tends to form more viscous pastes. However, the apparent viscosities of blends were reported to be higher in the corresponding diluted starch systems, indicating that protein-starch interactions also alter the gelatinisation and gelling properties (Bravo-Núñez et al., 2019; Bravo-Núñez & Gómez, 2019).

Breakdown viscosity has also been found to be lower for starch-protein mixtures compared to that of the corresponding sample containing only starch (Oñate Narciso & Brennan, 2018; Onwulata et al., 2014). In a study adding 10% extra protein, breakdown of starch-protein mixed pastes was no longer detected, suggesting that the protein gel network increased the resistance to mechanical shearing (Joshi et al., 2014). During cooling of starch dispersions, gelation occurs as amylose and amylopectin aggregate to form a gel network, but higher protein content has been found to correlate with a reduction in final viscosity (Bravo-Núñez et al., 2019; Bravo-Núñez & Gómez, 2019; Joshi et al., 2014; Oñate Narciso & Brennan, 2018; Onwulata et al., 2014; Ribotta et al., 2007). The amylose:amylopectin ratio in starch has been shown to have an effect on the viscosity and gel strength of composite gels, with the reduced viscosities associated with incorporation of protein being more pronounced for high-amylose starches (Joshi et al., 2014; Oñate Narciso & Brennan, 2018). Studies by Oñate Narciso and Brennan (2018) and Onwulata et al. (2014) revealed that proteins appeared to prevent molecular rearrangement of amylose, resulting in weaker gels. In contrast, the rigidity of high-amylopectin starch gel has been shown to increase with additional protein, as the protein acts as a filler (Onwulata et al., 2014).

Gels with higher solids content tend to be firmer and stronger, e.g. Yang et al. (2004) found that the reducing effect of protein on gel firmness became less marked as the solids content increased. Shim and Mulvaney (2001) observed a similar effect in gels with 15% solids content, where addition of whey protein isolate had a diluting effect on corn starch (Shim & Mulvaney, 2001). As the solids content increased to 30% in that study, separate phases in the mixtures were still present but the complex modulus (G*) increased, suggesting that the gel structure was maintained by the higher solids content.

The aim of the present study was to evaluate gel formation and pasting properties of different faba bean starch-protein gel mixtures. Gel microstructures were evaluated and continuous and discrete phases in the gels were identified. The gel structures observed were then compared against physical and textural attributes of the gels. By understanding the role of protein and starch in composite gel systems, products with tailored textures and functionality can be developed.

2. Materials and methods

To assess gel formation and properties, different analytical techniques were applied at different stages of gel production. The key steps in gel production are summarised, together with the analytical techniques applied during the different steps of gel formation, in Fig. S1 in Supplementary Information (SI).

2.1. Materials

The protein and starch fractions used in this study were isolated from dehulled and finely milled faba beans (*Vicia faba* var. Gloria) grown in central Sweden, harvested and dried in 2016. The beans were kindly provided by RISE (Research Institutes of Sweden). Based on previous characterisations by Johansson, Johansson, et al. (2022), the composition of the protein fraction was: protein 77.3%, starch 0.3%, fibre 1.0%, fat 3.4% and ash 8%, while that of the starch fraction was: protein 0.5%, starch 94.5%, fibre 2.2%, fat 0.3% and ash 0.2%.

Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were

purchased from Merck, ethanol from Solveco, osmium tetroxide and glutaraldehyde from Ted Pella, ruthenium red and light green from Sigma-Aldrich, Technovit 7100 from Kulzer and iodine from Fluka.

2.2. Extraction

Extraction of the protein and starch fractions was performed using methodology described previously (Johansson, Nilsson, Knab, & Langton, 2022). In brief, dehulled and milled faba beans were dispersed in de-ionised water with pH adjusted to 9. Protein was separated from starch and fibre by centrifugation and precipitated from the supernatant by adjusting the pH to 4. The mixture was further centrifuged and washed once before adjusting the pH back to 7 and freeze-drying. The pellet from the initial centrifugation was used to extract starch. First, the pellet was re-dispersed in de-ionised water and the pH was adjusted to 9.5. The mixture was then stirred for 24 h at room temperature and left to stand without agitation for an additional 24 h at 4 °C, followed by centrifugation and washing until pH 7 was reached. Finally, the starch was separated by filtering through a 70 μm nylon filter and dried at 40 °C.

2.3. Gel formation

Starch and protein flours were mixed well and then dispersed in distilled water to obtain a final concentration of 12% (g flour/g sample) The following starch:protein (S%P%) ratios were produced (values in brackets are percentage starch or protein in the total sample):

S100P0: Starch 100% (12%), protein 0% (0%)
S90P10: Starch 90% (10.8%), protein 10% (1.2%)
S80P20: Starch 80% (9.6%), protein 20% (2.4%)
S70P30: Starch 70% (8.4%), protein 30% (3.6%)
S60P40: Starch 60% (7.2%), protein 40% (4.8%)
S0P100: Starch 0% (0%), protein 100% (12%)

Initial trials conducted to identify the ideal solids content for the gels revealed that 12% solids content was a good intermediate concentration, as S60P40 samples were able to form freestanding gels and S90P10 samples did not form too viscous pastes for handling during the gel-making process.

To make the gels, the dispersions were stirred at 500 rpm at room temperature for 40 min and then stirred in a water bath at 65 °C for an additional 20 min, with the aim of avoiding sedimentation of the starch and maintaining a homogenous mixture for gelation. To produce gels of suitable size for compression tests and microscopy, 3-mL samples were loaded into hollow glass tubes with inner diameter 12 mm. The bottom of each glass tube was closed with a rubber lid and the top with thread tape punctured with a small hole to prevent pressure build-up. The samples were heated in a water bath (DYNEO DD-1000F Refrigerated/heating circulator, Julabo, Seelbach, Germany) from 65 to 95 °C at a rate of 1.5 °C/min. After a 30-min holding time at 95 °C, the samples were cooled to 25 °C at a rate of 1.5 °C/min. The gels were then left at room temperature for approximately 30 min before being stored at 4 °C overnight.

2.4. Hydration and water binding properties by centrifugation

Hydration and water binding properties were analysed before and after heat treatment, following the methodology of Bravo-Núñez and Gómez (2019) with some slight modifications. The hydration properties were assessed by measuring water binding capacity (WBC), i.e. the amount of water retained by samples before heating, which was determined by making 12% (w/v) flour mixture-water solutions. To ensure complete dispersion, the samples were quickly vortexed before being magnetically stirred for 40 min at 500 rpm. The samples were then centrifuged at 580×*g* for 10 min, the excess water was removed and the remaining hydrated solids were weighed. WBC was calculated as grams of water retained per gram of dry sample.

For further assessment of the water binding properties after heat treatment, water absorption index (WAI), swelling power (SP) and water solubility index (WSI) were determined (Bravo-Núñez & Gómez, 2019). For analysis, 120 mg of sample (Wi) were dispersed in 1 mL of de-ionised water and the samples were prepared in the same manner as described in section 2.3 until cooling to 25 °C. The samples were then left to stand overnight at 4 °C, prior to being centrifuged at 3000×*g* at 4 °C for 10 min. The supernatant was decanted and dried at 105 °C for 8 h, giving the dry solids weight (Ws). The remaining pellet was also weighed (Wr). WAI, WSI and SP were calculated using the following equations:

$$\textit{Water Absorption Index}\left(\frac{g}{g}\right)=\frac{Wr}{Wi} \tag{1}$$

$$\textit{Water Solubility Index}\left(\frac{g}{100g}\right)=\frac{Ws}{Wi}*100 \tag{2}$$

$$\textit{Swelling Power}\left(\frac{g}{g}\right)=\frac{Wr}{Wi-Ws} \tag{3}$$

2.5. NMR spectroscopy

Sample preparation for nuclear magnetic resonance (NMR) spectroscopy followed the method described by Larsen et al. (2013) with slight modification. For this, 50 μg of flour or flour mixtures with starch: protein ratio 60:40 and 90:10 were weighed out and packed into 4 mm ZrO_2 rotors. The moisture content in the flours was 4.0% (starch) and 6.4% (protein). Then 50 μL of D_2O were inserted into the rotor using a Hamilton Microliter® #810 syringe. To equilibrate the D_2O within the rotor, the samples were left to stand for 1 h prior to spinning at 9 kHz for 1 h to ensure proper mixing of flour and D_2O.

NMR spectra (carbon-13 (^{13}C) cross-polarisation magic angle spinning (CPMAS) and single-pulse excitation magic angle spinning (SPMAS) were obtained using a Bruker Avance III 600 MHz spectrometer equipped with a double-resonance 4 mm (1H&19F)/(15N–31P) CPMAS probe. The CPMAS spectra were recorded with a contact time of 1–2 ms and a repetition delay of 2.5–5 s. The NMR measurements were performed at a spinning frequency of 8 kHz and at three different temperatures; first at 25 °C before heating, then at 85 °C and finally at 25 °C after cooling. The maximum temperature was set to 85 °C, due to the limits of the instrument. All samples were analysed in triplicate.

2.6. Hot-stage microscopy

Swelling of the starch granules was visualised following the method described by Nilsson et al. (2022). Suspensions with starch-protein mixtures of 10 mg/mL were mixed for 40 min and then 80 μL of sample were pipetted onto a cover slip and 15 μL of diluted Lugol's stock solution were added to dye the starch. The samples were covered with a slightly smaller coverslip and sealed using nail polish. A tensile strength test stage (model TST350, Linkam Scientific Instruments, Surrey, UK) with heating capacity was attached to the light microscope (Nikon Eclipse Ni–U microscope, Tokyo, Japan) for temperature control. The heating rate was 5 °C/min within the range 30–95 °C. Samples were observed under bright-field light using a Plan Fluor 10 × (0.30 N.A.) objective. For observations under polarised light, samples were prepared in the same manner, with the exception of iodine staining. Micrographs were captured with a Nikon Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan) every 12 s. Captured micrographs had a pixel size of 1.21 μm (data size: 2560 × 1920 pixels). All micrographs acquired were analysed and compared visually, to pinpoint the temperature at which major changes in granules occurred.

2.7. Effect of heating on iodine dyeing of starch

To test the effect of heating on iodine dyeing, 200 mg flour were dispersed in 20 mL distilled water in capped heat-proof glass test tubes, 2 mL of Lugol's solution were added to each sample and the samples were heated in a water bath from 20 to 95 °C at 1.5 °C/min. Starting at 20 °C and at 10 °C intervals up to the final temperature (90–95 °C), the test tubes were shaken to ensure that flour and iodine were dispersed in the mixtures and 1-mL aliquots of each solution were collected in separate Eppendorf tubes and immediately placed on ice to cool. Duplicate 200 μL aliquots were then pipetted onto a 96-well plate. The samples were shaken for 2 min and absorbance was measured at 510 nm. Distilled water with iodine subjected to the same heat treatment was used as a control. All samples were analysed in duplicate.

2.8. Rheology

2.8.1. Pasting (95 °C)

Pasting data were obtained using a Discovery HR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with a Peltier pressure cell and steel starch paddle. Starch-protein mixtures with a solids content of 12% in solution were mixed at 20 °C for 40 min at the instrument maximum of 50 rad/s. For the remainder of the experiment the stirring rate was set to 16.75 rad/s, which is equivalent to the standard rotational rate of 160 rpm used in the rapid visco-analyser. As in the gelation process, samples were heated to 95 °C at a heating rate of 1.5 °C/min and kept at 95 °C for 30 min under constant stirring. Samples were cooled to 25 °C at a cooling rate of 1.5 °C/min and then kept for 30 min at 25 °C under constant stirring. All samples were analysed in duplicate.

2.8.2. High-temperature pasting (150 °C)

To investigate how the starch:protein ratio influenced peak viscosity, high-temperature pasting measurements were performed. Previous work has reported that peak viscosity of (pure) faba bean starch occurs at 119 °C (Nilsson et al., 2022). However, initial high-temperature pasting trials on the starch-protein mixtures in the present study revealed that the samples needed to be heated to 150 °C to guarantee that peak viscosity was achieved for all samples.

As in the lower-temperature pasting experiments (section 2.8.1), the flour solutions were mixed in the instrument for 40 min at 50 rad/s at 20 °C. During the pasting analysis, the heating cycle used was 20–150-25 °C with a heating and cooling rate of 5 °C/min and a constant stirring rate of 16.75 rad/s. The samples were kept for 5 min at the maximum temperature (150 °C) and 5 min at the end-set (25 °C) temperature. The main aim of the high-temperature pasting analysis was to determine how peak viscosity differed between the samples and identify any behavioural pattern present depending on the starch:protein ratio. The higher heating/cooling rate was applied to improve experimental efficiency, by reducing the time required for each trial, as overall relative pasting behaviour of the samples did not appear to change. Samples were analysed in duplicate.

2.8.3. Oscillatory rheology

The gelation process and viscoelastic properties of the gels were evaluated using a Discovery HR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with a 40-mm aluminium plate. Samples were prepared as described in section 2.3, excluding the final gelation step in the glass tubes. Paraffin oil, covering the exposed parts of the sample, was combined with a custom-made solvent trap to limit evaporation. The gelation process was monitored at 0.5% strain during a temperature ramp consisting of heating from 65 °C to 95 °C at a rate of 1.5 °C/min and 30 min holding time at 95 °C before cooling at 1.5 °C/min to 25 °C, followed by an additional holding time of 30 min. After the temperature ramp, a frequency sweep was run from 0.628 to 100 rad/s at 0.5% strain and 25 °C. Finally, an amplitude sweep was performed from 0.01 to 100% at 1 Hz and 25 °C to determine the linear viscoelastic region (LVR) and ensure that all measurements were performed within this region. The LVR was defined as the strain at which a 5% loss in storage modulus was observed. All oscillatory rheology measurements were performed in triplicate.

2.9. Compression tests

Gels prepared as described in section 2.3 were allowed to equilibrate to room temperature for 1 h before being cut into cylindrical pieces with height 14 mm and diameter 12 mm. The samples were compressed to 70% of their original height at a rate of 1 mm/s 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. A trigger force of 0.05 N was used to initiate measurement and data collection. Due to the weak nature of the 60:40 gels, a trigger force of 0.01 N was used to reduce the compression of these gels occurring before data collection started. To account for changes in cross-section during compression, true stress and true strain were calculated as described previously (Munialo, van der Linden, & de Jongh, 2014). True fracture stress and true fracture strain (hereafter referred to simply as fracture stress and fracture strain) were defined as the maximum true stress and corresponding true strain at the first clear peak before fracture. Young's modulus was calculated as the slope of the true stress-true strain curve during the initial 1–5% of deformation. This specific region (1–5%) was chosen to be within the initial linear region of the curve without including the potentially noisy first few measurements. Compression tests were performed for each sample, in three batches of 4–8 gels each. Statistical analysis was performed on the mean value obtained for each batch.

2.10. Microstructural characterisation of gels

Gels for microscopy were prepared as described in section 2.3. Samples for both light microscopy and scanning electron microscopy (SEM) were prepared similarly as described previously (Johansson, Johansson, et al., 2022).

2.10.1. Light microscopy

Gels were cut into approximately $2 \times 2 \times 2$ mm^3 cubes and fixated overnight in 2.5% glutaraldehyde and 0.1% ruthenium red. The gels were then further fixated in 1% osmium tetroxide for 2 h, followed by dehydration in a series of solutions with increasing ethanol concentration. Thereafter, the gels were embedded using Technovit 7100 and sectioned (Leica Microsystems GmbH, Leica EM UC6, Wetzlar, Germany) into 1 μm thick sections. The light microscope (Nikon, Eclipse Ni–U microscope, Tokyo, Japan) was equipped with a 60 × (1.4 N.A.) plan apochromatic objective and micrographs were captured using a Nikon Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan) with 0.08 μm/pixel (data size: 2560 × 1920 pixels).

2.10.2. Scanning electron microscopy

For SEM, samples were prepared as for light microscopy up to dehydration in ethanol. After dehydration, the samples were critical point-dried (Quorum Technologies Ltd, K850 Critical Point Dryer, East Sussex, UK), fractured and sputter-coated with gold (Cressington Scientific Instruments, Sputtercoater-108 auto, Watford, UK). Samples were then examined in the SEM device (Hitachi, FlexSEM 1000II, Tokyo, Japan) at 5 kV and micrographs were digitally recorded at two different magnifications (9.92 and 4.96 nm/pixel, data size: 2560 × 1920 pixels).

2.11. Statistical analyses

Data on hydration and water binding properties, rheological measurements and results of compression tests were analysed by analysis of variance (ANOVA) and pairwise comparison (Tukey) with a significance level of 95% ($p < 0.05$), using R studio (Version 1.2.5033, RStudio Inc., MA, USA). After evaluation of the residuals, G′, end of LVR, n-values and

fracture stress values were log-transformed to obtain normally distributed residuals before statistical analysis (ANOVA and pairwise comparison (Tukey)). Similarly, the inverse of tan δ values was used in statistical analysis. Pearson correlation coefficients were calculated to gain an overall understanding of parameters affected by the starch: protein ratio and to determine whether correlations between the parameters were significant.

3. Results and discussion

3.1. Hydration and water binding properties

The water binding capacity (WBC) increased with increasing starch content (Table 1). A negative WBC was observed for the sample containing only protein (S0P100), presumably because the protein dissolved in the water and was decanted with the supernatant.

The water absorption index (WAI) values of all samples increased dramatically after heat treatment but the gels with higher starch content still had higher water absorption, indicating that starch had a higher affinity for water than protein. The swelling power (SP) value also increased with increasing starch content and the correlation between WAI and SP was high ($r = 0.86$; $p = 0.002$). The swelling power of starch granules has previously been found to correlate with amylopectin content and faster heating rate (Nilsson et al., 2022; Tester & Morrison, 1990).

The WSI value of the samples increased with increasing protein content. The supernatant from both the hydration and gelation analysis was much clearer for the composite mixtures with higher starch content, corresponding to lower WSI.

3.2. NMR results

The ^{13}C-CPMAS NMR spectra at 25 °C of powder of starch, protein and a 50:50 starch-protein mixture are shown in Fig. 1, with assignments of the C1 to C6 resonances of the glucose-repeating unit in the starch indicated on top of the spectra. Assignments of the major protein signals are also shown.

Hydration often leads to large changes in the molecular mobility of starch. ^{13}C solid-state NMR has been used in a number of studies to follow hydration of the polysaccharide (Garbow & Schaefer, 1991; Larsen, Blennow, & Engelsen, 2008; Larsen et al., 2013; Morgan, Furneaux, & Larsen, 1995; Tang & Hills, 2003; Zhu, 2017). Two types of ^{13}C NMR spectroscopy, CPMAS and SPMAS, were used to monitor hydration in this study. Cross-polarisation (CP) depends on heteronuclear dipolar couplings between the ^{1}H and ^{13}C nuclei and if these couplings are averaged because of rapid molecular motion due to hydration, then CP will not occur. NMR signals from mobile components can be observed instead by a single pulse NMR experiment as performed in the solution-state. The CPMAS signals are interpreted as "rigid" carbons and SPMAS signals as "mobile" carbons. Thus, in CPMAS spectra, resonances

Table 1
Hydration and water binding properties of the starch-protein (S%P%) mixtures. Values shown are mean ± 1 st.dev. Different superscript letters indicate significant differences ($p < 0.05$).

	Water binding capacity (WBC)	Water absorption index (WAI)	Water solubility index (WSI)	Swelling power (SP)
S100P0	1.4 ± 0.0^{a}	11.7 ± 0.4^{a}	2.5 ± 0.1^{f}	12.0 ± 0.4^{a}
S90P10	1.2 ± 0.0^{b}	10.3 ± 0.2^{b}	5.7 ± 0.4^{e}	10.9 ± 0.2^{b}
S80P20	1.1 ± 0.0^{c}	9.5 ± 0.0^{c}	12.5 ± 0.5^{d}	10.8 ± 0.03^{b}
S70P30	0.9 ± 0.1^{d}	8.1 ± 0.1^{d}	20.0 ± 0.6^{c}	$10.2 \pm 0.1^{b,c}$
S60P40	0.8 ± 0.0^{e}	7.0 ± 0.3^{e}	29.7 ± 2.6^{b}	9.9 ± 0.1^{c}
S0P100	-0.4 ± 0.1^{f}	2.5 ± 0.2^{f}	73.6 ± 1.8^{a}	9.6 ± 0.4^{c}

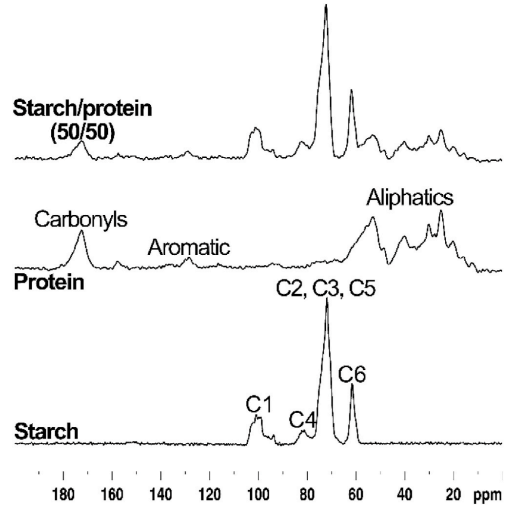


Fig. 1. Nuclear magnetic resonance ^{13}C-CPMAS spectra of powder of (top) 50:50 (w/w) starch-protein mixture, (middle) protein and (bottom) starch.

from carbons originating from immobile regions are enhanced, while in SPMAS spectra carbon resonances from immobile and mobile regions are enhanced.

Addition of water to starch resulted in attenuation of signal intensities in the amorphous region. After addition of water, the signals around 80–84 ppm, corresponding to C4, almost completely disappeared (Figs. 1 and 2). This is consistent with previous findings and derives from hydration-induced mobilisation of the amorphous starch, leading to reduced cross-polarisation efficiency. Hydration also resulted in an increase in spectral resolution for the ordered polysaccharide, due to reduced conformational distribution and substantial decreases in the signals from the amorphous region. The signals in the SPMAS spectra were sharp and the spectra were similar, although with broader resonances, to the solution-state spectra of soluble starch (Fig. 2). The increase in temperature to 85 °C led to a strong decrease in signal to noise ratio in the CPMAS spectra, but to a significant increase in spectral resolution in the SPMAS spectra. This indicates, as shown previously by others, that gelatinisation reduced the amount of immobile fraction of starch. Lowering the temperature back to 25 °C led to NMR spectra similar to those obtained before the temperature increase.

Addition of water to the S90P10 and S60P40 samples gave CPMAS and SPMAS spectra for starch that were similar to those obtained for starch alone (Figs. 2 and 3). However, closer inspection showed some small differences between the S90P10 and S60P40 samples, e.g. the C4 resonances at 80–84 ppm were not completely eliminated in the CPMAS spectra of the S60P40 samples at 25 °C. Narrower lines in the SPMAS NMR spectra are indicative of more efficient hydration and higher mobility. Thus, the narrower line width of the resonance in the SPMAS spectra of the S90P10 samples (60–90 Hz) compared with the S60P40 samples (130–140 Hz) suggests that hydration of starch was slightly more efficient in the S90P10 samples at 25 °C.

The differences between the S90P10 and S60P40 samples became more clear upon heating to 85 °C. The S90P10 samples showed results similar to that of pure starch, with a clear decrease in the signal to noise ratio in the CPMAS spectra and a significant increase in spectral resolution in the SPMAS spectra (Fig. 3). In contrast, only a slight decrease in the signal to noise ratio in the CPMAS spectra was observed for the S60P40 samples upon heating. Furthermore, the increase in spectral

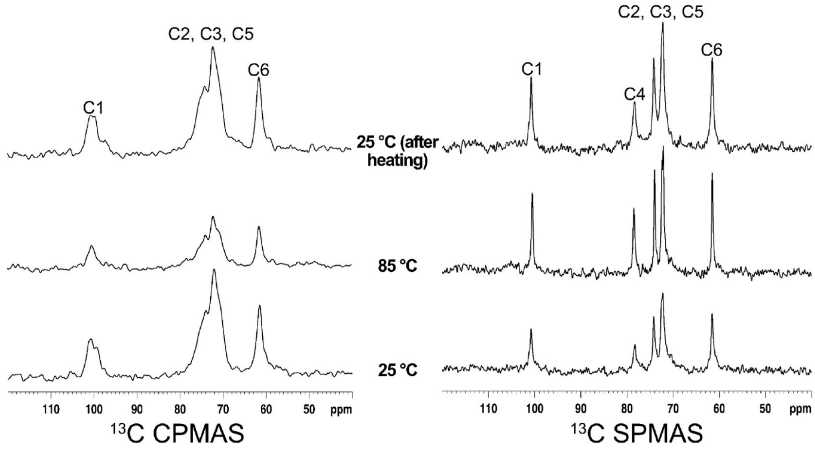


Fig. 2. Nuclear magnetic resonance ^{13}C CPMAS spectra (left) and SPMAS spectra (right) of hydrated starch (top) at 25 °C after heating to and cooling from 85 °C, (middle) at 85 °C and (bottom) at 25 °C. CPMAS signals interpreted as "rigid" carbons and SPMAS signals as "mobile" carbons.

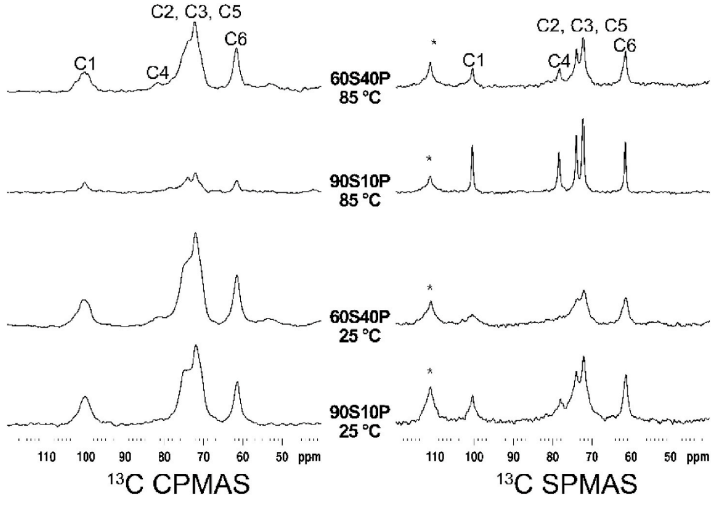


Fig. 3. Nuclear magnetic resonance ^{13}C CPMAS spectra (left) and ^{13}C SPMAS spectra (right) of (top to bottom) the samples S60P40 at 85 °C, S90P10 at 85 °C, 60S40P at 25 °C and 90S10P at 25 °C. *Background signal.

resolution was less pronounced for the S60P40 samples compared to the S90P10 samples. This indicates that the amount of immobile fraction was reduced less upon heating when more protein was present in the system. I.e. the hydration of starch seemed to be hampered by the addition of protein, which in turn could potentially influence the physiochemical properties of the starch. It is possible that, due to the relatively high solid to liquid ratio used for NMR experiments, the observed difference is simply a result of the protein absorbing water and reducing the water availability for the starch. However, further studies would be needed to clarify the mechanism behind this effect.

The assessments of water binding properties (Table 1) showed that WBC, WAI and SP of the mixtures increased as starch content increased, which may be because of increased starch content but also, as indicated by the NMR results, because of differences in hydration efficiency between the starch and protein. Addition of gluten (Eliasson, 1983), soy (Ribotta et al., 2007) and whey (Oñate Narciso & Brennan, 2018) protein has been shown to delay diffusion of water into starch due to its presence on the surface of starch granules. The decrease in hydration efficiency with increasing content of faba bean protein may have been the result of limited water diffusion.

3.3. Hot stage microscopy

Micrographs of the different starch-protein samples at 30, 67, 73 and 90 °C are shown in Fig. 4. At 30 °C, the colour intensity, size and shape of the starch granules were similar for all samples. As the temperature

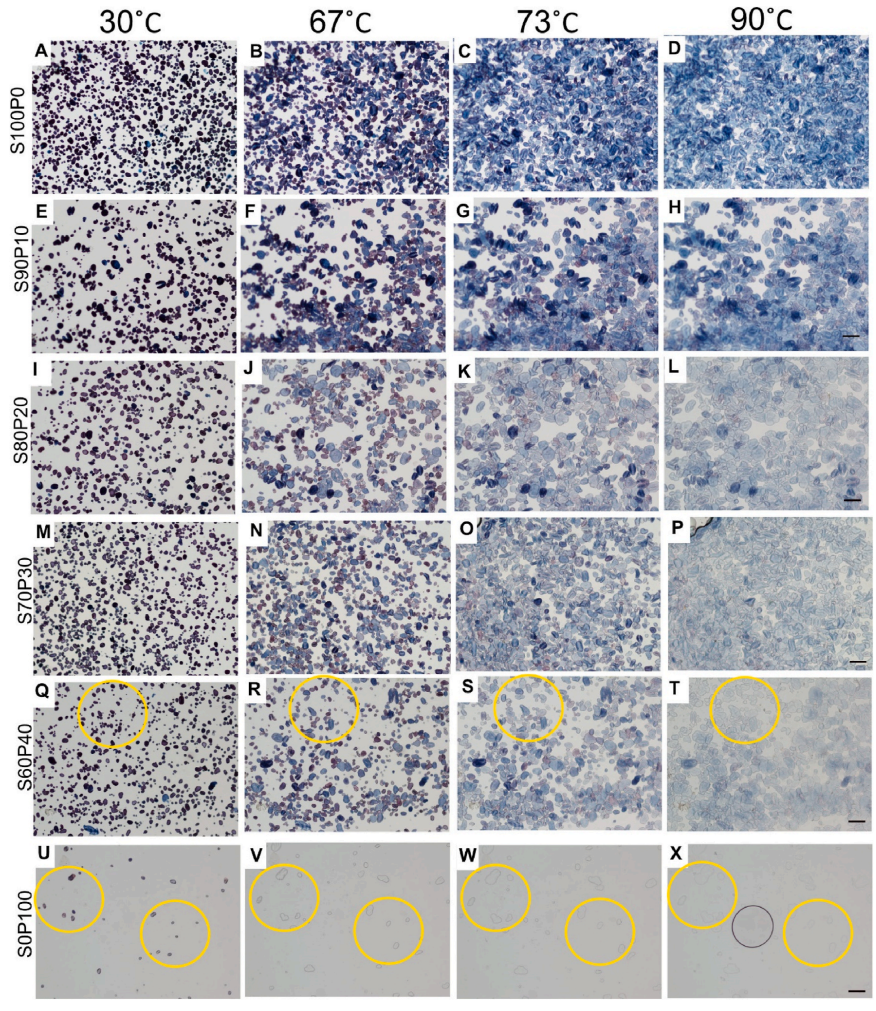


Fig. 4. Micrographs of samples S100P0 (A–D), S90P10 (E–H), S80P20 (I–L), S70P30 (M–P), S60P40 (Q–T) and S0P100 (U–X) under hot-stage microscopy at a temperature of 30 °C (first column A-U), 67 °C (second column B–V), 73 °C (third column C–W) and 90 °C (fourth column D-X). Scale bar = 100 μm. The starch granules were stained purple/blue by iodine, with the colour intensity decreasing for each sample as the temperature increased. The yellow circles for samples S60P40 and S0P100 indicate areas where starch granules had completely lost their colour. The grey circle, as seen in figure X, indicate air bubbles.

increased the starch granules began to swell, with swelling initiating at around 63 °C and the most pronounced swelling at 70 °C. There was a gradual increase in granule size as the temperature increased to 67 °C, 73 °C and 90 °C (Fig. 4). The gradual increase in the size of the starch granules during heating is also evident in Fig. S2 in SI. From the granule size distribution, the protein-rich sample S60P40 appeared to have the smallest granules at both 30 and 67 °C, which may tie in with the NMR observation that mixture S60P40 showed slightly less efficient starch hydration. There was a tendency for the largest starch granules to be found in samples S90P10 and S80P20, indicating that addition of protein may have had a small effect in delaying granule swelling. However, this tendency was very weak overall, with no distinct difference between the populations, making it challenging to draw any direct conclusions on whether starch swelling was delayed by the presence of protein. Furthermore, faba bean starch has a relatively high amylose content compared to mony other starches (Nilsson et al., 2022). High amylose content has previously been related to retarded swelling of starch granules (Nilsson et al., 2022; Sasaki & Matsuki, 1998; Vamadevan & Bertoft, 2020). It is possible that the effect of amylose on granule swelling was affected by the presence of protein and difference in starch content between samples.

Examination under polarised light revealed that samples with starch content ≥60% completely lost their birefringence at temperatures similar to that at which swelling occurred (around 70 °C). In the sample with no added protein (S0P100), birefringence was completely lost already at around 63 °C. Starch birefringence under polarised light is

caused by radial alignment of the crystalline amylopectin and is lost as the granules swell and lose their crystalline structure (Ambigaipalan et al., 2011; Li et al., 2019).

As the granules swelled, they became lighter and less intensely coloured. The loss of colour under progressive heating was more noticeable for the samples with higher protein content, indicating that the protein somehow interfered with or altered iodine dyeing of the starch, or that it interacted with the starch, inhibiting the formation of starch-iodine complexes. A possible explanation is that iodine formerly bound to and staining the starch granules bound to the protein instead at higher temperatures. At 95 °C, the S60P40 sample had completely lost its colour, with some of the starch granules in this sample losing their colour already at 90 °C (Fig. 4T). Areas where the starch granules in samples S0P100 and S60P40 lost their colour completely are indicated with yellow circles in the micrographs in Fig. 4. For the samples with starch content ≥70%, complete loss of colour of all granules was not observed. Samples S100P0 and S90P10 retained their colour relatively well throughout the heating (Fig. 4D and H). For sample S0P100 (Fig. 4U–X), where no starch was added to the mixture, any starch present was residual starch from protein isolation, which started to lose colour at 57 °C and lost its colour completely at around 60 °C in all micrographs.

3.4. Iodine binding capacity during heating

Fig. 5A shows the colouration of iodine staining in the different samples at different temperature intervals from 20 to 95 °C, while Fig. 5B shows the corresponding absorbance measured at 510 nm. For samples S90P10, S80P20, S70P30 and S60P40, the absorbance and observed colour intensity of the iodine staining decreased as the temperature of the mixture increased. As already observed in the hot-stage micrographs, the loss of colour was more prominent in the samples with higher protein content, with complete loss of colouration occurring at lower temperatures (50 °C for S60P40, 60 °C for S70P30, 70 °C for S80P20 and 80 °C for S90P10). For the pure starch sample S100P0, the measured absorbance remained constant up to and including 60 °C, while at 70 °C the absorbance peaked before decreasing. In a previous study, we found that gelatinisation of faba bean starch extracted from the same batch of raw beans occurred between 67 and 73 °C (Nilsson et al., 2022), so increased granule size might explain the observed peak in absorbance. As the temperature continued to increase measured absorbance decreased but, unlike in the other samples, there was no complete loss of colour for sample S100P0.

To verify that the loss in colour in the protein-rich samples was not due solely to the lower concentration of starch, a new sample S60P0 was prepared containing the same quantity of starch as the S60P40 sample but without any added protein (i.e. the overall solids content was lower). Sample S60P0 showed similar behaviour to the pure starch sample S100P0, with iodine colouration better maintained and not completely lost at higher temperatures. Hence, the loss in colour was not simply a consequence of the reduced starch content in the mixed samples.

Unheated starch in contact with iodine turns into a characteristic blue-black colour, as the iodine forms complexes inside the long helical chains of linear amylose. The highly branched amylopectin, with much shorter but more numerous chains, turns into a reddish-brown colour upon contact with iodine (Holló & Szejtli, 1958; Huber & Bemiller, 2017). Loss of colour of starch-iodine mixtures upon heating is a well-known phenomenon believed to be caused by the complex decomposing at elevated temperatures (Fonslick & Khan, 1989). The starch-iodine reaction has an exothermic equilibrium, with the iodine colouration recovering upon cooling. However, for the starch-protein mixtures in this study, the discolouration caused by heating did not recover after the samples were cooled, indicating that some interaction between protein-iodine and/or protein-starch prevented the reaction from returning to its original equilibrium. There was also no recovery of colour for the pure starch sample when tested at a lower iodine concentration (200 μL Lugol's solution instead of 2 mL) (Fig. S3 in SI). On adding iodine to the cooled samples, the mixtures turned blue-black again.

One possible explanation for the observed loss of colour is that the protein in the samples had a higher affinity for iodine than the starch, resulting in less iodine binding to starch in the presence of protein. The lower iodine concentration tested here resulted in loss of colour upon heating that was relatively similar to that seen for S60P40 (Fig. S3). Other possible explanations are complex formation between the starch and protein or protein adsorption on the surface of starch granules, preventing the iodine from binding to amylose. Encapsulation of corn starch granules by whey protein has been reported (Yang, Zhong, Goff, & Li, 2019). The iodine dyeing intensity may also be reduced if the amylose spiral cavity is occupied by guest molecules, thus hindering amylose-iodine complex formation. Further tests involving mixing the starch and Lugol's solution before addition of protein, compared with mixing the starch and protein before addition of Lugol's solution, resulted in a delay in observed colour loss as the temperature increased (Fig. S3). This indicates that competition for iodine is the more likely explanation for the observed loss of colour, with iodine previously complexed with starch instead bonding with the protein as the complex decomposed because of heating, and remaining bound to protein and no longer available to form a complex with starch again after cooling, resulting in the cooled mixture remaining discoloured. However, further studies are needed to fully identify the mechanism behind the observed loss in colour.

3.5. Pasting

Fig. 6 shows the pasting curves of the starch-protein mixtures during

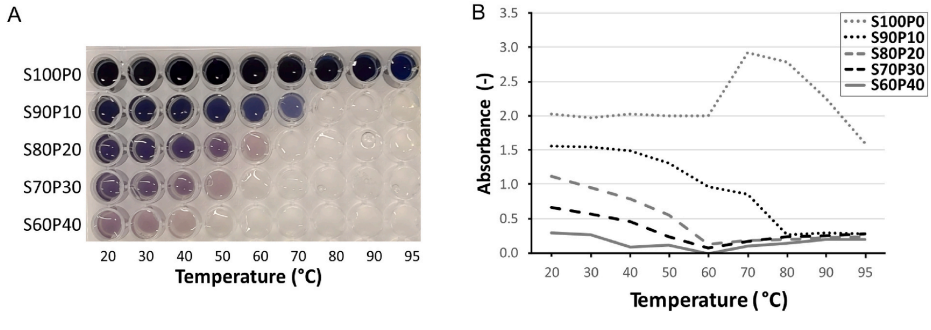


Fig. 5. (A) Image of iodine-stained starch-protein (S%:P%) mixtures at different temperatures and (B) corresponding absorbance measured at 510 nm.

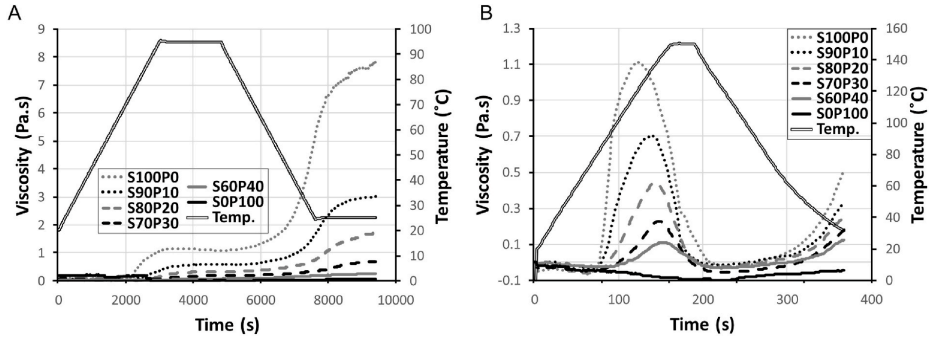


Fig. 6. Pasting curves of the starch-protein (S%P%) mixtures on heating to (A) maximum temperature 95 °C and (B) maximum temperature 150 °C.

heating to 95 °C and 150 °C. Although the shape of the curves differed, both graphs show that apparent pasting viscosities increased with higher starch content. This was observed from the thickness of the pastes already during analysis in the laboratory. At both temperatures, the S0P100 sample had the lowest final viscosity and did not form a gel in the pressure cell. Samples S60P40 and S70P30 formed viscous soft gels, while samples with starch content ≥80% formed cohesive gels. Another observation from the pasting analyses was that pasting was initiated at lower temperatures when the sample contained more starch.

In the pasting curves for heating to 95 °C (Fig. 6A), pasting commenced at around 73.2 °C for sample S100P0. For sample S90P10, the pasting temperature increased to 78.3 °C and for sample S60P40 it was 89.9 °C. For the pure protein sample S0P100, the pasting temperature could not be determined, as no pasting occurred. An absence of peak viscosity was observed during pasting experiments with heating to 95 °C. This is in line with previous results on faba bean starch and could possibly be related to the high amylose content which could help maintain the granular integrity (Hoover, Hughes, Chung, & Liu, 2010; Nilsson et al., 2022). Samples with a starch content of ≥70% showed a plateau during the holding time after the initial viscosity increase in the cooling phase (Fig. 6, Fig. S4 in SI). For sample S60P40, the viscosity increased steadily throughout the experiment. Increased protein content in the samples delayed the onset time for the second increase in viscosity in the samples. The second onset temperature, when the viscosity increased again during the cooling phase in the pasting analysis, was 31.6 °C for sample S70P30 and 42.1 °C for sample S100P0. From these results, it is not possible to conclude whether the difference in pasting temperature between the starch-rich and protein-rich samples was a result of the two components interacting or an effect of the starch-rich gels having overall higher viscosity.

Heating the samples to 150 °C was sufficient to achieve peak viscosity, followed by breakdown of the paste (Fig. 6B). The final viscosities in the 150 °C measurements were much lower than those in the 95 °C measurements (Table S1 in SI). The lower final viscosities in the high-temperature pasting analysis were presumably because of substantial granule breakdown on heating to 150 °C compared with 95 °C (Nilsson et al., 2022). However, the difference in heating/cooling rates and holding time at 25 °C limited comparison of the results of the different pasting experiments.

As the protein content increased, the temperature at which peak viscosity occurred also increased and the peak viscosity decreased (Fig. 6B, Table S1). The peak viscosity was highest (1.11 Pa s) for the pure starch sample S100P0 and was reached at 121 °C, while for sample S60P40 peak viscosity was 0.11 Pa s and was reached at 145 °C. Moreover, the breakdown viscosity of the samples increased with increasing starch content, most likely due to the higher peak viscosities of the more starch-rich samples. However, well-maintained viscosity during the heating and cooling may also suggest molecular entanglement between starch and/or protein molecules in the paste (Onwulata et al., 2014).

3.6. Oscillatory rheology

Storage modulus (G′) was monitored during gel formation (Fig. 7). The increase in G′ seen during initial heating seemed to occur earlier as the starch content increased. At a starch content of 80% (of total flour added) or higher, the gels showed a peak in G′ during heating and changes during cooling more similar to those in the pure starch gels. At lower starch contents, the peak observed during heating was not visible, and the changes in G′ were more similar to those in the pure protein gels.

The loss modulus (G′′) showed a similar pattern to G′ (Fig. S5 in SI). Except in the pure protein gels, G′′ was lower than G′ throughout the whole gelation process. In the pure protein gels, G′′ exceeded G′ during the initial part of the measurement, before gelation of the sample occurred. G′, G′′, tan δ and end of LVR of the final gels are summarised in Fig. 7B–E and Table S2 in SI.

Both G′ and G′′ decreased with increasing protein content. Tan δ of the final gels increased with increasing protein content, indicating less solid-like behaviour of the gels. Except for the pure protein gels, the amplitude sweep showed a decrease in LVR with increasing protein content. Large deformation properties, such as the structural breakdown occurring at high amplitudes during an amplitude sweep, may be affected by inhomogeneities and structural defects (Dille, Draget, & Hattrem, 2015; Munialo et al., 2014). Hence, the reduced LVR for the mixed systems could be related to inhomogeneities in the gel network created by either the starch, the protein or both. The amplitude sweep also showed a more distinct breaking point at the end of LVR with increasing starch content (Fig. S6 in SI), indicating more clear and brittle fracturing of the starch-rich gels. In contrast, the samples with higher protein content showed a more gradual decay in G′ as the strain increased, indicating more creamy behaviour.

The changes in G′ during gelation showed a relatively similar pattern to those of the pasting curves at 95 °C. However, the peak observed during heating of the starch-rich samples in the oscillatory measurements (highlighted with arrows in Fig. 7A) was not observed during pasting at 95 °C, which may be attributable to the continuous shearing occurring for the pasting measurements potentially being more disruptive for gel formation. The differences between pasting and oscillatory rheology data observed in the initial part of the measurements, as well as any potential differences between the pure protein gels, should be interpreted with caution, due to the erratic data obtained at low torques.

The changes in G′ observed for the pure protein gel were similar, but lower in absolute terms, to those previously reported for faba bean protein at higher protein concentrations (Johansson, Johansson, et al.,

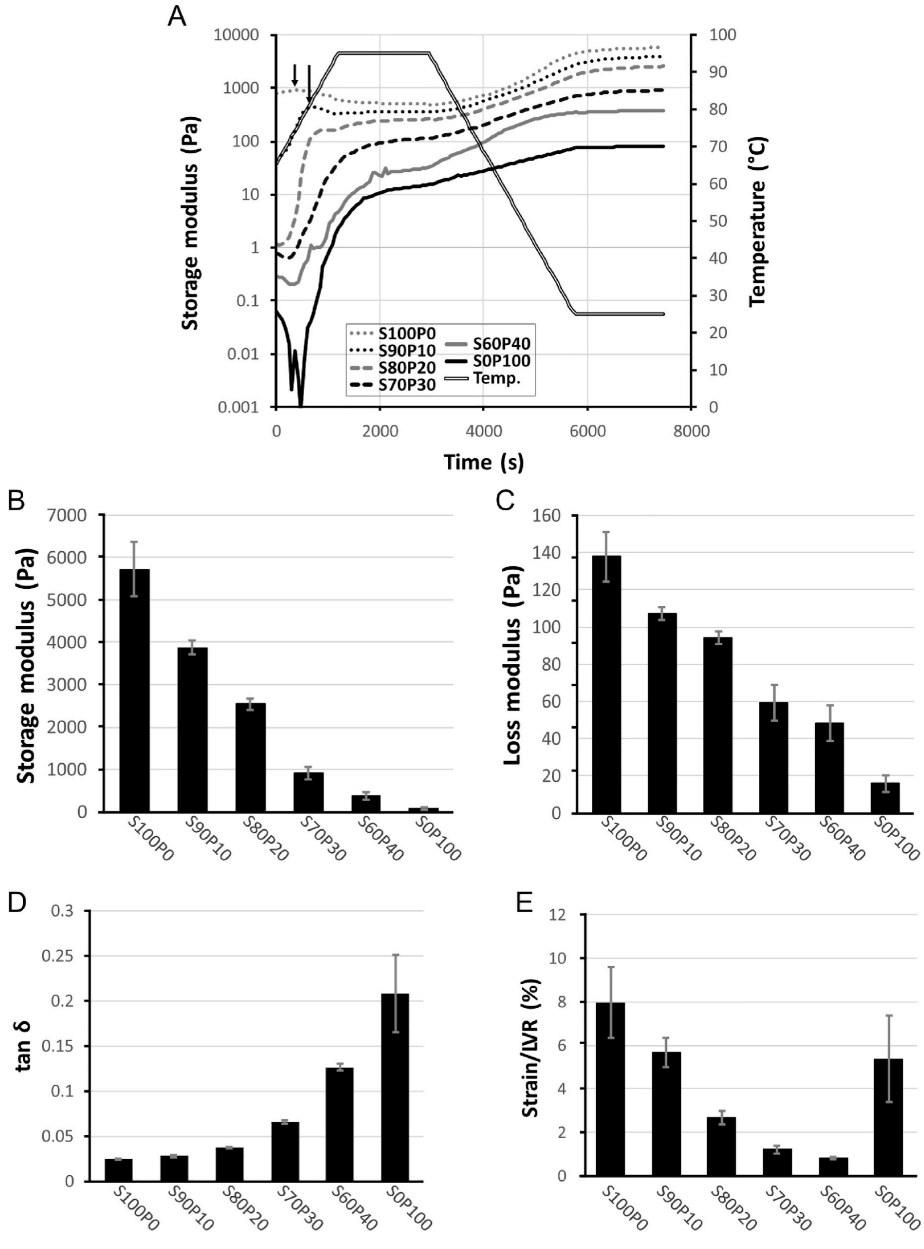


Fig. 7. (A) Storage modulus during temperature ramp for the different starch-protein (S%P%) composites. (B–D) Storage modulus, loss modulus and tan δ obtained from the final time point of the temperature ramp in A. (E) End of the linear viscoelastic region (LVR) measured on the final gels after the temperature ramp. Error bars represent ±1 st.dev.

2022; Langton et al., 2020). As the starch content in the mixtures here increased, G′ also increased and a gradual shift towards the behaviour of the pure starch sample was observed (Fig. 7A). A similar increase in G′ throughout the gelation process has been observed previously for faba bean starch-protein systems, although at higher total solids content and lower proportions of starch (Johanssonet al., 2022a).

An increase in G′ with increasing starch:protein ratio has previously been reported for lentil starch:lentil protein gels (Joshi et al., 2014). However, the opposite has been observed for potato protein:potato starch gels, with an increase in the proportion of starch reducing the G′ value of the gels (Zhang, Mu, & Sun, 2017). These results indicate that the properties and behaviour of starch-protein gel systems depend on the source and properties of the starch and protein used.

A study by Muhrbeck and Eliasson (1991) on the rheological properties of starch-protein mixtures from different sources revealed that the transition temperature and gelation rate of the two components were critical for the behaviour of the mixed system. In brief, it was shown here, that if the starch gelled before the protein, the rheological properties of the mixed gel system could be predicted by simple addition of the modulus values of its individual components. If the protein gelled before the starch, the system showed higher modulus values than predicted by simple addition of its components. The faba bean starch used in the present study has a gelatinisation temperature below the denaturation temperature of the protein (75–95 °C) (Kimura et al., 2008), and can be assumed to gel first. This assumption was supported by laboratory observations that gels with a higher fraction of starch were noticeably more viscous after the pre-heating step at 65 °C. Hence, the decrease in G′ in the gels as the starch content decreased seems to be in line with observations by Muhrbeck and Eliasson (1991), i.e. that increased protein concentration leads to lower G′ of the mixed system, since protein forms weaker gels than starch, and that G′ of the mixed system can be predicted by adding together the modulus values for its individual components.

The gels were further characterised by a frequency sweep (Fig. 8). The dependence of G′ on frequency was evaluated by calculating the relaxation exponent (n) after fitting the data to a power law equation, $G' = S\omega^n$, where S and n are constants and ω is the angular frequency (Chambon & Winter 1987; Tanger, Müller, Andlinger, & Kulozik, 2022; Winter & Chambon, 1986). A purely elastic material is frequency-independent, with n = 0 (Alting, Hamer, De Kruif, & Visschers, 2003), and gel networks formed by mainly chemical cross-links will have a relaxation exponent close to zero (Tanger et al., 2022). A gel network formed by mainly secondary bonds (e.g. hydrogen bonding and hydrophobic interactions) will have a slightly higher frequency dependence (Tanger et al., 2022). A small contribution from the viscous component (G′′) is typical for food gels. This contribution results in a frequency dependence of G′ reflecting relaxation of the viscous components (Alting et al., 2003).

In this study, G′ was higher than G″ for all samples over the full frequency range analysed (Fig. 8A and B), indicating mainly elastic deformations (Zhang et al., 2017). The n-values of the gels indicated that interactions within the gel network were mainly of a physical nature. The n-values increased with increasing protein content. This increased frequency dependence is in agreement with the increase in tan δ, reflecting the more viscous behaviour of the protein-rich gels, indicating weaker gel structure (Vogelsang-O'Dwyer et al., 2020).

The G′′ values indicated stronger frequency dependence compared with G′, as evidenced by the higher n-values (Table 2). Overall, similar trends were observed for n-values calculated for both G′ and G′′. As found for G′, both similar and opposing trends in n-values with increasing starch:protein ratio have been observed for other starch-protein sources (Gui et al., 2022; Joshi et al., 2014; Zhang et al., 2017). However, it could be noted that for all these cases, the gels with the highest G′ gave the lowest n-values. This is in line with the lower frequency dependence typically observed for stronger and less fluid like gels (Vogelsang-O'Dwyer et al., 2020).

3.7. Compression tests

Compression tests were used to investigate the textural properties of the gels. All characteristics evaluated (fracture stress, fracture strain, Young's modulus) showed a decrease with increasing protein content (Fig. 9, Table S3). The gels with 60% starch and 40% protein (S60P40) were not completely homogenous, with more solid-like behaviour towards the lower part of the gel due to partial sedimentation of the starch, so the middle part of these gels was used for compression tests. The gels containing only protein were not self-standing and could therefore not be analysed by compression tests.

Fracture stress, fracture strain and Young's modulus decreased with increasing protein content. The fracture and fracture point were more distinct for the gels with higher starch content, showing a clear drop in force as the gels fractured (Fig. S7 in SI). The gels with higher protein

Table 2
n-values (mean ± 1 st.dev.) for the different starch-protein (S%P%) gels. Values obtained after fitting frequency sweep data (storage (G′) and loss modulus (G′′)) to the equation: Storage/loss modulus = $S^*\omega^n$, where S and n are constants and ω is the angular frequency. Different superscript letters indicate significant differences within columns ($p < 0.05$).

Sample	n (G′)	n (G′′)
S100P0	0.016 ± 0.000^a	0.197 ± 0.004^a
S90P10	0.019 ± 0.001^a	0.198 ± 0.003^a
S80P20	0.024 ± 0.001^b	0.200 ± 0.005^a
S70P30	0.038 ± 0.001^c	0.234 ± 0.023^a
S60P40	0.069 ± 0.002^d	0.249 ± 0.032^a
S0P100	0.134 ± 0.022^e	0.291 ± 0.054^a

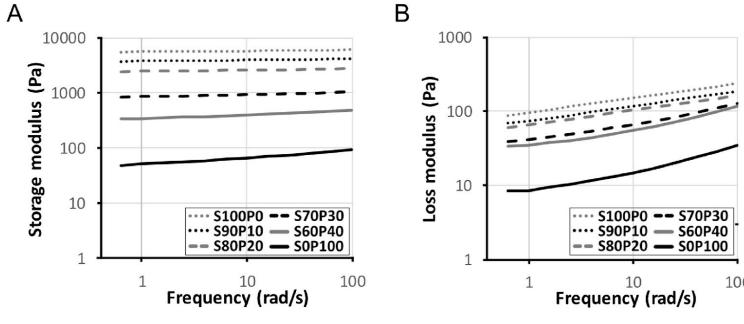


Fig. 8. (A) Storage modulus and (B) loss modulus as a function of frequency for the different starch-protein (S%P%) gels.

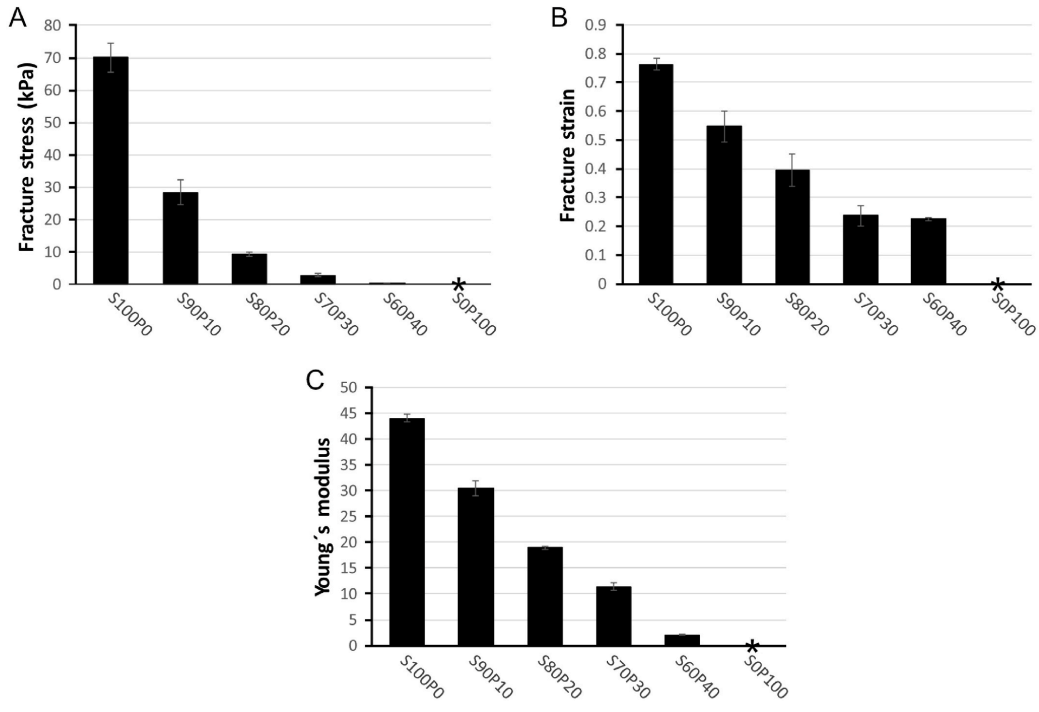


Fig. 9. (A) Fracture stress, (B) fracture strain and (C) Young's modulus of the different starch-protein (S%P%) gels analysed by compression tests. Error bars represent ±1 st.dev. *Sample did not form self-standing gels and could not be measured.

content showed more ductile behaviour and less clear fracture. The more distinct fracture of the starch-rich gels was also observed visually during compression tests, where starch-rich samples (≥80% starch) fractured into well-defined/separate pieces, whereas gels with ≤70% starch did not break into separate pieces and showed more paste-like behaviour upon fracture.

As expected, the Young's modulus and fracture strain values from compression tests showed similar trends to those observed for G′ and

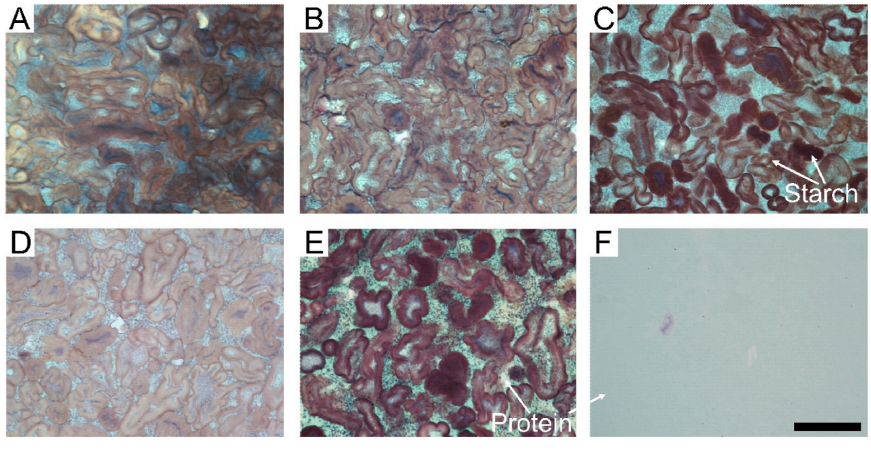


Fig. 10. Light microscopy micrographs of the starch-protein (S%P%) gels. (A) S100P0, (B) S90P10, (C) S80P20, (D) S70P30, (E) S60P40 and (F) S0P100. Scale bar = 50 μm.

LVR during oscillatory rheology measurements. The more clear and distinct fracture observed during compression of starch-rich gels (Fig. S7) was in agreement with the clearer drop in G′ at higher strains observed in the strain sweep (Fig. S6).

Previous studies on faba bean starch-protein gels have reported lower fracture stress and fracture strain with increasing starch content (Johansson, Johansson, et al., 2022). However, those gels contained mainly protein, with a maximum starch:protein ratio of 35:65, and had a higher solids content (20%) than in this study (12%). Firmer gel consistency with increasing starch:protein ratio has previously been reported for lentil starch:lentil protein gels (Joshi et al., 2014).

3.8. Microstructural characterisation of gels

3.8.1. Light microscopy

Gel microstructure was characterised by light microscopy with protein stained in blue/green and starch in brown/purple (Fig. 10). The micrographs indicated that starch-rich gels were more densely packed with starch, which could explain the observed firmness of starch-rich gels during compression tests, higher elastic modulus in oscillatory rheology and higher viscosity development in pasting analysis. The protein fraction was more evident in gels with higher protein content. The starch granules appeared to be the continuous phase for all gels with starch content ≥70%. For the S60P40 gel samples, the continuous phase was more difficult to define (Fig. 10E), with the starch and protein potentially forming two separate bicontinuous networks.

For the gels with higher protein content, the leaked amylose seemed to aggregate into more amylose-dense regions (darker blue spots) within the protein network (Fig. 11). Amylose aggregates within a continuous protein network have been observed previously in mixed faba bean protein:starch gels with higher protein content (protein ≥65%) and solids content (20%) (Johansson, Johansson, et al., 2022). For the gels with starch content ≥90%, the amylose seemed to form a gradually finer network, rather than the larger aggregates observed in the gels with higher protein content (Fig. 11). This amylose aggregation into clusters rather than a network could relate to the observed reduction in storage modulus, fracture stress and fracture strain with increasing protein content. Aggregation of amylose has been shown to occur in other mixed systems (starch-emulsifier), indicating that it is due to phase separation rather than amylose-protein complex formation (Richardson, Kidman, Langton, & Hermansson, 2004). The phase separation occurring between the amylose and protein might be related to differences in their hydrophobicity. Most plant proteins, including the majority of faba bean proteins, are globular proteins and tend to become more hydrophobic upon denaturation (Kim, Wang, & Selomulya, 2020). However, upon denaturation, the protein gel network will also start to form and mobility within the system will be reduced. Hence, any phase separation occurring before gelation might be permanently captured within the gelled structure (Yang, Liu, Ashton, Gorczyca, & Kasapis, 2013).

Previous studies have suggested that protein in mixture gels may perturb starch network formation, thereby weakening the gel (Bravo-Núñez et al., 2019; Bravo-Núñez & Gómez, 2019; Joshi et al., 2014; Oñate Narciso & Brennan, 2018; Onwulata et al., 2014). The compression test results here showed a strong correlation between high starch content and increased firmness, which could be explained by higher protein content disrupting the starch gel network and producing a heterogeneous and weaker gel. The sequence of gel formation has been found to influence the microstructure of lentil starch-protein mixed gels, with the starch becoming more viscous than the protein at lower temperatures (Joshi et al., 2014). Similar behaviour could be expected for faba bean gels, as faba bean starch has a lower gelatinisation temperature than the denaturation temperature of faba bean protein.

3.8.2. SEM

Gel microstructure characterisation by SEM revealed that gels with ≥80% starch had regions with a more porous network structure than gels with less starch (Fig. 12, Fig. S8 in SI). In general, the continuous network of these gels showed a structure more similar to the pure starch gels, while the network of gels with ≤70% starch showed a denser structure more similar to that of the pure protein gels. Examples of regions with porous and dense network structures are highlighted with arrows in Fig. 12. For gels containing starch, starch granules and cavities where starch granules had been present were evident throughout the structure. In general, a denser and finer network structure was observed around these cavities.

In the LM micrographs, starch was the most visible and dominant component of all samples except the pure protein gels. Despite this, the microstructure of S70P30 and S60P40 gels showed a greater resemblance to the pure protein gels when observed by SEM. The gels with ≥30% protein largely lacked the less dense and more fibrous and stranded structure observed in the starch-rich gels. This was possibly due to the fracturing of samples performed before analysis to expose the

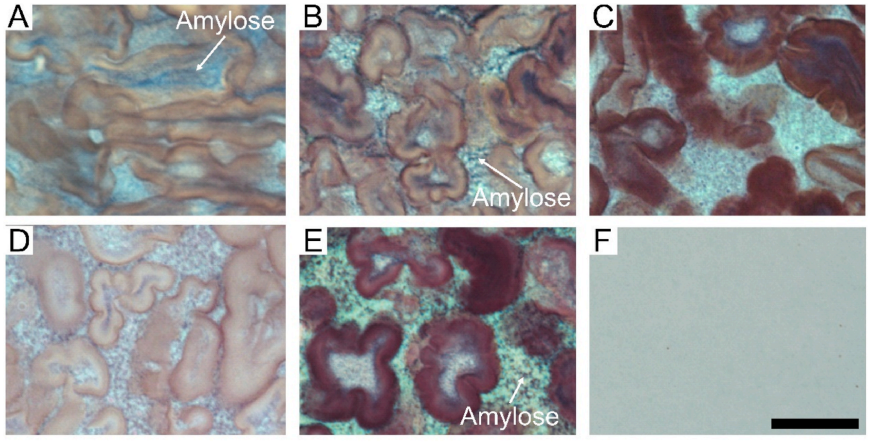


Fig. 11. Magnified views of the micrographs in Fig. 10. (A) S100P0, (B) S90P10, (C) S80P20, (D) S70P30, (E) S60P40 and (F) S0P100. Scale bar = 25 μm. Data size: 975 × 731 pixels.

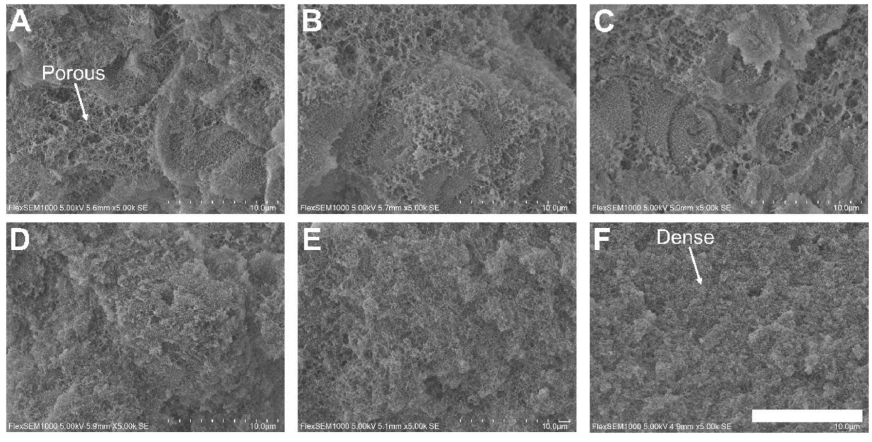


Fig. 12. Scanning electron microscopy (SEM) micrographs of the starch-protein (S%P%) gels. Examples of regions with porous or dense network structures are highlighted with arrows. (A) S100P0, (B) S90P10, (C) S80P20, (D) S70P30, (E) S60P40 and (F) S0P100. Scale bar: 10 μm.

inner part of the fixed gel, with fractures propagating mainly through the weakest regions of the structure. Since the protein-rich regions were likely to be weaker than the network formed by the starch, fracturing was more likely to occur through these regions. This could explain why the samples with ≥30% protein showed such high similarity to the pure protein gels despite their high starch content and why the starch was clearly evident in the LM micrographs of corresponding gel samples.

3.9. Overall trends and correlations

Overall, the results showed that gel morphology/structure and properties were highly dependent on the starch:protein ratio in the mixture. A comprehensive overview of the relationships between morphology/structure and properties of starch-protein mixtures is provided by Zhang et al. (2021). To summarise, higher protein content in the faba bean starch-protein samples resulted in lower pasting viscosities, less firm gels and a more compact and less fibrous gel structure, with the starch appearing to aggregate into clusters. Hydration was also less efficient for the high-protein mixtures. These effects became more pronounced as the protein content of the samples increased.

Apparent viscosity and firmness were strongly positively correlated for high-starch samples, e.g. there was a strong correlation ($r = 0.98$, $p < 0.001$) between peak pasting viscosity and elastic modulus in the rheological temperature ramp. Gels with a higher proportion of protein were also more frequency-dependent, which indicates a weakened gel structure (Joshi et al., 2014).

More efficient hydration and higher iodine absorbance were also positively correlated with higher paste viscosity and firmer gel ($r = 0.97$; $p < 0.001$ for the correlation between WAI and peak viscosity, $r = 0.85$; $p = 0.003$ for that between WAI and elastic modulus). The less efficient hydration properties of the gels containing protein could have affected the distribution of water within the starch-protein matrix and affected molecular interactions. A lower water holding capacity, as observed for the gels containing protein, can lead to lower textural stability (Boye, Zare, & Pletch, 2010). Proteins competing with starch for water may limit granule swelling by restricting water absorption, thereby reducing apparent viscosity (Eliasson, 1983; Oñate Narciso & Brennan, 2018; Ribotta et al., 2007). As indicated by the hot-stage microscopy micrographs (see Fig. 4 and Fig. S2), there was a tendency for slightly less intense swelling in the high-protein samples. The NMR results (Fig. 3) confirmed that hydration was slightly less efficient for sample S60P40 compared with S90P10. Slower absorption of water may also lead to delayed pasting because of retarded swelling of the starch granules causing less granule contact.

The more prevalent iodine colour loss associated with higher protein content (see Figs. 4 and 5) indicates that the protein formed a complex with the starch or bound the iodine, rendering it unavailable to form complexes with the starch. If protein-starch complexes are formed, consequences could be delayed granule hydration and swelling and a reduction or perturbation in starch-starch network development.

Another plausible explanation for higher protein content resulting in reduced paste viscosity (Fig. 6) and less firm gels (Fig. 9) is dilution of the starch by the protein, as starch often has better gelling properties, forming stronger gels, than protein (Bravo-Núñez et al., 2019; Bravo-Núñez & Gómez, 2019). The LM micrographs (Figs. 10 and 11) showed that starch granules were more tightly packed in the high-starch gels. However, viscosity differences exceeded actual starch dilution, suggesting that the protein somehow interfered with viscosity development of the starch.

In the SEM micrographs (Fig. 12), the starch-rich gels displayed regions with a more porous and fibrous network. The oscillating frequency results, with higher calculated n-value for samples with higher protein content, indicated greater presence of secondary bonds (Tanger et al., 2022), which in turn could lead to denser network structure because of less bonding between the starch chains. Starch gel matrices consist of swollen granules, entangled polymer chains and non-covalent interactions (Larrea-Wachtendorff, Del Grosso, & Ferrari, 2022; Walstra, 2002). Ribotta et al. (2007) concluded that soy protein can interact readily with amylose and exposed branches of amylopectin through non-covalent bonding, especially hydrogen bonds, causing further gel matrix weakening.

Proteins may act as an inert filler, hindering realignment of the starch chains during gelation. An effect of protein addition in altering microstructure and reducing viscosity and/or gel strength has been reported for numerous starch-protein mixtures, such as wheat starch-soy protein (Ribotta et al., 2007), starch-dairy (Yang et al., 2004) and various starch-plant/animal-based proteins (Bravo-Núñez et al., 2019; Bravo-Núñez & Gómez, 2019). A previous study analysing the same starch as used in this study revealed that the amylose content was 32.2% (Nilsson et al., 2022). The effect of protein in decreasing viscosity and gel strength has been shown to be greater for amylose-rich starches, as the proteins are more likely to interfere with re-ordering of the amylose

(Bravo-Núñez et al., 2019; Joshi et al., 2014; Oñate Narciso & Brennan, 2018). Amylose re-ordering into clusters, rather than developing into a network, in the presence of emulsifiers has also been found to have a weakening effect (Richardson, Kidman, et al., 2004; Richardson, Sun, Langton, & Hermansson, 2004). As observed from the LM micrographs (Figs. 10 and 11), similar re-ordering into clusters rather than a network seemed to occur for the gels in this study as the protein content increased.

The overall effect on pasting and gel structure matrix is also dependent on the type of protein incorporated (Zhang et al., 2021). The pure protein mixtures (S0P100) in this study formed very weak gels, as indicated by the rheological results (Figs. 6 and 7), compression results (Fig. 9) and LM micrographs (Figs. 10 and 11), so the protein in the mixed systems likely did not create any strong network contributing to the overall strength of the mixed systems. The lowest gelling concentration of faba bean protein is reported to be around 13–14% (Fernández-Quintela, Macarulla, del Barrio, & Martínez, 1997; Langton et al., 2020), which is higher than in the mixtures here (12% solids).

4. Conclusions

A decrease in the proportion of starch in faba bean starch-gel mixtures, while keeping the solids content (starch + protein) constant, resulted in lower measured viscosities during pasting. Gels with decreased starch content (and increasing protein content) also showed a decrease in storage modulus, fracture stress, fracture strain and Young's modulus. The water binding and hydration properties of the starch-protein mixtures improved with higher starch content, suggesting that water binding and gel rheological values may be related. Changes in textural and rheological properties appeared to occur gradually over the starch:protein ratios tested. Light microscopy indicated that starch was the continuous phase, consisting of swollen granules, for gels with starch content ≥70% of total solids and that starch and protein potentially formed two bicontinuous networks at a starch content of 60%. Scanning electron microscopy revealed a more porous starch network, with thicker strands than in the protein network. In general terms, it can be stated that the textural properties, and in turn also the mouthfeel and perception, of starch-protein gels depend largely on the starch to protein ratio in the system. To produce a stable and brittle gel a higher starch content may be desirable, while to produce a softer and perhaps pourable gel a higher proportion of protein may be preferred. However, if the solids content in the gels (12%) had exceeded the lowest gelling concentration of faba bean protein (14%), their properties may have been different.

CRediT authorship contribution statement

Klara Nilsson: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Mathias Johansson:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Corine Sandström:** Investigation, Methodology, Writing – review & editing. **Hanna Eriksson Röhnisch:** Investigation, Writing – review & editing. **Mikael S. Hedenqvist:** Writing – review & editing. **Maud Langton:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the FORMAS [grant numbers 2018–01869, 2017–00426]; and Trees and Crops for the Future (TC4F), a Strategic Research Area at SLU, supported by the Swedish Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2023.108494.

References

Alting, A. C., Hamer, R. J., De Kruif, C. G., & Visschers, R. W. (2003). Cold-set globular protein gels: Interactions, structure and rheology as a function of protein concentration. *Journal of Agricultural and Food Chemistry, 51*(10), 3150–3156. https://doi.org/10.1021/jf0209342

Ambigaipalan, P., Hoover, R., Donner, E., & Liu, Q. (2013). Retrogradation characteristics of pulse starches. *Food Research International, 54*(1), 203–212. https://doi.org/10.1016/j.foodres.2013.06.012

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., et al. (2011). Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. *Food Research International, 44*(9), 2962–2974. https://doi.org/10.1016/j.foodres.2011.07.006

Aschemann-Witzel, J., Gantriis, R. F., & Fraga, P. (2021). Plant-based food and protein trend from a business perspective : Markets , consumers , and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition, 61*(18), 3119–3128. https://doi.org/10.1080/10408398.2020.1793730

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414–431. https://doi.org/10.1016/j.foodres.2009.09.003

Bravo-Núñez, Á., Garzón, R., Rosell, C. M., & Gómez, M. (2019). Evaluation of starch-protein interactions as a function of pH. *Foods, 8*(5), 155. https://doi.org/10.3390/foods8050155

Bravo-Núñez, Á., & Gómez, M. (2019). Physicochemical properties of native and extruded maize flours in the presence of animal proteins. *Journal of Food Engineering, 243*(August 2018), 49–56. https://doi.org/10.1016/j.jfoodeng.2018.09.005

Chambon, F., & Winter, H. H. (1987). Linear viscoelasticity at the gel point of a crosslinking PDMS with imbalanced stoichiometry. *Journal of Rheology, 31*(8), 683–697. https://doi.org/10.1122/1.549955

Dille, M. J., Draget, K. I., & Hattrem, M. N. (2015). 9 - the effect of filler particles on the texture of food gels. In J. Chen, & A. Rosenthal (Eds.), *Modifying food texture* (pp. 183–200). Woodhead Publishing. https://doi.org/10.1016/B978-1-78242-333-1.00009-7.

Eliasson, A. C. (1983). Differential scanning calorimetry studies on wheat starch—gluten mixtures: I. Effect of gluten on the gelatinization of wheat starch. *Journal of Cereal Science, 1*(3), 199–205. https://doi.org/10.1016/S0733-5210(83)80021-6

Fernández-Quintela, A., Macarulla, M. T., del Barrio, A. S., & Martínez, J. A. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition, 51*(4), 331–341. https://doi.org/10.1023/A:1007936930354

Fonslick, J., & Khan, A. (1989). Thermal stability and composition of the amylose-iodine complex. *Journal of Polymer Science, 27*, 4161–4167.

Garbow, J. R., & Schaefer, J. (1991). Magic-angle 13c nmr study of wheat flours and doughs. *Journal of Agricultural and Food Chemistry, 39*(5), 877–880. https://doi.org/10.1021/jf00005a013

Gui, Y., Zou, F., Zhu, Y., Li, J., Wang, N., Guo, L., et al. (2022). The structural, thermal, pasting and gel properties of the mixtures of enzyme-treated potato protein and potato starch. *LWT, 154*, Article 112882. https://doi.org/10.1016/j.lwt.2021.112882

Holló, J., & Szejtli, J. (1958). The mechanism of starch-iodine reaction. *Periodica Polytechnica, Chemical Engineering, 2*(1), 25–37.

Hoover, R., Hughes, T., Chung, H. J., & Liu, Q. (2010). Composition, molecular structure, properties, and modification of pulse starches: A review. *Food Research International, 43*(2), 399–413. https://doi.org/10.1016/j.foodres.2009.09.001

Huber, K. C., & Bemiller, J. N. (2017). 3.3.6.8 starch complexes. In S. Damodaran, & K. L. Parkin (Eds.), *Fennema's food chemistry* (5th ed., pp. 140–141). CRC Press.

Johansson, M., Johansson, D., Ström, A., Rydén, J., Nilsson, K., Karlsson, J., et al. (2022). Effect of starch and fibre on faba bean protein gel characteristics. *Food Hydrocolloids, 131*, Article 107741. https://doi.org/10.1016/j.foodhyd.2022.107741

Johansson, M., Nilsson, K., Knab, F., & Langton, M. (2022). Faba bean fractions for 3D printing of protein-, starch- and fibre-rich foods. *Processes, 10*(3). https://doi.org/10.3390/pr10030466

Joshi, M., Aldred, P., Panozzo, J. F., Kasapis, S., & Adhikari, B. (2014). Rheological and microstructural characteristics of lentil starch–lentil protein composite pastes and gels. *Food Hydrocolloids, 35*, 226–237. https://doi.org/10.1016/j.foodhyd.2013.05.016

Kimura, A., Fukuda, T., Zhang, M., Motoyama, S., Maruyama, N., & Utsumi, S. (2008). Comparison of physicochemical properties of 7S and 11S globulins from pea, fava bean, cowpea, and French bean with those of soybean—French bean 7S globulin

exhibits excellent properties. *Journal of Agricultural and Food Chemistry, 56*(21), 10273–10279. https://doi.org/10.1021/jf801721b
Kim, W., Wang, Y., & Selomulya, C. (2020). Dairy and plant proteins as natural food emulsifiers. *Trends in Food Science & Technology, 105*, 261–272. https://doi.org/10.1016/j.tifs.2020.09.012
Langton, M., Ehsanzamir, S., Karkehabadi, S., Feng, X., Johansson, M., & Johansson, D. P. (2020). Gelation of faba bean proteins - effect of extraction method, pH and NaCl. *Food Hydrocolloids, 103*, Article 105622. https://doi.org/10.1016/j.foodhyd.2019.105622
Langton, M., & Hermansson, A.-M. (1992). Fine-stranded and particulate gels of β-lactoglobulin and whey protein at varying pH. *Food Hydrocolloids, 5*(6), 523–539. https://doi.org/10.1016/S0268-005X(09)80122-7
Larrea-Wachtendorff, D., Del Grosso, V., & Ferrari, G. (2022). Evaluation of the physical stability of starch-based hydrogels produced by high-pressure processing (HPP). *Gels, 8*(3), 152. https://doi.org/10.3390/gels8030152
Larsen, F. H., Blennow, A., & Engelsen, S. B. (2008). Starch granule hydration-A MAS NMR investigation. *Food Biophysics, 3*(1), 25–32. https://doi.org/10.1007/s11483-007-9045-4
Larsen, F. H., Kasprzak, M. M., Larke, H. N., Knudsen, K. E. B., Pedersen, S., Jorgensen, A. S., et al. (2013). Hydration properties and phosphorous speciation in native, gelatinized and enzymatically modified potato starch analyzed by solid-state MAS NMR. *Carbohydrate Polymers, 97*(2), 502–511. https://doi.org/10.1016/j.carbpol.2013.05.014
Li, L., Yuan, T. Z., Setia, R., Raja, R. B., Zhang, B., & Ai, Y. (2019). Characteristics of pea, lentil and faba bean starches isolated from air-classified flours in comparison with commercial starches. *Food Chemistry, 276*(October 2018), 599–607. https://doi.org/10.1016/j.foodchem.2018.10.064
Ma, K. K., Greis, M., Lu, J., Nolden, A. A., McClements, D. J., & Kinchla, A. J. (2022). Functional performance of plant proteins. *Foods, 11*(4), 594. https://doi.org/10.3390/foods11040594
Morgan, K. R., Furneaux, R. H., & Larsen, N. G. (1995). Solid-state NMR studies on the structure of starch granules. *Carbohydrate Research, 276*(2), 387–399. https://doi.org/10.1016/0008-6215(95)00173-Q
Muhrbeck, P., & Eliasson, A. (1991). Rheological properties of protein/starch mixed gels. *Journal of Texture Studies, 22*(3), 317–332.
Multari, S., Stewart, D., & Russell, W. R. (2015). Potential of fava bean as future protein supply to partially replace meat intake in the human diet. *Comprehensive Reviews in Food Science and Food Safety, 14*(5), 511–522. https://doi.org/10.1111/1541-4337.12146
Munialo, C. D., van der Linden, E., & de Jongh, H. H. J. (2014). The ability to store energy in pea protein gels is set by network dimensions smaller than 50nm. *Food Research International, 64*, 482–491. https://doi.org/10.1016/j.foodres.2014.07.038
Nicolai, T., & Chassenieux, C. (2019). Heat-induced gelation of plant globulins. *Current Opinion in Food Science, 27*, 18–22. https://doi.org/10.1016/j.cofs.2019.04.005
Nilsson, K., Sandström, C., Özeren, H. D., Vilaplana, F., Hedenqvist, M., & Langton, M. (2022). Physiochemical and thermal characterisation of faba bean starch. *Journal of Food Measurement and Characterization, 16*(6), 4470–4485. https://doi.org/10.1007/s11694-022-01543-7
Núñez-Santiago, M. C., Bello-Pérez, L. A., & Tecante, A. (2004). Swelling-solubility characteristics, granule size distribution and rheological behavior of banana (Musa paradisiaca) starch. *Carbohydrate Polymers, 56*(1), 65–75. https://doi.org/10.1016/j.carbpol.2003.12.003
Oñate Narciso, J., & Brennan, C. (2018). Whey and pea protein fortification of rice starches: Effects on protein and starch digestibility and starch pasting properties. *Starch/Staerke, 70*(9–10), 1–6. https://doi.org/10.1002/star.201700315
Onwulata, C. I., Tunick, M. H., & Thomas-Gahring, A. E. (2014). Pasting and extrusion properties of mixed carbohydrate and whey protein isolate matrices. *Journal of Food Processing and Preservation, 38*(4), 1577–1591. https://doi.org/10.1111/jfpp.12118
Punia, S., Dhull, S. B., Sandhu, K. S., & Kaur, M. (2019). Faba bean (Vicia faba) starch: Structure, properties, and in vitro digestibility—a review. *Legume Science, 1*(1), 3–7. https://doi.org/10.1002/leg3.18
Ribotta, P. D., Colombo, A., León, A. E., & Añón, M. C. (2007). Effects of soy protein on physical and rheological properties of wheat starch. *Starch - Stärke, 59*(12), 614–623. https://doi.org/10.1002/star.200700650
Richardson, G., Kidman, S., Langton, M., & Hermansson, A. M. (2004). Differences in amylose aggregation and starch gel formation with emulsifiers. *Carbohydrate Polymers, 58*(1), 7–13. https://doi.org/10.1016/j.carbpol.2004.06.013
Richardson, G., Sun, Y., Langton, M., & Hermansson, A. M. (2004). Effects of Ca- and Na-lignosulfonate on starch gelatinization and network formation. *Carbohydrate Polymers, 57*(4), 369–377. https://doi.org/10.1016/j.carbpol.2004.04.023
Röös, E., Carlsson, G., Ferawati, F., Hefni, M., Stephan, A., Tidåker, P., et al. (2020). Less meat, more legumes: Prospects and challenges in the transition toward sustainable diets in Sweden. *Renewable Agriculture and Food Systems, 35*(2), 192–205. https://doi.org/10.1017/S1742170518000443
Sasaki, T., & Matsuki, J. (1998). Effect of wheat starch structure on swelling power. *Cereal Chemistry, 75*(4), 525–529. https://doi.org/10.1094/CCHEM.1998.75.4.525
Schorsch, C., Wilkins, D. K., Jones, M. G., & Norton, I. T. (2001). Gelation of casein-whey mixtures: Effects of heating whey proteins alone or in the presence of casein micelles. *Journal of Dairy Research, 68*(3), 471–481. https://doi.org/10.1017/S0022029901004915
Sharan, S., Zanghelini, G., Zotzel, J., Bonerz, D., Aschoff, J., Saint-Eve, A., et al. (2021). Fava bean (Vicia faba L.) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color. *Comprehensive Reviews in Food Science and Food Safety, 20*(1), 401–428. https://doi.org/10.1111/1541-4337.12687
Shim, J., & Mulvaney, S. J. (2001). Effect of heating temperature, pH, concentration and starch/whey protein ratio on the viscoelastic properties of corn starch/whey protein mixed gels. *Journal of the Science of Food and Agriculture, 81*(8), 706–717. https://doi.org/10.1002/jsfa.869
Tanger, C., Müller, M., Andlinger, D., & Kulozik, U. (2022). Influence of pH and ionic strength on the thermal gelation behaviour of pea protein. *Food Hydrocolloids, 123*, Article 106903. https://doi.org/10.1016/j.foodhyd.2021.106903
Tang, H., & Hills, B. P. (2003). Use of 13C MAS NMR to study domain structure and dynamics of polysaccharides in the native starch granules. *Biomacromolecules, 4*(5), 1269–1276.
Tester, R. F., & Morrison, W. R. (1990). Swelling and gelatinisation of cereal starches I. Effect of amylopectin, amylose and lipids. *Cereal Chemistry, 65*(6), 551–557.
Vamadevan, V., & Bertoft, E. (2020). Observations on the impact of amylopectin and amylose structure on the swelling of starch granules. *Food Hydrocolloids, 103*, Article 105663. https://doi.org/10.1016/j.foodhyd.2020.105663
Vogelsang-O'Dwyer, M., Petersen, I. L., Joehnke, M. S., Sørensen, J. C., Bez, J., Detzel, A., et al. (2020). Comparison of faba bean protein ingredients produced using dry fractionation and isoelectric precipitation: Techno-functional, nutritional and environmental performance. *Foods, 9*(3), 322. https://doi.org/10.3390/foods9030322
Walstra, P. (2002). Physical chemistry of foods. In O. R. Fennema, Y. H. Hui, M. K. Rutgers, P. Walstra, & J. R. Whitaker (Eds.), *Physical chemistry of foods*. Marcel Dekker Inc. https://doi.org/10.1201/9780203910436.
Warsame, A. O., O'Sullivan, D. M., & Tosi, P. (2018). Seed storage proteins of faba bean (Vicia faba L): Current status and prospects for genetic improvement. *Journal of Agricultural and Food Chemistry, 66*(48), 12617–12626. https://doi.org/10.1021/acs.jafc.8b04992
Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the anthropocene: The EAT–lancet commission on healthy diets from sustainable food systems. *The Lancet, 393*(10170), 447–492. https://doi.org/10.1016/S0140-6736(18)31788-4
Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *Journal of Rheology, 30*(2), 367–382. https://doi.org/10.1122/1.549853
Yang, H., Irudayaraj, J., Otgonchimeg, S., & Walsh, M. (2004). Rheological study of starch and dairy ingredient-based food systems. *Food Chemistry, 86*(4), 571–578. https://doi.org/10.1016/j.foodchem.2003.10.004
Yang, N., Liu, Y., Ashton, J., Gorczyca, E., & Kasapis, S. (2013). Phase behaviour and in vitro hydrolysis of wheat starch in mixture with whey protein. *Food Chemistry, 137*(1), 76–82. https://doi.org/10.1016/j.foodchem.2012.10.004
Yang, C., Zhong, F., Goff, H. D., & Li, Y. (2019). Study on starch-protein interactions and their effects on physicochemical and digestible properties of the blends. *Food Chemistry, 280*, 51–58. https://doi.org/10.1016/j.foodchem.2018.12.028
Zhang, D., Mu, T., & Sun, H. (2017). Calorimetric, rheological, and structural properties of potato protein and potato starch composites and gels. *Starch - Stärke, 69*(7–8), Article 1600329. https://doi.org/10.1002/star.201600329
Zhang, B., Qiao, D., Zhao, S., Lin, Q., Wang, J., & Xie, F. (2021). Starch-based food matrices containing protein: Recent understanding of morphology, structure, and properties. *Trends in Food Science & Technology, 114*, 212–231. https://doi.org/10.1016/j.tifs.2021.05.033
Zhang, Z., Tian, X., Wang, P., Jiang, H., & Li, W. (2019). Compositional, morphological, and physicochemical properties of starches from red adzuki bean, chickpea, faba bean, and baiyue bean grown in China. *Food Sciences and Nutrition, 7*(8), 2485–2494. https://doi.org/10.1002/fsn3.865
Zha, F., Rao, J., & Chen, B. (2021). Plant-based food hydrogels: Constitutive characteristics, formation, and modulation. *Current Opinion in Colloid & Interface Science, 56*, Article 101505. https://doi.org/10.1016/j.cocis.2021.101505
Zhu, F. (2017). NMR spectroscopy of starch systems. *Food Hydrocolloids, 63*, 611–624. https://doi.org/10.1016/j.foodhyd.2016.10.015

IV

Food Hydrocolloids 131 (2022) 107741

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Effect of starch and fibre on faba bean protein gel characteristics

Mathias Johansson [a,*], Daniel Johansson [a], Anna Ström [b], Jesper Rydén [c], Klara Nilsson [a], Jakob Karlsson [b], Rosana Moriana [a,d], 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 Energy and Technology, Swedish University of Agricultural Sciences, Box 7032, SE-750 07, Uppsala, Sweden
[d] Bioeconomy and Health Division, RISE-Research Institutes of Sweden, Drottning Kristinas Väg 61, Stockholm, Sweden

ARTICLE INFO

Keywords:
Protein gelation
Faba bean
Starch
Fibre
Texture
Microstructure

ABSTRACT

Faba bean is a promising alternative to soybean for production of protein-rich plant-based foods. Increased understanding of the gelling behaviour of non-soy legumes can facilitate development of novel plant-based foods based on other legumes, such as faba bean. A mixture design was used in this study to evaluate the effect of different proportions of protein, starch and fibre fractions extracted from faba beans on gelation properties, texture and microstructure of the resulting gels. Large deformation properties, in terms of fracture stress and fracture strain, decreased as fibre and/or starch replaced protein. In contrast, Young's modulus and storage modulus increased with substitution of the protein. Light microscopy revealed that for all gels, protein remained the continuous phase within the region studied (65–100% protein fraction, 0–35% starch fraction, 0–10% fibre fraction in total flour added). Swollen and deformed starch granules were distributed throughout the mixed gels with added starch. Leaked amylose aggregated on starch and fibre surfaces and in small cavities (<1 μm) throughout the protein network. No clear difference between samples in protein network structure was observed by scanning electron microscopy. The reduction in large deformation properties was tentatively attributed to inhomogeneities created by the added starch and fibre. The increase in small deformation properties was hypothesised to be affected by water adsorption and moisture stability through the starch and fibre, increasing the effective protein concentration in the surrounding matrix and enhancing the protein network, or potentially by starch granules and fibre particles acting as active fillers reinforcing the gel structure.

1. Introduction

The food sector contributes significantly to the global environmental impact, accounting for e.g. 34% of total greenhouse gas emissions in 2015 (Crippa et al., 2021). Increasing consumption of locally produced plant-based foods have the potential to reduce the environmental impact of food consumption (Willett et al., 2019). Legumes, such as faba beans, can serve as good plant-based protein sources. Faba beans can be grown in most climate areas of Europe, including Sweden, where they are extensively produced but mainly used for animal feed (Crépon et al., 2010; Jansson, 2019). Faba beans are rich in protein, starch, fibre and micronutrients (Crépon et al., 2010). The major proteins in faba beans are the globular storage proteins legumin (11S) and vicilin (7S) (Warsame, O'Sullivan, & Tosi, 2018).

Protein gelation is an essential step in production of a range of foods. Today, many protein-rich plant-based foods are based on soy, but faba beans can serve as an alternative protein source, with promising properties in e.g. production of tofu (Jiang, Wang, Stoddard, Salovaara, & Sontag-Strohm, 2020). Protein gelation can be affected by multiple factors, such as protein source, pH, salt and extraction method (Nicolai & Chassenieux, 2019). The effect of extraction method has been investigated previously in terms of dry fractionation and dehulling on the functionality of faba bean protein fractions (Saldanha do Carmo et al., 2020; Vogelsang-O'Dwyer et al., 2020). It has been shown that dry fractionation has a lower environmental impact than wet fractionation, but yields less pure fractions (Vogelsang-O'Dwyer et al., 2020). Wet extraction and isoelectric precipitation have the benefit of almost completely removing the favism-causing glucosides vicine and convicine (Vioque, Alaiz, & Girón-Calle, 2012). Investigations of the effects of different wet protein extraction methods (alkali extraction and soaked extraction) on the texture and microstructure of faba bean protein gels have shown that gelation at neutral pH, compared with slightly acidic

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

https://doi.org/10.1016/j.foodhyd.2022.107741
Received 22 December 2021; Received in revised form 8 April 2022; Accepted 14 April 2022
Available online 19 April 2022

pH 5, results in less coarse gels (Langton et al., 2020).

Most foods have complex matrices containing more than one component. In addition to protein, faba beans also contain significant amounts of starch (40–44%) (Crépon et al., 2010). Protein and starch can be combined to create mixed gels, which have been studied previously for other protein and starch sources (Aguilera & Baffico, 1997; Joshi, Aldred, Panozzo, Kasapis, & Adhikari, 2014). The contribution from starch has been found to differ depending on whether the starch gels before or after the protein (Muhrbeck & Eliasson, 1991). If the starch in a mixed protein/starch gel is allowed to gel before the protein, the starch and protein will form two separate networks, supplementing each other without any specific interactions (Muhrbeck & Eliasson, 1991). If the starch has a higher gelatinisation temperature and the protein gels first, diffusion and network formation by the starch amylose is hindered and the starch instead acts mainly as a filler material. Studies on β-lactoglobulin/amylopectin gels have shown that amylopectin can influence protein aggregate density and connectivity at different length scales as observed by light microscopy (LM), confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) (Olsson, Langton, & Hermansson, 2002). These microstructural changes are correlated to the rheological properties of the gels (Olsson et al., 2002).

Another component present in significant amounts in faba beans is fibre (9–10% crude fibre) (Crépon et al., 2010). Dietary fibre is important for human health and can have multiple health benefits (Stephen et al., 2017). Nonetheless, average dietary fibre intake in most European countries is estimated to be below the recommended level (Stephen et al., 2017). Many side-streams from the food industry are rich in fibre and can be added to different food systems to modify their texture and functional properties (Lan et al., 2020; Niu et al., 2021). Faba beans contain mainly insoluble fibre (10.7–16.0%) and minor amounts of soluble fibre (0.6–1.1%) (Mayer Labba, Frøkiær, & Sandberg, 2021). Insoluble dietary fibre (IDF) from sugarcane has been shown to improve myofibrillar protein gelation properties through moisture stabilisation, due to its high water-holding capacity (Zhuang, Jiang, et al., 2020).

As food matrices are often complex, it is important to understand the effect of different macromolecules on the gelation properties of protein, particularly as dry fractionation, where the resulting fraction is less pure than in wet extraction, is increasing in popularity. In this study, the effect of starch- and fibre-rich fractions on faba bean protein gelation was investigated using a mixture design approach. This approach is widely applied in product formulation and can be used to study two or more components mixed in different ratios (Buruk Sahin, Aktar Demirtaş, & Burnak, 2016; Lawson, 2014). The effect of changes in the ingredient ratios can be studied and interaction effects can be identified. Surface or contour plots can be used to visualise how different product properties are influenced by the ratios of the raw materials.

The aim of this study was to determine the effect of substituting part of the protein for starch- and/or fibre-rich fractions on gel texture and microstructure in faba bean-based protein gels. Gel texture was analysed in terms of small and large deformations and microstructure, using light and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

The faba beans (*Vicia faba* L. var. *Gloria*) used for extraction were kindly provided by RISE (Research Institutes of Sweden). The beans were grown in central Sweden and harvested and dried in 2016. Before use in this study, the faba beans were dehulled (Hi-Tech Machinery Manufacturing Co. Ltd., China), separating the cotyledon and hull, and milled (Ultra-Centrifugal Mill ZM-1, Retsch, Germany) into flours using a mesh size of 0.5 mm. Only the cotyledon flour was used for further extraction.

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

2.2. Extraction of protein, starch and fibre fractions

The extraction process used to obtain the different faba bean fractions was based on isoelectric precipitation of protein and a starch extraction procedure described previously, with some modifications (Lee, 2007).

To extract the protein, the faba bean cotyledon flour (200 g) was dispersed in distilled water at a ratio of 1:10 (w/v). The pH was adjusted to 9.0 using 2 M NaOH and the dispersion was stirred for 1 h at room temperature before centrifugation (Thermo Scientific, Sorvall Lynx 4000, Waltham, MA, USA) at 3700×*g* (20 °C, 30 min). The supernatant was collected, pH-adjusted to 4.0 (a value chosen based on protein yield; (Herneke et al., 2021)) using 1 M HCl, stirred at room temperature for 1 h and then centrifuged at 3700×*g* (20 °C, 15 min). The pellet was collected and washed once by dispersion in distilled water at a ratio of 1:10 (w/v) with pH adjustment to pH 4.0, followed by centrifugation at 3700×*g* (20 °C, 15 min). The pellet was collected and freeze-dried (Martin Christ, Epsilon 2-6D LSC Plus, Osterode am Harz, Germany).

The starch and fibre fractions were extracted from the pellet obtained from the first centrifugation step during protein extraction. The pellet was dispersed in 750 mL 33.3 mM NaOH and stirred at room temperature for 24 h. The mixture was then left to stand, without agitation, at 4 °C for an additional 24 h before centrifuging at 3700×*g* (20 °C, 5 min). The resulting supernatant was discarded and replaced with distilled water. The washing process was repeated approximately five times until pH 7 was reached. After the final wash, the pellet was collected and mixed in a kitchen blender (Wilfa, XPLODE Vital, Hagan, Norway) with 1 L distilled water for 2 × 20 s on the 'smoothie' setting. Thereafter, the mixture was filtered through a 70 μm nylon filter. The filter cake was dispersed in 600 mL distilled water, mixed in the kitchen blender as previously described and filtered through the nylon filter. The mixing and filtering process was repeated 12 times, until further washing only recovered minor amounts of starch per additional washing step. After the last filtering step, the filter cake was collected and freeze-dried to obtain the fibre fraction. The filtrate was stored overnight without agitation at 4 °C to allow the starch to sediment. The supernatant was decanted and the sedimented starch dried in an oven at 40 °C for 48 h, or until fully dry. All three fractions were ground into flours using a pestle and mortar before use. The starch and fibre fractions were sieved (Retsch, AS200 basic, Haan, Germany) using a 250 μm mesh to remove any larger particles.

2.3. Characterisation of raw materials and extracted fractions

The analysis of protein, fat, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin content was performed by the Analysis Laboratory at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Ultuna. The crude protein content was analysed using the Kjeldahl method and a conversion factor of 5.4 (Mosse, 1990; Nordic Committee on Food Analysis, 1976). The measurements were performed using the digestion system Digestor 2520 combined with a Kjeltec 8400 analyser unit and an 8460 sampler unit (Foss Analytical A/S, Hillerød, Denmark). NDF and ADF were measured as described previously (Chai & Udén, 1998; Van Soest, Robertson, & Lewis, 1991). The fat content was determined according to the EU guideline method (EC) 152/2009 (Commission Regulation (EC) No 152/2009. H. Determination of Crude Oils and Fats, 2009), using a Hydrotec 8000 in combination with a Soxtec Extraction unit (Foss Analytical A/S, Hillerød, Denmark). For the fibre fraction, NDF, ADF, lignin, cellulose and hemicellulose were analysed using sequential

analysis (Robertson, Van Soest, James, & Theander, 1981), whereas the ADF and NDF in the protein and starch fractions were analysed separately (Chai & Udén, 1998; Van Soest et al., 1991). The cellulose content in the cotyledon and hull flours was calculated as the fraction remaining after subtracting the starch, protein, fat, hemicellulose, lignin and ash. All measurements were performed in at least duplicates, except for the protein and fat content in the cotyledon and hull flours, where only one sample was analysed.

Analysis of starch, resistant starch, moisture and ash content was performed in duplicate. Total starch content was determined using a Total Starch Assay Kit (Total starch HK assay kit, Megazyme Ltd, Wicklow, Ireland). Resistant starch was determined using a Resistant Starch Assay Kit (K-RSTAR, Megazyme Ltd, Wicklow, Ireland). Moisture content was determined by oven drying at 105 °C overnight and ash content according to the AOAC official method 942.05.

The monosaccharide composition of the faba bean cotyledon, hull and extracted fibre fraction was determined using a modified version of an existing method (Sluiter et al., 2008). First, 3 mL 72% H_2SO_4 was added to 200 ± 0.5 mg of sample in a 150 mL beaker and kept under vacuum for 15 min, before being placed in a water bath for 1 h at 30 °C with stirring every 20 min. Thereafter, 84 g of deionised water were added, followed by autoclaving at 125 °C for 1 h. The samples were filtered through a 1.6 μm filter (Whatman Grade GF/A Fine Retention Filter) with vacuum and diluted to a total volume of 100 mL. The filtrate was further diluted (1:10) with deionised water to remain within the calibration range, and fucose was added as an internal standard to obtain a total concentration of 400 mg fucose/L. Samples were then filtered through 0.2 μm filters (Acrodisc Syringe Filters with PVDF Membrane) into HPLC vials. The monosaccharide composition was analysed using an ion chromatograph (ICS 3000 Dionex) equipped with an AEC column (CarboPac PA 1 analytical 4 × 250 nm). Standards used were D (+) glucose, D (+) xylose, D (+) galactose, L (+) arabinose, L (+) rhamnose and D (+) mannose. All samples were measured in duplicate.

Uronic acid analysis was performed according to the Blumenkrantz-Asboe-Hansen method (Blumenkrantz & Asboe-Hansen, 1973) with modifications. In short, 10.6 ± 0.4 mg sample was weighed into a 10 mL glass tube. The samples were kept on ice and 0.5 mL 96% H_2SO_4 was added twice with 5 min of intermittent vortexing, after which 0.25 mL of deionised water was added twice with 5 min of intermittent vortexing. Deionised water was then added to a total volume of 10 mL. Next, 20 μL of 4 M sulfamic acid-potassium sulfamate (pH 1.6) were added to 1.5 mL plastic Eppendorf tubes, followed by 160 μL of the hydrolysed sample and 800 μL 12.5 mM Borax in 96% H_2SO_4. The tubes were oven-incubated at 95 °C for 20 min and the samples were cooled and pre-read on a UV–Vis spectrophotometer (Cary 60 UV–Vis Spectrophotometer, Agilent Technologies) at 525 nm, followed by addition of 40 μL of 0.15% (w/v) 3-phenylphenol in 0.5% (w/v) NaOH and reading again at 525 nm. A standard curve was created using D (+) galacturonic acid. The pre-read value was subtracted from the absorbance read after the colorimetric reaction to obtain the uronic acid concentration. All samples were analysed in duplicate.

2.4. Experimental design

An extreme vertices mixture design including axial blends and the overall centre point was used for analysis of small and large deformations (Fig. 1) (Lawson, 2014). Two additional mixtures, not included in the original design, were included along the protein-starch and protein-fibre axis for further investigation of the two-component systems. A total solids content of 20% was used unless otherwise stated. Of these 20% solids, the proportion of protein fraction (X_1) was kept in the range 65–100%, the starch fraction (X_2) was limited to 0–35% due to sedimentation and the fibre fraction (X_3) was limited to 0–10% due to layering during mixing at higher fibre content. The exact percentages of protein, starch and fibre fraction at each design point are listed in Table S1. Rheology experiments were run with two replicates at each design point. One replicate (mean value of 5–8 gels produced simultaneously from the same batch) was run at each point for the compression test, except for the centre point, at which six replicates were run to assess the variance.

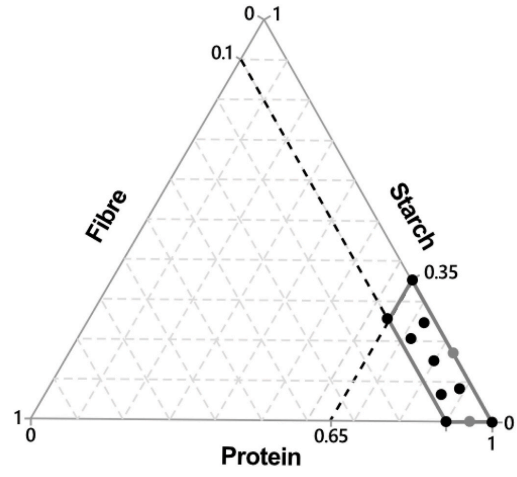


Fig. 1. The extreme vertices mixture design, including axial and centre points, used for the experiments with two additional experimental points (grey) added along the two-component axes.

2.5. Gel formation

Ingredients were dry mixed before being dispersed in distilled water and stirred for 15 min, followed by adjustment of the pH to 7.0 using 2 M NaOH. After pH adjustment, the mixture was stirred for 30 min with additional pH adjustments when needed and volume adjustment to give a final concentration of 20% (g flour/g sample). To limit sedimentation of the starch, the mixture was stirred for an additional 20 min in a water bath at 58 °C. Thereafter, 2.8 mL were loaded into hollow glass tubes with inner diameter 12 mm. The bottom of the glass tubes was closed with a rubber lid and the top with thread tape punctured with a small hole to prevent pressure build-up. The samples were heated in a water bath at 95 °C for 30 min, then cooled in tap water and stored in a fridge overnight for compression test and preparation for microscopy.

2.6. Rheology

A Discovery HR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with a 40 mm aluminium plate was used to study the gelation process of mixtures prepared as described in section 2.5, excluding the final heating step at 95 °C (Sun & Arntfield, 2011). Paraffin oil, coating the exposed parts of the sample, combined with a custom made solvent trap was used to limit sample evaporation (Saldanha do Carmo et al., 2020). Storage modulus (G') and loss modulus (G'') were recorded at a frequency of 1 Hz and a strain of 0.5% during a temperature ramp. A strain sweep was performed on each sample after gelation to confirm that the strain was within the linear viscoelastic region. The temperature ramp comprised heating from 60 to 95 °C at a rate of 1.5 °C/min followed by a holding time at 95 °C for 30 min and cooling to 25 °C at 1.5 °C/min followed by an additional holding time of 30 min at 25 °C.

2.7. Compression tests

Samples prepared as described in section 2.5 were allowed to

equilibrate to room temperature for 1 h before being cut into cylindrical pieces with height 14 mm and diameter 12 mm. Compression tests were performed 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. The samples were compressed to 60% at a rate of 1 mm/s. True stress and true strain were determined as described previously (Munialo, van der Linden, & de Jongh, 2014). True fracture stress and true fracture strain (hereafter referred to simply as fracture stress and fracture strain) were defined as the maximum true stress and corresponding true strain at the first clear peak before fracture. Young's modulus was calculated as the slope of the true stress-true strain curve during the initial 1–5% of deformation.

2.8. Regression analysis of compression tests and rheology

Regression analysis using the standard least squares method in JMP Pro version 16.0.0 (SAS Institute, Cary, NC, USA) was performed, fitting the data to a reduced version of the special cubic Scheffé model:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

where Y is the predicted response, X_1, X_2 and X_3 are the protein, starch and fibre content given as proportions of the total amount of each fraction added, β_1, β_2 and β_3 are the regression coefficients for each linear effect term, and β_{12}, β_{13}, β_{23} and β_{123} are the regression coefficients for the binary and ternary interaction effects terms. The L-pseudo component scaling in JMP was used for rescaling the components and to reduce multicollinearity. A dummy variable technique (Miller, 1984; Redgate, Piepel, & Hrma, 1992), combined with forward selection to minimise the Akaike information criteria (AIC), was used to decide what terms to include in the final model. In short, random and uniformly distributed dummy variables were generated and added to the list of potential candidate variables for the model. The selection process was stopped when either the first dummy variable entered the model or the AIC was minimised. The main effect terms (β_1, β_2 and β_3) were forced into the model. After model fitting, residuals were checked for homoscedasticity and normal distribution. Adjusted coefficient of determination ($R^2(adj)$) was used to determine how well the model fitted the sample data and the lack-of-fit was tested to evaluate whether the model adequately described the functional relationship between the experimental factors and the response variable. Contour plots of raw data were created using Origin Pro 2017 version 94E (OriginLab Corporation, Northampton, MA, USA).

2.9. Microscopy

Gels prepared as described in section 2.5 were cut into approximately 2 × 2 × 2 mm^3 pieces and fixated overnight in 2.5% glutaraldehyde and 0.1% ruthenium red solution (Langton et al., 2020). The samples were then further fixated in 1% osmium tetraoxide for 2 h, followed by dehydration in a series of ethanol with increasing concentration. For light microscopy (LM), the samples were infiltrated and hardened using Technovit 7100 and sectioned into 1-μm sections using an ultramicrotome (Leica Microsystems GmbH, Leica EM UC6, Wetzlar, Germany). The sections were double-stained with light green and iodine before being examined under a microscope (Nikon, Eclipse Ni–U microscope, Tokyo, Japan) equipped with a 40 × (0.75 NA) apochromatic objective. Images were captured with a Nikon Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan) with 0.12 μm/pixel.

For scanning electron microscopy, samples were critical point dried (Quorum Technologies Ltd, K850 Critical Point Dryer, East Sussex, UK) after the dehydration step, fractured and sputter-coated with gold (Cressington Scientific Instruments, Sputter coater-108 auto, Watford, UK) before being examined at 5 kV (Hitachi, FlexSEM 1000II, Tokyo, Japan). Images were recorded digitally at a magnification of × 10 000 (9.9 nm/pixel) and × 50 000 (1.98 nm/pixel).

3. Results and discussion

The design used in the study made it possible to investigate the effect of starch and/or fibre on protein gel textural and microstructural properties. It also allowed comparison of the textural properties of gels produced from faba bean protein isolate and gels produced from less pure, hypothetical protein fractions. Dry-fractionated protein concentrate from faba bean flour contains approximately 61% protein, 15% starch and 10% non-starch polysaccharides, while faba bean flour contains approximately 35% protein, 43% starch and 7% non-starch polysaccharides (Saldanha do Carmo et al., 2020). Understanding how the purity of a protein-rich material affects gel textural properties can help in choosing the most suitable source for a certain application.

3.1. Characterisation of raw materials

The chemical composition of the protein, starch and fibre fractions extracted from faba bean cotyledon for use in this study is summarised in Table 1. The cotyledon and hull flours obtained after dehulling of the beans are included for comparison. The protein fraction had a protein content of 77 g/100 g and a relatively high ash content (8 g/100 g). It also contained the highest amount of fat (3.35 g/100 g). The starch fraction contained 86 g starch per 100 g, with 8.1 g of this being resistant starch, and only minor amounts of protein, fibre, fat and ash. The fibre fraction was rich in fibre (28.94 g/100 g NDF and 15.17 g/100 g ADF), but also contained significant amounts of starch (22 g/100 g) and a minor amount of protein. The protein and starch fractions contained higher amounts of protein and starch, respectively, than the cotyledon flour used as the starting material for their extraction. The fibre fraction seemed to contain somewhat higher amounts of cellulose than the cotyledon starting material. However, the cellulose and hemicellulose contents in the fractions and the cotyledon flour were obtained using different methods, and can therefore not be compared directly. Based on literature values, the amounts of NDF and ADF in the fibre fraction were about 50% higher than the amounts normally found in whole (cotyledon plus hull) faba bean flour (Gdala & Buraczewska, 1997). The hull fraction contained less protein and starch, but larger amounts of fibre than the cotyledon flour. This highlights the potential of the hull fraction as a

Table 1
Chemical composition (g/100 g dry weight ± 1 st. dev.) of faba bean cotyledon, faba bean hull and protein, starch and fibre fractions extracted from the cotyledon. N.D. Not determined. N.A. Not applicable. *Expressed as g/100 g sample.

	Fractions extracted from cotyledon			Raw material	
	Protein fraction	Starch fraction	Fibre fraction	Cotyledon	Hull
Crude protein	77.28 ± 0.18	0.48 ± 0.03	5.27 ± 0.12	33.03 ± N.A.	20.38 ± N.A.
Starch	0.27 ± 0.05	86.43 ± 1.39	22.09 ± 0.21	40.31 ± 1.3	19.07 ± 0.63
Resistant starch	N.D.	8.1 ± 0.20	0.40 ± 0.02	N.D.	N.D.
NDF	0.99 ± 0.28	2.19 ± 0.28	28.94 ± 4.37	N.D.	N.D.
ADF	1.32 ± 0.03	1.37 ± 0.14	15.17 ± 0.02	N.D.	N.D.
Lignin	N.D.	N.D.	0.97 ± 0.11	2.97 ± 0.5	8.24 ± 1.07
Cellulose	N.D.	N.D.	14.20 ± 0.09	10.11 ± N.A.	26.88 ± N.A.
Hemicellulose	N.D.	N.D.	13.77 ± 4.34	8.79 ± 0.43	21.25 ± 0.85
Fat	3.35 ± 0.04	0.27 ± 0.03	0.35 ± 0.01	1.6 ± N.A.	1.24 ± N.A.
Ash	8.0 ± 0.72	0.2 ± 0.01	3.5 ± 0.03	3.19 ± 0.21	2.94 ± 0.03
Moisture*	5.3 ± 0.07	7.1 ± 0.01	6.3 ± 0.0	10.87 ± 0.15	10.45 ± 0.16

fibre-rich ingredient in various foods for increased fibre content. It should be noted that a large proportion of the starch and protein in the hull fraction most probably did not originate from the hull itself, but was rather a result of poor separation of the hull and the cotyledon (Frejnagel, Zduńczyk, & Krefft, 1997).

The monosaccharide composition of the fibre fraction, cotyledon and hull flours is shown in Table 2. Due to the presence of starch and cellulose, glucose was the major monosaccharide in all samples. The lower glucose levels in the fibre fraction were the result of starch removal during extraction.

The cotyledon contained higher amounts of arabinose and galactose, but lower amounts of rhamnose, xylose and uronic acids, than the hull. The arabinose and galactose levels in the cotyledon and hull may suggest presence of arabinan and arabinogalactan polysaccharides (Bhatty, 1990). Xylose was not detected in the cotyledon, whereas the hull contained relatively large amounts, suggesting presence of xylans in the hull (Gdala & Buraczewska, 1997). The differences in monosaccharide composition between the hull and the cotyledon indicate that inclusion of the hull during extraction of the fibre fraction could influence the composition of the fibre fraction.

The main difference in monosaccharide composition of the fibre fraction compared with the cotyledon and hull was the high content of arabinose and uronic acids. This suggests relatively high contents of arabinan and/or other arabinose-containing fibres, as well as pectin. Surprisingly, both xylose and rhamnose were detected in the fibre fraction, but not in the cotyledon from which the fraction was extracted. However, weak peaks were identified for both xylose and rhamnose in the cotyledon samples, indicating their presence in low concentrations. Hence, the higher contents in the extracted fibre fraction most likely resulted from up-concentration during extraction.

3.2. Rheology

For the two-component gels, an increase in storage modulus (G') was observed with increased starch and fibre content (Fig. 2a–b). The overall development in G' was similar for all the mixtures, indicating that protein played a dominant role in the gel network (Fig. S1). The loss modulus (G'') followed a similar pattern to G' (results not shown). The tan δ value (G''/G') was in the range 0.13–0.17 for all gels. All values recorded for G', G'' and tan δ of the final gels can be found in Table S2.

The main differences in the development of G' between the samples were the rate of increase in G' during heating and cooling. Both starch and fibre contributed to a higher rate of increase in G' during heating and cooling compared with the gels containing only protein. The G' value of the protein/fibre gels was higher than that of the pure protein gels throughout the whole gelation process. On the other hand, the initial G' value of the starch/protein gels was lower than the initial G' value of the pure protein gels, and overtook it only after reaching a temperature of 75–80 °C (Fig. S2). Thereafter, G' stayed higher for the remainder of the process. Faba bean starch gelatinisation, defined as the peak temperature from differential scanning calorimetry measurements, occurs at a temperature of approximately 70 °C (Ambigaipalan et al., 2011). The pasting temperature of faba bean starch is reported to be around 77 °C (Nilsson et al., 2022). However, one factor that might have influenced the functional properties of the starch used in this study was the alkaline conditions during extraction. Alkaline extraction or alkaline treatment of starch has previously been found to affect the structure, gelatinisation temperature and pasting properties of other starches (Cai et al., 2014; Dokić, Dapčević, Krstonošić, Dokić, & Hadnađev, 2010). Nonetheless, the previously reported functional properties of faba bean starch indicate that the increase in G' with addition of starch to the mixtures was a result of starch swelling, pasting and gelatinisation. The increase during cooling was likely due to starch amylose re-association and network formation. A similar effect has been observed for lentil starch during heating and cooling of pea protein gels (Johansson et al., 2021).

The consistently higher values for the protein/fibre gels compared with the pure protein gels indicates that the increasing effect from the fibre fraction was constant throughout the gelation process. This is consistent with the hydration properties of insoluble sugarcane fibres, which are reported not to change with temperature (Zhuang, Wang, Jiang, Chen, & Zhou, 2020). In general, the samples with increased addition of starch or fibre took longer to equilibrate during the holding time at 25 °C, as seen by the continued increase in G' at the end of the measurement (Fig. 2 and Fig. S1). This could be related to amylose network stabilisation by the starch and water migration due to water adsorption by the starch and/or fibre. However, for many of the starch and fibre containing samples, the increase in G' during the final holding time was not as large as in the 2-component systems (Fig. S1). Further research is needed to confirm and explain the observed increase.

Particles in a gel can be classified as either active or inactive fillers, depending on their interaction with the gel matrix (Dille, Draget, & Hattrem, 2015). Both modified cassava starch and insoluble dietary fibre from sugarcane have previously been shown to create thermodynamically incompatible and phase-separated gels with myofibrillar protein (Zhuang, Jiang, et al., 2020). Similar observations have been made for corn starch and soy protein gels (Li, Yeh, & Fan, 2007). Further studies of the investigated faba bean systems would be needed to specify the interaction between the starch/fibre surface and the protein matrix to elucidate whether they act as active or inactive filler particles. A decrease in G' has previously been correlated with addition of inactive filler particles and starch to protein gels (Dille et al., 2015; Yu, Ren, Zhao, Cui, & Liu, 2020). Despite this, we observed an increase in G' for the mixed gels compared with the pure protein gels. An increase in G' after heating of soy milk has been observed previously following inclusion of okara fibre with varying particle sizes (Lan et al., 2020). One possible explanation for the increase in G' with addition of fibre is water adsorption and improved moisture stability. This has been reported for myofibrillar protein gels with insoluble fibres from sugarcane (Zhuang, Wang, et al., 2020). Another potential explanation for the increase in G' could be that the starch granules and fibre particles act as active fillers. Active filler particles can be expected to reinforce the gel and increase the overall shear modulus if the filler particle has a higher modulus value than the surrounding gel matrix (Dille et al., 2015).

Contour plots at different temperatures visualised the differences in G' between samples during gelation (Fig. 2I–V). In contour plots at additional temperatures (data not shown), it was observed that a high proportion of the fibre fraction resulted in the highest G' values until the mixtures reached about 80 °C. Above that temperature, high starch content also resulted in comparably high G' and the highest values were observed when the proportions of fibre and starch were both high. During cooling, a high proportion of starch contributed more to the increase in G' than addition of fibre. This was apparent as a shift in the high-value region (shown in red in Fig. 2I–V) from the high fibre/high starch region towards the high starch region. The slight decrease in G' observed at high starch and high fibre, compared with only high starch or only high fibre, could be due to the large number of particles disrupting the continuous protein phase.

Table 2
Relative percentage of measured monosaccharides in the fibre fraction, the faba bean starting material (cotyledon) and hull. N.D. Not detected. N.Q. Detected but not quantified due to peaks being too vague for accurate quantification.

	Extracted fraction	Raw material	
	Fibre fraction	Cotyledon	Hull
Arabinose	20.39 ± 0.07	4.69 ± 0.01	3.22 ± 0.04
Rhamnose	2.34 ± 0.06	N.Q.	0.68 ± 0.02
Galactose	5.23 ± 0.04	4.70 ± 0.01	3.31 ± 0.02
Glucose	55.02 ± 0.03	88.70 ± 0.31	77.60 ± 0.71
Xylose	4.22 ± 0.02	N.Q.	7.79 ± 0.08
Mannose	N.D.	N.D.	N.D.
Uronic acids	12.80 ± 0.11	1.91 ± 0.33	7.40 ± 0.87

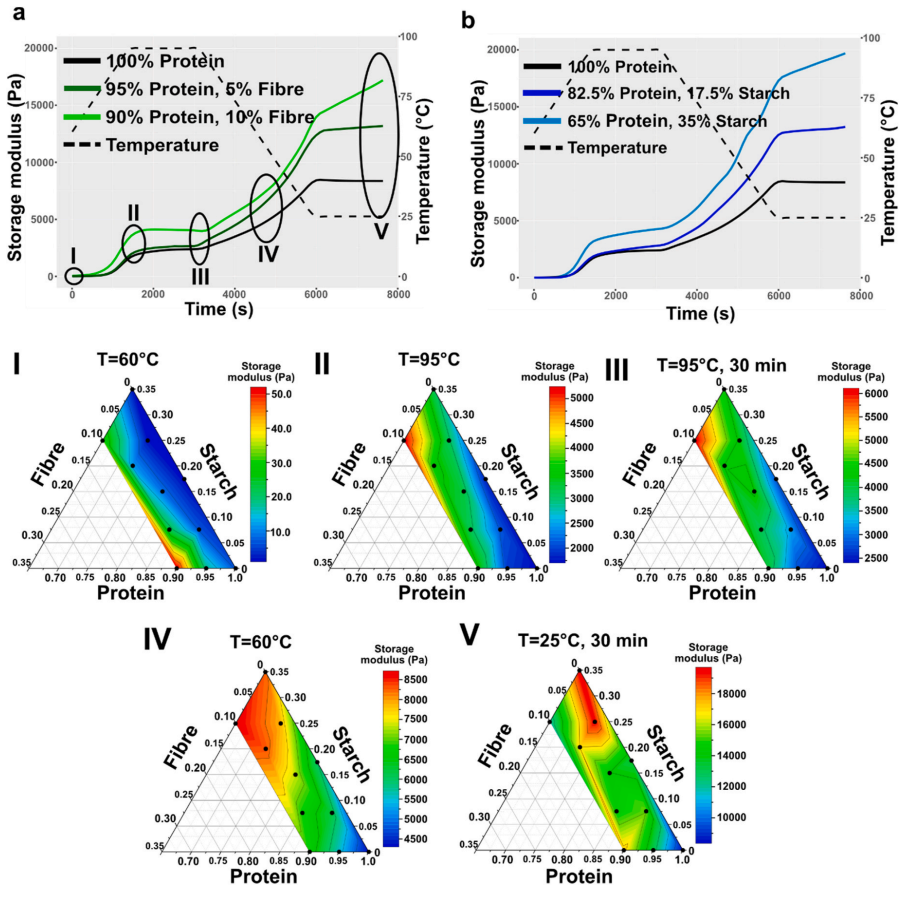


Fig. 2. Storage modulus (G′) throughout the gelation process of (a) protein/fibre gels and (b) protein/starch gels. Percentages of protein, starch and fibre in (a) and (b) are relative to the total amount of flour added. Panels I–V are contour plots of the storage modulus of all gels in the design at different time points during the gelation process, as also indicated in Fig. 2a. The black dots correspond to the measuring points. Axes correspond to proportion of protein/starch/fibre-fraction in total flour added.

3.3. Compression tests

The fracture stress, fracture strain and Young's modulus of gels prepared with different ratios of the extracted protein, starch and fibre fractions are visualised as contour plots in Fig. 3. The exact values with standard deviations are supplied in Table S3. The fracture stress of the gels increased with an increasing proportion of protein. The lowest gel strength was observed at a low protein content in combination with high fibre and high starch content. The fracture strain showed similar behaviour, with an increase with increasing protein content, but the lowest values were obtained for gels containing low amounts of protein and fibre combined with high amounts of starch. The lowest Young's modulus values were observed for gels high in protein and the highest values were obtained for gels with high starch content and/or high fibre content.

A similar decrease in hardness has been observed previously in texture profile analysis (TPA) of soy protein gels with an increased proportion of added native corn starch (Yu et al., 2020). This decrease in hardness was explained in that study by water adsorption by the starch, suppressing the protein aggregation during gelation, and leaked amylose disrupting the protein network formation. At higher starch concentrations, those authors also observed that starch induced formation of discontinuous gels, decreasing the TPA parameters. A reduction in the hardness of soy protein tofu after addition of okara dietary fibre with varying particle size has also been reported (Ullah et al., 2019; Wei et al., 2018). This decline in hardness of tofu with added IDF occurred despite an increase in water entrapment, which on its own could be expected to increase the strength of the gels. Instead, it was suggested that the addition of IDF reduced textural properties by creating a more inhomogeneous and unstable network structure. Large particles have also been suggested to explain the reduction in hardness of tofu gels by unfavourably affecting chain association during gelation, reducing the structural stability (Liu, Chien, & Kuo, 2013).

The larger fibre particles and gelatinised starch granules in our mixed gels most likely acted as inactive filler particles. A previous study examining gellan gum gels and the effect of adding gel particles

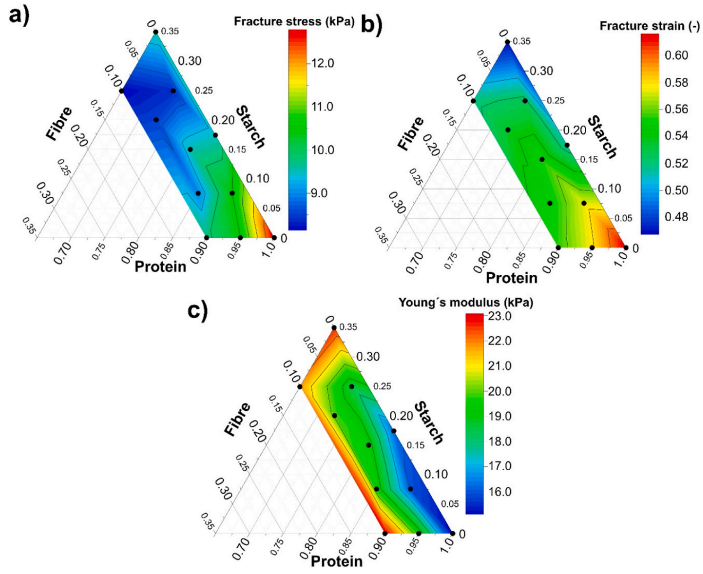


Fig. 3. Contour plots of (a) fracture stress, (b) fracture strain and (c) Young's modulus for gels prepared with different ratios of extracted protein, starch and fibre fractions. The black dots correspond to the measuring points. Axes correspond to proportion of protein/starch/fibre-fraction in total flour added.

approximately 100 μm in size found that addition of gel particles reduced the fracture stress and fracture strain of the gels, where the particles were assumed to act as inactive fillers (Moritaka, Takeuchi, Okoshi, & Fukuba, 2002). An earlier study of whey protein emulsion gels showed the importance of the interaction between filler particles and the gel matrix, with a decrease in gel strength with addition of inactive filler particles and an increase with addition of active filler particles (Dickinson & Chen, 1999).

3.4. Regression analysis of compression test and rheology

The results of regression analysis of the compression test and rheology results are shown in Table 3. Nonlinear blending effects were included in the models for all responses (fracture stress, fracture strain, Young's modulus and G'). However, it should be noted that including nonlinear blending terms in reduced models for mixture designs does not necessarily mean that the components included in these terms (e.g. Protein*Starch for fracture stress) are those chemically causing the nonlinear blending behaviour as a function of composition (Redgate et al., 1992). An additional reason for this is multicollinearity among linear and nonlinear terms (Redgate et al., 1992), where one of the components in the mixture can be written as a function of the others due to the constraint that their proportions should always add up to one.

The relatively high values of protein coefficient obtained for the fracture properties indicate that protein content was the main determining factor for fracture stress and fracture strain. On the other hand, the starch and fibre coefficients were higher for Young's modulus and G'. For G', the interaction term Starch*Fibre had the highest coefficient, indicating a strong interaction.

All models were statistically significant ($p < 0.05$) and did not exhibit any lack-of-fit as indicated by p-values above 0.05. Fracture stress, fracture strain and Young's modulus showed relatively high $R^2(adj)$ and low root mean square error (RMSE) in relation to the overall mean response. This indicates that the model fitted the data well and

Table 3
Regression coefficient (β_i) and R^2 for models fitted to the equation $Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} \times 1 \times_2 + \beta_{13} \times 1 \times_3 + \beta_{23} \times 2 \times_3 + \beta_{123} X_1 X_2 X_3$, where Y is the studied response and X_1, X_2 and X_3 are the proportions of protein, starch and fibre fractions, respectively. Forward selection and minimising of the AIC was used to remove insignificant model terms. Values within bracket are standard error of the regression coefficients. RMSE (root mean square error) is the standard deviation of the residuals.

	Fracture stress		Fracture strain		Young's modulus		Final G'	
	Coefficient (Std.Error)	p-value	Coefficient (Std.Error)	p-value	Coefficient (Std.Error)	p-value	Coefficient (Std.Error)	p-value
(Protein-0.65)/0.35	12.18 (0.38)	<0.0001	0.61 (0.01)	<0.0001	15.0 (0.8)	<0.0001	9300 (1300)	<0.0001
Starch/0.35	9.50 (0.44)	<0.0001	0.47 (0.01)	<0.0001	22.3 (0.8)	<0.0001	20900 (1400)	<0.0001
Fibre/0.35	4.24 (1.29)	<0.0001	0.41 (0.06)	<0.0001	43.9 (3.5)	<0.0001	37400 (6500)	<0.0001
Protein*Starch	−3.51 (1.56)	0.0445	–	–	−7.3 (2.7)	0.0210	–	–
Protein*Fibre	–	–	–	–	−32.2 (7.3)	0.0010	–	–
Starch*Fibre	–	–	0.39 (0.12)	0.0055	–	–	−58300 (13600)	0.0004
Protein*Starch*Fibre	–	–	–	–	–	–	–	–
Lack of fit (p-value)	0.26		0.57		0.33		0.12	
RMSE	0.52		0.014		0.89		2360	
R^2 (adjusted)	77.5%		81.0%		84.0%		56.4%	
p-value (model)	<0.0001		<0.0001		<0.0001		0.0004	

could be useful for understanding behaviour tendencies in the region studied. Lower R^2*(adj)* combined with high RMSE limited the usefulness of the model for the final G'. This poor fit of the model for the final G' was partly a result of the relatively large variation between replicates. Ternary plots of the final models are shown in Fig. S3.

3.5. Microstructure

The microstructure of the gels at the four vertex points, the centre point and an additional point on the starch-protein axis was evaluated by LM and SEM (Figs. 4 and 5). For all gels, LM revealed a continuous protein phase with homogeneous microstructure at the observed level of magnification (Fig. 4). Dense homogeneous protein microstructure has previously been observed for faba bean protein gels at pH 7 (Langton et al., 2020). The starch was present as swollen and deformed starch granules, as well as what were assumed to be small amylose aggregates from material leaked from the granules. Cell wall fragments were evident as white/non-stained parts throughout the gels including the fibre fraction (Fig. 4). Aggregation of amylose was observed on the surface of starch granules and, to a smaller extent, on the surface of larger fibre particles. Phase separation between the starch and protein with starch aggregated on surfaces and in smaller aggregates throughout the protein matrix has been reported previously in mixed protein/starch gels (Johansson et al., 2021; Li & Yeh, 2003).

The amylose aggregates observed by LM were also visible throughout the protein network in the SEM micrographs of gels containing starch (Fig. 5a). The amylose was present as aggregates inside spherical cavities (approximately 1 μm in diameter). Similar cavities were observed in the protein/fibre gels with what was assumed to be smaller fibre particles. No or few such cavities were observed in the protein gels. As observed by LM, SEM revealed amylose aggregates and amylose networks on the surface and in close proximity to starch granules and some of the fibre particles (micrographs not shown).

No major differences in the gel protein network were observed in the SEM micrographs after addition of fibre and/or starch fraction compared with the pure protein gel, despite differences in protein concentration (Fig. 5b). This could be a result of water adsorption by the starch and the fibre, which would increase the effective protein concentration in the continuous phase and keep it relatively constant for the different gels. Others have shown that starch and amylopectin can affect protein networks formed during heat-induced gelation (Olsson et al., 2002; Shim & Mulvaney, 2001). An additional reason for the lack of differences could be the high content of solid material in our gels, limiting diffusion of the leaked starch amylose. A previous study observed smaller differences in microstructure between starch/protein gels prepared at different pH and temperatures as the solids content was increased from 15% to 30% (Shim & Mulvaney, 2001). The high solids content in our gels and protein gelation, as indicated by a drastic increase in G', starting at a temperature close to the faba bean starch gelatinisation temperature, could both have limited the ability of amylose to form a continuous network.

3.6. Texture and microstructure

The general development in G' was similar for all gels, suggesting that protein was the dominant component during gelation. This is in agreement with protein forming the continuous phase for all gels, as observed by LM, and the protein network structure being very similar for all the gels, as observed by SEM.

Interactions between protein, starch and fibre in mixed gels influenced the textural properties and microstructure. Most heat-induced mixed protein-polysaccharide gels are obtained from thermal

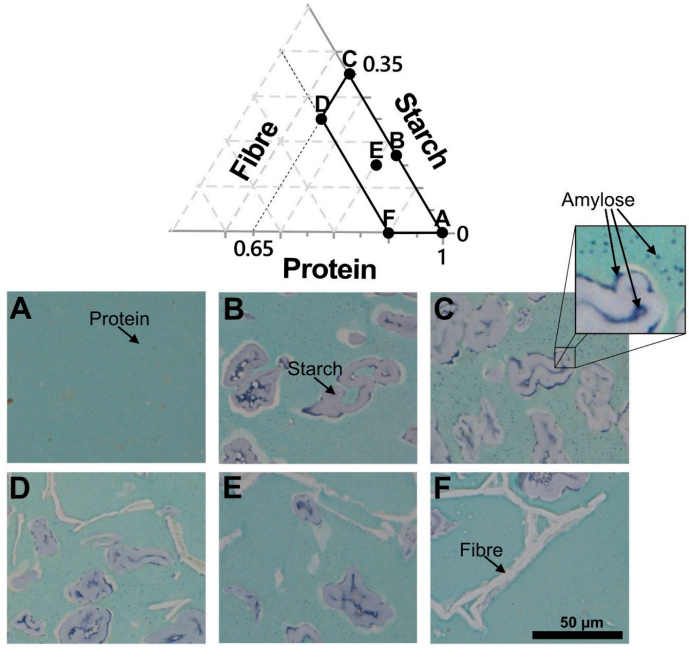


Fig. 4. Light micrographs of gels with different ratios of the extracted fractions (A–F) as indicated by the design points (filled circles) in the mixture triangle. Magnification: × 40. Pixels: 2560 × 1920.

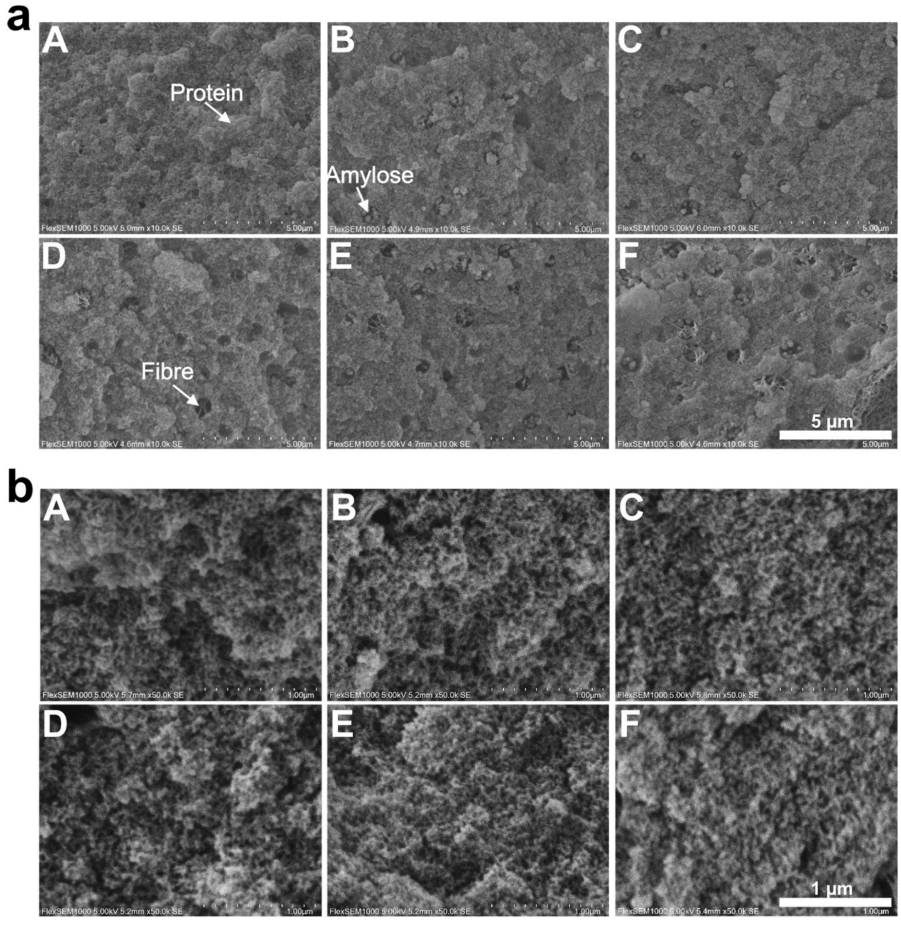


Fig. 5. Scanning electron micrographs of gels with different ratios of the extracted fractions corresponding to the design points marked in the mixture triangle seen in Fig. 4. Magnification: × 10.000 (a), × 50.000 (b). Pixels: 1280 × 960 pixels.

treatment under thermodynamic incompatibility conditions (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Starch and fibre have previously been identified as non-interacting or inactive fillers in other protein gels (Li et al., 2007; Zhuang, Jiang, et al., 2020). Addition of starch and fibre will increase the heterogeneity of the gel microstructure and affect the textural properties. However, the interaction between starch/fibre and protein depends on multiple factors such as source, surface properties, chemical modifications and the presence of other components. Interactions have been reported in e.g. wheat dough systems and fibre particles with modified surfaces (Ryan & Brewer, 2005; Wei et al., 2018).

Fracture of a gel is suggested to occur by propagation of cracks (Dille et al., 2015). Cracks are often formed at structural defects, such as filler particles, acting as stress concentrators during deformation (Dille et al., 2015). Similarly, microscale inhomogeneity based on apparent coarseness of the protein network has previously been correlated with lower fracture stress in pea protein gels (Munialo et al., 2014). The porous nature of the gel could cause step-wise crack growth, resulting in lower fracture stress (Munialo et al., 2014). Hence, inhomogeneities in the form of amylose/fibre-filled cavities, starch granules and larger fibre particles might explain the reduced fracture stress and fracture strain after addition of starch and/or fibre in this study.

While addition of fibre and starch fraction resulted in a decrease in fracture stress and fracture strain, an increase in Young's modulus and G' was observed. A similar increase in G' of heated soy milk after inclusion of large fibre particles (250–380 μm), accompanied by a decrease in textural properties of tofu prepared from the same raw materials, has been observed by others (Lan et al., 2020). Large deformation properties, such as fracture stress and fracture strain, are affected by inhomogeneities in the matrix. On the other hand, small deformation properties, such as Young's modulus and G', could be expected to be less dependent on inhomogeneity and inactive filler particles, and more dependent on the continuous phase. However, filler particles will also affect small deformation properties, especially active filler particles (Dille et al., 2015). Hence, the larger fibre particles, starch granules and small amylose/fibre aggregates throughout the protein matrix could explain the reduction in fracture stress and fracture strain seen in this study, while addition of fibre/starch could have increased G' through

water adsorption and moisture stability. The latter could have increased the effective protein concentration in the surrounding protein matrix and potentially overshadowed the negative effect of starch and fibre on G' and Young's modulus as filler particles.

Differences in heating and cooling rates for the rheology tests and gels prepared for compression tests and microscopy will influence the gelation and affect the comparability of the results from these measurements (Pelgrom, Boom, & Schutyser, 2015; Sun & Arntfield, 2011). The heating and cooling rates of gels prepared for compression and microscopy in this study exceeded the rates used during the rheology measurements by a factor of 10 or more. Hence, the lower heating rate during rheology measurements might have allowed starch gelatinisation to progress further before protein gelation occurred compared with the gels prepared for compression and microscopy. Heating and cooling rates have previously been shown to affect the gelatinisation of certain starches, with e.g. the pasting properties of wheat and semolina flour being affected by heating/cooling rate and those of rice flour being less affected (Mariotti, Zardi, Lucisano, & Pagani, 2005). For wheat and semolina flour, higher rates have been found to result in higher peak and end viscosity during viscoamylographic tests (Mariotti et al., 2005). A similar increase in viscosity at higher heating/cooling rates has been observed for faba bean starch, and can potentially be linked to more pronounced granular swelling at higher heating rates (Nilsson et al., 2022).

The cooling rate also significantly affects protein gelation, with a lower rate increasing gel strength (Sun & Arntfield, 2011). In mixed systems, slower cooling rates will give more time for the protein to arrange in a network, whereas fast heating rates can reduce phase separation (Pelgrom et al., 2015). It has been shown that the contribution from starch and the properties of the gel and gel network of starch/-protein mixed gels will differ depending on which component gels first (Muhrbeck & Eliasson, 1991). Those authors found that when the starch network forms before the protein network, the protein and starch supplement each other and form two continuous networks without any specific interaction. On the other hand, if the protein gels before the starch, this hinders diffusion and aggregation of starch amylose and the starch will not form a continuous network and will instead act as a filler material (Muhrbeck & Eliasson, 1991). Considering this, the slower heating rate in the rheometer in the present study gave more time for the amylose to leak into solution and aggregate compared with the fast heating during gel formation in the water bath. Hence, the starch might have had a larger positive effect on the G' value, compared with the effect on the fracture properties. The high solids content might also have limited amylose network formation due to reduced diffusion and steric hindrance.

4. Conclusions

Substituting protein for fibre and/or starch reduced gel fracture stress and fracture strain, but increased Young's modulus and storage modulus. Light microscopy revealed that protein formed the continuous phase for all mixtures studied (protein 65–100%, starch 0–35%, fibre 0–10%). Starch granules and fibre particles were distributed throughout the gels. Scanning electron micrographs indicated no clear differences between the protein matrix of the different gels, but revealed small cavities (<1 μm) in the protein matrix where fibre and amylose seemed to have aggregated. The reduction in fracture stress and fracture strain with addition of fibre and/or starch could be the result of inhomogeneities created in the protein matrix. The increase in Young's modulus and storage modulus was possibly more dependent on the continuous phase, where water adsorption by fibre and starch granules increased the protein concentration and moisture stability in the surrounding matrix, or possibly by starch granules and fibre particles acting as active fillers reinforcing the gel matrix.

In a broader perspective, our results indicate that the purity of the faba bean protein fraction can significantly affect the final textural properties of the gel. With a constant solids content, we observed significant differences in the textural properties of gels produced from a protein isolate compared with gels produced from a mixture of protein, starch and fibre with ratios more similar to those in a dry-fractionated protein concentrate. Differences in fracture properties and storage modulus were observed even as a small amount of protein was replaced with starch and/or fibre fraction. This highlights the possible usefulness of wet-fractionated protein isolates despite their higher environmental impact compared with dry-fractionated protein concentrates.

Credit authorship contribution statement

Mathias Johansson: Conceptualization; Formal analysis (statistics, data analysis); Investigation; Methodology; Validation; Visualization; Writing - original draft; Writing - review & editing. **Daniel Johansson:** Conceptualization; Methodology; **Anna Ström:** Writing - review & editing. **Jesper Rydén:** Formal analysis (statistics); Writing - review & editing. **Klara Nilsson:** Investigation; Writing - review & editing. **Jakob Karlsson:** Investigation. **Rosana Moriana:** Investigation; Writing - review & editing. **Maud Langton:** Conceptualization; Funding acquisition; Methodology; Supervision; Writing - review & editing.

Declaration of competing interest

None.

Acknowledgements

This work was supported by FORMAS [grant numbers 2018-01869, 2017-00426]; and C4F Trees and Crops for the Future (TC4F). The authors gratefully acknowledge Henrik Hansson for his efforts and help with the initial trials on monosaccharide analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2022.107741.

References

Aguilera, J. M., & Baffico, P. (1997). Structure-mechanical properties of heat-induced whey protein/cassava starch gels. *Journal of Food Science, 62*(5), 1048–1066. https://doi.org/10.1111/j.1365-2621.1997.tb15035.x

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., et al. (2011). Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. *Food Research International, 44*(9), 2962–2974. https://doi.org/10.1016/j.foodres.2011.07.006

Bhatty, R. S. (1990). Cooking quality of lentils: The role of structure and composition of cell walls. *Journal of Agricultural and Food Chemistry, 38*(2), 376–383.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*(2), 484–489.

Buruk Şahin, Y., Aktar Demirtaş, E., & Burnak, N. (2016). Mixture design: A review of recent applications in the food industry. *Pamukkale University Journal of Engineering Sciences, 22*(4), 297–304.

Cai, J., Yang, Y., Man, J., Huang, J., Wang, Z., Zhang, C., et al. (2014). Structural and functional properties of alkali-treated high-amylose rice starch. *Food Chemistry, 145*, 245–253. https://doi.org/10.1016/j.foodchem.2013.08.059

Chai, W., & Udén, P. (1998). An alternative oven method combined with different detergent strengths in the analysis of neutral detergent fibre. *Animal Feed Science and Technology, 74*(4), 281–288. https://doi.org/10.1016/S0377-8401(98)00187-4

Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P., & Duc, G. (2010). Nutritional value of faba bean (Vicia faba L.) seeds for feed and food. *Field Crops Research, 115*(3), 329–339. https://doi.org/10.1016/j.fcr.2009.09.016

Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F. N., & Leip, A. (2021). Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*. https://doi.org/10.1038/s43016-021-00225-9

Dickinson, E., & Chen, J. (1999). Heat-set whey protein emulsion gels: Role of active and inactive filler particles. *Journal of Dispersion Science and Technology, 20*(1–2), 197–213. https://doi.org/10.1080/01932699908943787

Dille, M. J., Draget, K. I., & Hattrem, M. N. (2015). 9 - the effect of filler particles on the texture of food gels. In J. Chen, & A. Rosenthal (Eds.), *Modifying food texture* (pp. 183–200). Woodhead Publishing. https://doi.org/10.1016/B978-1-78242-333-1.00009-7.

Dokić, L., Dapčević, T., Krstonošić, V., Dokić, P., & Hadnađev, M. (2010). Rheological characterization of corn starch isolated by alkali method. *Food Hydrocolloids, 24*(2), 172–177. https://doi.org/10.1016/j.foodhyd.2009.09.002

Frejnagel, S., Zduńczyk, Z., & Krefft, B. (1997). The chemical composition and nutritive value of low- and high-tannin faba bean varieties. *Journal of Animal and Feed Sciences, 6*(3), 401–412. https://doi.org/10.22358/jafs/69536/1997

Gdala, J., & Buraczewska, L. (1997). Chemical composition and carbohydrate content of several varieties of faba bean and pea seeds. *Journal of Animal and Feed Sciences, 6*, 123–135.

Herneke, A., Lendel, C., Johansson, D., Newson, W., Hedenqvist, M., Karkehabadi, S., et al. (2021). Protein nanofibrils for sustainable food–characterization and comparison of fibrils from a broad range of plant protein isolates. *ACS Food Science & Technology, 1*(5), 854–864. https://doi.org/10.1021/acsfoodscitech.1c00034

Jansson, K. (2019). På jakt efter den perfekta åkerbönan. *Ceralier, 1*, 10–11. https://www.slu.se/globalassets/ew/org/centrb/grogrund/dokument/cerealier_nr_1_2019.pdf.

Jiang, Z.-Q., Wang, J., Stoddard, F., Salovaara, H., & Sontag-Strohm, T. (2020). Preparation and characterization of emulsion gels from whole faba bean flour. *Foods, 9*(6), 755.

Johansson, M., Xanthakis, E., Langton, M., Menzel, C., Vilaplana, F., Johansson, D. P., et al. (2021). Mixed legume systems of pea protein and unrefined lentil fraction: Textural properties and microstructure. *LWT, 144*, 111212. https://doi.org/10.1016/j.lwt.2021.111212

Joshi, M., Aldred, P., Panozzo, J. F., Kasapis, S., & Adhikari, B. (2014). Rheological and microstructural characteristics of lentil starch–lentil protein composite pastes and gels. *Food Hydrocolloids, 35*, 226–237. https://doi.org/10.1016/j.foodhyd.2013.05.016

Langton, M., Ehsanzamir, S., Karkehabadi, S., Feng, X., Johansson, M., & Johansson, D. P. (2020). Gelation of faba bean proteins - effect of extraction method, pH and NaCl. *Food Hydrocolloids, 103*, 105622. https://doi.org/10.1016/j.foodhyd.2019.105622

Lan, Q., Lin, Z., Dong, H., Wu, D., Lin, D., Qin, W., et al. (2020). Influence of okara with varying particle sizes on the gelling, rheological, and microstructural properties of glucono-δ-lactone-induced tofu. *Journal of Food Science & Technology*, 1–12.

Lawson, J. (2014). *Design and analysis of experiments with R* (Vol. 115). CRC press.

Lee, H. (2007). *The isolation and characterisation of starches from legume grains and their application in food formulations*. RMIT University.

Liu, H.-H., Chien, J.-T., & Kuo, M.-I. (2013). Ultra high pressure homogenized soy flour for tofu making. *Food Hydrocolloids, 32*(2), 278–285. https://doi.org/10.1016/j.foodhyd.2013.01.005

Li, J.-Y., & Yeh, A.-I. (2003). Gelation properties and morphology of heat-induced starch/salt-soluble protein composites. *Journal of Food Science, 68*(2), 571–579. https://doi.org/10.1111/j.1365-2621.2003.tb05713.x

Li, J.-Y., Yeh, A.-I., & Fan, K.-L. (2007). Gelation characteristics and morphology of corn starch/soy protein concentrate composites during heating. *Journal of Food Engineering, 78*(4), 1240–1247. https://doi.org/10.1016/j.jfoodeng.2005.12.043

Mariotti, M., Zardi, M., Lucisano, M., & Pagani, M. A. (2005). Influence of the heating rate on the pasting properties of various flours. *Starch - Stärke, 57*(11), 564–572. https://doi.org/10.1002/star.200500425

Mayer Labba, I.-C., Frøkiær, H., & Sandberg, A.-S. (2021). Nutritional and antinutritional composition of fava bean (Vicia faba L., var. minor) cultivars. *Food Research International, 140*, 110038. https://doi.org/10.1016/j.foodres.2020.110038

Miller, A. J. (1984). Selection of subsets of regression variables. *Journal of the Royal Statistical Society: Series A, 147*(3). https://doi.org/10.2307/2981576

Moritaka, H., Takeuchi, M., Okoshi, H., & Fukuba, H. (2002). Particle and matrix gels of gellan gum: Effects of filler particles on rheological properties of matrix gels. *Food Hydrocolloids, 16*(2), 175–182. https://doi.org/10.1016/S0268-005X(01)00081-9

Mosse, J. (1990). Nitrogen-to-protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. *Journal of Agricultural and Food Chemistry, 38*(1), 18–24.

Muhrbeck, P., & Eliasson, A. (1991). Rheological properties of protein/starch mixed gels. *Journal of Texture Studies, 22*(3), 317–332.

Munialo, C. D., van der Linden, E., & de Jongh, H. H. J. (2014). The ability to store energy in pea protein gels is set by network dimensions smaller than 50nm. *Food Research International, 64*, 482–491. https://doi.org/10.1016/j.foodres.2014.07.038

Nicolai, T., & Chassenieux, C. (2019). Heat-induced gelation of plant globulins. *Current Opinion in Food Science, 27*, 18–22. https://doi.org/10.1016/j.cofs.2019.04.005

Nilsson, K., Sandström, C., Özeren, H., Vilaplana, F., Hedenqvist, M., & Langton, M. (2022). *Physiochemical and thermal characterisation of faba bean starch*. Manuscript in progress.

Niu, X., Zhao, C., Shi, Q., Wang, S., Zhao, Y., Li, S., et al. (2021). Effect of ultrasonic treatment on peanut protein isolate-oat dietary fibre mixture gels induced by transglutaminase. *International Journal of Food Science and Technology, 56*(5), 2233–2243. https://doi.org/10.1111/ijfs.14839

Nordic Committee on Food Analysis. (1976). *Nitrogen. Determination in foods and feeds according to Kjeldahl No 6* (3rd ed.). Statens Teknologiska Forskningscentral Esbo.

Olsson, C., Langton, M., & Hermansson, A.-M. (2002). Microstructures of β-lactoglobulin/amylopectin gels on different length scales and their significance for rheological properties. *Food Hydrocolloids, 16*(2), 111–126. https://doi.org/10.1016/S0268-005X(01)00069-8

Pelgrom, P. J. M., Boom, R. M., & Schutyser, M. A. I. (2015). Functional analysis of mildly refined fractions from yellow pea. *Food Hydrocolloids, 44*, 12–22. https://doi.org/10.1016/j.foodhyd.2014.09.001

Redgate, P. E., Piepel, G. F., & Hrma, P. R. (1992). Second-order model selection in mixture experiments. In *Proceedings of the Annual Meeting of the American Statistical Association* (pp. 104–110).

Regulations: Commision regulation (EC) No 152/2009. H. Determination of crude oils and fats. (2009). In Official Journal of the European Union.

Robertson, J. B., Van Soest, P. J., James, W. P. T., & Theander, O. (1981). In W. P. T. James, & O. Theander (Eds.), *The analysis of dietary fiber in food. The Detergent System of Analysis and its Application to Human Foods* (pp. 123–158). New York, NY: Marcel Dekker.

Ryan, K. J., & Brewer, M. S. (2005). Model system analysis of wheat starch-soy protein interaction kinetics using polystyrene microspheres. *Food Chemistry, 92*(2), 325–335. https://doi.org/10.1016/j.foodchem.2004.08.005

Saldanha do Carmo, C., Silventoinen, P., Nordgård, C. T., Poudroux, C., Dessev, T., Zobel, H., et al. (2020). Is dehulling of peas and faba beans necessary prior to dry fractionation for the production of protein- and starch-rich fractions? Impact on physical properties, chemical composition and techno-functional properties. *Journal of Food Engineering, 278*, 109937. https://doi.org/10.1016/j.jfoodeng.2020.109937

Schmitt, C., Sanchez, C., Desobry-Banon, S., & Hardy, J. (1998). Structure and technofunctional properties of protein-polysaccharide complexes: A review. *Critical Reviews in Food Science and Nutrition, 38*(8), 689–753. https://doi.org/10.1080/10408699891274354

Shim, J., & Mulvaney, S. J. (2001). Effect of heating temperature, pH, concentration and starch/whey protein ratio on the viscoelastic properties of corn starch/whey protein mixed gels. *Journal of the Science of Food and Agriculture, 81*(8), 706–717. https://doi.org/10.1002/jsfa.869

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., et al. (2008). Determination of structural carbohydrates and lignin in biomass. *Laboratory Analytical Procedure, 1617*(1), 1–16.

Stephen, A. M., Champ, M. M.-J., Cloran, S. J., Fleith, M., van Lieshout, L., Mejborn, H., et al. (2017). Dietary fibre in Europe: Current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. *Nutrition Research Reviews, 30*(2), 149–190. https://doi.org/10.1017/S095442241700004X

Sun, X. D., & Arntfield, S. D. (2011). Gelation properties of salt-extracted pea protein isolate induced by heat treatment: Effect of heating and cooling rate. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2010.07.063

Ullah, I., Hu, Y., You, J., Yin, T., Xiong, S., Din, Z., et al. (2019). Influence of okara dietary fiber with varying particle sizes on gelling properties, water state and microstructure of tofu gel. *Food Hydrocolloids, 89*, 512–522.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vioque, J., Alaiz, M., & Girón-Calle, J. (2012). Nutritional and functional properties of Vicia faba protein isolates and related fractions. *Food Chemistry, 132*(1), 67–72. https://doi.org/10.1016/j.foodchem.2011.10.033

Vogelsang-O'Dwyer, M., Petersen, I. L., Joehnke, M. S., Sørensen, J. C., Bez, J., Detzel, A., et al. (2020). Comparison of faba bean protein ingredients produced using dry fractionation and isoelectric precipitation: Techno-functional, nutritional and environmental performance. *Foods, 9*(3). https://doi.org/10.3390/foods9030322

Warsame, A. O., O'Sullivan, D. M., & Tosi, P. (2018). Seed storage proteins of faba bean (Vicia faba L): Current status and prospects for genetic improvement. *Journal of Agricultural and Food Chemistry, 66*(48), 12617–12626. https://doi.org/10.1021/acs.jafc.8b04992

Wei, F., Ye, F., Li, S., Wang, L., Li, J., & Zhao, G. (2018). Layer-by-layer coating of chitosan/pectin effectively improves the hydration capacity, water suspendability and tofu gel compatibility of okara powder. *Food Hydrocolloids, 77*, 465–473. https://doi.org/10.1016/j.foodhyd.2017.10.024

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the anthropocene: The EAT–lancet commission on healthy diets from sustainable food systems. *The Lancet, 393*, 447–492. https://doi.org/10.1016/S0140-6736(18)31788-4, 10170.

Yu, B., Ren, F., Zhao, H., Cui, B., & Liu, P. (2020). Effects of native starch and modified starches on the textural, rheological and microstructural characteristics of soybean protein gel. *International Journal of Biological Macromolecules, 142*, 237–243. https://doi.org/10.1016/j.ijbiomac.2019.09.095

Zhuang, X., Jiang, X., Zhou, H., Chen, Y., Zhao, Y., Yang, H., et al. (2020). Insight into the mechanism of physicochemical influence by three polysaccharides on myofibrillar protein gelation. *Carbohydrate Polymers, 229*, 115449. https://doi.org/10.1016/j.carbpol.2019.115449

Zhuang, X., Wang, L., Jiang, X., Chen, Y., & Zhou, G. (2020). The effects of three polysaccharides on the gelation properties of myofibrillar protein: Phase behaviour and moisture stability. *Meat Science, 170*, 108228. https://doi.org/10.1016/j.meatsci.2020.108228

Update

Food Hydrocolloids

Volume 144, Issue , November 2023, Page

DOI: https://doi.org/10.1016/j.foodhyd.2023.109004

Food Hydrocolloids 144 (2023) 109004

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Corrigendum to "Effect of starch and fibre on faba bean protein gel characteristics" [Food Hydrocolloids 131 (2022) 107741]

Mathias Johansson [a,*], Daniel Johansson [a], Anna Ström [b], Jesper Rydén [c], Klara Nilsson [a], Jakob Karlsson [b], Rosana Moriana [a,d], 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 Energy and Technology, Swedish University of Agricultural Sciences, Box 7032, SE-750 07, Uppsala, Sweden
[d] Bioeconomy and Health Division, RISE-Research Institutes of Sweden, Drottning Kristinas Väg 61, Stockholm, Sweden

The authors regret that the labels of subfigures in Fig. 5a and b were mismatched. The micrographs of subfigures D and F should swap places. The correct version of Fig. 5a and b is given in this corrigendum.

The authors would like to apologise for any inconvenience caused.

DOI of original article: https://doi.org/10.1016/j.foodhyd.2022.107741.
* Corresponding author.
E-mail address: mathias.johansson@slu.se (M. Johansson).

https://doi.org/10.1016/j.foodhyd.2023.109004

Available online 24 June 2023

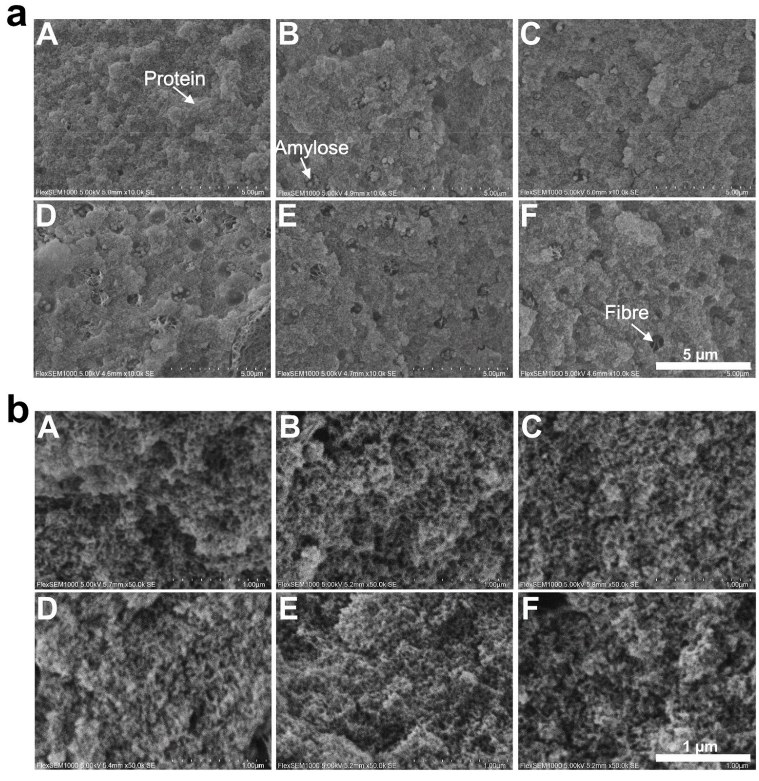


Fig. 5. Scanning electron micrographs of gels with different ratios of the extracted fractions corresponding to the design points marked in the mixture triangle seen in Fig. 4. Magnification: × 10.000 (a), × 50.000 (b). Pixels: 1280 × 960 pixels.

V

LWT - Food Science and Technology 144 (2021) 111212

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

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

Mathias Johansson [a,b], Epameinondas Xanthakis [a], Maud Langton [b], Carolin Menzel [c], Francisco Vilaplana [c], Daniel P. Johansson [b], Patricia Lopez-Sanchez [a,d,*]

[a] Agriculture and Food, Bioeconomy and Health, RISE-Research Institutes of Sweden, Box 5401, SE-402 29, Gothenburg, Sweden
[b] Molecular Sciences, SLU-Swedish University of Agricultural Sciences, Box 7015, SE-750 07, Uppsala, Sweden
[c] Division of Glycoscience, Department of Chemistry, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, AlbaNova University Centre, Stockholm, Sweden
[d] Food and Nutrition Science, Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

ARTICLE INFO

Keywords:
Pea protein
Lentil
Starch
Rheology
Microstructure

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

* Corresponding author. Food and Nutrition Science, Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden.
E-mail address: patlop@chalmers.se (P. Lopez-Sanchez).

https://doi.org/10.1016/j.lwt.2021.111212
Received 3 November 2020; Received in revised form 25 February 2021; Accepted 26 February 2021
Available online 1 March 2021

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

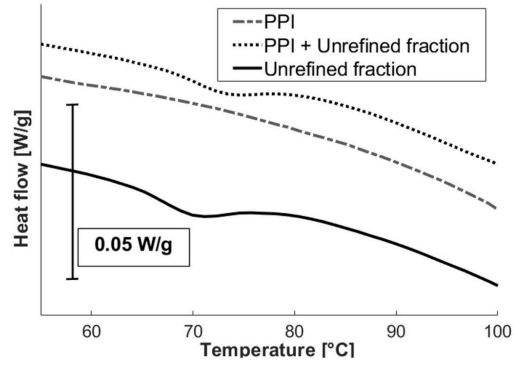


Fig. 2. Differential scanning calorimetry (DSC) measurements of lentil unrefined fraction, pea protein isolate PPI (13 wt %) and 0.3:1 unrefined fraction/PPI dispersions. All samples were measured at pH 3 in duplicates.

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

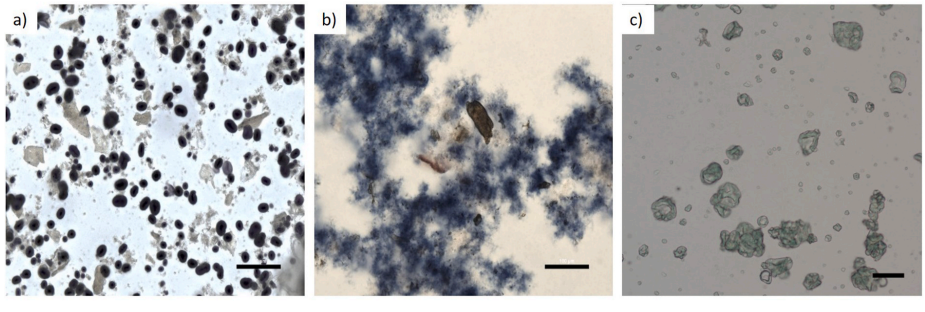


Fig. 1. Unrefined fraction obtained after lentil protein extraction, before (a) and after thermal treatment (95 °C, 30 min) and cooled down to 20 °C (b). Pea protein isolate in water 1% (w/v) pH 7.4 (c). Scale bars correspond to 100 μm. Staining: iodine for starch (purple) and light green for proteins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

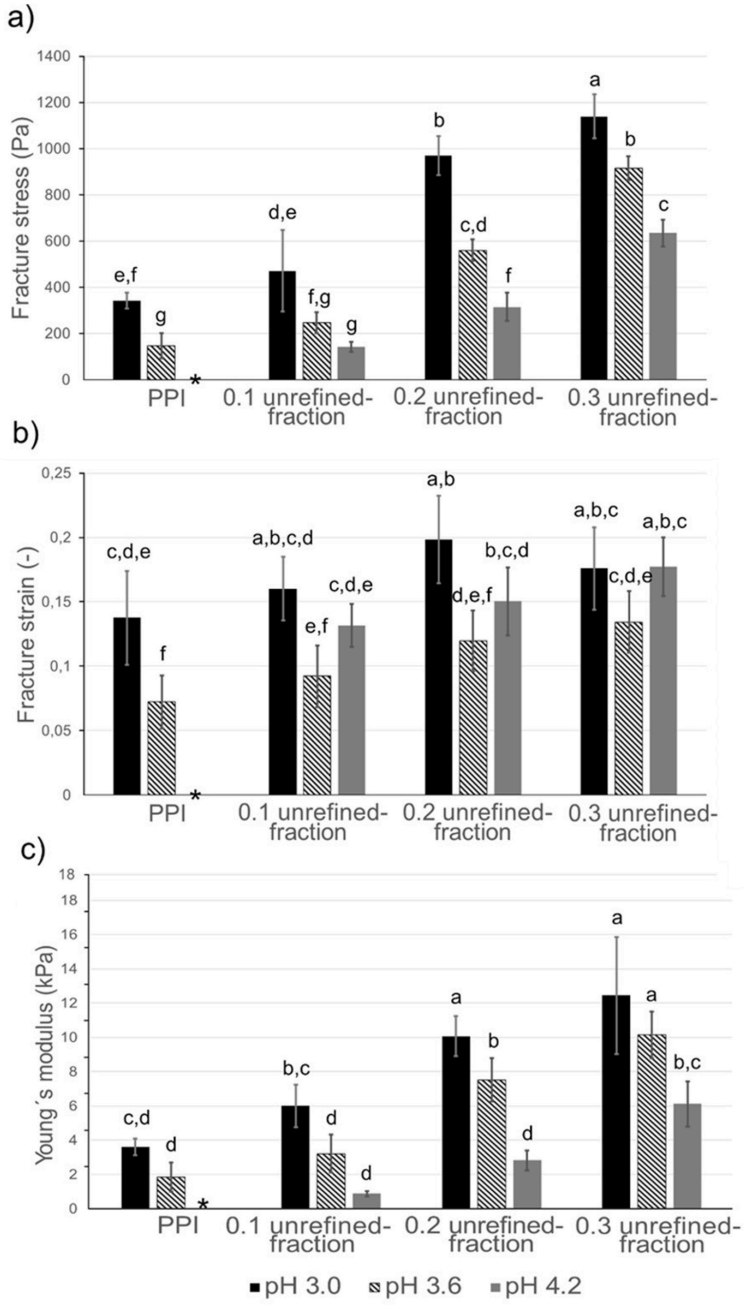


Fig. 3. Fracture stress (a), fracture strain (b) and Young's modulus (c) of 13 wt % pea protein gels (PPI) at different pH 3, 3.6 and 4.2, and with different lentil unrefined fraction (UF) contents (0:1, 0.1:1, 0.2:1 and 0.3:1, UF: PPI). Error bars represent the average and standard deviation of at least 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.

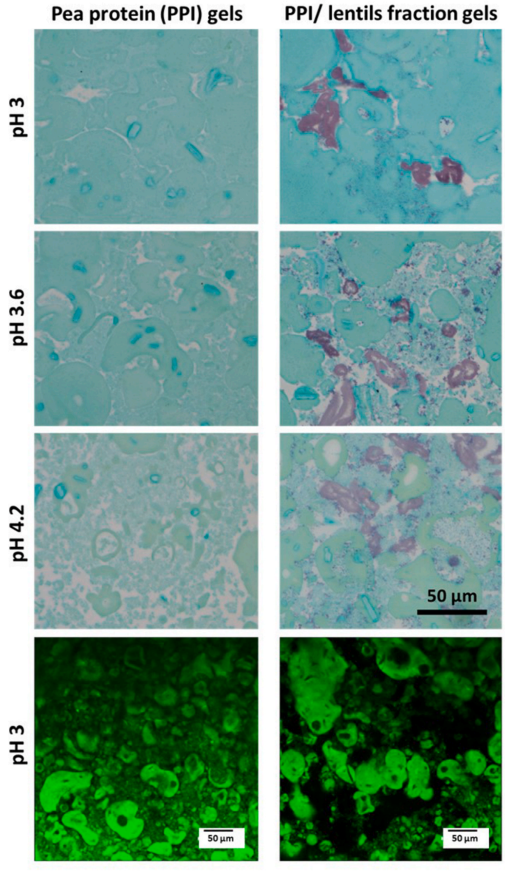


Fig. 4. Light micrographs of heat-induced pea protein gels (13 % wt) at different pH, and with added lentil unrefined fraction (0.3:1 UF: PPI). Scale bar corresponds to 50 μm in all images. Staining: light green for protein and iodine for starch (in purple). Confocal laser scanning micrographs of heat-induced 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.

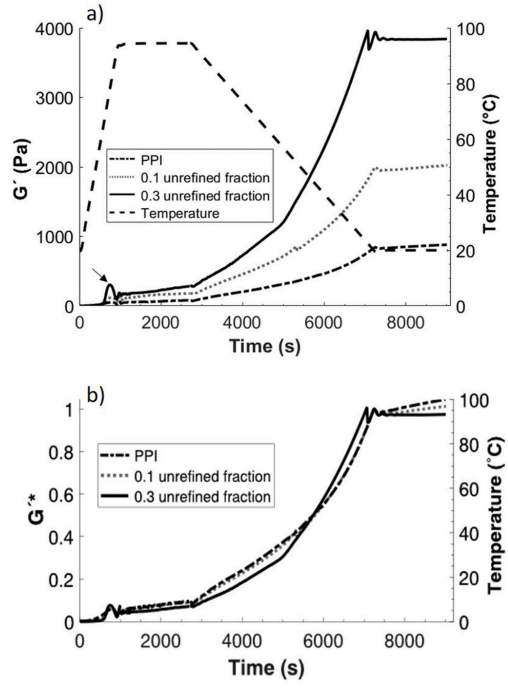


Fig. 5. Storage modulus (G′) evolution of 13 wt % pea protein isolate (PPI)) with and without the addition of lentil unrefined fraction (UF) at ratios 0.1:1 and 0.3:1 UF/PPI (a) and normalised G′ obtained using equation (1) (b). Samples were measured in duplicated.

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.

Funding

This work was partially supported by Swedish Research Council FORMAS grant number 2017-00426.

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.

Acknowledgements

The authors would like to acknowledge Lillie Cavonius for technical assistance with differential scanning calorimetry measurements. We gratefully thanked Ana Miljkovic and Lovisa Eliasson for technical assistance with gelation experiments.

References

Aguilera, J. M., & Baffico, P. (1997). Structure-mechanical properties of heat-induced whey protein/cassava starch gels. *Journal of Food Science, 62*(5), 1048–1066. https://doi.org/10.1111/j.1365-2621.1997.tb15035.x

Aguilera, J. M., & Rojas, G. V. (1997). Determination of kinetics of gelation of whey protein and cassava starch by oscillatory rheometry. *Food Research International, 30*(5), 349–357. https://doi.org/10.1016/S0963-9969(97)00058-6

Ako, K., Nicolai, T., Durand, D., & Brotons, G. (2009). Micro-phase separation explains the abrupt structural change of denatured globular protein gels on varying the ionic strength or the pH. *Soft Matter, 5*(20), 4033–4041. https://doi.org/10.1039/b906860k

Berghout, J. A. M., Pelgrom, P. J. M., Schutyser, M. A. I., Boom, R. M., & Van Der Goot, A. J. (2015). Sustainability assessment of oilseed fractionation processes: A case study on lupin seeds. *Journal of Food Engineering, 150*, 117–124.

Bertolini, A. C., Creamer, L. K., Eppink, M., & Boland, M. (2005). Some rheological properties of sodium Caseinate−Starch gels. *Journal of Agricultural and Food Chemistry, 53*(6), 2248–2254. https://doi.org/10.1021/jf048656p

Bhatty, R. S. (1990). Cooking quality of lentils: The role of structure and composition of cell walls. *Journal of Agricultural and Food Chemistry, 38*(2), 376–383.

Bildstein, M., Lohmann, M., Hennigs, C., Krause, A., & Hilz, H. (2008). An enzyme-based extraction process for the purification and enrichment of vegetable proteins to be applied in bakery products. *European Food Research and Technology, 228*(2), 177–186. https://doi.org/10.1007/s00217-008-0921-z

Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., et al. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International, 43*(2), 537–546. https://doi.org/10.1016/j.foodres.2009.07.021

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414–431. https://doi.org/10.1016/j.foodres.2009.09.003

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Brummer, Y., Kaviani, M., & Tosh, S. M. (2015). Structural and functional characteristics of dietary fibre in beans, lentils, peas and chickpeas. *Food Research International, 67*, 117–125. https://doi.org/10.1016/j.foodres.2014.11.009

Clark, A. H., Kavanagh, G. M., & Ross-Murphy, S. B. (2001). Globular protein gelation—theory and experiment. *Food Hydrocolloids, 15*(4), 383–400. https://doi.org/10.1016/S0268-005X(01)00042-X

Dalgetty, D. D., & Baik, B.-K. (2003). Isolation and characterization of cotyledon fibers from peas, lentils, and chickpeas. *Cereal Chemistry, 80*(3), 310–315. https://doi.org/10.1094/CCHEM.2003.80.3.310

Dang, H. V., Loisel, C., Desrumaux, A., & Doublier, J. L. (2009). Rheology and microstructure of cross-linked waxy maize starch/whey protein suspensions. *Food Hydrocolloids, 23*(7), 1678–1686.

Derbyshire, E., Wright, D. J., & Boulter, D. (1976). Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry, 15*(1), 3–24. https://doi.org/10.1016/S0031-9422(00)89046-9

Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *Journal of Biological Chemistry, 73*(2), 627–650. Go to ISI>://WOS:000187540700027.

García-Alonso, A., Goni, I., & Saura-Calixto, F. (1998). Resistant starch and potential glycaemic index of raw and cooked legumes (lentils, chickpeas and beans). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 206*(4), 284–287.

Hermansson, A.-M. (1983). Protein functionality and its relation to food microstructure. *Plant Foods for Human Nutrition, 32*(3), 369–388. https://doi.org/10.1007/BF01091195

Joshi, M., Aldred, P., McKnight, S., Panozzo, J. F., Kasapis, S., Adhikari, R., et al. (2013). Physicochemical and functional characteristics of lentil starch. *Carbohydrate Polymers, 92*(2), 1484–1496. https://doi.org/10.1016/j.carbpol.2012.10.035

Joshi, M., Aldred, P., Panozzo, J. F., Kasapis, S., & Adhikari, B. (2014). Rheological and microstructural characteristics of lentil starch–lentil protein composite pastes and gels. *Food Hydrocolloids, 35*, 226–237. https://doi.org/10.1016/j.foodhyd.2013.05.016

Lam, A. C. Y., Karaca, A. C., Tyler, R. T., & Nickerson, M. T. (2018). Pea protein isolates: Structure, extraction, and functionality. *Food Reviews International, 34*(2), 126–147. https://doi.org/10.1080/87559129.2016.1242135

Li, S., Wei, Y., Fang, Y., Zhang, W., & Zhang, B. (2014). DSC study on the thermal properties of soybean protein isolates/corn starch mixture. *Journal of Thermal Analysis and Calorimetry, 115*(2), 1633–1638. https://doi.org/10.1007/s10973-013-3433-4

Li, J.-Y., Yeh, A.-I., & Fan, K.-L. (2007). Gelation characteristics and morphology of corn starch/soy protein concentrate composites during heating. *Journal of Food Engineering, 78*(4), 1240–1247. https://doi.org/10.1016/j.jfoodeng.2005.12.043

McKee, L. S., Sunner, H., Anasontzis, G. E., Toriz, G., Gatenholm, P., Bulone, V., et al. (2016). A GH115 α-glucuronidase from Schizophyllum commune contributes to the synergistic enzymatic deconstruction of softwood glucuronoarabinoxylan. *Biotechnology for Biofuels, 9*(1), 2.

Munialo, C. D., van der Linden, E., Ako, K., & de Jongh, H. H. J. (2015). Quantitative analysis of the network structure that underlines the transitioning in mechanical responses of pea protein gels. *Food Hydrocolloids, 49*, 104–117. https://doi.org/10.1016/j.foodhyd.2015.03.018

Munialo, C. D., van der Linden, E., & de Jongh, H. H. J. (2014). The ability to store energy in pea protein gels is set by network dimensions smaller than 50nm. *Food Research International, 64*, 482–491. https://doi.org/10.1016/j.foodres.2014.07.038

Nayak, S. K., Makrariya, A., Singh, R. R. B., Patel, A. A., Sindhu, J. S., Patil, G. R., et al. (2004). Heat stability and flow behaviour of buffalo milk added with corn starch. *Food Hydrocolloids, 18*(3), 379–386.

Nunes, M. C., Raymundo, A., & Sousa, I. (2006). Rheological behaviour and microstructure of pea protein/κ- carrageenan/starch gels with different setting conditions. *Food Hydrocolloids*. https://doi.org/10.1016/j.foodhyd.2005.03.011

Peng, J., Calabrese, V., Ainis, W. N., Scager, R., Velikov, K. P., Venema, P., et al. (2019). Mixed gels from whey protein isolate and cellulose microfibrils. *International Journal of Biological Macromolecules, 124*, 1094–1105.

Peng, Y., Kersten, N., Kyriakopoulou, K., & van der Goot, A. J. (2020). Functional properties of mildly fractionated soy protein as influenced by the processing pH. *Journal of Food Engineering, 275*, 109875.

Poore, J., & Nemecek, T. (2018). Reducing food's environmental impacts through producers and consumers. *Science, 360*(6392), 987–992. https://doi.org/10.1126/science.aaq0216

Renkema, J. M. S., Gruppen, H., & Van Vliet, T. (2002). Influence of pH and ionic strength on heat-induced formation and rheological properties of soy protein gels in relation to denaturation and their protein compositions. *Journal of Agricultural and Food Chemistry, 50*(21), 6064–6071.

Ripple, W. J., Wolf, C., Newsome, T. M., Galetti, M., Alamgir, M., & Crist, E. (2017). World scientists' warning to humanity: A second notice. *BioScience, 67*(12), 1026–1028. https://doi.org/10.1093/biosci/bix125

Saeman, J. F., Moore, W. E., Mitchell, R. L., & Millett, M. A. (1954). Techniques for the determination of pulp constituents by quantitiative paper chromatography. *Tappi Journal, 37*(8), 336–343.

Shand, P. J., Ya, H., Pietrasik, Z., & Wanasundara, P. K. J. P. D. (2007). Physicochemical and textural properties of heat-induced pea protein isolate gels. *Food Chemistry, 102*(4), 1119–1130. https://doi.org/10.1016/j.foodchem.2006.06.060

Sharif, H. R., Williams, P. A., Sharif, M. K., Abbas, S., Majeed, H., Masamba, K. G., et al. (2018). Current progress in the utilization of native and modified legume proteins as

emulsifiers and encapsulants – a review. *Food Hydrocolloids, 76*, 2–16. https://doi.org/10.1016/j.foodhyd.2017.01.002

Sim, S. Y. J., & Moraru, C. I. (2020). High-pressure processing of pea protein–starch mixed systems: Effect of starch on structure formation. *Journal of Food Process Engineering, 43*(2), Article e13352. https://doi.org/10.1111/jfpe.13352

Sun, X. D., & Arntfield, S. D. (2011). Gelation properties of salt-extracted pea protein isolate induced by heat treatment: Effect of heating and cooling rate. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2010.07.063

Ullah, I., Hu, Y., You, J., Yin, T., Xiong, S., Din, Z., et al. (2019). Influence of okara dietary fiber with varying particle sizes on gelling properties, water state and microstructure of tofu gel. *Food Hydrocolloids, 89*, 512–522.

Wei, Y., Cai, Z., Wu, M., Guo, Y., Tao, R., Li, R., et al. (2020). Comparative studies on the stabilization of pea protein dispersions by using various polysaccharides. *Food Hydrocolloids, 98*, 105233.

Weijers, M., van de Velde, F., Stijnman, A., van de Pijpekamp, A., & Visschers, R. W. (2006). Structure and rheological properties of acid-induced egg white protein gels. *Food Hydrocolloids, 20*(2–3), 146–159.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., et al. (2019). Food in the anthropocene: The EAT–lancet commission on healthy diets from sustainable food systems. *The Lancet, 393*(10170), 447–492. https://doi.org/10.1016/S0140-6736(18)31788-4

Withana-Gamage, T. S., Wanasundara, J. P. D., Pietrasik, Z., & Shand, P. J. (2011). Physicochemical, thermal and functional characterisation of protein isolates from kabuli and desi chickpea (cicer arietinum L.): A comparative study with soy (Glycine max) and pea (pisum sativum L.). *Journal of the Science of Food and Agriculture, 91*(6), 1022–1031. https://doi.org/10.1002/jsfa.4277

Yu, B., Ren, F., Zhao, H., Cui, B., & Liu, P. (2020). Effects of native starch and modified starches on the textural, rheological and microstructural characteristics of soybean protein gel. *International Journal of Biological Macromolecules, 142*, 237–243. https://doi.org/10.1016/j.ijbiomac.2019.09.095

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2024:21

This thesis characterised mixed gels from laboratory-extracted faba bean protein, starch, and fibre, focusing on texture and microstructure. Results show that gels with particulate microstructures dominated by larger protein aggregates gave weak gels with low fracture stress. Addition of starch or fibre modified gel texture, resulting in increased fracture properties for gels with a particulate microstructure, decreased fracture properties and an increase in storage modulus for gels with a fine-stranded microstructure.

Mathias Johansson received his graduate education at the Department of Molecular Sciences, SLU, Uppsala. He attained his B.Sc. degree in Chemical Engineering with Engineering Physics and M.Sc. in Bioengineering at Chalmers University of Technology, Sweden.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-306-5
ISBN (electronic version) 978-91-8046-307-2