Texture and Microstructure of Mixed Gels From Faba Bean Protein, Starch and Fibre

Mathias Johansson[1], Klara Nilsson[1], Corine Sandström[1], Anna Ström[2], Daniel Johansson[1], Jakob Karlsson[2], Jesper Rydén[1], Hanna Eriksson Röhnisch[1], Mikael S. Hedenqvist[3], Rosana Moriana[1,4] and Maud Langton[1]

[1] Swedish University of Agricultural Sciences, Uppsala, Sweden
[2] Chalmers University of Technology, Gothenburg, Sweden
[3] KTH-Royal Institute of Technology, Stockholm, Sweden
[4] RISE-Research Institutes of Sweden, Stockholm, Sweden

ABSTRACT

Heat-induced protein gelation and starch gelatinization are two crucial processes in the production of a wide range of foods. We have studied different heat-induced mixed gel systems based on protein-, starch- and fibre-rich fractions extracted from faba beans. Oscillatory rheology was used to monitor the gel formation and to analyse the textural properties of the final gels. Light and electron microscopy was utilized to investigate the gel microstructure at different length scales. The aim was to evaluate the impact of different protein/starch/fibre-ratios on texture and microstructure of one starch- and one protein-rich system.

In the first study[1], starch-rich mixed gels were analysed at a constant solid content of 12%, with part of the starch replaced by protein. A decrease in the proportion of starch resulted in reduced water binding properties, lower viscosity during pasting and gels with lower storage modulus, fracture stress, fracture strain and Young's modulus. Scanning electron microscopy revealed a more porous starch network with thicker strands than in the protein network.

In the second study[2], protein-rich gels were analysed at a higher solid content (20%), with part of the protein replaced by starch and/or fibre. Fracture stress and fracture strain decreased while Young's modulus and storage modulus increased as the protein was replaced. Light and electron microscopy revealed that leaked amylose aggregated on the surfaces of starch granules and some of the fibre particles as well as in small cavities throughout the protein network. No clear difference in protein network structure was observed between samples. 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 more by water adsorption and moisture stability through the starch and fibre, increasing the effective protein concentration in the surrounding matrix.

INTRODUCTION

The food sector contributes significantly to the global environmental impact but this can be reduced by an increased consumption of locally produced plant-based foods[3]. Faba beans can serve as a good plant-based protein source and can be grown in most climate areas of Europe, including Sweden.

Heat-induced protein gelation and starch gelatinization are two crucial processes in the production of a wide range of foods. The majority of studies on these processes focus on pure systems containing only starch or protein. However, most foods contain a mixture of

components such as protein, starch and fibre. How these components interact will influence texture formation and an increased understanding of these processes can facilitate the development of novel plant-based foods.

This study aims to characterise the textural and microstructural properties and establish a relationship between texture and microstructure of mixed gels based on protein, starch and fibre extracted from faba beans.

MATERIALS AND METHODS

The methods used in this study are described in brief below. For further details, see Nilsson et al.[1] and Johansson et al.[2].

Extraction of faba bean protein-, starch- and fibre-fractions

The faba beans (*Vicia faba* L. var. *Gloria*) used for extraction were kindly provided by RISE (Research Institutes of Sweden). Before extraction of the different fractions, the beans were dehulled (Hi-Tech Machinery Manufacturing Co. Ltd., China) and milled (Ultra-Centrifugal Mill ZM-1, Retsch, Germany) into flours using a mesh size of 0.5 mm.

In short, the protein was extracted by solubilisation at pH 9 and removal of insoluble material by centrifugation followed by isoelectric precipitation at pH 4. The insoluble fraction obtained at the first centrifugation step was used for further extraction of the starch and fibre fractions. The starch was separated from the fibre fractions by filtration through a 70 μm nylon filter. The starch fraction was dried at 40 °C while the protein and fibre fractions were freeze-dried. The obtained materials were ground into flours and sieved using a 250 μm mesh (Retsch, AS200 basic, Haan, Germany).

Preparation and composition of studied gel systems

Mixtures and gels were prepared by first dispersing different ratios of the extracted starch, fibre and protein in distilled water according to **Table 1** (Additional samples with different compositions were studied[1,2] but not reported here). Samples were stirred and pH was adjusted to 7 if needed. After initial stirring, samples were preheated with continuous stirring at 65 or 58 °C to limit sedimentation. Samples were then used directly for rheological characterisation, or gels were prepared in 12 mm diameter glass tubes by heat treatment in a water bath at 95 °C for 30 min.

TABLE 1: Solid content and proportion (as the percentage of total solids added) of the different extracted fractions in each sample.

Total solid content (%)	Sample	Starch	Protein	Fibre
12	S0P100	0	100	0
	S60P40	60	40	0
	S80P20	80	20	0
	S100P0	100	0	0
20	P100	0	100	0
	P90F10	0	90	10
	P65S35	35	65	0
	P65S25F10	25	65	10

Characterisation of rheological and textural properties

The gel formation was monitored 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 the previous section. To limit evaporation from the sample, a custom-made solvent trap was used combined with the addition of paraffin oil around the edges of the sample. The gelation process was monitored by recording the storage modulus at 0.5% strain during a temperature ramp. The ramp consisted of heating from either 60 or 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.

Textural properties of gels prepared as described in the previous section were analysed by compression tests using a texture analyser (Stable Micro Systems, TA-HDi, Surrey, UK) equipped with a 500 N load cell and a 36 mm cylindrical aluminium probe. Samples were compressed until fracture at a rate of 1 mm/s.

Characterisation of microstructure

Gels for microstructure were prepared by fixation in 2.5% glutaraldehyde and 0.1% ruthenium red, followed by an additional fixation step in 1% osmium tetroxide. Samples were then dehydrated in a series of solutions with increasing ethanol concentration. For light microscopy, samples were infiltrated and hardened using Technovit 7100 and sectioned into 1-µm sections (Leica Microsystems GmbH, Leica EM UC6, Wetzlar, Germany). The sections were stained with light green and Lugol´s solution before being examined under the microscope (Nikon, Eclipse Ni–U microscope, Tokyo, Japan) and micrographs captured with a Nikon Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan) with 0.12 µm/pixel. For scanning electron microscopy (SEM), dehydrated samples in ethanol were critical point-dried (Quorum Technologies Ltd, K850 Critical Point Dryer, East Sussex, UK). Dried samples were fractured and sputter-coated with gold (Cressington Scientific Instruments, Sputtercoater-108 auto, Watford, UK) before being examined in the SEM device (Hitachi, FlexSEM 1000II, Tokyo, Japan) at 5 kV and micrographs recorded digitally with a pixel size of 4.96 nm/pixel and data size of 2560 × 1920 pixels.

RESULTS AND DISCUSSION

Starch-rich mixed gels were analysed at a constant solid content of 12%, with part of the starch replaced by protein. The protein-rich gels were analysed at a higher solid content (20%), with part of the protein replaced by starch and/or fibre.

For starch-rich gels at 12% solid content, there was a shift in gel formation occurring gradually as the starch content was decreased (**Fig. 1**). Gels with starch content ≥80% showed a development of the storage modulus more similar to the pure starch system, while gels with ≤70% starch were more similar to that of the pure protein system (data from all samples not shown in **Fig. 1**, see Nilsson et al.[1] for more details).

For protein-rich gels at 20% solid content, gel formation, as monitored by the storage modulus, occurred similarly for all gels studied (**Fig. 1**). This is in line with the protein being the continuous phase for all systems and suggests that the protein was the dominant component during gelation. However, some slight differences could be observed during the temperature ramp. Samples with fibre added had a higher initial storage modulus compared to samples without fibre. Samples including starch showed a larger increase in storage modulus during the initial heating and subsequent cooling compared to the pure protein sample.

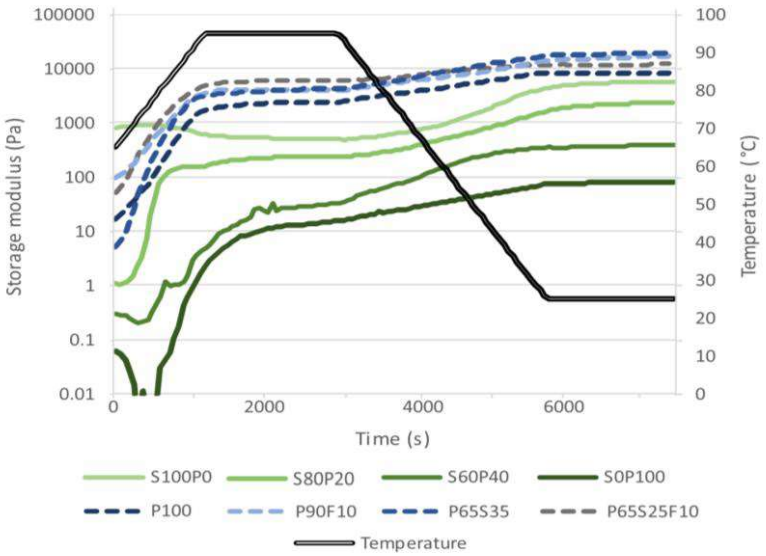

FIGURE 1: Storage modulus (G´) during temperature ramp. Samples with 12% solid content (continuous lines) and 20% solid content (dashed lines). Letters in labels correspond to P-protein, S-starch and F-fibre with the subsequent number representing the percentage of total solids added. Data adapted from Nilsson et al.[1] and Johansson et al.[2].

Overall, the starch-rich gels with >90% starch and a solid content of 12% had significantly higher fracture stress than the protein-rich gels at 20% solid content (**Fig. 2**). This highlights the good gel-forming properties of starch. However, as the proportion of starch was reduced to 80-60% of total solids added, the fracture stress of the gels at the lower solid content (12%) rapidly declined and were comparable to or lower than those of the protein-rich gels at a higher solid content (20%).

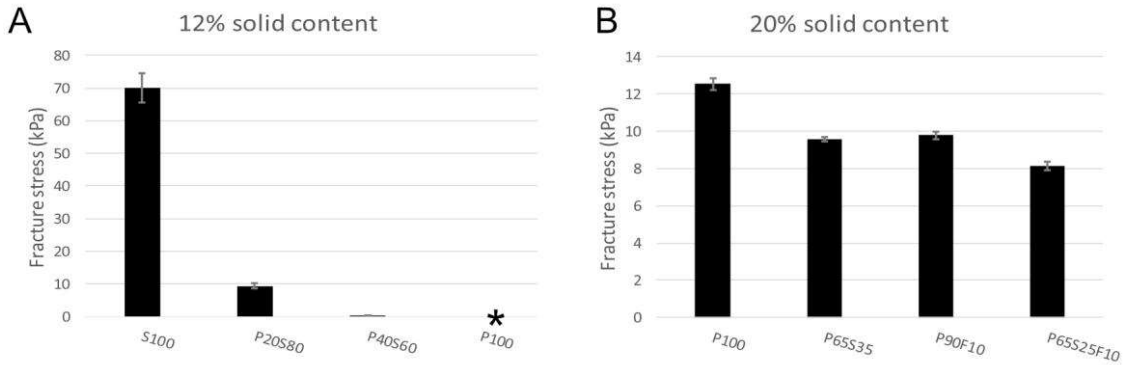

FIGURE 2: Fracture stress (kPa) of the different gel samples at 12% (A) and 20% (B) solid content. Letters in labels correspond to P-protein, S-starch and F-fibre with the subsequent number representing the percentage of total solids added. Data adapted from Nilsson et al.[1] and Johansson et al.[2]. *Did not form self-standing gels.

At the lower solid content, SEM revealed a more porous starch network with thicker strands in the pure starch sample (S100) compared to the protein network observed in the protein gels (S0P100) (**Fig. 3**). The protein network observed at a 20% solid content (P100) was denser than that at 12% solid content (S0P100). No clear difference in protein network was observed

between the different gels at the higher solid content (more clearly observed at a higher magnification, see Johansson et al.[2]). However, starch and fibre created inhomogeneities and cavities throughout the microstructure. Leaked amylose aggregated on the surfaces and in small cavities throughout the protein network (**Fig. 3-4**).

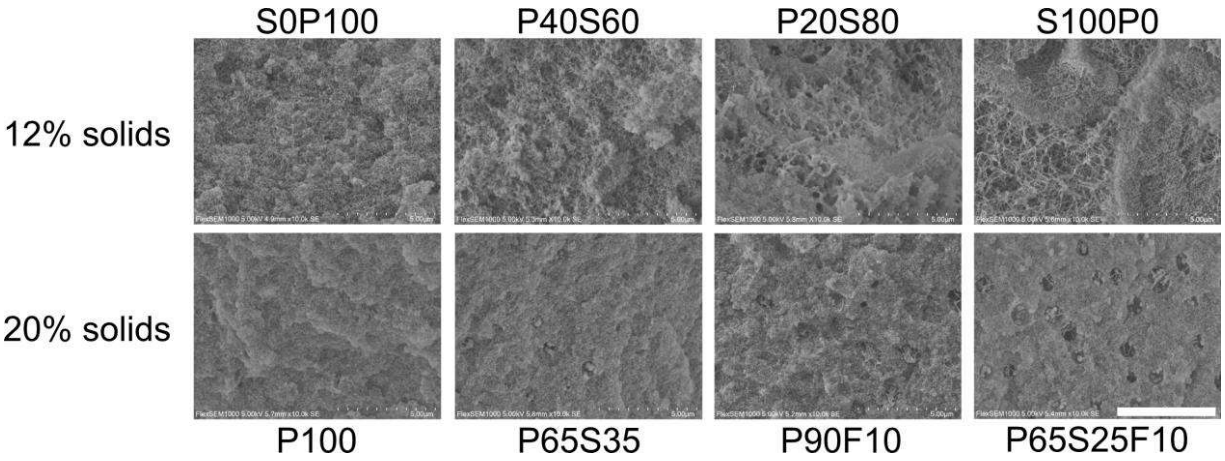

FIGURE 3: SEM micrographs of gels. Letters in labels correspond to P-protein, S-starch and F-fibre with the subsequent number representing the percentage of total solids added. Scale bar = 5 µm. Figures partly adapted from Nilsson et al.[1] and Johansson et al.[2].

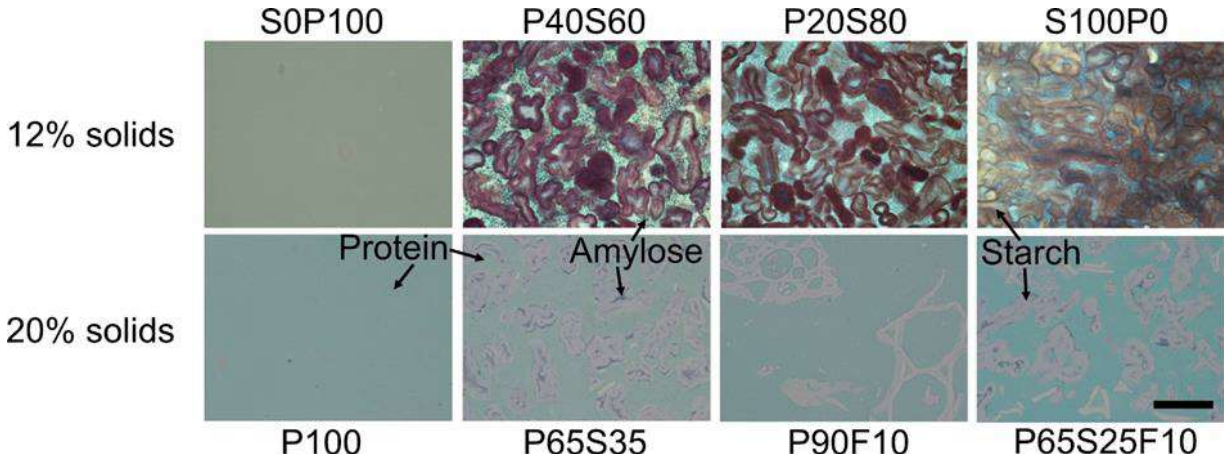

FIGURE 4: LM micrographs of gels. Letters in labels correspond to P-protein, S-starch and F-fibre with the subsequent number representing the percentage of total solids added. Scale bar = 50 µm. Figures partly adapted from Nilsson et al.[1] and Johansson et al.[2].

The reduction in large deformation properties for gels at 20% solid content upon the addition of starch and/or fibre was tentatively attributed to the introduction of inhomogeneities. The simultaneous increase in small deformation properties (G′) 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. Further studies would be needed to either dismiss or confirm these hypotheses.

CONCLUSION

The effect of changes in protein to starch ratio differed depending on the solid content and whether the starch or the protein was the dominant component in the system. In protein-rich gels at 20% solid content, inhomogeneities in the protein matrix created by the addition of starch/fibre seemed to affect large deformation properties while the small deformation properties seemed to be more affected by the properties of the continuous protein network.

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