Novel Uses of Bio-polymers in Composites

From Chemistry to Processing of Materials and Food

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Cover: Tray made from plasticised potato protein (above). Word “Bioplastic” is written with wheat gluten protein-starch composites produced in this thesis (below). (Photo: Faraz Muneer)

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Abstract

Plant bio-polymers obtained as industrial side-streams (wheat gluten and potato proteins) and specifically designed potato starch and hemp fibres were used to produce composite materials. Fractionated pea protein and pea dietary fibres were used to make pasta-like sheets for production of healthy food. During processing of composite materials and food, the blend composition, temperature and additives influenced protein chemistry and structure and caused physicochemical changes. These physicochemical changes in proteins and other blend components, and their interactions, influenced the functional performance of materials and food.

In wheat gluten protein-hemp fibre composites, the hemp fibres contributed to increasing stiffness, while higher temperatures increased protein cross-linking and thereby the mechanical strength of the composites. In wheat gluten protein-starch blends, high processing temperature (130 compared with 110 °C) induced a high degree of protein cross-linking and increased β-sheet formation, which increased both stiffness and strength in wheat gluten-starch blends and only strength in glutenin-starch blends. The gliadins showed a hierarchical hexagonal arrangement, observed for the first time in a gliadin-starch processed composite. The wheat gluten protein-starch composites also showed low oxygen permeability suitable for packaging applications. Combining glycerol with water improved composite processability and micro-structural morphology and also increased protein cross-linking and β-sheets. This increased the strength, stiffness and extensibility of wheat gluten- and glutenin-starch composites. Hot pressing at 130 °C induced a high degree of protein cross-linking and high amount of β-sheets and improved the mechanical properties of pea protein and pea protein-fibre at 90/10 and 80/20 blends. Pasta-like sheets with higher pea fibre content showed higher water uptake and reduced cooking losses. Chemical modification of wheat gluten and potato proteins de-polymerised the proteins, and thereby making them less cross-linked. In hot-pressed materials, less cross-linked proteins re-cross-linked and increased the tensile performance for potato proteins but not for wheat gluten. Processing conditions and blend component interactions in composites and foods governed the variation in protein cross-linking, structure and mechanical performance.

Keywords: wheat gluten, potato proteins, pea proteins, modified potato starch, hemp fibres, pea dietary fibres, bio-composites, protein modification, protein polymerization, protein-rich food, functional properties.

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Dedication

To my *late* parents.

"The more you challenge yourself, the more you discover greater reserves of strength within you."
Imran Khan

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

I **Muneer, F.**, Johansson, E., Hedenqvist, M.S., Gällstedt, M. & Newson, W.R. (2014). Preparation, Properties, Protein Cross-Linking and Biodegradability of Plasticizer-Solvent Free Hemp Fiber Reinforced Wheat Gluten, Glutenin, and Gliadin Composites. *BioResources,* 9(3)**,** 5246-5261.

II **Muneer, F.**, Andersson, M., Koch, K., Hedenqvist, M.S., Gällstedt, M., Plivelic, T.S., Menzel, C. & Kuktaite, R. (2015). Nanostructural Morphology of Plasticized Wheat Gluten and Modified Potato Starch Composites: Relationship to Mechanical and Barrier Properties. *Biomacromolecules,* 16(3)**,** 695-705.

III **Muneer, F.**, Andersson, M., Koch, K., Hedenqvist, M.S., Gällstedt, M., Plivelic, T.S., Menzel, C., Rhazi, L. & Kuktaite R. (2016). Innovative Gliadin/Glutenin and Modified Potato Starch Green Composites: Chemistry, Structure, and Functionality Induced by Processing. *ACS Sustainable Chemistry & Engineering,* 4(12)**,** 6332-6343.

IV **Muneer, F.**, Johansson, E., Hedenqvist, M.S., Plivelic, T.S., Markedal, K.E., Petersen, I.L., Sørensen, J.C. & Kuktaite, R. (2018). The impact of newly produced protein and dietary fiber rich fractions of yellow pea (Pisum sativum L.) on the structure and mechanical properties of pasta-like sheets. *Food Research International,* 106**,** 607-618.

V **Muneer, F.**, Johansson, E., Hedenqvist, M.S., Plivelic, T.S. & Kuktaite, R. (2018). Chemistry-mediated approach to impact structure-function behaviour and processing of industrial potato protein and wheat gluten composites. **Submitted**

Papers I-IV are reproduced with the permission of the publishers.

The contribution of Faraz Muneer to the papers included in this thesis was as follows.

I. Planned the experiments with supervisors, performed all the experimental work, evaluated and analysed the data and wrote the manuscript with input from the co-authors.

II. Planned the experiments with supervisors, performed most of the experimental work except production and characterisation of modified potato starch, evaluated and analysed the data and wrote the manuscript with contributions from the co-authors.

III. Planned the experiments with supervisors, performed most of the experimental work, evaluated and analysed the data and wrote the manuscript with input from the co-authors.

IV. Planned the experiments with supervisors, performed most of the experimental work, evaluated and analysed the data and contributed to writing the manuscript.

V. Planned the experiments with supervisors, performed all the experimental work, evaluated and analysed the data and wrote the manuscript with input from the co-authors.

Abbreviations

A4F	Asymmetrical flow field flow fractionation
FT-IR	Fourier transform infrared spectroscopy
Gli	Gliadins
GT	Glutenins
HMw	High molecular weight proteins
HMW-GS	High molecular weight glutenin subunits
LMw	Low molecular weight proteins
LMW-GS	Low molecular weight glutenin subunits
MM	Monomeric proteins
MPP	Modified potato proteins
MPS	Modified potato starch
MWG	Modified wheat gluten
OP	Oxygen permeability
PET	Poly(ethylene) terephthalate
PF	Pea fibres
PFW	Potato fruit water
PLA	Polylactic acid
PP	Polymeric proteins
PPI	Pea proteins
RH	Relative humidity
SAXS	Small angle X-ray scattering
SDS	Sodium dodecyl sulphate
SE-HPLC	Size-exclusion high performance liquid chromatography
SEM	Scanning electron microscopy
SH	Sulfhydryl cross-links
SS	Disulphide cross-links
WAXS	Wide angle X-ray scattering
WG	Wheat gluten

1 Introduction

Natural bio-polymers such as proteins and starch are an important part of the human diet. However, these bio-polymers in their structural make-up also possess suitable chemical characteristics and good processability for making bio-based materials, and could be a sustainable alternative to petroleum-based polymers. Petroleum-based polymers offer much versatility, durability and processing ease, but recent concerns about associated waste disposal issues, and environmental and health problems, combined with knowledge of depleting petroleum resources, make it necessary to find sustainable alternatives (Osswald & García-Rodríguez, 2011, Thompson *et al.*, 2009).

Starch is a promising alternative, owing to its thermoplastic behaviour and biodegradability. In 2017, starch-based plastics comprised about 29.1 % of the global bio-plastics market (of which 18.8 % were used in blends and 10.3 % as a precursor to make polylactic acid) (EU BP Market Data, 2017). Proteins obtained from plants are another attractive source to replace petroleum-based polymers, because of their interesting film-forming properties and biodegradability. However, use of proteins and starch in their native form to make materials poses challenges because of their poor mechanical performance and water sensitivity. Therefore, there is a need to understand the underlying chemical background and structural variations occurring in these bio-polymers during processing, and to devise suitable strategies to improve the functional performance of bio-based materials.

Proteins are comprised of several amino acids and each of these amino acids has special chemical characteristics. Under different processing conditions (*e.g.* heat, additives and pH), these amino acids react and form different chemical bonds, such as hydrogen, ionic and covalent bonds (Whitford, 2013, Rombouts *et al.*, 2010) (Figure 1). Since proteins are complex macromolecules, processing them into bio-based materials or foods requires an in-depth understanding of the protein interactions which maintain large protein networks and complex structures, in order to expand their use for various applications. Once knowledge

is available on chemical changes in plant proteins and how these changes affect structure and function, protein chemistry can be fine-tuned by modifying the processing environment in order to improve the properties of bio-based materials and foods. Recent studies have shown that the molecular structure of wheat proteins in protein-based films can be fine-tuned by changing the processing environment to influence their functional properties (Rasheed *et al.*, 2015a, Kuktaite *et al.*, 2012).

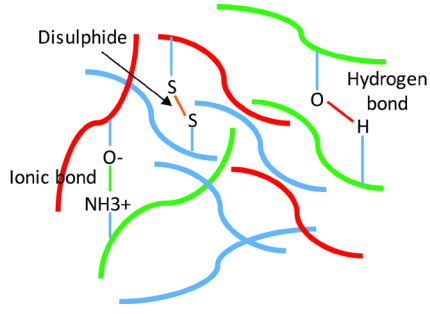

Figure 1. Schematic representation of interactions in a protein polypeptide chain.

However, use of bio-polymers for making materials leads to a food versus non-food debate, where many would argue that bio-polymers (such as proteins and starch) should only be used for food purposes due to food security issues. However, this problem can be solved by using industrial side-streams. Two such side-streams are wheat gluten and potato proteins, which are suitable for making bio-based materials. Whereas, in fact, starch is already well established as one of the main components for making bio-based materials (Zhang *et al.*, 2014).

1.1 Plant bio-polymers as source of bio-based materials and foods

1.1.1 Bio-polymers; chemistry and structure

Wheat gluten

Industrial wheat gluten is a side-stream of the bio-ethanol industry. Commercially produced wheat gluten contains around 80 % proteins on a dry weight basis, while the rest is starch, lipids, mineral fibre and ash (Gällstedt *et al.*, 2004).

Wheat gluten is composed of two major protein groups, gliadins and glutenins, which provide wheat gluten with its unique viscoelastic and

rheological properties (Wieser, 2007). In dough, the gliadins contribute to viscosity and extensibility, while the glutenins provide strength and elasticity. Gliadins are the ethanol-soluble monomeric fraction (molecular weight range 30-70 kDa) of wheat gluten, and can be divided into three subgroups of proteins (α/β, γ, and ω gliadins), based on their mobility in gel electrophoresis. The α/β and γ-gliadins contain six and eight cysteines, respectively, which are responsible for intra-disulphide cross-links in dough. The ω-gliadins lack the amino acid cysteine required to form disulphide bonds (Tatham & Shewry, 1985).

Glutenins are the ethanol non-soluble polymeric fraction of wheat gluten with molecular weight ranges between 30-160 kDa, although in aggregated/cross-linked form their molecular weight ranges from 500 kDa to several million kDa. The glutenins are subdivided in two distinct groups: low molecular weight (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). The LMW-GS contain similar types of amino acids to α/β gliadins in terms of their sequence and structure (Wieser, 2007). In contrast, the HMW-GS are very unique in their structure and amino acid composition and are rich in cysteine residues. These characteristics make the HMW-GS the main determinant for providing gluten with its unique strength and elasticity in dough (Johansson *et al.*, 2013, Shewry *et al.*, 2002). In dough or in aggregated form, both glutenins and gliadins are cross-linked with inter- and intra-chain disulphide bonds and other isopeptide bonds to maintain a three-dimensional structure (Kuktaite *et al.*, 2004).

The benefits of the viscoelastic properties of wheat gluten in bread making in improving bread volume and texture are well known (Malik *et al.*, 2013, Johansson *et al*., 2013, Khatkar *et al.*, 1995). However, during recent decades the viscoelastic properties of wheat gluten have also been exploited to produce bio-based films (Gällstedt *et al*., 2004), foams (Wu *et al.*, 2014, Blomfeldt *et al.*, 2012, Blomfeldt *et al.*, 2010, Blomfeldt *et al.*, 2011), nano-composites (Kuktaite *et al.*, 2014) and bio-composites (Wretfors *et al.*, 2009, Kunanopparat *et al.*, 2008b). These have reasonable mechanical and very good gas barrier properties (suitable for packaging applications).

Potato proteins

Potato proteins are a side-stream of the potato starch industry. After extraction of starch, potato fruit water (PFW), which contains about 5 % solids, is left behind. The 5 % solids in potato fruit water mainly consist of potato proteins and other constituents such as polyphenols, minerals, fibre and reagents, which are used to stop starch browning (Newson *et al.*, 2015, Løkra *et al.*, 2008). To recover the potato protein from potato fruit water, the pH is dropped to 3.5-4 to

coagulate protein and the coagulated mass is further spray-dried at 75-100 °C to obtain potato protein powder (Løkra *et al*., 2008, Knorr, 1980).

Potato proteins are composed of three major classes of protein: patatin (~40 %), with molecular weight ranging between 40 and 45 kDa, protease inhibitors (~50 %), with molecular weight ranging between 8 and 25 kDa, and other high molecular weight proteins (~10 %) 80 kDa in size *(Zhang et al., 2017, Løkra et al*., 2008, Pouvreau *et al.*, 2001, Pots *et al.*, 1999). Potato proteins also contain several essential and non-essential amino acids. High levels of essential amino acids such aspartic acid, glutamic acid, leucine, lysine, phenylalanine, valine and threonine have been observed, and potato proteins are rich in lysine, but low in the sulphur-containing amino acid cysteine (Zhang *et al*., 2017). However, the harsh acidic conditions and high temperature used in the potato starch industry during extraction of potato proteins from potato fruit water limit the activity of amino acids and induce a high degree of protein cross-linking (Zhang *et al*., 2017, Newson *et al*., 2015, Løkra *et al*., 2008).

A few recent studies have reported use of commercial potato protein concentrates for making bio-based materials (Newson *et al*., 2015, Du *et al.*, 2015). However, a pre-cross-linked protein material is not considered desirable for making bio-based materials, because of reduced availability of bonding sites for proteins to form new interaction (as seen in wheat gluten; (Rasheed *et al*., 2015a). All these factors limit the processing window and functional performance of the processed materials. Thus, there is a need to develop methods or chemical tools for treating industrially pre-cross-linked proteins to unfold and de-polymerise them, in order to improve their processability and functional performance.

Pea proteins

Pea proteins are an important meat-replacing protein source for human consumption because of their high content of the amino acid lysine, high nutritional value and low glycaemic index compared with wheat gluten (Choi & Han, 2001). Recently, particular attention and effort have been devoted to carefully milling and fractionating pea proteins and dietary fibre components from pea seed with minimal/or no damage to their primary structure (amino acid composition), because structural damage to the proteins could affect their functionality during food processing (Pelgrom *et al.*, 2013). This special fractionation of pea seed is aimed at producing pea protein-rich fractions/isolates that can eventually be used as protein fortifiers in modern day foods (Wang *et al.*, 1999) or to produce edible protein films (Choi & Han, 2001).

Pea proteins consist of albumins and globulins, with globulins accounting for more than 80 % of the total protein content, mainly stored in cotyledons. Pea

protein globulins consist of legumin, vicilin and convicilin, where legumin is the major and vicilin is the second major globulin fraction. Each of the legumin subunits (~60kDa) consists of disulphide-based acidic and basic polypeptides, with four methionine and two to seven cysteine amino acids (Chihi *et al.*, 2016). Vicilin is a trimeric protein with molecular weight ~150 kDa and convicilin consists of trimers or tetramers with molecular weight ~290 kDa (Choi & Han, 2001). Albumins account for 13-14 % of the total protein content and are mainly located as cytoplasmic proteins that include sulphur-containing amino acids (Chihi *et al*., 2016).

During processing of food, heat induces protein aggregation (increased protein-protein interactions), which determines the structural and textural features of the final food product. Therefore it is important to understand the impact of temperature on protein aggregation and pea protein interactions with other components (*i.e*. dietary fibre, starch or other proteins) in food when attempting to improve processing and produce nutritious food products with desirable functional properties (Chihi *et al*., 2016, Mercier *et al.*, 2011, Petitot *et al.*, 2010, Shand *et al.*, 2007, Choi & Han, 2001, Wang *et al*., 1999).

Starch

Starch is a polysaccharide of glucose molecules and is one of the main forms of energy storage in plants. It is usually produced in large quantities in plant storage organs such as seeds (*e.g*. cereals and legumes), tubers (*e.g*. potato, sweet potato *etc*.) and roots (*e.g*. cassava) (Avérous, 2004). Starch is stored in the form of granules and these granules are extracted during industrial processing. Starch is extracted in large quantities in the industry because of its extensive uses in different industrial applications (*e.g*. bio-based plastics, paper, textile adhesives *etc*.) and food applications.

Native starch granules are of different sizes (1-100 μm) and shapes (oval, round, spherical, elliptical, irregular *etc*.), depending on their origin (Hoover *et al.*, 2010). Starch consists of two major molecular components, namely amylose (~20-30 %) and amylopectin (~70-80 %). Amylose is a linear chain carbohydrate with molecular weight 10^4-10^8 Da consisting of D-glucose units which are linked together by α(1-4) linkages. Amylopectin is a highly branched carbohydrate with molecular weight 10^7-10^9 Da consisting of glucose molecules joined together by α(1-4) and α(1-6) linkages (Avérous, 2004, Flieger *et al.*, 2003). Starch granules are semi-crystalline in nature, with alternating crystalline and amorphous regions forming a lamellar arrangement.

Use of starch started in the 20th Century, when it was added as a filler to synthetic plastics to reduce the cost and enhance their disintegration in nature

(Flieger *et al*., 2003, Lourdin *et al.*, 1995). Today, starch is commonly used as a precursor for the production of a bio-based polymer called polylactic acid.

Materials made from native starch do not show impressive mechanical performance and are moisture-sensitive. Therefore starches are chemically and/or genetically modified to change their primary structure and ratio of amylose and amylopectin content, in order to improve interactions between glucose molecules and thus the functional performance of the product (Altskär *et al.*, 2008, Forssell *et al.*, 2002, van Soest & Essers, 1997). However, changing the ratio of amylopectin and amylose can lead to changes in the processing behaviour of starch. For example, a higher amount of amylose leads to higher viscosity and higher gelatinisation temperature during processing, but improves the strength and stiffness of the materials (Thunwall *et al.*, 2006).

1.1.2 Processing of bio-polymers into materials

The basic principle of processing proteins into films involves denaturation of the protein primary structure, followed by rearrangement of protein polymer chains and formation of new protein-protein interactions to create a stable three-dimensional structure (Lagrain *et al.*, 2010). To induce these structural changes in proteins to produce bio-based materials, different methods such as compression moulding (Gällstedt *et al*., 2004, Pommet *et al.*, 2004), extrusion (Ullsten *et al.*, 2010, Verbeek & van den Berg, 2010) and injection moulding (Cho *et al.*, 2011, Mohanty *et al.*, 2005, Huang *et al.*, 1999) are used. In these processing methods, the main determinants for denaturation of proteins are heat and mechanical shear (Cho *et al*., 2011, Pommet *et al*., 2004), which convert the protein into a solid phase that has certain functional properties.

Thermal processing of plant proteins leads to changes in the molecular and structural conformation of proteins and increases inter- and intra-molecular interactions between proteins (Johansson *et al*., 2013, Sun *et al.*, 2008). These molecular cross-links between proteins take place when amino acids become reactive due to application of heat, a change in pH or addition of chemical cross-linkers. These interactions between amino acids can take the form of covalent bonds such as disulphide, dityrosin and other non-reducible isopeptide, lanthionine and lysinoalanine interactions or non-covalent (hydrogen) bonding during processing (Johansson *et al*., 2013, Rombouts *et al*., 2010, Lagrain *et al*., 2010). Molecular interactions in wheat gluten are well understood, for example inter-molecular disulphide (SS) cross-links are formed by oxidation of sulfhydryl (SH) groups of cysteine during processing. Additional SH-SS interchange reactions also take place due to the availability of free sulfhydryl

groups present in glutenins (Lagrain *et al*., 2010), and these interactions are favourable for improved functional performance of protein-based materials.

To improve the molecular interactions between proteins and lower their glass transition temperature (*Tg*), additives such as plasticisers (*e.g*. glycerol) are included in the protein mix. Furthermore, chemical denaturants are added to improve the processability of the material (Du *et al*., 2015, Newson *et al*., 2015, Newson *et al.*, 2014, Rombouts *et al.*, 2013, Ture *et al.*, 2011). For example, during extrusion, addition of glycerol to the protein mix lowers the *Tg* and viscosity of the protein melt and increases the chain mobility of proteins for improved processing. However, in wheat gluten excessive protein aggregation in the extruder increases viscosity and reduces chain mobility, which results in localised dense protein association in the extruder, leading to poor functional performance of the materials. Therefore, careful selection of processing conditions such as temperature and type of additive are of the utmost importance to control protein aggregation and to steer the functional properties of the final product.

Processing proteins under alkaline conditions (at pH above the isoelectric point of most plant proteins) and high temperature also induces unfolding and re-organisation of proteins, leading to the formation of new protein-protein interactions during processing (Gerrard, 2002). However, extensive exposure to high pH and high temperature can result in breakdown of proteins (Newson *et al*., 2015, Newson *et al*., 2014, Johansson *et al*., 2013, Ullsten *et al.*, 2006). High pH can unfold and de-polymerise those proteins which are highly cross-linked due to industrial processing, *e.g*. potato proteins. In potato proteins, β-elimination of disulphide groups at high pH can lead to the formation of isopeptide bonds such as dehydroalanin, lanthionine and lysinoalanine (Newson *et al*., 2015, Gerrard, 2002).

To successfully process starch into bio-based materials, complete gelatinisation/destruction of the initial semi-crystalline structure of starch granules is important. The most common way to gelatinise starch is to use water and heat. With addition of water, the starch granules swell and become a viscous paste, due to destruction of inter-molecular hydrogen cross-links (Avérous, 2004). Solution casting to produce starch films is one such method, where a high amount of water is utilised to gelatinise starch along with heating of the solution. Another common method where minimal hydration is needed is extrusion. During extrusion processing, the plasticiser, heat and shear pressure are the main determinants to break down the starch granules (Altskär *et al*., 2008). During this gelatinisation process, new interactions between glucose molecules take place and a thermoplastic material with certain functional properties is obtained.

1.2 Bio-composite materials

Composite materials are composed of two or more polymers or polymer reinforced with fibrous materials (synthetic or bio-based fibres), with the aim of combining the properties of both components and achieving several functions. For example, synthetic glass fibres can be combined with epoxy resin, with the glass fibres adding stiffness and strength to the final composite material.

During recent decades, efforts have been made to produce bio-polymer composites with different functional properties. For example, wheat gluten has been combined with other polymers such as poly(vinyl alcohol) (Dicharry *et al.*, 2006), polycaprolactone (John *et al.*, 1998) and polylactic acid (Cho *et al.*, 2010) to enhance mechanical properties. For production of nano-composites, addition of clay particles to wheat gluten films has been found to improve their mechanical, gas barrier and thermal properties (Kuktaite *et al*., 2014, Cho *et al*., 2011). Other examples of bio-polymer composites include wheat gluten/rice proteins and egg albumin/potato starch blends in applications such as packaging materials with improved functional properties (Yang *et al.*, 2011, Gonzalez-Gutierrez *et al.*, 2010).

In addition, in order to produce fully biodegradable composites based on similar design principles as used to make synthetic composite materials, proteins from plant sources are being explored as a matrix to produce natural fibre-reinforced bio-composites. Here the protein matrix acts as a binder and the plant fibres provide stiffness and strength. In addition to offering similar added stiffness and strength as found in their synthetic counterparts, plant fibres are lightweight and fully biodegradable. There are several examples in the literature of wheat gluten and soy protein reinforced with hemp, jute and bamboo fibre displaying increased stiffness and strength compared with protein films without natural fibres (Wretfors *et al.*, 2010, Huang & Netravali, 2009, Sun *et al*., 2008).

Since the research area of bio-composites based solely on two bio-polymer or polymer/plant fibre composites is fairly new, there are still knowledge gaps to be filled for future understanding and improvement. Plant proteins are complex molecules and a better understanding of their interactions with other components, such as starch, other proteins and/or plant fibres, in the composite environment is needed to improve their material properties. Understanding of variations in bio-polymer chemistry and structure governed by processing conditions could play an important role for the development of bio-composites.

1.3 Protein and dietary fibre-rich foods

Changes in the eating patterns of modern society currently include an increasing trend to replace meat with plant protein-rich products and other modern food alternatives with a balanced content of nutrients. This relates to people's perception and desire for healthy eating, weight reduction and ethical and environmental concerns (regarding meat production) (McIlveen *et al.*, 1999). This calls for production of new, healthier protein-rich foods which can supply the daily requirement of proteins for the human body and which have a low glycaemic index. Plant proteins such as soybean, lupin, beans and peas are now commonly used in the production of modern protein-rich foods. All these proteins also possess a variety of essential amino acids necessary for human nutrition (Neacsu *et al.*, 2017).

Care and attention are needed during cooking of protein-rich foods, since the structural conformation of proteins is altered during cooking, affecting the final digestibility of the food. Therefore, controlled milling and fractionation techniques for legumes to obtain protein and dietary fibre fractions have been developed recently. One example is fractionation of peas to obtain protein-rich and dietary fibre-rich fractions for use in modern protein-rich and dietary fibre-rich foods (Pelgrom *et al*., 2013). Pea protein contains a high amount of lysine, an essential amino acid which is not produced in the human body, while the dietary fibre fraction is beneficial for gut microflora in the human body. Recent studies have reported use of pea protein-rich fractions as a protein-fortifier for traditional wheat pasta and use of the dietary fibre fraction to wheat-based pasta products to increase their nutritional value (Laleg *et al.*, 2017). However, the textural and structural properties are reported to be negatively impacted in some cases (Brennan *et al.*, 2004, Tudorica *et al.*, 2002). Therefore, when producing protein-rich and dietary fibre-rich foods, an in-depth understanding of the effects of *e.g*. heating/cooking on protein chemistry and structure and interactions with other components is needed to improve their processability.

2 Objectives

The overall aim of this thesis was to understand and identify chemical and structural changes taking place in bio-polymers during processing and the impact of those changes on the functional properties of composite materials and foods. A specific aim was to use protein-rich industrial side-streams (wheat gluten and potato proteins) in combination with either specifically designed potato starch or with plant fibres (from hemp) to produce composites with functional properties superior or different from their individual components. In addition, specifically fractionated pea proteins and dietary fibre from pea seed flour were used to produce protein and dietary fibre-rich pasta-like sheets that could be used as healthy food.

Specific objectives of the studies reported in this thesis were to:

- Evaluate the reinforcement of wheat proteins with hemp fibres in a plasticiser-free method to produce fully biodegradable composites with improved mechanical properties, and determine the impact of different processing temperature on protein polymerisation and its relation to mechanical performance.
- Use specifically modified potato starch in blends with wheat gluten proteins to produce composites with improved mechanical and gas barrier properties, and determine the influence of different temperatures and plasticisers on protein chemistry and structure-function relationships in protein-starch composites.
- Determine the impact of protein polymerisation and protein-dietary fibre interactions on the structural morphology, cooking quality and mechanical properties of pea protein and pea protein-fibre pasta-like sheets produced as a healthier food alternative.
- Evaluate and understand the impact on the chemistry and structure of industrial wheat gluten and potato proteins of chemical modification in order to improve the protein processing window and produce composites with improved mechanical performance.

3 Methodology

3.1 Raw materials and sample preparation

Wheat gluten
Industrial wheat gluten used in this thesis was purchased from Lantmännen Reppe AB, Lidköping, Sweden and contained 77.7 % protein, 5.8 % starch, 1.2 % fat and moisture content 6.9 %, according to the supplier. Gliadins, a monomeric protein fraction in wheat gluten, were separated by dispersing the wheat gluten in 70 % ethanol solution, followed by shaking for a certain time and centrifugation (Papers I-III). The gliadins, dissolved in ethanol, were recovered in the supernatant following centrifugation, while the residual pellet comprised a glutenin-rich fraction which also contained other components such as bran, fibre and residual starch. Solubilised gliadins were removed from solution by rotary evaporation of ethanol and thereafter freeze-dried and milled to powder. The glutenin-rich fraction was also freeze-dried and milled to powder.

Pea protein and pea dietary fibres
Pea protein isolate and pea dietary fibre (further referred as fibre) fractions were obtained by fractionation of commercial whole pea flour (Markedal *et al.*, 2016) at the Department of Food Science, University of Copenhagen, Denmark (Paper IV). The pea protein isolate contained 70 % protein, 18 % fibre and 7 % starch, and the pea fibre fraction contained 38 % fibre, 37 % starch and 21 % protein.

Potato protein
Potato protein was supplied by Lyckeby Starch AB. The protein content of the product was 82.2 % (Dumas method, Flash 2000 NC Analyser, Thermo

Scientific, USA, N X 6.25) and the moisture content was 8.1 % (dry basis, dried at 105 °C for 3 h) (Newson *et al*., 2015) (Paper V).

Wheat gluten and potato protein modification

Potato protein powder (50 g) was slowly dispersed in 600 ml distilled water while stirring. To adjust the pH 10, 5 M NaOH solution was added to protein suspension. The suspension was heated for 30 min at 75 (±3) °C. The suspension was cooled, lyophilized and milled to powder. Wheat gluten was modified in the same way as potato proteins (Paper V).

Hemp fibres

Industrially produced hemp fibre mat (of randomly arranged fibres) with an average fibre length of 14 mm was purchased from Hemcore, United Kingdom (Paper I).

Potato starch

Modified potato starch (MPS) was produced at the Department of Plant Breeding, Swedish University of Agricultural Sciences, Alnarp, Sweden (Papers II & III).

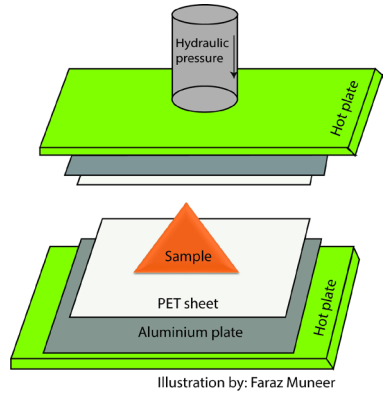

Figure 2. Illustration of the compression moulding set-up used in Papers I, IV and V.

Compression moulding

To prepare hemp fibre-reinforced samples, a pre-cut section of hemp fibre mat was placed in a plastic tray of similar size and protein powder (wheat gluten, glutenin or gliadin) was poured onto the surface of the mat. The tray was then shaken to allow the protein powder to infiltrate the empty spaces between the hemp fibres prior to compression moulding (Paper I). Blends consisting of pea protein-fibre and wheat gluten-potato protein (Papers IV and V) were hand-

mixed with glycerol in a small glass container before compression moulding. Each sample was placed between two polyethylene terephthalate (PET) films and thereafter placed between two pre-heated aluminium plates. The compression moulding set-up is illustrated in Figure 2.

Extrusion
The wheat gluten-, glutenin- and gliadin-starch blends were hand-mixed with either glycerol or a glycerol-water blend prior to extrusion (Papers II and III). For extrusion, a Haake minilab twin screw (Thermo Scientific Corporation, Germany) was used. Material was pushed by hand into the extruder, using a brass piston. An extrusion speed of 30 rpm was used to obtain rectangular strips (3.9-4.1 mm wide, 1.1-1.6 mm thick). A graphical representation of the extrusion set-up is presented in Figure 3.

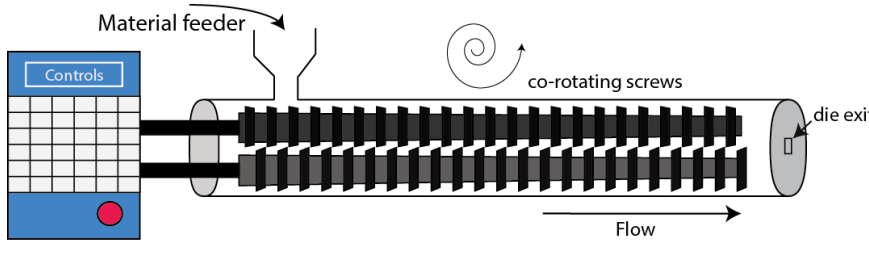

Figure 3. Illustration of the extrusion set-up used in Papers II and III.

3.2 Analysis

Size-exclusion high performance liquid chromatography (SE-HPLC)
To investigate the protein polymerisation and molecular size distribution of protein in the composite materials, a three-step extraction procedure with some modifications was used (Papers I-V). Proteins were extracted in sodium dodecyl sulphate (SDS)-phosphate buffer during the first extraction (no sonication), followed by two more extractions where 30 s (second extraction) and 30+60+60 s (third extraction) sonication intervals were used. The extractions were analysed by SE-HPLC (Papers I-V) to obtain protein molecular weight profile and solubility.

Fourier transform infrared spectroscopy (FT-IR)
The secondary structure of proteins in composites was investigated using FT-IR spectroscopy equipped with single reflection attenuated total reflectance (ATR) (Paper II-V). Structural changes in the amide-I spectra were studied.

Small-angle (SAXS) and wide-angle X-ray scattering (WAXS)
Changes in protein molecular and nano-structural morphology in the composites were studied by SAXS and WAXS. The MAX IV Synchrotron laboratory, Lund, Sweden, was used to investigate all samples (Papers II-IV).

Tensile testing
Tensile tests were performed to determine the mechanical performance of all processed materials (Papers I-V). Dumbbell-shaped specimens (Paper I, IV and V) and strips (Papers II and III) were tested using an Instron testing machine with a 100 N load cell. Stress-strain curves were used to calculate E-modulus (stiffness), maximum stress (strength) and strain at maximum stress (extensibility). At least 10 replicates were used for each sample.

Oxygen permeability
Oxygen permeability (OP) values for wheat gluten-, glutenin- and gliadin-MPS composites were analysed following an ASTM 1927-07 standard in an Ox-Tran 2/21 testing machine (Papers II and III). The samples were conditioned for at least 16 h prior to measuring the oxygen barrier properties (in duplicates).

4 Results and Discussion

4.1 Impact of bio-polymer type and blend composition on processing and functional properties of bio-composites and food

One of the main aims of this thesis was to use plant protein-rich residuals originating mainly as side-streams from industrial bio-ethanol or food/feed plant processing (wheat gluten and potato proteins) (Papers I-III and V) or proteins from fractionation processing (Paper IV). In a composite system, plant proteins were used either with specifically modified potato starch (altered amylopectin structure and higher amylose content) (Papers II-III) or with fibres (hemp and pea fibres) (Papers I and IV) to produce composites and foods with characteristics different from the individual components. The main focus was on proteins, as a major component of the blend forming a matrix in which starch or fibres acted as fillers in composites and foods. For pasta-like sheets, pea protein was combined with pea fibres, with the aim of developing a protein-rich food that is also functional and 'slow'.

During the course of the studies included in this thesis, it was found that the composition of the blend, in terms of the components used and their ratio, played a significant role during processing and for the final product properties in nearly all samples studied (Papers I-V). During processing of protein-fibre composites, the protein type essentially affected the processing behaviour, *e.g.* protein flow, as well as interactions between the protein and the filler. In wheat gluten- and gliadin-hemp fibres composites, protein flow was better than in glutenin-based composites (Paper I) (Figure 4a). These differences in protein flow can be explained by greater flexibility in protein chains, viscous nature and higher purity of the gliadin fraction compared with the glutenin fraction (which also contained bran, fibre, starch *etc*.). In contrast, for the pea protein-pea fibres

composites, addition of fibre and an increase in the amount of fibre led to weaker protein-fibre interactions that were affected by varying particle size and component purity (*e.g.* the pea fibre fraction contained a high amount of starch granules, which partly remained non-gelatinised after processing (Paper IV) (Figure 4b).

One explanation for differences in processability is variation in the chemical composition of the components and chemical reactions between the protein-protein, protein-fibre and other components. Pea protein consists of albumins and two globulins, legumin and vicilin, that are highly soluble in water and acidic environments (Tömösközi *et al.*, 2001). They can thus easily interact with each other during processing, as was found when the fibre-free pea protein composites were produced (Paper IV). Presence of insoluble dietary fibre (de Almeida Costa *et al.*, 2006) in a blend with pea protein, which also contained starch, non-structural polysaccharides (Paper IV) and other components (Dhingra *et al.*, 2012), affected pea protein chemistry and cross-linking interactions and resulted in weakening of the functional properties.

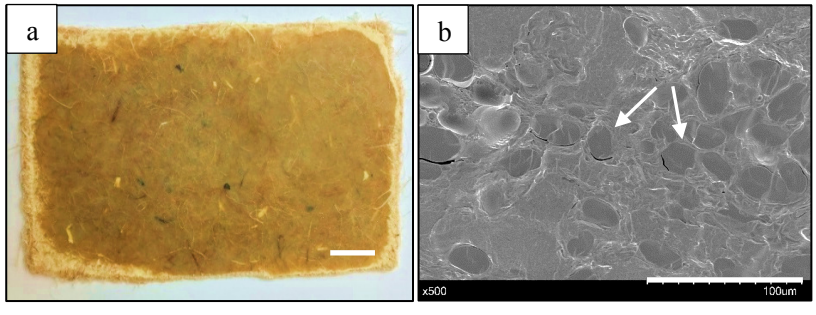

Figure 4. Protein-fibre composite and food. a) Gliadin-hemp fibre composite showing good protein melt and flow, bar = 100 mm, b) Scanning electron micrograph of a cross-section of a pea protein-fibre blend (50/50) pasta-like sheet, showing intact starch granules (indicated by arrows) imbedded in a protein matrix (bar = 100 µm). Figure 4b is taken from Paper IV, with kind permission of *Food Research International*.

Other examples of two components that were a good match in a composite with suitable properties were wheat gluten protein-MPS blends (Papers II and III). Both wheat gluten and glutenin in a blend with starch at equal ratios (50/50), with the help of plasticisers (glycerol and water) in the mix, showed excellent ability to interact/blend, which resulted in attractive functional properties (Papers II and III). During extrusion processing and heating, gliadins (present in wheat gluten) and high molecular weight glutenins (present in wheat gluten and glutenin) interacted with the debranched amylopectin (as opposed to non-

modified amylopectin, where the branched structure hindered close interaction with protein), amylose and smaller molecules from possible starch hydrolysis. From previous studies, it is known that amylose forms aggregates with gliadins and that gliadin-starch interactions of a hydrophobic nature exist (Guerrieri *et al.*, 1997). These interactions are governed by the structure and size of the molecules. Although, in the samples tested in this thesis, starch granules were incorporated into the protein matrix during extrusion and remained partly non-gelatinised.

Regarding the pea protein and fibres pasta-like sheets, compositional ratio was crucial in determining the functional properties (Paper IV). For pea protein, stronger protein-protein interactions were important for the improved functional behaviour. Addition of more than 20 % fibre in the pea protein-fibres composite had a negative impact on protein cross-linking and consequently decreased composite strength and extensibility. The composition of the pea protein and fibre fractions, as well as their particle size, also clearly affected protein cross-linking and microstructure (of the blend), which determined the quality characteristics of pasta-like sheets (mechanical performance and cooking quality).

4.2 Chemistry and cross-linking of proteins in bio-composites and food

4.2.1 Impact of processing temperature

Protein cross-linking is a process of chemically joining two or more protein molecules by a covalent, non-covalent or ionic bond. During processing under elevated temperature, relatively large-scale molecular changes promote protein-protein interactions. Protein cross-linking can be related to protein extractability/solubility, *e.g.* low protein solubility indicates a cross-linked or polymerised protein, and *vice versa*. In this thesis, a clear effect of processing temperature on protein cross-linking was observed in most of the composites studied (Papers I-III and V). In all wheat gluten protein-hemp fibres composites, a decrease in total protein extractability was observed with an increase in pressing temperature from 110 to 130 °C (Paper I). In addition, introduction of hemp fibre to the blend with the proteins induced variations in protein cross-linking, as also reported in previous studies (Wretfors *et al*., 2010, Kunanopparat *et al*., 2008b). High hot pressing temperature (up to 130 °C) was beneficial for gliadin-hemp fibres samples, where a large increase in protein polymerisation took place in comparison with glutenin-hemp fibres composites (Papers I). It was found that gliadins formed rather flexible protein-protein interactions,

where the disulphide bonds and their exchange chemistry, and hydrophobic and electrostatic interactions, played an important role (Papers I and III). This can partly be attributed to the chemical structure, *i.e*. small molecular size and viscous nature of gliadins.

Similarly, in wheat gluten- and glutenin-starch composites, higher temperature (130 compared with 110 °C) increased protein cross-linking (Figure 5). For these composites, the polymeric protein fraction became highly aggregated with an increase in processing temperature (Papers II and III). In addition, at 130 °C the monomeric protein fraction became more cross-linked (decreased solubility), suggesting monomeric protein incorporation into a larger polymer network via disulphide bonds, hydrogen bonds and hydrophobic and electrostatic interactions (total protein solubility decreased) (Rasheed *et al.*, 2014, Johansson *et al*., 2013). Across all wheat gluten protein-starch blends containing glycerol as a plasticiser, a processing temperature of 130 °C decreased protein extractability and increased cross-linking, except the glutenin-starch composite, which showed a small increase in protein solubility (Figure 5a). Although, an in-depth analysis of glutenin-starch composites by the asymmetrical flow field flow fractionation (A4F) method revealed that glutenins aggregated into large polymers (molecular weight up to 53 x 10^7 g/mol) at 110 °C and even larger polymers (169 x 10^7 g/mol) at 130 °C (Paper III). This suggests that the increase in temperature induced a high degree of protein aggregation in glutenins, maintained by covalent and non-covalent cross-links.

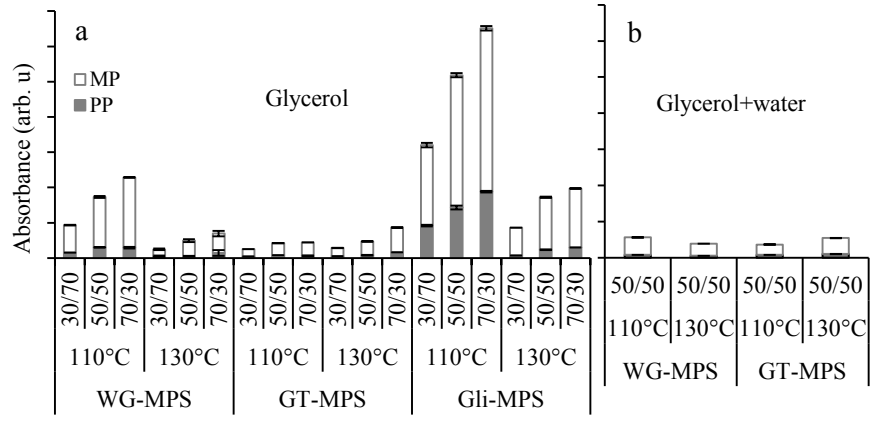

Figure 5. Impact of processing temperature and plasticiser on protein extractability in extruded wheat gluten (WG)-, glutenin (GT)- and gliadin (Gli)- modified potato starch (MPS) composites. PP = polymeric protein fraction, MP = monomeric protein fraction. Adapted from Papers II and III with permission, Copyright 2015, 2016 *American Chemical Society*.

For pea protein to polymerise, hot pressing (at 130 °C) was beneficial to induce protein-protein interactions and bonding through hydrogen, disulphide and isopeptide cross-links. With a high amount of pea protein in the pea protein-fibre pasta-like sheets, they showed decreased protein extractability and relatively better cross-linking compared with the 50/50 pasta-like sample (Paper IV). Among the pea protein-fibre blends, the lowest amount of high molecular weight proteins was extracted for the 80/20 and 70/20 blends, suggesting that the amount of protein in the blend contributed to increased polymerisation. The addition of ≥ 20 % pea fibre to the composite negatively affected protein polymerisation and resulted in a weak chemically bonded protein network with more total proteins being extracted (Figure 6).

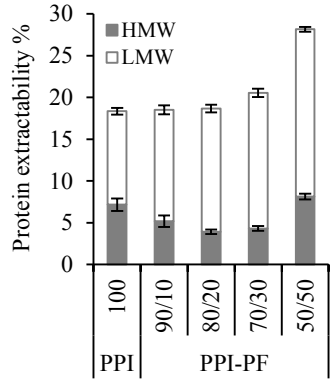

Figure 6. Impact of blend composition on protein extractability in pea protein isolate (PPI) and in pea protein isolate-pea fibre (PPI-PF) pasta-like sheets. HMW = high molecular weight, LMW = low molecular weight. Adapted from Paper IV, with kind permission of *Food Research International*.

4.2.2 Impact of additives

Additives such as plasticisers (glycerol or glycerol+water) and/or chemicals that bind to specific sites on protein effectively steered the polymerisation of proteins and the interaction of components of the blend (Papers II, III and V). With regard to protein plasticisation, in wheat gluten- starch blends, better protein plasticisation and polymerisation were achieved when a blend of glycerol and water was used at higher processing temperature (130 °C), while for glutenin-starch blends this effect was observed at lower processing temperature (110 °C) (Papers II and III). This suggests that chemical interplay between the proteins, starch and a blend of glycerol and water was able to induce protein-protein cross-linking and protein-starch interactions, compared with glycerol-only samples. This plasticisation process relies on water serving as a source of new hydrogen

bond formation in the proteins and increasing the mobility of protein chains by reducing extensive inter-molecular forces (Gontard *et al.*, 1993). The increase in mobility is due to gluten-water protein interactions and increased formation of supplementary hydrogen bonds between protein polypeptides (Mejri *et al.*, 2005, Gontard *et al*., 1993). The impact of glycerol and water plasticisation was also obvious on molecular weight build-up in glutenins, as glutenin-containing samples mixed with glycerol and water showed much smaller protein aggregates during processing than samples only containing glycerol (especially at 130 °C) (Paper III). A combination of glycerol and water also partly gelatinised starch granules, which improved the internal microstructure of the composite and resulted in better blending of protein-starch (Papers II and III). Improved plasticisation and polymerisation of proteins and partial gelatinisation of starch were reflected in increased mechanical performance, *e.g.* tensile strength, stiffness and extensibility, of wheat gluten-starch 50/50 composites at both processing temperatures (Paper II). For glutenin-starch composites, a positive effect of plasticisation in terms of improved mechanical performance was observed only at lower processing temperature (110 °C), possibly due to non-enzymatic glycation and oxidation reactions (Paper III) (Rasheed *et al.*, 2018).

In terms of processing, a combination of glycerol and water significantly improved physical processing conditions and allowed easy processing of wheat gluten-starch and glutenin-starch composites, also resulting in homogeneous composites (Figure 7).

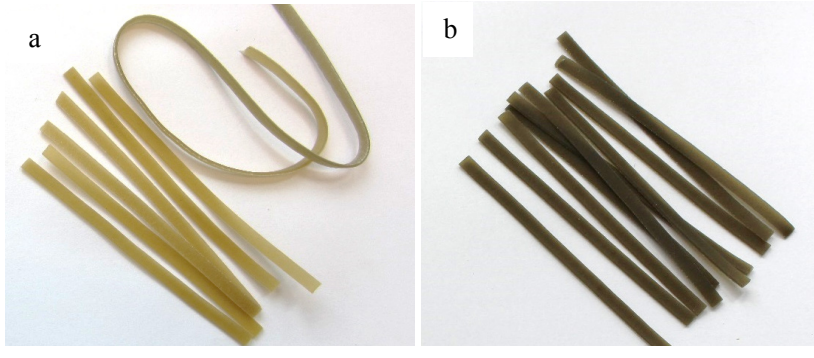

Figure 7. Representative images of a) a 50/50 blend of wheat gluten and modified potato starch (MPS) and b) a glutenin-MPS blend extruded with 30 % glycerol and 20 % water.

To improve processing, chemical modification of industrial wheat gluten and potato protein is needed to unfold their polymer aggregates and hydrophobic interactions, and later form new cross-links and improve the protein processing window (Rasheed *et al.*, 2015b, Newson *et al*., 2015, Du *et al*., 2015). The basic

pH treatment of wheat gluten and potato protein powders unfolded and de-polymerised the polymer aggregates that played a vital role in processing of the composites (Paper V). Processing of these basic pH-treated wheat gluten and potato proteins increased the protein-protein interactions, *e.g.* disulphide interactions, hydrogen bonding and other covalent bonds, at both 130 and 150 °C. This resulted in decreased extractability of high and low molecular weight proteins for both wheat gluten and potato proteins (Figure 8). A positive impact of chemical modification was observed for potato proteins, which showed increased protein polymerisation at 130 °C, although no such increase was observed at 150 °C. In contrast, wheat gluten was more prone to chemical modification, as it showed higher protein extractability at both processing temperatures. This high extractability from both wheat gluten and potato protein at 150 °C suggests that proteins formed a weak network, most likely due to breakdown of the high molecular weight fraction into low molecular weight fragments.

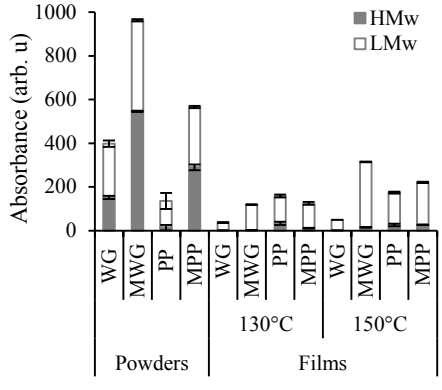

Figure 8. Impact of chemical modification on protein extractability of industrial wheat gluten (WG) and potato protein (PP), before and after modification (MWG and MPP), in unprocessed powders and films processed at 130 and 150 °C. HMW = high molecular weight, LMW = low molecular weight.

An impact of protein modification was also observed in modified wheat gluten-modified potato protein (MWG/MPP) composites, with more proteins being extracted at 150 °C, as seen for their individual processed modified protein films (Paper V). The increase in protein extractability was observed with increasing temperature from 130 to 150 °C and also with increasing proportion of modified wheat gluten in the blends (*i.e.* 70/30 MWG/MPP composite). The high protein extractability from MWG/MPP suggests that blending the two modified proteins in a composite did not improve protein polymerisation, due

e.g. to low amount of disulphide cross-links or covalent interactions between the proteins.

The wheat gluten-modified potato protein (WG/MPP) composites showed decreased protein extractability compared with the non-modified composites (WG/PP) at 130 °C. A decrease in protein solubility (or increased protein polymerisation) of WG/MPP processed at 130 °C and an increase when processed at 150 °C were observed, especially when amount of modified potato protein was increased to more than 50 % in the blend. This suggests that addition of modified potato protein in the blend favoured an increase in protein-protein interactions at 130 °C, although at 150 °C no such effect was observed.

4.3 Structure of bio-polymers in bio-composites and food

4.3.1 Variation in protein secondary structure in processed bio-composites and food

Understanding the mechanism that determines bio-polymer chemistry and structure during processing and in processed bio-composites and foods is important in order to steer the functional properties (Papers II-V). The secondary structure of a protein comprises a three-dimensional form of protein segments, known as α-helices, β-sheets, β-turns and random structures (Schermann, 2008). Analysis of amide-I band in the infrared spectrum indicated that the secondary structure of wheat gluten, gliadin and glutenin was influenced by processing temperature, plasticiser and composition of the blend in the protein-starch composites (Papers II-III). The changes in structure occurred at both extrusion temperatures (110 and 130 °C) compared with unprocessed protein powders, and led to increased protein cross-linking and formation of higher amounts of β-sheets in the composites (Papers II and III). The major difference between the wheat gluten-starch and glutenin-starch samples was the type and proportion of secondary structure, which were steered by the processing temperature and the type of plasticiser used. In wheat gluten-starch and glutenin-starch blends, an increase in processing temperature (from 110 to 130 °C) and the amount of protein in the blend increased the formation of β-sheet-related structures (Papers II and III). Furthermore, for wheat gluten-starch blends an increase in β-sheets was observed at 110 °C when glycerol and water were used to plasticise the blend, indicating favourable protein interactions in hydrated gluten. No such interactions were observed in proteins at 130 °C, as was shown by a decrease in β-sheets (Paper II). For gliadin-starch composites plasticised with glycerol, a high processing temperature as 130 °C, favoured the formation of higher amount of β-sheets structures compared with 110 °C. These higher amount of β-sheets

are formed due to gliadins ability to form intra-molecular cross-links between polypeptide chains in the presence of glycerol and other additives, as reported in several temperature-processed protein-based materials (Kuktaite *et al.*, 2016, Rasheed *et al*., 2015b).

Glutenins have a more complex structure than gliadins and can form inter- and intra-molecular cross-links maintained by hydrogen and disulphide interactions. These interactions were observed in wheat gluten-starch and glutenin-starch composites with glycerol-water blends at 110 and 130 °C (Papers II and III). A possible explanation is that, upon hydration and plasticisation with water and glycerol, and heating, glutenins formed inter-molecular cross-links where low molecular weight proteins became a part of this large protein network (Johansson *et al*., 2013). Non-covalent bonds such as hydrogen bonds, ionic bonds and hydrophobic bonds were also important for the amount of β-sheets formed, for both gliadins and glutenins. For glutenin-starch composites at 110 °C, the α-helix to β-sheet ratio was relatively similar, indicating a balance in elastic energy storage in the system compared with all other samples. This balance seems to be related to the very good mechanical properties of this composite.

Regarding the pea protein secondary structure in pea protein-fibre pasta-like sheets, the formation of β-sheets was induced by pressing and temperature (Paper IV), similarly to wheat gluten protein structure (Rasheed *et al*., 2015a, Kuktaite *et al*., 2014, Carbonaro *et al.*, 2012, Ture *et al*., 2011, Gällstedt *et al*., 2004, Gueguen *et al.*, 1998). Higher amounts of strongly hydrogen-bonded β-sheets (observed in pressed pea proteins at 130 °C) were reduced with addition of pea fibres to the blend (Figure 9, black arrows), suggesting weakened protein-protein interactions. SE-HPLC analysis showed that addition of more than 20 % pea fibres negatively affected protein cross-linking and that this was strongly driven by the chemical composition of the blend, already discussed in section 4.2.1. The use of both high temperature (130 °C) and glycerol facilitated chemical changes and transformation of pristine protein powders from less organised structures to more organised aggregated structures, as reported previously for other protein-based materials (Newson *et al*., 2015, Rasheed *et al*., 2015a, Kuktaite *et al*., 2014, Carbonaro *et al*., 2012, Ture *et al*., 2011, Gällstedt *et al*., 2004, Gueguen *et al*., 1998). Pea protein-fibre blends (70/30 and 50/50) had a low amount of total β-sheets content and β-turns, and a high amount of unordered structures, compared with the blends with more proteins, due to high amounts of starch, weakening the pea protein network. For a stable and cross-linked pea protein network, use of a more pure protein fraction is desired, where formation of hydrogen bonds and protein-protein cross-links could occur (Nowick, 2008).

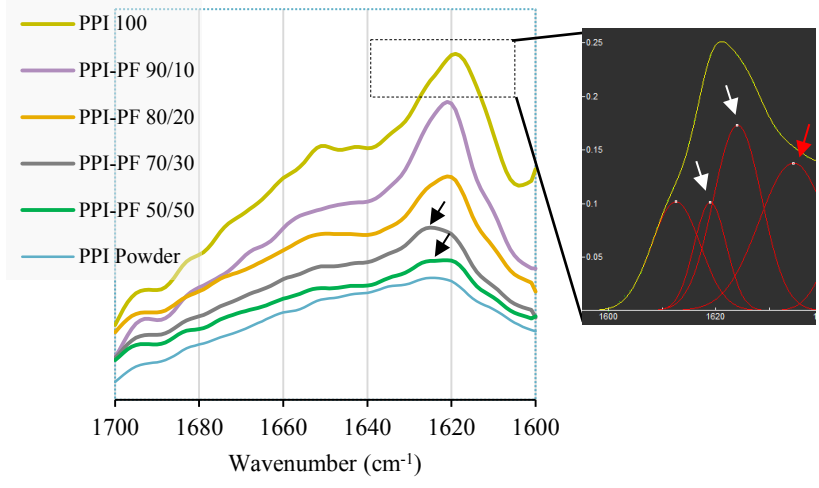

Figure 9. Secondary structure of pea protein isolate (PPI) and pea protein isolate-pea fibre (PPI-PF) pasta-like sheets. Spectral range 1645-1660 cm^{-1} corresponds to α-helices and random coils, and 1615-1635 cm^{-1} to β-sheets. Blown-up area indicates underlying strongly hydrogen-bonded (white arrows) and weakly hydrogen-bonded (red arrow) β-sheet structural peaks in PPI. Adapted from Paper IV, with kind permission of *Food Research International*.

4.3.2 Protein secondary structure in unprocessed and chemically modified protein composites

Comparing the unprocessed wheat gluten and potato protein powders, the potato protein showed strongly bonded β-sheet interactions, indicating a highly cross-linked protein network (as shown by low protein extractability; Figure 8). This was induced by the harsh industrial extraction conditions (high temperature and acidic pH) (Paper V). Unprocessed wheat gluten showed a low amount of β-sheets and greater amount of α-helices, random coils and unordered structures. These observations indicate that highly pre-cross-linked protein material limits the protein processing window and lowers the chances of formation of new protein-protein interactions, ultimately affecting the functional properties of the material. After alkaline modification, a favourable decrease in cross-linking and re-cross-linking took place in potato protein, but not in wheat gluten (Paper V). A certain proportion of β-sheets were presumably eliminated due to protein unfolding, and new protein interactions were formed, in potato protein. Chemical modification of wheat gluten powder resulted in an increase in β-sheets at the expense of α-helices, random coils and unordered structures, suggesting novel protein-protein interactions (maintained by hydrogen bonding due to the presence of hydrated alkaline conditions during the modification

process) and increased protein-peptide interactions (Nowick, 2008). This provides the information that the increase in β-sheets and formation of cross-linkages were governed by pH and became irreversible (Figures 8 and 10). Another possible explanation could be that alkaline pH favoured dissociation of wheat gluten monomeric proteins from oligomeric proteins (high solubility of the low molecular weight fraction was indicated by SE-HPLC; Figure 8). This was followed by non-reversible denaturation of proteins at the expense of secondary structure rearrangements (Lullien-Pellerin & Balny, 2002).

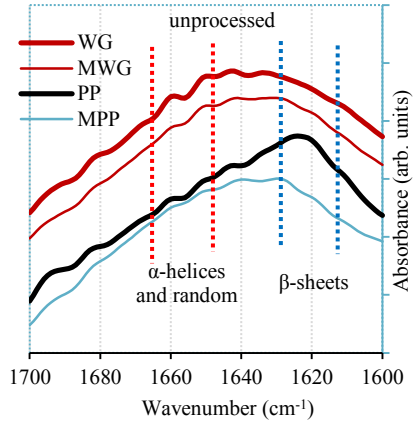

Figure 10. Secondary structure of pristine non-modified wheat gluten (WG) and potato protein (PP) powders and corresponding pH-modified powders (MWG and MPP). Area between blue dotted lines represents β-sheets and area between red dotted lines represents α-helices and random coils.

For wheat gluten-potato protein (WG/PP) composites, the decrease in protein-protein interactions and re-formation of stabilised interactions during processing suggests that the affinity of the hydroxyl (OH^-) group for specific sites on potato protein favours creation of multi-protein complexes (Kluger & Alagic, 2004) with wheat gluten (Figure 11). In modified wheat gluten-modified potato protein (MWG-MPP) composites, at 130 °C relatively high amounts of β-sheets, α-helices, random coils and unordered structures were found, while at 150 °C the amounts of these structures decreased (Paper V). This indicates large-scale secondary structure rearrangements in proteins, induced by high temperature (Diuk Andrade *et al.*, 2018).

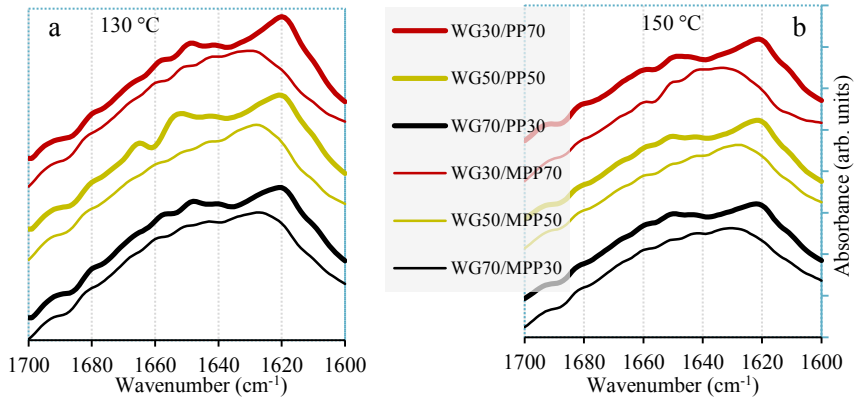

Figure 11. Fourier transform infrared (FT-IR) spectra of pressed wheat gluten (WG), potato protein (PP) and modified potato protein (MPP) blends at 130 and 150 °C.

4.3.3 Nano-structural morphology of bio-polymers in bio-composites and food

The nano-structural morphology of proteins and starch during processing can greatly influence the mechanical properties of the processed composites (Papers II and III) and act as an indicator for protein-fibre interactions in foods (Paper IV). During processing, gliadins in a blend with starch formed hierarchical hexagonal assemblies, observed for the first time for such a composite system in this thesis (Figure 12). Similar hexagonal structural assemblies have been found previously in wheat gluten films with various additives (ammonium hydroxide and salicylic acid or urea) (Kuktaite *et al*., 2012, Kuktaite *et al.*, 2011) and gliadins films processed with glycerol (Rasheed *et al*., 2014, Kuktaite *et al*., 2014). For some gluten protein films in those studies, the hexagonal structure complexity was correlated well with high amounts of β-sheets and good mechanical performance (Johansson *et al*., 2013, Kuktaite *et al*., 2011). In all previous studies except that by Rasheed *et al*. (2018), gliadins were shown to be responsible for formation of complex hierarchical structures (Rasheed *et al*., 2014, Kuktaite *et al*., 2012, Kuktaite *et al*., 2011). In the case of gliadin-potato starch composites in this thesis, at both extrusion temperatures (110 and 130 °C) hexagonal structures were formed, including at a protein concentration of 30 % in the blend with starch (light green curve in Figure 12). At the higher processing temperature (130 °C), additional structural peaks seemed to indicate higher protein aggregation (SE-HPLC data, see Figure 5) (Paper III). Due to the large amount of crystalline potato starch observed by SAXS and scanning electron microscopy (SEM) (which showed intact starch granules), it can be assumed that this is one of the reasons why gliadins interacted more with glycerol than with

starch. For hierarchical hexagonal assemblies of gliadins, the temperature and plasticiser used during processing induced chemical interactions in proteins, where starch was a filler in the semi-arranged and cross-linked protein network (Paper III), as observed previously in a similar gliadin-additive composite (Rafieian & Simonsen, 2015).

It is important to point out that, during wheat gluten-starch and glutenin-starch processing (into composites) with both glycerol (Figure 13a) and glycerol+water, no such hierarchical nano-assemblies as seen in gliadins were found (Figure 12). In addition, proteins and starch showed a combined scattering reflection of the lamellar structure of starch and a broad correlation distance of wheat proteins (d_{Broad} peak, observed in all wheat gluten-starch and glutenin-starch composites), and potato starch also showed a B-type crystalline structure (d_{100} peak) (Nishiyama *et al.*, 2009) (Figure 13a). Variation in the ratio of protein to starch and in processing temperature had a strong impact on the scattering components (Figure 13b). With decreasing protein content and increasing starch in the blend, the morphological distance d_{BROAD} increased, suggesting that protein peptides possibly became distant due to the presence of crystalline starch.

Starch lamellar structural arrangements were observed when studied with WAXS, where starch showed a number of crystalline structural peaks and an indication of B-type crystallinity (d_{100}) (Figure 14). However, addition of glycerol+water (compared with only glycerol) to wheat gluten-starch and glutenin-starch 50/50 blends reduced the crystallinity of the starch (Papers II and III). Addition of water contributed to chemical changes in starch (gelatinisation), leading to subsequent loss of crystallinity. Water also caused swelling of the amorphous regions of starch granules and, together with heat, melted the amylopectin crystals (Waigh *et al.*, 2000, Lai & Kokini, 1991). This loss of crystallinity in the starch helped to improve the processing and explains the improved tensile properties (stiffness, strength and extensibility) of the composites.

Compared with the nano-structural morphology of pea protein pasta-like sheets, a molecular distance of 85Å between the scattering objects remained the same in pea protein-fibre 90/10 and 80/20 blends, and increased with addition of $\geq$ 30 % pea fibre to the blend (Paper IV). The increase in inter-molecular distance to 90 Å suggests that protein-fibre (and component) interactions took place. Through protein-protein interactions (high amounts of proteins in the fibre fraction), pea protein and pea fibres seemed to form hydrogen and disulphide linkages and inter-molecular interactions, as shown by FT-IR and SE-HPLC.

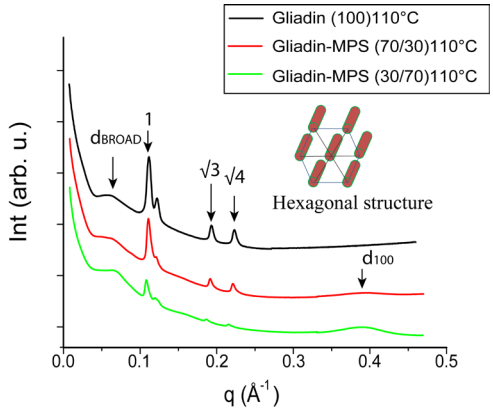

Figure 12. Nano-structural morphology of gliadin-modified potato starch (MPS) composites extruded at 110 °C and (insert) graphic showing hierarchically arranged hexagonal assemblies of gliadin. Adapted from Paper III with permission, Copyright 2016 *American Chemical Society*.

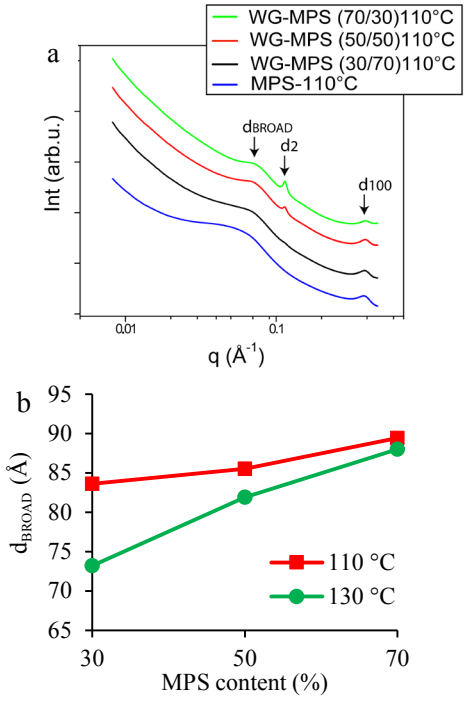

Figure 13. a) Morphology of wheat gluten (WG)-modified potato starch (MPS) blends and 100 % MPS extruded at 110 °C, b) Variation in combined scattering reflection of protein and starch (d_{BROAD}) with variation in MPS content at two processing temperatures. Adapted from Paper II with permission, Copyright 2015 *American Chemical Society*.

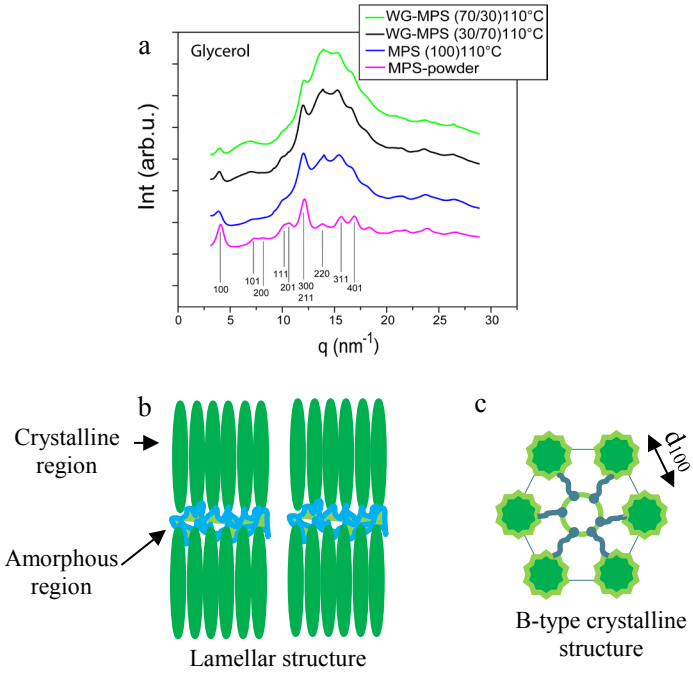

Figure 14. a) Nano-morphology of wheat gluten-modified potato starch (WG-MPS) composites extruded at 130 °C and unprocessed MPS powder (pink line). b) Characteristic lamellar structural arrangement and c) B-type crystalline structure of starch. Adapted from Paper II with permission, Copyright 2015 *American Chemical Society*.

4.4 Functional properties of bio-composites and food

4.4.1 Impact of processing temperature

The use of bioplastics and bio-composites as biodegradable alternative to petroleum plastics in food packaging, can be realistic (Siracusa *et al.*, 2008). The WG-and gliadin-hemp fibre composites showed a biodegradation rate of >30-40 % when buried in farmland soil under controlled environment, which was slightly faster compared to glutenin-hemp fibre composites after 90 days (Paper I). Moreover, protein-hemp fibre samples placed on soil surface showed the ability to fully degrade after 180 days, with no signs of physical sample components left (visually inspected) (Figure 15a, b), indicating the bio-composite potential to use in certain environment as packaging and other applications. Previously produced plasticized WG and soy films showed comparatively faster biodegradable rate (30-50 days) when subjected to farmland soil (Domenek *et al.*, 2004, Park *et al.*, 2000). A relatively slower

biodegradation rate of wheat protein-hemp fibre composites in this work could be due to the lack of plasticizer (hygroscopic in nature) which can reduce the ability of the samples to absorb moisture and increase micro-organisms' activity. Other explanation could be the high cross-linking and aggregation of proteins (in particular, for glutenin- composite's case) in the embedded hemp mat network. Wheat gluten proteins, seemed to degrade relatively quicker compared to hemp fibres, which contained structurally complex lignocellulosic compounds that were slowing down the degradation rate of wheat protein-hemp fibre blends.

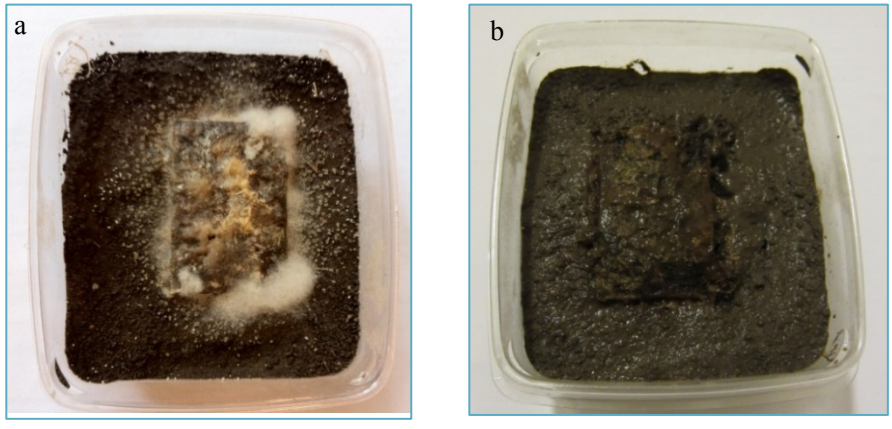

Figure 15. Wheat gluten-hemp fibres composite after a biodegradation period of (a) 45 and (b) 180 days in farmland soil. The sample in the picture was used for visual evaluation.

4.4.2 Oxygen permeability of wheat gluten-, gliadin- and glutenin-starch composites

In the wheat gluten-, gliadin- and glutenin-potato starch composites, the ratio of blend components strongly affected the oxygen permeability (OP) (Papers II and III). Low OP was observed in less protein-containing blends such as wheat gluten-starch 30/70 and glutenin-starch 30/70, but not the gliadin-starch samples, at ambient conditions (23 °C and 50 % relative humidity) (Table 1). These OP results, together with other protein cross-linking data (HPLC results, see Figure 5a), suggest that wheat gluten and glutenin proteins formed a more cross-linked network than gliadin. Glutenins form a protein matrix with inter-molecular and hydrogen bonds (β-sheets), as also observed in this study (Paper III, Figure 5), and the molecular interactions of the hydrated glutenins seem to depend on the length of protein chains (Feeney *et al.*, 2003). At higher gluten protein hydration levels, *i.e.* harsher testing conditions (38 °C, 90 % relative humidity), the OP values were very high due to an over-hydrated and weakened protein network. The modified potato starch samples showed OP values similar

to protein-starch blends with a high starch content (30/70), suggesting that the influence of starch in the protein blends was minimal. One explanation for increased OP values for the blends with higher protein content could be weak interactions of gluten protein with starch, and over-hydrated gluten proteins, leading to migration of glycerol from the protein-starch matrix and disintegration of the protein network. Overall, some of the OP values shown by protein-starch blends were better than those reported previously for wheat gluten composites (Rasel *et al.*, 2016, Kuktaite *et al*., 2014, Türe *et al.*, 2012).

Table 1. *Oxygen permeability of wheat gluten (WG)-, gliadin- and glutenin- modified potato starch (MPS) blends and of pure MPS samples, plasticised with 45 % glycerol, under different conditioning treatments. Standard deviation in brackets. Adapted from Papers II and III, with permission, Copyright 2015, 2016 American Chemical Society.*

Samples	23 °C, 50 % RH (mm mL/m^2 24 h atm)	38 °C, 90 % RH (mm mL/m^2 24 h atm)
WG-MPS 70/30	7.39 (0.98)	268.35 (18.73)
WG-MPS 30/70	2.68 (0.49)	OR>2000
Gliadin-MPS 70/30	10.62 (1.37)	226.3 (170.69)
Gliadin-MPS 30/70	12.01 (0.24)	260.4 (2.82)
Glutenin-MPS 30/70	3.02 (0.19)	OR>2000
MPS	3.29 (1.30)	OR>2000
WG-clay[a]	6 (0.4)	----

OR = over range values, RH = relative humidity, [a]Kuktaite *et al*. (2014)

4.4.3 Cooking quality of pasta-like sheets from pea protein and pea fibre

In terms of cooking behaviour, the pea protein and pea protein-fibre blends showed an increase in water uptake and decrease in cooking losses with increasing fibre fraction in the blends (Table 2), indicating ability of the pea fibre to capture and retain water. The lowest amount of water was absorbed by pea protein pasta-like sheets and the highest amount was absorbed by the pea protein-fibres 50/50 blend. This suggests that during processing, insoluble pea fibres, consisting of cellulosic and non-cellulosic polysaccharides, exhibited significant water binding and swelling ability, similar to that reported for fractionated fibres (Dalgetty & Baik, 2003). Large particle size and the less polymerised pea protein network allowed water molecules to penetrate into protein-protein and protein-fibre matrices. Cooking losses with pea protein and low fibre-containing blends were higher than for traditional pasta, which might be due to loss of water-soluble globulins (Petitot *et al*., 2010, Gueguen & Barbot,

1988) and starch (Marlies. *et al.*, 2017) during cooking. Another reason for the higher cooking losses might be loss of glycerol due to its hygroscopic nature (Müller *et al.*, 2008).

Table 2**.** *Water uptake and cooking losses of pea protein isolate-pea fibre (PPI-PF) samples after cooking. The wt. % of total wt. of sample, standard deviation in brackets. Taken from Paper IV, with kind permission of Food Research International.*

Sample	Water uptake [wt. %]	Cooking loss [wt. %]
PPI 100	22.5 (0.5)	45.8 (0.4)
90/10	35.0 (0.6)	43.6 (0.2)
80/20	38.4 (0.8)	43.9 (0.4)
70/30	45.7 (0.2)	44.9 (0.5)
50/50	67.7 (1.0)	41.2 (0.2)

4.4.4 Mechanical behaviour of bio-composites and food

4.4.4.1 Impact of processing temperature and blend ratio

Mechanical performance of wheat gluten protein-hemp fibres composites showed that the addition of hemp fibres primarily contributed to increase in stiffness and a decrease in extensibility compared to individual protein films (Paper I). In our study, the increase in stiffness in the composites was due to high stiffness of hemp fibres and due to possible interplay between the gluten protein network and hemp fibre mat, as observed in previous study (Kunanopparat *et al.*, 2008a).

Among all composites processed, the highest stiffness (~1.8 Gpa) was found for gliadin-hemp fibre composites produced at 130 °C. Part of the increase in stiffness and tensile strength in protein-hemp fibre-containing samples was due to the increase in pressing temperature (from 110 to 130 °C). Increasing tensile strength was a result of increased protein cross-linking and polymerisation, as shown in previous studies (Wretfors *et al*., 2010, Kunanopparat *et al*., 2008a). In general, poor protein to hemp fibre adhesion resulted in a hemp fibre 'pull out' effect, leaving clean holes in the protein matrix (Figure 16). The impurities (pectin and waxes) present on surface of hemp fibres (Mwaikambo & Ansell, 2002) might have hindered their bonding to the protein matrix and negatively affected protein polymerisation.

An increase in both stiffness and strength, or only strength, in wheat gluten-starch (Paper II) and glutenin-starch composites (Paper III) was observed with an increase in extrusion temperature from 110 to 130 °C. An increase in protein content in the composites resulted in an increase in extensibility in both wheat gluten-starch and glutenin-starch composites at 110 °C and in gliadin-starch

composites only at 130 °C. However, the most extensible composite studied in this thesis was wheat gluten-modified potato protein blend 70/30 processed at 130 °C (reaching an extensibility of close to 200 %) (Paper V).

The pea protein pasta-like sheets showed higher strength and extensibility than the pea protein-fibre blends (Paper IV), indicating weaker chemical interactions in pea protein-fibre blends. Among the pea protein-fibre blends studied, the highest strength and extensibility were observed for 90/10 blends. A possible explanation for the decrease in strength and elasticity with ≥20% pea fibres in the blends is the chemical composition of the fibre fraction contained residuals such as cotyledons, pea starch and large particles, impacting the protein-protein interactions, as confirmed by SE-HPLC (increased protein solubility). A large amount of non-gelatinised starch granules in the pea fibre fraction was possibly one of the reasons for the weakened interactions between the proteins and overall negative impact on the mechanical performance (Paper IV). Similarly, non-gelatinised starch was one of factors negatively impacting wheat gluten protein-potato starch composites extruded with glycerol (Papers II and III).

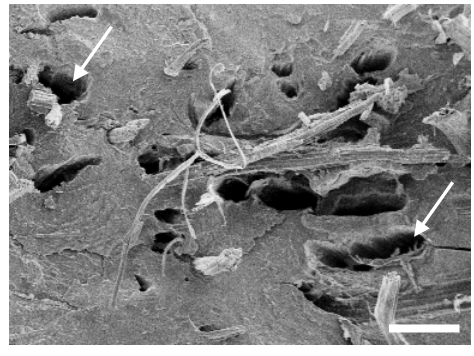

Figure 16. Representative SEM image showing fibre pull-out (white arrows) in wheat gluten protein-hemp fibres composites. Bar = 100 μm.

4.4.4.2 Impact of additives

The highest stiffness, strength and extensibility properties were obtained when a combination of glycerol+water was used for processing wheat gluten protein-starch blends rather than only glycerol (Figure 17). Among the composites, the wheat gluten-starch and glutenin-starch (glycerol+water) composites showed the highest stiffness, strength and extensibility at both extrusion temperatures (110 and 130 °C). However, slightly increased protein polymerisation, increased hydrogen bonding and greater amounts of β-sheets when processed at 110 °C were revealed by SE-HPLC (Figure 5b) and FT-IR (Papers II and III). This

suggests that lower extrusion temperature and glycerol+water blend favoured partial gelatinisation of starch and better plasticised the blend. In addition, modified potato starch with slightly higher amylose content and modified amylopectin structure had higher viscosity (1.7-fold higher) than native starch and required high hydration, which was difficult to achieve (Papers II and III).

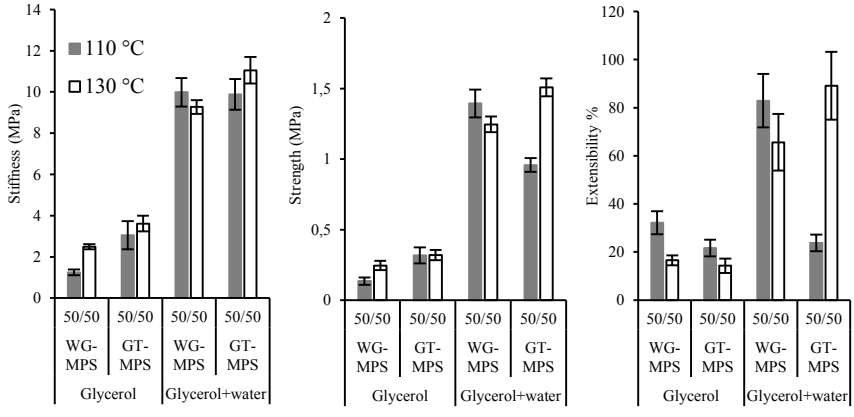

Figure 17. Mechanical performance of wheat gluten (WG)-modified potato starch (MPS) and glutenin (GT)-MPS extruded with glycerol (45%), and glycerol+water (30+20%). Adapted from Papers II and III with permission, Copyright 2015, 2016, *American Chemical Society*.

Furthermore, SEM revealed that the microstructure of wheat gluten protein-starch samples plasticised with glycerol and water showed somewhat more homogeneous morphology than samples with plasticised with glycerol alone (Figure 18), which improved the mechanical properties of the composites. The addition of water contributed to an increase in hydrogen bonding in proteins, which is known to improve the strength and elasticity of wheat gluten films (Gontard *et al*., 1993).

For glycerol-containing samples, large amount of non-gelatinised starch granules, excessive aggregation of proteins at higher temperature and high viscosity of starch were the main factors that negatively impacted the mechanical performance of the composites. This resulted in increased retention time of materials in the extruder barrel, leading to non-homogeneous microstructure (Figure 18b) and decreased mechanical performance, as shown in a previous study (Verbeek & van den Berg, 2010).

Different mechanical performance was observed for pH-modified wheat gluten and potato protein materials, indicating rather elastic behaviour for wheat protein and brittle properties for potato proteins when processed at 130 and 150 °C (Figure 19). High tensile strength shown by potato protein was correlated

with low protein extractability (as shown by SE-HPLC, see Figure 8), indicating that the high degree of protein cross-linking contributed to higher strength (Newson *et al*., 2015), whereas higher amounts of β-sheets contributed to higher stiffness and strength.

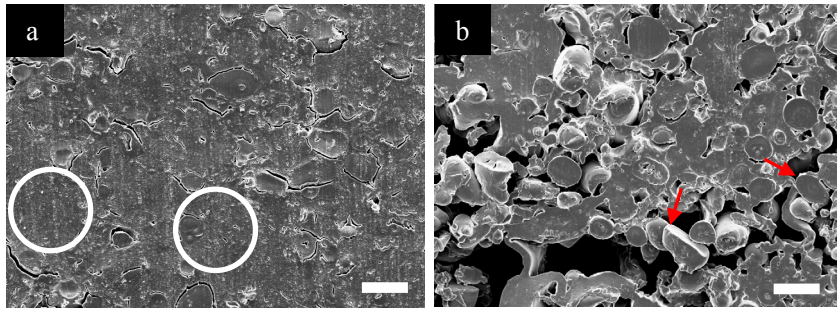

Figure 18. Microstructural morphology of wheat gluten-modified potato starch composites extruded with a) glycerol and water (encircled area showing homogeneous protein-starch matrix) and only b) glycerol (red arrows showing intact starch granules). Bars = 30μm. Taken from Paper II with permission, Copyright 2015 *American Chemical Society*.

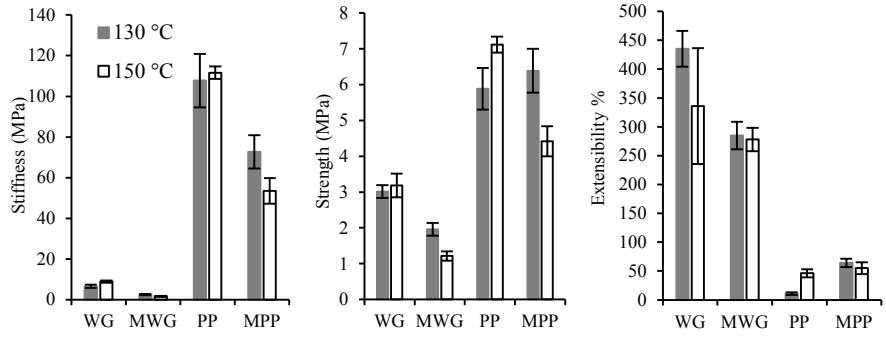

Figure 19. Impact of protein modification on mechanical properties of wheat gluten (WG) and potato protein (PP) films and their modified versions (MWG and MPP), pressed at 130 and 150 °C.

In general, chemical modification of wheat gluten did not improve the mechanical properties, and thus a decrease in stiffness, strength and elasticity was observed at both pressing temperatures, which corresponded well with the high protein extractability observed by SE-HPLC. However, the extensibility of the modified wheat gluten samples was still relatively high (around 300%). Comparing modified wheat gluten with modified potato protein materials, an increase in strength and extensibility was observed for potato protein materials

pressed at 130 °C. Thus pH modification of potato protein promoted the formation of new protein-protein cross-links during processing, which imparted flexibility to potato protein films, as observed in a previous study (Du *et al*., 2015). A noteworthy finding was that chemical modification of potato protein improved its mechanical performance in pressed films at lower pressing temperatures than found in a previous study (Newson *et al*., 2015).

Composites of wheat gluten and modified potato protein showed intermediate mechanical properties compared with individual protein films (both modified and non-modified). For example, an increase in potato protein in the composite contributed to increased stiffness (Paper V). The wheat gluten-modified potato protein (WG/MPP) composites showed similar tensile behaviour at both pressing temperatures, suggesting that blending pH-modified potato protein and non-modified wheat gluten did not induce protein-protein cross-links and did not improve mechanical properties. In the modified wheat gluten-modified potato protein (MWG/MPP) composites, their lowest stiffness, strength and extensibility occurred at 150 °C, which seemed to correlate with lower protein cross-linking shown by SE-HPLC (Figure 8) and smaller amounts of β-sheets shown by FT-IR (Paper V).

4.5 Relevance of chemistry and processing in bio-composites and foods

The results presented in this thesis clearly demonstrate that the chemistry of bio-polymers plays a key role during processing of bio-composites and foods. For example, gliadins, which were obtained as a purer fraction than glutenins, were easier to process because of their viscoelastic nature, lower molecular weight and ability to make flexible cross-links that were beneficial during processing of composite materials. During processing of gliadin-hemp fibres and gliadin-starch composites, gliadins showed excellent flow properties, which were beneficial in producing composites suitable for potential packaging applications. In contrast, glutenins with their higher molecular weight and their ability to make complex cross-links posed challenges during processing, due to excessive protein aggregation. During processing of glutenin-hemp fibres composites, a decreased flow of glutenins influenced the homogeneity of the samples. However, during processing of glutenin-starch blends with glycerol and water, protein flow was improved and the molecular weight of the aggregates did not increase to an extent that would negatively affect the material properties (Paper III). In fact, glutenin-starch processed with glycerol and water showed increased mechanical properties compared with other protein-starch blends produced in

this thesis, probably due to hydrogen bonding in the protein network and build-up of organised secondary structures. Successful gelatinisation of modified potato starch was also needed for improved processing and material properties, although due to its high viscosity this was not possible with only glycerol. Therefore, a glycerol+water blend helped to partly gelatinise starch, which was proven to be beneficial for improved processing and the functional properties of protein-starch composites.

It was also observed in this thesis that unfolded and de-polymerised proteins are desirable for improved processing and material properties. High pH treatment, especially of potato proteins, unfolded and de-polymerised the proteins, thus increasing the opportunities for formation of new protein-protein interactions (probably increased hydrogen bonding) during processing, which contributed to improved mechanical performance.

In pasta-like foods, blending pea protein and fibre in a suitable ratio and interactions between these components are important to achieve the required quality and functionality. In the pea protein-fibres pasta-like sheets produced in this thesis, the chemistry and particle size of both components were essential in determining the functional properties of the final product. For example, pea protein pasta-like sheets were stiffer, stronger and more elastic because of a stronger protein network formed with a combination of covalent and non-covalent cross-links. However, with addition of fibre this stronger protein network was weakened due to the presence of an impure fibre fraction (containing cotyledon fibres and a high amount of starch) and its larger fibre particles, factors impacting the functional performance.

Thus, to successfully produce bio-based materials or foods with required functionalities, factors such as initial bio-polymer chemistry, modification and factors affecting changes in chemistry need to be taken into account. In composite materials, interactions between different components and their response to various processing conditions also determine the functional performance of the final product.

5 Conclusions

Plant bio-polymers obtained either from industrial side-streams such as wheat gluten and potato protein or from specifically prepared pea protein, pea fibres and modified potato starch demonstrated good suitability to be processed into bio-based materials, composites and foods. Protein with either starch or fibres (from hemp or pea) were processed into composites and foods. Several factors, such as a ratio of components, protein chemistry, processing temperature and additives, determined the functional properties of the composites and foods. The processing temperature and the additive used were the main determinants of increased protein cross-linking and development of protein structural morphologies. The main conclusions and key findings are as follows:

- Hemp fibres-reinforced wheat gluten protein composites were successfully produced using a plasticiser-free processing method. The hemp fibres contributed to increased stiffness of the composites and the composites produced were fully biodegradable.
- An increase in hot pressing temperature from 110 to 130 °C caused an increase in protein cross-linking in all wheat gluten protein-hemp fibres composites, which resulted in an increase in mechanical strength.
- In wheat gluten protein-starch composites extruded with glycerol, an increase in extrusion temperature from 110 to 130 °C increased protein cross-linking/polymerisation and resulted in a higher amount of β-sheet structures, which contributed to increased stiffness and strength in wheat gluten-starch composites and increased strength in glutenin-starch composites.
- An increase in protein content decreased stiffness and strength at processing temperatures of both 110 and 130 °C, and increased extensibility at 110 °C, in wheat gluten-starch and glutenin-starch composites, respectively. For gliadin-starch composites, the highest extensibility was found for the composite with the highest amount of protein in the blend, processed at 130 °C.

- The wheat gluten protein-modified potato starch composites showed excellent oxygen barrier properties, and are thus suitable for packaging applications.
- A glycerol and water blend had a better plasticisation effect on proteins in wheat gluten-starch and glutenin-starch composites compared with only glycerol, and increased overall protein cross-linking and β-sheets in proteins.
- Use of a glycerol and water blend contributed to partial gelatinisation of starch granules, improved processing and better incorporation of protein-starch components (homogeneous microstructure), which in turn resulted in increased mechanical performance (stiffness, strength and extensibility) in wheat gluten-starch and glutenin-starch composites.
- The modified potato starch in protein-starch composites showed a characteristic lamellar structural arrangement and B-type crystalline structure. The crystallinity of starch was reduced with the use of glycerol+water blend and high processing temperature (130 °C).
- Factors such as high processing temperature, extrusion processing and the use of glycerol as a plasticiser increased protein-protein interactions, which favoured the formation of hierarchical hexagonal morphologies of gliadins in the gliadin-starch composites. This is the first observation of hexagonal morphology of gliadins in a blend with starch.
- Pasta-like sheets from pea protein and fibre showed strongly bonded proteins and organised protein secondary structure when a fibre fraction of up to 20% was used. More than 20% fibre fraction in the blend decreased the pea protein cross-linking due to the presence of non-protein plant components and their particle size, as well as high amount of non-gelatinised starch granules in the blend.
- Including more than 30% pea fibres in the pea pasta-like sheets had a positive effect on cooking quality, *e.g.* improved water uptake and reduced cooking losses.
- Basic pH treatment of industrial potato protein and wheat gluten unfolded and de-polymerised the initial proteins, favouring formation of new protein-protein interactions during processing into materials.
- Basic pH treatment of potato proteins had a major positive impact on their ability to cross-link and produce films with improved mechanical properties (up to 55-fold increased extensibility) at low processing temperature (130 °C) compared with non-modified potato proteins. No such impact was seen for wheat gluten, due to permanently pre-cross-linked proteins before processing.

6 Future prospects

- In this thesis, the processing conditions induced molecular and structural changes in proteins and these changes affected the functional properties of the materials. This structure-function relationship can further be explored by the packaging industry to produce targeted products with desired functional performance.
- The wheat gluten protein-hemp fibres reinforced composites showed good mechanical and biodegradability properties, which could be further explored for disposable packaging applications. It would also be of interest to develop and improve the wheat gluten protein-hemp fibre reinforced composites, by pre-treatment of hemp fibres with alkali to remove surface impurities, which could significantly improve protein matrix-fibre binding which will reduce fibre "pull-out" effect and improve mechanical properties.
- The excellent oxygen barrier properties shown by wheat gluten protein-starch composites were comparable to those of their synthetic counterparts. Therefore it would be of interest to further explore these materials in multilayer film packaging.
- A careful evaluation of plasticisers and traces of bio-polymers migrating to food in bio-based materials packaging is also needed.
- Pre-treatment/chemical modification of potato proteins prior to processing into materials improved their functional performance. Therefore it would be of interest to develop mild extraction methods to obtain these proteins without damaging their primary molecular structure. Such proteins would have added value for different applications such as materials and even in food applications due to their high nutritional value.
- The pea proteins acted successfully as a matrix to incorporate relatively high amount of fibre. Additional in-depth studies of protein-fibre interactions could be of interest to further improve the functionality of pasta-like foods and test their organoleptic and digestibility properties.

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