

Extruded Vegetable Proteins

An investigation of the influence of selected additives upon the texturization process of pea protein isolate during high moisture food extrusion

Master's Thesis in Materials Chemistry

JOHANNA ERIKSSON

MASTER'S THESIS 2019

Extruded Vegetable Proteins

An investigation of the influence of selected additives upon the texturization
process of pea protein isolate during high moisture food extrusion

JOHANNA ERIKSSON



Department of Chemistry and Chemical Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2019

Extruded Vegetable Proteins

An investigation of the influence of selected additives upon the texturization process of pea protein isolate during high moisture food extrusion

JOHANNA ERIKSSON

© JOHANNA ERIKSSON, 2019.

Supervisor: Sophia Wassén, RISE Agrifood and Bioscience

Examiner: Marie Alminger, Department of Biology and Biological Engineering at Chalmers University of Technology

Master's Thesis 2019

Department of Chemistry and Chemical Engineering

Chalmers University of Technology

SE-412 96 Gothenburg

Telephone +46 31 772 1000

Cover: Extrudate containing Pisane[®] C9, water and 10 % potato fibre (<1000 µm) with 100 µm scale bar. Black/purple areas are stained starch, grey to white areas are either potato fibre or voids and the continuous light brown network is pea proteins.

Extruded Vegetable Proteins

An investigation of the influence of selected additives upon the texturization process of pea protein isolate during high moisture food extrusion

JOHANNA ERIKSSON

Department of Chemistry and Chemical Engineering

Chalmers University of Technology

Abstract

The demand for vegetable protein sources as substitutes for animal protein is increasing in the world due to the significant contribution to climate change from livestock. One alternative is to produce textured vegetable protein products with extrusion, which is a high-productivity process used in food industry to manufacture products with specific texture and structure. Pea protein has shown possibility to form textured protein structures like those in meat-substitutes when processed using high moisture food extrusion.

In this project, formulations containing pea protein isolate together with four selected additives were processed with high moisture extrusion cooking. Additives investigated were potato fibre, wheat bran fibre, β -glucan fibre and rapeseed oil, in order to evaluate the influence of the additives upon the texturization process of pea protein during extrusion. The samples structure and texture was analyzed with light microscopy and texture analyzing tests together with information about the oil- and water holding capacities for the fibres and the pea protein isolate.

The analysis of all extruded formulations indicated on a reduced ability for the protein phase to form a textured structure upon addition of the investigated additives. Fibres functioned as a filler phase that interrupted the protein network, where smaller fibres interrupted the protein network to a larger extent. Potato fibre and β -glucan fibre tended to add soluble starch and β -glucan to the protein network, leading to further decreased texturization and decreased layering, while wheat bran fibre was more rigid and increased the layered structure. Rapeseed oil functioned as a lubricant that reduced the directionality of the protein network and addition of rapeseed oil together with small potato fibre or β -glucan fibre resulted in surface changes.

Keywords: Pea protein isolate, High moisture food extrusion, Textured vegetable proteins, Dietary fibres

Acknowledgements

I would first of all like to thank my supervisor Dr. Sophia Wassén from RISE Agrifood and Bioscience for the great support and guidance throughout the project. Secondly, I would like to thank Lovisa Eliasson, Lillie Cavonius, Patricia Lopez-Sanchez and Annika Altskär for all support and help with instruments and laboratory work, and the entire group at Product Design and Perception at RISE Agrifood and Bioscience for the warm welcome. I also want to thank my examiner Marie Alminger from Chalmers University of Technology for the support and interest in this project.

Additionally, I would like to thank my family and friends for all the love and support during this project and my years at Chalmers University of Technology.

Johanna Eriksson, Gothenburg January 30, 2019

Contents

| | | |
|----------|--|-----------|
| 1 | Introduction | 1 |
| 1.1 | Aim | 2 |
| 1.2 | Limitations | 2 |
| 2 | Theory | 3 |
| 2.1 | Peas | 3 |
| 2.1.1 | Protein Fractioning | 4 |
| 2.1.2 | Protein | 4 |
| 2.2 | Dietary Fibres | 5 |
| 2.2.1 | Dietary Fibre Components | 5 |
| 2.2.2 | Potato Fibre | 6 |
| 2.2.3 | Wheat Bran Fibre | 6 |
| 2.2.4 | β -glucan Fibre | 7 |
| 2.3 | Extrusion | 7 |
| 2.3.1 | The Extruder Elements | 8 |
| 2.3.2 | The Extruder Sections | 9 |
| 2.3.3 | Extrusion Cooking | 9 |
| 2.3.4 | Food Components in Extrusion Formulation | 10 |
| 2.4 | Analyzing Techniques | 12 |
| 2.4.1 | Light Microscopy | 13 |
| 2.4.2 | Instron Texture Analyzer | 14 |
| 3 | Method | 16 |
| 3.1 | Material | 16 |
| 3.2 | Extruded Samples | 17 |
| 3.2.1 | Preparing Formulations | 17 |
| 3.2.2 | Extrusion | 19 |
| 3.3 | Analysis | 20 |
| 3.3.1 | Light Microscope | 20 |

| | | |
|----------|---|------------|
| 3.3.2 | Instron Texture Analyzer | 21 |
| 3.3.3 | Water Holding Capacity (WHC) and Water Solubility Index (WSI) | 22 |
| 3.3.4 | Oil Holding Capacity (OHC) | 22 |
| 4 | Results and Discussion | 24 |
| 4.1 | Water- and Oil Holding Capacity and Water Solubility Index | 25 |
| 4.2 | Extruded Formulations | 26 |
| 4.2.1 | Pea Protein Isolate (PPI) | 26 |
| 4.2.2 | Potato Fibre | 27 |
| 4.2.3 | Wheat Bran Fibre | 32 |
| 4.2.4 | β -glucan Fibre | 36 |
| 4.2.5 | Rapeseed Oil | 38 |
| 4.2.6 | Fibre and Rapeseed Oil | 40 |
| 4.2.7 | Comparison Between Additives | 44 |
| 4.3 | Evaluation of Analysis | 45 |
| 5 | Conclusions | 46 |
| 6 | Future Work | 48 |
| | Bibliography | 56 |
| | Appendices | 57 |
| A | Sample Composition | i |
| B | Extrusion Data | iii |

1

Introduction

THE WORLD'S DEMAND FOR MEAT is increasing as an effect of the growing population on earth as well as increased income and urbanization. Today, land use for livestock stands for 30 % of the worlds ice-free terrestrial surface, including feed-crop production and grazing [1]. Simultaneously, the world faces a great threat from climate changes, causing global warming and rising sea levels [2]. Livestock is known to contribute significantly to climate change, along with for example land-based pollution and water use, due to the inefficient conversion from plant protein to animal protein [1]. Thereof, the demand for more sustainable protein sources is increasing. From life cycle assessment studies made by D. Nijdam *et al.* [3] it is concluded that vegetable products have lower impact on the climate and lower land use than food products of animal origin.

In order for vegetable protein sources to compete with meat alternatives, the vegetable products need to have both attractive texture and structure as well as sensory properties. Food extrusion, a continuous process that utilizes high temperatures, pressure and shear to process components, have the ability to restructure vegetable proteins into textured meat-like structures [4], known as *textured vegetable proteins* (TVP) [5].

Currently, soybeans are used in many studies of vegetable protein sources for extrusion, and in products on the market, due to their high protein content, suitable amino-acid composition and great ability to form textured structures [6],[7]. Though, a number of drawbacks, such as its allergenic potential and anti-nutritional factors, make other alternatives relevant [8]. Additionally, in Sweden most of the soybeans are imported [9]. As an alternative, pea proteins have shown ability to form textured protein structures

like those in meat products when processed using high moisture extrusion cooking [6]. Peas can be cultivated in Sweden [10] and the crop exhibit positive effects on human health [11], giving perquisites to create a vegetable protein product with good nutritional factors consisting of more locally grown raw products.

In order to produce an attractive texturized product containing pea protein isolate, it is interesting to investigate the influence of various additives, contributing to a lowered price as well as enhanced nutritious and sensory properties. With respect to above mentioned aspects, this project will focus on the ability of pea protein isolate to form textured structures with high moisture extrusion cooking in combination with added additives.

1.1 Aim

The overall aim of the project was to investigate how four, carefully chosen, additives influence the texturization process of pea protein isolate during high moisture extrusion cooking. The additives investigated were; potato fibre, wheat bran fibre, β -glucan fibre and rapeseed oil, both separately and in combination. The structural and textural properties of the extruded formulations was evaluated.

1.2 Limitations

This work only included the pea protein isolate Pisane[®] C9 (Cosucra Groupe Warcoing S.A., Belgium). In previous studies, a mixture of pea protein isolate and different concentrations of starch, fibres and water at certain temperatures have been extruded. These results was used to set the water content constant at 60 % and the temperature profile of the extruder to a constant of 30/80/120/135°C. No additional sensory analysis of the extruded products were evaluated.

2

Theory

THE AIM OF THE PROJECT was to understand how additives influence the texturization process of pea protein isolate during high moisture extrusion cooking. Therefore, in upcoming chapter, an introduction to peas and pea fractioning is presented along with information about the chosen additives. The extrusion process is presented, with process equipment and operation conditions, and the effect upon extruded components is introduced. The theory behind the analyzing techniques used in the project is also presented.

2.1 Peas

Peas, also known as the genus *Pisum*, belong to the plant family *Leguminosae*. *Pisum* includes three different species of peas, where *Pisum sativum* is widely known in the food industry. Already for early civilizations in Middle East and Mediterranean, peas were an important dietary product, and the locations are known to be the first place for cultivation and thus the origin of peas. Further on, evidence reveal that pea cultivation can be dated back to 10 000 BC in Central Asia and Near East and around Stone and Bronze Ages in Europe [12]. Today, peas are the second most cultivated legume in the world [13], cultivated widely in the temperate zones, where Europe, North America and Asia are big producers [12].

Peas can be divided into field pea and vegetable pea, including several subsets. Field pea, or dry pea, is round seeds with yellow, red or green varieties. The pea is mainly used

as protein or starch fractions in extruded snacks and baked products. Vegetable pea, or garden pea, include snow pea and snap pea [12]. Pea seeds are a valuable vegetable protein source, with a content of approximately 25 % protein, as well as a source for starch, fibre and soluble sugars [13].

2.1.1 Protein Fractioning

Compared to soy, peas have a low fat content that makes the protein fractioning from peas less extensive. The protein fraction occur either in the form of pea flour or pea protein concentrate, both prepared by dry separation, or pea isolate, prepared from wet separation. The methods differ in terms of yield, cost and purity of the end product [11].

In dry separation, the peas are dry milled into a fine flour containing fine protein powder and relatively intact starch granules forming a more crude fraction. By repeated milling and air classification, the two components can be separated. Dry separation involving air classification produces a product with lower purity, compared to wet separation. On the other hand, the process meets lower costs and no effluents [11].

The process of wet separation includes several steps using acidic and alkali conditions. The process starts with milling the peas, after which the pea flour is solubilized in water into a slurry. In order to separate solubilized and non-solubilized fractions, the slurry is centrifuged. After centrifugation, the proteins are extracted, either by pH shift or ultra filtration with membranes [11].

2.1.2 Protein

Proteins are big macromolecules consisting of amino acids linked together with peptide bonds into polypeptide chains [14]. Peptide bonds, or amino bonds, are formed between the amino acids carboxyl group ($C(=O)OH$) and amino group (NH_2), where the carboxyl group is activated [15]. The complex structure of proteins can be divided into four orders of structure, where the primary and secondary structure refers to the specific amino acid sequence in every polypeptide chain and the formation of α -helix and β -strands of the polypeptide stretches respectively. The polypeptide chain is further on arranged in three-dimensional arrangements, known as the tertiary structure. If the protein consist of more than one polypeptide chain, the three-dimensional arrangement of the whole protein is know as the quaternary structure [14].

Additionally, proteins can be divided into four different categories according to solubility; albumins, globulins, prolamines and glutelins. These proteins are soluble in water, saline solution, alcohol solution and acid or alkaline solution respectively [16]. Globulins is

the proteins with the best ability to texturize and form continuous structures during extrusion [16], [17] .

2.2 Dietary Fibres

According to regulation 1169/2011 (EU 2011), dietary fibre is defined as "carbohydrate polymers with three or more monomeric units, which are neither digested nor adsorbed in the human small intestine..." [18]. Dietary fibres can either be naturally occurring in food, extracted from food raw material or synthesized. There is a difference of opinion whether for example lignin should be counted as a dietary fibre due to the difference from polysaccharides. Previously mentioned regulation include lignin in the definition of dietary fibres. Though, due to the different opinions, labeling of dietary fibres in food differs around the world [18].

2.2.1 Dietary Fibre Components

Dietary fibres consist of components being both insoluble and soluble in water, where insoluble components include e.g. cellulose, hemicelluloses and lignin and soluble components include e.g. β -glucan and pectin [19]. Many fibre products, containing high amounts of dietary fibres, also contain some starch remains from the extraction processes [20], [21].

Cellulose exists in many fruits, vegetables, cereals and legumes as the main constituent of the plants cell walls [22]. Cellulose consist of β -1,4-glycosidic linked D-glucopyranose rings, with the general formula $[C_6H_{10}O_5]_n$ [23]. Cellulose can cross-link via hydrogen-bonding between hydroxyl groups and oxygen atoms to form a crystalline structure. This ability to form solid multi-chain complexes give cellulose the ability to ensure rigid construction in the plant [20]. Average degree of polymerization of cellulose vary between 1000 and 14000 monomeric units [22], and depending on the specific length in each plant cellulose can exhibit different properties [23].

In contrast to cellulose, **hemicellulose** is the collective name of a group of polysaccharides composed of combination of different sugar units. Hemicellulose can be highly branched with small side chains, leading to a more amorphous structure, and it has a degree of polymerization of about 50 to 300 [23]. Hemicellulose differ in composition depending on the plant of origin [20]. In general, a higher content of hemicellulose in the fibre give rise to a higher moisture adsorption [23].

Unlike cellulose and hemicellulose, **lignin** is not a carbohydrate, but it still counts as an

insoluble dietary fibre in most cases, due to its linkage with for example hemicellulose with covalent bonds. Lignin is a hydrocarbon polymer, based on phenylpropane, with a complex, highly cross-linked, three dimensional structure [22]. Due to the many different functional groups and bonds in lignin, the molecule is difficult to extract and is often seen linked to other organic molecules [24].

One of the soluble dietary fibres is **β -glucan**. β -glucan is a polysaccharide that is found in specific cell walls of the cereal seed, consisting of β -1,3 and β -1,4-linked D-glucopyranose. The portion of β -1,3-linkage is introducing bends in the chain, making β -glucan more soluble and flexible compared to cellulose [25], [26]. Due to the molecules solubility in water, it possesses a gelation capacity [22].

The second soluble dietary fibre is **pectin**, which exist in the cell walls of plants. Pectin can function as a gelling agent, used in e.g. yogurt and ketchup [27]. It is a very complex molecule, composed of up to 17 monosaccharides [28], varying in composition depending on source and treatments. Though, one common constituent is α -1,4-linked D-galacturonic acid [29].

During the extraction of products rich in dietary fibre, **starch** is often left as a residue [20], [21], and is therefore of importance when discussing fibre-rich products. Starch granules consist of predominant amounts of branched amylopectin and a smaller amount of linear amylose, which both are polyglucans. The amylopectin contributes to the semi-crystalline structure of the granule [30], and it consists of short chains of α -1,4-linked D-glucose residues. Some plants possess starch with higher amounts of amylopectin, named waxy starch, or higher amounts of amylose [31].

2.2.2 Potato Fibre

Potato fibre, varying in composition depending on producer, mainly consists of potato cell wall and some residue of e.g. starch. The potato cell wall is made up of parenchyma cells [32] and consists mainly of the polysaccharides pectin, hemicellulose and cellulose [20]. Parenchyma cells consist of large amounts of starch compared to cell walls [32], and the separation between the two are difficult to conduct, mainly due to the water-insolubility of both raw potato starch and cell walls. Hence, there is often some amount of starch contaminants in potato fibre [20].

2.2.3 Wheat Bran Fibre

The wheat seed can be divided into three parts, where the biggest part of the seed is the endosperm, containing big amounts of starch granules [33] surrounded by cell walls rich

in arabinoxylan and β -glucan [34]. Also included in the seed is the embryo, containing big parts of lipids, enzymes and proteins. The outer-most layer, surrounding the endosperm and the embryo, is called the bran. The bran can further be divided into several layers, including the pericarp, the testa, the nucellar layer and the aleurone layer [33]. The three outer-most layers, mentioned first, consist mostly of empty cells with cell walls made of xylan, cellulose and lignin. The inner most layer, the aleurone layer, contains cells filled with proteins [34]. During milling, the different parts of the seed are separated, producing different fragmented products including wheat bran [35].

2.2.4 β -glucan Fibre

β -glucan fibre, or β -glucan flour, is the name of a flour, originating from e.g. barley or oat, that is rich in β -glucans. In barley and oats, β -glucans is found in the cell walls surrounding the starch granules in the endosperm of the seed. The β -glucan rich cell walls can be extracted with several different methods, including dry milling or solvent extraction, where the main goal is to separate starch and protein from the cell walls. The product is seldom pure, certain amounts of starch and protein remains in the β -glucan fibre [21]. For example, the β -glucan fibre *DF25* (Lyckeby Culinar AB, Sweden) from barley contains approximately 30-35 % starch and 10-12 % protein [36].

2.3 Extrusion

Extrusion is a process used in the food industry to manufacture products with specific shape and form as well as texture and structure. The process have been utilized for over a hundred years in the plastic industry to produce thermoplastics and pipes, and in the 1930s, the extruders entered the food industry [37]. Extrusion is an efficient continuous process with the ability to simultaneously perform operations like homogenization, mixing, shearing and grinding among others [5].

There are many advantages of extrusion compared to other cooking and shaping processes. In general, it is an effective process with a lower processing cost compared to similar processes. It operates continuously and heat sensitive components can be retained due to short heating periods [17]. In general, the residence time in the extruder is 20-40 seconds, and the temperature and pressure can rise to approximately 180-190°C and 15-30 bars [38].

Extruders can be divided into different types depending on method of operation and barrel configuration. The method of operation varies between cold extrusion and extrusion cooking, where thermal energy is added in the second method, see chapter 2.3.3. The

extruder can also vary in barrel configuration, with single-screw and twin-screw [37]. Single-screw extruders operate with a single screw inside the barrel, and twin-screw extruders operate with two screws, which can be co-rotating or counter-rotating. Twin-screw extruders have the advantage of being more flexible in terms of extrudates and the system is exposed to less wear [37]. Henceforth, only single screw extruders are discussed.

2.3.1 The Extruder Elements

The extruder consists of different elements, see figure 2.1. The **barrel** is the cylinder for which the screw operates in and it can vary between smooth and grooved [5]. The length to diameter ratio is a crucial factor for extruders, where greater length gives longer residence time and greater heat transport surface. The ratio can vary greatly depending on type of extruder [39].

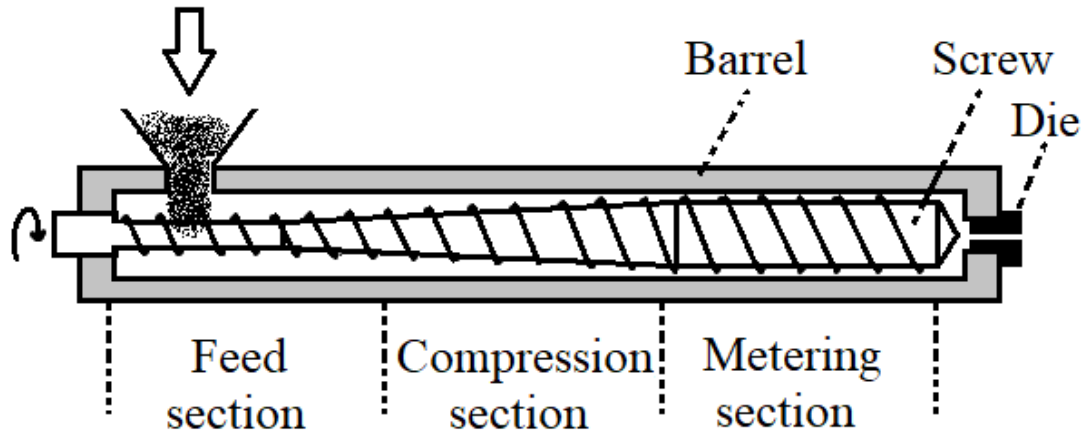


Figure 2.1: Basic model of a single screw extruder.

The **screw** is the element turning inside the barrel with flights pushing the material towards the exit [5]. Depending on application, the screw can have multiple designs including conventional screws and different sections. A conventional screw consists only of flights, as seen in figure 2.1. Other types of screws can include barrier melting sections and various mixing elements [40].

Between the barrel and the screw root the flow channel is formed. The area of the flow channel is reduced as the material is progressing in the barrel, leading to an increase in pressure [5]. In order for the material to move forward, the friction between the material and the barrel surface should be greater than the friction between the material and the screw [5]. Between the end of the flight and the barrel surface there is a clearance, that is usually sought to be small, that can create a back-flow in opposite direction of the drag flow due to the pressure gradient in the barrel. To compensate for the back-flow,

the barrel can be grooved, which although initiating increased leakage is compensated by reduced slip and better pump capacity [39].

The height of the screw flights vary depending on type of product produced and conditions requested. A screw operating at high speeds generally have shallow flights and therefore a smaller channel giving greater shear, pressure and mixing. When operating with heat-sensitive materials, extruders with shallow flights can damage the material [39].

The exit from the barrel is called a **die** and it can vary in design depending on the wanted structure [5]. The cross-section of the die is in general much smaller compared to that of the barrel. When the product leaves the barrel, the pressure drops rapidly, leading to quick evaporation of some of the water in the product [37]. This gives a puffing effect, which can be averted by cooling the mixture before the pressure release. The structure of the end product can therefore be controlled by altering e.g. temperature and form of the die [38].

2.3.2 The Extruder Sections

The extruder consists of three sections, see figure 2.1, which for a relatively long extruder represents approximately one third of the total length each [39]. In the **feed section**, the material enter the extruder and transport occur without considerable compression [5], due to that the screw usually inhibits deeper flights or flights with greater pitch in this section, giving a larger flow channel [39]. The material is starting to compress in section two, the transition or **compression section** [5]. Pressure and temperature increase as a consequence of a decreased flow channel and the characteristics of the ingredients change. The mixtures is converted from a flowing mixture to a plasticized dough. The third and last part is named **metering section** and here the flow channel decrease further leading to additional temperature and pressure increase and a thorough mixing. An extruder with longer metering section generally produce more homogeneous final extruded product with less variation [39]. This section is also refereed to as the cooking section [38].

2.3.3 Extrusion Cooking

As mentioned in the introduction of chapter 2.3, there are different methods of operation for extruders, including cold extrusion and extrusion cooking. Cold extrusion is a process where no additional thermal energy is added. This type of extrusion is frequently utilized to e.g. mix, knead and disperse products. Extrusion cooking on the other hand is a process using thermal energy to process the material. The thermal energy is added via

both conversion energy and conductive energy [37],[41]. Internally, a major part of the energy added to the shaft is dissipated as heat via friction between the barrel surface and the extruded ingredients, called conversion energy, contributing to thermal heating [5]. Externally, the heat is applied to the barrel surface and conducted through the barrel wall to the ingredients inside the extruder [37]. Due to generally short residence times during extrusion, the conductive energy is usually contributing to a smaller part of the added energy compared to conversion energy [41]. The extrusion process can further on be divided into low moisture food extrusion (<40 % moisture) and high moisture food extrusion (>40 % moisture). Low moisture food extrusion form other types of structures compared to high moisture food extrusion. The structures formed with high moisture food extrusion have demonstrated promising results in texturing vegetable protein into meat-like structures [6],[42].

2.3.4 Food Components in Extrusion Formulation

During extrusion, all ingredients in the extruded formulation are affected differently by the mechanical and thermal shear stress, giving rise to various kinds of structures [17]. In this chapter, the functional role of proteins, dietary fibres, starch and lipids and water during extrusion is presented.

Proteins

Proteins can function as a structure-forming material during extrusion, with the ability to form continuous textured structures upon extrusion at concentrations of >40 % protein [17]. There are several differences of opinion regarding the mechanisms behind protein texturing during high moisture food extrusion and the research field is highly topical. It is suggested that at high temperatures in the extruder during extrusion cooking, proteins denature and unfold due to high temperatures and shear forces [5],[17]. Denaturation occurs when the proteins are exposed to extreme environmental conditions, such as altered temperature or pH, leading to affected electrostatic and hydrophobic interactions within the the secondary, tertiary and quaternary structure of the protein [43]. When the denatured proteins are exiting through a die, a laminar flow and higher viscosity is obtained by lowering the temperature, and the proteins is believed to orient due to shear forces and cross-bond to create a new textured structure [5],[17]. The type of cross-bonding in the textured structure is debated, mainly including the effect from either disulphide linkages [16] or peptide bonds [44], where recent studies on pea protein isolate suggest that disulphide linkages outweigh the effect from peptide bonds [4]. Additionally, the importance of covalent bonds (e.g. peptide- or disulphide bonds) and non-covalent bonds (e.g. hydrogen or ionic linkages) are debated [4]. In contrast, another theory suggest that the texturization during extrusion is not due to mechanical and temperature shear stresses,

but mainly due to phase separation between protein-rich and water-rich phases due to cooling in the die. This would, according to the mentioned theory, explain the specific layered structure seen during extrusion of vegetable proteins [45]. So, there are several differences of opinion regarding the mechanism behind texturing of vegetable proteins.

Starch

Similar to proteins, starch can function as a structure-forming material during extrusion [17]. The behavior of starch during extrusion is determined mostly by the water content in the mixture [46]. When starch granules are exposed to heat and shear in addition to high moisture levels in the extruder, the granules start to swell and splinter [5] due to that thermal energy breaks the hydrogen bonds in the starch granule. In the initial step, the crystalline parts keep the granule stable while the amorphous parts collapse, causing a swelling effect that thickens the melt. During the swelling, gelation may be initiated by amylose leached from the granules, increasing the viscosity of the melt. After continued heating and shearing, also the crystalline parts melt, causing the granule to fragment, unfold and hydrate and thus the previously increased viscosity is somewhat lowered [30]. In this state, a starch paste is formed with a soluble continuous phase containing dispersed remains of starch granules [47]. This process is called gelatinization, where amount of heat energy applied varies depending on the water concentration in the slurry [17]. The temperature for which gelatinization occurs varies depending on heterogeneity in the starch granules and granular type among other factors, but for wheat and potato starch, the gelatinization temperature is approximately 58-85°C [47]. Generally, lower concentrations of water are required during extrusion compared to other processes for gelatinization to occur [5]. During extrusion at even lower moisture levels, the starch granules are instead disrupted and melted [46].

The structure obtained for extruded formulations containing starch is also determined by the type of die used. If the extrudate is cooled down before exiting the die, a rigid, firm and viscoelastic gel is formed [47]. In contrast, if the extrudate instead is not cooled down in the extruder die, a phenomenon called puffing can be observed, where starch retains the evaporated gases in the extrudate, creating a stabilized foam [46]. Lower levels of starch lead to a less extensive expansion and a smaller ability for the expanded state to stay stable [42].

Dietary Fibres

According to Guy [17], components rich in cellulose, hemicellulose and lignin, which are insoluble and generally remain firm during extrusion, can be counted as dispersed-phase filling materials when comprised in certain amounts in extruded formulations. It is stated

by Guy [17], that when extruding snack foods from starch-rich formulations, a dispersion is formed with starch as a continuous phase and both protein and fibrous materials as a dispersed phase. In an article by Áreas [48] it is also suggested, in accordance with Guy [17], that e.g. insoluble cell-wall material can be regarded as a filler material during extrusion, with either protein or starch as a continuous phase [48].

The ability for a formulation consisting of carbohydrates to extrude depend on the fraction of insoluble components, where insoluble components decrease the ability for the continuous phase to cross-bond. It is stated that a formulation containing insoluble material, making up the dispersed phase, give a melt with higher viscosity where the water phase, functioning as a plasticizer, is unevenly distributed. It is also proposed that there is a maximum level of possible filler material in the continuous phase, which for spherical particles would be a volume fraction of 0,64. With addition of filler material beyond this level, the molecules making up the continuous phase would have no ability to form a coherent structure [48].

Branched dietary fibres are though affected by shear during extrusion to some extent, creating various amounts of smaller fragments which may be soluble. Thus, the amount of insoluble dietary fibre generally decrease upon extrusion [49]. Soluble dietary fibre components behave differently upon extrusion. Both pectin and β -glucan is soluble components that possess a certain gelation capacity, leading to the components not forming a part of a filler phase [27],[22].

Water and Lipids

In the extrusion process, the shear exerted on both adjacent particles and between extrudates and the barrel surface can be decreased both by lubricants and plasticizers [17],[46]. Added lipids is dispersed in the continuous phase as fine droplets where they act as lubricants that reduce shear [17]. Both lipids and water function as plasticizers during extrusion, giving decreased viscosity of the melt as well as decreased shear [46]. The reduced shear reduce the mechanical input in the extruder and thus the forces exerted on for example starch and protein, decreasing the melting effect upon these. The loss of mechanical input in the extruder is generally attributed in decreased melt temperature and increased barrel pressure [46].

2.4 Analyzing Techniques

In the project, different analyzing methods were used in order to investigate the formed textured structure. In this section, the theory behind utilizing both a light microscope

with different effects and staining methods, as well as a texture analyzer, utilized for both compression test and cutting test, is presented.

2.4.1 Light Microscopy

Conventional light microscopy utilizes a light beam to illuminate a thin sample, letting an amount of light pass through the sample and get collected by a lens. The image is magnified by numerous lenses [50]. In order to enhance topographic details in the sample, differential interference contrast (DIC) microscopy can be used. A double quartz prism produces two light beams from the crossed polarized light that, on the image plane, creates two images with phase difference due to coherent interference. This gives rise to an image containing topographic information. On the other hand, in order to see anisotropic areas in the sample, polarized-light microscopy can be used. The light is polarized by a polarizer placed in front of the condenser, creating bright anisotropic areas and dark isotropic areas [51].

Cryostat Sectioning

When analyzing samples in a light microscope, the samples need to be thin. This in order for the light to pass through the sample and to improve the focus depth of the image. Thin pieces of samples can be achieved by cryostat sectioning. A cryostat consists of a chamber, a microtome and a sharp blade that section frozen samples. The chamber and the microtome can be cooled to approximately -35°C and the instrument can be set to section in thickness between 1-100 μm . Prior to sectioning, the samples can be frozen onto a circular specimen holder, using cryo-embedding compound, by immersing the sample into liquid nitrogen and then placing the sample in the cryostat [52].

Sample Staining

In order to increase contrast and distinguish different components in samples, staining agents can be utilized. In light microscope, staining agents such as iodine solution and osmium tetroxide can be utilized. By adding iodine (I_2) to the sample, a reaction occurs between iodine and glucans in the starch, giving the sample a black/purple colour [53]. In figure 2.2, stained starch is visible in a dark purple colour, indicated by an arrow in the image, and the light brown areas are proteins and the white voids are either fibre or voids. The metallic ion osmium tetroxide (OsO_4) selectively stains unsaturated lipids in a dark colour, turning from golden red to close to black [54]. In figure 2.3, the protein network is coloured light brown and the added fat is seen as darker brown droplets,

indicated by the arrow, dispersed in the continuous protein network.

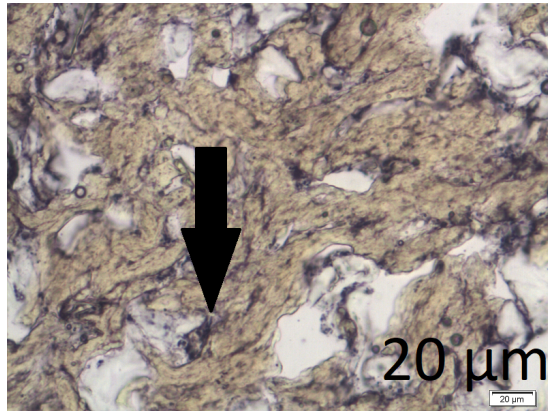


Figure 2.2: Iodine staining of 5 μm sections of sample containing Pisane C9, water and 20 % *Potex Crown* (potato fibre with a size of $<200\mu\text{m}$) presented with 20 μm scale bar. The dark purple areas, indicated by an arrow, is colored starch, the lighter brown network is protein and the white voids is both potato fibre and voids.

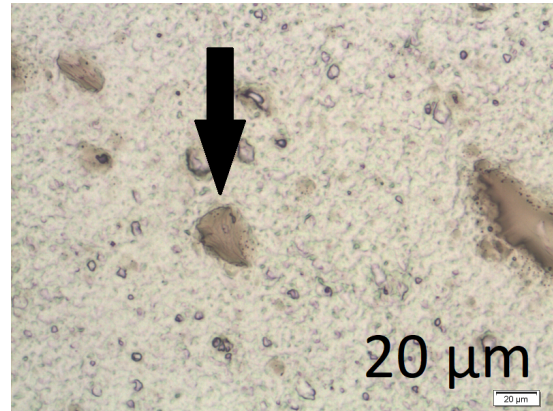


Figure 2.3: Osmium vapor staining of 5 μm sections of sample containing Pisane C9, water and 10 % rapeseed oil presented with 20 μm scale bar. The light brown continuous phase is stained protein network and the dark brown to almost black droplets, indicated by an arrow, in the continuous network is dispersed rapeseed oil.

2.4.2 Instron Texture Analyzer

Texture analyzers are instruments used for analyzing and measuring product texture and rheological properties in many industries where the texture is an important factor, for example cosmetics, pharmaceuticals and food products. In the food industry, the texture analyzer can be used to analyze factors affecting quality, shelf-life and handling [55]. The instrument can be adapted to a vast range of food products due to that fixtures, grips and other settings can be changed [56].

The texture is measured by applying forces using different fixtures, such as probes or knives, to deform a sample and measure the responses. There are several test methods specially designed to mimic a human interaction with the product while eating or pressing it. This in order to simulate how the product would interact with a consumer [55].

Rheological Properties

A texture analyzer can perform many different analyzes, commonly involving exerting a force on the sample analyzed. One common measurement is to conduct an uniaxial

compression, meaning that the probe is compressing the sample in a direction perpendicular to the surface. Though, during compression, the amount of deformation is not only affected by materials rheological properties but also by the size and shape of the sample analyzed. In order to conduct measurements that is not affected by the geometry of the sample, the applied force (F) can instead be converted to stress (σ) and the corresponding deformation (Δl) can be converted to strain (ϵ). Stress is defined as the force applied on the sample divided by the specific area (A) of the surface, see equation 2.1. In turn, strain is defined as the length of deformation divided by the initial length (l) of the sample, see equation 2.2 [57].

$$\sigma = \frac{F}{A} \quad (2.1)$$

$$\epsilon = \frac{\Delta l}{l} \quad (2.2)$$

Stress is expressed in the unit of force per square meter [N/m^2] which equals to the pressure unit of pascal [Pa]. Strain is, on the other hand, unitless [57].

3

Method

TO FULFILL THE AIM of investigating how additives influence the texturing process of pea protein isolate during extrusion, formulations containing the selected additives was prepared, extruded and analyzed. First, the materials used is presented with respect to composition, together with the composition of the extruded formulations. Secondly, the methods for extrusion is presented, including sample preparation and extruder operation. Finally, method for the analyzing techniques is presented.

3.1 Material

In the project, formulations containing four carefully selected additives were investigated. In all samples, the pea protein isolate *Pisane*[®] C9 (Cosucra Groupe Warcoing S.A., Belgium) was used as the protein source. It is a pea protein isolate isolated from yellow peas [58]. Additionally, four different additives were chosen, three fibres and one oil;

- Potato fibre *Potex* (Lyckeby Culinar AB, Sweden).
- Wheat bran fibre *Kruskakli* (Kungsörnen Lantmännen, Sweden).
- β -glucan fibre *DF25* (Lyckeby Culinar AB, Sweden).
- Rapeseed oil (Zeta, Sweden)

Two types of potato fibres were part of the project; *Potex* ($<1000\text{ }\mu\text{m}$) and *Potex Crown* ($<200\text{ }\mu\text{m}$). In order to compare the fibres well, the wheat bran was milled using a knife mill *Grindomix GM 200* (Retsch, Germany) and sieved with *Vibratory Sieve Shaker Analysette 3* (Fritsch, Germany), to provide fibres of two different sizes similar to those of potato fibre; larger wheat bran fibre ($710\text{-}1250\text{ }\mu\text{m}$) and small wheat bran fibre ($125\text{-}500\text{ }\mu\text{m}$). β -glucan fibre, with a non-specified size, originates from barley [59]. The compositions of all components are presented in table 3.1.

Table 3.1: Material compositions for the different components.

| Potato fibre [60] | Wheat bran fibre [61] | β -glucan fibre [36] | Rapeseed oil [62] | Pisane [®] C9 [58] |
|-----------------------|-----------------------|----------------------------|-------------------|-----------------------------|
| 25 % cellulose | 50 % fibre | 30-35 % starch | 91,6 % fat | 88 % protein |
| 25 % hemicellulose | 15 % carbohydrates | 25 % β -glucan | | 6 % ash |
| 20 % pectin | 14 % protein | 25 % arabinoxylan | | 3 % carbohydrates |
| 15 % starch | 6 % fat | 10-12 % protein | | 1,5 % fat |
| $<10\text{ }\%$ water | | 3 % fat | | |
| 5 % protein | | | | |
| 2 % lignin | | | | |

With the components mentioned in table 3.1, 26 sets of formulations were prepared and extruded. They can be divided into three categories; pea protein and fibre, pea protein and oil and finally pea protein, oil and fibre, see table 3.2. All formulations contained 60 % (weight/weight) water of total mass of sample. From tables 3.2, the weight of each component in the mixture was calculated, see table A.1 in appendix A. All samples had a total weight of 400 g.

3.2 Extruded Samples

With the components presented in section 3.1, formulations were prepared and extruded according to procedures presented in upcoming chapter.

3.2.1 Preparing Formulations

The formulations were prepared according to table A.1 in appendix A. For formulations containing fibre or fat, *Pisane*[®] C9 was weighted and added to a food processor *MultiTalent3* (Bosch Home Appliance AB, Germany). The additives were weighted and added and the materials were mixed for approximately one minute. Water was weighted

Table 3.2: Table of all formulations prepared containing fibres, rapeseed oil, *Pisane*[®] C9 and water. The concentrations are presented in percent (w/w) of the total amount of material in the mixture, thus excluding the 60 % water content.

| General composition | 40 % | | | 60 % |
|---------------------------------|-------|------|------------------------|-------|
| | Fibre | Oil | Pisane [®] C9 | Water |
| Pisane[®] C9 | - | - | 100 % | 60 % |
| Potex (>1000 µm) | 10 % | - | 90 % | ” |
| | 15 % | - | 85 % | ” |
| | 20 % | - | 80 % | ” |
| Potex Crown (>200 µm) | 10 % | - | 90 % | ” |
| | 15 % | - | 85 % | ” |
| | 20 % | - | 80 % | ” |
| Wheat bran (710-1250 µm) | 10 % | - | 90 % | ” |
| | 15 % | - | 85 % | ” |
| | 20 % | - | 80 % | ” |
| Wheat bran (125-500 µm) | 10 % | - | 90 % | ” |
| | 15 % | - | 85 % | ” |
| | 20 % | - | 80 % | ” |
| β-glucan fibre | 10 % | - | 90 % | ” |
| | 15 % | - | 85 % | ” |
| | 20 % | - | 80 % | ” |
| Rapeseed oil | - | 1 % | 99 % | ” |
| | - | 3 % | 97 % | ” |
| | - | 5 % | 95 % | ” |
| | - | 10 % | 90 % | ” |
| Potex Crown (>200 µm) | 15 % | 3 % | 82 % | ” |
| | 15 % | 10 % | 75 % | ” |
| Wheat bran (710-1250 µm) | 15 % | 3 % | 82 % | ” |
| | 15 % | 10 % | 75 % | ” |
| β-glucan fibre | 15 % | 3 % | 82 % | ” |
| | 15 % | 10 % | 75 % | ” |

and added to the blend and everything was mixed for one additional minute. The final mixtures were then stored over night in a fridge holding 6-8°C. Preparation of mixtures containing both oil and fibre were conducted similarly as stated above, a part from that a mixture of fibre and oil was prepared one day before adding it to pea protein isolate and water in order for the fibre to soak oil.

3.2.2 Extrusion

The extruder used in the project was a table-top single screw extruder *Collin Tech Line E20T* (Collin Lab & Pilot Solutions, Inc., Germany), see figure 3.1, with a cooling die (2 x 20 x 130 mm) and a conventional screw with compression ratio of 2:1 and a L/D ratio of 25.



Figure 3.1: The table-top single screw extruder *Collin Tech Line E20T* (Collin Lab & Pilot Solutions, Inc., Germany) used in the project.

The extruder consists of four heated temperature zones, zone one being located in direct contact to the feeding and zone four being closest to the die. The temperature chosen for each zone was 30°C, 80°C, 120°C and 135°C respectively. One additional temperature zone, temperature zone five, is the die. There is a sensor connected to zone four, which measured both melt pressure and melt temperature inside the barrel. All data was continuously monitored, including temperature of all zones, pressure and revolutions per minute of the screw, using the software *FeCon* (Feller Engineering, Germany). During extrusion, the temperature on all four temperature zones was held constant for all formulations, though the melt temperature differed depending on material extruded.

During initial feeding, the extruder was operated with a mixture of pea protein isolate and 60 % water. When the wanted temperature profile of 30/80/120/135°C was reached for the four temperate-controlled zones, feeding of the wanted formulation and cooling

of the die was initiated. The screw was operated at 60 rpm for all formulations. After extrusion, the samples were stored in a fridge holding 6-8°C and within the range of a couple of days from the extrusion date, the samples were cryo-sectioned and analyzed with a texture analyzer.

3.3 Analysis

After extrusion of the prepared formulations, the produced samples were analyzed regarding texture and structure using light microscope and a texture analyzer. Additionally, water and oil holding capacity measurements were conducted for the selected components.

3.3.1 Light Microscope

Thin samples were sectioned using *Leica CM1900 Cryostat* (Leica Biosystems, Germany). The samples were cut into pieces measuring approximately 10x10 mm and glued with *PELCO Cryo Embedding Compound* (Ted Pella Inc., U.S) onto a circular specimen holder. The samples were immersed into liquid nitrogen, to freeze the samples quickly, and placed in the sample holder in the cryostat. A sharp blade was set to a specific thickness, with most samples sectioned in 5 µm and some sectioned in 10 µm, depending on the rigidity of sample. The samples were placed on a slide *Superfrost™* (ThermoFischer Scientific, U.S), stained with staining agent and covered with a cover slip (ThermoFischer Scientific, U.S). Nail polish was used in the edges between the slide and the cover slip to seal in order to not expose the light microscope to iodine fumes.

The samples were stained with different staining agents, presented in chapter 2.4.1, depending on what they contained. Samples containing only pea protein isolate and fibre were stained with iodine solution. The staining agent was added to the samples and then rinsed off. Samples containing some part fat was instead stained using fumes from a solution containing 2% OsO₄ in water. Droplets of osmium solution were added to a petri dish and stands were placed in four corners of the dish. The slides with samples were placed up side down above the droplets for approximately 20-30 min.

The samples were then analyzed using a light microscope *Microphot-FXA Research Microscope* (Nikon, Japan), with a NikonFX-35DX camera and the images were processed in the software *cellSens Dimension* (Olympus, Japan). Images were taken with 4x, 10x and 20x magnification.

3.3.2 Instron Texture Analyzer

The texture analyzer *Instron 5542* (Instron, U.S) was used for two different measurements; compression test and cutting test. The instrument was operated with the software *Bluehill* (Instron, U.S).

Compression Test

For the compression test, a cylinder-probe was used measuring 30 mm in diameter. Fifteen circular replicates were cut from the extruded samples, measuring 15,3 mm in diameter. The height varied between 2,5-6 mm depending on the height of the extruded sample. The samples was compressed to 50 % of its original height at a speed of 0,1 mm/sec. Data for compressive stress (Pa) and compressive strain (mm/mm) was recorded.

Cutting Test

The cutting test was performed using a warner-bratzler blade with a triangular notch measuring 50x50 mm. The speed was set to 1 mm/sec and the blade started without touching the sample and ended when the sample was completely cut. The cutting test was conducted both perpendicular (longitudinal strength) and parallel (transverse strength) to direction of extrusion flow on ten sample pieces respectively, all measuring 20x20 mm. Data for compressive extension (mm) and compressive load (N) was measured.

Statistical Analysis

The arithmetic mean values and corresponding standard deviations were calculated using *MicrosoftTM Excel* in *Office 365*. Mean values were calculated using the function `=MEDEL`, where all values are summarized and then divided with the number of values [63], see equation 3.1, where n denotes the number of values and x_i corresponds to the observed value. The standard deviation was calculated with the function `=STDAV.S` [64], see equation 3.2.

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i \quad (3.1)$$

$$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}} \quad (3.2)$$

3.3.3 Water Holding Capacity (WHC) and Water Solubility Index (WSI)

Water holding capacity and water solubility index were measured for the fibres and the pea protein isolate, using a modified method originating from an article by J. Miedzinka *et al.* [65]. For measuring the water holding capacity and water solubility index, a solution of 0,1 g of the material in 10 ml milliQ-water was prepared in a 15 ml plastic test tube giving a solution of 1 % (weight/volume). The samples were stirred every 10 minute for 20 seconds during one hour using a *InterMed Whirli VIB 2* (Labassco, Sweden) vortex mixer. The solutions were centrifuged in a *Hettich Universal 320* (Hettich Instruments, Germany) centrifuge for 25 min at 4000g. Afterwards, the supernatant was added to a glass petri dish and the pellet was left in the test tube and inserted to an oven for 25 min at 50°C to evaporate excess water. The pellet was then weighted and the water holding capacity was calculated according to equation 3.3.

$$\text{WHC} = \frac{\text{wet pellet (g)} - \text{initial material (g)}}{\text{initial material (g)}} = \frac{\text{bound water (g)}}{\text{initial material (g)}} \quad (3.3)$$

The water solubility index was measured by drying the supernatant in an oven in 85°C for one day. The weight of the dried solubilized material was recorded and the water solubility index was calculated according to equation 3.4.

$$\text{WSI (\%)} = \frac{\text{dried supernatant (g)}}{\text{initial material (g)}} \cdot 100 \quad (3.4)$$

3.3.4 Oil Holding Capacity (OHC)

Oil holding capacity was, similar to water holding capacity, also measured on the pea protein isolate and on all fibres. This method too originated from an article by J. Miedzinka *et al* [65], used with some modifications. A solution of 0,1 g of the material in 10 ml rape-seed oil was prepared in a 15 ml plastic test tube. The samples were stirred for 30 seconds using a *InterMed Whirli VIB 2* vortex mixer and left undisturbed for 30 minutes. The solutions were centrifuged in a *Hettich Universal 320* centrifuge for 25 minutes at 4000g.

Afterwards, the supernatant was removed and the remaining pellet was weighted. The oil holding capacity was calculated using equation 3.5.

$$\text{OHC} = \frac{\text{soaked pellet} - \text{initial material (g)}}{\text{initial material (g)}} = \frac{\text{bound oil (g)}}{\text{initial material (g)}} \quad (3.5)$$

4

Results and Discussion

THE AIM of the project was to understand how additives influence the texturing process of pea protein isolate during high moisture extrusion. Four additives were investigated; potato fibre, wheat bran fibre, β -glucan fibre and rapeseed oil. The properties of the isolate and respective added fibre were analyzed with emphasis on water- and oil holding capacity as well as water solubility index. Formulations with fibre (10 %, 15 %, 20 %), rapeseed oil (1 %, 3 %, 5 %, 10 %) and combination of fibre and rapeseed oil (15 % fibre with 1 %, 3 % oil) were prepared, together with pea protein isolate and 60 % water, see table 3.2 in chapter 3.1. The extruded samples were analyzed regarding macrostructure, microstructure and texture properties. The results for the analyses as well as a discussion of the findings are presented in current chapter.

4.1 Water- and Oil Holding Capacity and Water Solubility Index

For the fibres and the pea protein isolate *Pisane*[®] C9 (PPI), the water holding capacity (WHC) and water solubility index (WSI), see table 4.1 and the oil holding capacity, see table 4.2, was analyzed. *Potex* and PPI possessed high values for water holding capacity, but great difference in water solubility index, while β -glucan possessed the lowest value for water holding capacity. PPI had the lowest oil holding capacity while *Potex* and β -glucan fibre had the highest values.

Table 4.1: Water holding capacity (g water/g material) and Water Solubility Index (%), presented with standard deviation, for (a) pea protein *Pisane*[®] C9, (b) potato fibre *Potex* (<1000 μ m), (c) potato fibre *Potex Crown* (<200 μ m), (d) wheat bran fibre (710-1250 μ m), (e) wheat bran fibre (125-500 μ m) and (f) β -glucan fibre *DF25*.

| | Water holding capacity (g water/g material) | Water solubility index (%) |
|-----------------------------------|---|----------------------------|
| (a) <i>Pisane</i> [®] C9 | 9,2 \pm 0,13 | 28,0 % \pm 0,9 % |
| (b) <i>Potex</i> | 9,6 \pm 0,12 | 5,1 % \pm 0,40 % |
| (c) <i>Potex Crown</i> | 7,9 \pm 0,27 | 6,0 % \pm 0,32 % |
| (d)Wheat bran (710-1250 μ m) | 5,5 \pm 0,13 | 13,9 % \pm 0,32 % |
| (e)Wheat bran (125-500 μ m) | 4,6 \pm 0,07 | 9,5 % \pm 1,3 % |
| (f) β -glucan fibre | 2,6 \pm 0,14 | 14,6 % \pm 1,0 % |

Table 4.2: Oil holding capacity (g oil/g material), presented with standard deviation, for (a) pea protein *Pisane*[®] C9, (b) potato fibre *Potex* (<1000 μ m), (c) potato fibre *Potex Crown* (<200 μ m), (d) wheat bran fibre (710-1250 μ m), (e) wheat bran fibre (125-500 μ m) and (f) β -glucan fibre *DF25*.

| | Oil holding capacity (g oil/g material) |
|-----------------------------------|---|
| (a) <i>Pisane</i> [®] C9 | 2,0 \pm 0,12 |
| (b) <i>Potex</i> | 5,5 \pm 0,23 |
| (c) <i>Potex Crown</i> | 3,5 \pm 0,51 |
| (d)Wheat bran (710-1250 μ m) | 5,6 \pm 0,16 |
| (e)Wheat bran (125-500 μ m) | 3,9 \pm 0,21 |
| (f) β -glucan fibre | 5,7 \pm 0,05 |

4.2 Extruded Formulations

Extrusion formulations were prepared according to table 3.2 in chapter 3.1, and the results obtained as well as a discussion of the findings are presented below. The results and discussions are divided into chapters with regards to formulations, including *Pea Protein Isolate*, *Potato Fibre*, *Wheat Bran Fibre*, *Rapeseed Oil* and *Fibre and Rapeseed Oil*. The chapter is ended with a comparison of the analyzed additives and an evaluation of the analyzing methods. The data recorded during extrusion of the different formulations, including temperature for the temperature zones and screw speed, is presented in appendix B.

4.2.1 Pea Protein Isolate (PPI)

A formulation containing 100 % PPI together with 60 % water was first extruded to investigate the ability of the isolate to form texturized structures upon high moisture extrusion cooking. The analysis of the macrostructure and microstructure of the extruded formulation are presented in figure 4.1, and the mechanical properties together with extrusion data are presented in table 4.3.

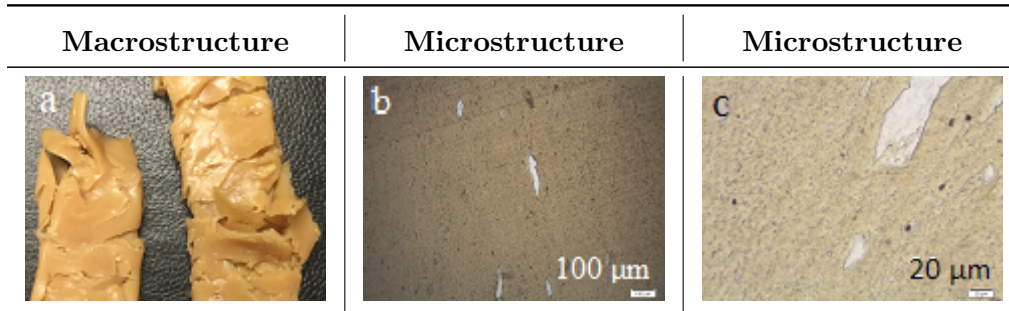


Figure 4.1: Extrudates containing 100 % Pisane[®] C9 (a). Light microscopy images of the microstructure of the extrudate, proteins shown in yellow/brown (b-c). Scale bars are 100 μm (b) and 20 μm (c).

The sample extruded were compact with a layered and rubbery structure, see figure 4.1.a. The microstructure display a continuous protein network without interruption, coloured light yellow/brown both in figure 4.1.b and 4.1.c. Any directionality in the protein network is difficult to interpret.

The values of compressive stress (kPa) and cutting strength (N), presented in 4.3, were utilized to analyze the formed structure. The compressive stress analysis displayed a big standard deviation presumably originating from insecurities of test. The probe was moved manually to where it touches the surface of the sample, the samples analyzed

Table 4.3: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing Pisane[®] C9.

| | 100 % Pisane [®] C9 |
|--|------------------------------|
| Compression stress (kPa) | 339,5±132,8 |
| Longitudinal cutting strength (N) | 8,8±2,2 |
| Transverse cutting strength (N) | 8,4±2,2 |
| Melt temperature (°C) | 122,1±1,6 |
| Melt pressure (bar) | 8,0±1,0 |

exhibited varying surface structures and the samples also possessed varying degree of coherency, leading to some samples falling apart during compression. In general, the large standard deviation presumably indicate on inhomogeneity in the extrudates.

The cutting strength values display almost equal longitudinal and transverse cutting strength, implying a similarity in structure in both directions. One previous study, conducted by Osen *et al.* [6], investigated textural properties of different extruded pea proteins with 55 % moisture content upon different process parameters, using a twin-screw extruder, and found that the cooking temperature greatly affected the texturization of PPI and thus the cutting strength of the extrudate. They presented that for Pisane[®] M9, the longitudinal and transverse cutting strength was similar up to a certain temperature where after mainly the longitudinal cutting strength increased, indicating a more longitudinally aligned protein network. This in accordance with literature, where it is presumed that proteins align during cooling under directed shear forces [16], [5]. In two other studies by Akdogan [42] and Chen *et al.* [66], on protein from soy bean, a fibrous structure of proteins is formed at extrusion temperature of 140-160°C. It is probable that a more longitudinally aligned structure would appear at elevated extrusion temperatures in the project.

4.2.2 Potato Fibre

Extrusion formulations containing two different potato fibres were analyzed, *Potex* (<1000 µm) and *Potex Crown* (<200 µm). The fibres are of different size but have the same composition, see table 3.1 in chapter 3.1. This in order to investigate both the impact of the fibre itself and the impact of different fibre sizes upon the texturing process of proteins during extrusion. Formulations were prepared with *Potex* and *Potex Crown* (10 %, 15 %, 20 %) together with PPI and 60 % water, see composition in table 3.2 in chapter 3.1. The analysis of the macrostructure and microstructure of the extruded formulations are presented in figures 4.2 and 4.3, and the mechanical properties together

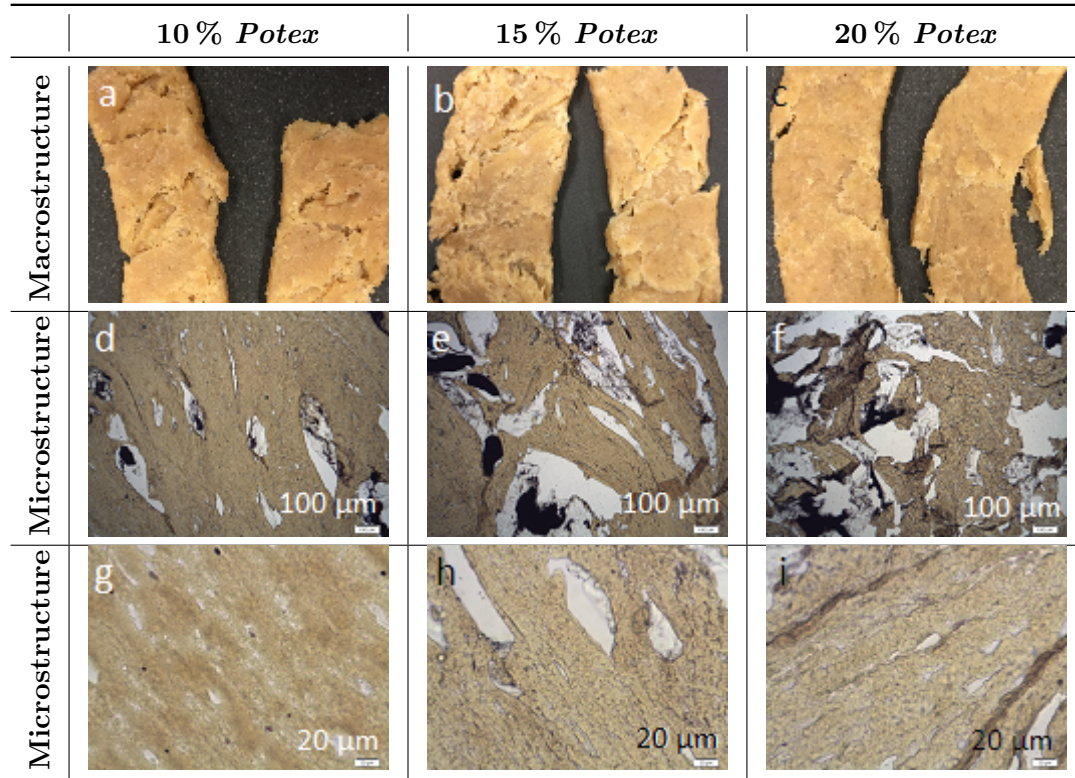


Figure 4.2: Extrudates containing 10 %, 15 %, 20 % *Potex* (a-c). Light microscopy images of the microstructure of the extrudate, proteins shown in yellow/brown and starch in purple (d-i). Scale bars are 100 μm (d-f) and 20 μm (g-i).

Table 4.4: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing 10 %, 15 % and 20 % *Potex*.

| | 10 % <i>Potex</i> | 15 % <i>Potex</i> | 20 % <i>Potex</i> |
|---|-------------------|-------------------|-------------------|
| Compression stress (kPa) | 139,1 \pm 46,4 | 159,0 \pm 77,2 | 288,1 \pm 86,9 |
| Longitudinal cutting strength (N) | n.a. | n.a. | n.a. |
| Transverse cutting strength (N) | n.a. | n.a. | n.a. |
| Melt temperature ($^{\circ}\text{C}$) | 118,7 \pm 2,6 | 120,4 \pm 1,1 | 121,9 \pm 0,9 |
| Melt pressure (bar) | 12,1 \pm 3,8 | 13,3 \pm 3,1 | 13,1 \pm 2,6 |

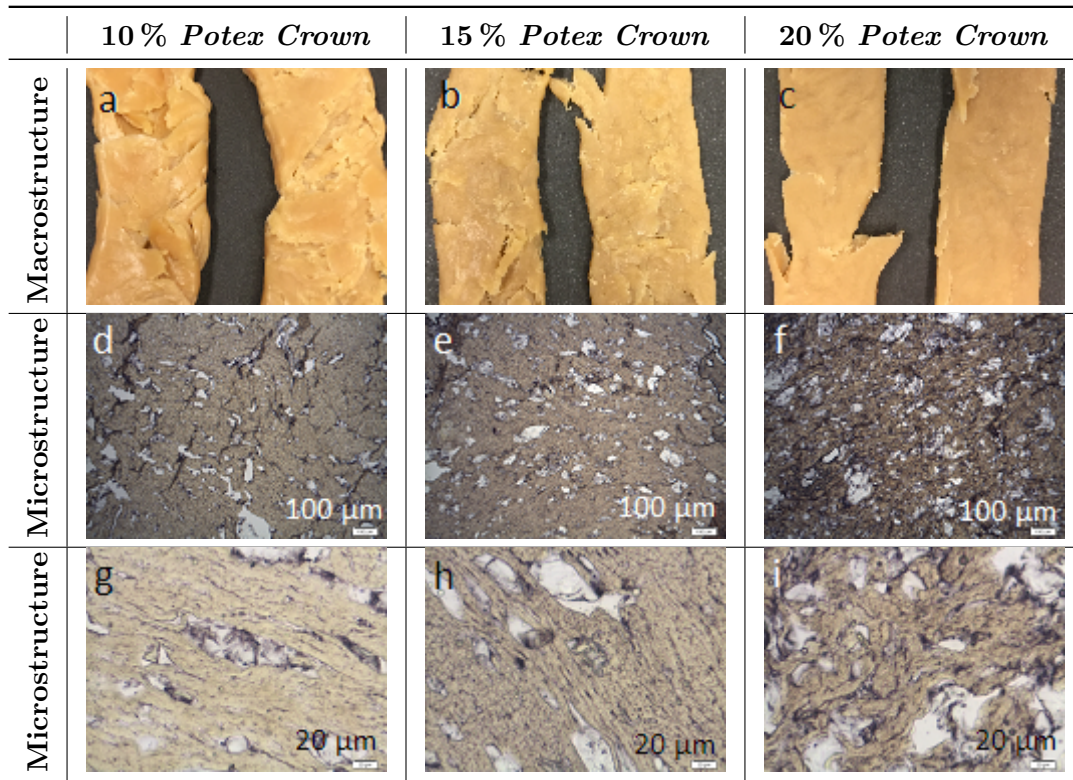


Figure 4.3: Extrudates containing 10 %, 15 %, 20 % *Potex Crown* (a-c). Light microscopy images of the microstructure of the extrudate, proteins shown in yellow/brown and starch in purple (d-i). Scale bars are 100 μm (d-f) and 20 μm (g-i).

Table 4.5: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing 10 %, 15 % and 20 % *Potex*.

| | 10 % <i>Potex Crown</i> | 15 % <i>Potex Crown</i> | 20 % <i>Potex Crown</i> |
|---|-------------------------|-------------------------|-------------------------|
| Compression stress (kPa) | 167,1 \pm 93,1 | 263,1 \pm 83,3 | 373,9 \pm 111,1 |
| Longitudinal cutting strength (N) | n.a. | n.a. | n.a. |
| Transverse cutting strength (N) | n.a. | n.a. | n.a. |
| Melt temperature ($^{\circ}\text{C}$) | 122,9 \pm 0,6 | 121,6 \pm 0,8 | 121,2 \pm 1,0 |
| Melt pressure (bar) | 13,1 \pm 1,4 | 11,3 \pm 2,2 | 12,8 \pm 4,3 |

with extrusion data are presented in table 4.4 and 4.5.

The macrostructure of the extruded formulation containing 10 % *Potex* is grooved and layered, see figure 4.2.a, and the sample fell easily apart. The corresponding microstructures reveal a continuous protein network where a tendency of a directional structure can be observed, see figure 4.2.d and 4.2.g. Both potato fibres and voids are displayed as white dispersed areas in the yellow/brown protein network and the purple areas is starch inside the fibres.

The macrostructure for the extrudate containing 20 % *Potex* is smooth and compact, see figure 4.2.c. The corresponding microstructure reveal a continuous though interrupted protein network with a lacking directionality, see figure 4.2.f. However, the microstructure at higher magnification in figure 4.2.i display a directional protein network, indicating that the protein structure was only interrupted on a larger scale as seen in figures 4.2.d-f.

The values of compressive stress (kPa) indicated on an increase in value upon increase of fibre content in the formulation, see table 4.4, implying that more force is needed to compress the sample. Simultaneously, the microstructure shift from layered to smooth, as seen in figures 4.2.a-c. This implies that an increased smoothness and decreased layering of the samples gave increased compressive stress values, suggesting denser and harder samples. The standard deviation for the compressive stress values were though significant. Analysis of the cutting load were unavailable due to inadequate samples.

The melt pressure for formulations containing *Potex*, presented in table 4.4, is higher compared to the melt pressure for the formulation containing 100 % PPI, see table 4.3 in chapter 4.2.1. An increased melt pressure could be due to increased friction in the barrel as an effect of added fibres to the formulation. It could also be due to the specific water holding capacity for the fibres, which for *Potex* is higher compared to PPI, see table 4.1 in chapter 4.1, indicating that some amount of moisture in the protein network could be soaked by the fibre, thus reducing the water content and increasing the viscosity of the melt.

The samples containing *Potex Crown* behaves in a similar way as the *Potex* samples. The sample containing 10 % *Potex Crown* was perceived as dry, with a macrostructure revealing layers that was easily separating the sample into smaller pieces, see figure 4.3.a. The corresponding microstructure display a protein network that is highly interrupted by both potato fibres and voids, however still continuous, see figure 4.3.d. The same trend is seen in the microstructure at higher magnification, as seen in figure 4.3.g.

The macrostructure of sample containing 20 % *Potex Crown* was more compact and soft, with a smooth surface, see figure 4.3.c. The microstructure in figure 4.3.f reveal that the the previously continuous protein network is instead replaced by a bi-continuous protein network, interrupted by potato fibre, lacking directional order. The same lack of

directional order is seen in the microstructure at higher magnification, see figure 4.3.i, where also purple areas of starch is visible, indicating that starch from the potato fibre sample affected the protein network.

Starch can function as a structure-forming continuous material [17] when gelatinized, and the starch seen in sample containing 20 % *Potex Crown*, see figure 4.2.i, is most certainly gelatinized, since starch gelatinize at approximately 58-85°C [47] and the sample was extruded with a melt temperature of 121°C. When the melt containing starch is cooled down, a rigid and viscoelastic gel is formed [47]. Presumably, this is the reason for the smooth macrostructure seen in figure 4.3.c.

The values for compressive stress for samples containing *Potex Crown* indicate on an increase upon increase in fibre content, similar to formulations containing *Potex*. Together with previous trend of increase in smoothness and compactness of the samples upon increase of fibre content in the formulation, seen in figure 4.3.a-c, it is presumed that high values of compressive stress points to a more compact and smooth sample. Similar to formulations containing *Potex*, the melt pressure for formulation containing *Potex Crown* is higher compared to the melt pressure for the formulation containing 100 % PPI, see table 4.3 in chapter 4.2.1, presumably due to the same reasoning as mentioned earlier.

In general, samples containing *Potex* was feeling more dry compared to samples containing *Potex Crown*. The water holding capacity was higher for *Potex* than for *Potex Crown*, 9,6 g/g compared to 7,6 g/g, see table 4.1, indicating that *Potex* might hold a bigger portion of water in the formulation compared to *Potex Crown*. Though, the water holding capacity for PPI is comparable, 9,2 g/g, so it would be interesting to investigate the partitioning of water in the formulation containing both fibre and PPI.

The formulations containing *Potex* and *Potex Crown* both exhibit a shift from layered to smooth macrostructure upon increase of fibre content in the formulation. Also an increase in compressive stress upon increase of fibre content was displayed, though the increase was larger for formulations containing *Potex Crown*. It is indicated that starch in *Potex Crown* gelatinize during extrusion, giving rise to a more smooth sample. The texturization of the protein isolate seemed to decrease upon addition of both *Potex* and *Potex Crown* since the fibres interrupted the network at different length scale. *Potex Crown* was seemingly affecting the protein network to a larger extent, where directionality in the protein network was lacking at concentrations of 20 % fibre in the formulation. Comparing observations between samples containing 20 % *Potex* and 15 % *Potex Crown*, both the macrostructure, see figures 4.2.c and 4.3.b, and compressive stress, see table 4.4 and 4.5, is comparable. This is implying that there is a shift in effect upon addition of fibre between *Potex* and *Potex Crown*.

4.2.3 Wheat Bran Fibre

Two different wheat bran fibres were analyzed, a larger sized wheat bran fibre (710-1250 μm) and a smaller wheat bran fibre (125-500 μm). The aim was to investigate how the wheat bran, both regarding composition and size, affect the texturization of pea protein isolate during extrusion. Formulations were prepared, similar to potato fibre formulations, with both bigger wheat bran fibre and smaller wheat bran fibre (10 %, 15 %, 20 %) together with PPI and 60 % water, see composition in table 3.2 in chapter 3.1. The analysis of the macrostructure and microstructure of the extruded formulations is presented in figure 4.4 and 4.5, and the mechanical properties together with extrusion data are presented in tables 4.6 and 4.7.

The macrostructure of the extruded formulations containing larger wheat bran fibre shifted from a layered surface, see figure 4.4.a, to a more porous structure with smaller layers, see figure 4.4, upon increase of fibre content. The larger wheat bran fibre tended to fall out of the structure of the samples. The microstructure of the sample containing 10 % big wheat bran show a continuous protein network, interrupted by large wheat bran fibre seen as a large pericarp including brown protein cells, see figure 4.4.d. The microstructure of higher magnification seen in figure 4.4.g show a seemingly directed protein network. A higher fibre content in the formulation show a similar microstructure, seen in figure 4.4.f, due to the size of the added fibers. The microstructure of larger magnification seen in figure 4.4.i show a seemingly directional protein network. The magnification is presumably too large in figures 4.4.d-f to see a distinct difference in the microstructures of the different formulations. However, the increased amount of layers seen in the macrostructure in figure 4.4.c could be an effect of a more interrupted protein network. An analysis with lower magnification of the microstructure of the formulation containing big wheat bran fibre is recommended in future work.

The compressive stress was tending to decrease upon increase of fibre content in the formulation, see table 4.6. This indicate that the compressive stress decrease upon increased porosity and decreased compactness of the samples, giving a less stress resistant material. Analysis of the cutting load were unavailable due to inadequate samples.

The macrostructure of the samples containing small wheat bran fibre shifted from layered and porous, see figure 4.5.a, to a more layered and porous structure, see figure 4.4.c, upon increase of fibre content. The microstructure of the formulation containing 10 % small wheat bran fibre revealed a continuous protein network where fibres interrupt, see figure 4.4.d. The microstructure of larger magnification seen in figure 4.4.g reveal a tendency of a directional network. Upon increase of fibre content, the protein network got more interrupted though still being continuous, which can be see in figure 4.4.f. In the microstructure seen in figure 4.4.e, small parts of the wheat bran is dispersed in the protein network, indicating that the small wheat bran fibre is partly splintered, probably

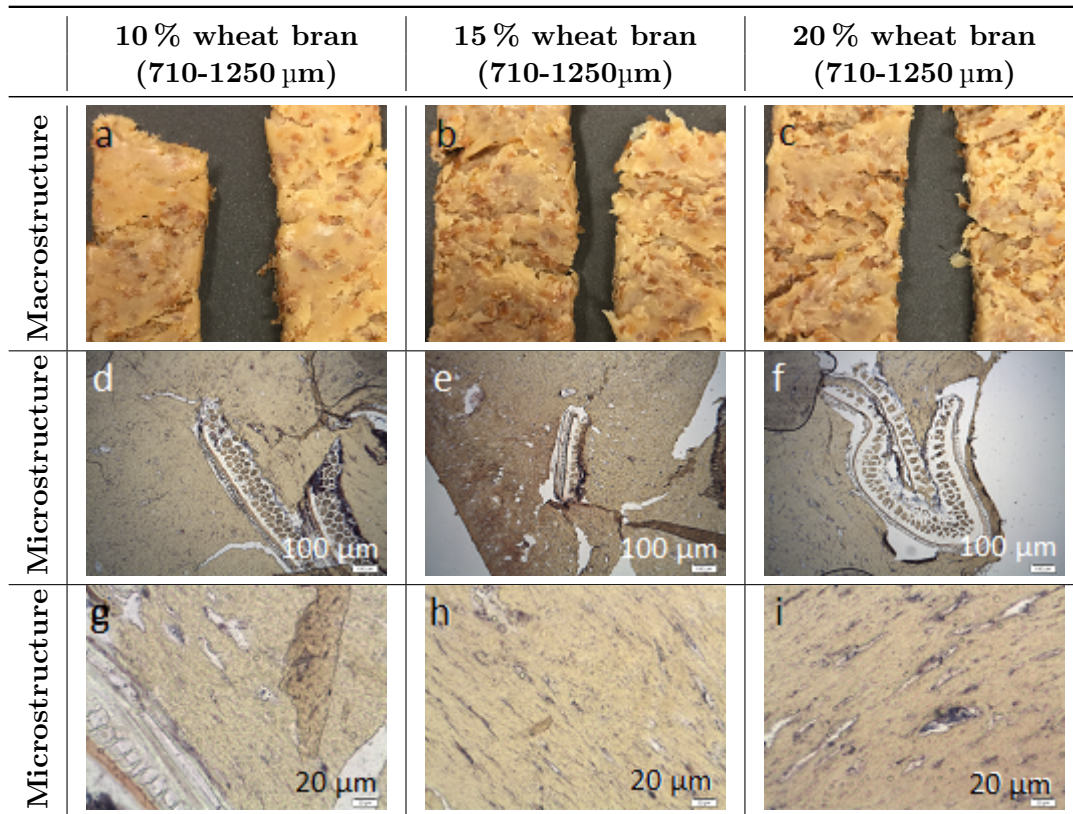


Figure 4.4: Extrudates containing 10 %, 15 %, 20 % wheat bran (710-1250 μm) (a-c). Light microscopy images of the microstructure of the extrudate, proteins shown in yellow/brown, fibres unstained (d-i). Scale bars are 100 μm (d-f) and 20 μm (g-i).

Table 4.6: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing 10 %, 15 % and 20 % wheat bran (710-1250 μm).

| | 10 % wheat bran (710-1250 μm) | 15 % wheat bran (710-1250 μm) | 20 % wheat bran (710-1250 μm) |
|---|--|--|--|
| Compression stress (kPa) | 244,3 \pm 59,5 | 208,4 \pm 73,4 | 124,0 \pm 60,6 |
| Longitudinal cutting strength (N) | n.a. | n.a. | n.a. |
| Transverse cutting strength (N) | n.a. | n.a. | n.a. |
| Melt temperature ($^{\circ}\text{C}$) | 122,3 \pm 1,0 | 124,1 \pm 0,6 | 124,1 \pm 0,8 |
| Melt pressure (bar) | 14,1 \pm 2,3 | 12,6 \pm 1,1 | 12,9 \pm 1,9 |

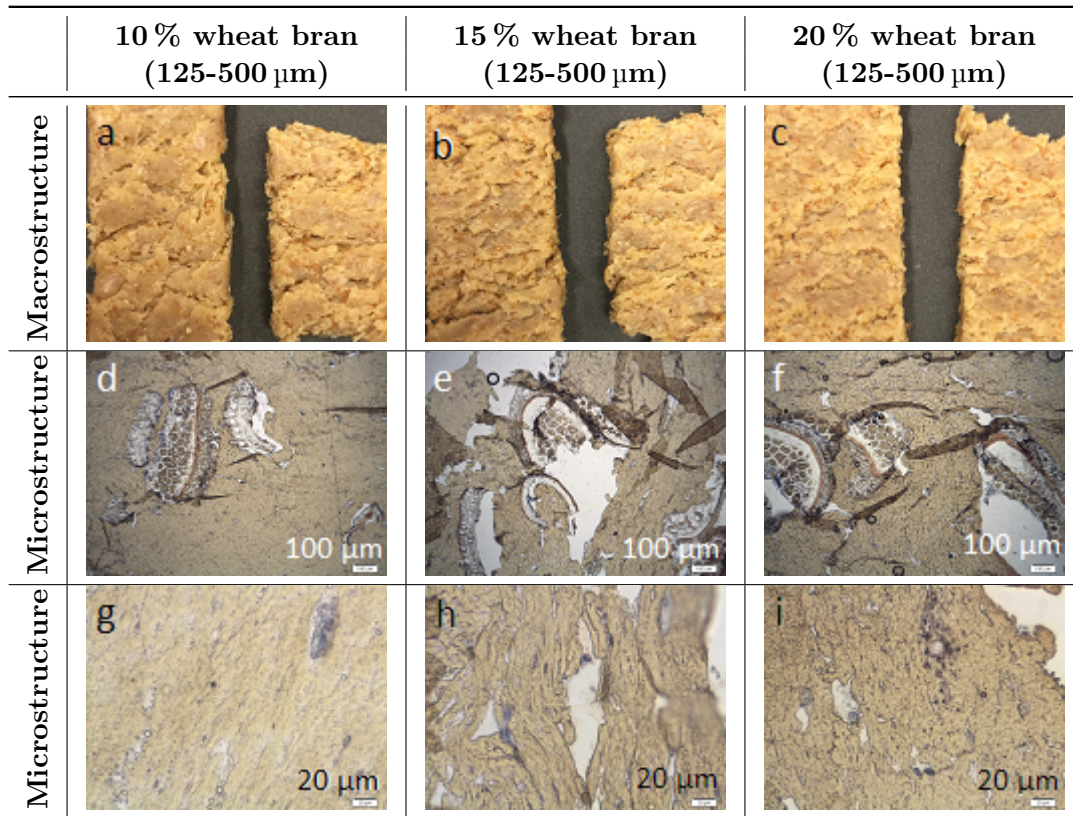


Figure 4.5: Extrudates containing 10 %, 15 %, 20 % wheat bran (125-500 μm) (a-c). Light microscopy images of the microstructure of the extrudate, proteins shown in yellow/brown, fibres unstained (d-i). Scale bars are 100 μm (d-f) and 20 μm (g-i).

Table 4.7: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing 10 %, 15 % and 20 % wheat bran (125-500 μm).

| | 10 % wheat bran (125-500 μm) | 15 % wheat bran (125-500 μm) | 20 % wheat bran (125-500 μm) |
|---|---|---|---|
| Compression stress (kPa) | 264,9 \pm 73,9 | 150,4 \pm 60,0 | 164,4 \pm 80,2 |
| Longitudinal cutting strength (N) | 2,8 \pm 0,7 | 2,3 \pm 0,4 | 2,4 \pm 0,6 |
| Transverse cutting strength (N) | 7,8 \pm 1,7 | 5,9 \pm 1,8 | 5,7 \pm 1,0 |
| Melt temperature ($^{\circ}\text{C}$) | 120,7 \pm 0,8 | 120,1 \pm 0,3 | 120,3 \pm 1,7 |
| Melt pressure (bar) | 19,4 \pm 2,2 | 18,9 \pm 2,1 | 17,0 \pm 2,0 |

during the milling of the wheat bran fibre.

Similar to formulations containing the larger wheat bran, the compressive stress was decreased upon increase of fibre content for small wheat bran. This indicate that the compressive stress decrease upon increased porosity and decreased compactness of the samples, giving a less stress resistant material.

The values for cutting strength for formulations containing small wheat bran is higher in the transverse direction compared to the longitudinal direction, see table 4.7. This indicate that the structure is more textured in the direction perpendicular to the extrusion direction. Chen *et al.* [66] suggested that extrusion settings below 150°C and 60 % moisture content tend to display a more textured structure in the direction perpendicular to the extrusion direction, in accordance with observed results. A similar theory is presented by Osen *et al.* [6], where it is suggested that an increased melt viscosity due to decreased temperatures give a less textured structure in the longitudinal direction. In this project, the extrusion temperature was 135°C, which is below the temperature mentioned by Chen *et al.* [66] of 150°C. During extrusion, a water content of 60 % was utilized in this project. Though, small wheat bran had a water holding capacity of 4,6 g/g, see table 4.1 in chapter 4.1, indicating that some portion of the water added could be sought up by the fibre and thus decrease the total moisture content in the protein network. The water holding capacity for PPI was though higher, indicating that the rate of which PPI and wheat bran fibre soak up water is an interesting factor. Though, the fibre formation for small wheat bran could be dominant in the transverse direction due to low temperatures and possibly also reduced moisture levels in the protein network [6].

What is interesting is the close to constant longitudinal cutting strength and the decreasing transverse cutting strength for small wheat bran, see table 4.7. Comparing the cutting strength values for small wheat bran with those for 100 % PPI, see table 4.3 in chapter 4.2.1, the transverse cutting strength is initially comparable but the longitudinal cutting strength is lower for samples containing small wheat bran fibre compared to the samples containing 100 % PPI. One suggestion is that the addition of fibres reduce the strength of the textured protein network, which for small wheat bran is transversely directed, due to interruption. It is stated that addition of fibre reduce the ability for the continuous material to cross-bond, in this case referring to protein [48]. Upon further increase of fibre, the transverse cutting strength continue to decrease, probably due to more fibres interrupting the formed network.

The melt pressure for formulations containing small wheat bran is tending to be higher compared to the melt pressure for big wheat bran, see table 4.6 and 4.7. Compared to the melt pressure for the sample containing 100 % PPI, both the fibres have a high melt pressure, indicating that PPI flow more easily in the barrel. The increased pressure is presumably due to the same reasoning as mentioned for potato fibre. During addition of small wheat bran fibre, more components is added to the formulation compared to the

amount during addition of larger wheat bran fibres, presumably enhancing the suggested effect.

4.2.4 β -glucan Fibre

β -glucan fibre is a soluble fibre [21], in contrast to the main components of potato fibre and wheat bran fibre which is non-soluble. The β -glucan fibre was therefore used in order to investigate how a soluble fibre affect the texturing process of protein isolate. Formulations were prepared as previously with fibre (10 %, 15 %, 20 %), PPI and 60 % water, see composition in table 3.2 in chapter 3.1. The analysis of the macrostructure and microstructure of the extruded formulations is presented in figure 4.6, and the mechanical properties together with extrusion data are presented in table 4.8.

The macrostructure shift from layered and rubbery for 10 % β -glucan, see figure 4.6.a, to smooth and soft for 20 % β -glucan, see figure 4.6.c, upon addition of fibre. The microstructure for the formulation containing 10 % β -glucan fibre display a directional continuous protein network, seen in figure 4.6.d, containing enclosures of fibre and voids seen as white. The microstructure of larger magnification in figure 4.6.g also show a directional network. Upon addition of fibre to 20 % β -glucan, the protein network get more interrupted by fibres, and less directional, see figure 4.6.f. In figure 4.6.i, of larger magnification, the protein phase consist of more round and less stretched protein clusters, and no clear directionality is visible. This microstructure indicate that something is interrupting the structure in the protein network. A small amount of dark edges of the protein network can be seen in figure 4.6.i indicating that starch is present. Though, not in large amounts. Presumably, another component, such as β -glucan, which is a soluble dietary fibre that possess a certain gelling capacity [22], affect the protein structuring process too. The samples are stained with iodine, visualizing starch and protein, though other components are not visible. It would be interesting to stain β -glucan for microscopy analysis in future studies.

The analyzed compressive stress, in table 4.8, indicate a trend in increased compressive stress upon increased fibre content, though significant standard deviation is presented for all values. An increased compressive stress indicate a more stress resistant material, which presumably is more compact. This in accordance with observed macrostructure seen in figure 4.6.a-c, as well as in accordance with reasoning in chapter 4.2.2 and 4.2.3.

The cutting strength indicated a trend where increased fibre content gave a decrease in cutting strength, both longitudinally and transversely, see table 4.8. The cutting strengths is also lower compared to cutting strength for the sample containing 100% PPI, see table 4.3 in chapter 4.2.1. The decreased cutting strength could be due to the increased interruption from fibre during increased concentration, decreasing the ability

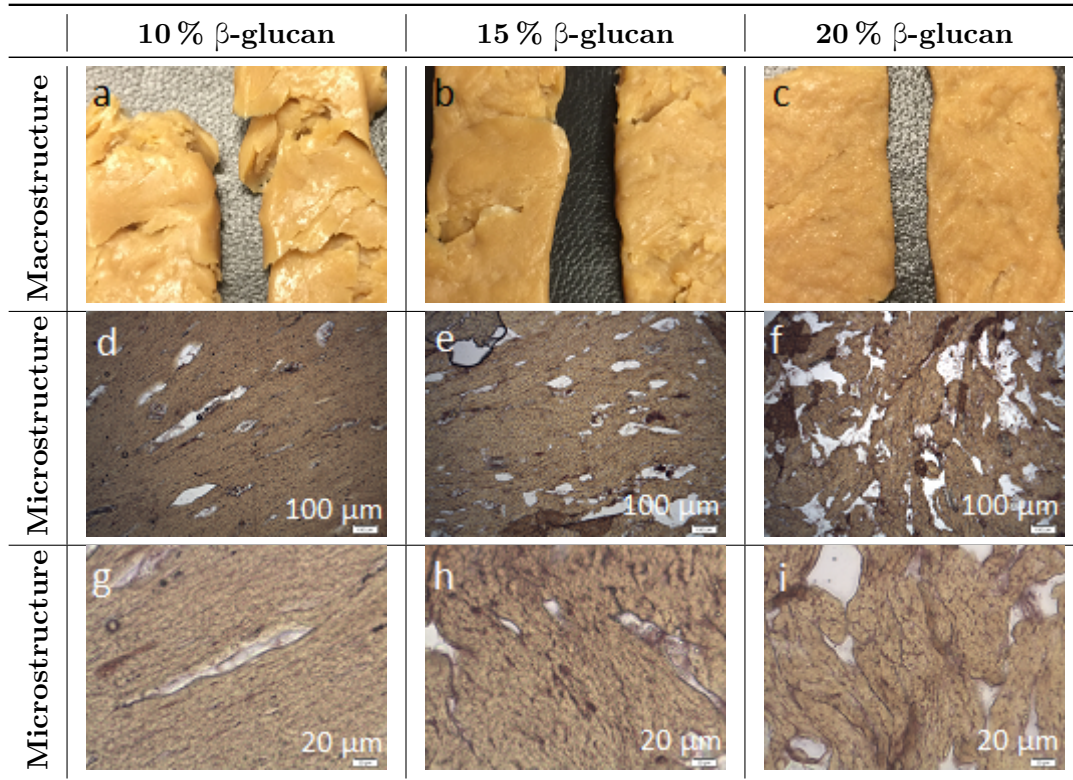


Figure 4.6: Extrudates containing 10 %, 15 %, 20 % β -glucan fibre (a-c). Light microscopy images of the microstructure of the extrudate, proteins shown in yellow/brown, fibres unstained (d-i). Scale bars are 100 μ m (d-f) and 20 μ m (g-i).

Table 4.8: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing 10 %, 15 % and 20 % β -glucan fibre.

| | 10 % β -glucan fibre | 15 % β -glucan fibre | 20 % β -glucan fibre |
|-----------------------------------|----------------------------|----------------------------|----------------------------|
| Compression stress (kPa) | 100,3 \pm 59,9 | 125,3 \pm 64,1 | 137,0 \pm 76,8 |
| Longitudinal cutting strength (N) | 5,3 \pm 1,3 | 4,6 \pm 0,6 | 2,6 \pm 0,7 |
| Transverse cutting strength (N) | 6,6 \pm 0,9 | 5,2 \pm 1,2 | 3,4 \pm 1,3 |
| Melt temperature ($^{\circ}$ C) | 120,7 \pm 0,5 | 121,7 \pm 0,7 | 120,7 \pm 1,4 |
| Melt pressure (bar) | 10,7 \pm 2,6 | 8,8 \pm 1,1 | 7,7 \pm 2,6 |

for the continuous phase to texturize [48] and thus presumably giving a decreased strength in the protein network. The reduced cutting strength could also be due to the increase of soluble components from the fibre, such as β -glucan, that presumably affect the texturing of protein during extrusion by contributing to the molten phase, reducing the friction in the barrel and thus reducing the texturing process of pea protein.

The melt pressure for β -glucan fibre samples was similar to the melt pressure seen for PPI sample in table 4.3. Fibres can function as a filler phase which increase the viscosity of the melt [48] and presumably give a higher melt pressure. The reason for this not being observed when adding β -glucan fibre is presumably the high concentrations of starch and β -glucan in the fibre, which are soluble components that generally do not increase the viscosity of the melt.

4.2.5 Rapeseed Oil

Rapeseed oil was used as a component in certain formulations in this project in order to investigate how an added lubricant [17] affect the texturing process of pea protein isolate. Formulations were prepared containing rapeseed oil (1 %, 3 %, 5 %, 10 %), PPI and 60 % water, see composition in table 3.2 in chapter 3.1. The analysis of the macrostructure and microstructure of the extruded formulations is presented in figure 4.7, and the mechanical properties together with extrusion data are presented in table 4.9.

The macrostructure of the sample containing 1 % rapeseed oil, see figure 4.7.a, is relatively similar to the macrostructure of the sample containing 100 % PPI, see figure 4.1.a, with a rubbery and layered surface. The sample containing 1 % rapeseed oil did though have an oily surface. Upon addition of rapeseed oil in the formulation, the macrostructure turn from layered and rubbery, see figure 4.7.a, to more compact though still layered for the sample containing 10 % rapeseed oil, see figure 4.7.d.

The microstructures in figures 4.7.e-h, display no rapeseed oil in the sample. The protein network is coloured light grey/yellow in all the mentioned figures, with areas of darker colour, presumably arising from irregularities in the sample section that is analyzed. The magnification in figures 4.7.e-h, is probably too small to display any rapeseed oil content. The macrostructure displayed for 1 % rapeseed oil in figure 4.7.i, with a larger magnification compared to figure 4.7.e-h, indicate no rapeseed oil content either. Upon increase of rapeseed oil content in the formulation to 3 % and 5 %, brown shades of stained rapeseed oil is seen in the microstructure in figure 4.7.j-k. An increase to 10 % rapeseed oil in the formulation resulted in distinct oil droplets, displayed as brown dispersed droplets in the protein network, being visible in the microstructure, as seen in figure 4.7.l. This in agreement with literature [17], where it is stated that lipids is dispersed in the continuous phase as fine droplets during extrusion.

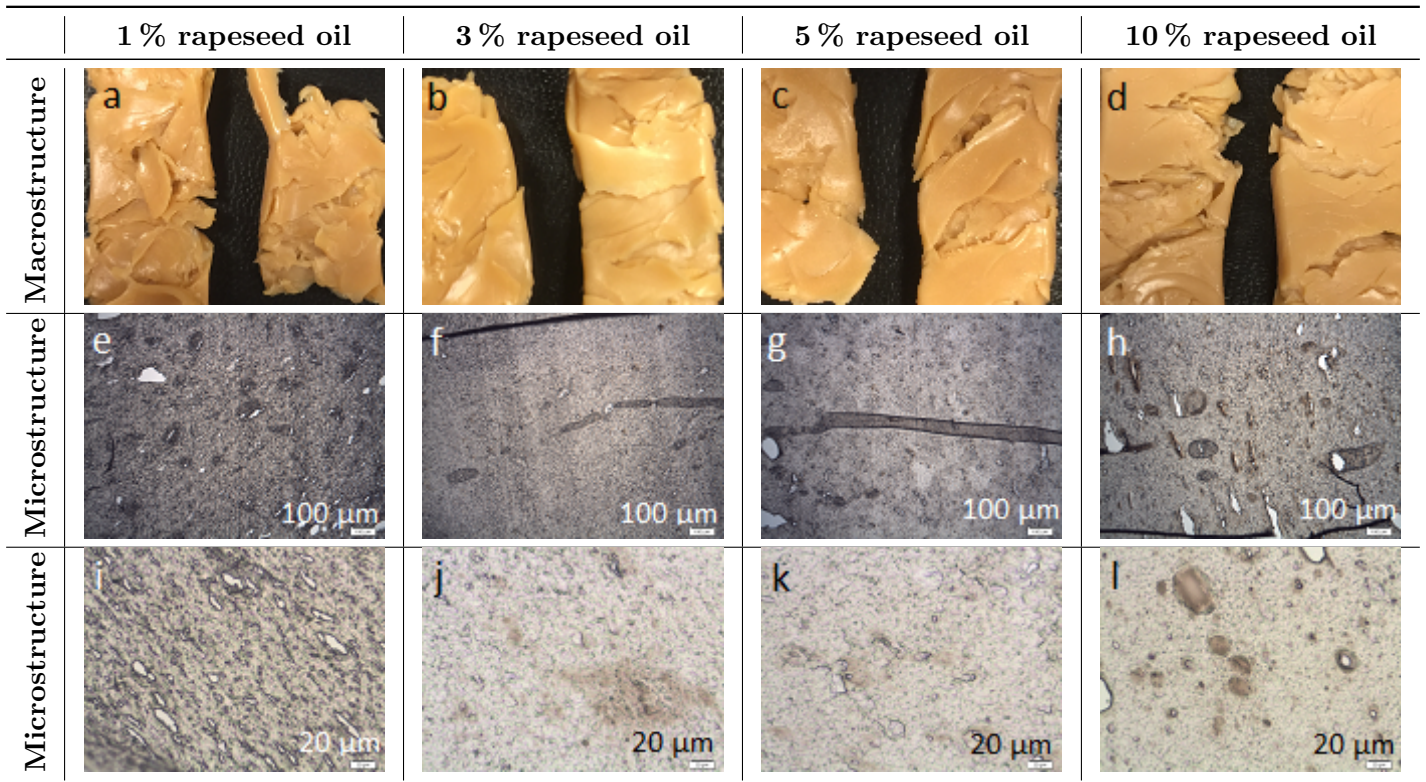


Figure 4.7: Extrudates containing 1 %, 3 %, 5 %, 10 % rapeseed oil (a-d). Light microscopy images of the microstructure of the extrudate, proteins shown in light brown/yellow. Oil seen in darker brown (e-l). Scale bars are 100 μm (e-h) and 20 μm (i-l).

Table 4.9: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing 1 %, 3 %, 5 %, 10 % rapeseed oil.

| | 1 % rapeseed oil | 3 % rapeseed oil | 5 % rapeseed oil | 10 % rapeseed oil |
|---|------------------------|------------------------|------------------------|-------------------------|
| Compression stress (kPa) | 207,6 \pm 141,1 | 249,8 \pm 152,4 | 280,5 \pm 142,6 | 218,5 \pm 109,6 |
| Longitudinal cutting strength (N) | 8,1 \pm 2,6 | 7,5 \pm 2,8 | 8,5 \pm 3,4 | 4,8 \pm 2,0 |
| Transverse cutting strength (N) | 13,0 \pm 2,3 | 9,9 \pm 3,1 | 10,2 \pm 1,8 | 6,7 \pm 1,5 |
| Melt temperature ($^{\circ}\text{C}$) | 121,4 \pm 1,5 | 124,7 \pm 0,5 | 123,3 \pm 1,7 | 118,1 \pm 7,9 |
| Melt pressure (bar) | 8,9 \pm 1,7 | 7,1 \pm 1,0 | 9,6 \pm 4,9 | 11,1 \pm 5,8 |

The compressive stress, in table 4.9, is implying a breaking point at 5 % rapeseed oil, where it is indicated that the compressive stress increase for formulations up to 5 % rapeseed oil and then decrease to the formulation containing 10 % rapeseed oil. A significant standard deviation is though present. The melt temperature, see table 4.9, vary without obvious trend for formulations containing 1 % to 5 % rapeseed oil but tend to decrease between formulations containing 5 % and 10 % rapeseed oil. In literature [46], it is stated that a reduced shear as an effect of added lipids in the extruded formulation can give rise to decreased melt temperature and increased melt pressure, due to decreased melt capacity of the extruded components. In literature [42], it is further suggested that concentrations over 5 % of lipids in extruded formulations is decreasing the fibre formation significantly, presumably meaning that the lubrication effect is enhanced, in agreement with observed trends for melt temperature.

The cutting strength, see table 4.9, is decreasing upon increase of rapeseed oil content from 5 % to 10 % in the formulations, both in the longitudinal and transverse direction, presumably indicating that the material get less textured. It is suggested in literature [42] that lipids decrease the ability to form textured structures, especially in the direction of extrusion, due to the lubricant effect that reduces shear, in accordance to the observed results in the project.

4.2.6 Fibre and Rapeseed Oil

In addition to investigation of formulations containing either fibre or rapeseed oil, the effect of combining fibre and rapeseed oil is investigated. The addition of *Potex Crown*, larger wheat bran fibre (710-1250 μm) and β -glucan fibre were investigated. Formulations were prepared containing fibre (15 %), rapeseed oil (3 %, 10 %), PPI and 60 % water, see composition in table 3.2 in chapter 3.1. The analysis of the macrostructure of the extruded formulations is presented in figure 4.8, and the analysis of the macrostructure and microstructure for samples containing 15 % fibre and 10 % rapeseed oil is presented in table 4.9. The mechanical properties together with extrusion data are presented in table 4.10.

The analysis of the macrostructure for formulations containing *Potex Crown* and rapeseed oil display a wavy and smooth surface with lack of layers, see figure 4.8.a and 4.8.d, where the structure get more tattered with more pronounced wavy attributes in the centre of the sample upon increase of rapeseed oil content. The macrostructure for samples containing wheat bran fibre and rapeseed oil display a layered surface with visible fibres, see figure 4.8.b and 4.8.e. No significant difference between addition of 3 % or 10 % rapeseed oil is observed. For formulations containing β -glucan and rapeseed oil, the macrostructure display a wavy and smooth surface, similar to the sample containing *Potex Crown* and rapeseed oil, as seen in figure 4.8.c and 4.8.f. No significant difference

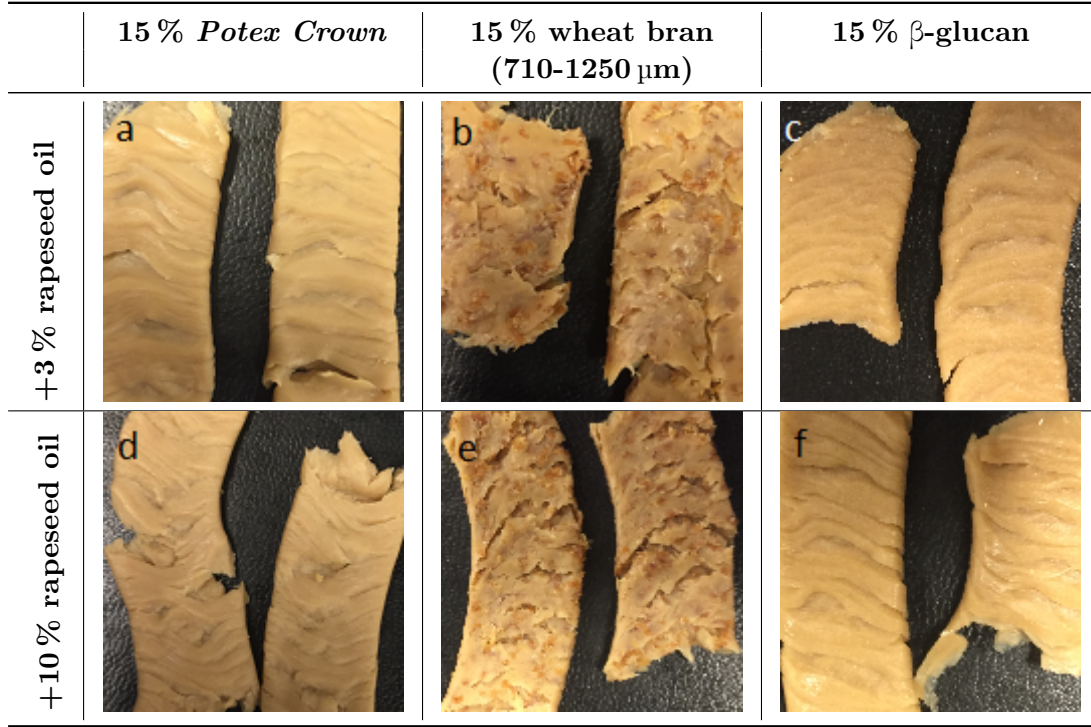


Figure 4.8: Extrudates containing 15 % fibre and either 3 % or 10 % rapeseed oil (a-f).

between the sample containing 3 % and 10 % rapeseed oil is observed.

The displayed wavy structure for formulations containing rapeseed oil together with *Potex Crown* or β -glucan fibre is probably a surface change [67]. One possible explanation to the wavy surface could be that due to the added lubricant in form of rapeseed oil to the formulation, together with starch and β -glucan from the fibres that presumably reduce the viscosity, the friction between the melt and the die surface is reduced, causing an increased velocity of the melt in the centre of the die. The increased velocity of the melt lead to an lacking ability for the melt to cool down, possibly giving a pronounced parabolic flow profile that could cause the wavy surface. The lack of wavy surface for the formulations containing rapeseed oil and the bigger wheat bran fibre could be explained by the lower amount of either free starch or β -glucan in the protein network, increasing the viscosity of the melt.

The microstructure for formulations containing 10 % rapeseed oil together with fibre was analyzed, see figure 4.9. In all the microstructures presented, see figures 4.9.(d-i), it is though difficult to see distinct areas of oil, determine exactly where the oil is situated and to analyze the directionality of the protein network. It would instead be interesting to analyze the oil in a confocal microscope using another staining agent, that is added before extrusion of the formulation.

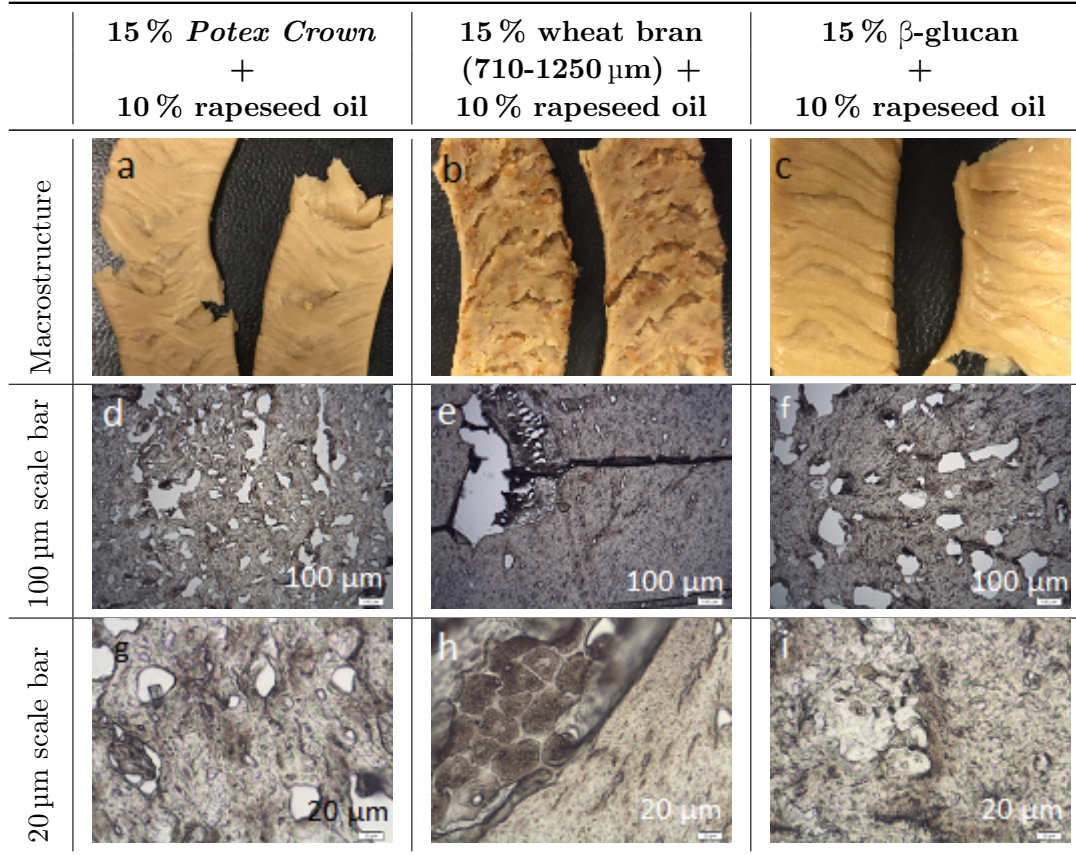


Figure 4.9: Extrudates containing 15 % fibre and 10 % rapeseed oil (a-c). Light microscopy images of the microstructure of the extrudates, proteins shown in grey/yellow, fibres unstained, oil dark brown (d-i). Scale bars are 100 μm (d-f) and 20 μm (g-i).

The compressive stress for the formulations containing *Potex Crown* or larger wheat bran fibre together with rapeseed oil indicate a decrease of compressive stress upon addition of rapeseed oil, see table 4.10, indicating that the material get less stress resistant upon addition of rapeseed oil. In contrast, the compressive stress for the sample containing β -glucan fibre and rapeseed oil display no significant change upon addition of oil in the formulation, indicating that the material is equally stress resistant independent of oil content.

The cutting strength for all formulations is lower compared to the cutting strength for 100 % PPI, see table 4.3 in chapter 4.2.1, and is tending to decrease upon addition of rapeseed oil, both in the longitudinal and transverse direction, presumably due to the addition of lubricant that generally reduce the ability to form a textured structure [42].

The melt pressure for the samples containing *Potex Crown* or β -glucan fibre in addition

Table 4.10: Texture analyzing properties and extrusion temperature and pressure, presented with standard deviation, for samples containing 15 % fibre (*Potex Crown*, big wheat bran (710-1250 μm) and β -glucan) and either 3 % or 10 % rapeseed oil.

| | Compression stress (kPa) | Longitudinal cutting strength (N) | Transverse cutting strength (N) | Melt temperature ($^{\circ}\text{C}$) | Melt pressure (bar) |
|--|--------------------------------|---|---------------------------------------|---|---------------------------|
| 15 % <i>Potex Crown</i> + 3 % rapeseed oil | 270,5 \pm 100,5 | 4,6 \pm 1,5 | 4,8 \pm 0,9 | 124,1 \pm 0,8 | 8,5 \pm 1,5 |
| 15 % <i>Potex Crown</i> + 10 % rapeseed oil | 154,8 \pm 83,0 | 2,8 \pm 0,8 | 2,6 \pm 0,4 | 124,0 \pm 0,7 | 6,6 \pm 1,1 |
| 15 % wheat bran (710- 1250 μm) + 3 % rapeseed oil | 238,5 \pm 75,4 | 6,4 \pm 1,3 | 6,8 \pm 1,2 | 123,6 \pm 0,5 | 10,3 \pm 1,5 |
| 15 % wheat bran (710- 1250 μm) + 10 % rapeseed oil | 160,7 \pm 75,4 | 4,3 \pm 0,6 | 4,7 \pm 0,5 | 123,0 \pm 0 | 8,7 \pm 1,0 |
| 15 % β -glucan fibre + 3 % rapeseed oil | 232,2 \pm 68,4 | 7,3 \pm 0,6 | 6,2 \pm 0,4 | 121,5 \pm 1,3 | 9,0 \pm 2,0 |
| 15 % β -glucan fibre + 10 % rapeseed oil | 235,5 \pm 39,4 | 3,4 \pm 0,6 | 4,1 \pm 0,9 | 125,7 \pm 1,9 | 5,0 \pm 1,4 |

to 10 % rapeseed oil is lower compared to the melt pressure for the formulation containing 100 % PPI, as seen in table 4.3. According to literature [46], added lubricant can decrease the melt pressure due to decreased friction in the barrel, in accordance with observed results. The melt pressure for big wheat bran in addition to 10 % rapeseed oil is though similar as compared to 100 % PPI, presumably due to the decreased amount of soluble components, such as starch and β -glucan, in the protein network.

The melt temperature for the sample containing β -glucan fibre in addition to rapeseed oil is tending to increase upon addition of rapeseed oil, while no significant change is observed for the formulations containing *Potex Crown* or bigger wheat bran in addition to rapeseed oil. The reason for increased melt temperature, in addition to the indicated constant compressive stress, is interesting. β -glucan fibre contain 30-35 % starch and 20 % β -glucan [36], soluble components which presumably affect the extrusion differently compared to insoluble fibres which function as filler material in the melt [48]. The increased amount of melted phase could affect the properties of the melt during

extrusion and therefore effect the temperature and also the final stress resistance of the extruded material. Further investigation of soluble components in the formulations during extrusion is recommended in future works.

4.2.7 Comparison Between Additives

The texturization of pea protein isolate was analyzed regarding macrostructure, microstructure, compressive stress, cutting strength, melt temperature and melt pressure for the different formulations. The macrostructure divided the samples into two; wheat bran fibre that enhanced the layered structure and potato fibre and β -glucan fibre that reduced the layered structure and created more smooth and compact samples. This difference was believed to be due to the soluble components that was added when adding potato fibre and β -glucan fibre to the formulation, where starch was suggested to contribute to the smooth surface. When adding wheat bran fibre, the fibre instead only functioned as a filler phase, reducing the amount of non-interrupted protein areas, enhancing the layered structure. During addition of both rapeseed oil and fibre, a surface defect was observed for formulations containing β -glucan fibre and the small potato fibre, presumably due to the high content of lubricant, including both the added oil and the soluble starch and β -glucan that is included in the two fibres. The same defect is not seen for the formulation containing big wheat bran and rapeseed oil, presumably due to the lack of starch and soluble dietary fibres in the protein network.

The difference in smoothness in the macrostructure was also observed when conducting the compressive stress, where it is believed that a more compact and smooth sample is more stress resistant. It was observed that wheat bran fibre reduced the stress resistance in the material and potato fibre and β -glucan fibre increased the stress resistance in the material upon increased addition of additive. Though, all fibres except *Potex Crown* reduce the stress resistance compared to the stress resistance observed for the sample containing 100% PPI.

The microstructures revealed differences in directionality and interruption. Small fibres showed to interrupt the protein network to a larger extent compared to bigger fibres. The fibres contributing with soluble components to the protein network, here *Potex Crown* and β -glucan, decreased the directionality of the protein network, indicating a further reduced ability for the protein phase to texturize. The microstructure for rapeseed oil was difficult to analyze regarding directionality, but it was suggested that an increased amount of lubricant reduce the shear forces during extrusion and thus the ability for the protein phase to texturize.

The cutting tests generally displayed reduced cutting strengths upon increase of additive for all formulations analyzed with cutting test, indicating on a reduced texturization of

protein. This was believed to be due to either interruption from insoluble fibre components, which function as a filler phase, or the addition of soluble fibre components or lubricants, which reduce shear in the extruder, and thus reduce the ability for the protein phase to texturize.

So, the ability for the protein phase to form a textured structure is indicated to decrease upon addition of all analyzed additives in this project; including potato fibre, wheat bran, β -glucan fibre and rapeseed oil.

4.3 Evaluation of Analysis

Analysis of the macrostructure and microstructure were conducted in order investigate how the additives changed the visible structure of the extruded samples. The analysis of the structures proved useful since differences between the extruded formulations were observed. Iodine staining was appropriate for analysis of fibre content in the samples and displayed starch content for some sectioned samples. Osmium vapour staining was utilized for samples containing rapeseed oil. The staining was though relatively vague, so another staining agent for oil content would be interesting to investigate in future work.

Compressive stress values were correlated to the observed macrostructure of the extruded samples, where an increased compactness and smoothness of the macrostructure indicate increased compressive stress values, implying that the formed material was more stress resistant. Compressive stress analysis did though display big standard deviation originating from insecurities of test and variation in sample structure. The probe was moved manually to where it touches the surface of the sample, where after the test was initiated. The samples analyzed in compressive stress test exhibited varying surface structures, and a skewed and grooved surface gave deviating results. The samples also possessed varying degree of coherency, leading to some samples falling apart during compression. Additionally, the samples that broke before compression were not used in the analysis, giving some offset of results. Thereof, the compression test gave a significant standard deviation and uncertainty in results. In future work, it is recommended to instead evaluate the possibility of using tensile test or breaking test.

Analysis of cutting strength was found most useful in evaluating the texture of the material. The cutting strength varied for different additives, but generally decreased upon increase of all additives in the formulation for the formulations analyzed with cutting test. The samples that broke before cutting were not used, giving some offset of results, similar to the compression test. In future works, it is recommended to conduct a cutting test on all formulations analyzed.

5

Conclusions

THE GOAL of this project was to investigate the influence of different additives upon the texturing process of pea protein isolate during high moisture extrusion cooking. The main conclusion from this project is that the analysis of all additives included in the project indicated on a reduced ability to form a textured structure of pea protein during extrusion.

All fibres functioned as a filler material to some degree, interrupting the protein network to varying extent, reducing the ability for the protein network to texturize, in accordance with literature.

The effect from potato fibre varied depending on size. The bigger potato fibre, *Potex*, interrupted the protein network but the directionality in the network remained, while the smaller potato fibre disturbed the directionality in the protein network. The smaller potato fibre also brought starch to the protein network which is believed to reduce the ability to form a textured structure further. Increased concentrations of potato fibre lead to larger interruption of the protein network and the protein network was eventually broken up.

Formulations containing wheat bran fibre displayed a similar directionality in the protein network regardless of fibre size or fibre concentration. The fibres were interrupting the protein network more upon increase of fibre content, where smaller wheat bran (125-500 μm) interrupt the protein network further, reducing the continuity in the protein network.

When including β -glucan fibre in the formulation, the directionality of the protein network was reduced upon increase of fibre content, simultaneously as the cutting strength of the material was reduced. It is suggested that the protein network is effected by the soluble β -glucan fibre, which presumably could form a second molten phase that reduce the ability for the protein to form a texturized structure.

Rapeseed oil in the formulation functioned as a lubricant and it was indicated that the ability to form a texturized structure was reduced upon increase of rapeseed oil. The effect was mainly seen at concentrations of 5 % and 10 %. During addition of both rapeseed oil and fibre, the formulations containing β -glucan fibre and the small potato fibre displayed a surface defect, indicating extrusion difficulties. The cutting strength of the formed material containing fibre and rapeseed oil was lower compared to the strength of the material containing 100 % pea protein isolate. The cutting strength got further reduced upon increase of rapeseed oil in the formulations, indicating that addition of fibre and rapeseed oil reduce the ability to form a textured structure of pea protein.

So, to conclude, the ability of the pea protein to form a textured structure is indicated to be reduced upon addition of potato fibre, wheat bran fibre, β -glucan fibre or rapeseed oil. The amount of soluble components in the fibres proved important, where soluble components presumably form a separate molten phase that tended to reduce the ability for the pea protein to form a textured structure.

6

Future Work

THE MAIN SETTING TO INVESTIGATE FURTHER is the temperature of extrusion. In this project, it was seen that the temperature of 135°C created a texture without significant difference in longitudinal or transverse direction for pea protein isolate. According to literature, higher temperatures can form longitudinally aligned protein textures from pea protein isolate [66].

When analyzing formulations containing wheat bran, especially the formulations containing the larger wheat bran (710-1250 µm), using a light microscope, it is recommended to either use a smaller magnification or use the technique of multiple image alignment (MIA). The magnification used in this project to analyze the microstructure of the formulations containing wheat bran fibre was too large to determine a difference between the different fibre concentrations.

The microstructure for the formulations containing 20 % β -glucan displayed a protein network that was presumably affected by a compound not visible with iodine staining. The suggestion is that the 25 % of β -glucan present in the fibre affects the protein network. Thus, it is recommended in future studies to use a staining agent that stains β -glucan in light microscopy or use another microscopy technique. The same goes for samples containing rapeseed oil, another analyzing method than osmium staining in light microscopy is recommended in future studies.

Compressive stress implicated large standard deviations and the stress values were not directly correlated to the degree of texturization of the pea protein. An evaluation of using tensile test or breaking test is instead recommended.

During the course of this project, investigations of the effect of freezing and frying the extruded samples was initiated. The results were though not summarized and analysed, so for future studies it is recommended to continue the work of investigating the ability to freeze and fry the samples.

In a broader scope, other additives as well as sensory properties, nutritional factors and appearance is interesting to investigate, in order to create an attractive product for consumers. Additives, such as color enhancers, sugars, salt and pH-adjusters, affect the formed protein fibre structure [68]. Salt is known to shield the charged sites on proteins and other biopolymers and thereof affect the interactions between the two [7]. pH-value also greatly affect the reaction system between proteins and polysaccharides, affecting the surface charge, and therefore it is of interest to also investigate the pH of the specific system [7].

Bibliography

- [1] Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, de Haan C. Livestock's long shadow. Rome: FAO of the UN; 2006. Available from: <http://www.fao.org/docrep/010/a0701e/a0701e00.HTM>.
- [2] Nations U. Climate Change;. Available from: <http://www.un.org/en/sections/issues-depth/climate-change/>.
- [3] Nijdam D, Rood T, Westhoek H. The price of protein: Review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. Food Policy. 2012;37:760–770. Available from: https://ac-els-cdn-com.proxy.lib.chalmers.se/S0306919212000942/1-s2.0-S0306919212000942-main.pdf?_tid=383ecf26-c2e3-47fc-871d-ab9de2160118&acdnat=1540976985_73699ea848deb65913dcac849cab5639.
- [4] Osen R, Toelstede S, Eisner P, Schweiggert-Weisz U. Effect of high moisture extrusion cooking on protein-protein interactions of pea (*Pisum sativum* L.) protein isolates. International Journal of Food Science & Technology. 2015 6;50(6):1390–1396. Available from: <http://doi.wiley.com/10.1111/ijfs.12783>.
- [5] Berk Z. Extrusion. In: Food Process Engineering and Technology. 2nd ed. London: Elsevier Inc; 2013. p. 373–391. Available from: <http://link.springer.com/10.1007/978-3-319-54217-1>.
- [6] Osen R, Toelstede S, Wild F, Eisner P, Schweiggert-Weisz U. High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. Journal of Food Engineering. 2014;(127):67–74. Available from: <http://dx.doi.org/10.1016/j.jfoodeng.2013.11.023>.
- [7] Yuan Y, Wan ZL, Yang XQ, Yin SW. Associative interactions between chitosan and soy protein fractions: Effects of pH, mixing ratio, heat treatment and ionic strength. Food Research International. 2014;55:207–214. Available from: <http://dx.doi.org/10.1016/j.foodres.2013.11.016>.

- [8] Martínez-Villaluenga C, Piotr AE, Ae G, Frias J, Krzysztof AE. Assessment of protein fractions of three cultivars of *Pisum sativum* L.: effect of germination. *Eur Food Res Technology*. 2008;226:1465–1478. Available from: <https://link-springer-com.proxy.lib.chalmers.se/content/pdf/10.1007%2Fs00217-007-0678-9.pdf>.
- [9] Jordbruksverket. Export och import av livsmedel;. Available from: <https://www.jordbruksverket.se/omjordbruksverket/statistik/statistikomr/utrikeshandel/basfaktaomsverigeshandelmedlivsmedel.4.116e9b9d159b31e6cb943e47.html>.
- [10] Olsson Y. Jordbruksmarkens användning 2018. Statens Jordbruksverk; 2018. Available from: <https://www.jordbruksverket.se/webdav/files/SJV/Amnesomraden/Statistik,%20fakta/Arealer/J010/J010SM1801/J010SM1801.pdf>.
- [11] Sandberg AS. Developing functional ingredients: a case study of pea protein. In: *Functional Foods - Concept to Product*. 2nd ed. Woodhead Publishing; 2011. p. 563,564. Available from: https://app.knovel.com/web/toc.v/cid:kpFFCPE001/viewerType:toc//root_slug:functional-foods-concept/url_slug:standard-procedures-preparation?b-q=pea&toc-within=pea&issue_id=kt0093LIN1.
- [12] Warkentin TD, Smýkal P, Coyne CJ, Weeden N, Domoney C, Bing DJ, et al. Pea. In: *Grain Legumes*. Springer, New York, NY; 2015. p. 37–83. Available from: http://link.springer.com/10.1007/978-1-4939-2797-5_2.
- [13] Smýkal P, Konečná E. Advances in Pea Genomics. In: *Legumes in the Omic Era*. New York, NY: Springer New York; 2014. p. 301–337. Available from: http://link.springer.com/10.1007/978-1-4614-8370-0_15.
- [14] Walsh G. Proteins and proteomics. In: *Proteins: Biochemistry and Biotechnology*. John Wiley & Sons, Incorporated; 2014. p. 1–2. Available from: <https://ebookcentral.proquest.com/lib/chalmers/reader.action?docID=1647734&ppg=1>.
- [15] Goodman M, Toniolo C, Moroder L, Aurthur F. The Peptide Bond. In: *Houben-Weyl Methods in Organic Chemistry, Volume E22, - Synthesis of Peptides and Peptidomimetics, Volumes 1-5 (Workbench Edition) - Knovel*. 22nd ed. Thieme Medical Publishers Inc.; 2004. p. 18–19. Available from: https://app.knovel.com/web/toc.v/cid:kpHWMOCV02/viewerType:toc//root_slug:houben-weyl-methods-in/url_slug:the-peptide-bond?&issue_id=kt00APX9L6.
- [16] Mauraya AK, Said PP. Extrusion processing on physical and chemical properties of protein rich productsan overview. *Cancer Causes & Control*. 2014;12:461–475.

- [17] Guy R. Raw Materials for Extrusion Cooking. In: Extrusion Cooking: Technologies and Applications. Cambridge: Woodhead Publishing; 2001. p. 2–21. Available from: https://app.knovel.com/web/toc.v/cid:kpECTA0006/viewerType:toc/root_slug:extrusion-cooking-technologies?kpromoter=federation.
- [18] Dietary Fibre; 2018. Available from: <https://ec.europa.eu/jrc/en/health-knowledge-gateway/promotion-prevention/nutrition/fibre>.
- [19] Arendt E, Zannini E. Dietary Fibre. In: Cereal Grains for the Food and Beverage Industries - Knovel. Elsevier; 2013. p. 80–81. Available from: https://app.knovel.com/web/toc.v/cid:kpCGFBI003/viewerType:toc/root_slug:cereal-grains-food-beverage/url_slug:maize-carb-starch?&issue_id=kt010XNSB1.
- [20] Ralet MC, Buffetto R, Capron I, Guillon F. Cell Wall Polysaccharides of Potato. In: Singh J, Kaur L, editors. Advances in Potato Chemistry and Technology. 2nd ed. Elsevier; 2016. p. 33–57. Available from: https://app.knovel.com/web/view/khtml/show.v/rcid:kpAPCTE001/cid:kt010WKCD2/viewerType:khtml/root_slug:2-cell-wall-polysaccharides-of-potato/url_slug:cell-wall-polysaccharides?kpromoter=federation&b-toc-cid=kpAPCTE001&b-toc-root-slug=&b-toc-url-slug=cell.
- [21] Lee SH, Jang Y, Kim MY, Hwang IG, Kim HY, Woo KS, et al. Optimization of extraction conditions of barley β -glucan by oxidation with hydrogen peroxide and heat treatment. Journal of Cereal Science. 2015;65:147–153. Available from: <http://dx.doi.org/10.1016/j.jcs.2015.07.003>.
- [22] Delcour J, Poutanen K. The range of dietary fibre ingredients and a comparison of their technical functionality. In: Fibre-Rich and Wholegrain Foods: Improving Quality. Elsevier Science & Technology; 2013. p. 96–116. Available from: <https://ebookcentral.proquest.com/lib/chalmers/reader.action?docID=1575054&ppg=125>.
- [23] Verma D, Jain S, Zhang X, Gope PC. Natural Fibers for the Production of Green Composites. In: Green Approaches to Biocomposite Materials Science and Engineering. IGI Global; 2016. p. 8–11. Available from: https://app.knovel.com/web/toc.v/cid:kpGABMSE03/viewerType:toc/root_slug:green-approaches-biocomposite/url_slug:hemicellulose?&issue_id=kt010YSEX3.
- [24] Xiao Z, Li Y, Wu X, Qi G, Li N, Zhang K, et al. Utilization of sorghum lignin to improve adhesion strength of soy protein adhesives on wood veneer. Industrial Crops & Products. 2013;50:501–509. Available from: <http://dx.doi.org/10.1016/j.indcrop.2013.07.057>.
- [25] Havrlentová M, Petruláková Z, Burgárová A, Gago F, Hlinková A, Šturdík E. Cereal β -glucans and their Significance for the Preparation of Functional Foods-A Review;

2011. 1. Available from: https://www.agriculturejournals.cz/publicFiles/162_2009-CJFS.pdf.
- [26] Sadler MJ. Authorised EU Health Claims for Barley and Oat Beta-Glucans. In: Foods, Nutrients and Food Ingredients with Authorised EU Health Claims. Elsevier; 2014. p. 25–28. Available from: https://app.knovel.com/web/toc.v/cid:kpFNFIAEUQ/viewerType:toc//root_slug:foods-nutrients-food/url_slug:authorised-eu-health?&issue_id=kpFNFIAEUQ.
- [27] Nyman M, Haskå L. Vegetable, fruit and potato fibres. In: Delcour J, Poutanen K, editors. Fibre-rich and wholegrain foods: Improving quality. Cambridge: Woodhead Publishing; 2013. p. 193–207. Available from: <https://ebookcentral-proquest-com.ezproxy01.rhul.ac.uk/lib/rhul/reader.action?docID=3095323&ppg=41>.
- [28] Vincken JP, Schols HA, Oomen RJFJ, Beldman G, Visser RGF, Voragen AGJ. Pectin the Hairy Thing. In: Advances in Pectin and Pectinase Research. Dordrecht: Springer Netherlands; 2003. p. 47–59. Available from: http://link.springer.com/10.1007/978-94-017-0331-4_4.
- [29] Damodaran S, Parkin KL. Polysaccharides. In: Fennema's Food Chemistry. 5th ed. CRC Press; 2017. p. 158,160. Available from: https://app.knovel.com/web/toc.v/cid:kpFFCE001G/viewerType:toc//root_slug:fennemas-food-chemistry/url_slug:carbohydrates?&issue_id=kpFFCE001G.
- [30] BeMiller JN, Whistler RL. Starch Use in Foods. In: Starch : chemistry and technology. Burlington: Academic; 2009. p. 748–751. Available from: <https://ebookcentral.proquest.com/lib/chalmers/detail.action?docID=428579>.
- [31] Bertoft E. Fine Structure of Amylopectin. In: Starch. Tokyo: Springer Japan; 2015. p. 3–40. Available from: http://link.springer.com/10.1007/978-4-431-55495-0_1.
- [32] Harries P. Cell-Wall Polysaccharides of Potatoes. In: Singh J, Kaur L, editors. Advances in Potato Chemistry and Technology. Elsevier; 2009. p. 63–81. Available from: https://app.knovel.com/web/toc.v/cid:kpAPCT0018/viewerType:toc//root_slug:advances-in-potato-chemistry/url_slug:cell-wall-polysaccharides?&issue_id=kpAPCT0018.
- [33] Edwards WP. The Wheat Seed. In: Science of Bakery Products. Royal Society of Chemistry; 2007. p. 31–32. Available from: https://app.knovel.com/web/toc.v/cid:kpSBP00001/viewerType:toc//root_slug:science-bakery-products/url_slug:the-wheat-seed?&issue_id=kt00BYP811.
- [34] Jääskeläinen AS, Holopainen-Mantila U, Tamminen T, Vuorinen T. Endosperm and aleurone cell structure in barley and wheat as studied by optical and Raman microscopy. Journal of Cereal Science. 2013;57:543–550. Available from: <http://dx.doi.org/10.1016/j.jcs.2013.02.007>.

- [35] Wheat Bran. In: Dietary Supplements Compendium. 1st ed. The United States Pharmacopeial Convention; 2009. p. 741–742. Available from: https://app.knovel.com/web/toc.v/cid:kpDSCE0001/viewerType:toc//root_slug:dietary-supplements-compedium/url_slug:official-monographs-wheat-bran?&issue_id=kt008JG0I1.
- [36] Johansson K. Ungefärlig komposition av nytt betaglukan. Lyckeby Culinar [email]; 20160621.
- [37] Singh RP, Heldman DR. Extrusion Process for Foods. In: Introduction to Food Engineering. Elsevier Inc; 2014. p. 743–766.
- [38] Riaz MN. Selecting the Right Extruder. In: Extrusion Cooking: Technologies and Applications. Cambridge: Woodhead Publishing; 2001. p. 29–50. Available from: https://app.knovel.com/web/view/khtml/show.v/rcid:kpECTA0006/cid:kt002QST72/viewerType:khtml//root_slug:3-selecting-the-right-extruder/url_slug:selecting-right-extruder?&issue_id=kpECTA0006&b-toc-cid=kpECTA0006&b-toc-root-slug=&b-toc-url-slug=selecting-ri.
- [39] Harper JM, Clark JP. Food extrusion. C R C Critical Reviews in Food Science and Nutrition. 1979 2;11(2):155–215. Available from: <http://www.tandfonline.com/doi/abs/10.1080/10408397909527262>.
- [40] Kanai T, Campbell GA. Extruder and Screw Design for Film Processing. In: Film Processing Advances. Hanser Publishers; 2014. p. 1–26. Available from: https://app.knovel.com/web/toc.v/cid:kpFPA00011/viewerType:toc/root_slug:film-processing-advances?kpromoter=federation.
- [41] Kazemzadeh M. Introduction to Extrusion Technology. In: Maskan M, Altan A, editors. Advances in Food Extrusion Technology. 1st ed. Contemporary Food Engineering. Boca Raton: CRC Press; 2011. p. 1–22. Available from: <https://www.taylorfrancis.com/books/9781439815212>.
- [42] Akdogan H. High moisture food extrusion. International Journal of Food Science & Technology. 1999;34:195–207.
- [43] Damodaran S, Parkin KL. Protein denaturation. In: Fennema's Food Chemistry. 5th ed. CRC Press; 2017. p. 269. Available from: https://app.knovel.com/web/toc.v/cid:kpFFCE001G/viewerType:toc//root_slug:fennemas-food-chemistry/url_slug:protein-denaturation?&issue_id=kt011MEUH5.
- [44] Burgess LD, Stanley DW. A Possible Mechanism for Thermal Texturization of Soybean Protein. Ontario: Department of Food Science; 1976. Available from: <https://eurekamag.com/pdf/004/004609400.pdf>.
- [45] Sandoval Murillo JL, Osen R, Hiermaier S, Ganzenmüller G. Towards understanding the mechanism of fibrous texture formation during high-moisture extrusion of meat

- substitutes. *Journal of Food Engineering*. 2019 2;242:8–20. Available from: <https://www.sciencedirect.com/science/article/pii/S0260877418303406>.
- [46] Ilo S, Schoenlechner R, Berghofe E. Role of lipids in the extrusion cooking processes. *Grasas y Aceites*. 2000;51(1-2):97–110.
- [47] Damodaran S, Parkin KL. Starch. In: Fennema's Food Chemistry. 5th ed. CRC Press; 2017. p. 132–149. Available from: https://app.knovel.com/web/toc.v/cid:kpFFCE001G/viewerType:toc//root_slug:fennemas-food-chemistry/url_slug:granule-gelatinization?&issue_id=kt011MEQR4.
- [48] Arêas JAG. Extrusion of food proteins. *Critical Reviews in Food Science and Nutrition*. 1992 1;32(4):365–392. Available from: <http://www.tandfonline.com/doi/abs/10.1080/10408399209527604>.
- [49] Camire ME. Extrusion cooking. In: *Nutrition Handbook for Food Processors*. Woodhead Publishing; 2002. p. 314–330. Available from: https://app.knovel.com/web/toc.v/cid:kpNHFP0004/viewerType:toc//root_slug:nutrition-handbook-food/url_slug:extrusion-cooking?&issue_id=kpNHFP0004.
- [50] L Flemming F. Microscopy. Salem Press; 2013. Available from: <http://eds.a.ebscohost.com/eds/detail/detail?vid=5&sid=9767cb09-2421-4c10-b28c-f90cebabf435%40sessionmgr4007&bdata=Jmxxhmc9c3Ymc2l0ZT1lZHMtbGl2ZSZZY29wZT1zaXRl#AN=87690582&db=ers>.
- [51] Davis JR. Differential Interference Contrast Microscopy. In: *Metals Handbook, Desk Edition*. 2nd ed. ASM International; 2015. p. 1362. Available from: https://app.knovel.com/web/view/khtml/show.v/rcid:kpMHDEE004/cid:kt010QW1F6/viewerType:khtml//root_slug:metals-handbook-desk/url_slug:differential-interference?&kpromoter=federation&page=7&view=collapsed&zoom=1.
- [52] Hyam P. Understanding and Maintaining the Cryostat. New York: New Jersey Medical School; 2010. Available from: <file:///C:/Users/johannaer/Downloads/APracticalGuidetoFrozenSectionTechnique.pdf>.
- [53] Santacruz S, Andersson R, Man PA. Characterisation of potato leaf starch with iodine-staining. *Carbohydrate Polymers*. 2004;(59):397–400. Available from: www.elsevier.com/locate/carbpol.
- [54] Belazi D. Visualizing and profiling tissue lipids by ToF-SIMS imaging mass spectrometry. Stockholm: Karolinska Institutet; 2009. Available from: <https://openarchive.ki.se/xmlui/bitstream/handle/10616/40194/thesis.pdf;sequence=1>.
- [55] Food Technology Corporation. How a Texture Analyzer Works; 2016. Available from: <https://www.foodtechcorp.com/how-texture-analyzer-works>.

-
- [56] Kolmin R. What is a Texture Analyzer?; 2015. Available from: <http://www.instron.se/sv-se/our-company/press-room/blog/2016/june/what-is-a-texture-analyzer>.
- [57] Figura LO, Teixeira AA. Rheological Properties. In: Food Physics. Berlin, Heidelberg: Springer Berlin Heidelberg; 2007. p. 117–206. Available from: <http://link.springer.com/10.1007/978-3-540-34194-9>.
- [58] Cosucra Groupe Warcoing. Product Sheet Pisane C9; 2008. Available from: <http://chefpeters.com/pict/Pisane%20C9%20-%20Spec.pdf>.
- [59] Stora Smakupplevelser till konsumenter. Fjälkinge: Lyckeby Culinar AB; 2015. Available from: https://www.culinar.se/wp-content/uploads/2016/06/Culinar_Bageri_NEWS_SE.pdf.
- [60] Johansson K. Komposition Potex. Lyckeby Culinar [email]; 20140206.
- [61] Kungsörnen Lantmännen. Nyckelhålsmärkt Kruskakli; 2018. Available from: <https://www.kungsornen.se/produkter/mjol/kli--kross/kruskakli-350g/>.
- [62] Zeta. Rapsolja; 2018. Available from: <https://www.zeta.nu/produkter/rapsolja/>.
- [63] Microsoft. Funktionen MEDEL;. Available from: <https://support.office.com/sv-se/article/funktionen-medel-047bac88-d466-426c-a32b-8f33eb960cf6>.
- [64] Microsoft. STDAV.P;. Available from: <https://support.office.com/sv-se/article/stdav-p-funktionen-stdav-p-6e917c05-31a0-496f-ade7-4f4e7462f285>.
- [65] Miedzianka J, Peksa A, Pokora M, Rytel E, Tajner-Czopek A, Kita A. Improving the properties of fodder potato protein concentrate by enzymatic hydrolysis. Food Chemistry. 2014;159:512–518.
- [66] Chen FL, Wei YM, Zhang B, Ojokoh AO. System parameters and product properties response of soybean protein extruded at wide moisture range. Journal of Food Engineering. 2010 1;96(2):208–213. Available from: <https://www.sciencedirect.com/science/article/pii/S0260877409003653>.
- [67] Rauwendaal C. Troubleshooting Extruders. In: Polymer Extrusion. 5th ed. Hanser Publishers; 2014. p. 821–822. Available from: https://app.knovel.com/web/toc.v/cid:kpPEE00022/viewerType:toc//root_slug:polymer-extrusion-5th/url_slug:extrusion-instabilities?&issue_id=kt010RRK41.
- [68] Yada RY. Effect of Additives on Texturized Vegetable Protein. In: Proteins in Food Processing. Woodhead Publishing; 2004. p. 531–534. Available from: https://app.knovel.com/web/toc.v/cid:kpPFP00004/viewerType:toc//root_slug:proteins-in-food-processing/url_slug:effect-additives-texturized?&issue_id=kt003R03H1.

Appendices

A

Sample Composition

In the following appendix, the exact sample composition in gram is presented, see table A.1.

APPENDIX A. SAMPLE COMPOSITION

Table A.1: In the table, the mass of fibres, fat, pea protein isolate and water for all the samples prepared is presented. The content are presented in (g). The total weight of each sample is 400g.

| General composition | 160 g | | | 240 g |
|------------------------------------|-----------|---------|----------------------------|-----------|
| | Fibre (g) | Oil (g) | Pisane [®] C9 (g) | Water (g) |
| Pisane[®] C9 | - | - | 160 | 240 |
| Potex (>1000 µm) | 16 | - | 144 | ” |
| | 24 | - | 136 | ” |
| | 32 | - | 128 | ” |
| Potex Crown (>200 µm) | 16 | - | 144 | ” |
| | 24 | - | 136 | ” |
| | 32 | - | 128 | ” |
| Wheat bran (710-1250 µm) | 16 | - | 144 | ” |
| | 24 | - | 136 | ” |
| | 32 | - | 128 | ” |
| Wheat bran (125-500 µm) | 16 | - | 144 | ” |
| | 24 | - | 136 | ” |
| | 32 | - | 128 | ” |
| β-glucan fibre | 16 | - | 144 | ” |
| | 24 | - | 136 | ” |
| | 32 | - | 128 | ” |
| Rapeseed oil | - | 1,6 | 158,4 | ” |
| | - | 4,8 | 155,2 | ” |
| | - | 8 | 152 | ” |
| | - | 16 | 144 | ” |
| Potex Crown (>200 µm) | 24 | 4,8 | 131,2 | ” |
| | 24 | 16 | 120 | ” |
| Wheat bran (710-1250 µm) | 24 | 4,8 | 131,2 | ” |
| | 24 | 16 | 120 | ” |
| β-glucan fibre | 24 | 4,8 | 131,2 | ” |
| | 24 | 16 | 120 | ” |

B

Extrusion Data

Data, collected during extrusion with the software *FeCon* (Feller Engineering, Germany), is presented for all extruded samples in table B.1. The data is presented as the mean value from for all data points collected for each sample composition, though the data points for the first and last minute of extrusion is excluded. The mean value is calculated using *Microsoft™ Excel* in *Office 365* and the function *=MEDEL*.

Table B.1: A table of all extruder properties, presented as the mean value for all data collected during extrusion for each sample composition.

| | Melt pressure (bar) | Melt temperature (°C) | Screw speed (rpm) | Zone 1 (°C) | Zone 2 (°C) | Zone 3 (°C) | Zone 4 (°C) | Zone 5 (°C) |
|------------------------|---------------------|-----------------------|-------------------|-------------|-------------|-------------|-------------|-------------|
| Pisane [®] C9 | 7,8 | 122,1 | 60,0 | 29,9 | 79,6 | 119,6 | 135,5 | 59,0 |
| 10 % <i>Potex</i> | 12,1 | 118,7 | 60,0 | 30,0 | 79,7 | 120,6 | 137,0 | 53,6 |
| 15 % <i>Potex</i> | 13,3 | 120,4 | 60,0 | 29,9 | 80,1 | 120,0 | 134,9 | 57,5 |
| 20 % <i>Potex</i> | 13,1 | 121,9 | 60,0 | 30,0 | 80,0 | 120,0 | 135,0 | 59,2 |

APPENDIX B. EXTRUSION DATA

| | Melt pressure (bar) | Melt temperature (°C) | Screw speed (rpm) | Zone 1 (°C) | Zone 2 (°C) | Zone 3 (°C) | Zone 4 (°C) | Zone 5 (°C) |
|--|---------------------|-----------------------|-------------------|-------------|-------------|-------------|-------------|-------------|
| 10 % <i>Potex Crown</i> | 13,1 | 122,9 | 60,0 | 30,0 | 80,0 | 120,0 | 135,0 | 62,3 |
| 15 % <i>Potex Crown</i> | 11,3 | 121,6 | 60,0 | 30,0 | 80,3 | 120,0 | 135,0 | 58,6 |
| 20 % <i>Potex Crown</i> | 12,8 | 121,2 | 60,0 | 30,0 | 81,1 | 120,0 | 135,1 | 56,6 |
| 10 % wheat bran (710-1250 µm) | 14,1 | 122,3 | 60,0 | 29,8 | 80,1 | 120,0 | 135,1 | 63,4 |
| 15 % wheat bran (710-1250 µm) | 12,6 | 124,1 | 60,0 | 30,0 | 80,0 | 120,0 | 135,0 | 67,7 |
| 20 % wheat bran (710-1250 µm) | 12,9 | 124,1 | 60,0 | 29,9 | 80,0 | 120,0 | 135,0 | 68,1 |
| 10 % wheat bran (125-500 µm) | 19,4 | 120,7 | 60,0 | 30,0 | 79,9 | 120,0 | 135,0 | 59,2 |
| 15 % wheat bran (125-500 µm) | 18,9 | 120,1 | 60,0 | 30,0 | 80,0 | 120,1 | 135,0 | 58,5 |
| 20 % wheat bran (125-500 µm) | 17,0 | 120,3 | 60,0 | 30,0 | 79,9 | 119,6 | 134,5 | 60,0 |
| 10 % β -glucan fibre | 10,7 | 120,7 | 60,0 | 30,0 | 80,1 | 119,9 | 135,0 | 58,4 |
| 15 % β -glucan fibre | 8,8 | 121,7 | 60,0 | 30,0 | 80,2 | 120,1 | 135,0 | 59,7 |
| 20 % β -glucan fibre | 7,7 | 120,7 | 60,0 | 29,9 | 81,4 | 119,9 | 134,9 | 57,8 |
| 1 % rapeseed oil | 8,9 | 121,4 | 60,0 | 29,9 | 79,9 | 119,9 | 134,9 | 61,4 |
| 3 % rapeseed oil | 7,1 | 124,7 | 60,0 | 30,0 | 79,9 | 119,8 | 135,0 | 67,5 |
| 5 % rapeseed oil | 9,6 | 123,3 | 60,0 | 30,0 | 80,6 | 120,1 | 134,9 | 66,0 |
| 10 % rapeseed oil | 11,0 | 118,1 | 60,0 | 30,0 | 80,0 | 113,5 | 128,8 | 63,9 |
| 15 % <i>Potex Crown</i> + 3 % rapeseed oil | 8,5 | 124,1 | 60,0 | 30,0 | 80,2 | 120,0 | 134,7 | 65,2 |
| 15 % <i>Potex Crown</i> + 10 % rapeseed oil | 6,6 | 124,0 | 60,0 | 30,0 | 79,8 | 119,9 | 135,0 | 64,2 |
| 15 % β -glucan + 3 % rapeseed oil | 9,0 | 121,5 | 60,0 | 30,0 | 80,1 | 120,0 | 135,4 | 63,1 |

APPENDIX B. EXTRUSION DATA

| | Melt pressure (bar) | Melt temperature (°C) | Screw speed (rpm) | Zone 1 (°C) | Zone 2 (°C) | Zone 3 (°C) | Zone 4 (°C) | Zone 5 (°C) |
|--|---------------------|-----------------------|-------------------|-------------|-------------|-------------|-------------|-------------|
| 15 % β -glucan + 10 % rapeseed oil | 5,0 | 125,7 | 60,0 | 29,8 | 79,9 | 119,9 | 135,0 | 69,7 |
| 15 % wheat bran (710-1250 μ m) + 3 % rapeseed oil | 10,3 | 123,6 | 60,0 | 30,0 | 80,0 | 120,0 | 135,0 | 63,9 |
| 15 % wheat bran (710-1250 μ m) + 10 % rapeseed oil | 8,7 | 123,0 | 60,0 | 30,0 | 80,0 | 120,1 | 135,0 | 63,4 |