Analyzing potato protein isolates for their potential in developing meat analogues

A Master's Thesis by

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Abstract

To keep up with the rapidly increasing global population, a significant increase in production of protein foods is required. The environmental impact of the meat industry, in combination with consumer awareness, calls for this food production to become more sustainable. One solution is development of meat analogues, which are meat-like texturized products based on vegetable proteins.

In this project, potato proteins were analyzed for their potential in developing these meat analogues. Currently, potato proteins with great nutritional value often go to waste as a by-product of the potato starch industry. Therefore, finding ways to utilize these proteins in the food industry is both economically and environmentally beneficial.

High moisture extrusion is a promising technique for obtaining fibrous products from vegetable proteins. Here, a Rapid Visco Analyzer (RVA) was used to mimic the process conditions inside an extruder, and to study the behavior of the potato proteins during high temperature (>100°C) heating and subsequent cooling. Various parameters were analyzed, such as heating temperature, cooling rate, shear rate, protein concentration, addition of potato starch, and addition of rapeseed oil. It was found that potato protein isolates were able to form compact texturized products in the RVA, when heated to 140°C. The best structure was obtained for a 6% protein concentration (w/w on wet basis) with addition of 1% starch and 1.5% rapeseed oil. Overall, this research shows that potato proteins show great potential for developing meat analogues, already at low protein concentrations.

Preface

I would first of all like to thank my supervisor Eva Tornberg, from Lund University and Veg of Lund AB, for her guidance and enthusiasm during the entire project, and for believing in me from day one. Her feedback and ideas have contributed greatly to all aspects of this project. Secondly, I would also like to thank Karolina Östbring, from Lund University, for taking the time to read and examine my thesis, and for her insightful feedback.

Since moving to Sweden, I have changed my diet to (almost entirely) vegetarian in an attempt to lead a more sustainable lifestyle. Growing up as an avid meat-eater, who is not very skilled at cooking, I felt a bit lost in my new found diet. Especially in the beginning, meat analogues were a great solution for me, since I could simply substitute meat with these meat alternatives in recipes I was already familiar with. After a while, I also found many recipes without the need for meat alternatives, but I definitely think they are a great option for clueless meat-eaters trying to lower their meat consumption, like I was. For this reason, this project immediately sparked my interest and it has been very exciting to dive deeper into the world of these "fake meats".

Working on a thesis project and trying to stay motivated during the 2020 Covid-19 pandemic was challenging at some times. However I am thankful that, living in southern Sweden, I was still able to finish my research in the lab and to complete my thesis without any delay.

Lastly, I would like to give a big thank you to all of my family and friends, near and abroad, who have supported me throughout these past two years while obtaining my master's degree at Lund University in Sweden.

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List of Abbreviations

DM	Dry Matter
HME	High Moisture Extrusion
HT	High Temperature
LME	Low Moisture Extrusion
n/a	Not Available
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PFJ	Potato Fruit Juice
pI	Iso-electric point
PPI	Potato Protein Isolate
PPI1	Lyckeby AB Potato Protein Isolate (Lyckeby AB, Sweden)
PPI2	Solanic®200 (Avebe, The Netherlands)
PPI3	Solanic®300 (Avebe, The Netherlands)
RVA	Rapid Visco Analyzer
w/w	Weight per weight

1 Introduction

The growing global population, in combination with its economic growth, demands a significant increase in production of protein foods, such as meat products. However, production of meat products has a substantial impact on the environment since it requires a large amount of water, land use and energy. Furthermore, the conversion from plant protein into animal protein through animal feed is highly inefficient (Steinfeld, et al., 2006). As sustainability is becoming increasingly important to consumers, in combination with ethical aspects and health concerns, a promising solution is the partial replacement of meat proteins in the human diet with plant proteins (Smil, 2002).

While consumers are generally aware of the issues surrounding meat consumption, only a fraction of the world population identifies as vegetarian (Bartz, 2014). One reason could be that meat is strongly embedded in many cultures and is often seen as a key aspect of a nutritious diet. Another reason is that consumers feel a lack of familiarity with cooking vegetarian meals (Schösler, de Boer, & Boersema, 2012). This is where meat analogues provide a possible solution. Meat analogues are plant-based "meat" products which can be prepared and consumed in the same manner as their meat-counterpart (Hoek, et al., 2011).

A promising technique for obtaining a meat-like fibrous structure from plant proteins is high moisture extrusion (HME). In the long cooling die of a high moisture extruder, the moisture, temperature, pressure and shear are varied to plasticize and texturize the plant proteins (Noguchi, 1989). Molecular transformations in combination with chemical reactions between the protein molecules can then result in a stabilized three-dimensional network, which resembles a meat-like structure (Chen, Wei, & Zhang, 2011; Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014; Palanisamy, Franke, Berger, Heinz, & Töpfl, 2019).

Meat analogues can be produced from many sorts of protein sources, but are currently mostly produced from vegetable proteins such as soy protein (Kumar, et al., 2017). Proteins from soybean are cheap, have a comparable biological value to meat (Hoffman & Falvo, 2004), and are great at forming textured structures (Lin, Huff, & Hsieh, 2000). However, drawbacks of soy protein include that soy is a common food allergen, concerns regarding its anti-nutritional factors (Hurrell, et al., 1992), and that soybean has to be imported to Europe (EC, 2019). For these reasons, it remains interesting to continue researching other possible protein sources for developing meat analogues.

A product which is harvested in enormous quantities in Europe is the potato (De Cicco & Jeanty, 2019). Potato proteins have a nutritious amino acid composition, rich in the essential amino acids lysine, methionine, threonine, and tryptophan (OECD, 2002). Furthermore, potato protein is a waste product in industrial starch production, which makes it a sustainable plant protein source (Alt, et al., 2005). In this project, therefore, potato protein isolates are analyzed for their potential in developing texturized structures. Three different potato proteins will be considered in this project, namely Lyckeby Potato Protein (Lyckeby AB, Kristianstad, Sweden), Solanic®200 and Solanic®300 (Avebe, Veendam, Netherlands).

1.1 Aim

The aim of the project is to analyze whether potato protein isolates can be used as a protein source in developing meat analogues, resembling chicken meat. Firstly, the potato protein isolates were characterized and analyzed for gelling properties. Then, their ability to form texturized structures was further analyzed with a high temperature Rapid Visco Analyzer (HT-RVA) under various conditions.

1.2 Limitations

The main limitation in this project was the limited prior knowledge about the potato protein isolates in regards to their potential in developing texturized structures. Another limitation was that the Department of Food Technology in Lund, where this project was executed, did not have sufficient available staff with experience in high moisture extrusion of meat analogues to analyze the potato protein isolates in an extruder. Experiments to analyze the texturization of potato protein isolates were, therefore, executed using an HT-RVA. The RVA is a common empirical instrument used to predict behavior inside an extruder (Elliott, Dang, & Bason, 2012). Even though the HT-RVA can give a good indication of protein behavior inside an extruder, it needs to be noted that it is still a different machine than the extruder.

Furthermore, due to the global Covid-19 pandemic, the project was halted for a short period of two weeks, since it was uncertain whether student researchers were able to go to the lab during this time, and how this pandemic in Sweden would pan out.

2 Theoretical Background

2.1 Meat analogues

Meat analogues, also known as meat alternatives or vegetarian meat, are plant-based food products which resemble texture, flavor, and appearance of meat products (Kumar, et al., 2017). Some Asian traditional meat alternatives include texturized products, such as tempeh and tofu. However, these products are not as popular in Western cultures (Asgar , Fazilah, Huda, Bhat, & Karim, 2010), since most Western countries have meat embedded in their culture (Schösler, de Boer, & Boersema, 2012), and find it important that meat analogues resemble actual meat in order to find these products attractive (Hoek, et al., 2011).

2.1.1 Importance of meat alternatives

The reason that meat alternatives are important, can be summarized by four major concerns related to meat production and consumption (Kumar, et al., 2017; Post, 2012).

Firstly, the meat industry has a huge environmental impact, as it is responsible for a substantial portion of greenhouse gas emissions, depletion of natural resources, and water and energy consumption (FAO, 2006).

Secondly, due to the growing world population and increasing wealth in developing countries, meat resources will become scarce (FAO, 2011). More efficient production and sustainable food sources are required in order to keep up.

Another concern related to the meat industry, is animal welfare. The public is becoming increasingly aware of unethical treatment of animals during the entire chain of production, and more consumers are pursing diets with a lower meat intake.

Lastly, consumption of meat has also been linked to various health issues, such as cardiovascular diseases, diabetes and colorectal cancer (Larsson & Wolk, 2006; Song, Manson, Buring, & Liu, 2004). A study in the United States also linked meat consumption to an increased risk for obesity and central obesity, related to the higher intake of total fat and saturated fats from meat products (Wang & Beydoun, 2009; Leitzmann, 2005).

2.2 High moisture extrusion

Several techniques exist to produce meat analogues, depending on the type of meat one is trying to imitate. One of the most common techniques for developing meat analogues is extrusion, which can be divided into low-moisture and high-moisture extrusion (Dekkers, Boom, & van der Goot, 2018). Low moisture extrusion (LME), with a moisture content below 40%, results in products with a sponge-like texture, which fails to mimic the texture of real meat (Guy, 2001). High moisture extrusion (HME), however, is a promising technique for developing fibrous structures, similar to meat (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). During HME, the products are not expanded when they leave the extruder barrel, by using a long cooling die which reduces the exit temperature of the extruded product. In this long cooling die, the proteins are plasticized and texturized by varying the moisture, temperature, pressure and shear. Combining these parameters results in molecular transformation of the protein molecules, which contribute to stabilization of the three-dimensional network formed after the extrusion step (Noguchi, 1989; Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). The products formed during HME are characterized by their fibrous structure and enhanced appearance and texture compared to LME products (Osen, 2017).

Extrusion can be described as a biopolymer melt which forms two phases, a homogenous continuous phase and a dispersed, insoluble phase (Fig. 2.1). The dispersed phase can either be formed during processing in the barrel at high temperatures, or it could have been present in the raw material already. At high temperatures during the extrusion, the dispersed phase can undergo macromolecular transformations, such as deformation and re-alignment into a more solid and stable 3D-network (Dekkers, Boom, & van der Goot, 2018; Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014)

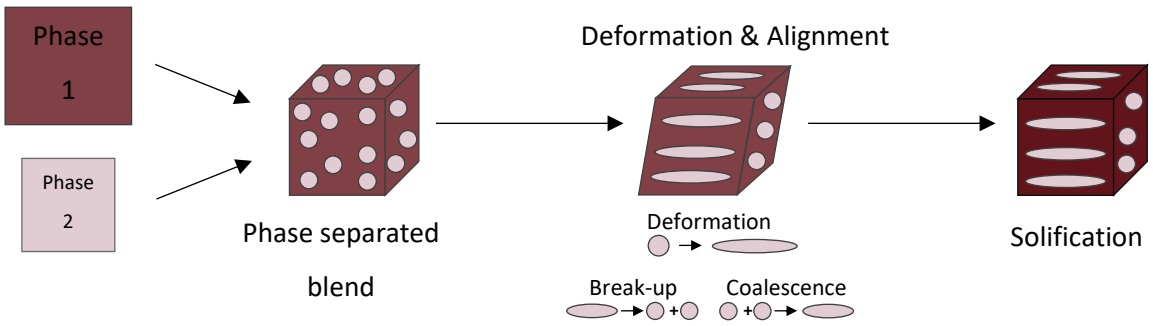

Figure 2.1. Schematic description of the extrusion process (Dekkers, Boom, & van der Goot, 2018)

A schematic figure depicting the actual protein changes during extrusion is shown in Figure 2.2. First, the native protein is denatured in a process involving heat and moisture. In the extruder barrel, heat, moisture, and shear are applied to the denatured proteins which forces the proteins to align. In the long cooling die, cross-linking of the aligned proteins results in texturization. These cross linkages can be non-covalent interactions or disulfide bonds, of which the latter has been proposed to be most important in formation of rigid fibrous structures . However, controversy exists in the literature whether this is true and it remains uncertain (Liu & Hsieh, 2008; Dekkers, Boom, & van der Goot, 2018; Osen, Toelstede, Eisner, & Schweiggert-Weisz, 2015).

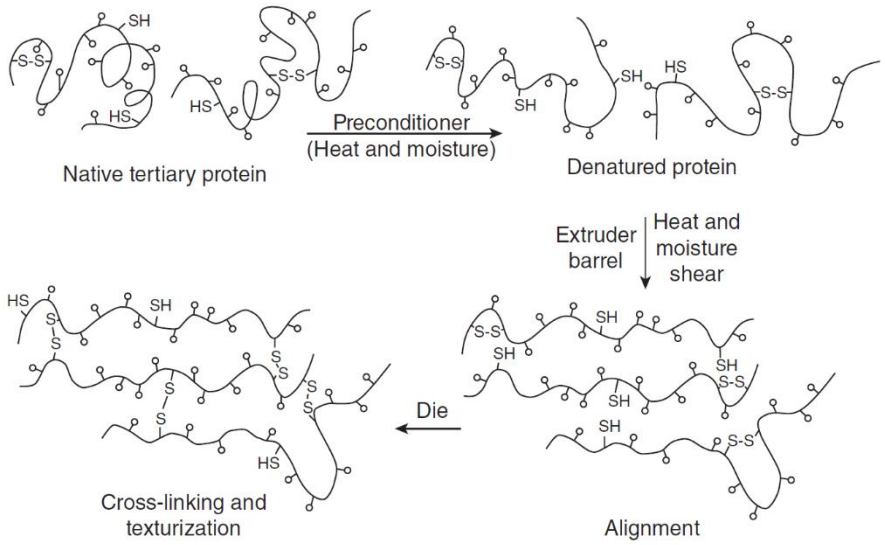

Figure 2.2. Schematic figure illustrating the protein changes during extrusion (Riaz, 2011).

One of the main challenges regarding extrusion is that it is mostly based on empirical knowledge. Although it has been studied for many years, the extruder is still seen as a "black box", where raw material goes in and a final extruded product comes out, while the processes inside the extruder barrel remain poorly understood (Emin & Schuchmann, 2017; Chen, Wei, & Zhang, 2011).

2.3 High Temperature Rapid Visco Analyzer (HT-RVA)

The high temperature RVA (HT-RVA) is a stirring viscometer with variable shear capability, which can heat samples up to 140°C. It is able to analyze the viscosity during various heating-cooling cycles, making it very versatile, and allowing for small scale simulations under controlled conditions (Martinez, 2020). Similarly to the HME process, the samples inside the HT-RVA undergo high temperature heating (>100°C), shear, and a long cooling process to prevent expansion. For these reasons, the RVA is a common empirical instrument used to predict behavior inside an extruder (Elliott, Dang, & Bason, 2012).

2.4 Potato protein

2.4.1 Extracting proteins from potato fruit juice

Compared to other vegetables, potatoes are not usually seen as a good dietary source of proteins, as they only contain around 1-1.5% protein. However, potato proteins remain an interesting raw material, since it is largely found in potato fruit juice (PFJ), which is a waste product of the potato starch industry. Over 6 million tons of this PFJ is produced annually in the EU, which contains over 80,000 tons of potato protein, and is also rich in minerals and free amino acids (Alt, et al., 2005; Waglay, Karboune, & Alli, 2014). It is both environmentally and economically beneficial to convert PFJ into high value ingredients, because the profit is marginal when used as animal feed or fertilizer, and the PFJ requires costly downstream processing steps in order to be safely disposed in the environment (Vikelouda & Kiosseoglou, 2004; Waglay, Karboune, & Alli, 2014).

The challenge of recovering highly functional potato proteins is in the complex composition of the PFJ. Industrially, PFJ often undergoes thermal coagulation and acidic precipitation to isolate the potato proteins, which is a high yield process, but often results in low quality potato proteins with no functionality (Cheng, Xiong, & Chen, 2014; Miedzianka, Pęksa, & Aniolowska, 2012). Other techniques for recovering functional proteins, include salt, acid, ethanol, and ammonium sulphate precipitations, carboxymethyl cellulose (CMC), complexation, and chromatographic techniques (Bárta, Heřmanová, & Diviš, 2007; Bártová & Bárta, 2009; van Koningsveld, et al., 2001; Vikelouda & Kiosseoglou, 2004). Solanic® potato protein isolates used in this project are isolated from PFJ using a combination of adsorption processes. This method results in highly functional potato proteins, because modification by chemical or enzymatic reactions is prevented (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011).

2.4.2 Potatoes and nutritional value of potato proteins

Potatoes are produced in many European countries in large quantities (De Cicco & Jeanty, 2019). Proteins from potatoes have an excellent amino acid composition full of essential amino acids, as well as a high biological value (BV), which indicates how well proteins can be absorbed in the body. The BV of potato proteins is 90-100, which is similar to that of eggs (100) and higher than the BV of soy proteins (84) (Camire, Kubow, & Donnelly, 2009; OECD, 2002). The amino acid composition can be scored using the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which for potato proteins is among the highest compared to other vegetable protein sources. Potato proteins obtained by Avebe (Solanic®, Veendam, the Netherlands) have a PDCAAS of 0.99, which is higher than that of wheat (0.43), soy (0.91), and slightly lower than that

of whole egg (1.19) and cow's milk (1.21) (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011).

Potato proteins can be divided into three classes: the 40 kDa patatin family (40% of total soluble proteins); the 5-25 kDa protease inhibitors (50% of total soluble proteins); and the others, which are mostly high molecular weight proteins (van Koningsveld, et al., 2006). Similar to soy protein, potato protease inhibitors have certain anti-nutritional properties, however these are not widely described in literature. Moreover, they show potential as anti-carcinogenic agents and positive dietary agents as they release cholecystokinin (CCK), which stimulates digestion of proteins and fats (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011; Hill, Peikin, Ryan, & Blundell, 1990; Kissileff, Pi-Sunyer, Thornton, & Smith, 1981).

2.4.3 Functionality of potato proteins

Functional properties of potato proteins can differ substantially depending on how they are recovered, their purity, salt content and composition. Generally, an important characteristic for successful application in food, is the solubility of the protein. This often determines other functional properties of a protein such as, gelling, emulsifying and foaming. The solubility of potato proteins is vastly dependent on the extraction technique from PFJ (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011).

Patatins, one class of potato proteins, have an iso-electric point (pI) between 4.5 and 5.2, which at a neutral pH and room temperature exist as a dimer bound mostly through hydrophobic interactions. It has a denaturation temperature of 60°C (at pH 7.0), and is relatively unstable as a function of pH (Pots, De Jongh, Gruppen, Hessing, & Voragen, 1998).

Protease inhibitors are the most abundant group of potato proteins found in PFJ, and are a more diverse group than patatins. They consists of proteins with a wider range of molecular weight and pI values. The pI of protease inhibitors is higher than that of patatins, and varies from 5.7 to 9.0. Protease inhibitors are mostly stabilized by disulfide bonds, which results in a good stability of the proteins as a function of pH (Pouvreau, et al., 2001).

Potato proteins have a good foamability, which is better than egg albumins and caseins, but worse than whey proteins (Jackman & Yada, 1988; Partsia & Kiosseoglou, 2001; Ralet & Gueguen, 2001). The foam capacity of patatins is lower than that of protease inhibitors, even though the latter is more unstable.

Emulsifying properties of potato proteins are not well described in literature, but it has been shown that protease inhibitors form more stable emulsions than patatins, especially at low pH values. Emulsions based mostly on patatins displayed droplet aggregation and a relative fast creaming rate, which is thought to be caused by the lipid acyl hydrolase (LAH) activity of patatins (van Koningsveld, et al., 2006)

Gelation behavior of potato proteins is also poorly described in literature, especially for protease inhibitors. However, research by Creusot et al. (2011) showed that patatins have excellent gelation properties, as it is able to gel at concentrations of 6% (w/v), due to its exposed hydrophobicity relative to other proteins.

3 Materials and Methods

All experiments were performed as described in this chapter. Any additional detailed notes of the procedures, including difficulties which were encountered, can be found in Appendix 1. All protein concentrations described in this thesis are on wet basis.

3.1 Characterization of potato protein isolates

Three potato protein isolates were used during these experiments: Lyckeby Potato Protein (Lyckeby AB, Kristianstad, Sweden), Solanic®200 and Solanic®300 (Avebe, Veendam, Netherlands). These potato protein isolates were labeled PPI1, PPI2, and PPI3, respectively.

3.1.1 pH value of 1% PPI solution

Firstly, the pH value of 1% PPI solutions in distilled water (w/w) was determined in order to get a general view of the PPIs. Prior to the experiment, the pH meter was calibrated using two standard buffers with pH 4.00 and pH 7.00. The samples were stirred with a magnetic stirrer at medium speed during the measurements, and the pH was determined for triplicate samples.

3.1.2 Dry matter and protein content determination

The total dry matter (DM) was determined according to the AOAC method (AOAC, 2000). Empty dishes were first dried in an oven at 105°C for five hours. After cooling the dishes in a desiccator, 5g of sample was weighed onto the dishes and dried in the oven at 105°C for another five hours. The dry matter was then calculated based on the weight difference of the sample before and after drying, using Equation 3.1. The measurements were performed in triplicates.

$$DM\ (\%) = \frac{weight\ of\ powder\ after\ drying\ (g)}{weight\ of\ powder\ before\ drying\ (g)} * 100\% \qquad (3.1)$$

The protein content of the PPIs was calculated based on the dry matter, according to the Dynamic Flash Combustion technique, also known as the modified Dumas method (Krotz, Leone, & Giazzi, 2016) using the FlashEA®1112 N/Protein Analyzer (Thermo Fisher Scientific, Waltham, United States). Pressed Tin capsules containing approximately 30 mg of dried protein powder were prepared and loaded into the attached autosampler, which automatically drops one capsule into the combustion reactor at a time. The measurements start with four calibration samples, one blank Tin capsule, two standards containing ascorbic acid, and one reference sample with a known protein content.

In the machine, samples are combusted and the amount of nitrogen (N) is measured by a Thermal Conductivity Detector, which is converted to the protein content based on a standard conversion of 6.25 (Van Gelder, 1981). Triplicate measurements were carried out for each protein powder.

3.1.3 Protein solubility

The protein solubility experiment is derived from the procedure described by Osen et al. (2014), which is based on the standard procedure by Morr et al. (1985). Solutions of 2% (w/w) protein in 50 mL 0.1 M NaCl were prepared for pH values 3 to 8, using 1 M HCl or 1 M NaOH to adjust the pH, respectively. The samples were mixed in a rotator at room temperature, for one hour. After centrifugation at 4750 rpm for 30 min, 5 g supernatant was taken from each sample and dried in the oven at 105°C for five hours. Protein content was then determined as described in Section 3.1.1 "Dry matter and protein content determination". This experiment was performed in duplicates.

First, the protein solubility in g protein per 100 g supernatant (sn) was determined using Equation 3.2. Then, based on the assumption that a 2% protein solution, with a 100% protein solubility would have a protein concentration in the supernatant of 2 g per 100 g supernatant (sn), the protein concentration in percentages was calculated using Equation 3.3.

$$Protein\ solubility\ ({}^{g\ protein}/_{100\ g\ sn}) = 100 * \frac{DM\ sn\ (g)\ *\ protein\ conc.sn\ (\%)}{weight\ sn\ before\ drying\ (g)} \quad (3.2)$$

$$Protein\ solubility\ (\%) = protein\ solubility\ ({}^{g\ protein}/_{100\ g\ sn}) * \frac{100\ g\ sn}{2\ g\ protein} \quad (3.3)$$

3.1.4 Particle size distribution

Particle sizes of the protein powders were determined using a Malvern Mastersizer 2000 and the Mastersizer 2000 software (Malvern Panalytical, Malvern, United Kingdom). Solutions of 2% protein (w/w) in 0.1 M NaCl were prepared for pH values 3 to 8, and 0.1 M NaCl was also used as the dispersant with a pH value corresponding to the pH of the sample. A refractive index of 1.53 was used for the potato protein, based on that of pea protein, and a refractive index of 1.335 was used for the 0.1 M NaCl dispersant (Zhu, Mai, & Zhao, 2017). The pH was adjusted using 1 M HCl or 1 M NaOH, respectively. The stirrer speed was set to 2000 rpm, and sample was added until a laser obscuration of 5% was reached. The particle volume distributions were used to determine the volume diameters D(v, 0.1), D(v, 0.5), and D(v, 0.9) for each protein isolate at pH values 3 to 8. Duplicate samples were prepared for each pH value, of which 2 measurements were taken per sample. The Mastersizer then made three recordings per measurement.

3.2 Gelation experiments

3.2.1 Small volume gelation experiments at different concentrations at 72°C

The minimum gel concentration was determined based on the procedure described by Creusot et al. (Creusot, Wierenga, Laus, Giuseppin, & Gruppen, 2011). Samples of 300 µL were prepared ranging in protein concentration from 12% to 28% for PPI1, 2% to 14% for PPI2, and 4% to 28% for PPI3. The dilutions were prepared by making a protein mix of the highest concentration, and diluting with distilled water to obtain the other desired concentrations. Duplicate samples were prepared for each concentration. Then, the samples in sealed Eppendorf tubes were heated to 72°C for 20 min in a warm water bath, and then cooled to 20°C for approximately 20 min, before determining visually whether a gel had formed by turning the Eppendorf tube upside down, and shaking it lightly. Samples that did not flow down were considered to be "gelled".

3.2.2 Small volume gelation experiment with various pH values at 72°C

The small volume gelation experiment was also performed on samples with various pH values, namely pH 5, 5.5, and 6, since the pH of chicken meat is close to this range (Ristic & Damme, 2013). Solutions of 12% protein (w/w) were prepared for PPI2 and PPI3, of which the pH was adjusted using 1 M HCl or 1 M NaOH, respectively. Dilutions of 2, 4, 6, 8, and 10% were obtained from the 12% protein mix, with distilled water. The rest of the procedure remains as described above, in duplicates.

3.2.3 Small volume gelation experiment with mixed PPIs at 72°C

The small volume gelation procedure described above was also performed on mixes of PPI2 and PPI3 for the unadjusted pH value and for pH 5.5. The mixes were prepared and denoted as described in Table 3.1. Solutions of 12% protein (w/w) were prepared for each mix, of which the pH was adjusted to pH 5.5 using 1 M HCl or 1 M NaOH, respectively. Dilutions of 2, 4, 6, 8, and 10% were obtained from the 12% protein mix, with distilled water. This experiment was performed in duplicates.

Table 3.1. PPI mixes and their composition.

Name	PPI mix (w/w protein)
Mix A	50% PPI2 and 50% PPI3
Mix B	70% PPI2 and 30% PPI3
Mix C	80% PPI2 and 20% PPI3
Mix D	90% PPI2 and 10% PPI3

3.2.4 Gelation at 72°C and gel strength measurements

To analyze gelation in a larger volume, first, 12% protein solutions (w/w) of Mixes A-D and PPI2 were prepared, and mixed thoroughly on a magnetic stirrer for approximately 30 min. Half of each 12% protein solution was set aside, and for the other half the pH was adjusted to pH 5.5 using 1 M HCl or 1 M NaOH, respectively. Protein concentrations of 4 and 8% were derived from the 12% protein mixes through dilution with distilled water. The pH was then measured again for all samples after dilution, for both the adjusted pH and the unadjusted pH, while stirring at a medium speed on a magnetic stirrer. If diluting the samples had a strong effect on the pH values, then the pH was again adjusted to pH 5.5. Duplicate samples of 30 mL were prepared in 50 mL Falcon tubes for each PPI at pH 5.5 and at the unadjusted pH value. The samples were, then, heated in a warm water bath at 72°C for 20 min, and cooled down to 20°C, for around 20 min. Holes of 5 mm were made in the lids of the Falcon tubes with scissors, to relieve the pressure building up inside the tubes during heating.

Gel strength was measured using a penetrometer (Texture Analyzer TVT-300XP, Perten Instruments, Stockholm, Sweden), which measures the peak force that occurs during the initial compression of the gel.. The trigger force, test speed and starting distance were set to 5 g, 1 mm/s and 10 mm, respectively. Falcon tubes of 50 mL were secured using a rig, and a cylinder probe was used with a 7 mm diameter and 11 cm length. The probe was centered above the sample surface, and the test began when the trigger force was reached, and ran until a target compression of 25 mm was made. The peak force was obtained from the TexCalc software (Perten Instruments, Stockholm, Sweden). The gel strength (Pa) was calculated by dividing the peak force by the area of the used probe. Gel strength was measured in duplicates.

3.2.5 Gelation experiment for a higher heating temperature at 90°C

The effect heating temperature on the gel strength was determined by executing the experiment as described in Section 3.2.2, with the exception of heating the samples in a warm water bath up to 90°C for 20 minutes. The samples were analyzed at pH 5.5 for protein concentrations of 12% (w/w). Measurements were performed for PPI2, Mixes A-D, in triplicates.

3.3 HT-RVA measurements

A high temperature Rapid Visco Analyzer (HT-RVA) was used to measure the viscosity of various protein solutions as a function of temperature (>100°C)(RVA 4800, Perten Instruments, Stockholm, Sweden). In all experiments, protein slurries of the desired concentrations were prepared by first mixing the PPIs with distilled water and mixing thoroughly for 30 min, using a magnetic stirrer at high speed. While still stirring at high speed, the pH was adjusted to 5.5 using 1 M HCl or 1 M NaOH, respectively. After thorough mixing, 25 g of the slurry was weighed into a high temperature can and placed in the HT-RVA. Various program settings were used to analyze parameters such as the cooling rate, maximum heating temperature, and stirring speed, which are described below. The various mixes of PPIs, Mixes A-D, described in Table 3.1, were analyzed during these experiments as well. Results were analyzed using the TCW3 Software (Perten Instruments, Stockholm, Sweden). The HT-RVA was re-zeroed every time a new HT can and paddle combination was used.

3.3.1 Effect of maximum heating temperature of 100, 120, and 140°C

Heating temperatures of 100, 120, and 140°C were analyzed to study the effect of the maximum heating temperature on the HT-RVA results. In this HT-RVA program, slurries of 12% (w/w) protein were stirred at 960 rpm for 10 s at 20°C for thorough dispersion, after which the stirring speed was lowered to 160 rpm. The slurry was held at 20°C for 1 min before being heated to either 100, 120 or 140°C at 12°C/min, and held at the maximum temperature for 5 min, cooled to 50°C at 6°C/min and held at 50°C for 5 min, then cooled to 20°C at 6°C/min and held there for 5 min.

3.3.2 Effect of various parameters in the program settings

Variations of the program settings described in Section 3.3.1. were used to analyze the effects of various parameters on the RVA outcome, such as cooling and heating rate, stirring speed, and protein concentrations.

To analyze the effect of the cooling rate on the RVA outcome, the cooling rate was adjusted to 3°C/min compared to the 6°C/min. For these experiments, the maximum heating temperature was kept at 140°C.

Another parameter which can be varied in the RVA is the stirring speed. This was varied from 160 rpm to 760 rpm, to determine how the RVA results would be influenced by different shear rates. Remaining parameters were set to a maximum heating temperature of 140°C, 12°C/min heating, and 6°C/min cooling.

3.3.3 Effect of protein concentration

Slurries of protein concentrations varying from 6%, 8%, 10% (w/w) were compared according to a program with a maximum heating temperature of 140°C, 12°C/min heating, 6°C/min cooling, and a stirring speed of both 160 rpm and 760 rpm.

3.3.4 Effect of potato starch addition

The effect of potato starch on the RVA outcome was determined by adding 1% or 2% starch (w/w) in the form of potato flakes (Ekologiska potatisflingor, Kobelco AB, Tingsryd, Sweden) to a 6% PPI2 solution (w/w protein). The potato flakes, PPIs and distilled water were all added together at once, and mixed at high speed for ~30 min using a magnetic stirrer. As in all the previous experiments, the pH was then adjusted to pH 5.5 using 1M HCl, and 25 g of the mix was used for the RVA measurement. A maximum heating temperature of 140°C was used according to the program described in Section 3.3.1. "Effect of maximum heating temperature" , and a stirring speed of either 160 or 760 rpm. Measurements were performed in duplicates for a stirring speed of 160 rpm, and once for 760 rpm.

3.3.5 Effect of oil droplet addition

To analyze the effect of oil on the RVA results, either 1.5% or 3% rapeseed oil (AAK, Karlshamn, Sweden) was added to a 6% PPI2 solution (w/w protein). The oil was added to the mix prior to stirring thoroughly for 30 min, using a magnetic stirrer. Similar to the experiment on starch addition, the samples with oil were tested under the same program settings of a maximum heating temperature of 140°C, and a stirring speed of either 160 or 760 rpm. Measurements were performed in duplicates for a stirring speed of 160 rpm, and once for 760 rpm.

3.3.6 Combined effect of potato starch and oil droplet addition

A combination of oil droplets and potato starch was added to a 6% PPI2 solution (w/w protein), to determine the combined effect of these ingredients. Two different samples were prepared, one with 1% starch and 1.5% oil droplets, and one with 2% starch and 3% oil droplets. The oil and starch were added to the PPIs prior to adding the distilled water and mixing thoroughly for 30 min at a high speed, using a magnetic stirrer. The RVA measurements were performed with a maximum heating temperature of 140°C and a stirring speed of 160 rpm. The measurements with performed in triplicates.

4 Results and Discussion

4.1 Characterization of potato protein isolates

4.1.1 pH value of 1% PPI solution

To get a basic idea of the PPIs, the pH values of 1% solutions (w/w) were determined, which are shown in Table 4.1 from triplicate measurements. PPI1 and PPI3 have a pH around 3.2, whereas PPI2 has a neutral pH of 7.0. For PPI2 and PPI3, these pH values are in accordance with the values given by the producers. This information was not provided by the producers for PPI1.

Table 4.1. The pH values of 1% PPI solutions in distilled water (n=3).

Potato protein	Sample	pH
Lyckeby	PPI1	3.24 ± 0.01
Solanic®200	PPI2	7.00 ± 0.02
Solanic®300	PPI3	3.27 ± 0.00

4.1.2 Dry matter and protein content determination

An important characterization step of the PPIs is to determine their total dry matter (DM) as well as the protein content. The results from the dry matter determination, as well as the protein content based on the DM are shown in Table 4.2. The results show that all PPIs have both a high total dry matter and a high protein concentration (>85%). Especially PPI2 and PPI3 are almost pure potato protein isolates, which is in accordance with the values given by the producers. This information was not provided by the producers for PPI1.

Table 4.2. Chemical composition of the PPIs (n=3).

Label	Dry matter (DM)(%)	Protein content per DM (%)
PPI1	92.84 ± 0.16	86.02 ± 3.07
PPI2	90.77 ± 0.06	92.59 ± 2.00
PPI3	92.49 ± 0.00	97.09 ± 0.13

4.1.3 Protein solubility

The protein solubility was measured for the PPIs because it gives a good indication of the protein functionality (Aryee, Agyei, & Udenigwe, 2018). The protein solubility was measured at various pH values between pH 3 and 8, of which the results are shown in Figure 4.1. As Figure 4.1 shows, PPI1 was not soluble at any of the pH values. The actual solubility of PPI1 is most likely not zero, but because of the small amount of dry matter in the 5 g supernatant which was taken to determine the solubility, it was hard to analyze these samples with the protein analyzer.

On the other hand, PPI2 and PPI3 have a high solubility, with especially PPI3 showing excellent solubility for all pH values. The solubility of PPI2 is more dependent on the pH value, with a minimal solubility for pH 4 and 5, which can be explained by the iso-electric point (pI) of PPI2. Proteins are least soluble at their pI, and the pI of PPI2 is mainly 4.8-5.2 (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011; Aryee, Agyei, & Udenigwe, 2018). A similar solubility curve for PPIs was observed by Van Koningsveld et al. (2001)

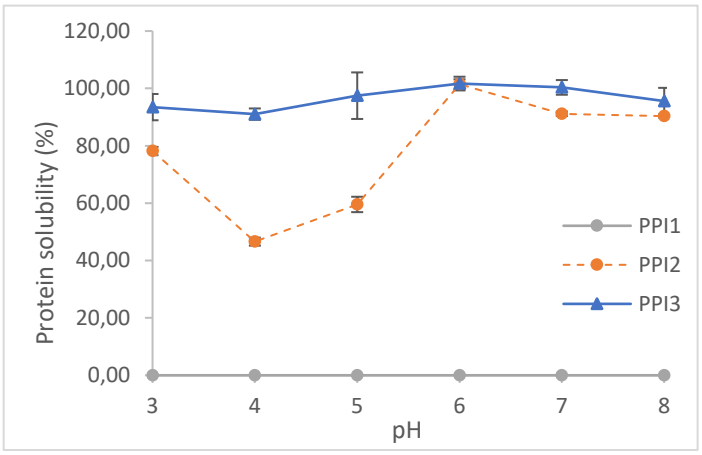

Figure 4.1. Protein solubility (%) of PPIs as a function of pH (n=2).

While preparing the samples for the protein solubility experiment, some observations of the PPIs at various pH values were made (see Figure 4.2). PPI1 has a the same opaque light brown color in all samples. The color of PPI2 samples changed drastically for different pH values. For pH 3-5, the color of PPI2 was white, with the sample at pH 3 being slightly more translucent than the milky samples at pH 4 and 5. At pH 6-7, the samples are dark grey/brown, and at pH 8 the color is more translucent and orange/brown. PPI3 has a white color for pH 4-8, and has an increasing opacity as the pH value becomes higher. At pH 3, PPI3 is transparent. These observations are of interest, because the color of the product is important in developing a meat analogue which resembles chicken meat. For this project, the desired color of the final project is white, or very light brown.

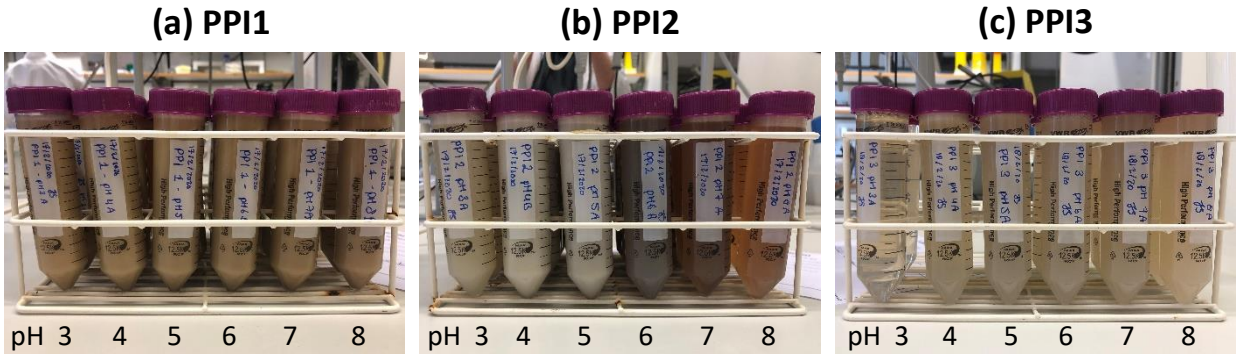

Figure 4.2. Solutions of 2% protein at various pH values for **(a)** PPI1, **(b)** PPI2, and **(c)** PPI3.

4.1.4 Particle size distribution

Particle size distribution is an important factor in any extrusion process. If the particles are too large, they might be harder to hydrate, disperse, and plasticize. However, if the particles are too small they might agglomerate and cause dispersion problems in the extruder barrel. For soy protein and wheat gluten, a particle size of around 74-149 μm is a common characteristic in producing meat analogues (Strahm, 2006).

The average particle size of PPI2 and PPI3 are shown in Table 4.3 and Table 4.4, respectively. Values d(v, 0.1), d(v, 0.5), and d(v, 0.9) give the particle diameters where 10, 50 or 90% of the volume distribution is below, respectively. The particle size distribution was not measured for PPI1, since its low solubility might have clogged the machine. Results show that the average particle size for PPI2 was lowest at pH 4, followed by pH 5, which are the pH values where PPI2 is least soluble. The average particle sizes of PPI2 and PPI3 were comparable, but with PPI2 having a slightly higher average particle size at most pH values, with the exception of pH 3 and pH 4. The particle sizes of the PPIs are close to the acceptable range described by Strahm (2006).

Table 4.3. Average particle size (volume diameter) of PPI2 at various pH values (n=12).

pH	d(v, 0.1)(μm)	d(v, 0.5)(μm)	d(v, 0.9)(μm)
pH 3	12.0 ± 4.5	83.1 ± 37.4	247.4 ± 42.3
pH 4	2.6 ± 0.1	6.5 ± 1.7	56.5 ± 55.2
pH 5	3.1 ± 0.3	31.0 ± 12.2	109.0 ± 17.3
pH 6	12.2 ± 2.5	63.8 ± 22.8	200.0 ± 62.4
pH 7	17.8 ± 6.5	94.4 ± 40.6	216.5 ± 65.7
pH 8	8.1 ± 4.6	58.2 ± 52.6	118.1 ± 106.1

Table 4.4. Average particle size (volume diameter) of PPI3 at various pH values (n=12).

pH	d(v, 0.1)(μm)	d(v, 0.5)(μm)	d(v, 0.9)(μm)
pH 3	100.0 ± 6.4	174.8 ± 9.4	292.1 ± 17.4
pH 4	59.8 ± 29.3	172.5 ± 36.3	288.6 ± 40.0
pH 5	4.5 ± 0.3	7.9 ± 0.6	18.1 ± 14.0
pH 6	5.9 ± 0.9	60.8 ± 37.3	150.3 ± 84.2
pH 7	6.6 ± 1.4	45.0 ± 31.8	124.8 ± 67.5
pH 8	10.8 ± 5.2	107.5 ± 25.9	222.0 ± 31.0

The particle size for PPI2, according to Table 4.3, is smallest when PPI2 is least soluble. This is interesting, because it was expected that the average particle size would be smaller for proteins with a high solubility, also for PPI3. However, it needs to be noted that there was a large variation of the particle size between triplicate recordings of one measurement, and between duplicate samples. This is shown in the tables by the large error bars, and makes it difficult to interpret these results accurately.

4.2 Gelation experiments

4.2.1 Small volume gelation experiments with varying protein concentration at 72°C

To determine whether potato proteins were able form a solid network, their gelation capability was analyzed. First, a wide range of protein concentrations was analyzed in small volumes, to determine around what concentration they would start to gel. All samples were heated to 72°C for 20 min, then cooled down to 20°C, and gelation was determined visually by placing the tubes upside down. The results are summarized for all PPIs in Table 4.5, including the range of protein concentrations the samples were tested in. PPI1 did not gel at any of the concentrations, and was therefore not included in any of the subsequent experiments. The minimal gelling concentrations for PPI2 and PPI3 in this experiment were 4% and 8%, respectively. A reason for the enormous difference in functionality of PPI1 compared to PPI2 and PPI3 is most likely related to the different producers. As mentioned previously, the functionality of potato proteins is strongly depended on the extraction technique from the PFJ, so the producer from PPI1 (Lyckeby AB) most likely used a different extraction technique than the producer of PPI2 and PPI3 (Avebe).

Table 4.5. Minimal gelling concentration of PPIs from a wide range of concentrations (n=2).

	Tested concentrations (%)	**Minimal gelling concentration (w/w protein)**
PPI1	12, 16, 20, 24, 28%	Did not gel
PPI2	2, 4, 6, 8, 10, 12 14%	4%
PPI3	4, 8, 12, 16, 20, 24, 28%	8%

After the approximate minimal gelling concentration had been established, a similar experiment was executed for a narrow range of protein concentrations, to obtain a more accurate result. These results are shown in Table 4.6, which show that the minimal gelling concentration for PPI2 is 4%, and 8% for PPI3. This low gelling concentration can be partially attributed to the high hydrophobicity of patatin, which causes patatin to aggregate quite easily (Creusot, Wierenga, Laus, Giuseppin, & Gruppen, 2011). PPI2 largely consists of these patatins, whereas PPI3 consists mostly of protease inhibitors. For protease inhibitors, disulfide bonds are mostly responsible for network formation (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011; Pouvreau, Gruppen, van Koningsveld, van den Broek, & Voragen, 2005).

Table 4.6. Minimal gelling concentration of PPIs from a narrow range of concentrations (n=2).

	Tested concentrations (%)	**Minimal gelling concentration (w/w protein)**
PPI2	2, 3, 4, 5, 6, 7, 8%	4%
PPI3	4, 5, 6, 7, 8, 9, 10, 11, 12%	8%

4.2.2 Small volume gelation experiment with various pH values at 72°C

In the previous experiment, the pH of the PPIs was not adjusted. From Table 4.1 it is known that the unadjusted pH of PPI2 and PPI3 are around pH 7 and pH 3.2, respectively. Since the pH of meat is around pH 5 to pH 6, the same small volume gelation experiment is also done for the PPIs at pH 5, 5.5, and 6. All samples were analyzed with a protein concentration ranging from 2-12% (w/w) with 2% increments. Results are shown in Table 4.7. The minimal gelling concentration of PPI2 is not affected by the change in pH, even though the solubility is lower at pH 5. The minimal gelling concentration of PPI3 is slightly higher at pH 5 and pH 5.5, but only by 2%.

Table 4.7. Minimal gelling concentration of PPIs for pH 5, 5.5, and pH 6 (n=2).

	pH 5	**pH 5.5**	**pH 6**
PPI2	4%	4%	4%
PPI3	10%	10%	8%

The color of the gels was also observed, as an almost white color was desired to resemble chicken meat. As was seen in Figure 4.2, the color of PPI2 solutions varies drastically for different pH values. Figure 4.3 shows the gels formed by PPI2 and PPI3 at pH 5.5. The gels formed by PPI2 had slightly grey color, whereas the samples from PPI3 were white. Since both PPI2 and PPI3 formed gels with the desired light color at pH 5.5, it was decided to continue the experiments using a pH value of 5.5.

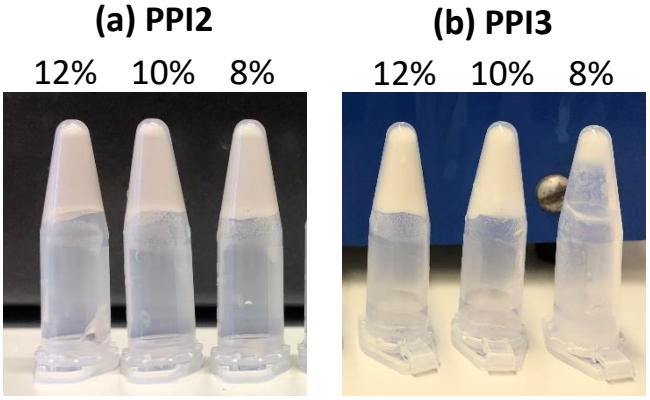

Figure 4.3. Gels formed by **(a)** PPI2, **(b)** PPI3, with pH 5.5, after heating to 72°C for various protein concentrations between 8-12%

4.2.3 Small volume gelation experiment with mixed PPIs at 72°C

Thus far, results show that both PPI2 and PPI3 show promising gelling behavior. Especially, PPI2 has a low minimal gelling concentration, which is why it was decided not to continue the experiments with pure PPI3. However, because the two PPIs carry an opposite charge at most pH values, it was interesting to analyze how the two PPIs function together. These opposite charges are caused by the different iso-electric points of the PPIs, namely a pI around 5.0 for PPI2, and >8 for PPI3 (Alting, Pouvreau, Giuseppin, & van Nieuwenhuijzen, 2011). The various mixes of PPI2 and PPI3 are described in Table 4.8, including the resulting pH for samples with a 12% protein concentration (w/w).

Table 4.8. Mixes of PPI2 and PPI3 and their pH values at a 12% protein concentration (w/w)(n=1).

Name	Mix	pH
Mix A	50% PPI2 and 50% PPI3	4.66
Mix B	70% PPI2 and 30% PPI3	5.23
Mix C	80% PPI2 and 20% PPI3	5.63
Mix D	90% PPI2 and 10% PPI3	6.14

The minimal gelling results for the solutions with an unadjusted pH and pH 5.5 can be seen in Table 4.9. The mix with the lowest minimal gel concentration was Mix D, for both pH values. This was expected, since Mix D contains the highest proportion of PPI2. All mixes had a lower minimal gelling concentration compared to samples only containing PPI3. This shows that addition of PPI2 had a positive effect on the gelling behavior of PPI3, and that gelling behavior seems to be dominated by PPI2. The formed gels all had an almost white color, independent of the pH.

Table 4.9. Minimal gelling concentrations of PPI mixes at pH 5.5 and for their unadjusted pH (n=2).

	Mix A	Mix B	Mix C	Mix D
pH unadjusted	6%	6%	4%	4%
pH 5.5	6%	4%	6%	4%

4.2.4 Gelation at 72°C and gel strength measurements

In the previous experiments, gelation was only determined visually and in small volumes, and no comparisons could be made between the various gels. Therefore, gelation experiments were completed in higher volumes so the actual gel strength could be measured. Firstly, the gel strength of PPI2 and all mixes was measured at an unadjusted pH for concentrations 4, 8, and 12% (w/w). The gel strength (Pa) of the samples is shown in Table 4.10. Some samples did not gel, and thus no gel strength could be measured. This is denoted by n/a (not available) in Table 4.10.

Table 4.10. Gel strength (Pa) for PPI2 and PPI mixes A-D at various concentrations (n=2).

Protein conc. (w/w)	Gel strength (*10^3 Pa)				
	Mix A	**Mix B**	**Mix C**	**Mix D**	**PPI2**
4%	n/a	n/a	n/a	3.4 ± 0.1	6.8 ± 0.1
8%	9.6 ± 0.6	3.1 ± 0.0	7.0 ± 0.1	54.5 ± 0.5	66.4 ± 1.2
12%	45.5 ± 1.2	12.0 ± 0.3	32.6 ± 10.5	230.0 ± 4.2	224.2 ± 12.0

It is observed that the gel strength drastically increases with an increasing protein concentration. The lowest gel strength is seen for Mix B, while the highest gel strengths are obtained by PPI2 and Mix D. This indicates, again, that the highest gel strength is obtained by a higher proportion of PPI2. No major difference was found between PPI2 and Mix D regarding their gel strength, even though Mix D contained 10% PPI3.

Interestingly, Mix A had a higher gel strength than Mix B and C, even though it contained more PPI3. This might be related to the pH value of the mixes, since the pH of Mix A (pH 4.66) is lower than the pI of PPI2 (5.0), whereas the pH values of Mix B (5.23) and Mix C (5.63) are above the pI of PPI2. When the pH is below the pI, the proteins predominantly carry a net-positive charge. The pI of PPI3 is >8, so the charge of PPI3 molecules in all the mixes is mostly positive. Thus, in Mix A, both PPI2 and PPI3 both mainly carry a positively charge. However, in Mixes B and C the PPI2 molecules mostly carry a net-negative charge, which results in electrostatic interactions between the two PPIs. This results in an increased hydrophilicity of the proteins, which then causes lower gelation abilities of the proteins, or a lower gel strength (Creusot & Gruppen, 2007). Another possible explanation for the low gel strength of Mix B is that the sample started to precipitate quite quickly, inhibiting formation of a strong protein network.

The color of the gels was also analyzed, and is shown in Figure 4.4. Mixes A-C all formed gels with a light, almost white, color. Gels formed by Mix D and PPI2 had a darker color, namely dark brown for PPI2, light grey/brown for Mix D. In the attempt to lighten the color of the samples, mainly Mix D and PPI2, an experiment with the adjusted pH of 5.5 was done for protein concentrations of 12% (w/w). Photos of these gels are also shown in Figure 4.4.

Figure 4.4 shows the effect of adjusting the pH on the color of the gels formed by Mix D and PPI2. These gels turned from a grey/brown color before the pH was adjusted, to a creamy color at pH 5.5.

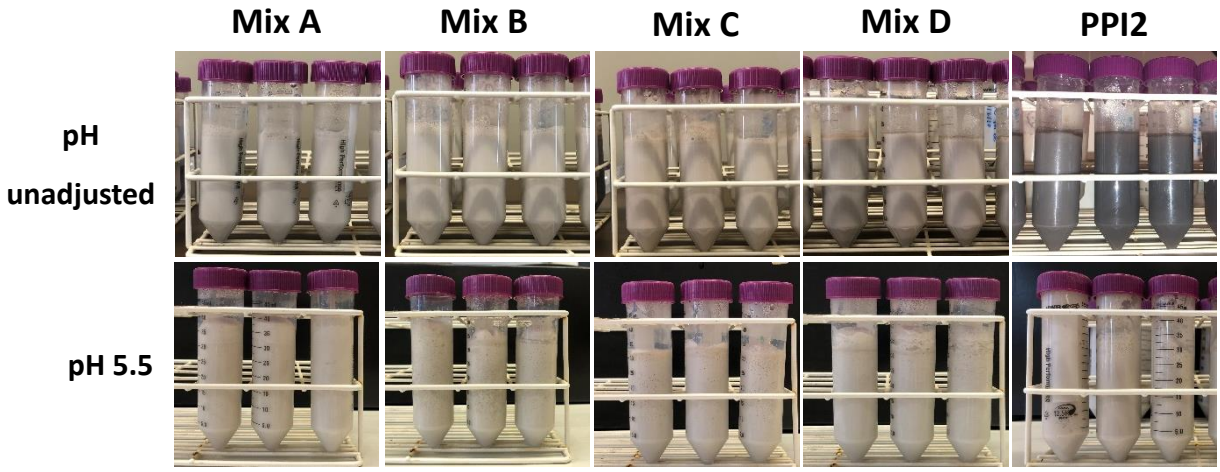

Figure 4.4. Gels formed after heating to 72°C by Mixes A-D and PPI2, for both the unadjusted pH and for pH 5.5. The protein concentration for the samples with the unadjusted pH are 12, 8, 4% (from left to right). All samples with pH 5.5 had a protein concentration of 12%.

Besides evaluating the color of the gels at pH 5.5, the gel strength of Mixes A-D and PPI2 was also determined, and is shown in Table 4.11. Compared to the gel strength of the mixes with unadjusted pH (see Table 4.10), the gel strength is higher for Mix B and C at pH 5.5, but much lower for Mix A, D, and PPI2.

Table 4.11. Gel strength (Pa) of 12% PPI mixes (w/w) at pH 5.5 (n=3).

	Mix A	Mix B	Mix C	Mix D	PPI2
Gel strength (*10^3 Pa)	15.2 ± 0.3	21.6 ± 1.4	51.4 ± 3.2	50.5 ± 3.5	153.2 ± 8.1

The results show that the gel strength of Mix A is much lower for pH 5.5, which can be attributed to the change in pH from below the pI of PPI2 to above the pI, increasing the hydrophilicity of the proteins. At pH 5.5, it can be seen that the gel strength considerably increases with the amount of PPI2 in the mix, with pure PPI2 demonstrating the highest gel strength.

A possible explanation for the lower gel strength of Mixes A, D and PPI2 at pH 5.5, compared to the unadjusted pH could be related to the volume expansion of these samples during mixing. After mixing for 30 min, Mixes A, D and PPI2 with pH 5.5, had almost doubled in volume due to foaming. For the gel strength measurements, samples of 30 ml were taken from these mixes, which due to the expansion of the volume essentially contained less protein than Mix B and C, which had not changed in volume.

4.2.4.1 Gelation experiment for a higher heating temperature of 90°C

In the HT-RVA and extruder, samples can be heated to temperatures much higher than 72°C. For this reason, another similar experiment was performed with a heating temperature of 90°C. The gel strength was measured for the mixes and PPI2, at pH 5.5 for a protein concentration of 12% (w/w). As the results in Table 4.12 show, the gel strength of all samples increased substantially after heating to 90°C, compared to 72°C (Table 4.11).

Table 4.12. Gel strength (Pa) of 12% PPI mixes (w/w) at pH 5.5, heated to 90°C (n=3).

	Mix A	**Mix B**	**Mix C**	**Mix D**	**PPI2**
Gel strength (*10^3 Pa)	87.5 ± 5.9	249.5 ± 9.9	220.2 ± 37.1	203.6 ± 56.5	527.6 ± 16.6

These results indicate that the heating temperature has an enormous effect on the gel formation, and the functionality of the proteins. By far the highest gel strength was observed for PPI2, followed by Mix B, C, and D. The lowest gel strength at pH 5.5 is found for Mix A.

The observation that gel strength increases with an increasing heating temperature, is also found in literature, until a certain maximum temperature range is reached. This is related to the level of protein unfolding, as more protein unfolding results in formation of a stronger gel (Damodaran, 1996).

For several samples, namely Mixes B, C, and D it was observed that the gel partially detached from the flask after heating to 90°C, due to the strong protein bonds. After heating to 90°C, PPI2 completely detached from the flask, as is shown in Figure 4.5. It was possible to remove the gel from the flask as a whole, which is also shown in the figure. This gel had a fibrous structure and showed great promise for the HT-RVA, where the samples can be heated to even higher temperatures.

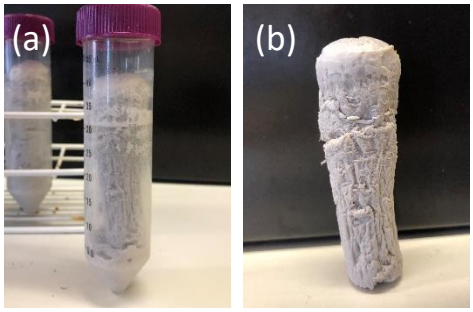

Figure 4.5. Gel formed by 12% PPI2 after heating to 90 °C, **(a)** in the flask and **(b)** removed from the flask.

4.3 HT-RVA measurements

The Rapid Visco Analyzer (RVA) is a great instrument for imitating the processes inside an extruder. Similarly to extrusion, the samples inside the RVA can undergo high temperature heating, shear from the stirring paddle, and a long cooling process to prevent expansion.

4.3.1 Effect of maximum heating temperature of 100, 120 and 140°C

In the first RVA measurements, PPI2 and mixes A-D were analyzed for a protein concentration of 12% at various maximum heating temperatures of 100, 120, and 140°C. The paste profiles for the PPIs with a maximum heating temperature of 100 and 120°C are shown in Figure 4.6.

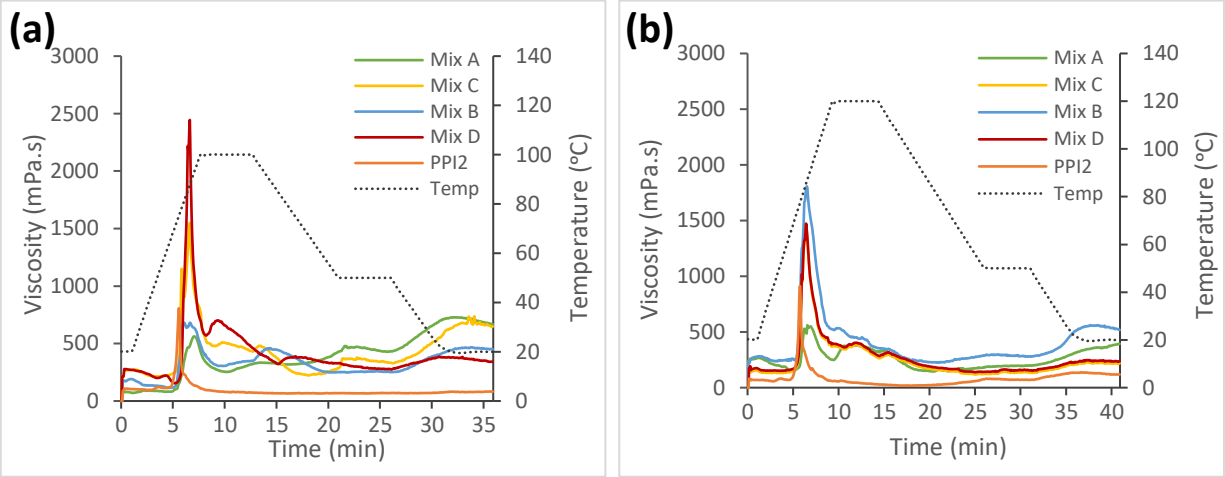

Figure 4.6. RVA viscosity curves of the 12% PPIs (w/w) for a maximum heating temperature of **(a)** 100°C and **(b)** 120°C (n=1).

As the results show, the PPIs have a low initial viscosity of ~200 mPa·s, after which a peak is reached at a temperature of 75-80°C, after around 6 minutes. This peak is related to the denaturation temperature of the PPIs, which is between 55 and 75°C (van Koningsveld, et al., 2001). When all the native potato proteins have denatured, the protein solubility quickly drops and causes the viscosity to increase. This peak could also be the result of starch remains in the PPIs, because as is seen in Table 4.2, the PPIs are not 100% pure potato protein. Swelling of the hydrated starch granules has been related to a viscosity peak around the same temperature and time, followed by a decrease in viscosity as the granules rupture (Copeland, Blazek, Salman, & Tang, 2009; Osen, 2017). After this peak, the viscosity of the PPIs decreased drastically and remained constant until low cooling temperatures (<50°C) were reached, where the viscosity increased again for most PPIs due to gelling and formation of a three-dimensional protein network (Osen, 2017).

At a maximum heating temperature of 100°C, the final viscosity is the highest for Mix A and Mix C, followed by Mix B and Mix D, while PPI2 has the lowest viscosity. For a maximum heating temperature of 120°, Mix B had the highest final viscosity, followed by Mix A, Mix C, Mix D, and again the lowest final viscosity was found for PPI2.

Inside the RVA cans, the structures of the samples were also analyzed. Mixes A-C all formed soft wet sponge-like textures for both heating temperatures, with the samples from 120°C being slightly sturdier. However, none of these samples were sufficiently coherent to hold in your hand. Mix D and PPI2 formed stronger spongy structures, of which those of PPI2 are shown in Figure 4.7 for both 100 and 120°C. These spongy structures were found on top of the stirring paddle inside the RVA, leaving the bottom of the can almost empty, which is thought to be the cause of the low measured viscosity for PPI2 in Figure 4.6.

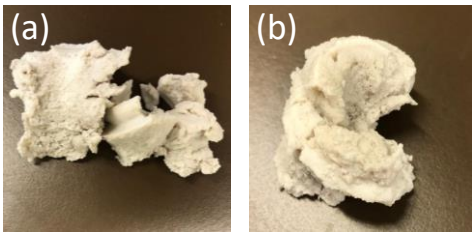

Figure 4.7. Sponge-like structures formed in the RVA by 12% PPI2 at **(a)** 100°C and **(b)** 120 °C.

The mixes were also heated to 140°C, of which the pasting profiles are shown in Figure 4.8. Mix D and PPI2 are shown separately, because the magnitude of the viscosity was much higher. Heating up 140°C best resembles the cooking temperature during HME, which is usually between 130 and 170°C (Palanisamy, Franke, Berger, Heinz, & Töpfl, 2019; Zhang, Liu, Jiang, Faisal, & Wang, 2020; Liu & Hsieh, 2008).

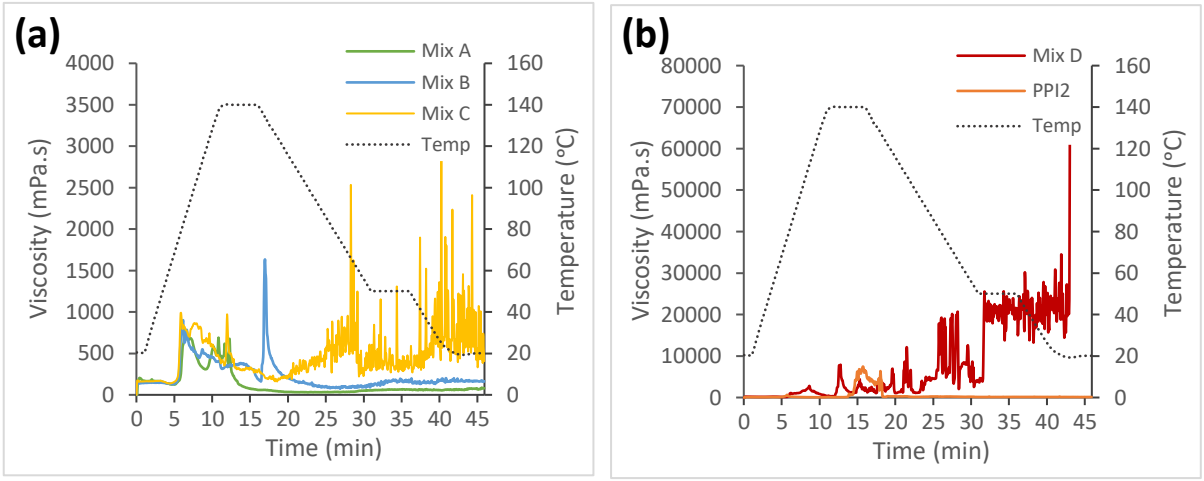

Figure 4.8. RVA viscosity curves for a maximum heating temperature of 140 °C for **(a)** Mix A, B, and C, and **(b)** for Mix D and PPI2, with a protein concentration of 12% (w/w) (n=1).

The graph in Figure 4.8A shows multiple peaks besides the peak observed before, namely between 5 and 12 minutes, and at 17 minutes, corresponding to temperatures between 80-140°C. For Mix C, it can be seen that the viscosity increased drastically upon cooling, which is not the case for Mix A and Mix B. The increase in viscosity for Mix C is due to the higher amount of PPI2 in this mix, compared to Mix A and B. As seen in Figure 4.8B, the RVA detected a final viscosity of around 64,000 mPa·s for Mix D, which resulted in an error by the RVA machine. The first major peak was observed right at the maximum temperature of 140°C. Following peaks for Mix D were observed as the protein slurries were cooled, reaching a maximum viscosity peak as the cooling temperature of 20°C was reached.

By comparing the results from the various maximum heating temperatures, it can be seen that the pasting profiles from 100 and 120°C are quite similar, while heating the samples up to 140°C results in very different graphs. This can be explained by the minimal temperature for extrusion texturization, which is around 130-140°C, and seems to be equivalent for all globular native and denatured proteins. This temperature range also corresponds to the melting temperature of many protein gels (Tolstoguzov, 1993). Around 140°C, the destabilization action of hydration is zero, or in other words, hydrophobic interactions are at a maximum. This is thought to be the cause for compact protein structure formation during extrusion. These cross-linkages between denatured proteins increase molecular rigidity and lead to rapid material conversion from liquid to rubber-like state during cooling (Privalov & Gill, 1989; Osen, 2017; Tolstoguzov, 1993). The high amount of cross-linkages also causes expulsion of water from the solid product, resulting in a hardened protein shell. This phenomenon is known is syneresis (Mizrahi, 2010).

Examples of syneresis and formation of compact protein structures are shown in Figure 4.9, which were formed by Mix D and PPI2 in the HT-RVA for a heating temperature of 140°C. Besides these solid products, a remaining liquid was found inside the RVA cans. These dense structures were dark in color, hard, and very difficult to pull apart.

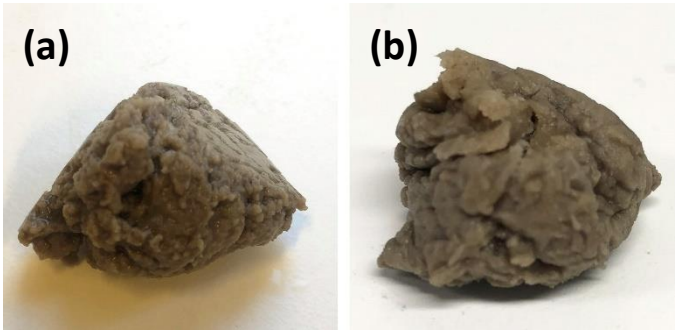

Figure 4.9. Dense protein balls formed during RVA experiment with 140°C maximum heating from 12% protein concentrations (w/w) of **(a)** Mix D, and **(b)** PPI2.

Even though similar protein structures were formed, the pasting profile of PPI2 and Mix D (Fig. 4.8B) looked very different. Perhaps, the dense PPI2 structure remained above the stirring paddle, which resulted in viscosity measurements of only the residual liquid inside the RVA can. However, as Mix D and PPI2 resulted in formation of similar textured products, while having completely different pasting profiles, it was decided not to compare the graphs of the remaining experiments at 140°C, but rather to compare the effects of various parameters on the structure of the final RVA protein structure, resembling a 'black box' experiment. The RVA graphs associated with the protein structures described in this project are shown in Appendix 2 "RVA graphs".

4.3.2 Effect of various parameters in the program settings

Many parameters can be varied in the settings of the RVA program. One of these is the heating and/or cooling rate. Since results from previous experiments indicated that most of the changes in viscosity occur during the cooling phase, several experiments were conducted with a slower cooling rate of 3°C/min, instead of 6°C/min. This experiment was conducted for Mixes B, C, and D, for a maximum heating temperature of 140°C. The results are shown in Figure 4.10. The RVA graph for Mix D can be found in Appendix 2 (Fig. A1), for which the reason is described above.

Figure 4.10A, again, shows various peaks during the heating phase, between 80 and 140°C. During cooling, the viscosity increased less notably compared to the mixes with the higher cooling rate (Fig. 4.8A). Also, the final viscosity of the samples is lower for the mixes with a slower cooling rate. Figure 4.10B shows the protein structure formed by Mix D under the same conditions. The formed structure was equally hard and dense as the structures formed with a faster cooling rate.

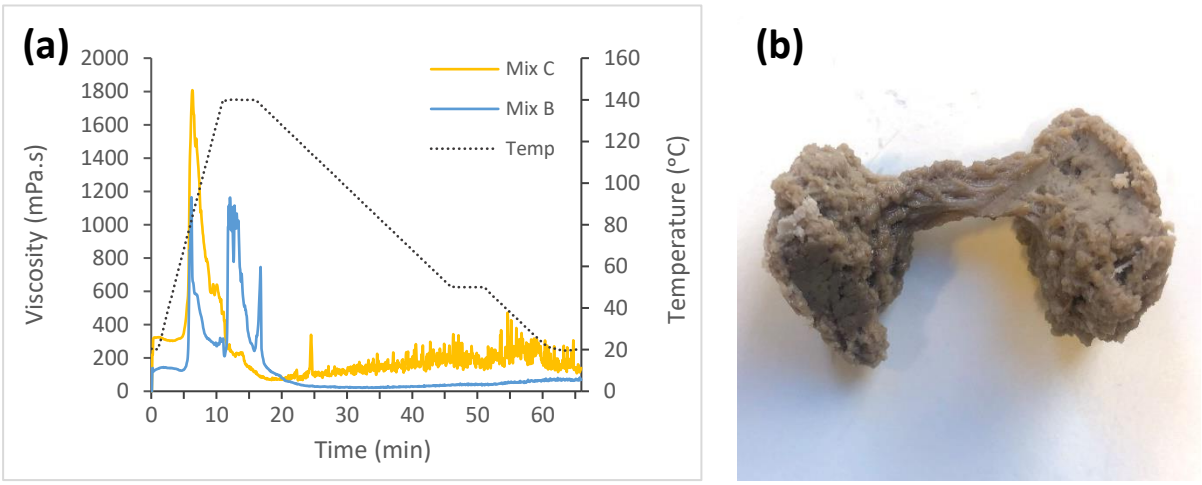

Figure 4.10 Effect of slower cooling rate. 12% PPIs heated up to 140°C, with a cooling rate of 3°C/min, and a stirring speed of 160 rpm. **(a)** RVA paste profile for Mix B and C (n=1), **(b)** protein structure formed by Mix D under the same conditions.

Since the results show that a slower cooling rate lowers the final viscosity of Mix B and C, and does not alleviate the compact protein structure formed by Mix D, it was determined that the cooling rate did not have a major effect on the RVA outcome. Therefore, it was decided to continue the remaining experiments with the original cooling rate of 6°C/min.

Another parameter that can be changed in the settings of the RVA is the shear rate, or the stirring speed (rpm). Several experiments were performed with a stirring speed of 760 rpm, instead of the 160 rpm from the previous experiments. The paste profiles of the protein slurries at a higher shear rate are shown in Figure 4.11A, for Mixes A, B, and C. These results, again, show multiple peaks during the heating phase of the RVA. Both the peak and final viscosity of the mixes are much lower compared to the samples which endured a lower shear rate (Fig. 4.8A). This is probably due to the high shear rate breaking up the protein network.

Dense structures formed in the RVA by Mix D and PPI2 at a high shear rate are shown in Figure 4.11B,C. Compared to the dense ball structures formed in previous experiments, the high shear resulted in protein structures which were slightly less dense, with a seemingly more fibrous structure, which was easier to pull apart. Therefore, it was hypothesized that a higher shear rate in the RVA results in a visibly more fibrous protein structure. The color remained a similar brown for a high stirring speed.

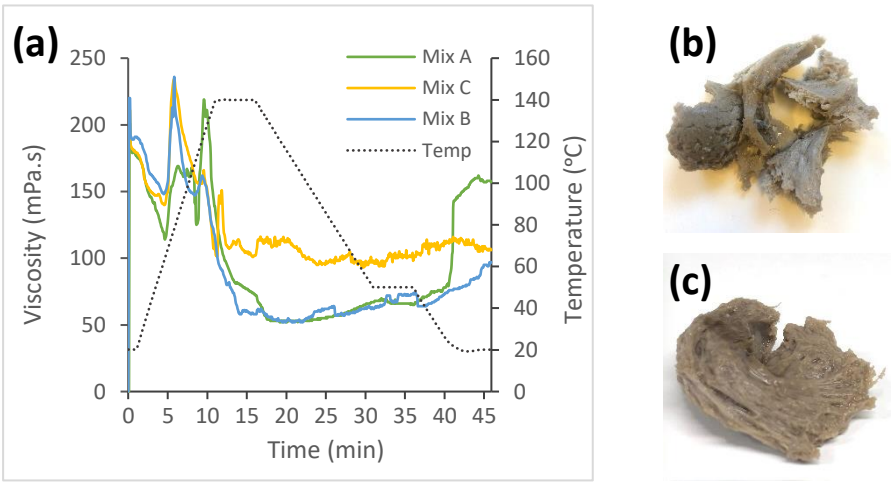

Figure 4.11. Effect of increased shear rate. 12% PPIs heated up to 140°C, with a cooling rate of 6°C/min, and a stirring speed of 760 rpm. **(a)** RVA paste profile for Mix A, B and C (n=1). And protein structures formed by **(b)** Mix D and **(c)** PPI2, under the same conditions.

4.3.3 Effect of various protein concentrations

As previous results have shown, the HT-RVA is able to form texturized products when using a cooking temperature of 140°C. Since the other protein mixes were not able to form solid protein structures, the focus was shifted towards Mix D and PPI2. The protein structures formed with a 12% concentration were very dense, so in these next experiments the protein concentration was varied to analyze whether this would open up the structures. Protein concentrations of 8% and 10% (w/w) were used in the RVA, heated to 140°C, and analyzed for both a stirring speed of 160 and 760 rpm. The texturized products of PPI2 and Mix D are shown in Figure 4.12, for both stirring speeds and concentrations. The RVA graphs are shown in Appendix 2 (Fig. A2).

As the results in Figure 4.12 show, many different kinds of structures can be obtained from the RVA independent of the variables, as balls or stretched shapes are seen for both shear rates and both protein concentrations. Lower protein concentrations did not result in more opened structures, which can be clearly seen in Figure 4.12A and C, as these balls are comparable to the dense protein balls obtained in previous experiments. Instead of protein products with a less dense structure, the lower protein concentration only resulted in a smaller amount of protein structures formed inside the RVA.

The observed color difference between Mix D and PPI2 is solely caused by the change of the background color. The structure color shown with the brown background was more true to reality, as the camera automatically color-corrected for the white background, making the structures appear darker than observed with the eye.

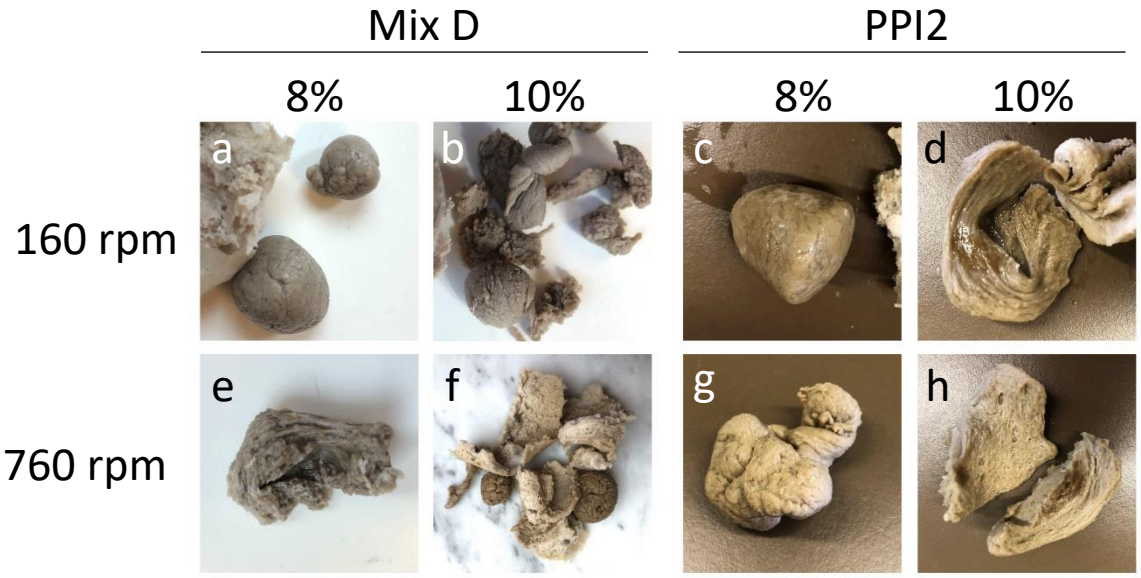

Figure 4.12. Protein structures obtained from the RVA for Mix D and PPI2 for stirring speeds of 160 and 760 rpm, protein concentrations of 8% and 10% (w/w), and a maximum heating temperature of 140°C.The texturized products have been (partially) pulled apart on the photos F and H.

As no considerable difference was established between the protein structures from Mix D and PPI2, it was decided to focus on PPI2. In the attempt to use less protein powder for the consequent experiments, a quick experiment was done for a 6% PPI2 protein concentration (w/w). As Figure 4.13 shows, a dense structure is still formed for a protein concentration of 6% (w/w).Therefore, consequent experiments are executed with a 6% PPI2 protein concentration, instead of 12% (w/w).

Figure 4.13. Protein structure from the RVA experiment with 6% PPI2 (w/w), heated to 140°C with 160 rpm.

4.3.4 Effect of potato starch addition

In an attempt to open up the dense structures formed inside the RVA at 140°C, potato starch was added to 6% protein slurries (w/w protein). Starch was added to the samples, because it has been observed before that starch granules in hydrophobic protein networks can destroy or weaken the protein network (Zeng, et al., 2011), and PPI2 is highly hydrophobic (Creusot, Wierenga, Laus, Giuseppin, & Gruppen, 2011). Protein structures formed during the RVA with addition of 1% or 2% potato starch, at a stirring speed of 160 rpm (in duplicates) or 760 rpm are shown in Figure 4.14. Corresponding RVA graphs are shown in Appendix 2 (Fig. A3).

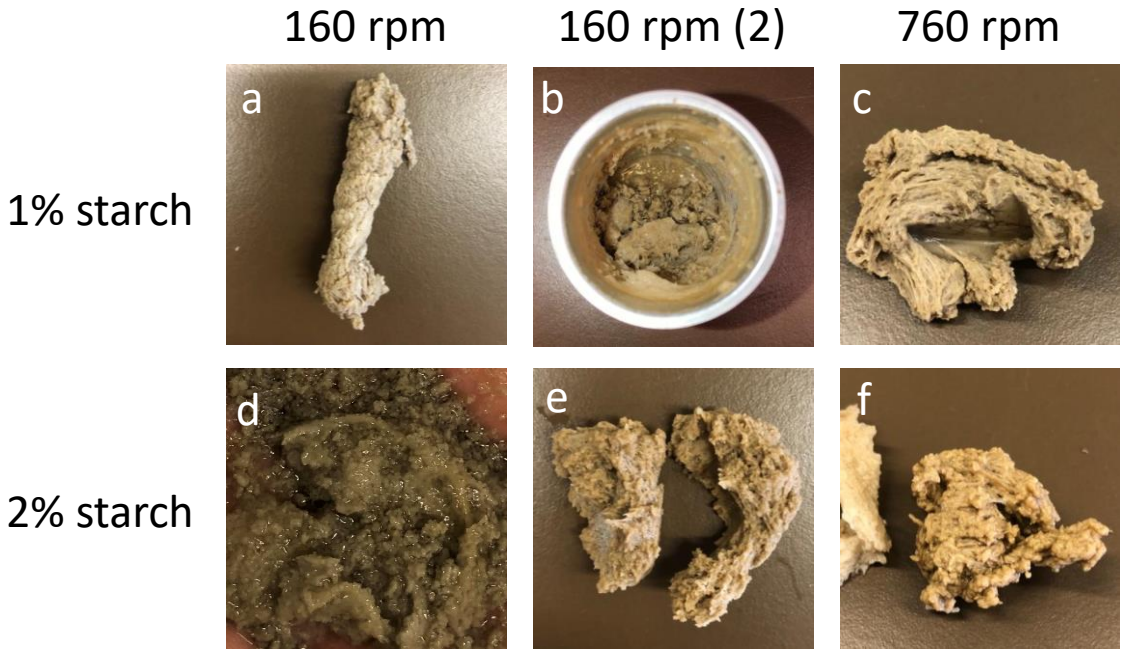

Figure 4.14. Protein structures formed in the RVA of 6% PPI2 (w/w) with addition of 1% or 2% potato starch (w/w), heated to 140°C with a stirring speed of either 160 (in duplicates) or 760 rpm.

The results show that addition of potato starch could prevent formation of dense structures at a low shear rate, and instead result in formation of many incoherent small grains (Fig. 4.14B,D). However, this effect was seen once for each duplicate, while the other duplicate did form a coherent protein structure making it hard to draw conclusions. The structures that did form, as seen in the rest of Figure 4.14, were less dense, more fibrous and easier to pull apart than those without the starch. It appeared that 1% starch addition resulted in products with a slightly lighter color, however no other considerable differences were observed between 1 or 2% starch addition.

It was previously hypothesized that a higher shear rate resulted in formation of more fibrous structures. However, with the addition of starch, this was not observed in the same manner. Equally fibrous products were formed regardless of the stirring speed. This is likely caused by the starch interfering with the protein network formation to a greater extent than the stirring speed.

4.3.5 Effect of oil droplets addition

The effect of oil droplets on the final protein structure of the RVA product was analyzed by adding 1.5% or 3% rapeseed oil to 6% PPI2 (w/w). Oil is able to form bonds with proteins, which can lower the amount of protein-protein interactions (de Vries, Gomez, van der Linden, & Scholten , 2017). It was, therefore, theorized that this would lead to lower aggregation, which could result in

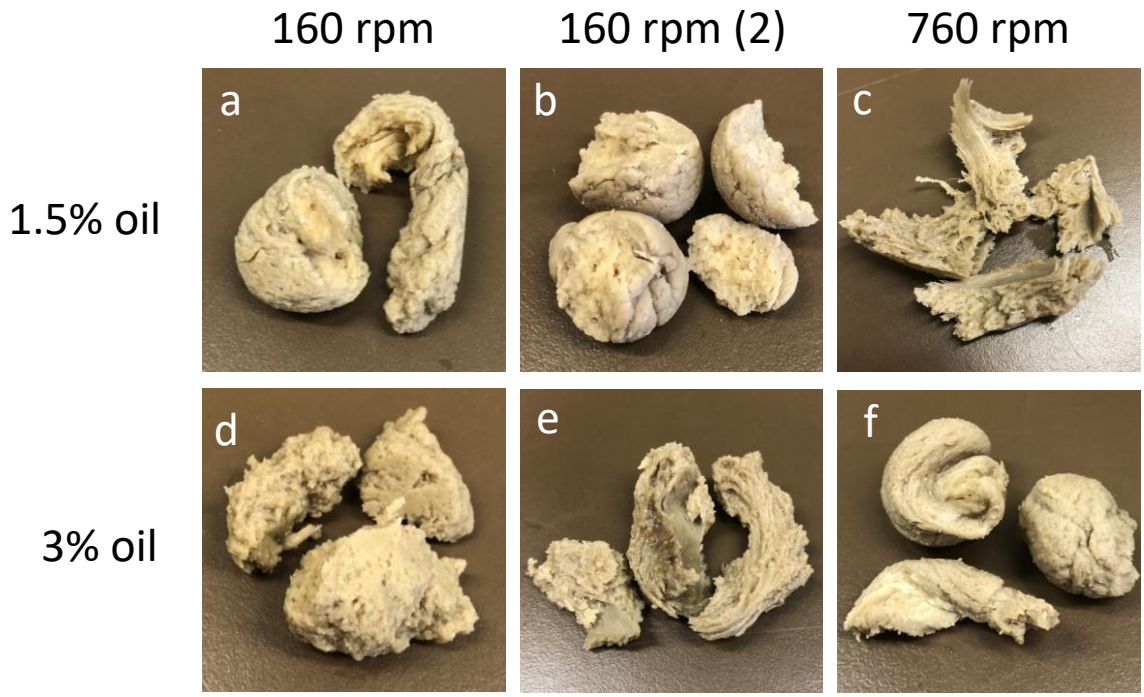

Figure 4.15. Protein structures formed in the RVA of 6% PPI2 (w/w) with addition of 1.5% or 3% rapeseed oil (w/w), heated to 140°C with a stirring speed of either 160 (in duplicates) or 760 rpm. The texturized products have been (partially) pulled apart on the photos.

protein structures which were less dense. The results are shown in Figure 4.15, and the RVA graphs can be found in Appendix 2 (Fig. A4).

The structured RVA products with oil addition were lighter in color, less dense and much easier to pull apart than products from previous experiments, while still maintaining a fibrous structure. Samples with 3% oil had a slightly more grainy structure than samples with only 1.5% oil, however overall the differences between the two samples were not substantial. Moreover, addition of oil resulted in samples with a more pleasant smell.

Again, the hypothesis that a higher shear rate resulted in a more fibrous protein structure was not observed in this experiment. The protein structures all had a similar fibrous structure independent of the stirring speed.

4.3.6 Combined effect of potato starch and oil droplet addition

As a final experiment, the combined effect of adding potato starch and oil droplets was analyzed for two combinations, 1% starch with 1.5% oil, and 2% starch with 3% oil, in triplicates at a stirring speed of 160 rpm. The results are displayed in Figure 4.16, and the accompanying RVA graphs can be found in Appendix 2 (Fig. A5).

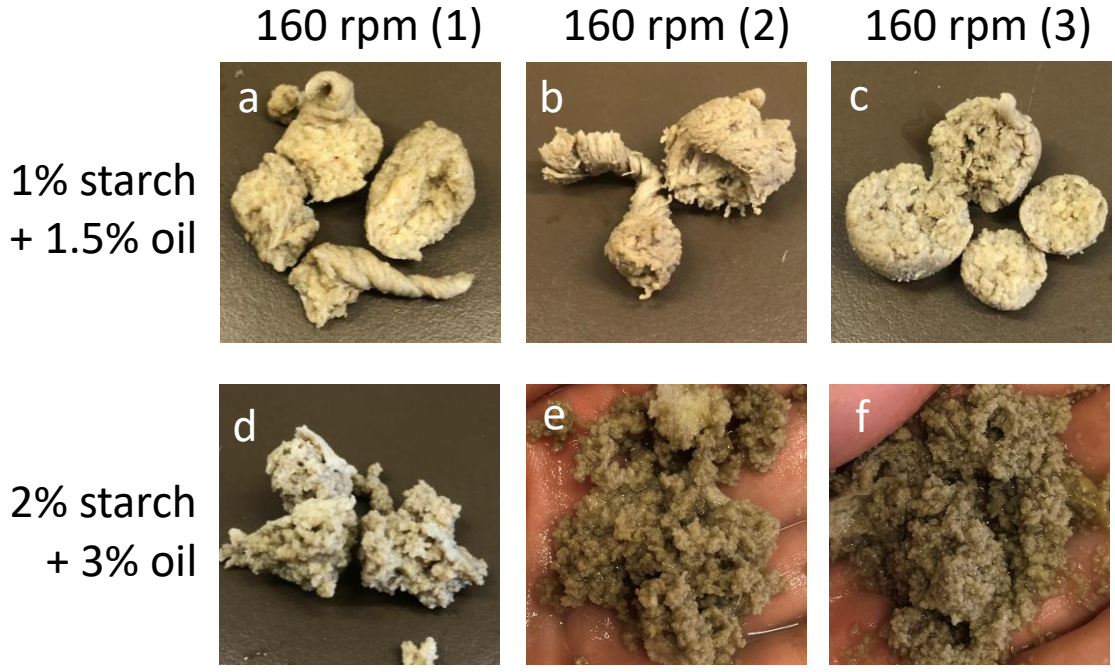

Figure 4.16. Protein structures formed in the RVA of 6% PPI2 (w/w) with a combination of 1% starch and 1.5% rapeseed oil, or 2% starch and 3% rapeseed oil, with a stirring speed of 160 rpm in triplicates. The texturized products have been (partially) pulled apart on the photos.

Figure 4.16 shows that the combination of 2% starch with 3% oil did not result in formation of a structured product in two of the triplicates. This is most likely due to excessive disruption of the protein-protein interactions leading to network formation. Since these grainy results were also observed in the experiment with starch addition (Fig. 4.14B,D), it is believed that the starch is mostly accountable for the disruption of the protein network. One possible explanation for this could be that the average particle size of starch granules is much larger than that of rapeseed oil droplets (Akhtar & Dickinson, 2001; Grommers & van der Krogt, 2009). The larger starch molecules interfere more with the protein network formation.

The combination of 1% starch with 1.5% oil did result in formation of coherent products. The structures were fibrous, light in color, without an unpleasant smell, and less dense than the results with only oil addition. The fibrous structure is especially notable in Figure 4.16B. This shows that a combination of potato starch and rapeseed oil is able to open up the compact structures formed inside the RVA, by lightly disrupting the protein network formation.

5 Future Directions

The scope of this project was to investigate whether potato protein isolates could be used to produce novel meat analogues. RVA results indicated that PPI2 show great potential in forming texturized products at 140°C, however it needs to be remembered that the RVA is not the machine that would be used for actual production of meat analogues. The RVA works as a great tool to mimic the process during high moisture extrusion, however since HME is still mostly based on empirical knowledge, it remains difficult to completely predict behavior inside an extruder. Therefore, an obvious next step for this research is to analyze the potato proteins in the extruder.

In order to get a deeper understanding of the protein interactions responsible for the network formation, protein solubility patterns can be analyzed using various solvents as described by Liu and Hsieh (2008). Results from such experiments indicate which type of interactions, such as disulfide bonds or non-covalent interactions, are responsible for the network formation by potato proteins.

Addition of 1.5% rapeseed oil together with 1% starch had a positive effect on the RVA product, as the structure became less dense and lighter in color. Only two different combinations of starch and rapeseed oil were examined, so in the future more variations could be analyzed. Other parameters which can be studied include, but are not limited to, faster heating rate, adding potato fiber, or other types of fiber, or combining potato proteins with other vegetable proteins.

6 Conclusion

The goal of the project was to determine whether potato protein isolates can be used as a protein source in developing texturized structures, resembling chicken meat. Three potato protein isolates were analyzed in this project, namely PPI1 (Lyckeby potato protein), PPI2 (Solanic®200), and PPI3 (Solanic®300). First, they were characterized by their protein concentration, particle size distribution, and solubility. The gelation abilities of the PPIs were also analyzed. It was determined that PPI1 had a low functionality, and was therefore excluded from most of the experiments. PPI2 and PPI3, both exhibited great functional properties, with especially PPI2 having the ability to form gels with high strength at low protein concentrations. Mixes of PPI2 and PPI3, Mixes A-D, were also analyzed for their joint functionality, but it was concluded that the samples containing mostly PPI2 gave the most promising results regarding their ability to form strong protein networks.

PPI2 and the potato protein mixes were then analyzed in an RVA, because the RVA can give an indication of the protein behavior in an extruder, as it also involves high temperature heating (>100°C), shear, and a long cooling process. It was discovered that when heated to 140°C, Mix D and PPI2 formed dense protein structures in the RVA already at protein concentrations of 6% (w/w on wet basis), mostly during the cooling phase. The effect of various parameters on the final protein structure was studied, such as heating temperature, cooling rate, shear rate, and protein concentration. Here, it was determined that the heating temperature had the most drastic effect on formation of texturized products, since they were only formed when heated to 140°C, not when heated to 100 or 120°C, which is most likely due to the melting temperature of the proteins in the range of 130-140°C. Initially, it was hypothesized that a high shear rate resulted in formation of more fibrous structures, but this hypothesis was rejected in later experiments. Mixes A-C were not able to form protein structures in the RVA, not even when heated to 140°C.

Since the formed protein structures were quite hard and dense, the effect of starch and oil addition on the texturized products was analyzed. The best results were observed for addition of 1.5% and 3% oil, or the combined addition of 1.5% oil with 1% starch. The structures obtained under these conditions were void of an unpleasant smell, lighter in color, and most importantly had a less dense structure which was easier to pull apart. This was caused by the starch and oil interfering with the compact protein network formation. However, too much disruption of the network, namely 3% oil together with 2% starch prevented development of a coherent product.

All in all, potato proteins show great potential for developing texturized products, which, in the future, could be used to develop novel meat analogues. The key next step is to analyze the potato protein isolates in an extruder.

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8 Appendices

Appendix 1: Additional details of Materials and Methods

In this appendix, additional information supporting Section 3 "Materials and Methods" is given. This information is not required to understand the method, but gives more detailed information about difficulties faced when executing the experiments, intended for people interested in continuing this research.

Protein solubility

It took a long time to prepare the samples for the protein solubility experiment, because some of the samples were super hard to dissolve. It was important that the protein samples were well mixed prior to adjusting the pH, since the protein powder had a large effect on the pH value. Especially PPI1 had to be stirred at a high speed for at least 15 minutes, in order to mix properly. After mixing PPI1 samples thoroughly, the samples would quickly sediment again. PPI2 was also difficult to mix and had to be stirred at a high speed for at least 10 minutes. However, once PPI2 was mixed, the samples were much more stable than the PPI1 samples. PPI3 samples were the easiest to handle, they were mixed for approximately 5-10 minutes at a high speed, but remained stable for the longest compared to the other PPIs.

Furthermore, the procedure required to centrifuge the samples at 20,000 g for 15 mins, but unfortunately the ultracentrifuge in the lab was not suitable for the large tubes used in this experiment. The protein solubility (%) was determined with the following assumption:

- Initially, 2% protein solutions (w/w) were prepared for the experiments
- Theoretically, if the protein was 100% soluble, then during the centrifugation, all of the protein would remain in the supernatant.
- The protein content results from the supernatant would, therefore, be 2g protein/100 g supernatant (2% w/w) for a solubility of 100%.
- Using this correlation, the protein content in % was determined for all samples

Small volume gelation experiments

Samples with different concentrations were obtained by diluting the protein mix of the highest concentration with distilled water. These dilutions were made inside the Eppendorf tube for each sample. For example, a 4% protein dilution from a 12% protein mix was obtained as follows:

- Mix 100 µl 12% PPI mix + 200 µl dH_2O
- Pipette up and down several times to ensure thorough mixing

In experiments with adjusted pH values, the pH was only adjusted of the main protein mix with the highest concentration, prior to the dilutions for the other samples. This was done because the volumes were too small to adjust the pH for each 300 µl sample at the diluted concentrations.

PPI1 was tested for a maximum concentration of 28%, where it did not gel. It was attempted to analyze PPI1 for higher concentrations starting with 36%, but the sample was too thick to mix well and dilute.

One challenge in the small volume gelation experiments was that some samples started to sediment while still preparing other samples, because of the time it took to deal with a large amount of samples.

Large volume gelation experiments and gel strength determination

In the large volume experiment, some challenges were faced which were not there during the small volume gelation experiments.

- It was very hard to get the PPIs to mix at larger volumes, and some mixes had to be stirred at a high speed for at least 30 min.
- Mixes A, B, D and PPI2 all expanded in volume drastically while mixing, from a volume of 150 ml to 200-250 ml.
- Mix B separated the fastest after mixing.
- Mix C foamed in a different way, where a separate foam layer was created on top of the mix while stirring.
- Mix A and D were very thick after mixing and adjusting the pH to 5.5, which sometimes made it hard to stir thoroughly on a magnetic stirrer, and also made it more difficult to accurately pipette the desired volume.
- It was found that the easiest way to prepare the mixes was by pre-mixing the PPI powders before adding the water.
- After heating to 90°C, the gel volume had increased for Mix A, D and PPI2. Also, the gels from Mix B, C, D and PPI2 had detached from the side of the flask.

RVA measurements

Each HT-RVA can was used 3-6 times, before discarding it for a new one, depending on the deformation of the can from the clamping inside the RVA machine. The HT stirring paddles were used for more experiments, until it became too difficult to clean the paddle properly. In order to adjust the pH, the protein mixes were first prepared by adding almost all the distilled water, except for around 5 g. The amount of 1 M HCl or 1 M NaOH required to adjust the pH was measured, and subtracted from the 5 g of water that still needed to be added. This way, the adjustment of the pH did not change the final weight of the mixture, to obtain an accurate 12% w/w protein solution.

In order to compare various RVA results, the moisture content needs to be equal. However, because for me it was also important to keep the protein content similar, the results from the dry matter experiment were combined with the protein content values, to determine a "moisture content" of PPI2 and PPI3, as described in Table A1. The approximated "moisture content" of Mixes A-D based on the values for PPI2 and PPI3 is then shown in Table A2.

Table A1. Approximation of the 'moisture content' (%) of PPI2 and PPI3

Sample	Average dry matter (DM) (%)	Protein content average of DM (%)	Protein content (corrected for DM) (%)	'Moisture content' based on corrected protein conc. (%)
PPI 2	90.77	92.59	84.04	15.96
PPI 3	92.49	97.09	89.80	10.20

Table A2. Approximation of the 'moisture content' (%) for mixes A-D.

Sample	Mix A	Mix B	Mix C	Mix D
Approximated 'moisture content'(%)	13.08	14.23	14.81	15.38

The sample calculator built into the RVA TexCalc software was used to prepare the protein concentrations, using the moisture contents described in the table above. The amount of protein required to prepare a sample of the desired concentration was determined first, and this value was entered into "Sample Calculator" function of the RVA software, together with the approximated moisture content (Table A2). The moisture basis was set to 0%, which was kept consistent for all measurements, and is a parameter which needs to be set in order to compare various RVA results. The "Sample Calculator" function then showed the amount of distilled water that needed to be added to the PPI powders to prepare the samples. These values would be used to make ~200 g of one sample at once, and from this mixture, 25 g was taken into the RVA can for the measurements. All RVA experiments were performed accordingly.

Appendix 2: RVA graphs

In this appendix, the RVA graphs are shown which are not included in the main body of the report, as it had been decided to compare the dense structures formed in the RVA, rather than their RVA graphs. Because the quality of the smalls graphs, unfortunately, is not very high, larger photos of the RVA graphs can be found online using the following link: https://tinyurl.com/y868ev6n

Appendix 2A: RVA graphs from experiments with various program settings

The RVA graphs from Mix D and PPI2 heated to 140°C with a stirring speed of 760 rpm are shown in Figure A1

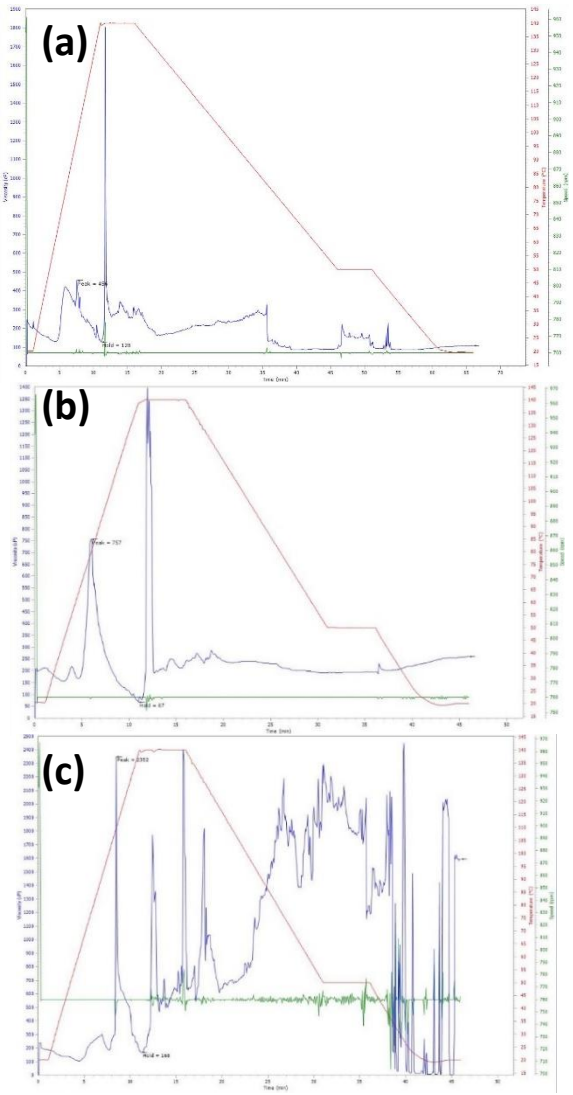

Figure A1. RVA viscosity curves for heating to 140°C with various program parameter settings. **(a)** 12% Mix D with a cooling rate of 3°C/min. Then, with a stirring speed of 760 rpm the pasting profiles for **(b)** PPI2 and **(c)** Mix D, are shown.

Appendix 2B: RVA graphs from experiments with various concentrations

These graphs accompany the results from Section 4.3.3 "Effect of various protein concentrations".

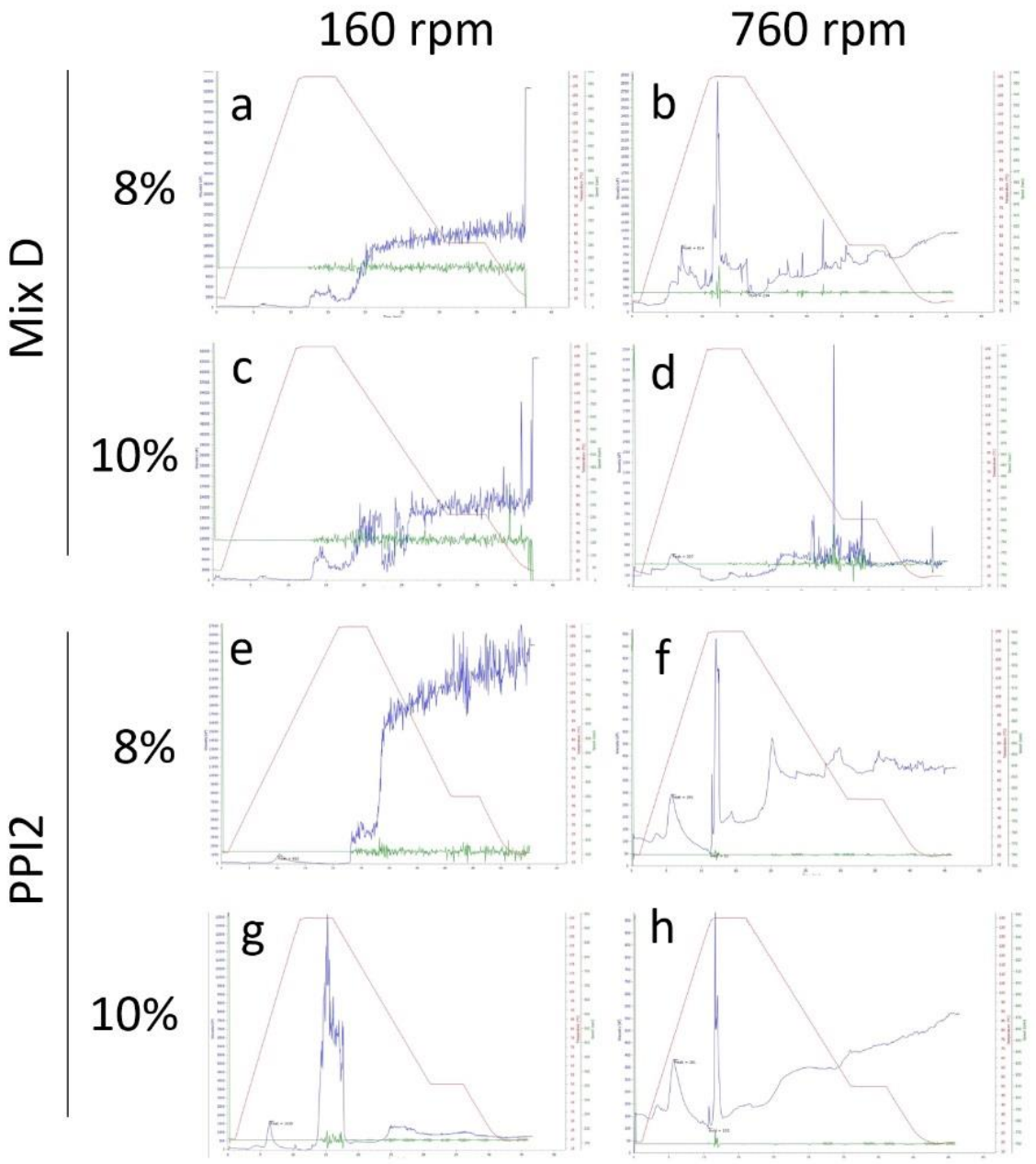

Figure A2. RVA viscosity curves of PPI2 and Mix D for protein concentrations 8% and 10% (w/w) and stirring speeds 160 and 760 rpm.

Appendix 2C: RVA graphs from experiments with potato starch addition

These graphs accompany the results from Section 4.3.4 "Effect of potato starch addition".

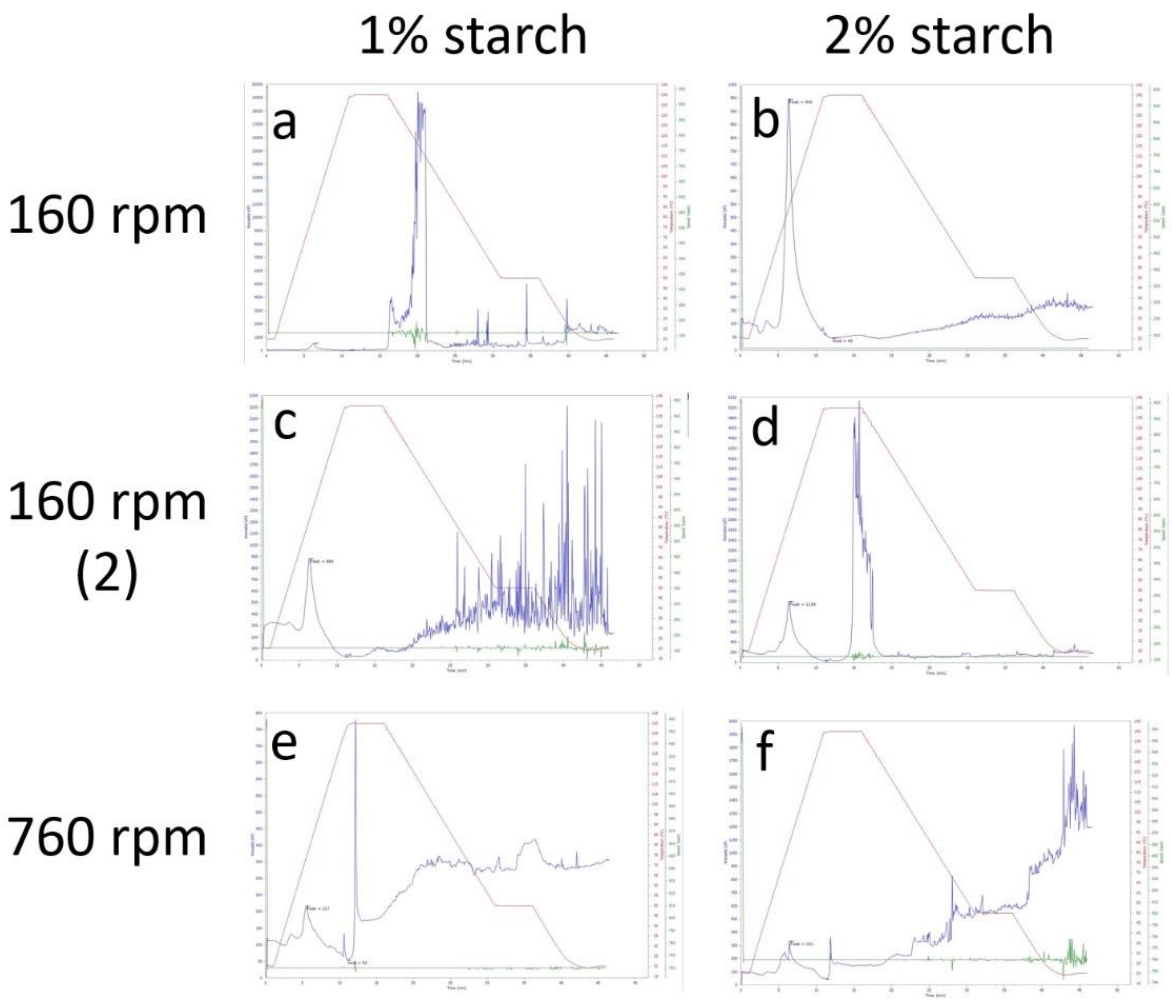

Figure A3. RVA viscosity curves of 6% PPI2 (w/w), with 1% or 2% potato starch addition, and stirring speeds 160 and 760 rpm.

Appendix 2D: RVA graphs from experiments with rapeseed oil addition

These graphs accompany the results from Section 4.3.5 "Effect of rapeseed oil addition".

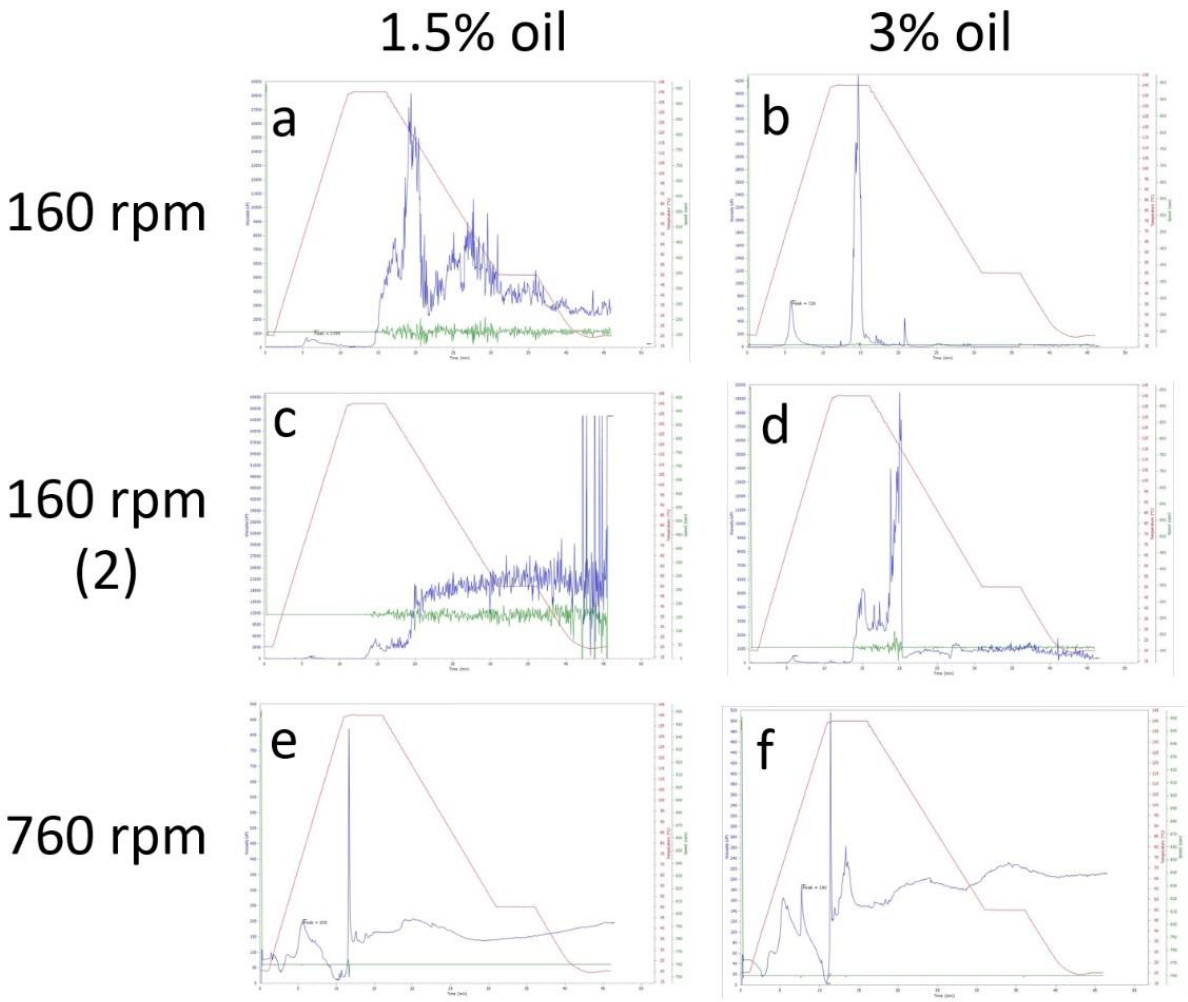

Figure A4. RVA viscosity curves of 6% PPI2 (w/w), with 1.5% or 3% rapeseed oil addition, and stirring speeds 160 and 760 rpm.

Appendix 2E: RVA graphs from experiments with combined starch and rapeseed oil addition

These graphs accompany the results from Section 4.3.6 "Combined effect of potato starch and oil droplet addition".

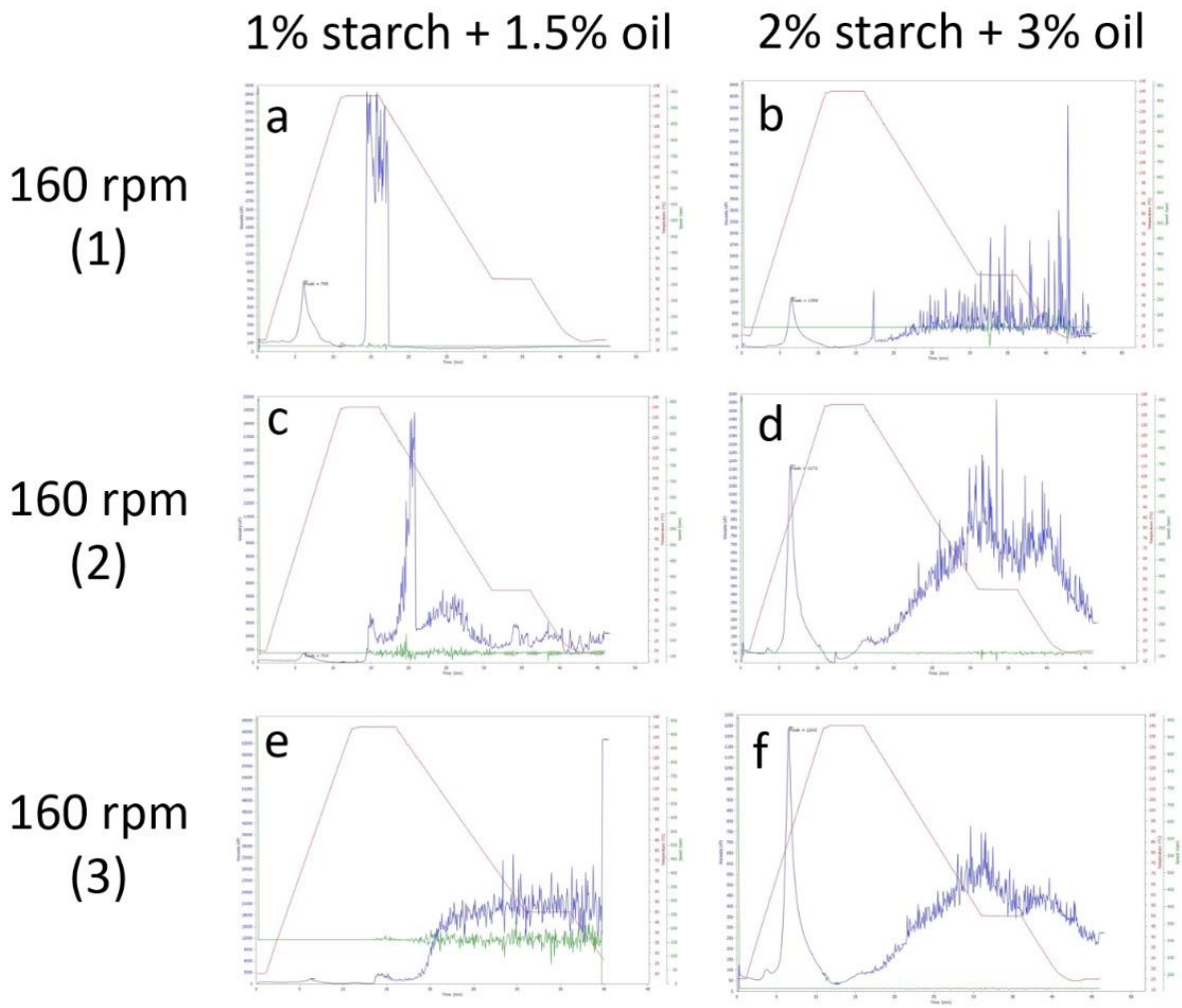

Figure A5. RVA viscosity curves of 6% PPI2 (w/w) with either a combination of 1% starch and 1.5% rapeseed oil, or 2% starch and 3% rapeseed oil, in triplicates.

Appendix 3: Popular Science Summary

In this thesis project, it was analyzed whether potato proteins could be used to develop a "fake meat".

The world population keeps growing rapidly, is becoming more affluent, and has a strong desire to eat meat. According to the Food and Agriculture Organization (FAO), approximately 72 billion land animals are killed for food per year (FAOSTAT, 2017), and this is estimated to double by 2050 (FAO, 2019). Double! The environmental impact of the meat industry is enormous, and combined with animal welfare issues, consumers are looking for a change. Instead of stuffing animals with plant proteins, to later eat their meat as a source of protein and energy for humans, why not find ways to eat the plant proteins directly? One reason is that meat is embedded in many Western cultures as a staple food. But what if we can make products that resemble meat from plant proteins? A-ha, this is where meat analogues come in, also known as vegetarian or "fake" meats.

Potato proteins are great vegetable proteins to use in the food production industry. They are very nutritious, and are already found in enormous quantities all over the world. This is because potato proteins are a byproduct of the potato starch industry. Currently, however, a lot of the potato protein goes to waste, which is why finding a use for these potato proteins in food is both sustainable and economically beneficial.

First, the potato proteins were analyzed for their functionality and ability to form gels. It was determined that the potato proteins could indeed form stable structures. Then, a promising technique to produce meat analogues is called high moisture extrusion. During this process, protein mixes are first heated up, after which pressure and shear are applied during cooling, which results in formation of fibrous products. Unfortunately, it was not possible to use an actual extruder in this project, so instead a machine called a Rapid Visco Analyzer (RVA) was used to predict behavior of the potato proteins inside an extruder. The RVA measures the viscosity of the proteins as a function of time. The effect of many different parameters on the outcome of the RVA was analyzed, namely shear rate, heating temperature, cooling rate, protein concentration, addition of oil, and addition of starch. It was determined that potato proteins show great potential in producing "fake meat" when heated up to 140°C, for a low protein concentration of 6%. Especially combined addition of starch and oil have beneficial effects on the texture of the formed protein product. An exciting next step of this research is to use an actual extruder to make meat analogues based on potato proteins.