AN INVESTIGATION OF RAPESEED PROTEIN AS A NEW FOOD PRODUCT

Improvements of the isolation method to increase the protein yield and sensory parameters as well as investigating its ability to form a meat-analogue

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Abstract

The combintemperaation of a growing global population and a limiting amount of farmland creates a need for new innovative food products to achieve a global sustainable development and a healthy population. Rapeseed press cake is a low-cost by-product of rapeseed oil production and is very high in protein and fiber. The rapeseed protein contains high concentrations of S-amino acids as methionine and cysteine, comparable with soy. Most of the rapeseed press cake is sold as animal feed. If the press cake instead could be used as human food directly, one step in the food chain could be eliminated and more of the plant nutrients could be utilized by humans. As there is a growing demand of meat-analogues due to the increase of the vegan scene in Sweden, there seem to be a market for at meat-analogue based on locally produced rapeseed protein. By up-cycling an existing agricultural by-stream and recover high quality protein for human consumption, the import of soy can be decreased in Sweden.

In previous master's thesis and studies a method to isolate proteins from cold-pressed rapeseed press cake has been developed. The current thesis has been focusing on improving the isolation method to achieve an edible food product as well as investigating different processing methods of the isolated rapeseed protein paste (RSPP). To optimize the protein yield two different approaches has been investigated, the incubation time and its performance in the leaching phase as well as recirculation of the first sediment at different pH. To decrease the content of anti-nutrients and discoloring compounds in the RSPP, ultrafiltration and diafiltration have been investigated.

Drum drying, freeze drying and vacuum evaporation was investigated to decrease the water content. Finally, thermoplastic extrusion has been used to investigate the abilities of rapeseed proteins to be texturized. A single-screw extruder with a conventional screw (20 mm Ø x 25) was used. Two different dies were used, a heated round die (3 mm Ø) and a cooled flat die (2x20 mm)

The results of the process optimization indicated that incubation in 60 min with stirring, instead of the previous incubation time of 180 min without stirring saved two hours of processing time without reducing the protein yield. By recirculation of the first sediment the total protein yield was increased by at least 7.2% (from 38% to 45.2%). Further it was showed that addition of $Na_2S_2O_5$ in the diafiltration improved the color and taste of the rapeseed protein paste. The freeze- and drum-dried products achieved a water activity below 0.6, which is suitable for food applications. The result of the extrusion indicated that a single-screw extruder at an extrusion temperature of 100°C-120°C and water content of 50% did not seem to form the desired fiber structure. As the fat content was high and as a higher temperature could not be tested in the single-screw extruder, extrusion trials in a twin-screw extruder should be performed to further investigate the ability of rapeseed protein to form an elastic texture by thermoplastic extrusion.

Contents

1. Introduction

As the global population is growing and there is a limiting amount of farmland, more plant-based protein sources are needed to be developed (Kumar et al., 2017). To convince people used to the meat texture to eat more plant-based proteins, more products in the meat analogue segment should be developed.

Rapeseed press cake is a protein-rich residue of rapeseed oil production. Year 2014 the harvested production of rapeseeds in Sweden and Europe was 356 500 ton respectively 24 252 700 ton (Ländell and Funcke, 2016) (Eurostat, 2015). Today, rapeseed meal is mainly used as animal feed and it is the second largest feed protein source (Wanasundara et al., 2017) (Mohamad et al., 2002). Besides the high protein content (generally 30% per weight rapeseed) its protein quality is excellent for human consumption (Wanasundara et al., 2017) (Bos et al., 2007). As the rapeseed press cake is a by-product of the rapeseed oil production a further utilization of the press cake would not contribute to any further use of farmland. In 2013, the first protein isolate derived from rapeseeds proteins was granted as a novel food ingredient by EFSA, European Food Safety Authority (Nutrition and Allergies, 2013).

There is a range of different kinds of vegetable protein powders (isolates and concentrates) on the market but these products do not contribute to any meat-analogue texture. Thermoplastic extrusion can be used to produce a meat-alike texture. Thermoplastic extrusion has been used to produce soybean based meat-analogues since the 1970's and today there is a range of different kinds of products such as minced soy and soy chunks (Berk, 1992b) (Honeyville, 2017). Soybean based products has in general less impact on the environment compare to animal-based foods, however there is still issues associated with soy products (Reijnders and Soret, 2003). Soybean has been established as one of the eight foods that are associated with the most frequently human food allergies (Cordle, 2004). As the soybeans are not grown in Sweden, it also needs to be transported long distances. Producing a meat-analogue based on rapeseed protein would give a nationally produced plant-based protein food without compromising on the texture or mouth feeling, which also would be convenient for consumers allergic to soybeans. A national production of rapeseed protein based meat-analogues would also contribute to strengthen the Swedish labor market and the national economy as new job opportunities would arise.

1.2 Rapeseed oil

Rapeseed oil contributes for the second largest part of the global oilseed industry (after soybean) and third largest in the global vegetable oil production (after palm oil and soybean oil) (Wanasundara et al., 2017).

1.2.1 Production of warm-pressed rapeseed oil

The seeds are cleaned from foreign materials and dried to a moisture level of maximum 10%. Normally the seeds are pre-heated to 35 °C and then they are flaked by a flaking unit to rupture the seed coat. The ruptured seeds then undergo a cooking step at a temperature of around 80°C-105°C. During the cooking step, the non-cracked seeds are thermally ruptured and enzymes are inactivated. The cooked ruptured seeds are then pressed by a series of screw presses (Canola council of Canada, 2016). The remaining oil in the press cake is extracted by a solvent, normally hexane (AAK, 2017).

1.2.2 Production of cold-pressed rapeseed oil

5-10% of the rapeseed production in Sweden is being cold-pressed (Nilsson, 2017). First, the rapeseeds are cleaned in the same way as in the warm-pressed process. The mature and dried rapeseeds are then pressed in a screw-press process at a maximum temperature of 35 °C. The crude oil is pressed and filtered to remove seed coat residuals. The residue of this cold-pressed rapeseed oil production is the press cake (Nilsson, 2017). In this thesis, rapeseed press cake from cold-pressed production has been used as those proteins have not been denatured by heat.

1.3 Rapeseed press cake

1.3.1 Proteins

The rapeseed press cake consists of approximately 30 % proteins and the main proteins in rapeseeds are storage proteins, which respond to 80%-90% of the total protein content. The storage protein found in rapeseed are 12S cruciferin and 2S napin (12S globulin and respectively 2S albumin) (Mohamad et al., 2002) (Campbell et al., 2016). In mature rapeseeds, cruciferin stands for approximately 60 % of the total protein content and napin for 25% (Mohamad et al., 2002). Minor proteins in the rapeseeds are oil body proteins such as oleosins (75-80 % of the oil body proteins) and caleosins, see the distribution of the different kinds of proteins in rapeseed and soybeans in Fig 1. These oil body proteins prevents coalescence of oil droplets in the cellular structure inside the rapeseeds (Wanasundara et al., 2017). The nutritional value of the rapeseed protein is very high as it contains high levels of essential amino acids, including the sulfur-containing amino acids methionine and cysteine. The amino acid composition of rapeseed protein is closer to the human reference pattern (defined by FAO/UNU/WHO) compare to all other plant-based proteins (Wanasundara et al., 2017).

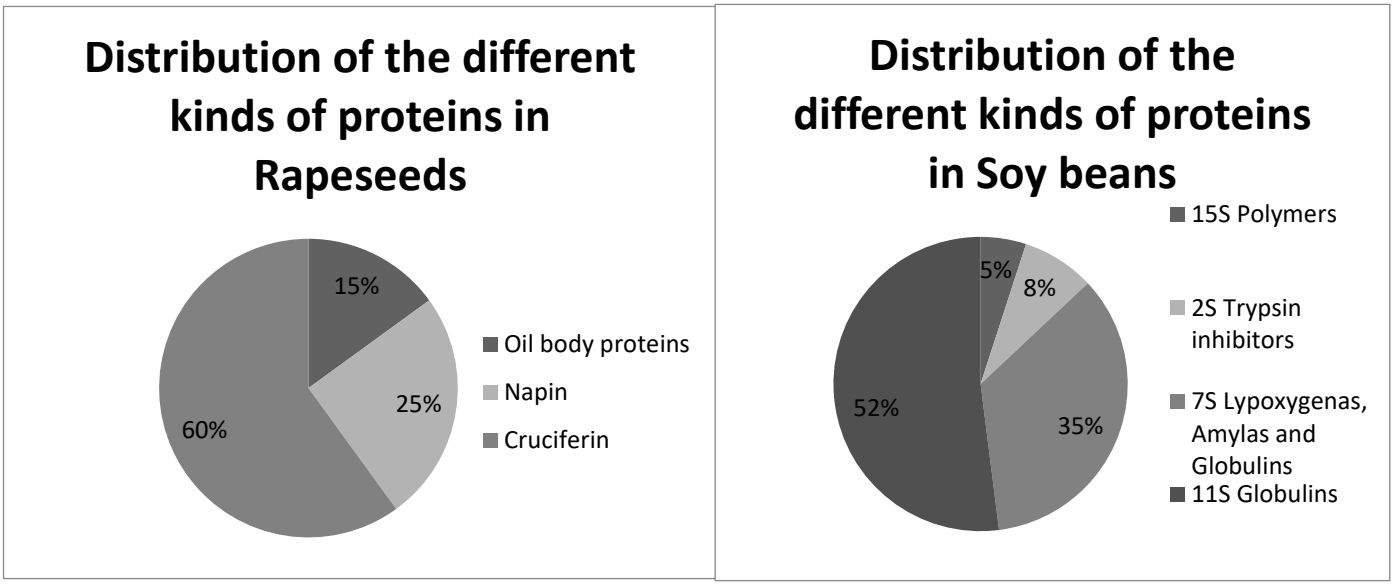


Figure 1. The distribution of the different kinds of proteins in rapeseeds (Wanasundara et al., 2017) and soy beans (Kinsella, 1979).

1.3.1.1 12S cruciferin - 12S globulin

The cruciferin is a globular protein, which is a group of protein that have shown to form the desired continuous structure by extrusion (Tan et al., 2011). Cruciferin has a denaturation temperature of 86.6 ˚C, compare to 93.9 ˚C of the globulin protein glycinin in soybeans (Mohamad et al., 2002). Cruciferin has a molecular mass of approximately 48-56 kDa, see its 3D-structure in Fig. 2 (Wanasundara et al., 2017).

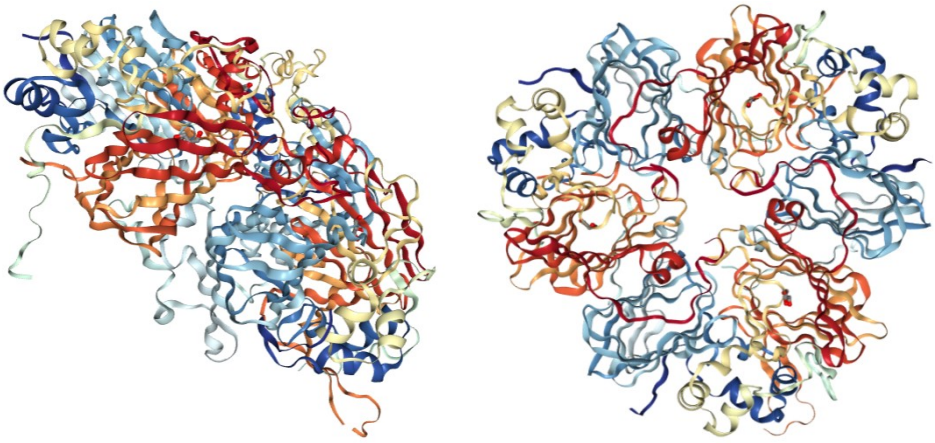


Figure 2. The 3D-structure of 12S Cruciferin (Tandang-Silvas et al., 2010) .

1.3.1.2 2S napin- 2S albumins

2S napin is a 2S albumin that belongs to the family of prolamin proteins. European rapeseed cultivars contain 25-45 % 2S albumin of the total amount of seed proteins. Rapeseed napins are hydrophilic proteins and remain stable when heated up to 75˚C. The napins contain a large amount of alkaline and sulfur amino acids (Wanasundara et al., 2017). Napin consists of two subunits, one small unit of approximately 4.5 kDa and a larger unit of approximately 10 kDa (Wanasundara et al., 2017) . The two subunits should give napin a total molecular mass of 14.5 kDa, see the 3D-structure in Fig. 3.

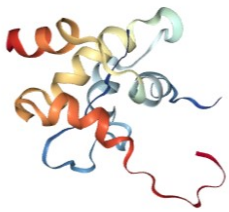


Figure 3. The 3D-structure of 2S Napin (Rico et al., 1996).

1.3.1.3 The Isoelectric point of rapeseed proteins

The literature describes many different isoelectric points (pI) for the rapeseed proteins. Das Purkayastha et al. (2015) gives the rapeseed proteins a pI of around pH 10 and Wanasundara et al. (2017) claimed that the pI of cruciferin is at pH 7.25. Von Der Haar et al. (2014) also stated that the pI of cruciferin is at pH 7.25 and that napin has an isoelectric point above pH 10. In the same article it is described that the mixture of proteins in rapeseed protein concentrate (approximately 80% protein) has a pI of pH 6.3. Since the literature most frequently report the pI of cruciferin to be at pH 7.25 and the pI of napin to be above pH 10, these values will be used as the true values in this thesis. It has been demonstrated that different varieties of rapeseed/canola contains proteins that precipitation at different pH values (Xu and Diosady, 1994). This phenomenon may explain why different pI has been found in the literature.

1.3.1.4 The processing effects on the proteins

During warm-pressed rapeseed oil the seeds are heated to 80-105 °C (Canola council of Canada, 2016). This means that the proteins will be denatured to some extension since they seem to start to denature in a temperature range of 75-86 °C, see sections 1.3.1.1 12S cruciferin - 12S globulin and 1.3.1.2 2S napin- 2S albumins above (Wanasundara et al., 2017).

1.3.2 Anti-nutrients in rapeseed press cake

Rapeseeds contain anti-nutrients that will remain in the rapeseed cake after the oil extraction, for example phytates, phenolic compounds and glucosinolates (Wanasundara et al., 2017). Phytate decreases the bioavailability of several minerals in humans, such as zink and iron (Lopez et al., 2002).

1.3.2.1 Phenolic acid esters

Phenolic acid esters such as sinapate esters, sinapoylcholine (sinapine) and sinapoylglucose are anti-nutrients that exists in higher amounts in rapeseeds compared to soybeans. The sinapate esters cause a bitter flavor to rapeseed meals and extracted protein products. Sinapate do also have negative effects on the digestibility of the rapeseed meal. It forms complexes with the proteins through oxidation during the rapeseed oil pressing, which prevents proteases to reach the proteins (Tan et al., 2011). Interactions of polyphenolic compounds with proteins and oxidation of phenolic compounds forms a dark, brown-grey color. This discoloration can be prevented by addition of sodium sulfite, sodium bisulfite or polyvinyl pyrrolidine (Wanasundara et al., 2017).

1.3.2.2 Glucosinolates

Glucosinolates are a group of thioglucosides that naturally occurs in rapeseeds and many other plants and vegetables in the Brassica family such as radish, broccoli, mustard seeds and some varieties of cabbage. The glucosinolates can be degraded by an enzyme called myrosinase or by enzymes from the human gastrointestinal bacteria. The glucosinolates and the myrosinase are kept in separate compartments in the rapeseed, unavailable to each other until the seeds are being damage, such as oil pressing or chewing (EFSA, 2008). After cell damage, the myrosinase and glucosinolate can get in contact with each other due to breakage of the cell compartments. The enzyme thereby hydrolyses the glucosinolate to a glucose unit and potential toxic compounds such as nitrile and isothiocyanate (Das S. et al., 2000), see Fig 4. The general products formed from hydrolysis of glucosinolates can be seen in Fig. 4.

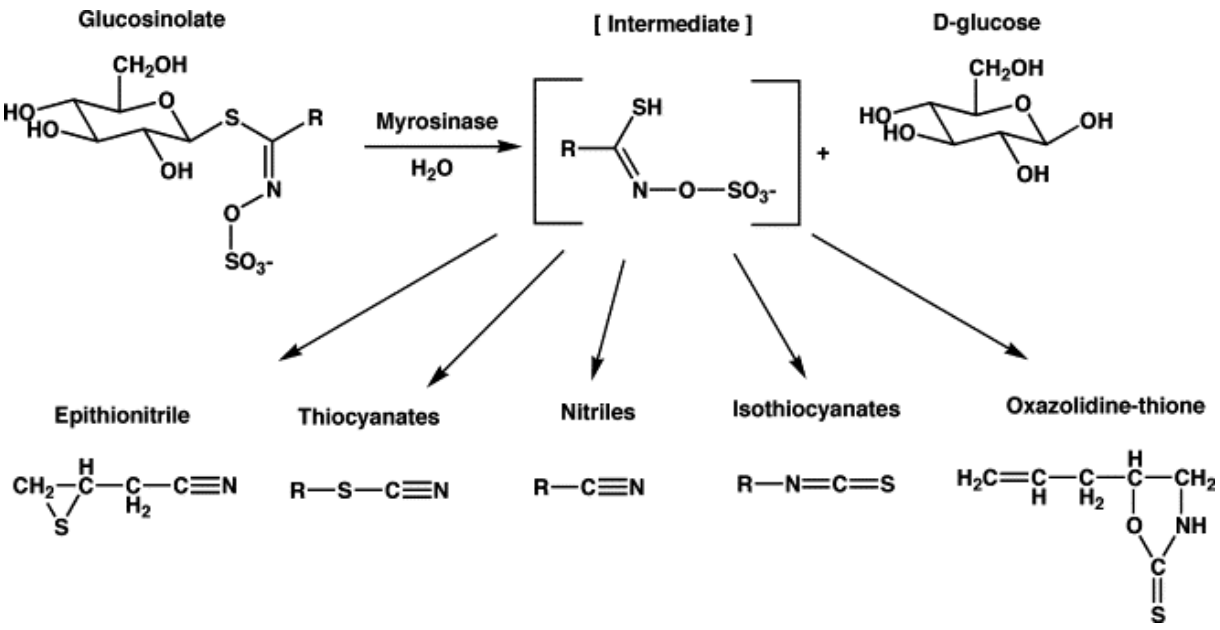


Figure 4. The general structure of glucosinolates and its hydrolysis products (Vaughn and Berhow, 2005).

The effect of the degradation products of glucosinolates is disputed in the literature and has been demonstrated to have both beneficial- and adverse health effects. Sulforaphane, which is a breakdown product of the glucosinolate glucoraphanin, have shown to activate enzymes that have shown to be strongly linked to prevention of different kinds of cancer (Vaughn and Berhow, 2005). On the other hand, different kinds of adverse health effects have been observed after intake of glucosinolates in animal studies. Observed adverse effects are liver- and kidney enlargement and decreased egg production in birds. In humans, higher levels of metabolites of glucosinolates have shown adverse effect on the thyroid gland, leading to reduced iodine uptake (Wanasundara et al., 2017).

Today only double low rapeseed varieties are allowed to be cultivated in EU due to the negative health effects of glucosinolates. Double low means that the seed contains maximum- 1% erucic acid and 25 µmol/g glucosinolates (on dry basis). Since the glucosinolates are water soluble it remains in the press cake while the oil is pressed out and the remaining concentration of glucosinolates is probably higher than 25 µmol/g in the rapeseed press cake. The prevalence of glucosinolates in rapeseeds differs from different kinds of varieties and is affected by the geographic region, soil and agriculture practices (EFSA, 2008).

1.4 Background of the processing methods

1.4.1 Protein recovery method

The rapeseed protein (RSP) was isolated from the rapeseed press cake by a water based extraction method by several steps including change of pH and centrifugations, see section 2.2 Isolation of proteins from rapeseed press cake. The composition of the rapeseed press cake (RSPC) can be seen in Table 1.

Table 1. Composition of rapeseed press cake received from Gunnarshög, (K. Nilsson, 2015).

Compound	g/100g press cake
Water	10.3 ± 10%
Ash	6.04 ± 10%
Protein	27.3 ± 10%
Fat	15.5 ± 10%
Carbohydrates (calculated)	10.8
Fibers	30.1 ± 15%

1.4.2 Processing methods

1.4.2.1 Ultrafiltration (UF)

Ultrafiltration (UF) is used to separate and/or concentrate different components in an aqueous solution based on differences in their molecular weight and it uses a pressure of up to 10 bars. The feedstock is fed to the system, when the feedstock meets the membrane a part of the fluid will pass through, the permeate. The rest of the fluid is rejected by the membrane and will not pass, the retentate, see Fig 5. The specific fraction that is cut off by the membrane is determined by the porosity of the membrane, generally ranging between 1 to 1 000 kDa for UF. As the given porosity of the membrane is set by measurement of the molecular weight of the passed molecules there will not be a sharp cut off size. Generally, molecules with both slightly lower and higher molecular weight than the set cut off size will be found in both the retentate and permeate. Ultrafiltration is widely used in the dairy industry to concentrate proteins (Bird, 1996). To concentrate the rejected molecules in the retentate several diafiltrations can be used. Diafiltration means that the retentate is diluted, in general with clean water or what else the continuous phase consists of and the feed is again filtered (Bird, 1996).

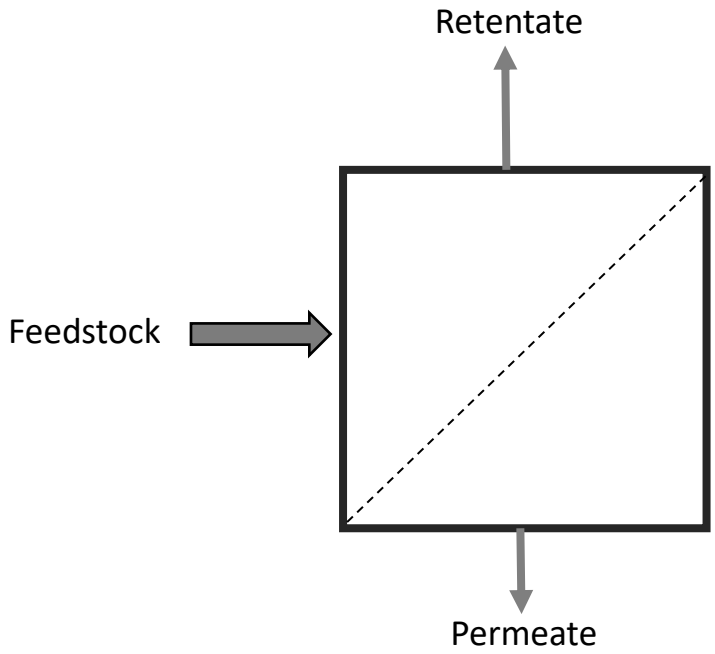


Figure 5. The membrane divides the feedstock into retentate and permeate, the dashed diagonal line is the membrane.

1.4.3 Drying methods

1.4.3.1 Drum drying

Drum drying is used for drying of food were the product are to be powders or flakes, it is widely used for baby foods with low moisture, fruit powders and for gelatinization of starches (Caparino et al., 2012) (Colonna et al., 1984). The drum dryer can consist of either a single- or double cylindrical drums rotating in contrary directions to each other. The large cylindrical drum/s is/are filled with hot saturated vapor with a temperature of approximately 120-170°C. The feed to be dried is poured in the adding line, see Fig. 6. The feed is spread out to a thin layer over the drums and finally scraped off in the form of flakes. The high temperature can cause a cooked aroma and potentially change the properties of proteins due to heat denaturation (Caparino et al., 2012).

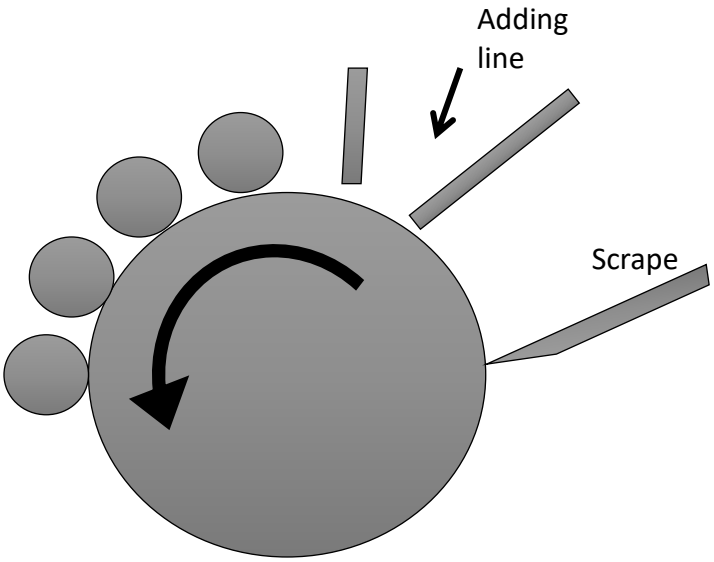


Figure 6. A single drum dryer with four smaller rotating cylinders.

1.4.3.2 Vacuum evaporation

Vacuum evaporation is used to dehydrate a sample while minimizing thermal damage such as change in color and flavor. Vacuum evaporation dehydrates a sample by letting moisture evaporate at a lower temperature due to reduced pressure. The used pressure is usually in the range of 7.5-85.0 kPa and the corresponding evaporation temperature is 40-95°C. (Brennan, 2006)

1.4.3.3 Freeze drying

Freeze drying is a process that dehydrates a frozen sample by sublimation of frozen water at reduced pressure (Caparino et al., 2012) . The freeze drying produces a high quality product since most of the deteriorations and microbiological reactions can be avoided by low temperatures and lack of water (Ratti, 2001). Both freeze-drying and spray drying are preferable drying methods for proteins. However, freeze-drying better protects the proteins from denaturation (Haque and Adhikari, 2014). On the other hand, it is a high energy consuming process that has low productivity (Caparino et al., 2012).

1.4.4 Texturization method

1.4.4.1 Extrusion

There are different kinds of extruders used for texturizing of plant protein such as soy flours, but they follow the same principle. Defatted soy flour (see composition in Table 2) is blended with water to achieve a specific moisture level and is then fed into the extruder.

Table 2. The macro components of defatted soy flour (Berk, 1992a).

Component	Percentage (%)
Moisture	7.0
Ash	6.4
Protein	59.0
Fat	0.9
Fiber	2.6

An extruder consists of a large screw inside a cylindrical barrel, which force the soy paste forward towards the die-end, see Fig 7. The melt (soy paste) is compressed since the free volume in each screw shaft is decreased along the flow direction.

Figure 7.The single-screw extruder used in the current thesis.

Inside the extruder, the shafts of the screw also commonly contain heating or cooling elements to regulate the temperature. The paste is also heated by friction along the extruder and the temperature of the paste reaches 150-180˚C (Berk, 1992b) . Due to the friction, the temperature is usually increased through the extruder (Camire, 1991). The process is also called “thermoplastic extrusion” since the material is being thermoplastic melted by the high temperature and pressure. When the proteins are heat denatured, they align to each other by the direction of the shear forces in the barrel, see Fig. 8. When the melt reaches the end-opening (the die) of the extruder, the pressure is suddenly decreased which allows the water to rapid evaporate and the alignment of the proteins have created porous and laminar formed chunks, see Fig. 9. The porosity of the product depend on how fast the water evaporates when it enters the end-opening, if a less porous product is desired the melt is cooled down a bit before it reaches the end-opening (Berk, 1992b).

The texturized structure of the extrusion product is formed by the protein polymers forming laminar cross-links as they flow in the streamlines (Steel et al., 2012). During extrusion, hydrophobic amino acids are being displayed which decreases the water solubility of the extruded product. The

intermolecular bonds that mainly are formed during extrusion are disulfide and hydrophobic bonds (Camire, 1991), see Fig. 8. Proteins shown to form a continuous fiber structure by extrusion are typically the globular proteins from oilseeds, in rapeseed that is cruciferin (Steel et al., 2012).

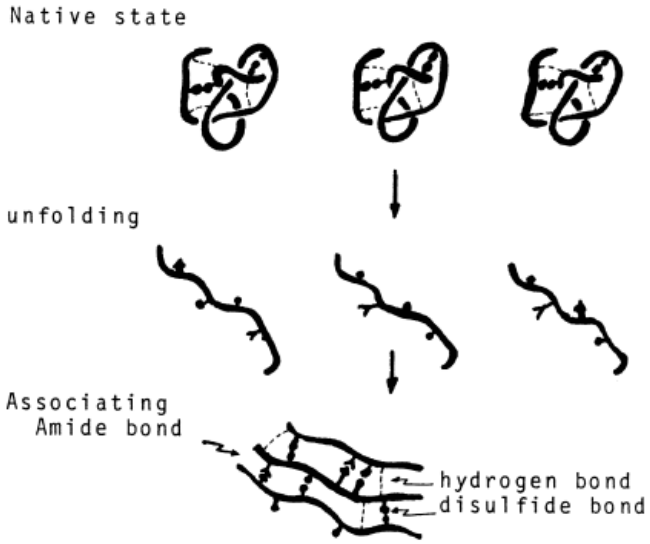
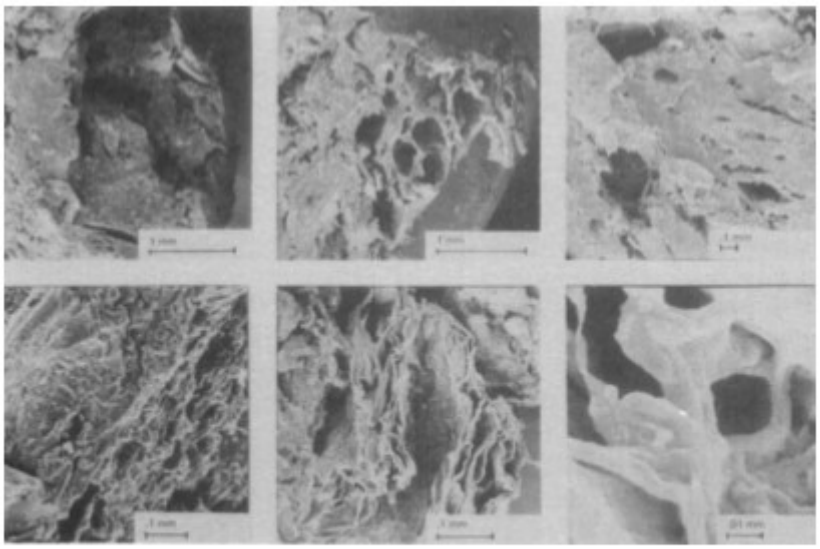


Figure 8. Reconstruction and aligning of proteins during extrusion (Hendrowarsito, 1984) .



CROSS SECTIONS AND LONGITUDINAL SECTIONS OF SOY-MEAL EXTRUDED AT DIFFERENT TEMPERATURES

(l) 150°C; (c) 165°C; and (r) 180°C. Twin-screw commercial extruder.

Figure 9. Picture of dry extruded soy flour. Left: It is demonstrated that no fiber structure has been formed, only that the material is denser packed. Center: A fiber structure starts to form, cavities and a structure with structural integrity. Right. A structure with distinct structural integrity has been formed (D. W. Stanley and J. M. deMan, 1978).

1.4.4.1.1 Wet- and dry extrusion

There is both dry- and wet extrusion and the moisture content of the feed governs what type of products that is produced. In dry extrusion, the feed contains maximum 35 % moisture whereas in wet extrusion the moisture content is over 50%. Dry extrusion generates a product that usually expands and losses most of its water when it reaches the die and consequently needs to be re-hydrated before consumption. Wet extrusion on the other hand generates a product that maintains more of its moisture and can be consumed without re-hydration. The product from wet extrusion is mainly used as totally meat replacement (meat analogues) whereas the product from dry extrusion is more used as meat extenders. Soy products on the Swedish market that has been either wet- or dry extruded are Food for progress's "Oumph" (Food for Progress, 2017) and Anamma's "Vegofärs" (Anamma, 2017). The dry extruded product can be added to patties together with minced meat to enhance the nutritional value or to improve the texture. The dry extrusion product has much lower water activity than products from wet extrusion, hence the shelf-life of the dry extrusion product is significantly longer (Steel et al., 2012).

2. Aim

The aim of this Master's thesis is to investigate the abilities to create a rapeseed protein based meat analogue for human consumption. To achieve this, the protein yield, color and taste of the recovered rapeseed protein must be improved. Finally, the rapeseed protein will be thermoplastic extruded to investigate the ability to create chewy and elastic texture characteristics that mimic meats.

3. Materials and methods

3.1 Chemicals and materials

The cold-pressed rapeseed press cake was a kind gift from Gunnarshög Jordbruks AB. The rapeseed press cake (RSPC) was stored in the freezer, -20°C.

3.1.2 Chemicals

Chemicals used in different experiments are listed in Table 3.

Table 3. Name of used chemicals in the conducted experiments.

Name	**Producer, City, Country**
Aspartic acid	ThermoElectron, Milan, Italy
Citric acid (powder)	Merck, Darmstadt, Germany
Sodiumdisulfite ($Na_2S_2O_5$)	Merck, Darmstadt, Germany
Petroleum Benzine	Merck, Darmstadt, Germany
Ultrasil 53 (enzymatic detergent)	Ultrasil 53, Taastrup, Denmark
0.3% Urea solution	ThermoElectron, Milan, Italy

3.2 Isolation of proteins from rapeseed press cake

The rapeseed protein (RSP) was isolated from the RSPC by a water based extraction process by several steps including change of pH and centrifugations based on a procedure described by (Wijesundera et al., 2013).

The RSPC was dry milled (Retsch Grindomix GM 200, Haan, Germany) at five 4-seconds-intervals to a powder. The RSPC powder was dispersed with tap water (1:10 w/w) and pH was adjusted to X1 by 2 M NaOH. Typically, 200 g RSPC was used as raw material for extraction. The pH adjusted slurry was stirred for 10 min (IKA Labortechnik Eurostar digital, Staufen, Germany) at 180 rpm and the pH was then reset to X1 and incubated at 4°C for 3 hours, this phase of the process is called leaching phase.

The slurry was centrifuged (Beckman Coulter, Indianapolis, USA) for 30 min at 4°C at 5000*g. The supernatant was collected and the sediment was discarded. The pH of the supernatant was set to X2 by citric acid (Merck, Darmstadt, Germany). The slurry was heated to X°C on a hotplate during continuously stirring. After thermal treatment, the slurry was cooled to ambient temperature in a cold water bath. The slurry was again centrifuged and the sediment was collected as rapeseed protein paste (RSPP). See the flow chart of the recovery process in Fig 10.

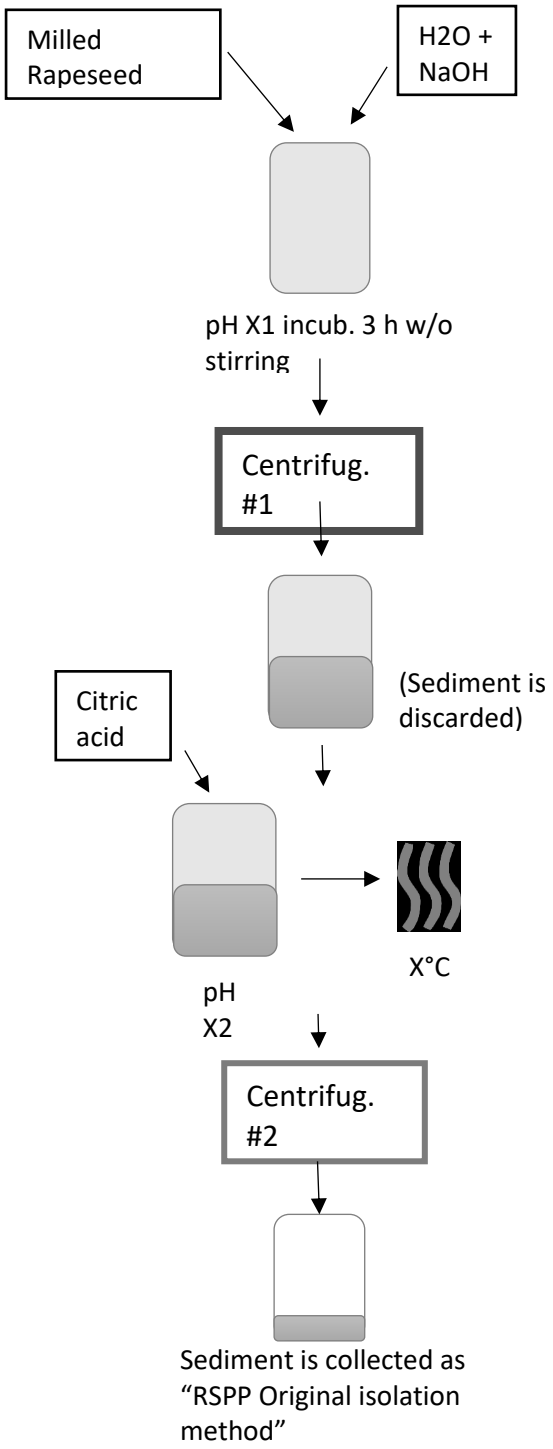


Figure 10. The flow chart of the isolation process of RSPP.

3.3 Process optimization

As isolation of rapeseed protein still is a new research field there seemed to be potentialities to optimize the isolation process. Not many studies are conducted to investigate the effect of incubation time in alkali condition on protein yield. To investigate the impact of incubation time in alkaline condition on leaching of proteins, trials with three different incubation times were made.

3.3.1 Change of alkaline incubation time and performance

The effect of the protein yield due to incubation time and its performance of the initially used process (see section 2.2) was investigated by testing three different incubation times with two duplicates, 30 min 60 min and 180 min, all during constantly stirring. The purification process was conducted in order of the original description (section "2.2 Isolation of proteins from rapeseed press cake)", except for following adjustment:

- 100 g rapeseed cake for each replicate
- 30 min/ 60 min / 180 min incubation at 4°C at constantly stirring.
- The pH was measured at 15 minutes intervals during the alkali incubation.

3.3.2 Recirculation of Sediment #1

The protein yield of the original isolation method (see section "2.2 Isolation of proteins from rapeseed press cake") reveled that lots of proteins were lost in the first centrifugation step (Centrifugation #1 in Fig. 10) were the sediment is discarded. Thereby re-dispersion of that sediment was investigated.

To investigate if the product yield could be increased, trials were conducted were a recirculation step of the first sediment was included in the process (Fig. 11). The sediment was dispersed in water (1:8 wt/wt) and pH was adjusted to X3, X4 and X5 (Fig 11). After the re-dispersion of the sediment, the same procedure as described in section "2.2 Isolation of proteins from rapeseed press cake" was used, except for the following changes:

- No stirring for 10 min and readjustment to pH X1 when alkali conditions was set before refrigerated incubation.
- Re-adjustment to pH X1 after incubation in alkaline solution for 60 min. Then the slurry was stirred for 30 min on the bench at room temperature and then 30 min more at 4°C due to a mistake in the booking system for the centrifuge. The experiments were conducted in duplicates.

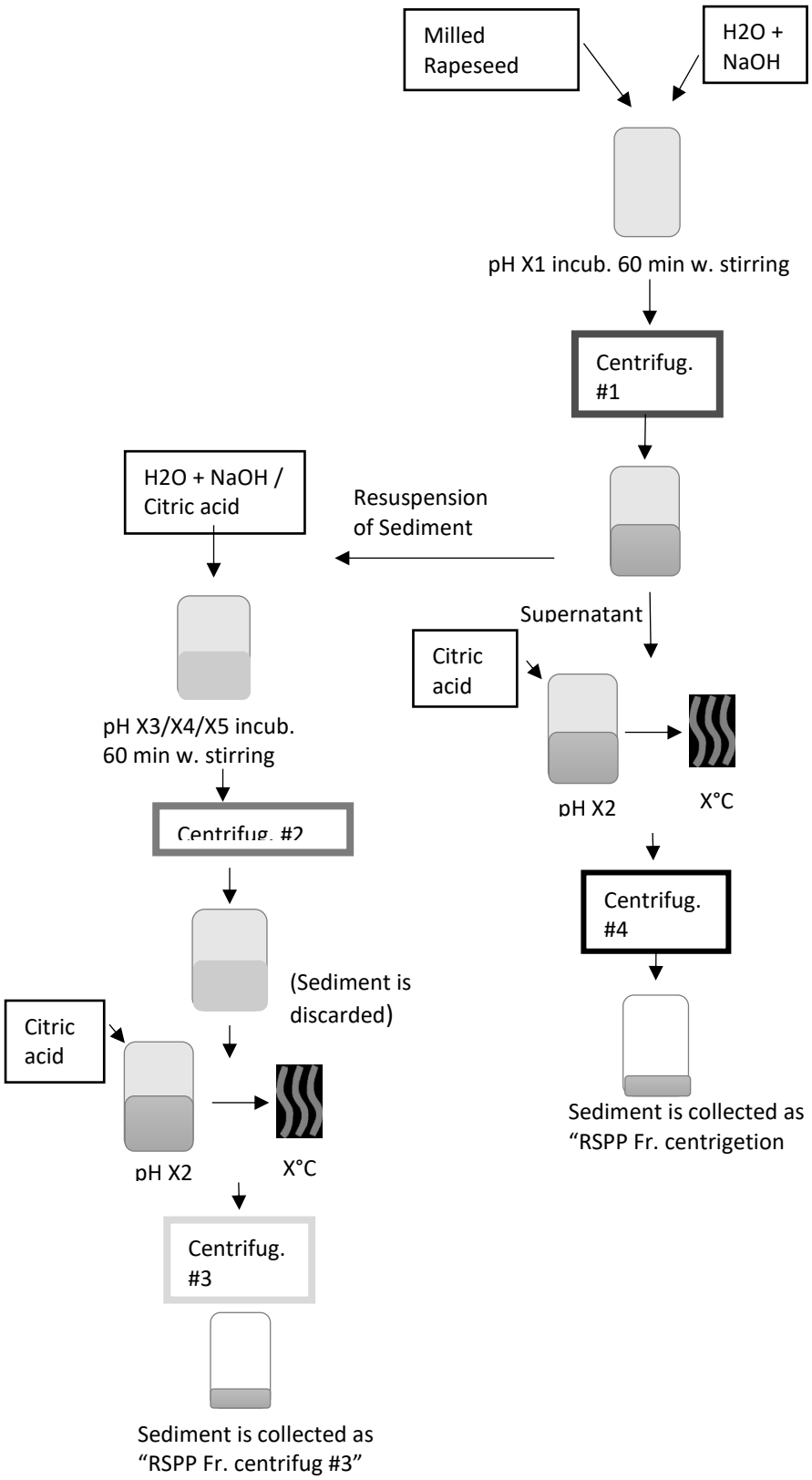


Figure 11. The flow sheet of the recirculation process.

3.3.3 Separation of anti-nutrients from RSP by ultrafiltration

Since no studies were performed by this institution to separate anti-nutrients and discoloring compounds such as glucosinolates and polyphenols from proteins in the RSPP, ultrafiltration and diafiltration was conducted.

The protein was recovered in accordance with the method described in section “2.2 Preparation of the Rapeseed protein paste (RSPP)” except for some adjustment:

- In the first step where milled rapeseed press cake is dispersed in tap water, 0.04 wt% sodiumdisulfite ($Na_2S_2O_5$) was added.
- After centrifugation #1 (Fig. 11) the supernatant was diafiltrated once (see section “2.4.1.1 Ultrafiltration (UF)” below and see Fig 12).
- After the diafiltration, the produced retentate was further processed by citric acid, heating and centrifugation according to “2.2 Preparation of the Rapeseed protein paste (RSPP)”, see Fig 12.

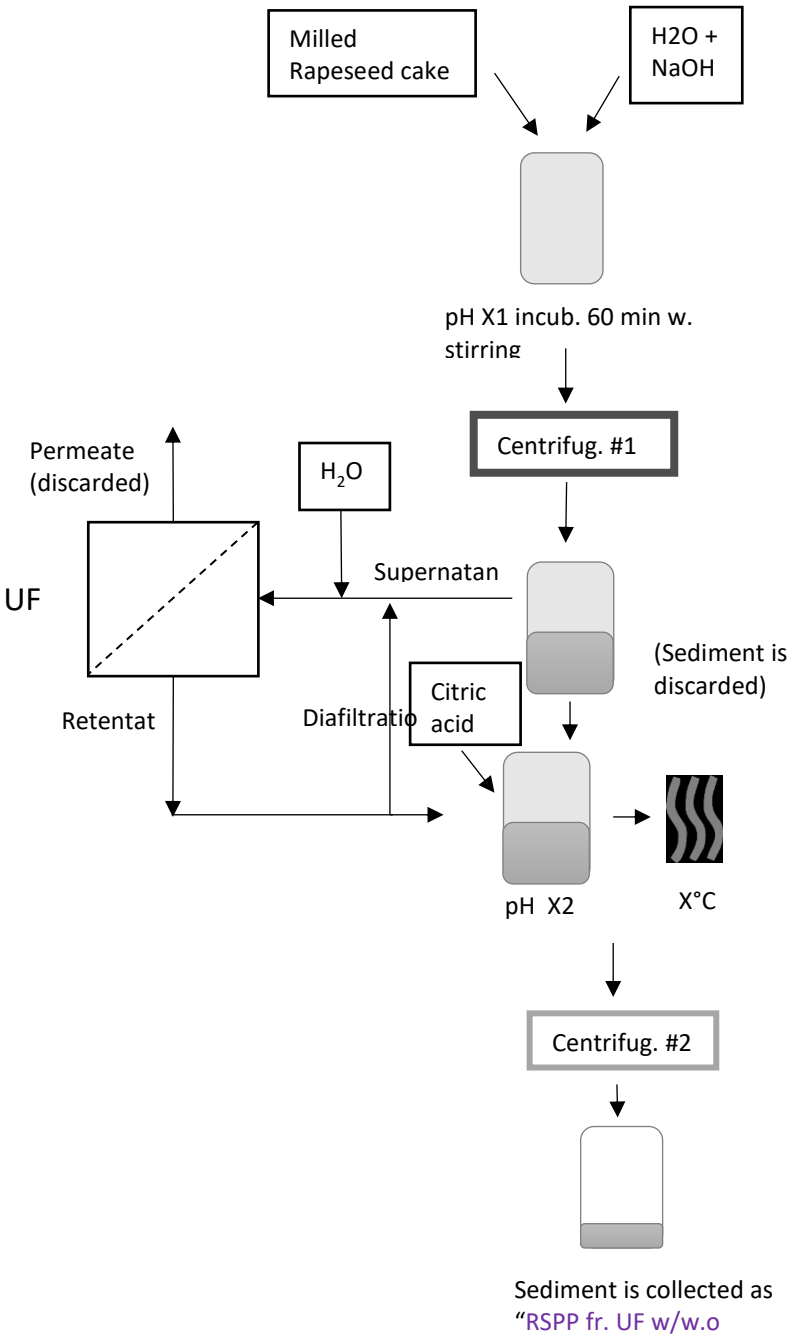


Figure 12. Flow chart of the isolation of the RSPP with diafiltration.

3.4 Processing methods

3.4.1 Separation

3.4.1.1 Ultrafiltration

A plate-and-frame module LabStak M10 (Alfa Laval, Lund, Sweden) with filter of 10 kDa was used to separate the proteins and larger molecules from smaller molecules such as salts, sugars, glucosinolates, phytates and polyphenols, see Fig 13, (Diosday et al., 1984) . The plate-and-frame module consist of four plates in series, the retentate is recirculated and the permeate is discarded, see Fig 14. When the UF-system was assembled, it was cleaned by the enzymatic detergent (Ultrasil 53, Taastrup, Denmark) and finally filled with deionized water. The initially flow rate of deionized

water in the system was measured, both the retentate and the permeate. The used feed was the supernatant of the first centrifugation (centrifugation #1, Fig. 12) in the protein isolation process. The feed was added to the system and the pressure was set to 2 bar and flow rate to approximately 3.5 L/min. A water bath of 50°C was used to heat the system. The permeate rate was continuously measured every fifth minute. When the permeate volume had reached 500 mL (half of the initial feed volume) 50°C deionized water was added to the retentate (diafiltration). When the permeate had reach 500mL once again, the UF was ended and the resulting retentate was collected. The system was cleaned by enzymatic detergent and deionized water.

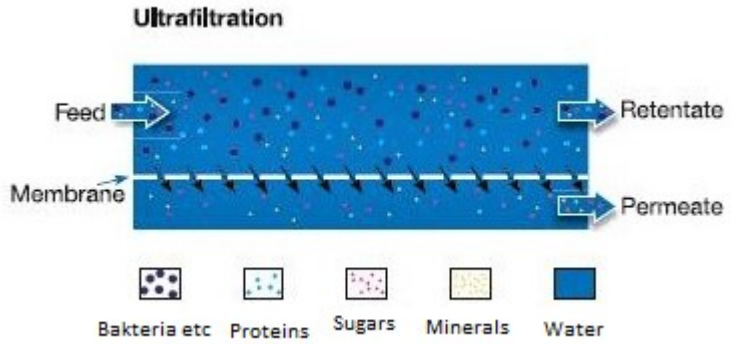


Figure 13. The principle of the Ultrafiltration flat sheet, (AlfaLaval, 2017b).

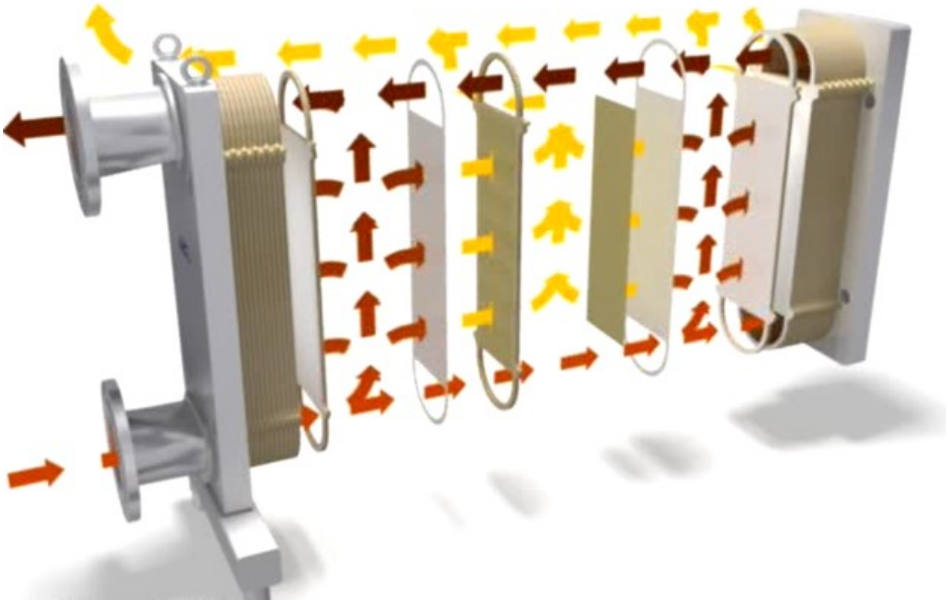


Figure 14. A picture of a typical plate-and-frame module used for UF. The red arrows represents the retentate and the yellow arrows the permeate (AlfaLaval, 2017a).

3.4.2 Post protein recovery processing methods

3.4.2.1 Drum drying

To test-dry the RSPP made by SwePharm AB, a drum-dryer was used, (Goudsche Machinefabriek, Waddinxveen, Netherlands). This drum-dryer consists of a single cylindrical large drum in contact with four smaller cylinders rotating in contrary directions to the large one. As the RSPP became a pourable slurry after defrosting it was used without any pre-treatment. The temperature of the steam filled drum was measured and the RSPP was added when the temperature reached 118 °C. The RSPP slurry was poured slowly on to the large cylinder in the adding line. The drum drying was conducted at three different speeds, 16, 25 and 45 sec/lap and the finished product of each round was collected separately.

When the drying was cancelled, water and detergent was used to clean the drum dryer manually.

3.4.2.2 Vacuum evaporation

A vacuum evaporator (Heidolph, Schwabach, Germany) was used to evaporate the RSPP for 30 min, 60 min, 120 min and 180 min. The RSPP was put in a spherical flask and attached to the evaporator and a negative pressure of 0.8 bar was applied. Every set time a sample was taken from the flask.

3.4.2.3 Freeze-drying

The RSPP to be freeze-dried were evenly spread out on trays and frozen at -18°C for 14 hours prior to freeze-drying. The trays were transferred from the freezer into the freeze-dryer (Labconco, U.S, Kansas City) and left there for seven days.

3.4.2.4 Extrusion

RSPP produced by SwePharm AB (delivered 2017-05-19) was freeze-dried (as described in section 2.4.2.3 Freeze-drying) prior to extrusion trials. Freeze-dried (FD) RSPP and milled RSPC were separately mixed with water for pretest of different water contents in a mixing chamber (Brabender, Duisburg, Germany). The mixtures were mixed in the mixing chamber for 5 min with and without heating, the temperature of the heating tests was 80°C. The dough of FD RSPP pretested without heating containing 35%, 50%, 60% and 70% water. The FD RSPP dough was then tested with heating at 35%, 50% and 60 % water content. The RSPC powder was pretested with a water content of 35 % with and without heating. A RSPC dough of 50 % water was also prepared but was too sticky for further treatment.

The most convenient mixtures were then tested in a single-screw extruder (Dr Collin Teach Line E20 T, Ebersberg, Germany). The extruded mixtures were only the FD RSPP with 50% and 60% water content. A conventional screw (20 mm Ø x 25 D (3:1)) and two different dies were used, a heated round die (3 mm Ø) and a cooled flat die (2x20 mm). The extruder had five different heating/cooling zones and the set temperature was applied in zone four. Each dough was generally initiated into the extruder at a lower screw speed of 15 rpm, 40 rpm or 45 rpm and then increased to 70 rpm as the melt continuously run out of the die. Three different extrusion temperatures were applied when the heated round die was used, 100°C, 120°C and 110°C. When the cooled flat die was used, the set temperatures were 100°C, 120°C, 123°C, 130°C and 135°C, see the used screw speed for each temperature in Table 4 and 5.

Table 4. The set temperature, screw speed and die temperature of the round die.

	Heated round die (3 mm Ø)		
Set Temp. (°C)	100	120	118-110
Screw speed (RPM)	40	45	70
Die Temp. (°C)	100	120	120

Table 5. The set temperature, screw speed and die temperature of the flat die.

	Cooled flat die (2x20 mm)						
Set Temp. (°C)	100	100	120	123	130	130	135
Screw speed (RPM)	45	70	45	70	45	70	70
Die Temp. (°C)	65	65	65	65	65	65	65

3.5 Analyzing methods

Several analyses were conducted to evaluate the performance of the protein isolation process and to evaluate the effect of the added process steps (incubation time in alkaline solution, recirculation of sediment and ultrafiltration to mention a few). Analyses were also conducted on physiochemical properties and macronutrient composition to characterize the RSPC and different RSPPs.

All measurements in all analyses were performed in triplicates if not stated otherwise.

3.5.1 Protein content

Protein content was analyzed by an element analyzer FlashEA 1112 Series (FlashEA 1112 Series, Waltham, U.S). The analysis was conducting according to the user manual and aspartic acid (ThermoElectron, Milan, Italy) was used as standard for all measurement except for liquids were 0.3% urea solution (ThermoElectron, Milan, Italy) was used as standard. A nitrogen conversion factor of 6.25 was used to convert the nitrogen content to protein.

3.5.2 Water activity

The water activity of the drum dried-, extruded- and freeze-dried products was quantified by an AquaLab Series 3TE (AquaLab, Washington, U.S.). A thin layer of the milled sample was evenly distributed in the measuring cup covering the bottom. Water activity was measured.

3.5.3 Moisture content

By measuring the dry matter (DM) the moisture content was calculated. The samples were put in an oven (Termaks AS, Bergen, Norway) at 105 °C until constant weight (approximately 16 hours) and let cool in a desiccator. By comparing the initial sample weight (approximately 2 grams) with the final sample weight, the moisture content was calculated, see Eq 1. X_{H2O} is the moisture content (percentage), X_{Si} is the initial sample weight (g) and X_{Sf} is the final sample weight (g).

$$X_{H2O} = \frac{X_{Si} - X_{Sf}}{X_{Si}} * 100 \qquad (1)$$

3.5.4 Energy content

The bomb calorimeter Parr 6200 Isoperibol Calorimeter (Parr, Moline, U.S.) was used to determine the energy content in the rapeseed samples and to calculate the carbohydrate content. Samples with high moisture content (e.g. rapeseed protein paste, 82% moisture) was dried in the oven (Termaks AS, Bergen, Norway) at 105°C for 2 hours prior analysis to reduce the moisture content. Non-cohesive samples and samples with a powder like structure (e.g. rapeseed powder) was pressed to a tablet before analysis to enable complete combustion of the samples. The analysis was performed according to the user manual.

3.5.5 Ash content

2-5 grams of sample was first heated on a hotplate to eliminate all smoke. The samples were put in an oven (Heraeus, Hanau, Germany) for 16-20 hours at 550°C. The ash content was calculated according to Eq.2. X_{Ash} is the ash content (percentage), X_{Si} is the initial sample weight (gram) and X_{Sf} is the final sample weight (gram)

$$X_{Ash} = \frac{X_{Sf}}{X_{Si}} * 100 \qquad (2)$$

3.5.6 Fat content

The fat content was measured by SOXTEC 2055 Manual Extraction Unit and associated 2055 SOXTEC Manual Control unit (FOSS, Warrington, United Kingdom). The sample was mixed with sand in a porous paper collet, which was put in an aluminum tube dish. The fat extraction was achieved by four operation steps in the following order, boiling, rinsing, evaporation of solvent and drying. Petroleum Benzine (Merck, Darmstadt, Germany) was used as solvent. The extracted oil remains on the bottom of the tube dish. The fat content was determined by the weight of the tube dish containing the extracted oil, Eq 3. M_{Fat} is the extracted fat (gram), M_{TE} is the weight of the empty tube and M_{Twfat} is the weight of the tube containing the extracted fats.

$$M_{Fat} = M_{TwFat} - M_{TE} \qquad (3)$$

4. Results and discussion

4.1 Original recovery process

4.1.1 Macronutrient content

The composition of the rapeseed protein paste recovered by the original isolation method (section 2.2) was analyzed, see Table 6. The protein content was 11.9%, fat 3.6%, water 82.1 % and carbohydrates 4.1%. The used processing parameters were alkaline incubation at pH X1 and at 4°C for three hours without stirring. Precipitation by adjusting the pH to X2 and heating to X°C.

Table 6. Macronutrients of RSPP recovered by the original purification method.

Compound	g/100g RSPP incl. std dev.
Water	82.1 ± 0.75
Ash	0.47 ± 0.02
Protein	11.9 ± 1.2
Fat	3.6 ± 0.6
Carbohydrates (calculated)	4.1

4.2 Change in alkaline incubation time and performance

The effect of incubation time in alkaline conditions was investigated. By dispersing the milled RSPC in an alkaline solution, the proteins are charge and separate from the cell walls and other parts of the meal and thereby dissolved into the solution (Shen et al., 2008).

It was found in the literature that the incubation in alkaline condition often was conducted for a shorter time than three hours (which is the time used in the original isolation method, section 2.2) and sometimes together with stirring (Tan et al., 2011) (Das Purkayastha et al., 2015). To save time and potentially increase the protein yield, three different incubation times that were found in the literature or previously used were investigated. Stirring were used in all three cases as mixing were thought to increase the chance for the hydroxide molecules in the alkaline solution to get in contact with the proteins and thereby charge and disperse them. As seen in Fig. 15, the trial with a stirring time of 180 min gave the highest protein yield.

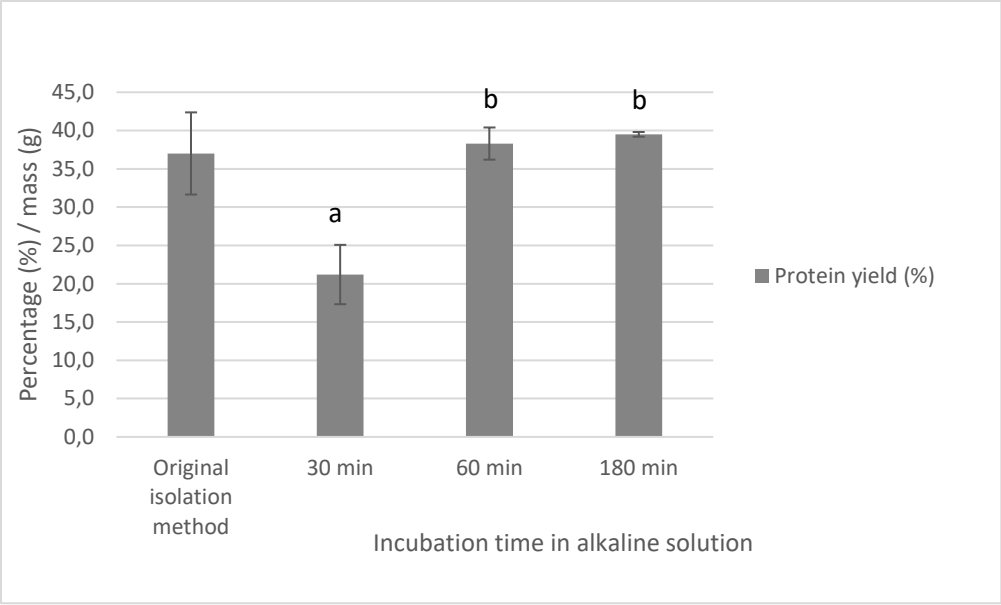


Figure 15. The protein yields in total mass (g) and percentage of the initial protein in the used RSPC due to different incubation times. T-test was performed and showed that the 30 min trial (a) gave a significantly lower protein yield than the 60 min.

The original isolation method also uses 180 min incubation time in alkaline conditions but without stirring. As seen in Fig. 15, the largest differences in protein yield is between 30 min and 60 min incubation time whereas the protein yield do not increase with the same rate between 60 min and 180 min incubation time. T-test was performed and showed that the 30 min trial's protein yield was significant lower than the protein yield of 60 min and 180 min stirring ($p<0.05$). There was no significant differences of the 60 min and 180 min trials. These trials also gave an indication of that the trial with 60 min incubation time with stirring give at least as high protein yield as the three hour's trial used in the original isolation method, see Fig 15. As the 60 min's trial saves two hours of production time compare to the original isolation process it has been chosen to be continued with as the new standard method.

4.3 Recirculation of Sediment #1

The analysis of the protein yield in the RSPP reveals that lots of the proteins are lost in the sediment in the first centrifugation step (see Fig. 11). Thereby the effect of the protein yield by re-dispersion of the first sediment was investigated. The isoelectric point (pI) of napin (the second major protein in rapeseeds) are thought be above pH 10 (Von Der Haar et al., 2014). As the alkaline incubation has been at pH X1 (see section 2.2) it is thought that the napins might have been uncharged at that pH, and thereby precipitated during the first centrifugation and has further on been discarded (see Fig. 10). To recover the potential napins in the first sediment and other undispersed proteins the sediment was re-dispersed at different pHs. Other studies conducted to isolate rapeseed proteins have often used an alkaline solution of pH 11 or above and received a higher protein yield than what has been achieved by the used methods in this thesis (Das Purkayastha et al., 2015) and (Xu and Diosady, 2012). The alkaline pH of the used isolation method has been limited to X1 due to the increased health risk associated to handling of solutions at extreme pH. Due to scientific curiosity and the hypothesis that napin might have ended up in the sediment, re-dispersion of the sediment at pH X5 was conducted. It was desired to investigate the effect of the protein yield by re-dispersion of the sediment in pH both above and below the first alkaline dispersion step (see Fig. 11). At the same time, it was necessary to avoid setting the pH of the re-dispersion to the pI of cruciferin (the major

protein in rapeseeds) which is at pH 7.25 (Wanasundara et al., 2017). Thereby the three different re-dispersion pHs to be investigated were decided to be pH X3, X4 and X5, see Fig. 11 and Fig. 16.

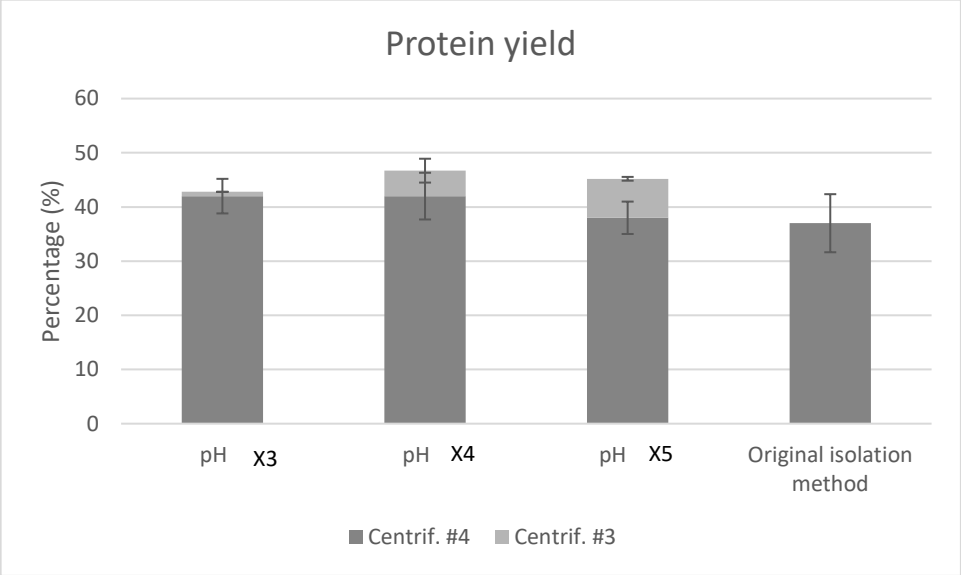


Figure 16. The protein yield of the product of centrifugation #3 (after re-dispersion of the sediment) and centrifugation #4 (original isolation method).

As seen in Fig. 16 the re-dispersion at pH X5 gave the highest increase in protein yield (centrifugation #3) of 7.2 % of the initial protein content in the RSM. Re-dispersion at pH X4 gave an increase in protein yield of 4.7 % of the initial protein content in the RSM. However, the increase in protein yield achieved by re-dispersion at pH X3 was 0.8 % and can thereby be neglected. pH X5 gave the highest increase in protein yield which is reasonable as other studies previously shown that pH X5 or above seems to give the highest protein yields (Xu and Diosady, 2012). Over all the protein recovery was increased by recirculating the sediment but the method could be improved to collect even more protein. A previous study has shown that a large protein yield can be obtained (total 69%) by one recirculation of the sediment if the ratio water:RSM is increased to 30:1 and if the RSM is pre-soaked in methanol or acetone (Das Purkayastha et al., 2015). However, the use of solvents such as methanol and acetone should be avoided due to the negative environmental and economically caused by increased resources for waste handling and manufacturing.

4.4 Purification of RSPP by Ultrafiltration

4.4.1 Separation of anti-nutrients

There was a noticeable difference of the color of the retentate and permeate of the trial were $Na_2S_2O_5$ was added compare to the trial were $Na_2S_2O_5$ not was added, see Fig. 17 and 18. Most importantly, also the final precipitated and centrifuged product of the two trials had a distinct noticeable difference in color, see Fig. 19.

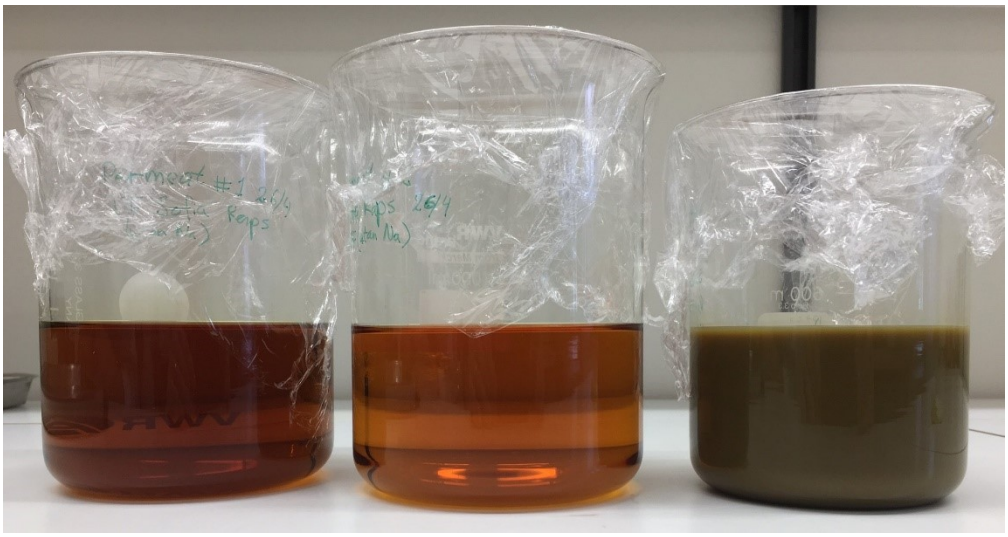


Figure 17. The permeate and retentate from the UF-trial without $Na_2S_2O_5$. From left, Permeate #1, Permeate #2 and retentate.

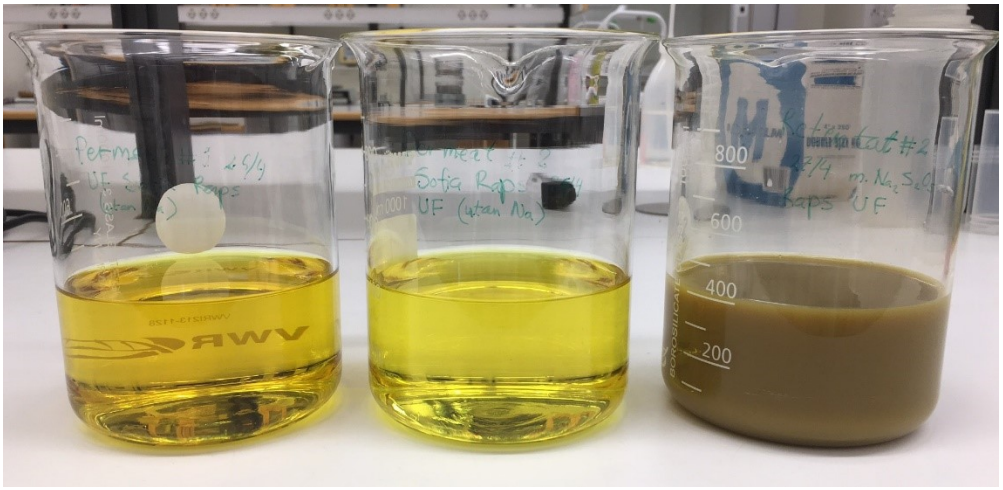


Figure 18. The permeate and retentate from the UF-trial with $Na_2S_2O_5$. From left, Permeat #1, Permeat #2 and retentate.

As seen in Fig. 17 and 18 the first permeate has a darker color than the second permeate which indicates that some kind of colorful compound/s is/are being washed out. The content of glucosinolates could not be measured as the lab performing those testes was fully booked. However, as the glucosinolates are water soluble and smaller than the porosity of the used membrane (10kDa) they should penetrate the membranes and gradually end up in the permeates.

Figure 19. The products of the two UF-trials with and without $Na_2S_2O_5$. Above: UF product w/o $Na_2S_2O_5$, below: UF product with added $Na_2S_2O_5$.

As seen in Fig. 19 there is a distinct color difference of the two products. This indicates that treatment with $Na_2S_2O_5$ improves the color of the RSPP. When the taste of the two UF products were compared, the UF product with $Na_2S_2O_5$ seemed to taste less of the grassy off-flavor compared to the UF product without $Na_2S_2O_5$ (also commonly associated with the product of the original isolation product). On the other hand, the UF product with $Na_2S_2O_5$ had a metallic or salty off-flavor that probably is due to residuals of $Na_2S_2O_5$. When the ash content was measured, a white powder appeared in the crucible of the sample containing UF product with $Na_2S_2O_5$ but not in the crucible containing the product without $Na_2S_2O_5$. This observation indicates that there is a residual of $Na_2S_2O_5$ in the product and explains the metallic off-flavor. It also shows that more than one diafiltration is needed to wash out the added $Na_2S_2O_5$.

4.4.2 Components of the RSPP recovered by Ultrafiltration

The components of RSPP recovered by diafiltration with and without $Na_2S_2O_5$ (see Fig. 12) can be seen in Table 7. The observed residuals of $Na_2S_2O_5$ in the crucibles will probably give an incorrect ash content, as the amount $Na_2S_2O_5$ left in the UF-product cannot be determined, see Table 7. In Fig. 20 it looks like the ultrafiltration without added $Na_2S_2O_5$ recover more protein than the trail with added $Na_2S_2O_5$. However, the protein content of the final products of the two trials show no greater differences, 11.5 % protein with $Na_2S_2O_5$ and 11.1% protein without $Na_2S_2O_5$, see Table 7.

Table 7. Components of the UF products w. and w/o. $Na_2S_2O_5$.

Measurement	UF w. $Na_2S_2O_5$	UF w/o. $Na_2S_2O_5$
Moisture content (%)	80.7 ±0.85	79.2 ±0.60
Protein (%)	11.5 ±0.32	11.1 ±0.52
Ash (%)	0.48	0.44

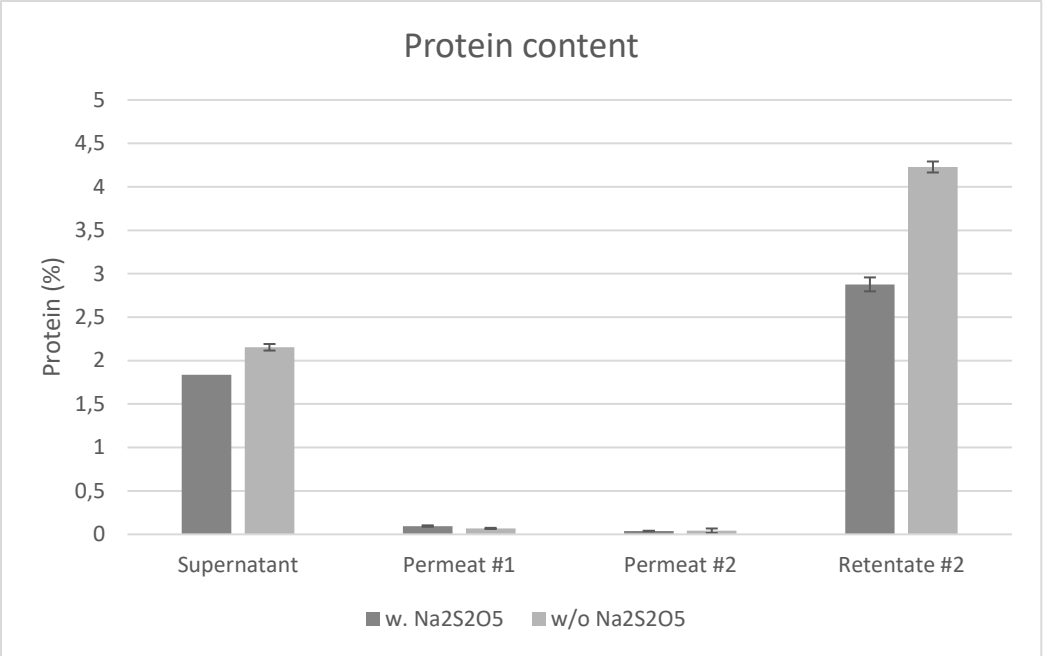


Figure 20. The protein content in the permeate and retentate from the UF trials.

To thoroughly investigate the amount of the recovered protein of the two UF products the protein yields should be compared. Not the whole volume of the produced supernatant in the first centrifugation step (Fig. 12) continue to the ultrafiltration due to lack of space. Thereby the protein yield cannot fairly be investigated as an uneven amount of protein might was continued to the ultrafiltration. Anyway, in Fig. 20 it is seen that no protein seems to be lost in the permeate in any of the two cases. The very small amount of proteins detected in the permeate flows (Fig. 20) are likely to be other nitrogen compounds. The reason is that the used method determines the nitrogen content in the sample and then converts that to protein by the general nitrogen conversion factor of 6.25. Instead, the small amounts of determined proteins in the permeate flows might be an indication of that glucosinolates has been washed out as they contain nitrogen.

4.5 Drying methods

4.5.1 Vacuum evaporation

The RSPP recovered by the original isolation method (section 2.2) was evaporated to decrease the water content, see Fig. 21.

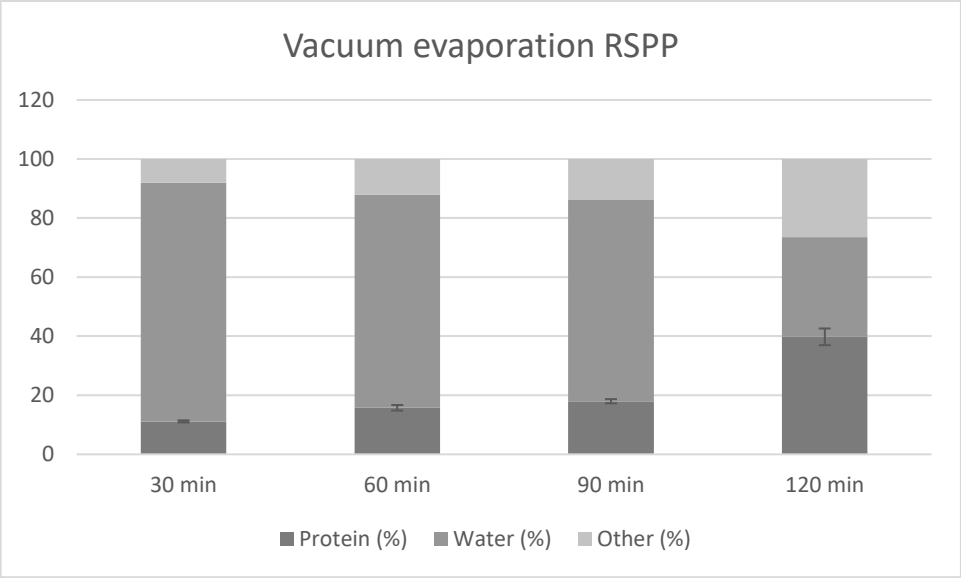


Figure 21. The water- and protein content due to vacuum evaporation time.

By increased time in the vacuum evaporator more water is evaporated leading to a higher protein content which is reasonable as the RSPP is exposed to heat and decreased pressure for a longer time. However, the lab scale vacuum evaporator was not very efficient as the samples were not evenly dried and the trial should be repeated with a more efficient equipment.

4.5.2 Different drying methods

The water content, water activity (a_w) and protein content of the products of the used drying methods are seen in Table 8. The freeze-dried was clearly the drying method that achieved the lowest water activity (a_w) of 0.075, although drum drying also achieved water activities below 0.6, see Table 8 and Fig. 22 and Fig. 23. The protein content does not differ too much due to type of drying method. Since the freeze-dried product contained less water than the other products it could be expected to have a higher protein content than what has been observed.

Table 8. The water activity (a_w), water content and protein content of the products of the used drying methods.

Measurement	Freeze drying	Drum drying High viscosity Cooled down, 45 sec/lap	Drum drying Low viscosity Cooled down, 45 sec/lap	Vacuum evaporated 120 min
a_W	0.075 ±0.008	0.32	0.45	---
Moisture content (%)	1.3 ±0.20	3.4 ±0.31	4.9 ±1.2	33.8
Protein (%)	40.4 ±1.5	43.5 ±0.30	42.1 ±0.11	39.8 ±2.8

4.5.3 Drum drying

The water activity (a_w) of drum dried products with high viscosity feed can be seen in Fig. 22. In Fig. 23 the water activity of the drum dried products produced by the low viscosity feed can be seen. The rapeseed protein slurries used in this trial were produced by SwePharm. The rapeseed protein paste had been frozen after they were manufactured and thereby lost its quark-like structure. The low viscosity feedstock seemed to contain more water than the feedstock called “high viscosity feed”, thereof the notations. However, no measurement was conducted to distinguish the two different feedstock content, as the low viscosity feedstock at first were not thought to be tested.

Not all materials can be drum dried. As the product of the drum dried rapeseed protein slurries (Fig. 22 and 23) formed nice flakes in different sizes this trial showed that rapeseed protein is able to be drum dried.

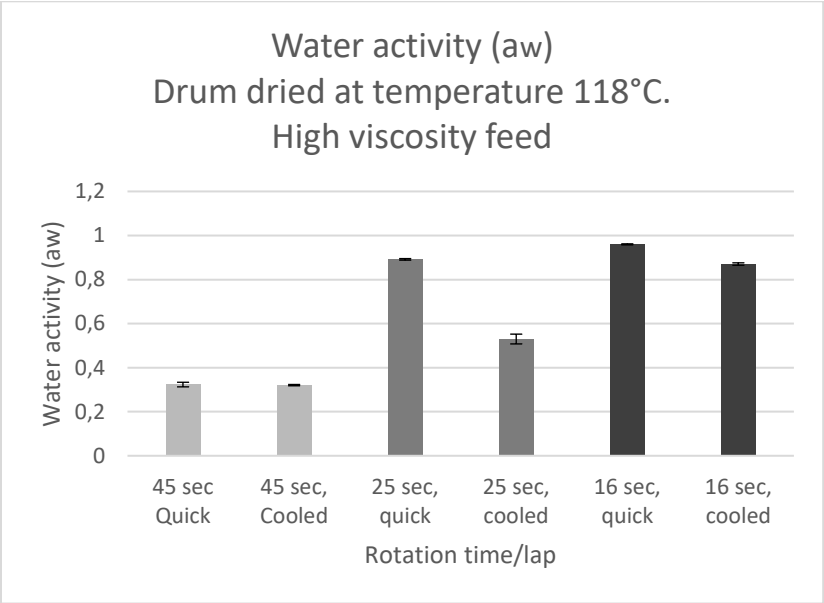


Figure 22. The water activity (aw) of the drum dried products produced by the high viscosity feed. Quick= the product was sealed in a container immediately, cooled= the product were cooled down to ambient temperature before it was put and sealed in a container.

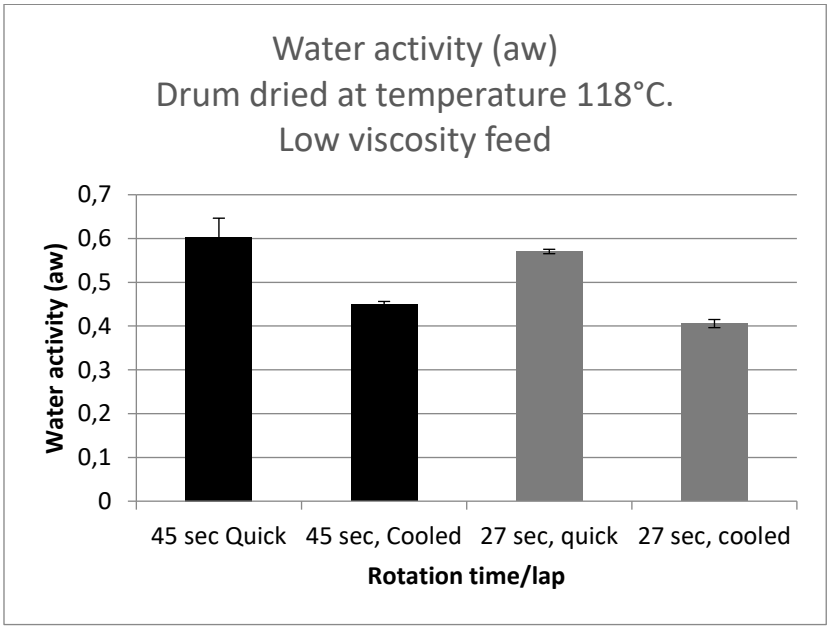


Figure 23. The water activity (aw) of the drum dried products produced by the low viscosity feed. Quick= the product was sealed in a container immediately, cooled= the product were cooled down to ambient temperature before it was put and sealed in a container.

Letting the product cool to ambient temperature before sealing it in a container decreases the water activity as more water were allowed to evaporate to the surrounding air. The rotation time was a crucial factor affecting the water activity. A slower rotation time allowed more water to evaporate and yielded a powder with lower water activity. On the other hand, a shorter rotation time allowed less time for the water to evaporate and yielded a product with higher water activity, see Fig. 22 and

23. In general, a water activity of 0.6 or less is regarded to be a limit to aim for to avoid microbial growth (Labuza, 1980). Regarding the microbial safety, a rotating time of 45 sec/ lap is suitable for both the high- and low viscosity feedstock, however it is important that the low viscosity feed are allowed to cool down afterwards to gain a water activity less than 0.6, see Fig. 23. Even though the drum-dried products achieved a water activity that is less than 0.6 the smell of it was not yet pleasant. Since the used material was one of SwePharm's worse smelling batches it might increased to grassy smell. Another point of view is how the proteins are affected by the treatment. Since the proteins are being heat denatured, drum drying cannot be used as a drying method prior to thermoplastic extrusion of RSP.

The protein content of the drum-dried products produced by the high viscosity feedstock seem to increase by decreased rotation time, see Fig. 24. That is reasonable as more water is allowed to evaporate, leading to an increase in percentage of the remaining component such as protein. The same pattern cannot be seen for the protein content between the samples of the low viscosity products (Fig. 25). Even though the result probably is not significant, the trial indicates that the protein content of the low viscosity products seem to be less affected by differences in the used rotation speeds, 27 sec/lap or 45 sec/lap. The protein content of the sample"16 sec/lap quick sealed" could not be measured due to growth of mould.

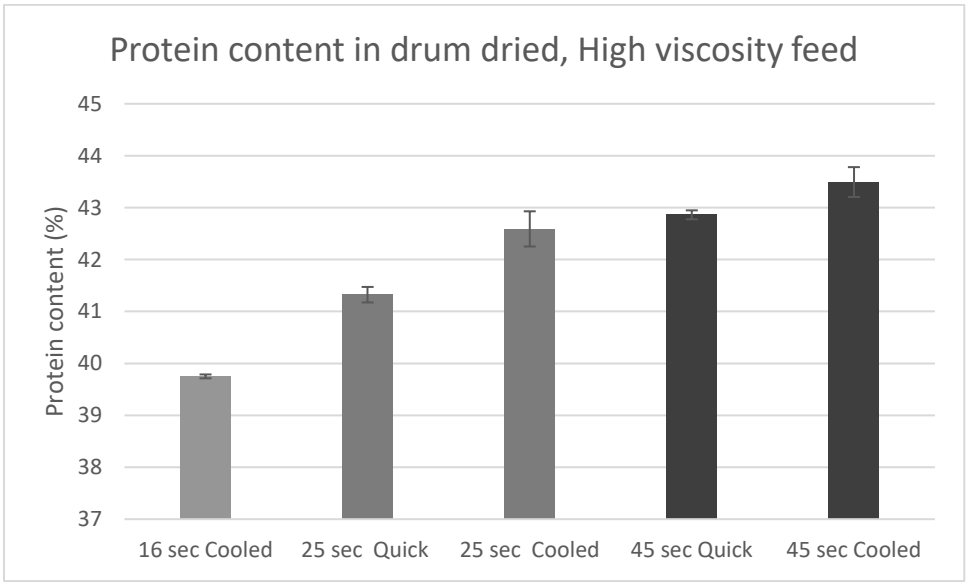


Figure 24. The protein content (%) of the drum dried products produced by the high viscosity feed. Quick= the product was sealed in a container immediately, cooled= the product were cooled down to ambient temperature before it was put and sealed in a container.

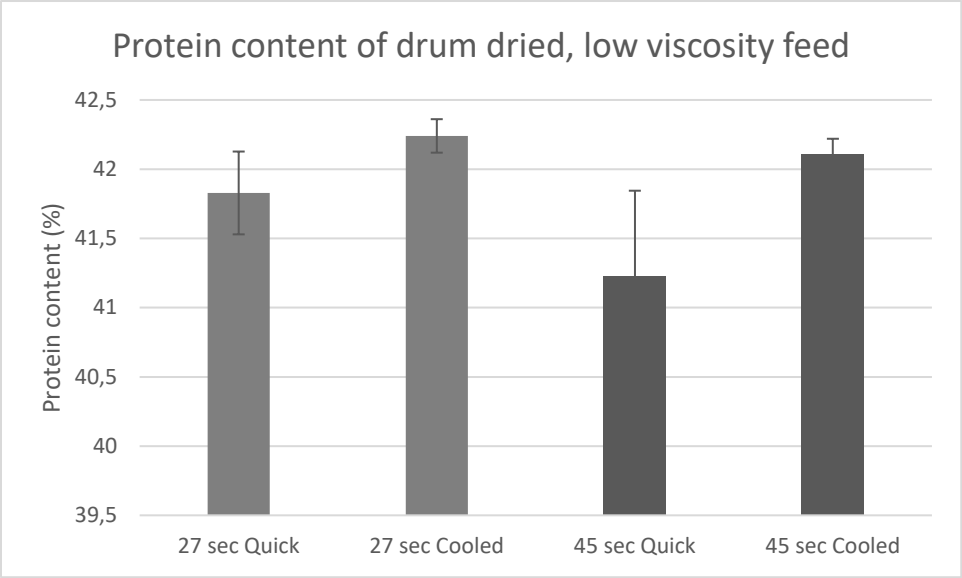


Figure 25. The protein content (%) of the drum dried products produced by the low viscosity feed. Quick= the product was sealed in a container immediately, cooled= the product were cooled down to ambient temperature before it was put and sealed in a container.

4.5.4 Freeze drying

Freeze drying showed to be a very efficient way to reduce the water content. The FD products only contained 1.3 % water, see Table 9. Since the water content is low, the protein content could be assumed to be higher if the values are compared to the other drying methods, see Table 8. As the RSPP used for the freeze-drying was produced by Swepharm and arrived just before the samples were needed to be put in the freeze-dryer, the other macronutrients of the RSPP was not analysed. Therefore, it is possible that the content of fat or carbohydrate are greater than the last produced batches.

Table 9. The water content, water activity and protein content of the freeze-dried product.

Measurement	Freeze drying
a_w	0.075 ±0.008
Moisture content (%)	1.3 ±0.2
Protein (%)	40.4 ±1.5

4.6 Extrusion

4.6.1 Pre-tests in mixing chamber

4.6.1.1 Freeze-dried rapeseed protein with different water content

No noticeable differences of the structure of the 35% water content was discovered when comparing samples mixed with and without heating to 80°C, see left and right cup in a) in Fig. 26.

When the water content was 50% and 60% the dough was dried out and crumbled when mixed at 80°C, see b (50%) and c (60%) in Fig. 26. If proteins have properties that can obtain a fiber structure by thermoplastic extrusion they generally form a jelly or elastic structure while being premixed in a mixing chamber at 80°C (Wassén, 2017). The rapeseed protein mixtures instead started to fall apart from each other (a, b and c in Fig. 26) and that is a sign of that they do not have properties that allow fiber formation by thermoplastic extrusion. The FD RSPP with 70% water were too sticky to be put down in the mixing chamber, far right in d) in Fig. 26. Thereby the 70 % dough was also thought to be

too sticky to be able to put down in the extruder and further experiments with this dough was not conducted.

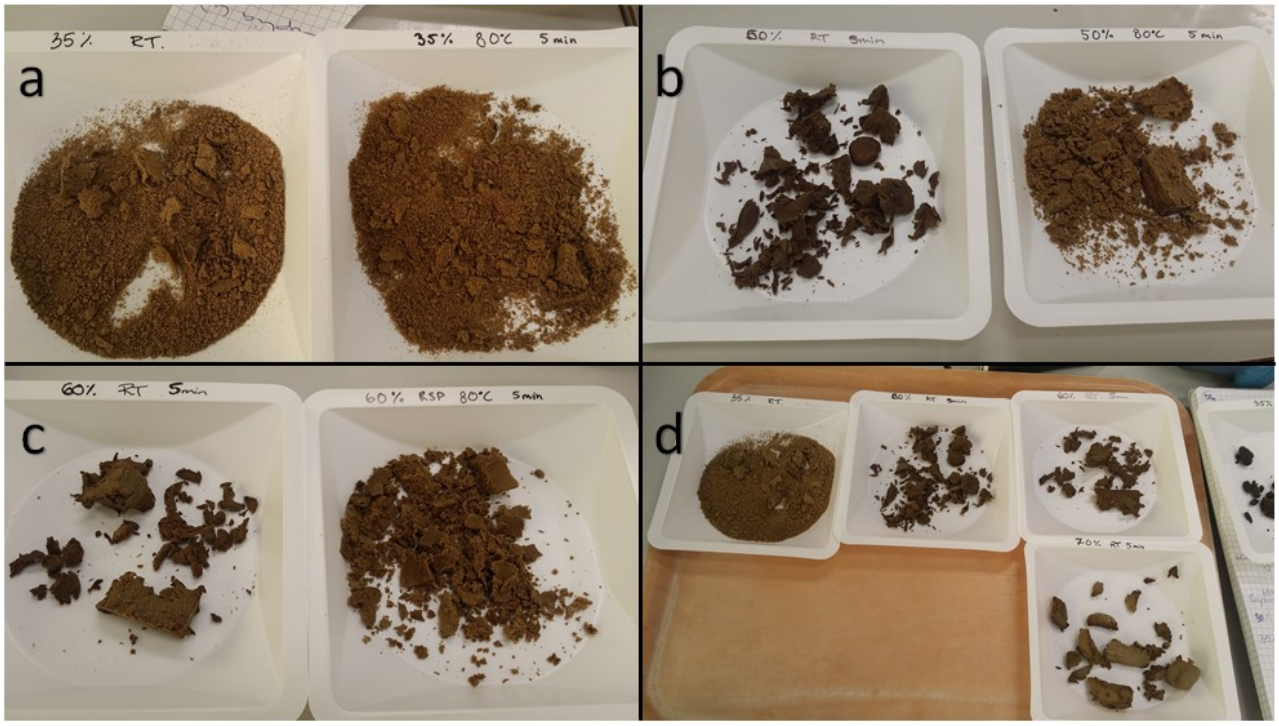


Figure 26. The result of the premixing of mixtures of FD RSPP. a) 35%, left: without heating and right: 80°C. b) 50%, left without heating and right: 80°C. c) 60%, left: without heating and right: 80°C. d) Different water content without heating, upper: left to right: 35%, 50% and 60% water. Below: 70% water.

4.6.1.2 Rapeseed press cake powder with different water content

The dough with 50% water content was too sticky to put into the mixing chamber. The dough with 35% water content was mixed in the mixing chamber with and without heating. Without heating the dough did not stick too much to the walls and held nicely together, see Fig. 27. With 80°C heating it was very sticky and got stuck on the walls as well as it dried out, see Fig. 27. This result contributed to that no mixtures with RSPC was tested in the extruder as there was thought to be a risk that it would get stuck and burnt inside. Anyhow, as the RSPC contained so much fat (15.5%) it was not thought to be able to form a fiber structure during thermoplastic extrusion.

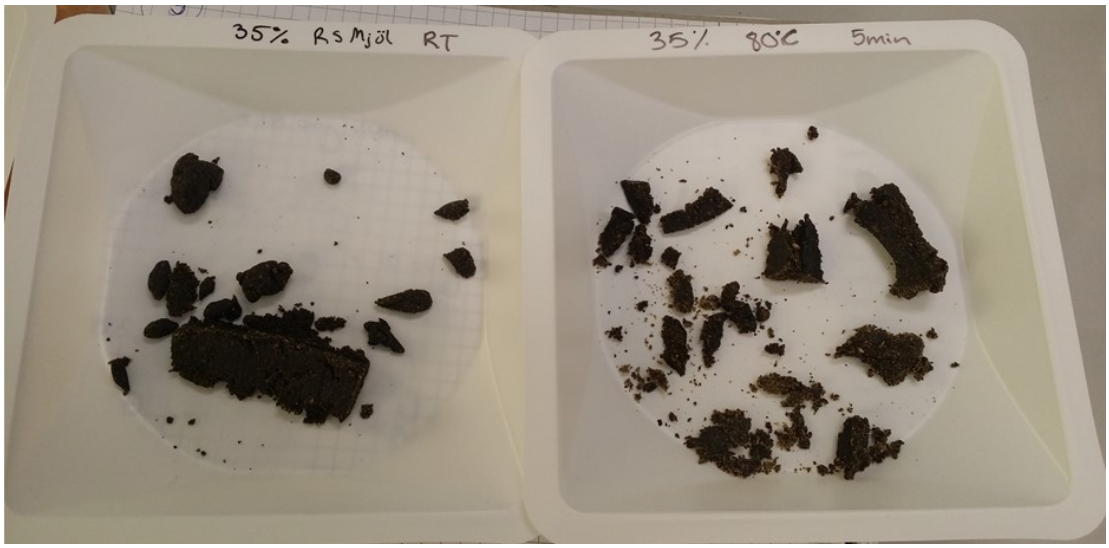


Figure 27. Premixed mixtures of RSPC and 35% water. Left: without heating, Right: with 80°C heating.

4.6.2 Thermoplastic extrusion with a single-screw extruder

4.6.2.1 Trials with heated round die (3 mm Ø)

4.6.2.1.1 Dough with 50% water content

The first run with a temperature of 100°C and screw speed of 40 RPM gave a product that nicely held together while exiting the die, see Fig 28. See the used extruder in Fig. 7. However, when it was pulled by the fingers it was discovered to not be elastic. Elasticity is a property that indicates that a fiber structure has been formed. When the temperature was increased to 120°C the melt fell apart and was "puffed" out from the die and the product was easily crumbled to crumbles when softly squeezed by the fingers, see Fig 28. These results indicated that the rapeseed proteins do not have properties that can create a fiber structure by thermoplastic extrusion. However, there is lots of parameters that can affect whether a material can form a fiber structure due to thermoplastic extrusion and small changes can be critical (Wassén, 2017). Other plant-based proteins have shown to be able to form fiber structures by specific equipment settings but not at others. Parameters that affect the outcome is for example the water content, fat content, extrusion temperature, what kind of screw and extruder that it used and pretreatment of the material (Wassén, 2017).

Figure 28. Left: The extruded product with the round die and 100°C with a screw speed of 40 rpm. Right: The extruded product with the round die and 120°C with a screw speed of 45 rpm.

The melt temperature and pressure of the different runs can be seen in Table 10.

Table 10. The received pressure and melt temperature of the runs with the round die.

	Heated round die (3 mm Ø)		
Set Temp. (°C)	100	120	118-110
Screw speed (RPM)	40	45	70
Die Temp. (°C)	100	120	120
Mean Pressure (bar)	16	9	9
Mean Melt Temp. (°C)	96	114	111

4.6.2.1.2 Dough with 60% water content

The dough with 60% water content was too sticky to run through the extruder and the dough only rolled on the top of the screw instead of being entrained by it. Thereby no extrusion product with 60% water was received.

4.6.2.2 Trials with cooling flat die (2x20 mm)

As the dough with 60% water could not be put down in the extruder it was not investigated with the cooled flat die either. The only mixture put through the extruder with flat die was the dough of FD RSPP with 50% water content.

Even with the cooling die (65°C) the extruded products showed to form a non-elastic structure. Already when the melt exited the die it started to cleave off into fragments, see Fig. 29. The received pressures and melt temperatures of all runs with the flat die can be seen in Table 11.

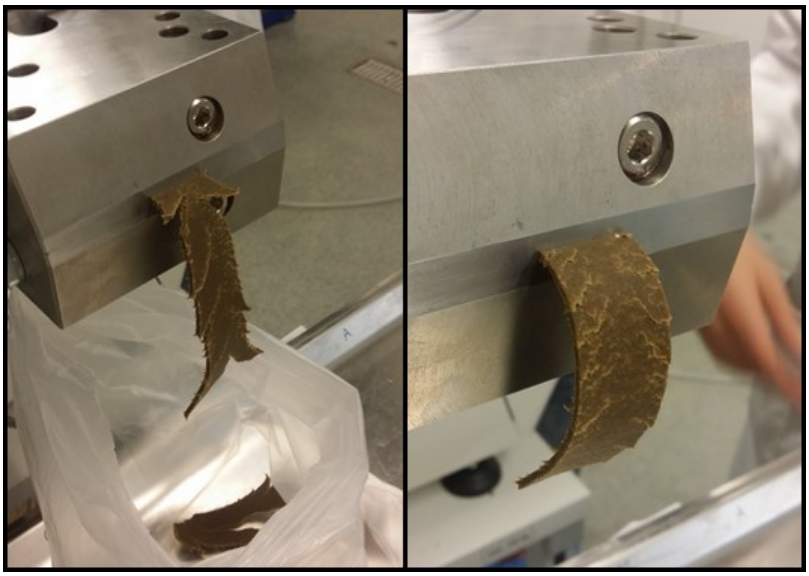


Figure 29. Extruded products with cooled flat die. Left: At 100°C and screw speed 70 RPM. Right: 120°C and screw speed of 70RPM.

Table 11. The received pressure and melt temperature of the runs with the flat die.

	Cooled flat die (2x20 mm)						
Set Temp. (°C)	100	100	121	123	130	130	135
Screw speed (RPM)	45	70	45	70	45	70	70
Die Temp. (°C)	65	65	65	65	65	65	65
Mean Pressure (bar)	37	32	25	25	21	22	22
Mean Melt Temp. (°C)	89	90	108	109	118	118	125

Rapeseed proteins did not form a fiber structure at the settings tested. There are still many factors that can be investigated to form rapeseed proteins in a fiber structure (which is desired to produce meat analogue) by thermoplastic extrusion. There is both single-and twin-screw extruders. In a twin-screw extruder more shear forces are produced, higher temperatures can be achieved and shorter residence time can be obtained. These factors have shown to favour fiber structure formation of some plant proteins. Thereby some plant proteins have shown to form a fiber structure in a twin-screw extruder but not in a single-screw extruder (Wassén, 2017). However, previous studies have shown that a wet and elastic extrusion product based on vegetable proteins is favourably produced by a twin-screw extruder with a cooled die (Applewhite, 1989). Trials at different temperatures and water contents should therefore be conducted in a twin-screw extruder. The tested rapeseed protein material has also been freeze-dried. It might have affected the protein structure. Another possibility is to reduce water from the RPSS by vacuum evaporation, to avoid changes in the protein structure.

Most often thermoplastic extrusion is conducted with defatted flours that thereby have very low fat contents, see Table 2. Since the FD RSP probably has a relatively high fat content it increases the lubrication during the extrusion, which otherwise is regulated by the specific amount of water content. It is possible that the high fat content increases the lubrication in such a way that the shear forces that normally occur, -do not arise to the same extension as for samples with low fat content (Applewhite, 1989). The shear forces affects the denaturation of the proteins and thereby the ability to form a fiber structure, see section 1.4.4.1 Extrusion. Presumable the fat content in the FD RSPP is around 18 % if the water content is 1.3% and it is assumed that the proportion of the rest of the

macronutrients of the SwePharm product is the same as the RSPP produced by the original isolation method, section 2.2 Isolation of proteins from rapeseed press cake. See the estimated values in Table 12. To minimize that a too high fat content in the FD RSP causes a too high lubrication effect, the DF RSP should be defatted prior to extrusion or lower water content should be used. As the measured protein content of the FD RSP is 40.4 %, compare to 58.5 % that is the calculated, the calculated values may not be too accurate, see Table 12. However, even if the actual fat content is as little as 10 % it is still considerably high compared to defatted soy flour.

Initially, lower water content of 10% and 20% were considered to be investigated by extrusion. However, during the pre-tests in the mixing chamber by water levels of 10% and 20% their behavior raised the worry of that they would get stuck or burn to the walls in the extruder. The reason is that the water evaporates inside a single-screw extruder and travels faster than the melt and thereby the melt can dry out. This does not happen in a twin-screw extruder and a lower water content would therefore be interesting to investigate in a twin-screw extruder (Wassén, 2017).

Table 12. The calculated values of the macronutrients in the FD RSP and the macronutrients of the RSPP produced by the original isolation method. The values of the FD RSP are based on the assumption that the rest of the macronutrients in RSPP produced by SwePharm are the same as the analyzed values of the RSPP produced in lab scale (Original isolation method).

Compound	g/100g RSPP incl. std dev.	g/100 g FD RSPP (calculated)
Water	82.1 ± 0.75	1.3 ±0.2
Ash	0.47 ± 0.02	2.3
Protein	11.9 ± 1.2	58.5 (measured: 40.4 ±1.5)
Fat	3.6 ± 0.6	18
Carbohydrates (calculated)	4.1	20.2

Another thing to take into consideration while analysing the extrusion result is that the used extruder in these trials initially was not built for food materials. Maybe an extruder constructed for food materials would handle the complexity that can occur in food solutions and would not allow the melt to dry out during the extrusion.

Another reason why an elastic fiber structure not was achieved could be that the temperature was not high enough. Defatted soy flour seem to form the most elastic (maximum tensile strength) extrusion products at higher temperatures, such as 180°C (Applewhite, 1989). Defatted soy flour have shown to not even form a fiber structure during extrusion at 150°C even though the denaturation temperature of glycinin (the major proteins in soybeans) has been determined to 93.9°C (D. W. Stanley and J. M. deMan, 1978) (Wanasundara et al., 2017). Cruciferins, the major proteins in rapeseeds, have a denaturation temperature of 86.6°C (Wanasundara et al., 2017). Therefore, FD RSP might not need as high extrusion temperature as defatted soy flour but still likely to need higher temperature than 125°C, which was the maximum temperature of the melt during the extrusion trials, see Table 10 and Table 11.

5. Conclusion

There was no significant difference ($p<0.05$) in protein yield between the 180 min incubation time and 60 min incubation time. This confirmed the hypotheses that a shorter time with stirring would be at least as efficient as three hours incubation time without stirring and thereby the production time could be decreased by two hours.

The recirculation of the first sediment at different pH confirmed the hypotheses that more proteins could be leached out from the RSPC if it was re-suspended. pH X5 gave the highest increase, 7.2% more of the protein in the RSPC could be recovered

Coloring compounds was removed by ultrafiltration. Addition of $Na_2S_2O_5$ reduced the discoloring of the RSPP product and the color of the received product was appealing ivory colored. Also, the grassy taste seemed to decrease by the addition of $Na_2S_2O_5$. On the other hand, the addition of $Na_2S_2O_5$ gave the product a slightly metallic or salty taste, which probably were due to residuals of the $Na_2S_2O_5$. The protein analysis indicated that no proteins were lost during the filtration.

Moisture could be evaporated using a vacuum evaporator but the equipment was not very efficient as lots of the material was lost inside the chamber and the samples were not evenly dried. The RSPP worked well to drum dry and a satisfying water activity was received. However, the proteins were denatured and drum drying is therefore not a suitable drying method prior to extrusion. The freeze-drying achieved the lowest water activity, which is favorable while dealing with foods. Freeze-drying is also suppose to restore the proteins properties more than drying methods were heating is used. Therefore, it is thought to be a suitable drying method prior to extrusion.

The conducted single-screw extrusion trials did not indicate that RSP has the ability to form a fiber structure by thermoplastic extrusion. However, many factors affect whether a material forms a fiber structure due to thermoplastic extrusion or not and the results of these extrusion trials should not be seen as a proof of that RSP cannot be thermoplastic extruded. A twin-screw extruder and higher temperatures are factors that earlier studies have shown to favor texturization of plant-based protein by thermoplastic extrusion. Therefore, trials with a twin-screw extruder should be used. If trails with a twin-screw extruder also show that RSP not have the ability to form an elastic texture by thermoplastic extrusion, another well-extruded raw material (such as legumes) can be mixed with RSP and then extruded to create a Swedish based meat-analogue.

6. Future outlook

More ultrafiltration trials should be done to further investigate the optimal salt concentration and number of diafiltrations that should be done to wash out all the salt but still maintain an appealing taste and color of the product.

Previous studies have shown that higher protein yield can be achieved if the RSPC is dispersed in more water during the leaching phase (preferably a ratio of water:RSPC of 30:1), that would be an interesting factor to investigate further. Another trial with re-dispersion of the first sediment at the same pH as the first dispersion, X1, would have been interesting in a health risk point of view. That could have shown if an increase to pH X5 was necessary to achieve the same increase in protein yield.

The functionality of the proteins after the isolation methods should be studied to investigate if any proteins have started to denature during the heating in the precipitation step. Denatured proteins cannot be thermoplastic extruded.

The proteins ability to form a gel structure should be investigated as that is a property that indicates that the proteins can form an elastic texture due to thermoplastic extrusion. It would also be interesting to do differential scanning calorimetry of the rapeseed proteins. Those measurements could tell at what temperature that the proteins denature depending on the water level. This temperature would give an indication of what temperature that is needed to melt the proteins during thermoplastic extrusion and thereby form a fiber structure.

Intrinsic factors such as salt concentration and pH can also affect the extruding abilities of a material and those would be interesting to variate in new extrusion trials. Lower water levels of the FD RSP paste should be tested as that might compensate the high fat content and decreases the lubrication.

Thermoplastic extrusion trials using a twin-screw extruder and a cooled die at different water levels and temperatures should be conducted as those equipment and factors has shown to be favorable to form an appealing elastic texture out of plant-based proteins.

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9. Appendix

Table A1. The raw data of the first run with the heated round die. Setting temperature (zone 4) was 100 °C, and screw speed set to 40 rpm. The measured temperatures of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	17	96	40	29	65	80	100	100
	17	96	40	29	65	80	100	100
	17	96	40	29	65	79	100	100
	17	96	40	29	65	79	100	100
	17	96	40	29	65	79	100	100
	17	95	40	29	65	79	100	100
	16	95	40	29	65	79	100	100
	16	95	40	29	65	79	100	100
	16	95	40	29	64	79	100	100
	16	95	40	29	65	79	100	100
	15	95	40	29	65	79	99	100
	16	95	40	29	65	80	99	100
	16	95	40	29	65	80	99	100
	17	95	40	29	65	80	99	100
	17	96	40	29	65	80	99	100
	17	96	40	29	65	80	99	100
	17	96	40	29	65	80	99	100
	17	96	40	29	65	80	99	100
	16	96	40	29	65	80	99	100
	17	96	40	29	65	80	100	100
	16	97	40	29	65	80	100	100
	17	97	40	29	65	80	100	100
	17	97	40	29	65	80	100	100
	17	97	40	29	65	80	100	100
	16	97	40	29	65	80	100	100
	16	97	40	29	65	80	100	100
	16	97	40	29	65	79	100	100
	15	97	40	29	65	79	100	100
	14	97	40	29	65	79	100	100
	14	97	40	29	65	79	100	100
	14	97	40	29	65	80	100	100
	15	96	40	30	65	80	100	100
	15	96	40	30	65	80	100	100
	15	96	40	30	65	80	100	100
	16	96	40	30	65	80	100	100
	16	96	40	30	65	80	100	100
	16	96	40	30	65	80	100	100
	16	96	40	30	65	80	100	100
	16	96	40	30	65	80	100	100

	16	96	40	30	65	80	100	100
	16	96	40	30	65	80	100	100
	17	97	40	30	65	80	100	100
	16	97	40	30	65	80	100	100
	16	97	40	30	65	80	100	100
	17	97	40	30	65	80	100	100
	16	97	40	30	65	80	100	100
	16	97	40	30	64	80	100	100
	16	97	40	30	64	80	100	100
	16	97	40	30	65	80	100	100
	16	97	40	30	65	80	100	100
	16	97	40	30	65	81	101	100
	16	97	40	30	65	80	101	100
	16	97	40	30	65	80	101	100
	16	97	40	30	65	80	101	100
	16	97	40	30	65	80	101	100
Mean value	16	96	40	29	65	80	100	100
Std dev	0,8	0,7	0	0,5	0,2	0,5	0,5	0

Table A2. The raw data of the second run with the heated round die. Setting temperature (zone 4) was 120 °C, and screw speed set to 45 rpm. The measured temperatures (°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	8	115	45	30	65	90	120	121
	9	115	45	30	65	90	121	121
	8	115	45	30	65	89	121	121
	9	115	45	30	65	89	121	121
	10	114	45	30	65	89	121	121
	11	114	45	30	65	89	121	120
	7	114	45	30	65	89	121	120
	7	114	45	30	65	89	120	120
	8	113	45	30	65	89	120	120
	7	113	45	30	65	89	120	120
	7	113	45	30	65	89	120	120
	10	113	45	30	65	90	120	120
	11	113	45	30	65	90	120	120
	7	113	45	30	65	90	119	120
	8	113	45	30	65	90	119	120
	11	113	45	30	65	90	119	120
	8	113	45	30	65	90	119	120
	12	113	45	30	65	90	119	120
	12	113	45	30	65	90	119	120
	8	113	45	30	65	90	119	120
Mean value	9	114	45	30	65	90	120	120
Std dev	1.7	0.8	0	0	0	0.5	0.8	0.4

Table A3. The raw data of the third run with the heated round die. The screw speed set to 70 rpm. The measured temperatures of the different zones are shown. The pressure and the temperature (°C) of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	11	113	70	30	65	89	118	120
	9	113	70	30	65	89	118	119
	8	113	70	30	65	89	118	119
	9	113	70	30	65	89	118	119
	9	113	70	30	65	89	118	119
	8	113	70	30	65	89	118	119
	8	113	70	30	65	89	118	119
	8	113	70	30	65	89	117	119
	9	113	70	30	65	89	117	119
	12	112	70	30	65	90	116	120
	8	112	70	30	65	90	116	120
	11	111	70	30	65	90	116	120
	8	111	70	30	65	90	115	120
	8	111	70	30	64	90	115	120
	9	110	70	30	64	90	114	120
	11	110	70	30	64	90	114	120
	8	110	70	30	64	90	113	120
	9	110	70	30	65	90	113	120
	10	110	70	30	65	90	113	120
	10	110	70	30	65	90	112	120
	7	110	70	30	65	90	112	120
	12	109	70	30	65	90	112	120
	10	109	70	30	65	90	112	120
	9	109	70	30	66	90	111	120
	9	109	70	30	66	90	111	120
	10	109	70	30	66	90	111	120
	9	108	70	30	66	90	111	120
	9	108	70	30	65	90	110	120
	9	108	70	30	65	90	110	120
	9	108	70	30	65	90	110	120
Mean value	9.2	111	70	30	65	90	114	120
Std dev	1.2	1.8	0	0	0.5	0.5	2.9	0.4

Table A4. The raw data of the first run with the cooling flat die. The setting temperature was 100 °C and the screw speed set to 45 rpm. The measured temperatures(°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	37	89	45	30	65	80	100	65
	38	89	45	30	65	80	100	65
	39	89	45	30	65	80	100	65
	38	89	45	30	65	80	100	65
	38	89	45	30	65	80	100	65
	38	89	45	30	65	80	100	65
	40	89	45	30	65	80	100	65
	40	89	45	30	65	80	100	65
	39	89	45	30	65	80	100	65
	38	90	45	30	65	80	100	65
	38	90	45	30	65	80	100	65
	37	90	45	30	65	80	100	65
	37	90	45	30	65	80	100	65
	36	90	45	30	65	80	100	65
	35	90	45	30	65	80	101	66
	34	90	45	30	65	80	101	66
	33	90	45	30	65	80	101	66
Mean value	37	89	45	30	65	80	100	65
Std dev.	1.9	0.5	0	0	0	0	0.4	0.4

Table A5. The raw data of the second run with the cooling flat die. The setting temperature was 100 °C and the screw speed set to 70 rpm. The measured temperatures (°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw speed (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	33	90	70	30	65	80	101	66
	32	90	70	30	65	80	101	66
	32	90	70	30	65	80	101	66
	31	90	70	30	65	80	101	65
	31	90	70	30	65	80	101	65
	31	90	70	30	65	80	101	65
	31	90	70	30	65	80	100	65
	32	90	70	30	65	80	100	65
	32	90	70	30	65	80	100	65
	32	90	70	30	65	80	100	65
	32	90	70	30	65	80	100	65
	32	90	70	30	65	80	100	65
	31	90	70	30	65	80	100	65
	31	90	70	30	65	80	100	66
Mean value	32	90	70	30	65	80	100	65
Std dev.	0.6	0	0	0	0	0	0.5	0.5

Table A6. The raw data of the third run with the cooling flat die. The setting temperature was 120 °C and the screw speed set to 45 rpm. The measured temperatures (°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	26	106	45	29	66	93	118	65
	26	106	45	29	66	93	119	65
	26	107	45	29	66	93	119	65
	26	107	45	30	66	93	120	65
	26	107	45	29	65	93	120	65
	25	108	45	30	65	93	120	65
	26	108	45	30	65	93	121	65
	25	108	45	30	65	92	121	66
	25	108	45	30	65	92	121	66
	25	109	45	30	65	92	122	66
	25	109	45	30	65	92	122	66
	24	109	45	30	65	92	122	66
	24	109	45	30	65	92	122	66
	24	109	45	30	65	91	122	66
	24	109	45	30	65	91	123	66
	24	109	45	30	65	91	123	65
	23	109	45	30	65	91	123	65
	21	109	45	30	65	91	123	65
	23	109	45	30	65	91	123	65
Mean value	25	108	45	30	65	92	121	65
Std dev.	1.3	1.1	0	0.4	0.4	0.8	2	0.5

Table A7. The raw data of the forth run with the cooling flat die. The setting temperature was 123 °C and the screw speed set to 70 rpm. The measured temperatures (°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	26	108	70	30	65	91	123	65
	28	108	70	30	65	91	123	65
	27	108	70	30	65	91	123	65
	24	109	70	30	65	91	123	65
	26	109	70	30	65	91	122	65
	24	109	70	30	65	90	123	65
	25	109	70	30	65	90	123	65
	26	109	70	30	65	90	123	65
	25	109	70	30	64	90	123	65
	24	110	70	30	64	90	123	65
	21	110	70	30	64	90	123	65
	26	110	70	30	64	90	123	65
	24	110	70	30	64	90	123	65
	26	110	70	30	65	89	123	65
	26	110	70	30	65	89	123	65
	25	110	70	30	65	89	123	66
Mean value	25	109	70	30	65	90	123	65
Std dev.	1.6	0.8	0	0	0.5	0.7	0.3	0.3

Table A8. The raw data of the fifth run with the cooling flat die. The setting temperature was 130 °C and the screw speed set to 45 rpm. The measured temperatures (°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw speed (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	24	119	45	30	73	102	130	66
	22	119	45	30	73	102	130	66
	20	119	45	30	73	102	131	66
	23	119	45	30	73	101	131	66
	23	118	45	30	73	100	132	66
	23	118	45	30	73	100	132	66
	23	118	45	30	73	100	132	66
	21	118	45	30	73	99	132	66
	17	118	45	29	73	99	132	66
	21	118	45	29	72	99	132	66
	21	117	45	29	72	99	132	66
	22	117	45	29	72	99	132	66
	18	117	45	29	72	99	132	66
	16	117	45	29	72	99	132	66
	20	117	45	29	72	99	132	66
	21	117	45	29	72	99	132	66
	21	117	45	29	72	98	131	66
	21	117	45	29	72	98	131	66
	21	117	45	29	72	98	131	66
	21	118	45	29	72	97	131	67
	21	118	45	29	72	97	132	66
	21	118	45	29	71	97	132	67
	22	119	45	29	71	97	132	67
	21	119	45	29	71	96	132	67
	19	119	45	29	71	96	132	67
Mean value	21	118	45	29	72	99	132	66
Std dev.	1.9	0.8	0	0.5	0.7	1.7	0.6	0.4

Table A9. The raw data of the sixth run with the cooling flat die. The setting temperature was 130 °C and the screw speed set to 70 rpm. The measured temperatures (°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw speed (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	23	119	70	30	70	96	133	67
	24	119	70	30	70	95	133	67
	23	119	70	30	70	95	133	67
	21	119	70	30	70	95	133	67
	24	119	70	30	70	95	133	67
	20	119	70	30	70	95	133	68
	22	119	70	30	70	94	133	68
	21	118	70	30	70	94	132	68
	23	118	70	30	70	94	132	68
	17	118	70	30	70	94	132	68
	24	118	70	30	70	94	131	68
	22	118	70	30	70	94	131	68
	22	118	70	30	70	94	131	69
	18	118	70	30	70	93	130	69
	22	118	70	30	70	93	130	69
	23	118	70	30	70	93	130	69
	24	118	70	30	70	93	130	69
	23	119	70	30	70	93	130	69
	24	119	70	30	70	93	130	69
Mean value	22	118	70	30	70	94	132	68
Std dev.	2.0	0.5	0	0	0	0.9	1.3	0.8

Table A10. The raw data of the seventh run with the cooling flat die. The setting temperature was 135 °C and the screw speed set to 70 rpm. The measured temperatures (°C) of the different zones are shown. The pressure and the temperature of the melt is measured right before it reaches the die.

	Pressure (bar)	Melt temp (°C)	Screw speed (rpm)	Zone 1 (feed)	Zone 2	Zone 3	Zone 4	Zone 5 (die)
	24	124	70	30	70	94	134	69
	24	124	70	30	70	94	134	69
	23	124	70	30	70	94	135	69
	23	125	70	29	70	94	135	69
	24	124	70	29	70	94	135	69
	23	124	70	29	70	94	135	69
	23	124	70	29	70	94	135	69
	22	124	70	29	70	94	135	69
	23	124	70	29	70	94	135	69
	19	124	70	29	69	94	135	69
	23	124	70	29	69	94	135	69
	22	124	70	29	70	94	135	69
	24	124	70	29	70	94	134	69
	24	124	70	29	70	94	134	69
	21	125	70	29	70	94	134	69
	24	125	70	29	70	94	134	69
	24	125	70	29	70	94	134	69
	22	126	70	30	70	95	135	69
	24	126	70	30	70	95	135	69
	23	126	70	30	70	95	135	69
	21	127	70	30	70	95	135	69
	23	127	70	30	70	95	136	69
	23	127	70	30	70	96	136	69
	23	128	70	30	70	96	136	69
	19	128	70	30	70	96	136	69
	23	128	70	30	70	96	137	69
	19	128	70	30	70	96	137	69
	23	127	70	30	70	96	137	69
	20	126	70	30	70	96	136	69
	20	126	70	30	70	96	136	69
	24	125	70	30	70	95	135	69
	22	124	70	30	70	94	135	69
	17	124	70	30	70	94	134	69
Mean value	22	125	70	30	70	95	135	69
Std dev.	1.8	1.5	0	0.5	0.2	0.9	0.9	0

Figure A1. The dough used in the extruder. This is right after it had been mixed with water in the mixer. The FD RSP mixed with 50% water.