An investigation of high moisture meat analogues as mince - The influence of process parameters and ingredients on the final texture

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Abstract

The goal of this master thesis project was to investigate and develop a minced meat analogue through high moisture extrusion and shredding of soy protein and vegetable fat. Soy protein in the form of soy protein concentrate (SPC) and soy protein isolate (SPI), vegetable fat in the form of high oleic canola oil and a blend containing partially and fully hydrogenated canola oil was utilized in the study. Texture analysis was performed to determine hardness, chewiness and springiness. The samples were also analyzed on water and oil holding capacity as well as through structural and chemical analysis to get a deeper understanding on the structure and chemical bonds responsible for the texture. The structure and texture of the minced meat analogue was compared to commercially available products produced using textured vegetable protein (low moisture extrusion).

Acknowledgements

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Contents

1

Introduction

According to European consumer research, the acceptance of meat-analogues can be linked to four major consumer groups: those reducing their meat consumption, convenience oriented and cost-conscious consumers, consumers who are conscious of animal welfare, sustainability, indulgence and innovation-oriented consumers. The term "meat-analogue" describes a meat-free food with similar taste, appearance, haptic experience and nutritional value compared to traditional meat products [Sun et al., 2020].Results from life-cycle assessments comparing meat-analogues with animal derived meat show considerably lower greenhouse gas emissions in favor of meat-analogues [Sun et al., 2020].

Extrusion is a versatile processing technology consisting of various process operations. Extruders are generally high temperature and short time cooking systems which provide product qualities such as nutrient retention, texture, improved starch and protein digestibility as well as anti-nutrient reduction. As a result of shear generated during the extrusion process, food material undergoes compaction, shearing, particle size reduction, phase transition and molecular breakdown. Residence time is typically less than a minute during which the food material is exposed to high temperature and pressure, generated by the mechanical shear and the die restriction [Ek and Ganjyal, 2020].

A common ingredient used in structured plant protein products is soy protein including soy protein concentrate and soy protein isolate due to low costs, abundance, meat-like texture after hydration and similar protein quality to that of animal proteins because of the amino acid profile [Sun et al., 2020].

Developing plant-based meat analogues is believed to be a way to conserve natural resources, improving human health and maintain animal welfare. A challenge when creating meat substitute products using extrusion technology is matching the fibrous structure, juicy mouth-feel and bite of meat. As a result the process most likely requires optimization of processing conditions and special designs for meat substitute formulation [Sun et al., 2020]. With respect to the aforementioned as-

pects, this study will focus on the ability to produce high moisture meat analogues using soy protein and additives such as vegetable oil and fat.

1.1 Aim

The aim of the study was to investigate the addition of lipids in the form of vegetable oil and fat to high moisture extrusion (HME) of soy protein with the objective of creating a high moisture meat analogue (HMMA) in a minced form. In addition, there were other specific aims focused on the production process and characteristics of the final product such as:

- Identifying potential challenges in the process of high moisture extrusion due to the increase in lubrication when adding lipids.
- Comparing the characteristics of the extrudate containing hydrogenated canola oil and a mixture of non-hydrogenated canola oil with partially hydrogenated canola oil.
- Investigating the physicochemical properties of the high moisture extruded soy protein with and without the addition of lipids and comparing it to commercially available low moisture extruded minced meat analogues.

2

Theory

2.1 Extrusion

Extrusion is a multi-step, multi-functional and thermal/mechanical process. By a combination of temperature, pressure, moisture and mechanical shear moistened proteinaceous foods are plasticized and cooked resulting in molecular transformations and chemical reactions. Among one of several applications is the production of textured meat-like materials from defatted high-protein flours [Singh et al., 2007].

As a result of the intense mechanical shear, extrusion has some unique features compared to other heat processes. The process results in modification of functional properties of food ingredients, inactivation of certain anti-nutritional factors, denaturation of undesirable enzymes and sterilization of the finished product while retaining natural flavors [Singh et al., 2007].

The food extrusion process can be divided into two processes, distinguished by the moisture content and die type. These are referred to as high-moisture extrusion and dry extrusion. High-moisture extrusion generally contains 40%-80% of water compared to 10%-30% in dry extrusion.

The extruder

The extruder consists of several essential parts as illustrated in figure 2.1. The barrel is typically divided into equally sized zones which provides flexibility as these can be heated or cooled independently, illustrated by the heating blocks. The screws can be divided into three distinct sections. At the conveying zone the screw has a larger pitch for the material to be transported. In the compression section the pitch is shorter, in this section the material is compacted and mixed. This generates heat because of the shear created between the food particles as well as between the particles and the barrel and particles and the screw. The third section is the melting/metering, where the material generally transforms into a melt due to the heat supplied by the heating blocks and additional heat built up from the mechanical

shear and pressure. Pressure is particularly high due to compaction and restriction from the die. The die provides the restriction and helps to form the final product [Ek and Ganjyal, 2020].

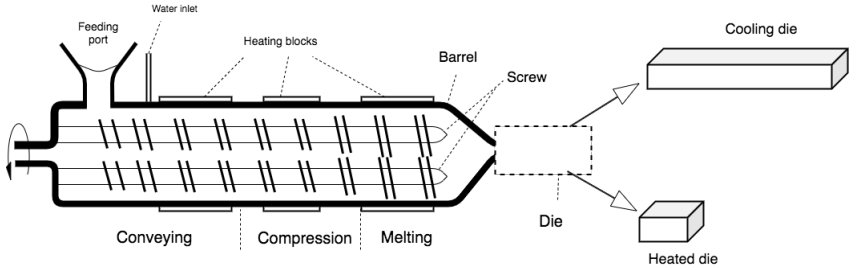

Figure 2.1 Schematic model of a generic twin screw extruder.

Twin-screw extruders Twin-screws are have great flexibility and efficiency in mixing and kneading which makes them a versatile processing equipment. They are classified on the direction of rotation which is either co or counter and on whether they intermesh or not. The difference being that the screws squeeze the product forward vs wiping each other in moving the product forward. The versatility is a consequence of the modular aspect of most screws used in twin-screw extruders, meaning that the screw elements are mounted on the screw shaft and can be changed to meet the conditions of the material operated in the extruder. The screw elements serve different purposes like conveying, mixing and kneading [Ek and Ganjyal, 2020].

High Moisture Extrusion

High moisture extrusion (HME) is used for texturing vegetable proteins to products with fibrous texture similar to animal meat. The mechanism of texturizing vegetable protein correlates to pressure and flow of material which depend on the feed moisture, cooking temperature and screw rotational speed. For vegetable proteins to denature through the shearing force of the rotating screw the minimum temperature needed is approximately 130°C, this is highly dependent on the type of protein and water content. The three dimensional protein structures are destroyed due to the effects of temperature and moisture causing the polypeptide chains to unfold. Crosslinks are formed between denatured chains, stabilized by different bonds such as amide, disulfide and hydrogen bonds. These changes occur as the protein melt is passing through the barrel. The protein melt continues through the die, flowing in a laminar manner, the lower temperature in the die cools the melt. This combination between the laminar flow and decrease in temperature causes the melt to texturize

in the longitudinal direction [Ryu, 2020]. Despite several studies on the molecular interactions, there is no consensus on the mechanism of fiber formation [Dekkers and Goot, 2018].

Low Moisture Extrusion

In low-moisture extrusion (LME) the protein matrix undergoes an expansion as it exits the die. This is due to the superheated vapor inside the viscoelastic melt encountering a sudden pressure drop. The expansion effect is dependent on pressure, moisture content and physical properties of the melt. The texturized products are then cut and dried. Once dried the product can be stored and has a longer shelf life compared to high-moisture analogues. Further processing such as rehydration, seasoning and coloring is however needed previous to consumption. Low-moisture analogues have a higher nitrogen solubility index compared to high-moisture extrusion analogues. The nitrogen solubility index (NSI) is used as a reference to determine the degree of protein solubility [Ryu, 2020]. Increased protein solubility suggests increased protein digestibility. If a greater amount of protein is digested, the quality of the absorbed protein will in turn be higher. NSI is also an indirect indicator of the amount of heat damage to the protein. According to Twombly high NSI ingredients are necessary for protein texturization with the exemption of soy. Texturized soy will have a low NSI [Twombly, 2020].

2.2 Soybean

Soy ingredients are commonly used in HME and LME because of their functional properties such as efficient water-holding capacity, fat-absorbtion, gelling and emulsifying properties to produce texturized vegetable proteins [Kyriakopoulou et al., 2019]. The protein fraction of soy contributes to the structure and nutritional aspects [Twombly, 2020]. As a source of protein, soybeans have the advantage of containing all essential amino acids required for human development. 60% - 80% of the protein is located in two major storage proteins glycin (11S) and β-conglycin (7S). At a neutral pH, glycin has a denaturation temperature of 90°C while β-conglycin denatures at 74°C [Renkema et al., 2000]. Both proteins are globulins, meaning they are soluble in salt solution. Glycin consists of two polypeptide chains, one acidic with a molecular weight between 37-40 kDa and a basic with molecular weight of approximately 20 kDa. β-conglycin has a trimeric structure with subunits α', α and β with molecular weights of 83-53 kDa, 76-57 kDa and 53-42 kDa respectively [Arrese et al., 1991]. The remaining proteins are oleosins 8%-20% and trypsin inhibitors 0% - 1.7%. Trypsin inhibitors can cause negative effects on the digestive system by forming a complex with trypsin in the gastrointestinal tract. However, trypsin inhibitors are commonly denatured by heat inactivation during the extraction process. Excessive exposure to high temperatures during the extraction

process can have a detrimental effect as the protein extraction yield decreases with increased protein denaturation. Protein solubility will also depend on the isoelectric point which on average is pH 4.5 [Preece et al., 2017].

Extraction

The most common component utilized from the oilseed, such as soybeans, is the oil which for the most part is extracted through mechanical and solvent extraction. This process yields defatted soy flakes as a byproduct [Preece et al., 2017]. Further on soy protein concentrate is produced by leaching away solubles while the protein remains insoluble following up with a drying step. The different extraction alternatives are illustrated in figure 2.2 [Deak et al., 2008].

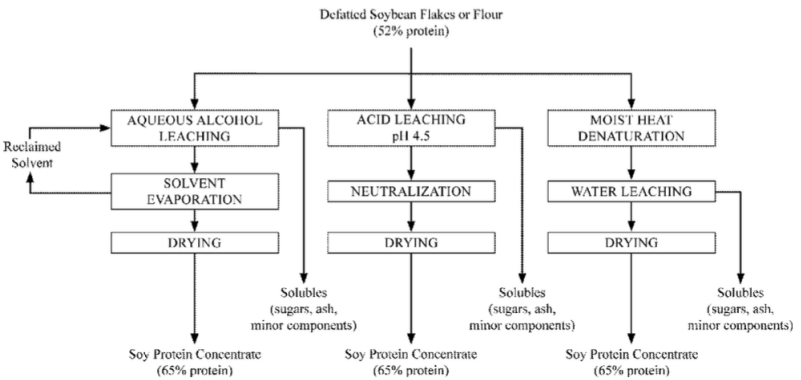

Figure 2.2 Method of soy protein concentrate production.[Deak et al., 2008]

Soy protein isolate is prepared by utilizing its solubility properties. A traditional process consists of; solubilizing protein from the defatted flakes, centrifugation to remove insoluble fiber, precipitation of protein at pH 4.2−4.5, centrifugation to separate the curd followed by washing and finally drying.

The production process is illustrated in figure 2.3.[Deak et al., 2008]

Soy protein concentrate

As previously illustrated soy protein concentrate (SPC) is produced by adding alcohol or water to soybean flakes in order to remove soluble carbohydrates and other compounds [Preece et al., 2017]. The overall compositions of SPC may vary slightly depending on the process, the ash content will for example be higher when extracting with alcohol, an indication of less removal of minerals [Deak et al.,

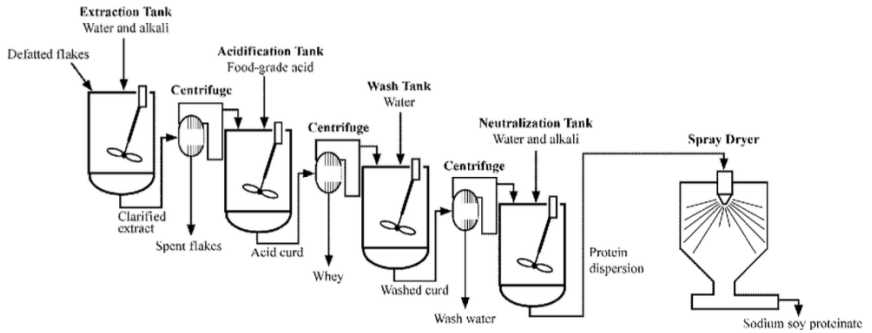

Figure 2.3 Method of soy protein isolate production.[Deak et al., 2008]

2008]. Protein content will be of at least 65% [Deak et al., 2008]. It is reported that anisotropic structures achieved using SPC compared to soy protein isolate (SPI) are more pronounced and the extrusion process is easier using SPC compared to SPI under the same conditions[Akdogan, 1999]. It is argued that polysaccharides contribute to formation of a dispersed phase [Pietsch et al., 2019].

Soy protein isolate

Soy protein isolates contains a minimum of 90% protein, throughout the extraction process, fibers and sugars from dehulled and defatted soy flour are removed [Deak et al., 2008]. Advantages of using SPI is the high purity, bland flavor and light color [Kyriakopoulou et al., 2019]. Comparing the typical composition of SPI with that of SPC presented in figure 2.4 by [Deak et al., 2008], SPI has a higher protein percentage but lower fiber and carbohydrate percentage.

2.3 Lipids

Addition of lipids in the form of oil or fat will act as lubricants in the extrusion system resulting in lower specific mechanical energy (SME) and lower pressure drop between the barrel and the die [Twombly, 2020]. This will affect the formation of fibers, making it more difficult for macromolecules to align since the shear will be weakened and slippage can take place. The maximum amount of oil reported to have been added without negatively affecting the extrusion process varies from 8% - 15% in different studies [Twombly, 2020][Kyriakopoulou et al., 2019]. On the other hand adding oil or fat may be an advantage by contributing to juiciness and tenderness. Improved retention of volatile flavor compounds when oil or fat is

Constituent	Defatted Flours and Grits		Protein Concentrates		Protein Isolates	
	as is	mfb[a]	as is	mfb[a]	as is	mfb[a]
Crude protein (N × 6.25)	52–54	56–59	62–69	65–72	86–87	90–92
Crude free lipid (pet. ether)	0.5–1.0	0.5–1.0	0.5–1.0	0.5–1.0	0.5–1.0	0.5–1.0
Crude fiber	2.5–3.5	2.7–3.8	3.4–4.8	3.5–5.0	0.1–0.2	0.1–0.2
Ash	5.0–6.0	5.4–6.5	3.8–6.2	4.0–6.5	3.8–4.8	4.0–5.0
Moisture	6–8	0	4–6	0	4–6	0
Carbohydrates (by difference)	30–32	32–34	19–21	20–22	3–4	3–4

[a]mfb: moisture-free basis.
Source: Endres (2001).

Figure 2.4 Composition of soy protein products (%) [Deak et al., 2008].

present is seen in meat products, a similar effect can be expected in meat analogues [Dekkers and Goot, 2018].

High oleic canola oil

High oleic canola oil (HOCO) was developed using conventional plant breeding techniques as a solution to avoid *trans* fats. Through breeding techniques it has been possible to decrease linoleic and linolenic acid to produce more stable oils without partial hydrogenation and production of *trans* fats. This reduction is crucial since linoleic and linolenic acid are both polyunsaturated fatty acids and have an oxidation rate between 12-25 times higher compared to oleic acid, a monounsaturated fatty acid. A desirable trait in ingredient oils is to possess extremely high oxidative stability and no flavor, achieved by reducing linoleic acid to less than 20% and increasing oleic acid to over 70%. This does not apply for frying oil were taste is more important, for this reason linoleic acid is $>$20% in oils intended for frying because of its importance in flavor development. In addition to increasing stability through plant breeding, canola oil contains high levels of tocopherols which further enhances stability by containing antioxidative properties [DeBonte et al., 2012].

Hydrogenated canola oil (HCO)

Hydrogenation of vegetable oils is a modification method used to obtain oils/fats with improved texture and oxidative stability. Using hydrogen gas and nickel as a catalyst double bonds in the unsaturated fatty acids are saturated. In the process some double bonds can isomerize and convert from *cis* to *trans*, this is unwanted because of the negative health effects associated with *trans*-fatty acids [Hashempour-Baltork et al., 2016]. However, formation of *trans*-fatty acids is suppressed when

hydrogenation is carried out under non-selective conditions including, lower temperature, higher pressure, increased agitation and higher catalyst loadings [List and Jackson, 2011].

2.4 Analysis

In an effort to better understand the parameters that give rise to the texture and structure of the extrudate, several techniques for analysis are utilized. Some with the aim of giving insight on the chemical aspects while others focus on the physical structure.

Water Holding Capacity

Water holding capacity (WHC) describes the ability of a food matrix to retain fluid in its semi-solid matrix. It is commonly analyzed by a centrifugal method [McClements et al., 2021]. WHC can be modified by altering the cross-link density, reducing cross-link density results in improved WHC and increased cross-link density reduces WHC [Cornet et al., 2021]. As the extrusion process progresses, protein will undergo denaturation. This leads to the aggregation of protein as a result of changes in surface hydrophobicity. Aggregation of protein forms three-dimensional structures with increased WHC [Leonard et al., 2020].

Chemical analysis

The role of proteins is critical in understanding the contribution to physicochemical and sensory attributes of meat analogues [McClements et al., 2021]. Protein characterization, solubility and protein determination enhance the understanding of the structure.

Protein solubility Protein solubility is used to investigate the forces in the structure stabilizing the extrudate [Lin et al., 2000]. This is done by using buffer systems to extract the protein in the extrudate. The buffer solutions are combined using the four following solutions that act on different bonds; phosphate, urea, sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (2-ME).Urea, SDS and 2-ME disrupt, hydrogen bonds, inter-molecular hydrophobic interactions and disulfide bonds respectively [Chen et al., 2011]. According to Lin, the phosphate buffer only dissolves protein molecules in their native state [Lin et al., 2000]. After several centrifugation steps the total protein content is determined using the Kjeldahl method, the soluble protein in the supernatant is analyzed using the Lowery method. The protein solubility is then calculated according to the following;[Chen et al., 2011]

$$Solubility = \frac{Soluble\ protein}{Total\ protein} \tag{2.1}$$

Protein concentration The protein concentration in the supernatant can be determined using a spectrophotometric method. Using the Bradford method, Coomassie Brilliant Blue G-250 (CBB) dye reacts with the protein causing a shift in the absorbance maximum at 595 nm. The absorbance corresponding to the protein content can be read using a spectrophotometer and compared to the known protein standard [Bradford, 1976].

SDS-PAGE electrophoresis SDS-PAGE is used to investigate the aggregation of protein subunits in the extrudate [Chen et al., 2011]. It can provide a visual representation of the changes in protein composition during processing. The proteins are separated on a polyacrylamide gel, several studies use the conventional Laemmli buffer containing sodium dodecyl sulfate and 2-mercaptoethanol (2-ME) to solubilize the proteins and cleave the disulfide bonds. Once the electrophoresis is performed the gel is stained with Coomassie brilliant blue to visualize the proteins [McClements et al., 2021].

Texture analysis

According to Szczesniak texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics [Szczesniak, 2002]. Texture can be analyzed using a texture profile analyser (TPA) which provides controlled and well defined conditions for measuring texture by applying uni-axial compression to determine the fracture properties of systems. The method applied involves a sample of standard size and shape placed on a base plate and compressed/decompressed twice by an upper plate [Kasapis and Bannikova, 2017]. A force vs. deformation curve is recorded from which several textural parameters can be extracted, such as; hardness, elasticity and chewiness among others. Elasticity measures how much the original structure is broken down by the initial compression. Samples with high cohesiveness values are perceived as tough and difficult to masticate. Chewiness is defined as the energy needed to chew a solid food until it is ready for swallowing [Kasapis and Bannikova, 2017]. Elongation tests provide insight into the extend of anisotropic structures in the sample [McClements et al., 2021].

Hardness Hardness is obtained from the force vs. deformation curve as the maximum force that occurs during the first compression cycle. It is in most cases correlated to the rupture strength and expressed in Newtons [Kasapis and Bannikova, 2017].

Confocal scanning laser microscopy

Is a technique that uses non-simultaneous illumination by having a laser that concentrates onto a determined spot at a specific depth of the sample. The objective lens and scanning system collects the fluorescence from the focal plane and reflects off the dichroic mirror. A filter containing a slit inside the optical pathway eliminates the out-of-focus light, this is the reason only fluorescence signals from the illuminated spot are able to enter the detector resulting in excellent resolutions in the section plane. Using staining it is possible to analyze several components simultaneously [Sharif et al., 2020].

3

Materials and experimental method

3.1 Materials

Three different extrusion trials were performed in order to determine optimal parameters and conditions for the production of a minced meat analogue. In total the formulations consisted of four different ingredients; soy protein concentrate, soy protein isolate, high oleic canola oil and a blend of fully hydrogenated canola oil and non hydrogenated canola oil. The equipment used was a TwinLab-C 20/40 (Brabender® GmbH & Co. KG, Duisburg, Germany) twin extruder and a shredder.

Soy protein concentrate

Arcon® (ADM), a water-washed soy protein concentrate with a low flavor profile and high protein solubility. Functionally it provides good water binding and dispersibility properties as well as fat emulsification capacities.

Soy protein isolate

SUPRO® EX 33 IP (Solae, Geneva, Switzerland). The product hydrates rapidly, provides texture and high emulsion stability. It is also effective in emulsifying fat and has clean flavor characteristics.

High oleic canola oil

Fritex HORO (AAK) is suitable in applications with high temperature and regarded as having high stability due to the low linoleic acid content.

Fully hydrogenated canola oil + non hydrogenated canola oil

A blend consisting of fully hydrogenated and non hydrogenated vegetable oils and fats. It contains a maximum of 0.1% oleic acid and 1% trans fatty acids and has a natural flavor profile.

Extruder

The twin screw extruder used was a Brabender twin extruder. It consists of five zones that can be independently heated.

Shredder

Once the protein mix had been extruded it was shredder to achieve a mince like structure. The extrudate was manually fed and the shredder was woven by hand. The shredding was repeated five times in order to get smaller and even sized pieces. The equipment can be observed in figure 3.1.

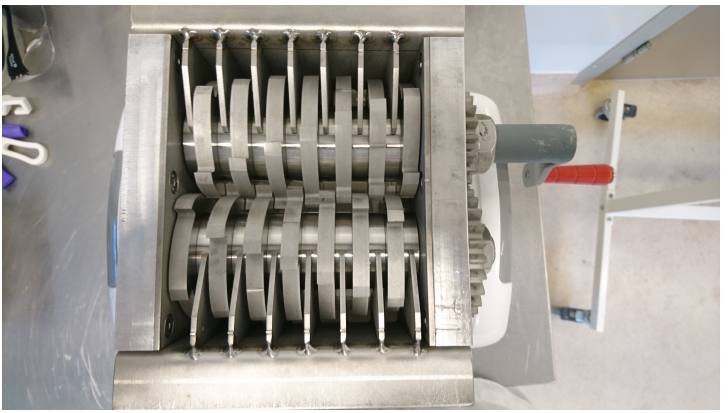

Figure 3.1 Shredder used for processing the extrudate.

3.2 Extruded Samples

Three different extrusion trials were performed. The purpose of the first two trials was to determine the conditions for the third extrusion where fat was added. The parameters what were investigated in the first two trials were, moisture, temperature and protein content.

Temperature and moisture content

The aim of the first extrusion trial was to investigate the impact of moisture and temperature on the texture of the minced meat analogue. Two temperature profiles were investigated; 40/80/120/125/125/35 °C and 40/90/130/135/135/40 °C were the first 5 represent the zones in the extruder and the last is the temperature of the die. A sensor located at the exit of zone 5 records pressure and temperature of the melt. Each temperature profile was evaluated along with three different moisture contents 70%, 65% and 60%. The screw speed was held constant at 400 rpm. Soy protein concentrate was used throughout the first run. The different trials are illustrated in table 3.1.

Table 3.1 Temperature profile in °C and targeted moisture content %.

Temperature profile °C	**Targeted moisture content** %		
40/80/120/125/125/35 °C	70	65	60
40/90/130/135/135/40 °C	70	65	60

The trials were performed by starting with the lower temperature profile and the highest moisture content. The extrudate was cut and placed in bags that were sealed and stored at -18°C.

Soy protein concentrate and isolate

The purpose of the second extrusion trial was to determine how the consistency was affected by increasing the protein content. This was done by extruding three different combinations of SPC and SPI at with the following temperature profile 40/90/130/135/135/40°C and 65% moisture in the order as illustrated by table 3.2.

Table 3.2 Extrusion conditions for the second trial. The indicated temperature refers to zone 5 .

SPC/SPI	**Targeted moisture content** %	**Temperature** °C
75/25	65	135
50/50	65	135
25/75	65	135

Soy protein concentrate, isolate, vegetable oil and fat

The final extrusion trial was designated to determine the differences obtained when including vegetable oil in the extrusion mixture, both in a solid and liquid state. The vegetable fat and oil was fed into the extruder along with the soy protein. A mixing

step of soy protein and oil was therefore performed previous to feeding it into the extruder. Using a food processor (Bosch MCM3200W) approximately 500 grams of soy protein and the required amount of vegetable fat and oil needed to achieve the desired concentration were weighed. The soy protein was mixed at the highest speed while feeding the vegetable fat and oil. The food processor was then shaken while running at the highest speed in order to achieve a homogeneous mixture, this was performed for approximately 5 minutes.
Six samples were produced with fat concentrations form 5% to 15% at the previously used temperature profile including 135°C in zone 5 and 65% moisture with a ratio of SPC/SPI of 75/25 as illustrated by table 3.3. The samples were extruded according to the order in the table.

Table 3.3 Extrusion conditions for the third trial including Hydrogenated canola oil (HCO) and high oleic canola oil (HOCO). The temperature refers to zone 5.

SPC/SPI	**HCO/HOCO**	**Targeted moisture content** %	**Temperature** °C
75/25	5% HOCO	65	135
75/25	5% HCO	65	135
75/25	10% HOCO	65	135
75/25	10% HCO	65	135
75/25	15% HOCO	65	135
75/25	15% HCO	65	135

3.3 Shredding

The extruded samples from the first extrusion trial were thawed at room temperature for a couple of hours before being shredded. The samples from the second trial were shredded directly after being extruded in order to mimic an industrial situation and determine if any difference could be observed visually. Samples from the third trial were removed from refrigeration and placed at room temperature for a while before being shredded.

3.4 Analysis

Texture analysis

Hardness was analyzed using a Kramer shear cell mounted on an Instron 5542 texture analyzer and operated with the software Bluehill (Instron Ltd Norwood,

MA). The shear cell was loaded with approximately 10 grams of shredded extrudate and compressed once at 20 mm/min. Five replicates were performed and the maximum compressive load (N) and the maximum compressive extension (mm) were recorded. The setup used is illustrated in figure 3.2. Hardness as defined in 2.4 was the most relevant texture parameter to analyze given that the extrudate was shredded.

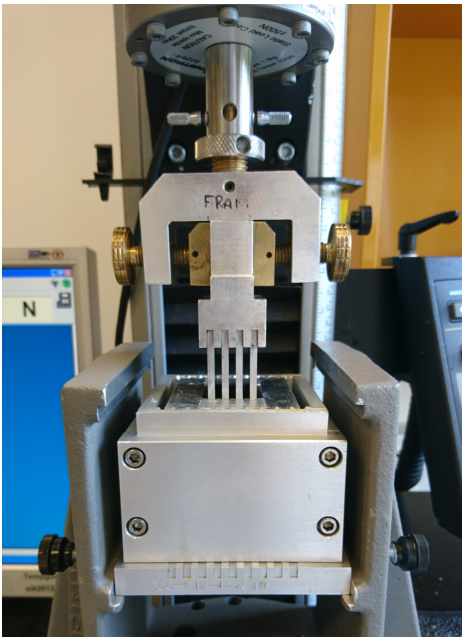

Figure 3.2 Kramer shear cell used for texture analysis.

Water holding capacity

Water holding capacity was measured on different protein combinations as well as the extrudates in replicates of three. This was done using the method stated by [Miedzianka et al., 2014] with slight modifications. Approximately 0.1 grams of sample with 9.9 ml milliQ-Water were measured in a 15 ml centrifuge tube, vortexed and placed on a swivel roller mixer for 60 minutes. The solutions were then centrifuged at 4000 g for 25 minutes.The supernatant was discarded and the weight of then pellet was recorded. The WHC was calculated according to equation 3.1. Where m_b is the mass of the protein sample and m_a is the mass of the pellet after the oven.

$$WHC = 100 \times \frac{m_b - m_a}{m_b} \tag{3.1}$$

Oil holding capacity

Oil holding capacity was performed similarly to WHC also using the method stated by [Miedzianka et al., 2014]. It was however only performed on four different soy protein combinations in replicates of three. Approximately 0.1 g of sample were collected in centrifuge tubes and vortexed with 10 ml of canola oil after which they were left standing for 30 minutes. The samples were then centrifuged at 3000 g for 25 min followed by removal of the supernatant. The weight of the oily pellet was recorded and the OHC is given by equation 3.2.

$$OHC = 100 \times \frac{oily\ pellet - initial\ material}{initial\ material} \tag{3.2}$$

Protein solubility

The protein solubility was determined on the extrudates according to the method stated by Chen in [Chen et al., 2011] with slight modifications. Four different buffers were used to extract the protein; 0.035 mol/L pH 7.6 phosphate buffer solution, 1.5 g/100 ml sodium dodecyl sulfate (SDS) in the phosphate buffer, 8 mol/L urea in the phosphate buffer solution and 0.1 mol/L dithiothreitol (DTT) in the phosphate buffer. 0.5 grams of protein sample was collected in a centrifuge tube with 10 ml of buffer solution and placed on a shaker for 2 hours. It was then centrifuged at 4000 g for 10 minutes after which the supernatant was collected to determine the protein concentration through a spectrophotometric method.

Spectrophotometry

The protein concentration in the supernatant obtained from the buffer treatment in section 3.4 was analyzed using the Bradford method [Bradford, 1976]. A standard standard series was prepared using bovine serum albumin (BSA) with the following concentrations 1.0, 0.8, 0.6, 0.4 and 0.2 mg/ml diluted in distilled water. 980 μL Bradford reagent and 20 μL of the standard/sample were mixed in a 1 mL cuvette, incubated for 5 min before the absorbance was measured at 595 nm by a spectrophotometer. By subtracting the absorbance for the blank from all samples a standard curve was created using the BSA absorbance with the known protein concentrations to determine the protein concentration in the analyzed samples.

SDS-PAGE

Protein was extracted using a phosphate buffer containing 8 mol/L urea, 1.5 g/100 ml SDS and 0.1 mol/L DTT according to the method stated by Chen in [Chen et al., 2011]. This was performed for three high moisture extruded samples; SPC, 75/25 and 25/75 two low moisture extruded samples 2126 and 2117 and non extruded SPI. Once the protein solutions had been on a shaker for 2 hours it was diluted 5 times in eppendorf tubes and heated in a water boiling bath for 5 min, followed by centrifugation at 10,000 rpm for 10 minutes. 10 μL of supernatant were loaded in individual lanes of the gel and electrically separated. After which the gels were stained with CBB and de-stained using water. The concentration of protein loaded in the lanes was unknown and most likely varies between lanes. This is important to keep in mind when interpreting the electrophoretic pattern.

Confocal microscopy

The confocal laser scanning microscope was used to analyze the extruded samples. Samples were stained with BODIPY and Texas Red in order to visualize lipids and proteins respectively. Staining highlights lipids as green and protein as red. Samples were sliced perpendicularly to the flow direction into approximately 3 mm thick pieces before staining. The analyzed samples were HCO 10%, HOCO 10% and 75/25 as a reference.

Statistical analysis

Microsoft™Excel in Office 365 was used to calculate arithmetic mean values, standard deviation and T-tests used for comparing if samples show significant difference.

4

Results and Discussion

The aim of the study was to produce a HME minced meat analogue containing vegetable oil or vegetable fat. Leading up to the addition of the lipids in the extrudate, other parameters of the HME process were evaluated. These included temperature, moisture and protein content. The trials and evaluation of these parameters are presented below. Recorded data of the three runs is presented in figures A.1−A.9.

4.1 High moisture extrusion

Temperature and moisture trials

The purpose of the first trials was to analyze how temperature and moisture content affected the structure and firmness of the extrudate. This was done by studying two temperature profiles with three moisture contents.

Decreasing moisture content while maintaining the same temperature profile led to an increase in melt temperature, pressure and main load drive, this holds true for both temperature profiles as presented in table 4.1.

Table 4.1 Averages for melt temperature, pressure and main load drive for six high moisture extrusion trials at two different temperature profiles and three different moisture contents.

Temperature profile [°C]	40/80/120/125/125/35			40/90/130/135/135/40		
Targeted moisture content %	70	65	60	70	65	60
Melt temperature [°C]	131.6	134.5	136.3	139.0	142.2	144.7
Pressure [bar]	4.96	6.59	10.93	5.43	6.55	11.35
Main load drive %	7.83	9.28	11.54	7.20	8.37	10.57

When comparing the two temperature profiles at the same moisture content it is possible to see that the melt temperature increased and the main load drive

decreased when the temperature was increased. Pressure was also greater at the higher temperature profile, except for when the moisture was at 65% were it was slightly lower.

It is possible to observe that the melt temperature which is recorded at the exit of zone 5 is higher than the temperature of any of the zones in the extruder. Which can be explained by the heat generated through mechanical energy and as a consequence of the shear forces in the melt. Decreasing moisture content results in a more dense melt, the higher viscosity of the melt will flow less freely and therefore results in an increase in pressure and temperature.

The combination of 70% moisture and 135 °C profile resulted in shout outs, were the pressure built up inside the extrudate as it exited the die and would cause it to burst.The WHC of SPC and other soy protein combinations were analyzed and is presented in section 4.3, the findings suggest that SPC can hold 9 times its weight in water previous to being extruded. This suggests that the WHC decreases when exposed to the combination of high temperature and moisture content causing the structure to burst. According to the WHC analysis represented in figure 4.2 it is possible to see that the WHC decreases with approximately 50% post extrusion. Several burnt sections were observed in some of the extrudate containing 65% and 60% moisture, which followed the 70% moisture run.

SPC and SPI trials

Using the information obtained from the first extrusion trial it was decided to perform the subsequent extrusions at 135°C with 65% and 70% moisture. The SPC/SPI ratios of 75/25 were run at 65%, 50/50 at 65% and 70% and 25/75 at 70% . It was hypothesized that as the SPI content increased the extrudate obtained would be more firm and potentially be able to retain more water without bursting. This proved partially correct as the obtained samples were more rubber-like compared to previous trials, however it was not possible to perform the extrusion with 70% moisture as it proved to be unstable causing several shoot outs. The following analysis is therefore based on the three runs with 65% moisture. Figure A.10 illustrates the composition of the attempted runs.

Protein concentration increased as a consequence of adding more SPI, causing the melt temperature and pressure to increase while the main load drive decreased as can be observed in table 4.2.

In regards to the texture and physical characteristics, Flier states similar observations in a patent regarding the extrusion process of plant proteins aimed at producing texturized meat-like materials. Flier used defatted soy flour with 35% - 70% protein content, extruded at 20%- 40% moisture content and observed that the

Table 4.2 Averages for melt temperature, pressure and main load drive for three HME trials at 135°C and 65% targeted moisture content with varying protein concentrations.

Temperature profile [°C]	40/90/130/135/135/40		
SPC/SPI	75/25	50/50	25/75
Targeted moisture content %	65	65	65
Melt temperature [°C]	141.7	143.2	143.2
Pressure [bar]	6.48	6.56	6.70
Main load drive %	8.82	8.49	8.38

lower protein content did not hold well during hydration while the product produced using higher protein content was described as "gummy" [Twombly, 2020].

As discussed further on in section 4.3 the WHC is greatest for SPC and decreases with increased amount of SPI. WHC can therefore be adjusted by for example changing the amount of SPI or adjusting the pH [Vatansever et al., 2020].

HOCO and HCO trials

The third extrusion was run using a SPC/SPI combination of 75/25 at 135°C and moisture content of 65%. It was decided to run with a slightly higher protein concentration to achieve more stable processing with a more firm structure as the addition of vegetable oil and fat has a plasticizing effect. The addition of lipids was performed by mixing it in to the protein powders previous to filling the feeding machine. The different trials with average melt temperatures, pressures and main load drives can be observed in table 4.3. Composition of the extrudates is illustrated in figure A.11.

Table 4.3 Averages for melt temperature, pressure and main load drive for six HME trials at 135°C, 75/25 SPC/SPI ratio at 65% targeted moisture content.

Temperature profile [°C]	40/90/130/135/135/40					
HOCO and HCO %	5 HOCO	10 HOCO	15 HOCO	5 HCO	10 HCO	15 HCO
Melt temperature [°C]	142.8	141.3	142.0	142.3	142.3	142.4
Pressure [bar]	5.23	7.70	6.86	5.68	5.58	6.77
Main load drive %	7.82	6.65	6.41	7.74	6.77	6.54

Compared to the previous runs the processing did not show any of the problems witnessed earlier, however as the concentration of lipids increased the feeding of the protein and lipid mixture was more troublesome as the flow properties of the powders changed causing bridging in the feeding port. As a consequence of the bridging there was lack of protein powder entering the extruder causing water to

pour out instead of extruded protein. This was manually controlled by pushing the protein in the feeding port to avoid bridging. This was evident when running the trials containing 10% HOCO and HCO. This is seen in ingredients that have high protein or lipid content, resulting in bridging or "rat-hole" formation in the feeder resulting in unstable feed rates [Alvarez, 2020]. This problem can be controlled by having a specific feeding port similar to how water is fed.

There was not a great difference between the melt temperature of the six different extrusions, a slight decrease can be observed when comparing 5% with 15% HOCO. This can be explained by the lubricating effect of the lipids which decrease the shear forces that otherwise lead to an increase in temperature. When comparing this to the previous trial at 65% moisture and 75/25 SPC/SPI presented in table 4.2. It is possible to see that the average temperatures for all trials is slightly higher when the extrudate contains lipids, which contradicts the idea of the lipids lubricating and preventing the temperature increase.

Similarly to the melt temperature there is no large difference in the average pressures of the trials, with the exception of the one containing 10% HOCO which is higher and also stood out when comparing melt temperatures. Curiously the pressure obtained in the previous trial that did not contain lipids falls between the pressures obtained with 10% and 15% lipids.

The main load drive is the set of values that shows a more clear relationship between lipid content and its effect. Comparing the set of HOCO and HCO it is possible to see a decrease in main load drive in both groups. However it is not possible to observe a great difference when comparing HOCO and HCO to each other. As opposed to the previously discussed parameters the trial 75/25 in figure 4.2 shows a higher value for the main load drive compared to the ones containing lipids.

The extrudates appearance changed as lipids were added in greater quantity, this will be discussed in more detail in section 4.5.

4.2 Texture analysis

Texture analysis was performed on all samples from the second and third extrusion trials. The results are presented as the maximum compression load (N/kg) indicating the hardness of the samples. Of the previously mentioned texture parameters in section 2.4, hardness was the most relevant to focus on because of the minced structure of the extrudate.

As predicted, the hardness of the extrudate decreases as the amount of lipids increase. When comparing the extrudates containing HOCO and HCO the hardness is higher for all samples containing the HCO, with the greatest difference for the

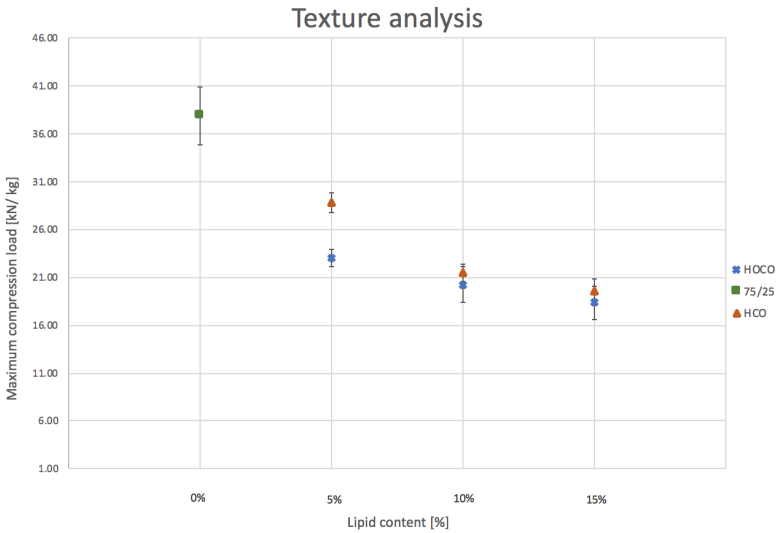

Figure 4.1 Average maximum compression load of seven shredded high moisture extrusion samples containing 0%, 5%, 10% and 15% fat or oil. 75/25 ■, HOCO ∗, HCO ▲

samples containing 5% lipids. T-tests were performed to determine whether the difference in hardness of the samples with 5%-15% HOCO or HCO, were significant. The results showed that there is significant difference for the sample containing 5% and 10% HCO and 5% and 15% HCO. There was only significant difference between the sample containing 5% and 15% HOCO.

T.tests comparing the results of HCO and HOCO revealed that the samples containing 5% oil or fat were the only ones with a significant maximum compression load difference.

Further texture analysis, including the other parameters mentioned in section 2.4 can be performed on the extrudate previous to shredding.

4.3 WHC and OHC

Investigating the WHC of soy protein as concentrate and isolate revealed that on average SPC has a higher WHC. SPC was able to retain approximately 9 times its mass in water while SPI was able to hold approximately 7 times its mass. Three combinations of SPC/SPI were also investigated both before and after HME (marked with E in the figure label), the average WHC are shown in figure 4.2.

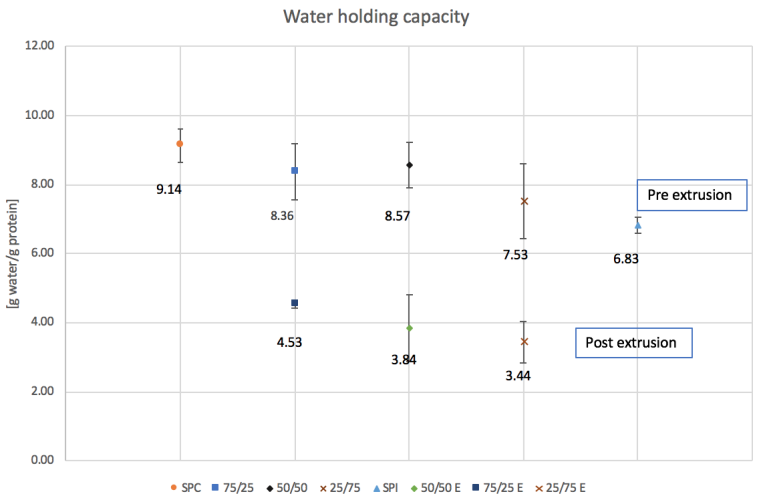

Figure 4.2 Average water holding capacity of soy protein with different protein combinations, before and after high moisture extrusion with error bars based on standard deviation. E represents the extruded sample

Statistically analyzing the data using t-tests revealed significant difference between the WHC of SPI and SPC as well as between 50/50 and SPI. In regards to the HME samples, t-tests revealed no significant difference. Observing the combinations of SPC/SPI it is possible to see that the WHC decreases as the amount of SPI increases, this holds true for both HME and non HME samples with the exception of the 50/50 sample. This deviation from the pattern is most likely due to experimental and human errors, as can be seen by the error bars the method used lacked precision. It should also be mentioned that the pellet formed after centrifugation was not solid as expected, this was the case for all samples and the analysis was not repeated due to lack of time.

The findings in a study using a soybean protein-gluten blend extruded with a co-rotating twin extruder report that the WHC after extrusion were between 3-4 times the initial weight [Wu et al., 2018]. WHC was analyzed on 30 different samples with a gluten content ranging from 20%- 32% and moisture from 40%-60%. In an other study using similar extrusion conditions and with proteins including SPI and wheat gluten the WHC after extrusion was 5.2 and 3.0 times the initial weight respectively [Samard and Ryu, 2019]. It is important to note that the WHC will be impacted by the extrusion process utilized, with this in mind and taking into account the potential experimental error in the results. The obtained results

are comparable by the ones obtained in the previously mentioned studies. WHC is sensitive to pH as well as certain additives, an aspect that can be used to modify texture [Vatansever et al., 2020].

Another comparison of the properties between SPC and SPI was the oil holding capacity, analyzed on non extruded protein samples. The four analyzed samples were; SPC, 75/25, 25/75 and SPI. On average they were able to hold 2.5-2.7 g of oil / g of protein as shown in figure 4.3. T-tests revealed no significant difference between the samples.

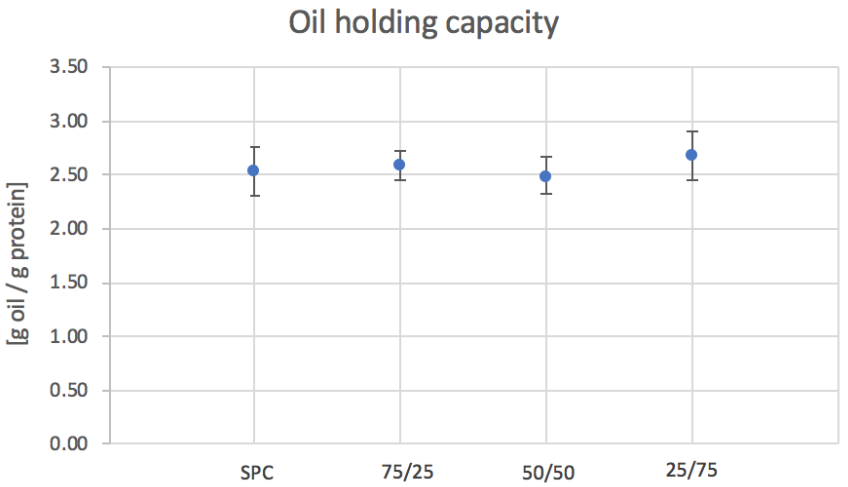

Figure 4.3 Average OHC of non extruded protein blends. Holding capacity measured in [g oil/g protein].

4.4 Protein determination

Using different buffer solutions the soy protein in the extrudate and raw material was analyzed to get a deeper understanding in regards to the changes within the bonds that occur during processing using high and low moisture extrusion.

SDS-PAGE Using SDS-PAGE it was possible to obtain a visual representation of the protein subunits within the extrudates and possible differences between how these are affected by different extrusion methods, as seen in figure 4.4.

Five different protein mixture were analyzed. SPI was used as a standard, representing the protein in the raw material, meaning the protein in this lane was not processed using extrusion. Lanes 2126 and 2117 in figure 4.4, represent soy protein used in commercially available minced meat analogues processed with LME. The remaining lanes; SPC, 75/25 and 25/75 were all processed were HME with the same moisture and temperature conditions.

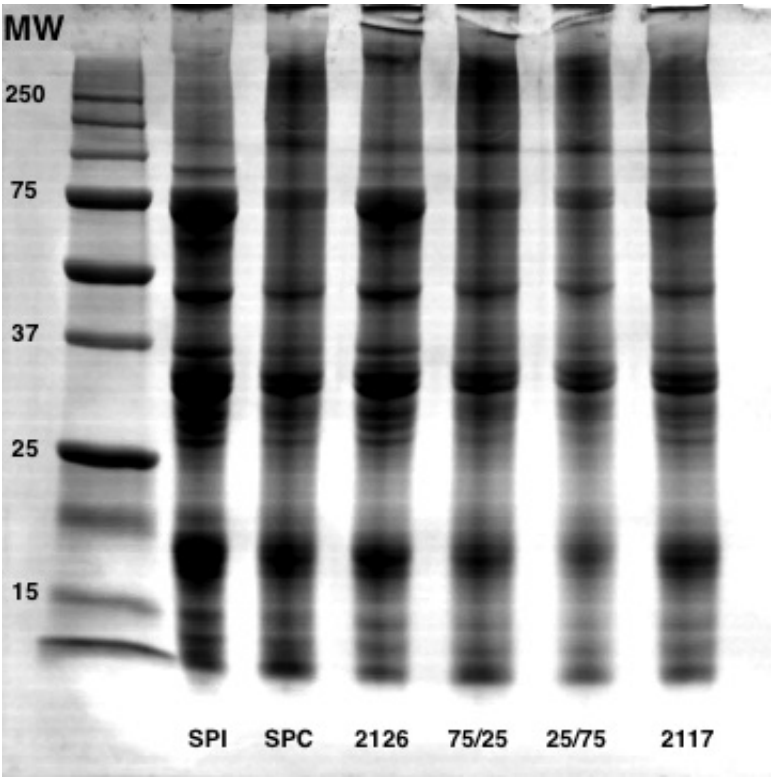

Figure 4.4 SDS-PAGE of five different extruded soy proteins where the first lane from the left indicates the markers in kDa. SPI is non extruded representing the raw material. SPC is extruded at 135°C and 65% moisture. Likewise 75/25 and 25/75 are extruded under the same conditions as SPC but with different SPC/SPI ratios. 2126 and 2117 are samples of purchased low moisture extruded soy protein.

All proteins were treated with the same buffer solution with the aim of disrupting hydrogen bonds, disulphide bonds and hydrophobic interactions. Comparing the bands from lanes SPI, 2126 and 2117 with the marker it is possible to see that the majority of the protein aggregates are approximately 75, 37 and 17 kDa. Which

is to be expected, as mentioned in section 2.2 the two globulins that make up most of the protein in soy are build up by 5 different subunits with size that match the location of the bands.

The high moisture extruded samples show lighter and more narrow bands around the 75 kDa, compared to the low moisture extruded samples there is also protein present above the 250 kDa band, suggesting large molecular weight protein-protein compounds with low electrophoretic mobility through the gel. Looking at the bottom of the lanes, all present a band with varying intensities, these are a sign of depolymerization of protein into smaller sub-units while undergoing LME or HME as seen in [Fang et al., 2014]. Compared to other studies the lane representing SPI in 4.4 is not consistent with other findings such as [Fang et al., 2014], [Chen et al., 2011],[Wu et al., 2018] were the smallest sub-unit is at 20 kDa. The remaining bands are consistent with previous findings.

The low and high moisture extrusion Chen performed used SPI with 28% and 60% moisture. In regards to the high moisture extrusion the closest available comparison is the lane containing 25/75 since it was the greatest amount of SPI that underwent HME. Comparing the two lanes there are resemblances such as a darker band present at approximately 20 and 35 kDa with some lighter bands at approximately 40 and 74 kDa. The main difference is that the SDS-PAGE by Chen reveals a darker band at the bottom of the lane, suggesting depolymerization of proteins. while figure 4.4 reveals a darker band above the 250 kDa marker.

There was no total protein determination performed on the extracted protein solutions once they had been treated with the buffers. Because of this it has been assumed that the lanes have been loaded with the same amount of protein. This is important to keep in mind since the thickness of the bands is a result of the amount of protein.

Overall the obtained results are not consistent with previous discoveries by [Chen et al., 2011] where the lane representing LME shows no distinct bands and the HME lane shows narrow faded bands. This could be attributed to differences such as Chen using SPI, 60% moisture and 80/110/150/135 and 80°C from the first to the fifth zone of the extruder barrel to the fifth as well as a screw rotating at 160 rpm.

Spectrophotometry Six different samples of extruded soy were treated with buffers and analyzed with a spectrophotometer according to the Bradford method. Two of the samples (2126 and 2117) were LME while (SPC, 75/25, 10% HOCO and 10 % HCO) were HME at 135 °C and 65% moisture.

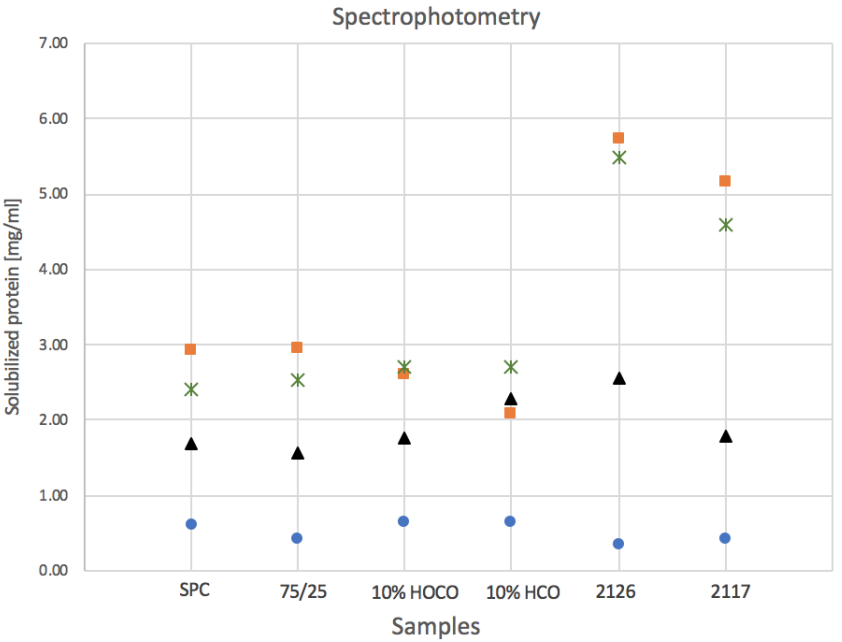

Figure 4.5 Protein solubility in different buffers for high and low moisture extruded soy protein. ■ (Urea), ∗ (SDS), ▲ (DTT), ◯ (Phosphate)

Observing the samples representing SPC and 75/25 in figure 4.5 it is possible to see how the buffers have had the same impact on the bonds in these two extrudates which is as expected. Since the only difference between the two samples is the protein concentration. 75/25 contains a combination of SPC/SPI and therefore has a higher protein concentration than pure SPC, they have otherwise undergone the same HME.

When observing the two low moisture extruded samples it is possible to see a different relationship in the protein solubility. Compared to all other samples, the urea and SDS buffers have solubilized a greater concentration of protein. As mentioned previously in section 2.4 urea and SDS will disrupt hydrogen bonds and inter-molecular hydrophobic interactions. It is therefore possible to assume that these forces and bonds play a more important role in the structure of texturized vegetable protein compared to the structure obtained through high moisture extrusion.

Lastly the two extrudates containing lipids in the form of oil and vegetable fat also demonstrate a slightly different relationship. Compared to the other four samples the SDS buffer has solubilized the greatest amount of protein. Non polar

substances such as fat molecules prefer to interact with each other by aggregating and minimizing their surface area that is in contact with water. This type of interaction is most likely present in the structures containing HOCO and HCO which explains the greater impact of the SDS-buffer on these extrudates. The buffer containing urea has had slightly less impact on these structures, possibly suggesting the hydrogen bonds have not been present to the same extent as in the other structures possibly due to interference caused by the oil and fat.

Comparing the results to the ones obtained by Chen in [Chen et al., 2011] it is possible to see that the buffers have had similar impacts on disrupting the protein structure obtained by low moisture extrusion. Comparing the buffers used, Chen has opted for using 2-Me to disrupt disulphide bonds. The structure obtained through high moisture extrusion also show a similar reaction to the buffers. With a smaller difference between the protein solubilized with SDS and 2-Me buffer in Chen's results compared to the ones presented in 4.5. A major difference to be kept in mind is that Chen has processed to examine the total amount of protein present in the sample and is able to present the solubility as a fraction of the total protein.

4.5 Confocal laser scanning microscopy

The structure of the extrudates containing lipids was visually analyzed using a confocal microscope and compared to the extrudate without added lipids. The aim was to identify the lipid interaction within the structure and potentially identify differences in the way fat interacted with the structure compared to how oil did. Images of the structure and surface are presented in figure 4.6. The bright red sections represent dense protein networks while the black and darker voids represent less dense protein networks. The green shades indicate presence of lipids.

Comparing the surfaces from left to right it is possible to see how 75/25 has a smooth surface with a slight color gradient, 10% HCO is also very smooth except for a mark in the corner which might be indicative of some slip caused by the increased lubrication. 10% HOCO has a distinct surface compared to the previous two, here the surface has several curved shaped slits in the direction of the flow, also an indication of increased lubrication.

When observing the images captured using the confocal microscope it is possible too see the green die, indicating presence of lipids present in all images. As presented in A.10 and A.11 the composition data indicates the amount of fat as 0.88%, 11% 11% respectively.

Comparing the images from left to right in row b), it is possible to see how

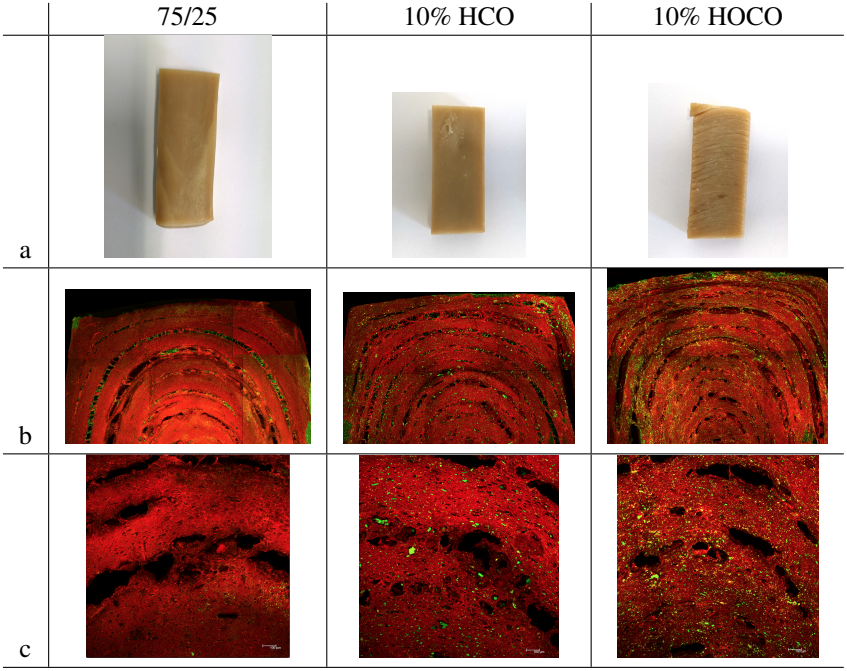

Figure 4.6 Extrudates with and without added lipids. (a) illustrates the surfaces of the extrudates. (b) and (c) are confocal images magnified 5 and 10 times respectively. The red represents protein, green represents lipids.

the presence of lipids is greater in the voids. In the second image the lipids are dispersed throughout the whole sample with some greater particles in the voids. The third image has a lot or resemblance to the previous ones when comparing the location of the lipids. There is however a difference in the shade of the lipid indicative die, compared to the previous were the die is bright green the die in this image has several shades of yellow and green. This may be an indication of how the oil has interacted differently and may be mixed into the protein in a different way compared to the other two.

The same color differences can be observed in the images at 10 times the magnification. In regards to the appearance of the magnified structure, all three show a mix of voids and more dense protein networks. It would require a greater sample of images to fairly compare the appearance of the structure.

4.6 Limitations

Some of the aspects of the performed analysis could be improved such as the WHC. Firstly the WHC was analyzed for five different samples which of three were analyzed before and after extrusion, this could be improved by analyzing all five samples before and after extrusion. Gathering more data would give more comprehensive results to determine a pattern on the effect of HME on WHC. A larger sample group would be suggested in order increase the confidence level. The obtained pellet after centrifugation did not have the dense appearance as expected due to lack of time this analysis could not be repeated.

The protein determination analysis could be improved by having a method to determine the total protein concentration in the samples.

Increasing the number of replicates would also improve the structure analysis performed with confocal laser scanning microscopy.

5

Conclusion

The aim of this project was to investigate the possibility of producing a minced HMMA when adding oil and vegetable fat to the HME of soy protein. While analyzing the influence of lipids on the physicochemical properties of the extrudate. The main conclusion is that it is possible to produce HMMA with up to 10% added vegetable fat or oil. At 15% the flow properties complicate the feeding, creating an unreliability in the process. Equipment that permits feeding of fat or oil through a separate port is therefore crucial in order to avoid bridging or rat-hole formation of the protein powder.

By analyzing the texture, more specifically hardness it was evident that, increasing the amount of fat or oil in the extrudate decreased the hardness. The difference in hardness was significant when compared to the sample without added lipids. However, there was no significant difference between the hardness of the extrudates containing 10% HCO or HOCO.

Another important aspect for texture and mouthfeel is the WHC and OHC. SPC had a higher WHC compared to SPI, the combination of SPC and SPI had less WHC compared to SPC. OHC however did not differ between SPC and SPI.

The structure was analyzed using spectrophotometry, confocal laser scanning microscopy and SDS-PAGE. Comparing the spectrophotometric results of the different HME and LME products revealed how the hydrogen-bonds were not as prevalent in the structures containing 10% lipids compared to the other extrudates. SDS-PAGE was used to compared the subunits found in LME and HME using SPI as a reference and did not reveal major differences as seen in previous studies.

Confocal microscopy revealed the location of the lipids within the extruded structure. Showing resemblance in the location of the lipids between 75/25 and 10% HCO. The images of 10% HOCO differ slightly from the other two because of the shade of the lipid indicator die. Suggesting oil differs in its interaction with the structure compared to fat. The sample size is not sufficiently large to suggest

possible impact of the added lipids on the structure, the surface of 10% HOCO however shows the consequence of added lubrication.

6

Future work

An important aspect to investigate further is the feeding of fat or oil into the extruder using a specific feeding port designed for this purpose. In this project the fat or oil were mixed with the protein and the mixture was then fed into the extruder. As the lipid content increases, the flow properties of the protein powder mixture changes. Resulting in increased risk of bridging causing uneven feed rates, in extreme conditions feeding may stop [Alvarez, 2020].

An other possibility is to further investigate the shredding process, either using the same equipment and changing the size of the blades or using other techniques to produces a minced meat analogues.

This project did not include any method of preparation such as frying or heating in the oven. It is therefore suggested to perform such analysis to determine how this affects the product. Impacting WHC, OHC, color and overall appearance of the extrudate. Further on it is important to perform a sensory evaluation.

Furthermore, when considering costs of raw materials using SPI will increase costs. It is therefore of interest to investigate how an extrudate produced using only SPC would compare to the ones investigated in this study.

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A

Appendix

A.1 Temperature and moisture extrusion data

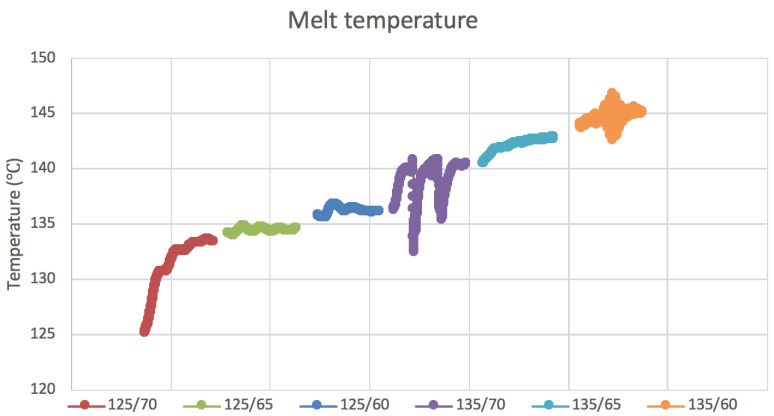

Figure A.1 Recorded melt temperature data for temperature and moisture trials. Label indicates temperature/moisture of the six different runs.

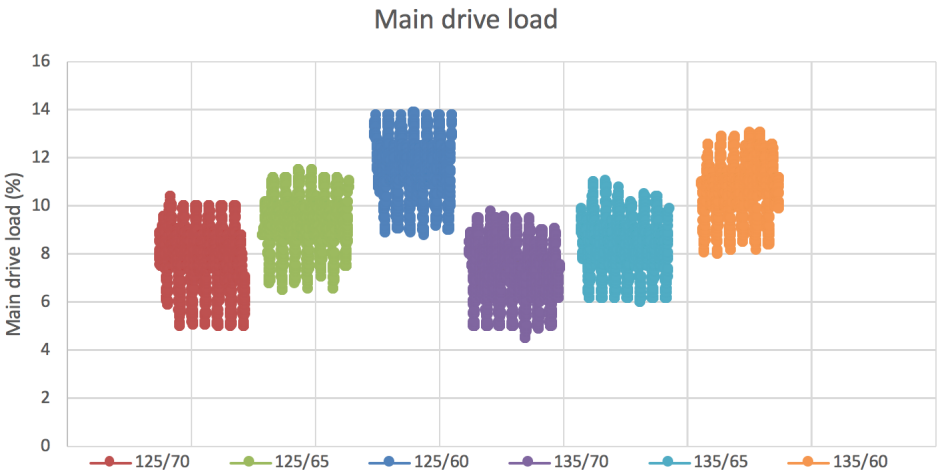

Figure A.2 Main drive load data for temperature and moisture trials. Label indicates temperature/moisture of the six different runs.

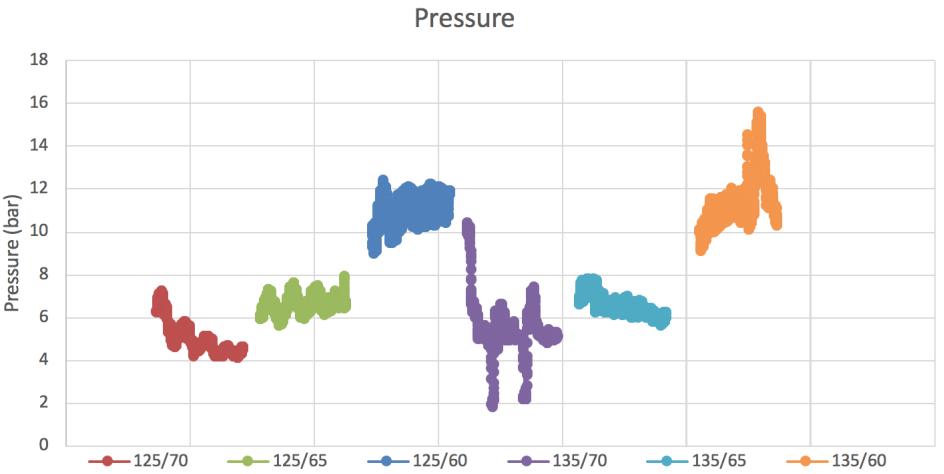

Figure A.3 Recorded pressure data for temperature and moisture trials. Label indicates temperature/moisture of the six different runs.

A.2 SPC/SPI extrusion data

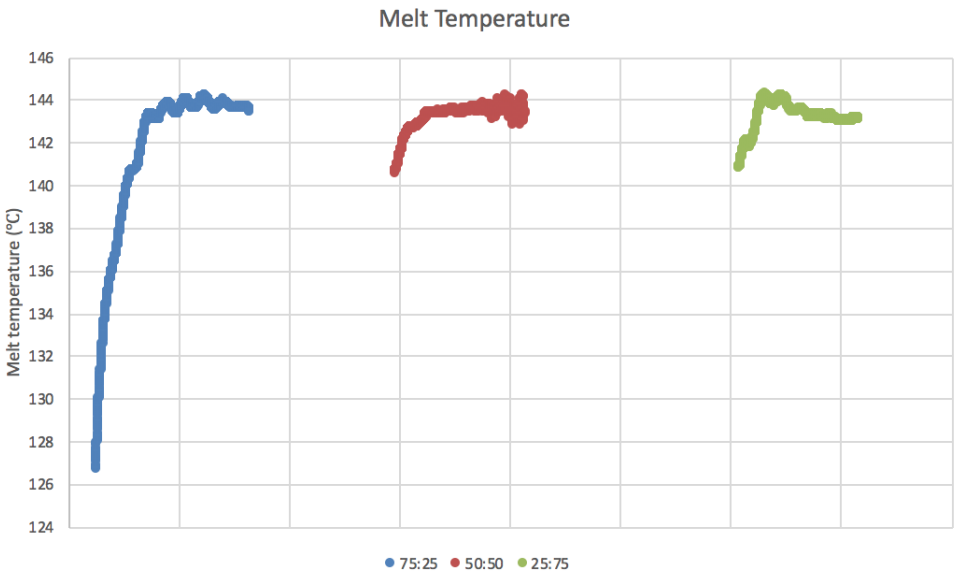

Figure A.4 Recorded melt temperature for SPC/SPI trials. Label indicates amount of SPC/SPI in the three different runs.

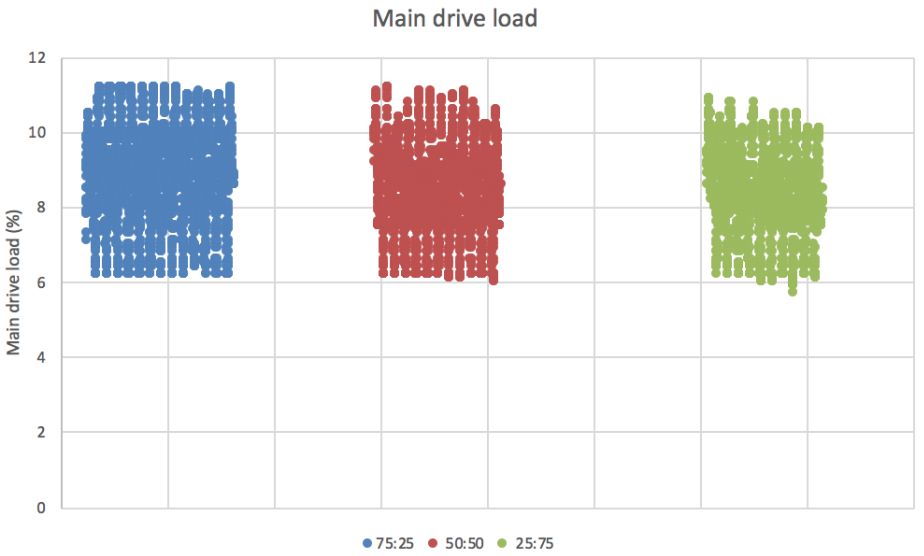

Figure A.5 Recorded main drive load for SPC/SPI trials. Label indicates amount of SPC/SPI in the three different runs.

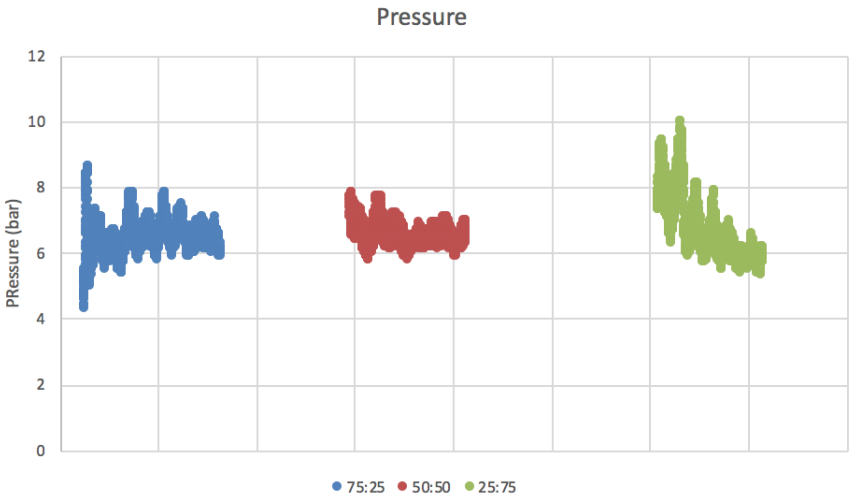

Figure A.6 Recorded pressure for SPC/SPI trials. Label indicates amount of SPC/SPI in the three different runs.

A.3 HOCO and HCO data

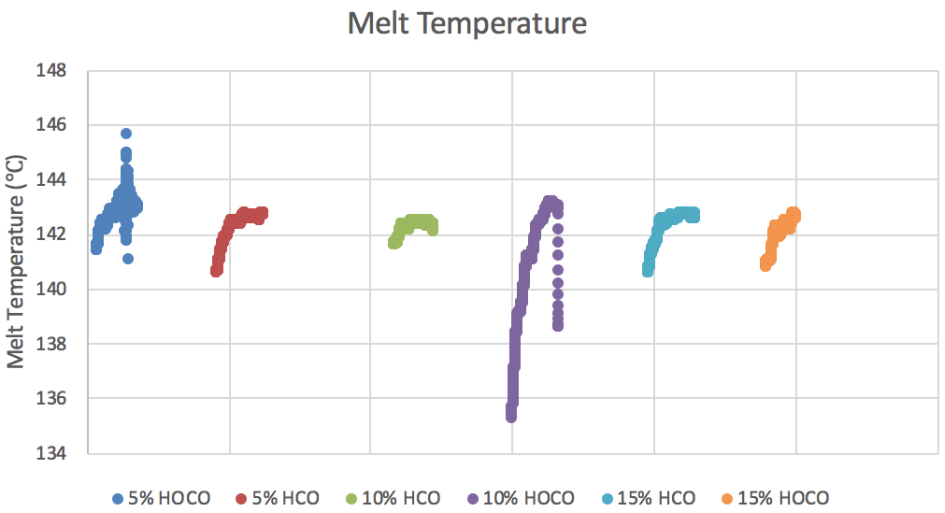

Figure A.7 Recorded melt temperature data for HCO and HOCO trials. Label indicates type and % of fat added.

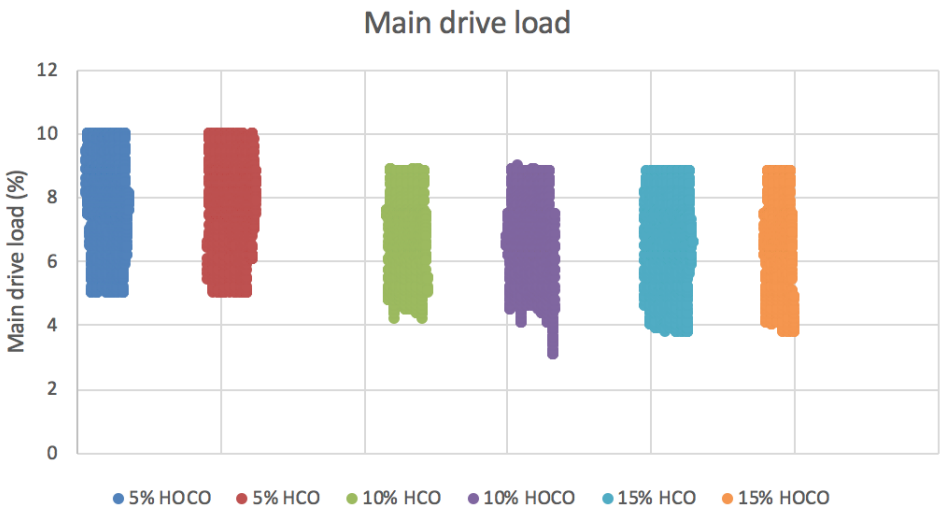

Figure A.8 Recorded main drive load data for HCO and HOCO trials. Label indicates type and % of fat added.

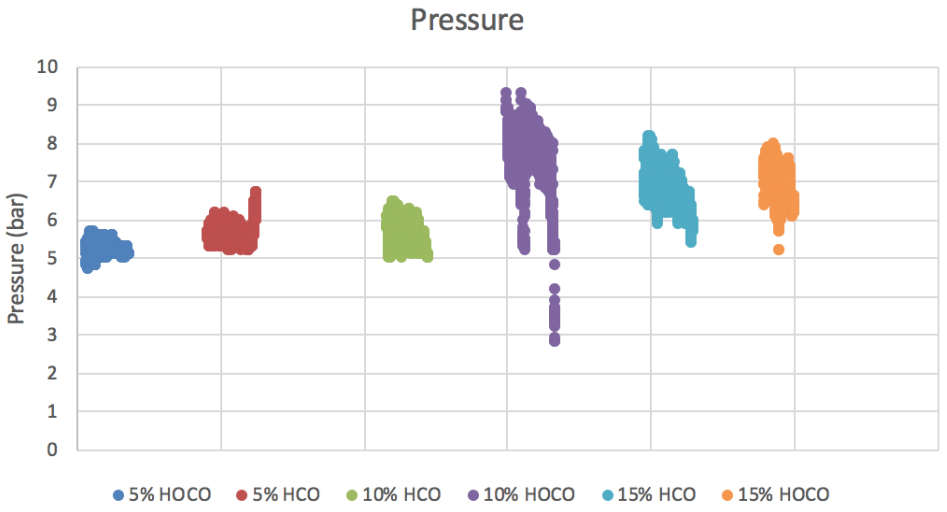

Figure A.9 Recorded pressure data for HCO and HOCO trials. Label indicates type and % of fat added.

A.4 Extrudate composition

The composition has been calculated theoretically using the suppliers specification sheets.

Extrudates	Moisture max %	Protein %	Ash %	Fat %	Dietary fiber %
25/75	66.81	29.18	1.75	0.52	1.74
50/50	66.98	27.08	1.75	0.70	3.49
50/50*	71.69	23.22	1.50	0.60	3.00
75/25	67.16	24.96	1.75	0.88	5.25
75/25*	71.85	21.39	1.50	0.75	4.50

Figure A.10 Composition of HME produced in trial 2 with varying SPC/SPI content at 135°C and 65% moisture. * denote the composition of the HME that were not successful due to unstable product formation.

Extrudates	Moisture max %	Protein %	Ash %	Fat %	Dietary fiber %
Extrudate 75/25 Moisture 65 + 5% HOCO	63.06	23.43	1.64	6.93	4.93
Extrudate 75/25 Moisture 65 + 10% HOCO	60.29	22.41	1.57	11.01	4.72
Extrudate 75/25 Moisture 65 + 15% HOCO	57.76	21.47	1.51	14.74	4.52
Extrudate 75/25 Moisture 65 + 5% HCO	63.06	23.43	1.64	6.93	4.93
Extrudate 75/25 Moisture 65 + 10% HCO	60.29	22.41	1.57	11.01	4.72
Extrudate 75/25 Moisture 65 + 15% HCO	57.76	21.47	1.51	14.74	4.52

Figure A.11 Composition of HME produced in trial 3 with varying HOCO or HCO content with 75/25 SPC/SPI at 135°C, 70% and 65% moisture.