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Abstract

The main aim for this thesis was to develop products with defined texture and viscosity properties for elderly people and dysphagia patients at Stavanger Universitets Sykehus (SUS). Two products were developed: 1) a texture modified cod product and 2) a protein enriched fish soup, both stored frozen (-30 °C) to obtain longer shelf life. The products were enriched with the dairy proteins whey protein concentrate (WPC) and caseinate, and with fish protein hydrolysate (FPH).

The products were characterised in accordance with texture levels in the International Dysphagia Diet Standardisation Initiative (IDDSI). The texture modified products (TMP) produced were between level 5-"minced and moist" and level 6-"soft and bite size". Texture analysis showed that TMP with a higher total protein content was significantly firmer than non-enriched or less enriched products. Also, the IDDSI fork pressure test of the texture modified products revealed that they got firmer with increasing protein content and with cooling during 15- and 30 min standstill, from eating temperature at 55 °C. Rheology measurements of TMP showed that there could be a correlation between protein content, temperature and storage modulus. High-pressure processing of TMP was tested to see if combination of pressure and chilled storage (4 °C) could give prolonged shelf life. It showed that bacteria survived at 600 MPa for 10 min. The numbers where <1000 bacteria/g, and no growth was observed when the products were stored for 35 days at 4 °C. This indicate that HPP products could have an acceptable safety for 5 weeks at chilled storage. The texture analyses of HPP showed that the firmness changed significantly with different high-pressure treatments and different protein content. Colour analysis showed that the protein enrichment in TMP gave a more yellow product.

The non-enriched soup belonged to IDDSI level 1 at 55 °C, while the low- and high-enriched soup both were at level 3 at 55 °C. The viscosity was not significantly different at 25 °C and 55 °C for the non-enriched soup and measurements showed increased viscosity with increasing protein enrichment. Rheology measurements of the soup showed that at 55 °C, the yield stress increased with increasing protein content and the most viscous soup was the high-enriched at 25 °C. Colour analysis showed that a higher protein enrichment yielded a significantly less light-colored soup product, but not increased yellowness as in TMP.

Digestion of proteins in INFOGEST models showed that protein enrichment gave more protein available for absorption in the intestine for both adults and elderly. Protein enriched products had approximately the same digestibility as non-enriched products. The protein digestibility was higher in the soup than the TMP and the raw material cod fillet. All products showed slightly reduced protein digestibility in the elderly model, mainly due to lower amounts of soluble protein in the intestinal phase.

The pilot products developed in this work showed that it was possible to enrich foods using sustainable fish protein hydrolysate, in addition to dairy proteins. The non-enriched TMP contained 17.4 % protein, the low-enriched contained 18.8 % protein, and the high-enriched contained 20.2 % protein. The soups from pilot production were enriched up to 8 % of protein. The non-enriched soup contained 4 %, and the low-enriched 6 % protein.

Acknowledgement

I would like to express my deepest appreciation to my supervisors at Nofima Jan Thomas Rosnes and Aase Vorre Skuland for their outstanding aid and guidance during my master thesis. They both spent many hours correcting my writing of the thesis.

I would like to extend my sincere thanks to other employees at Nofima for guiding me through different methods. Stian Gjerstad Iversen aided me in the making of texture modified products and the enriched soup, as well as providing a base recipe to further develop. Izumi Sone provided me with knowledge about rheological measurements and the operation of the rheometer. Sigurd Øines educated me about the texture analyser and provided a deeper understanding of texture measurements. I am also grateful to Laila, Tone Mari, Leena and Karin for always being available with their expertise.

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Abbreviations

ANOVA	Analysis of variance
CIE	Commission Internationale de l´Eclairage
DRI	Dietary reference intake
FPH	Fish protein hydrolysates
HPP	High-pressure processing
IDDSI	International Dysphagia Diet Standardisation Initiative
LVR	Linear viscoelastic region
PCA	Plate count agar
PN	Personalised nutrition
RPM	Revolutions per minute
SEC	Size exclusion chromatography
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SSF	Simulated salivary fluid
SUS	Stavanger Universitets Sykehus / Stavanger University Hospital
TMP	Texture modified product
TSA YE	Trypticase soy agar with yeast extract
WPC	Whey protein concentrate
WPI	Whey protein isolate

Table of content

1 Introduction

Personalised nutrition has great potential to improve the health of people. The field has yet to be clearly delineated and a consensus definition of the term "personalised nutrition" (PN) has not been developed. In general, the term PN is based on the understanding that one size does not fit all due to e.g. difference in biochemistry, metabolism, genetics, and microbiota (Bush et al., 2020). Products tailored to the tastes and preferences of specific consumer groups, such as protein bars for athletes have been available for a long time. Personalised nutrition takes that a step further, with developing products and compounds that are tailored to the needs of specific groups such as people with chronic diseases, elderly or athletes within a specific sport (Reinders et al., 2020).

In Nofima, personalised food is so far defined as food with properties that are adapted to special needs of different consumer groups, such as protein enrichment of food for sports nutrition and the elderly. It differs from personalised nutrition in that it is not based on gene expression in the individual but looks at the different needs of groups. There are some groups, e.g., the elderly people, that will increase remarkedly in the next decade both in Norway and worldwide, and the industry has shown an increasing interest for a growing market of personalised food products. During the last 5-10 years several projects regarding personalised foods and nutrition have been initiated in Norway (e.g., Matlyst) and in EU (e.g., Performance, The personalised nutrition project, Eit food Quisoer project, Food4me).

To efficiently market these products, it is necessary to first learn more about the preferences, needs and resources of the heterogenous groups of elderly consumers. To accommodate the markets requirements, technological solutions for product composition and characteristics must be developed, together with packaging and logistics systems. The technological solutions required for the retail market may differ remarkedly from the official health care system and may lead to completely different individual technological solutions. There are few products in the commercial market that are adapted to the groups of elderly and dysphagia patients. Nofima has for several years worked to increase the competence in texture modified products through the internal project called "VårMat". Pilot products have been developed with fish raw materials, with the focus on different protein enrichments, texture adaptions and processing, packaging and storage systems.

The main aim for this thesis was to develop products for dysphagia patients at SUS (Stavanger Universitets Sykehus). A request was directed from the hospital kitchen (through the project "Måltidsglede"), to develop a texture modified product and a soup with defined texture levels. Elderly and people who have suffered a stroke or those undergone treatments such as neck and head surgeries may suffer from swallowing difficulty, called dysphagia, and such patients require special foods and drinks that are easier to ingest and swallow (Sungsinchai et al., 2019).

In order for the food industry, foodservice facilities and caregivers to provide adequate nourishment and food safe for swallowing, some quality control benchmarks are needed. The International Dysphagia Diet Standardisation Initiative (IDDSI) is proposing such a framework and testing methods, that makes it possible to described food used in nutritional care plans to prevent dysphagia (Côté et al., 2020). This framework is in the process of being implemented worldwide and it is important that the research communities now follow up with analyses and documentation that confirm the justified use of the framework.

Protein enrichment and texture modification are key factors in products for elderly and dysphagia patients. However, to achieve successful products for use in different applications, it is necessary to specify the amounts and types of proteins that can be used, which processing methods that will work and how this affects the digestion in the intestine of the user group. Therefore, this work contains examination of new combinations including high-pressure processing, use of fish hydrolysates and digestion in an *in vitro* intestine model.

The global demand for protein is expected to double by the year 2050 (Aspevik et al., 2017). Using sustainable protein sources may therefore be necessary in the years to come, and alternative protein sources or better utilisation of existing resources are therefore important. Globally, in the fish processing industries it is estimated that up to 60 % of the harvested biomass is discarded every year (Siddik et al., 2020). One way to increase utilization of these by-products is by e.g., extracting proteins and further hydrolyse them into smaller peptides, referred to as fish protein hydrolysates (FPH) (Aspevik et al., 2017). These sustainable proteins can subsequently be used as an enrichment in texture modified foods and drinks.

The effect on the human body of protein enrichment in food, the type of protein used, and the process the proteins are subjected to before eaten, is still not well understood. Different *in vitro* models have been used to document digestibility of proteins, and the INFOGEST model have been developed as a promising model for this purpose (Brodkorb et al., 2019; Minekus et al., 2014). In cooperation with Nofima Ås, texture modified products and soups were investigated for protein digestion in an adult and elderly model. An overview of the work is shown in Figure 1.1.

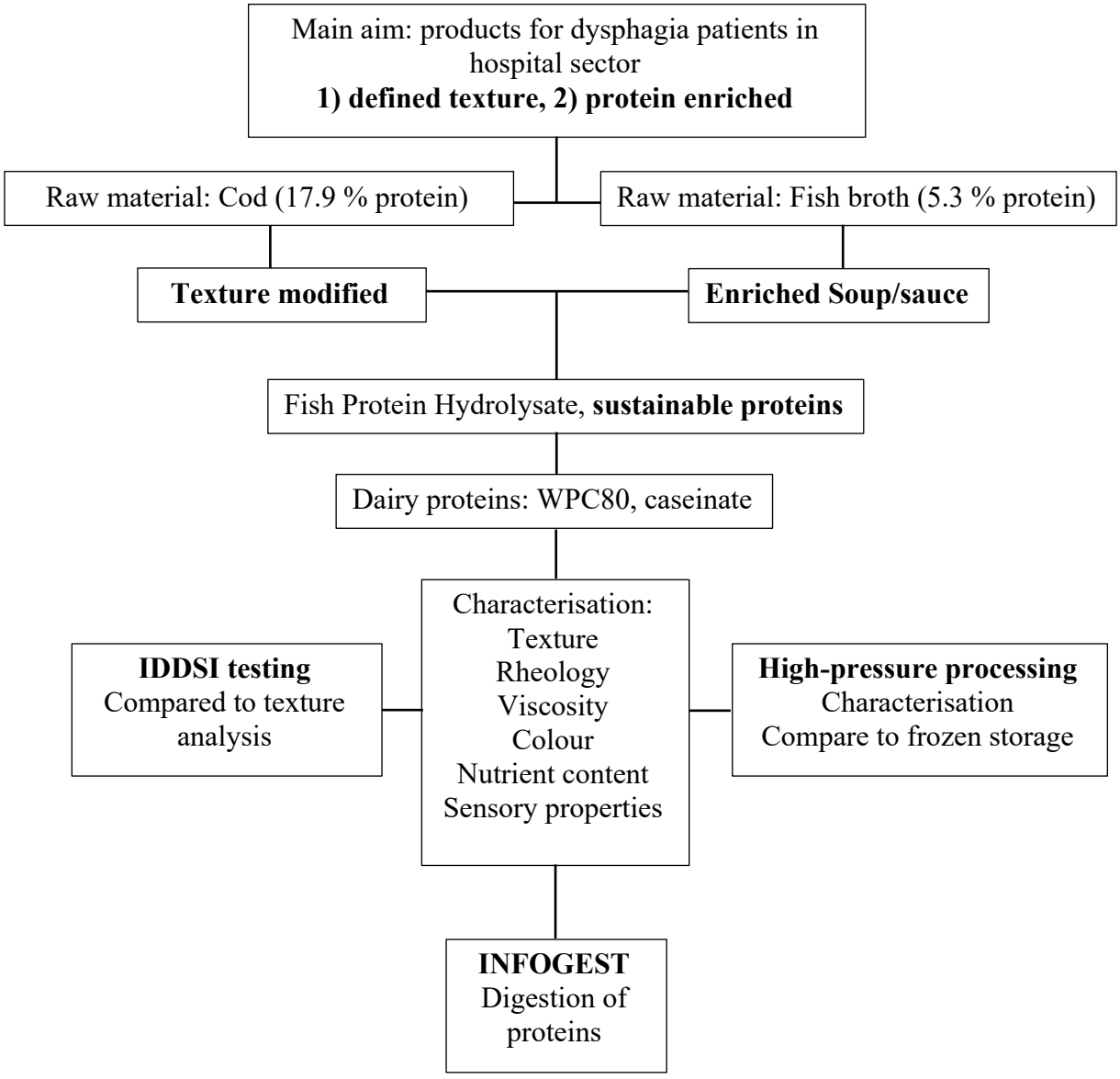

Figure 1.1 Overview of the different parts in the work. The text with bold font in the figure, are the main focal points.

2 Theory

2.1 Personalised nutrition versus personalised food

Personalised nutrition (PN) is developed from the concept that one size does not fit all (Bush et al., 2020). The objective of personalised nutrition is to improve dietary habits of people, which contributes to improve the overall public health by prevention or treatment of chronic diseases (Biesiekierski et al., 2019). While personalised nutrition can be defined as developing specific nutritional recommendations for each individual; precision nutrition takes it a step further by giving advices based on gene expression in the individual as well as environmental and lifestyle factors (Betts & Gonzalez, 2016). However, it is important to take into account that personalised nutrition is very specific, and it is more common with PN products targeted towards bigger groups such as elderly or people with dysphagia.

Personalised food can be defined as food with properties that are adapted to special needs of different consumer groups, such the elderly or athletes within a specific sport (Ueland et al., 2020). Functional foods is a term used to describe processed foods that could provide an additional function by adding new ingredients or more of existing ingredients (Granato et al., 2020). Bioactive compounds are considered the source of functional food effectiveness. Bioactive compounds include phenolic compounds, lipids, proteins and peptides and carbohydrates (Martirosyan & Singh, 2015). Addition of these bioactive compounds to food targeted towards elderly are expected to decrease the risk of diet-related disorders and diseases such as obesity, type 2 diabetes, hypertension, and some types of cancer (Jędrusek-Golińska et al., 2020). The essence of personalised nutrition is to "assist individuals in achieving a lasting dietary behaviour change that is beneficial for health" (Eggersdorfer et al., 2016). Personalised nutrition can therefore be beneficial to everyone.

2.1.1 Protein consumption and the elderly

The health of elderly adults is greatly influence by proper nutrition. Insufficient nutrition can contribute to the development of both sarcopenia and obesity. Protein has been identified as key nutrient for elderly in several studies. Protein intake greater than recommended for healthy adults < 50 years, could improve muscle health, prevent sarcopenia, help maintain energy balance and cardiovascular function in elderly (Baum et al., 2016). The recommended amount of protein intake may vary from country to country. The World Health Organization (WHO) recommend healthy adults to consume at least 0.8 g protein/kg bodyweight per day (Joint

Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition et al., 2007). The Norwegian Directorate of Health (Helsedirektoratet) recommend healthy adults consume 1 g protein/kg bodyweight (Norge & Helsedirektoratet, 2016). For elderly <65 Helsedirektoratet recommends an intake of 1-1.5 g protein/kg bodyweight per day, which equals 15-20 % of the total energy intake.

Insufficient protein intake to satisfy the daily requirements leads to negative protein balance and results in skeletal degradation and impaired muscle growth. Elderly eat slower, are less hungry and consume smaller portions compared to adults (Deer & Volpi, 2018). Undernutrition affects over 44 % of hospitalized older people, who often dislike oral nutritional supplements (Mills et al., 2018). Therefore, might an increase of nutritional content of food, such as adding more protein, help elderly reach their daily recommended amounts, even if they eat small portions.

2.1.2 Protein, fat and salt recommendations

Salt

The body needs approximately 1.5 g salt per day for optimal function, but it is not recommended to heat more than half a teaspoon or 5 g per day. According to Helsedirektoratet, the population eat around 10 g of salt each day which equals a topped teaspoon. Salt (NaCl) consists of chloride (Cl) and sodium (Na), and it is the latter that has the most documented negative effects on health. Over 30 % of the Norwegian population has high blood pressure (Helsedirektoratet, 2017), and cardiovascular diseases are the most common cause of death. High intake of salt is proven to increase the risk of high blood pressure, which in turn can lead to cardiovascular diseases. As people age there is also and increased salt sensitivity, meaning that that salt has a greater effect on blood pressure (AlGhatrif et al., 2017). Elderly should therefore be cautious about the amount of salt consumed, even if they do not have high blood pressure.

Fat

Fat is an important part of a healthy diet, not only providing energy but also essential fatty acids, fat-soluble vitamins and phospholipids (Ueland et al., 2020). Fat is known to be the most concentrated energy source in foods. Helsedirektoratet recommends that fat should contribute with 25-40 % of the total energy intake (Helsedirektoratet, 2016a). Many nutrients are deficient in the diets of elderly, while other nutrients, such as saturated fats, are consumed in excess,

contributing to overnutrition and the risk of chronic diseases and obesity. Protein intake of elderly is often increased at the expense of other macronutrients (Volpi et al., 2004). A product targeted towards elderly should therefore contain energy as well as proteins. The macronutrient fat is a great source of energy in such a product.

Protein

Protein recommendations that are based on minimum protein intake in order to maintain nitrogen balance do not take any physiological outcome relevant to healthy aging, such as muscle function into account. Dietary reference intakes (DRI) for macronutrients are therefore included in the recommendations for protein intake (Baum et al., 2016). Helsedirektoratet recommend that proteins make up 10-20 % of the energy intake for adults. Protein intake for elderly (people over 65) should be between 15-20 % of total energy intake, this is equal to 1-1.5 g protein/kg bodyweight per day (Helsedirektoratet, 2016b).

2.1.3 Protein digestion and absorption

A key factor responsible for the age-related decline in skeletal muscle mass is thought to be the reduced muscle protein synthetic response in elderly. Since the basal (fasting) muscle protein synthesis rates do not seem to considerably differ between adults and elderly, mainly the muscle protein synthetic response toward food intake and physical activity is researched (Koopman et al., 2009).

Digestion is the breakdown of food into smaller components that can be easily absorbed into the bloodstream. The digestive process includes degradation of proteins and larger peptides into smaller peptides and amino acids. Normally only di- and tripeptides, and free amino acids can be actively absorbed by the intestinal epithelial cells for transportation into the bloodstream. Ageing is associated with changes in gut functions that may influence food digestion. There is less secretion of gastric fluid causing higher pH and reduced pepsin levels in the stomach, as well as lowered bile and reduced levels of pancreatic enzymes in the intestine (Rémond et al., 2015; Shani-Levi et al., 2017). This may lead to reduced digestibility and uptake of proteins which again may cause loss of muscle mass and strength. Therefore, elderly people are recommended to increase their protein intake. Protein quality differ based on their amino acid content, digestibility and bioavailability and there is a need to understand how various protein sources are digested in the elderly gastrointestinal tract (Gibson, 2007; Hiolle et al., 2020).

It is possible to measure the absorption of protein using different models. One model is called INFOGEST, which is a static *in vitro* simulation of gastrointestinal food digestion. Static *in vitro* digestion models have been shown to be very useful in predicting outcomes of *in vivo* digestion (Brodkorb et al., 2019). To enable quantification and comparison of protein digestibility in different foods the INFOGEST model needs to be combined with adequate analytical methods. Size exclusion chromatography (SEC) with UV-detection is a well-recognized analytical tool for measuring molecular weight distributions of protein digests (Wubshet et al., 2017). and has been applied to estimate the proportion of peptides with specific size ranges generated during simulated digestion by partial area integration (Le Roux et al., 2020). In addition to that, SEC can provide an overall qualitative fingerprint of a given digest in the form of chromatograms (Rieder et al., 2021).

2.2 Anatomy and physiology of normal and abnormal swallowing

The ability to swallow food and fluid is essential to the process of eating, but also one's quality of life. Swallowing is an important useful ability to enable adequate nutrition and hydration, but also part of the enjoyment of eating. The swallowing process is highly complex and involves muscles in the mouth, pharynx, larynx, and esophagus (Sasegbon & Hamdy, 2017). Any impairment to the process of swallowing can have a negative effect on quality of life.

2.2.1 Phases of swallowing

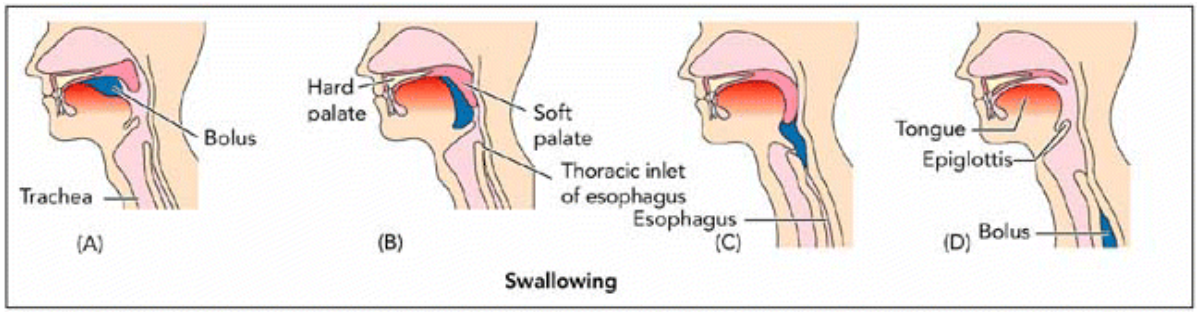

Figure 2.1 The swallowing process is a dynamic process which can be described into four phases, starting by making bolus in the mouth to moving the bolus down the esophagus. Copied from (Wysong, 2007).

Swallowing is a complicated sequence of both voluntary and reflex movements, which needs several areas of your brain to coordinate the more than 30 muscles and nerves involved (Matsuo & Palmer, 2008). Swallowing is an important part of food digestion, and also prevention of food and/or other materials into the lower respiratory tract (Nishino, 2013).

Usually, people will swallow between 600-1000 times per day, and it happens automatically without thinking about it. The brain consists of twelve nerves, and five of them are involved in the swallowing process which can be divided into different phases (Solberg Mathisen, 2016). The swallowing process is commonly divided in the oral, pharyngeal, and esophageal stages according to the location of the bolus, but the oral stage was later subdivided into oral preparatory and oral propulsive stages. While solid and liquids share common pharyngeal and esophageal phases, they differ slightly in the processing and transport of boluses during the oral phase (Matsuo & Palmer, 2008).

The oral phase is a voluntary process where the tongue, palate, lips, teeth and cheeks are used together with saliva to process the food and forming a bolus which is suitable for swallowing. In the oral preparatory phase hunger and the smell of food also increase the saliva production preparing for food intake. For liquids, the bolus is first sealed in the oral cavity by the tongue anteriorly, and hard palate posteriorly. For solids, the bolus is not sealed in the oral cavity as it undergoes processing via mastication (Figure 2.1 A) (Panara et al., 2021). In the oral propulsive phase, the tip of the tongue elevates to move the bolus into the oral cavity (Matsuo & Palmer, 2008). The third, pharyngeal phase is rapid sequential movement, occurring within a second. During this phase the soft palate closes the nasopharynx at about the same time that the bolus comes into the pharynx and the muscles in the pharynx push the food further down. The pharyngeal phase serves to protect the airway. The vocal cords close off the laryngeal opening, and the epiglottis moves to cover the trachea and lastly the esophagus opens (Figure 2.1 B and C) (Matsuo & Palmer, 2008; Solberg Mathisen, 2016). The last and fourth phase is called the esophageal phase and is a reflex movement. The bolus of food moves further down the esophagus by peristatic contractions all the way to the stomach. Once the food or drink have passed the epiglottis it goes back to its original position and covers the esophagus while the trachea opens, so that it is possible to breathe again (Figure 2.1 D).

Difficulty with swallowing can occur in different parts of the swallowing process. Impaired sensibility and muscle weakness in face, lips and tongue can affect the oral phase and lead to a

poorly formed bolus and food gathering (hoarding) in the mouth. Coughing, mushy/hoarse voice, longer meals, weight loss, dehydration and pneumonia are some of the symptoms associated with dysphagia (Solberg Mathisen, 2016).

2.2.2 Dysphagia

Dysphagia or swallowing difficulty is most commonly experienced by elderly, people who have suffered a stroke or those undergone treatments such as neck and head surgeries. The main problems deriving from dysphagia are aspiration pneumonia, malnutrition and dehydration. Dysphagia patients require special foods and drinks that are easier to ingest and swallow (Matsuo & Palmer, 2008; Sungsinchai et al., 2019).

An understanding of the physiology of swallowing is important in helping the clinicians determine the cause of dysphagia (Panara et al., 2021). Dysphagia can be classified as oropharyngeal and esophageal dysphagia. Oropharyngeal dysphagia can cause bolus remaining in the oral cavity or food aspiration. Common symptoms lead by this are difficulty initiation swallowing, coughing, choking, and gagging during swallowing. The main symptoms of esophageal dysphagia are sensation of food sticking in the chest and symptom of gastroesophageal reflux disease such as heartburn and belching. This is caused by the retention of food and liquid in the esophagus after swallowing. (Sungsinchai et al., 2019).

There are several ways to manage with dysphagia, such as feeding tubes, swallowing therapy and texture modified foods. For individual who require long-term dysphagia treatment texture modification of foods and fluids is the most commonly used method. It has been reported that thicker liquids increase duration of swallowing, thus allowing an adapted reflex response time while swallowing (Hadde & Chen, 2019).

2.2.3 Presbyphagia versus dysphagia

Presbyphagia refers to gradual changes in the swallowing mechanism of healthy older adults that result from the normal aging process (McCoy & Desai, 2018). An older adult´s swallow can be increasingly more challenging without it being impaired. Changes that may impact swallowing with ageing include missing teeth making it difficult to chew, changes related to the muscles and tissue in the body and sensory changes (Matsuo & Palmer, 2008). Dysphagia is different from presbyphagia in that it does not only affect older adults. The group of people

having dysphagia is often cognitively impaired due to dementia, trauma, disease, surgery or other additional diagnoses (Okkels, 2018).

2.3 Essential ingredients in texture modified product

A food product consists of several ingredients that give rise to different properties, such as gelling, thickening abilities and nutritional content. The main ingredient used for the texture modified product in this thesis is fish fillet (cod) which offers great nutrients and a high protein content (17.9 %). In addition, fish protein hydrolysate and dairy proteins may be used to enrich products. One commonly used group of texture modifiers is hydrocolloids. The type of hydrocolloid used in this thesis is a modified corn starch. The chapters below will go more in depth about some of the ingredients that were used in this thesis (See 2.3.1-2.3.5).

2.3.1 Atlantic cod (*Gadus morhua)*

Atlantic cod *(Gadus morhua)* is a saltwater fish of the family *Gadidae.* Cod is only present in the northern Atlantic Sea between Vizcaya in south and Novaja Zemlja and Spitsbergen in the north, by Iceland, South-Greenland, Newfoundland and U.S. east coast to Cape Hatteras in North Carolina. The *Gadus morhua* mainly resides in water with temperatures between 0 and 10 °C, but some seem to thrive in significant higher temperatures (Vøllestad, 2020). Up to 96 % of the calories in a portion of cod come from protein. Cod is an excellent source of protein, providing all of the essential amino acids the human body needs. The main nutritional content of the Atlantic cod is protein with 17.9 g/100 g. Cod also has high energy content with 343 kJ/81 kcal. It has low salt content with only 0.1 g/100 g. Cod is also known to be a lean fish, with 1.1 g of fat/100 g (*Matvaretabellen*, n.d.).

2.3.2 Fish protein hydrolysates

The global demand for protein is expected to double by the year 2050 (Aspevik et al., 2017). Fish and also meat products are important sources of protein in the human diet and contain essential minerals, vitamins and amino acids. When processing fish and meat on an industrial level, the main goal is to process the main products, such as fillets, trimmings and mince. However, these processes generate huge amounts of protein-rich residual raw material. About 40-60 % of the total weight of animals and fishes are classified as residuals, such as heads, bones and skin (Aspevik et al., 2017).

Globally, in the fish processing industries it is estimated that up to 60 % of the harvested biomass is discarded every year (Siddik et al., 2020). These large quantities of processing by-products are commonly converted into low-value products such as animal feed and fertilizer. There is significant potential to utilize these protein-rich waste materials by converting them into more valuable, bioavailable nutritional food products such as fish protein hydrolysate (FPH) (Siddik et al., 2020). It has been reported that FPH has excellent physicochemical properties including increased solubility, emulsifying properties, foaming properties, water-holding capacity and fat binding capacity, which in turn increase feed palatability and simplify the biological nutrient uptake. Also, peptides derived from FPH have shown various physiological benefits including antioxidant, antihypertensive (blood pressure lowering), antimicrobial, immunomodulatory and anticancer activities when consumed *in vivo*. FPH can be produced in either liquid or powdered (dried) form (Siddik et al., 2020). Fish protein hydrolysates can be manufactured from the decomposition of fish proteins from by-products into simple peptides (2-20 amino acids) through hydrolysis by adding enzymes, acids, or bases. The quality and characteristics of FPH are highly influenced by several factors, such as type of proteases or chemicals used, temperature, pH, and duration of hydrolysis (Prihanto, 2019). The liquid FPH is a watering mixture of hydrolysed proteins, which contains up to 90 % moisture. The FPH in liquid form is highly unstable for a long-term storage. Thus, dried FPH is preferable due to a longer shelf-life, easier storage and transportation. FPH has a huge potential use as a protein source for human consumption, but the step of dehydration demands a big energy supply and can be very costly (Petrova et al. 2018). In this thesis a powder hydrolysate made from salmon backbone was used. It was preferred to use a powder FPH when making the texture modified products, to ensure a homogenized product.

Fish protein hydrolysates are highly nutritious and sustainable, but there are limitations. The sensory properties of hydrolysates are important as taste-neutral products are highly desired. Bitter taste of fish protein hydrolysates has been associated with the formation of small peptides containing hydrophobic amino acids during hydrolysis (Steinsholm, 2021). In order to increase the use of these sustainable hydrolysates, the reduction of bitter taste intensity is much studied. In a study by Steinsholm 2021 NMR spectroscopy, a predictive tool that measures sensory descriptors of foods, was used to determine which chemical substances produce flavour. There was found to be a link between metabolites and flavour. The study concluded that it was possible to remove the smallest metabolites via nanofiltration. Bitterness, however, increased as small peptides associated with bitter taste were rejected by the membrane. The intensity of

bitterness is produced by small peptides and depends on the choice of enzyme and the degree of hydrolysis (Steinsholm, 2021).

2.3.3 Dairy proteins

Milk proteins or dairy proteins have many functionalities, mainly due to their molecular structures and interactions with other food components. Textural, rheological and sensory properties of food products could be altered with dairy proteins. Because of their high nutritional quality and versatile properties, dairy proteins are widely used in many foods such as, desserts, nutritional beverages, ice cream, yoghurt, meat products and baked goods. Key functions of dairy proteins include emulsification, thickening, gelling and foaming (Andiç & Boran, 2015).

Dairy protein is mainly divided into two types of protein: casein and whey protein. Casein is the most abundant representing 80 % of dairy proteins and whey represent the remaining 20 %. Caseins are a group of phosphoproteins that precipitate at pH 4.6 and temperature 20 °C. At these conditions' whey protein remain soluble. All the amino acids that are essential to humans are present in casein in high amounts, with the exception of cysteine. Casein is therefore considered a highly nutritious protein. In milk, casein exists in complex groups of molecules called micelles. The micelles consist of calcium, inorganic phosphate and citrate ions in addition to the casein molecules (Petrotos et.al 2014).

Whey protein is a collection of globular proteins with a high level of α-helix structure and the acidic-basic and hydrophobic-hydrophilic amino acids are distributed in a balanced form.
The most important whey proteins are beta-lactoglobulin, alfa-lactalbumin, serum albumin, immunoglobulin, lactoferrin and protease-peptones. Whey proteins have significant levels of secondary, tertiary and quaternary structures, and are heat-labile stabilizing their protein structure through intermolecular disulphide bonds (Davoodi et al., 2016).

Whey protein and casein are both known to be high quality proteins because of their digestibity and high content of essential amino acids (McGregor & Poppitt, 2013). Two most common types of whey protein are whey protein concentrate (WPC) and whey protein isolate (WPI). WPC is produced by separation techniques such as precipitation, filtrations and dialysis. In this thesis WPC with a concentration of 80 % was used.

2.3.4 Hydrocolloids

Hydrocolloids are a heterogenous group of long chain polymers. A large number of hydroxyl (-OH) groups in starches markedly increases their affinity for binding water molecules. Hydrocolloids are known to have a wide array of functional properties in food. These include thickening, gelling, emulsifying, stabilization. Starch is the most commonly used hydrocolloid thickener, the reason being its cheap, abundant and does not impart any noticeable taste at low concentrations (Saha & Bhattacharya, 2010). The denaturation process has an important role in the making of texture modified products using hydrocolloids. Food hydrocolloid work as a filler and interact with denatured protein, inducing crosslinking and intra-protein reactions. Which in turn leads to better gel formations in the product (Ramírez de León et al., 2011). Figure 2.2 shows a possible interaction between the hydrocolloids and proteins.

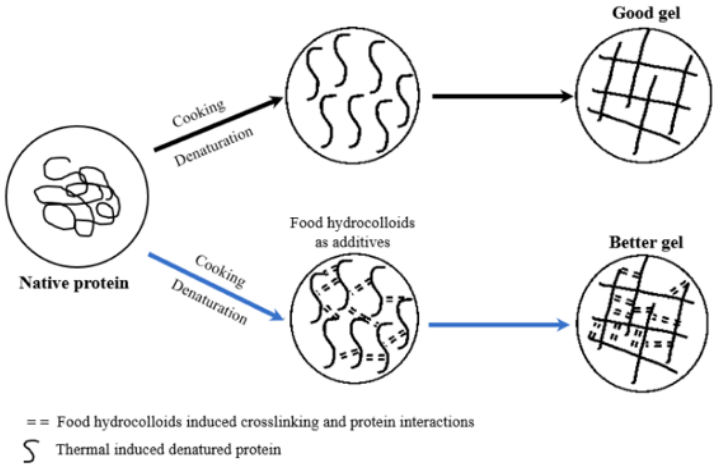

Figure 2.2 Possible interaction between proteins and food hydrocolloids. Adaption from (Prabhu, 2018; Ramírez de León et al., 2011).

2.3.5 Modified corn-starch

Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primary by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin). Each glucose unit possess a maximum of three hydroxyls that can undergo chemical substitution (FAO, WHO, 2016). Starch is an important ingredient for the food industries, whereas starches with specific properties are necessary to add functionality desirable attributes to foods (Yousif et al., 2012). Starch is used both in the native and modified form. Starch is not dissolved in cold water, but when heat is applied it allows for the granules to swell up, thus, thicken occurs (Saha & Bhattacharya, 2010). Native starches provide viscous, cohesive and sticky pastes when they are

heated and form gels when these pastes cool off. Starch modification can be done by physical or chemical methods. Physical modifications are made using heat and moisture, while chemical treatments involves the introduction of functional groups into the starch molecule using reactions of derivatization (etherification, esterification and crosslinking) or decomposition (hydrolysis and oxidation) (Yousif et al., 2012).

The modified corn starch used in this thesis is Farinex™ WM 55 (Appendix A), which is an acetylated distarch adipate of waxy maize origin. Acetylated distarch adipate is a modified starch that is obtained by esterification of food starch with acetic anhydride and esterification/cross-linking with adipic anhydride (FAO, WHO, 2016). Chemical modification as acetylation allows better functional properties such as high solubility, water absorption, swelling power and lower gelatinization temperature than native starches, which have wide applications mainly in the food industry (Han et al., 2005). Acetylated distarch adipate can be used as a thickener, stabilizer and binder in food (Tian et al., 2018).

2.4 Food safety

The texture modified products (TMP) produced in this thesis used fish as raw material. Fish and fish products are often associated with human disease, especially when raw or undercooked fish are consumed. Fish are known to harbour various bacterial species. Due to constant exposure to contaminated water, bacterial colonisation can often be observed on fish skin and gills. Along with human non-pathogenic bacteria and natural microflora of aquatic environments, pathogenic bacteria are also widely found in fish. *L. monocytogenes, Vibrio spp., Salmonella, Yersinia spp., and C. botulinum* are pathogenic bacteria of special interest due to high mortality rates in humans through diseases such as listeriosis and botulism, but also the abundant distribution in aquatic environments (Novoslavskij et al., 2016).

To increase the shelf life of fish products it is possible to include processing steps e.g., freezing or heat treatment. Processing by high-pressure processing HPP is a relatively new technology in the seafood industry worldwide (Singh et al., 2018), and not used for retail seafood products in Norway (Personal communication: Tone Mari Rode). The use of HPP to extend the shelf life of chilled products and reduce the number of pathogens in fish makes it advantageous. The processing is milder than traditional heat processing, causing higher nutritional retention and better sensory properties of the products. Mainly the proteins, lipids and enzymes in fish are

affected by HPP (Singh et al., 2018). Primary structure of large molecules is minimally affected by pressure. Hydrogen bond formation is stabilised by HPP, along with the breaking of ions, as this leads to a decrease in volume. HPP modifies secondary, tertiary and quaternary structure of proteins. Alongside its high efficiency in prevention of fish spoilage, HPP does not affect vitamins and flavour compounds, allowing preservation of nutritional value and sensory appeal (Rode & Rotabakk, 2021; Singh et al., 2018)

2.5 IDDSI - International Dysphagia Diet Standardisation Initiative

The International Dysphagia Diet Standardisation Initiative (IDDSI) framework provides a common terminology to describe food textures and drink thickness. A main objective is to improve the lives of over 590 million people worldwide suffering with dysphagia. The IDDSI framework consists of 8 levels (0-7), where drinks are measured from levels 0-4, while foods are measured from levels 3-7 (Figure 2.3). IDDSI tests are used to confirm the flow or textural characteristics of a particular product. Testing should be done on food and drinks as it appears at the time of service. This means reheated to an eating temperature of the food at approximately 60 °C. To mimic the current eating situation in a hospital ward, IDDSI suggest that subsequent measurements should be taken fifteen and thirty min after serving (elapsed time for eating) as the texture will change during chilling (*IDDSI - IDDSI Framework*, n.d.)**.** The IDDSI framework uses simple tools to define consistency levels. The utensils are normally found in the kitchen system and consist of fork test, spoon test, and the time it takes for liquid to flow through a 10 ml plastic syringe. It is important to note that in order to obtain correct measurements for the different texture levels, the instructions from IDDSI must be followed strictly, e.g., with respect to temperatures and times used.

As an assisting tool, IDDSI has created a form that can be followed. Here, time aspects are also taken into account, such as i) immediately at service, ii) 15 min after service and iii) 30 min after service. In this way on can get a picture of how texture and viscosity change with standing over time in room temperature with a probable temperature drop.

The figure below (Figure 2.3) shows the separation into classes for food (left) and liquids (right), respectively. The IDDSI standard as proposed is based mainly on the existing international texture standards. The work in the professional group started with collecting standards from different countries and then merging these to a common framework on how to

define the individual texture levels needed. Table 2.1 shows an example from this work, where viscosity measurements in liquid is defined into IDDSI levels 1-4.

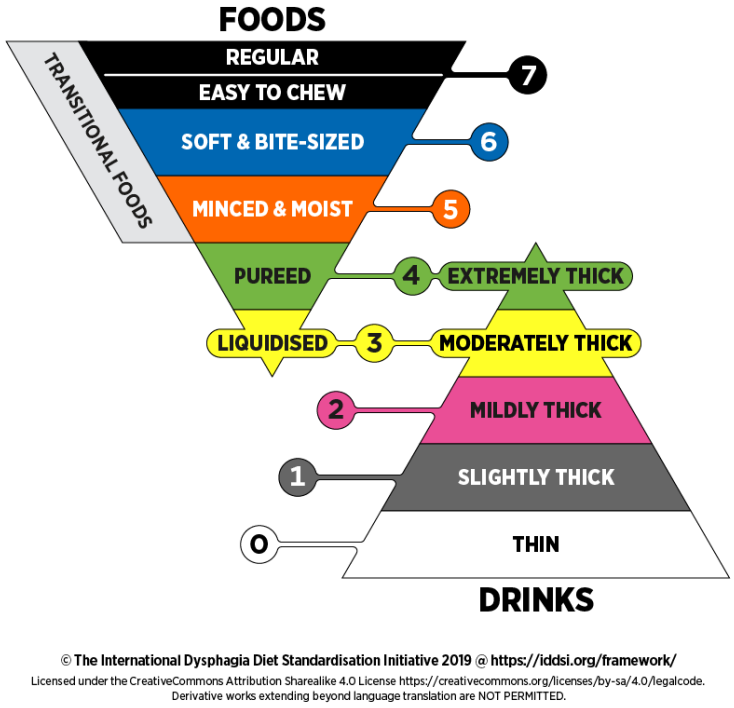

Figure 2.3 IDDSI levels with specific numbers from 0-7 combined with colours. Foods have the triangle top downwards, and drinks have the triangle top upwards. The figure is copied from (*IDDSI - IDDSI Framework*, n.d.)

Table 2.1 Corresponding IDDSI drink levels to measured viscosities. 1 centipoise (cP) is equal 1 mPa.s (Cichero et al., 2013).

Level 1 – slightly thick	Level 2 – mildly thick	Level 3 – moderately thick	Level 4 – extremely thick
50-150 mPa.s	150-300 mPa.s	300-500 mPa.s	>500 mPa.s

The IDDSI framework offers international terminology which will help choose the right food regardless of tradition and culture. It mainly helps people suffering from dysphagia and clinicians and food serving staff in facilities and hospitals. To classify the food or drink into the IDDSI framework, the products must pass certain tests. The tests are simple, fast and demand little equipment. Some of the tests include the flow test, or the syringe test, fork drip test, spoon tilt test and fork pressure test (Cichero et al., 2017). In this thesis only the fork pressure test and flow test were carried out since they were thought to be the most suitable for the products made. The IDDSI tests are described in more detail in chapters 3.2.1 and 3.3.3.

Although the food and liquid description for each level of IDDSI (hardness, moisture, stickiness, flow behaviour etc.) is described qualitatively, it can still be difficult and subjective to categorise the food for patient and care givers. Ensuring that thickened fluids have suitable rheological properties is an important part of dysphagia management to secure safe swallowing. Too thin fluids could potentially lead to pneumonia, while too thick fluids may become a choking risk due to residue getting inhaled (Hadde & Chen, 2019).

2.6 Methods to analyse properties of texture modified products

Texture modified foods refers to foods with soft textures and/or reduced particle size as well as thickened liquids. Texture modification of food is applied for consumer groups with specific needs, ranging from babies to people suffering from injuries and elderly (Ueland et al., 2020). A texture analyser determines the firm or soft texture of a food. While both a rheometer and a viscometer can measure viscosity, a viscometer can be used for tests that require simple flow measurements of Newtonian materials (where viscosity depends on shear rate), however the performance of a rheometer allows far greater characterization of flow and deformation of a material (for Newtonian and non-Newtonian materials) (Sariyerli et al., 2018). Methods to analyse different properties of texture modified products, such as firmness, viscosity or colour are described in more detail in the chapters below.

2.6.1 Texture analysis of food

Textural properties are related to the deformation and the flow of food under force. The study of texture is a branch of rheology. Rheology measures both viscosity and texture. Viscosity can be defined as the internal friction of a fluid or its tendency to resist flow. The difference between viscosity and texture is simple; viscosity relates to fluid foods or foods that flow, while texture relates to solid or semisolid foods. Texture instruments such as the TA-XT Plus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) may be used to measure firmness, springiness, adhesiveness and more in different types of food (Giese, 2003). The texture analyser determines the firm or soft texture via a puncture test, were a probe penetrates the surface of a product with a given distance under constant load (Bourne, 2002).

2.6.2 Rheology measurements

Rheology is the study of the deformation and flow of matter. As mentioned, a commonly used term in rheology is viscosity; its resistance of flow due to internal friction caused by interactions between molecules in a fluid, semifluid or solid. Food is usually a solid or a semifluid but may behave as a fluid when sufficient stress is applied. Stress is defined as the forcer per unit area. The result of applied shear stress can be shear thinning or shear thickening. Shear thinning behaviour is when the viscosity decreases with increasing shear rate and is also referred to non-Newtonian behaviour. The viscosity of a Newtonian fluid will not be affected by shear stress (Bourne, 2002; Janmey & Schliwa, 2008). When it comes to rheological measurements, temperature is an important parameter. The rheological parameters might be affected by the temperature, e.g., the viscosity of a calibration oil changes approx. 7 % when there is a temperature increase of just 1 °C (*Rheological Measurements*, n.d.).

In published studies of texture modified products, shear viscosity is the most often-reported measure. However, it has been found that two liquids with equal apparent viscosity at one shear rate can have very different viscosity at other shear rates. This can cause liquids with the same reported viscosity to behave differently when ingested, e.g cause swallowing difficulties, and is therefore important clinically. The process of swallowing involve a range of shear rates, and unlikely to be purely shear as in a rheometer (Hanson et al., 2019).

After viscosity, yield stress is a commonly measured parameter. The yield stress or yield point is the lowest shear stress value that needed to break down the sample's structure, and make it flow. When at rest, the interacting forces between particles form a stable, three-dimensional network. After the yield point has been exceeded, the structure breaks down and the material might start to flow (Sun & Gunasekaran, 2009; Varchanis et al., 2020). Therefore, could a higher yield stress prevent a material to undergo phase separation, sedimentation or aggregation. Yield stress is usually determined by fitting the stress/rate curve with Bingham, Casson or Herschel-Bulkley models.

2.6.3 Colour measurements

The surface of foods may be glossy, diffuse, irregular, porous or flat. They may be transparent, hazy, translucent or opaque and their colours may be uniform, patchy or multi-layered (Macdougall, 2010). Colour is an important quality attribute in the food industries. The surface

colour of food is part of the first impression and can be used as a tool to either accept or reject food (Pathare et al., 2013).

Colour can be measured by CIELAB, a visually uniform colour space (Macdougall, 2010). The CIELAB colour space or CIE L* a* b* colour system represents quantitative relationship of colours on three axes: L* is represented on a vertical axis with values from 0 (black) to 100 (white). The a* value indicates red-green components of a colour, where þa* (positive) and Àa* (negative) indicated red and green values. The yellow and blue components are represented on the b* axis as þb* (positive) and Àb* (negative) values. The centre of the plane is neutral or achromatic. The distance from the central axis represents the chroma (C), or saturation of the colour (Ly et al., 2020). The CIE L* a* b* colour system is shown in Figure 2.4.

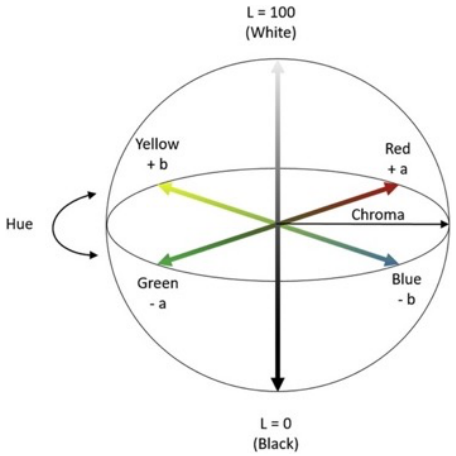

Figure 2.4 The CIE L* a* b* colour system. (Ly et al., 2020)

3 Materials and methods

3.1 Workflow Texture modified products (TMP)

The workflow diagram in Figure 3.1 shows the steps used to produce the protein enriched texture modified products (TMP). A list of ingredients used for the products is found in Appendix B. The aim was to develop texture modified products that were suitable for elderly and people with swallowing difficulties. A high protein content was desired, and an aim was to exceed the normal protein content of the fish raw material while maintaining a soft product. The products were enriched using whey protein hydrolysate (WPC), sodium caseinate (caseinate) and fish protein hydrolysate (FPH). Analysis of colour, texture, rheology and IDDSI testing were used to describe the characteristics of the products.

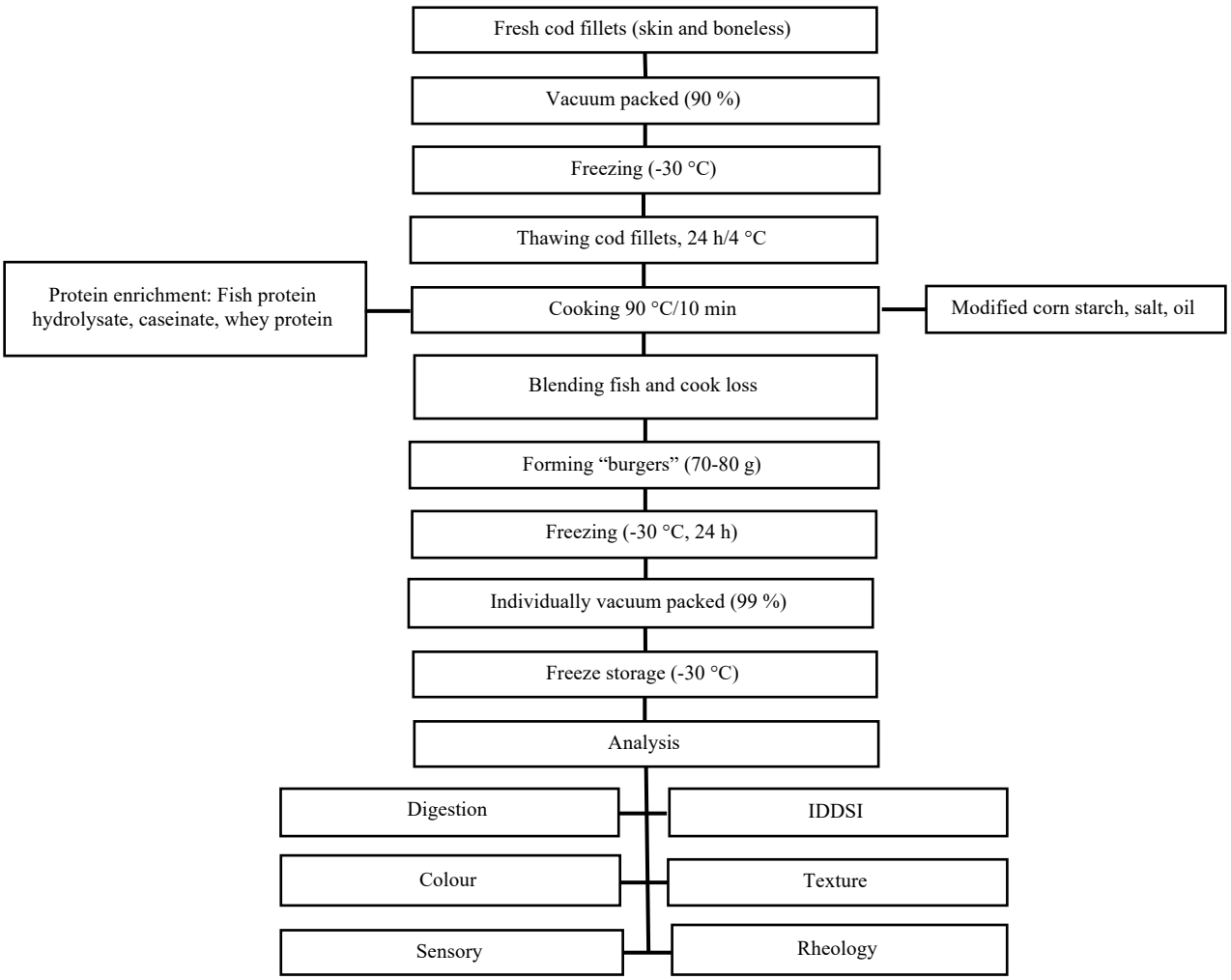

Figure 3.1 Workflow of the production and analysis of texture modified products.

The work in this thesis was done in two steps: Preliminary and pilot production. In the preliminary step, different recipes of texture modified products were developed. Analyses used to describe the functional properties of the different recipes were texture analysis using a TA.XT Plus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) and colour measurements using a DigiEye system (VeriVide Ltd., UK), in addition to sensory evaluations performed by a semi-trained panel (4 assessors). Based on the conclusions from the preliminary step, three recipes continued into the pilot step and testing. The recipes were chosen based on the desired attributes, such as high protein content, high energy content and low salt content (See 2.1.2). The product also had to be soft (IDDSI level 5 or 6) in order to be suitable for elderly and dysphagia patients (See 2.5). Pilot testing included analyses, such as rheology, IDDSI, digestion and sensory evaluation in addition to the analyses done in the preliminary step.

3.1.1 Preparation of fish

Commercially fresh skin and boneless wild caught cod fillets (caught: 15.02.21, Northwest Atlantic) were obtained from Domstein Sjømat AS (Stavanger, Norway) and prepared the day of arrival (18.02.21). The fillets were first removed of excess bones and skin and then cut into smaller pieces (200-300 g). During this process the ready prepared fillets were stored on a tray of ice covered with plastic clingfilm (Global Plastics International, France) to ensure that the fish did not absorb any excess water that could affect the nutrient content.

Figure 3.2 Vacuum packed cod fillets ready to be frozen.

The cod fillets were vacuum packed at 99 % vacuum, into portions of 1000±1 g in plastic bags (250 x 300 mm, PA/PEK 20/50, Lietpack, Lithuania) using a vacuum machine (Supermax C, Webomatic, Germany) (Figure 3.2). Then the packed fillets were stored in a freezer room (Huurre, Porkka Finland Oy, Finland) at -30 °C until further use.

3.1.2 Production of texture modified product

In the next step in production of texture modified products (TMP) the vacuum-packed fish was thawed (24 h, 4 °C) and then cooked in a preheated, convention oven (MSCC61, Metos system Intl., Germany) at 95 °C, 100 % steam. The core temperature was monitored using temperature probes (Testo AG, 176T4, Germany) placed into the thickest part of the fish fillet (Figure 3.3 A). The cod was cooked for 10 min after reaching a core temperature of 90 °C. Immediately after the heat treatment, the fish and cooking loss was transferred to a blender (Thermomix tm5, Vorwerk, France) (Figure 3.3 B). The fish was mixed at high speed for 1 min and 30 sec before adding the remaining ingredients and mixed as described in Table 3.1. The modified starch was mixed for 1 min in order to be activated.

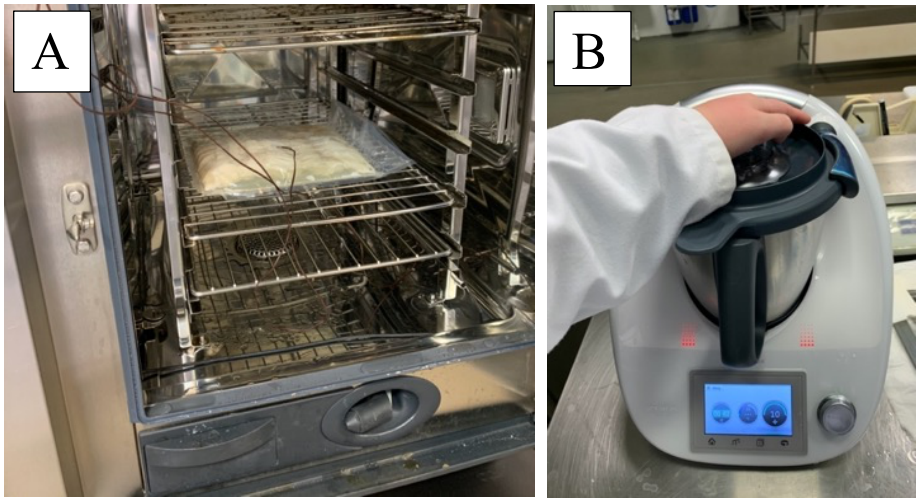

Figure 3.3 A) Heat treatment of fish as a first step in making texture modified products. The fish was cooked at 90 °C for 10 min, and temperature was monitored using a probe as shown in the picture. B) Thermomixer used to blend the texture modified product.

Table 3.1 Order of added ingredients, mixing speed and times used during production of texture modified product.

Order	**Ingredient**	**Mixing time (min:sec)**	**Mixing speed**
1	Cod fillet	01:30	10
2	Salt	00:15	8
3	Modified starch	01:15	10
	Hydrolysate		
	Caseinate		
	WPC80		
4	Rapeseed oil	00:30	8

Initially, custom made steel moulds (Ø80 mm) were placed on a tray with baking sheet and greased with vegetable fat (Melange Form Fett, Mills DA, Norway). The mixture was carefully pressed into the mould and made even using a spatula. The mixture was allowed to cool for a couple of minutes before being pressed out of its mould (Figure 3.4 A). The tray was wrapped in plastic cling film (Global Plastics International, France) and placed in the freezer (-30 °C, 24 h). Some of the batches made were too liquid to form a "burger" and had to be frozen in the mould, and where thus difficult to remove from the steel-moulds when frozen. To facilitate the process, it was eventually switched to using silicone moulds (Moul'flex, 6 tartelettes, SAS de Buyer Industries, France) (Figure 3.4 B). This allowed for the mixture to be frozen in its mould and easily removed. The method was kept the same, except that the silicone moulds where not greased beforehand.

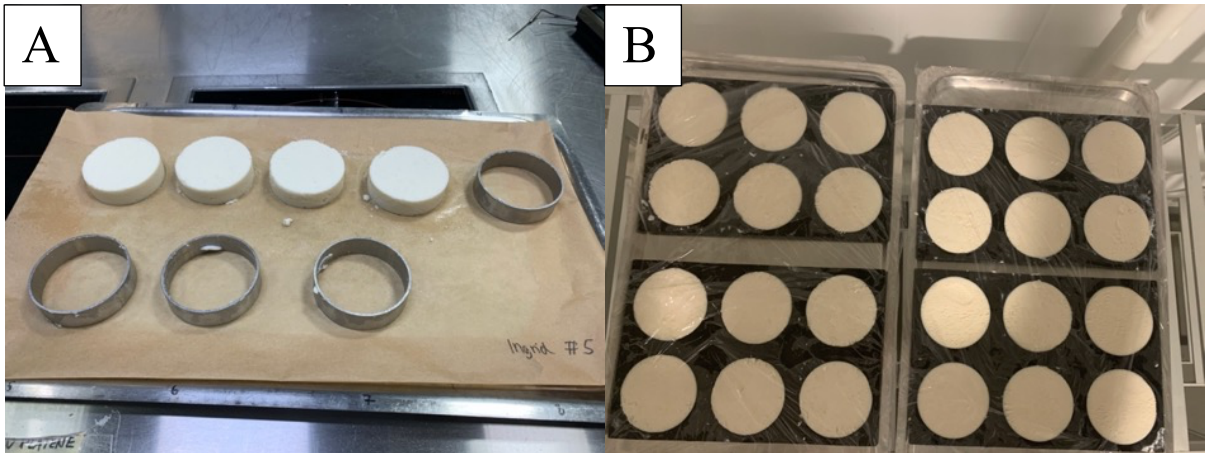

Figure 3.4 A) Steel rings used initially to shape the texture modified product. B) Silicone forms used throughout the rest of the preliminary and all pilot production.

After freezing, the products were individually vacuum packed (99 % vacuum) using vacuum bags (160 x 200 mm, PA/PEK 20/50, Lietpack, Lithuania). All the products were weighed, marked with recipe type and date of production. The individually packed products were stored in a freezer room (Huurre, Porkka Finland Oy, Finland) at -30 °C until further analysis. The same procedure for production was used in both preliminary and pilot production.

3.1.3 Nutrient calculations

A key component in developing recipes for texture modified products (TMP) was calculation of the nutrient content. Information about the nutrient content is especially crucial when developing products toward elderly and people with dysphagia. In general elderly need nutrient rich food containing high amounts of protein. Due to the loss of appetite often observed among

elderly, the products should preferably be additionally energy dense (See 2.1.2). Fish itself is among the raw materials rich in protein (Table 3.2), but the texture may cause problems for people with chewing disabilities. In this thesis it was desired to further increase the protein-level, as well as energy content. Fish protein hydrolysate (FPH) together with whey protein and caseinate were added to the fish to enrich the product (Appendix C, Appendix D). The aim was ultimately to get a product with more protein than the fish raw material, without comprising the soft texture. This was applied when developing the recipes of the texture modified products. In addition to calculating the total amount of protein, total amount of salt and fat was also included. Too much salt could cause diseases for both young and elderly people, so it was important to keep the level low (See 2.1.2). Since the TMP was mostly aimed towards elderly a high energy content was desired. Carbohydrates and kJ/Kcal were also included in the calculations. Table 3.2 below shows the nutritional values of cod used when calculation nutritional content during recipe development in both preliminary and pilot step.

Table 3.2 The nutrient content of 100 g raw, wild caught cod fillet (*Matvaretabellen*, 2021)

Material	**Protein**	**Salt**	**Fat**	**Carbohydrate**	**kJ/Kcal**
Raw, wild caught cod	17.9	0.1	1.1	0	343/81

3.1.4 Recipes developed in preliminary production

The preliminary production was used to obtain an improved understanding of how different ingredients like added protein, modified corn starch and oil influenced the texture of the product. The overall aim was to exceed the total protein content of the fish raw material, while also targeting the texture towards people with dysphagia and elderly. Different adjustments of mainly fish protein hydrolysate (FPH), whey protein, caseinate and oil were tested. The relationship between whey protein, FPH and caseinate was kept at 2:1:1. The recipes developed in the preliminary production are listed in Table 3.3 and are also colour coded according to which recipes were produced together.

Recipe 1 and 2 both had a grainy texture reminiscent of mashed potatoes. Due to the enrichment the recipe 2 was slightly firmer than recipe 1, and also had a bitter taste that could be related to the use of a commercially available fish protein hydrolysate (Hofseth, Appendix C). To obtain a smoother, less grainy texture, it was decided to add oil, and reduce the amount of modified corn starch (50 %) in the next step of preliminary testing. A new recipe (3) without protein

enrichment was made with less modified corn starch and addition of oil was tested. Recipes 4 and 5 had the same amount of protein enrichment, but recipe 5 had more oil and also less modified corn starch than the previous recipes. The added oil in recipe 5 gave a smooth and soft texture and it was decided to keep the oil content (1.4 %) at this level for the next step in recipe testing.

Recipes 6 and 7 was a replica of recipe 5, except for in recipe 7 a new hydrolysate was used (Nofima hydrolysate, Appendix D). Recipe 7 with the Nofima hydrolysate obtained a more distinct bitter aftertaste than recipe 6. Also, when making recipe 6 and 7, the fish was cooked at core temperature 75 °C instead of 90 °C. This led to a runnier mixture, and the products also slightly lost their shape during reheating. This may be due to insufficient activation of the modified corn starch. The heat treatment of the fish was therefore maintained at 90 °C for 10 min throughout the rest of preliminary and pilot production.

Similarly, to recipe 6 and 7, recipe 8 contained Hofseth hydrolysate, while recipe 9 contained Nofima hydrolysate. Oil content was increased further (4 % oil) to obtain a smoother texture, which in turn lead to slightly less protein content. When making the product, recipe 9 which contained Nofima hydrolysate became firmer and less sticky. Based on these recipes it was concluded that increased oil content gave a texture more suited for the target group/use group. The Nofima hydrolysate still gave a distinct bitter taste and was therefore not used further in the thesis.

Recipe 10 and 11 was made to compare different amounts of oil. Recipe 10 had 5 % oil and recipe 11 had 7 % oil. Recipe 10 had a drier mouthfeel, but still smooth. Recipe 11 was softer than the previous enriched products made in preliminary production. For recipe 12 and 13 it was decided to keep fish content at the same level as recipe 11 (88.3 %) to avoid having to many varying factors. Starch and salt were also kept constant. Recipe 12 did not contain any enrichment and had the highest amount of oil of all recipes and was therefore very soft but did not keep shape. Recipe 13 had the highest amount of protein content and was made with the intention to maximize possible protein enrichment. Recipe 13 had a soft texture, while keeping its shape well.

Table 3.3 Recipes tested during the preliminary production. The recipes are named by a number representing the order of production. The recipes marked with * contains salmon hydrolysate produced by Nofima. The total nutritional values are calculated, and all values are given in percentage (%).

Ingredient	1	2	3	4	5	6	7*	8	9*	10	11	12	13
Fish	97.3	93.6	97.3	93.6	93.6	93.6	93.6	91.2	91.2	90.3	88.3	88.3	88.3
Oil	0.0	0.0	1.0	0.9	1.4	1.4	1.4	4.0	4.0	5.0	7.0	10.6	5.4
FPH	0.0	0.9	0.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0	1.3
Caseinate	0.0	0.9	0.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0	1.3
WPC80	0.0	1.9	0.0	1.9	1.9	1.9	1.9	1.8	1.8	1.8	1.8	0.0	2.6
Salt	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Modified corn starch	1.9	1.9	1.0	0.9	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4
Total protein	17.4	19.9	17.4	19.9	19.9	19.93	19.87*	19.42	19.35*	19.2	18.8	15.8	20.2
Total fat	1.1	1.2	2.0	2.1	2.6	2.6	2.6	5.1	5.1	6.1	8.2	11.6	6.6
Total salt	0.9	0.8	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8

The total protein of recipe 6,7,8 and 9 are given with two decimals to emphasise that there was a slight difference. This was due to the difference in protein content of the Hofseth- (97 %) and Nofima* (89.8 %) hydrolysate. When the numbers were rounded to nearest tenth, there was no difference in calculated protein content between recipes that share a colour code (Table 3.3).

3.1.5 Texture analysis of TMP

Elderly and people suffering from dysphagia requires texture modified foods that are soft. A rapid method to determine the texture of food can be performed via a penetration/puncture test. A probe penetrates the surface of a product with a given distance under constant load (See 2.6.1). During the recipe development the firmness of the modified products were measured using TA.XT Plus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) to identify effects of the various compositions of ingredients. A 5 kg loading cell and a cylinder probe (Delrin cylinder P/0.5R, Stable Micro Systems Ltd., Godalming, UK) was used. Data was collected by the software Exponent (Version 6.1.16.0, Stable Micro Systems Ltd., Godalming, UK). The TA.XT instrument was operated through this software and the selected project of specific settings. The products were penetrated using test mode compression. The firmness was measured as the force (N, newton) required to penetrate the product for a specified distance (10 mm). Pre-test and test speed were set to 1.50 mm/sec while the post-test sped was 10.00 mm/sec. The analysis initiated when trigger force of 5 g was reached.

Prior to analysis instrument force and probe height was calibrated. The force was calibrated first, using a calibration weight (2000 g). The probe height was calibrated by zeroing the height

against the base. The measuring data was presented in a graph, further analysed by selected a *macro*; a list of instructions preformed automatically. The selected macro was made to find the maximum force (N). Results and graphs were then copied into Excel (v16.0, 1909, Microsoft Corporation, US.) for further analysis.

3.1.5.1 Sample preparation and analysis

Texture modified products were thawed (24 h, 4 °C) prior to the texture analysis. The products were weighed to observe potential fluid release. Before heating products were placed in plastic containers (Dynopack PE-HD 2, RPC Bebo food packaging, Kristiansand, Norway) and wrapped with plastic cling film (Global Plastics International, France). The convention oven (MSCC61, Metos system Intl., Germany) was set on 90 °C, 100 % steam. The core temperature was monitored using temperature probes (Testo AG, 176T4, Germany). The products were heated until reaching a core temperature of 75 °C due to food safety recommendations.

After heating, the products were transferred directly to a food warming trolley (Termia 950 H, Metos, Finland) with temperature set at 75 °C, to keep temperature in the sample constant until analysis. Before analysis, plastic cling film (Global Plastics International, France) around the plastic container (Dynopack PE-HD 2, RPC Bebo food packaging, Kristiansand, Norway) was removed and the sample placed on the base of the TA.XT instrument (Figure 3.5). The core temperature was measured once more before analysing the texture in three parallel products of each recipe performed by three penetrations on each sample (3 x 3 measurements per recipe).

Figure 3.5 TA.XT Plus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) with a cylinder probe (Delrin cylinder P/0.5R, Stable Micro Systems Ltd., Godalming, UK) attached.

3.1.6 Colour analysis of TMP

Possible difference in colours of products with variations in protein enrichment were analysed. The same products as for the texture measurements were used for colour analysis, only let cooled down (20 °C) and flipped over to have an even surface. A DigiEye system (VeriVide Ltd., UK) consisting of a camera, imaging cube and applications were used to perform the imaging and colour analysis. Before calibration and analysis, the light box had to be turned on 30 min prehand, to make sure the lamp has the correct brightness. The DigiEye system was calibrated using a white- and coloured calibration board (DigiTizer Calibration Pack, VeriVide Ltd., UK). The products were placed on a tray, transferred into the light box (standardised day light 6500 K) and photographed by a digital camera (Nikon D90, AF, Nikkor 35 mm f/2D, Nikon, Japan) (Figure 3.6). The camera settings were aperture: 10 and shutter: 1/5. For the colour measurement, DigiPix (VeriVide Ltd., UK) was used. The extracted colour coordinates were CIEL*a*b* (See 2.6.3).

Figure 3.6 Photograph taken of texture modified products by the Nikon camera and Digieye system.

3.1.7 High-Pressure processing of TMP

As an alternative to freezing the texture modified products, recipes 6, 8 and 11 were subjected to high-pressure processing (HPP). Prior to processing the mixture was filled in round plastic containers (C 0086-1C Black CPET, Faerch Plast UK), each containing approximately 115 g of mixture. For recipe 6 and 8, the products treated to HPP were from 1-2 batches, whereas for recipe 11, 5-6 batches were randomised to even out possible differences. After cooling (30-40 °C) each of the containers were vacuum-packed (95 % vacuum) using vacuum bags (160 x 200 mm, PA/PEK 20/50, Lietpack, Lithuania). HPP was carried out in a high hydrostatic pressure machine QFP 2L-700 (Avure Technologies Inc., Columbus, USA). Products were

pressurized at 400 MPa for 2 min and 600 MPa for 2- and 10 min. Prior and after treatment, products were stored in the chilled room (4 °C). Reference samples were non-pressurised products.

3.1.7.1 Texture analysis

The product was removed from the container before heated (75 °C) prior to analysis. As it was quite difficult remove the HPP product from the container without pressing the sides, it could have interfered with the texture results. The reference (non-HPP) was stored chilled (4 °C), except for recipe 6 which was frozen (-30 °C). The method for measuring the texture of high-pressure processed products was the same as the for non-processed texture modified products (TMP), except that the products did not need to be thawed (See 3.1.5.1). The products were measured at 55 °C.

3.1.7.2 Microbiology

Microbiological analyses of recipe 11 on day 0 was carried out prior to the texture measurements. A ten-fold dilution was made by diluting approximately 5 g of sample with 45 g of peptone water and homogenized in the stomacher for 2 min. The diluted product was then manually spread (100 µl) on Plate Count Agar (PCA) and TCA Yeast Extract (TSA YE) plates. In addition, an automatic plater (Eddy Jet ver. 1.23, model J000J/00/764, IUL Instruments, Spain) was used to plate the control samples (non-pressurised) and the products treated with the lowest pressure (400 MPa for 2 min) on PCA plates. The plates were incubated for 2-4 days at 37 °C. The detection level was 10^2. For plates with no colonies detected, the level was set to half of the detection limit. The microbial analysis was performed on day 0, 17 and 35.

3.2 Pilot production of TMP

A total of thirteen different recipes were developed in the preliminary production. The different products were measured in terms of colour and texture, and also evaluated by a simplified visual and taste testing performed by a semi-trained panel (4 assessors). Based on these results, three recipes were chosen to move forward with. These were 1) recipe without enrichment of FPH, whey protein or caseinate, 2) recipe with low protein enrichment and 3) recipe with high protein enrichment. The total calculated protein content in these recipes was respectively 17.4 %, 18.8 % and 20.2 %. The non-enriched recipe had the highest content of fish (97.3 %) due to few other ingredients, while both enriched recipes had 88.3 % fish. Ingredients like salt and modified corn starch was kept at the same level for all three recipes in the pilot production (See Table 3.3 for

fulfilled recipes). Adding oil influenced on softening the texture. The recipe with low enrichment had highest amount of oil (7 %), while the non-enriched recipe contained 1 % oil. To enabling high level of protein in combination of soft texture the recipe with high enrichment was added 5.4 % oil. Recipes used in the pilot production are presented in Table 3.4.

Table 3.4 The recipes used in pilot production of texture modified fish product. Calculated total protein, fat and salt content are included below the ingredients. The values are given in percentage.

Ingredient	**3**	**11**	**13**
Fish	97.3	88.3	88.3
Oil	1.0	7.0	5.4
FPH	0.0	0.9	1.3
Caseinate	0.0	0.9	1.3
WPC80	0.0	1.8	2.6
Total protein	17.4	18.8	20.2
Total fat	2.0	8.2	6.6
Total salt	0.9	0.8	0.8

3.2.1 IDDSI testing of TMP

The International Dysphagia Diet Standardisation Initiative (IDDSI) developed global, standardised terminology and definitions to describe texture modified food and drinks for people with dysphagia. IDDSI have developed a framework consisting of 8 texture and viscosity levels (0-7). A main aim has been to use easy testing methods that could be utilized by people with dysphagia, nurses, clinicians, people in the catering industry, to confirm what level a type of food or drink belongs to (See 2.5).

An easy way to control the firmness of food is by using IDDSI fork pressure test. This test is best used to assess foods in level 4-7. Blanching of the fingernail indicates food with firmer texture. As shown in Figure 3.7 a standard metal fork with four prongs was used in this test. The thumb was lightly placed right above the prongs. The fork was then pressed through the product until there was ¼ left at the bottom. The whole prong area was used when doing this test, not just the tip of the fork. Blanching or whitening of the fingernail was then observed or not. Additionally, the test was performed after 15 min and 30 min as suggested by IDDSI to examine how the firmness changes as food cools when eating (See 2.5).

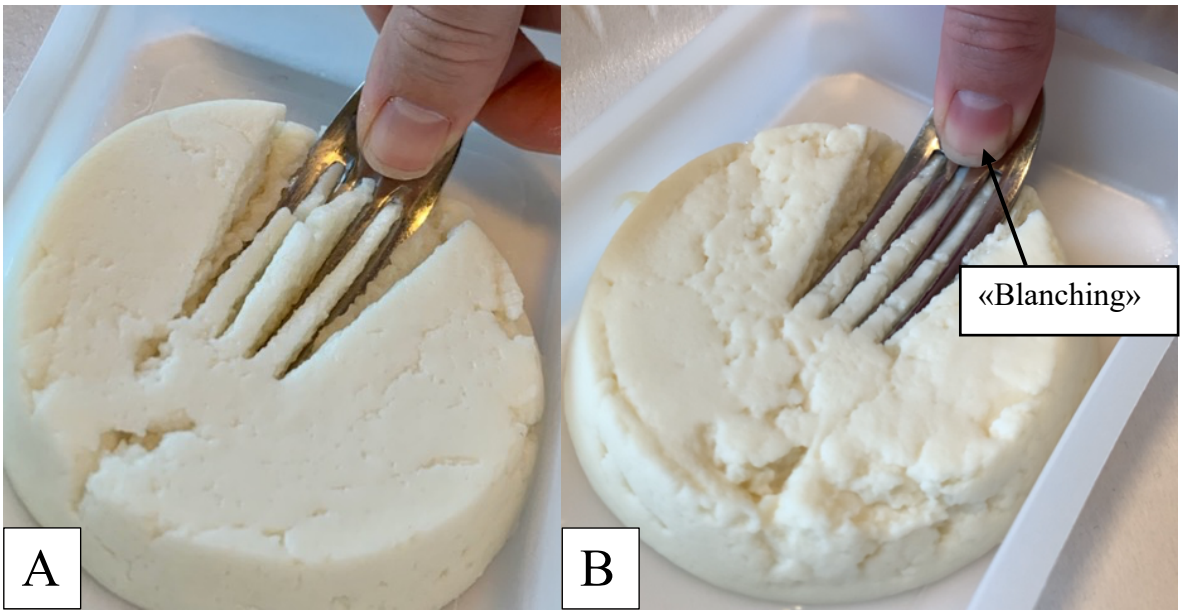

Figure 3.7 The figure shows examples of how the IDDSI fork pressure test was performed on texture modified products. Figure A is an example with no blanching of the fingernail, while figure B shows blanching.

3.2.2 Texture analysis of TMP

The method used when measuring firmness in the preliminary step is described in chapter 2.6.1 and was also used in the pilot step with some modifications that are described below.

The distance the probe would penetrate the sample, was initially set at 10 mm, but had to be reduced to 7 mm, which was about halfway into the product. This was crucial as when set at 10 mm, the probe sometimes reached the bottom and gave a much higher firmness (N) than anticipated. The products were measured at 55 °C to simulate the service temperature.
Additionally, the texture was measured after 15- and 30 min storage of the products in room temperature (20 °C) adapted from IDDSI (See 2.5). Mainly to examine effects on the firmness when the food cools during an eating situation.

3.2.3 Rheological measurements of TMP

Foods may be classified in terms of their rheological properties and sensory attributes as e.g., liquids, semi-solids or soft solids. Fluid foods flow and do not require chewing, semi-solid foods are processed in the mouth by squeezing the tongue and palate and soft-solid foods require chewing but do not have “crispy” attributes. Studying the rheology can therefore be useful when developing texture modified products.

A hybrid rheometer (Discovery HR-2, TA Instruments, US) was used for the rheological measurements of texture modified products (TMP). Air supply and fluid circulation was first turned on due to the installed temperature system Peltier Plate (New advanced, TA Instruments, US). Next, the cap on the rheometer was removed, and then the equipment was switched on. The rheometer was run on a computer via the software Trios (Trios, v4.3.0.38388, TA Instruments, US). The geometry was then attached to the instrument and calibrated.

The method applied for rheological measurements of the texture modified products (TMP) was also used in the MSc thesis by Østebrød (Østebrød, 2020), but with some modifications. The used geometry was a parallel plate (XHATCH, 20 mm, Serial number 111670, TA Instruments) shown in Figure 3.8.

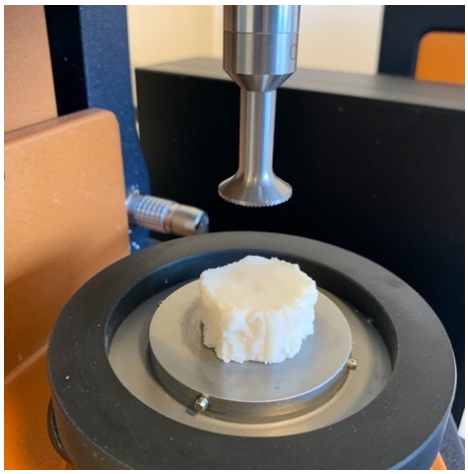

Figure 3.8 Parallel plate (XHATCH, 20 mm, Serial number 111670, TA Instruments) geometry attached to the rheometer (Discovery HR-2, TA Instruments, US). The sample is placed on the Peltier plate.

The products were thawed overnight at 4 °C. Before analysis, the products were left on the bench to reach room temperature (20 °C). Meanwhile, the instrument and software were initiated and calibrated. Since the products had a larger diameter than the Peltier plate, the sample was divided in smaller pieces (3 cm). An amplitude sweep was first performed on the three recipes produced in the pilot production: non, low and high enrichment of proteins. The output gave storage modulus (Pa) G′ and G′′. The storage modulus G′ was above the G′′ graph which indicated that the sample was a solid. The aim was to determine the linear viscoelastic region (LVR), and therefore storage modulus G′ (Pa) was plotted against oscillation strain (%). The strain (%) was increased to 1 % which was within the LVR-region for all the recipes and

temperatures. The soak time was also increased from 10 sec to 5 min as this was recommended by the Trios software (Trios, v4.3.0.38388, TA Instruments, US), and due to the thickness of the sample (14 mm). The gap at which the texture modified product was measured was 500 µm. After the geometry had reached the gap, the sample was trimmed, and a solvent trap was used to keep temperature stable. The selected temperatures were kept constant at 25 °C and 55 °C, and the frequency at 1 Hz. The oscillation strain varied from $3.33*10^{-3}$ to 100 %. Points per decade were set to ten and the axial force was set on compression mode with force 0,25 and sensitivity 0.1 N.

Based on the selected strain (1 %) from the amplitude sweep, a temperature ramp was performed. The temperature started at 25 °C, then up to 55 °C and back down to 25 °C. A total of six ramps were done for each recipe. The frequency was kept constant at 1 Hz. The storage modulus G` (Pa) was plotted against temperature (°C).

3.2.4 Colour analysis of TMP

The colour analysis was done on the same products that the texture was measured on. The colour analysis was done the same way as in the preliminary production (See 3.1.6), except in the pilot production a total of 9 parallels for each recipe were measured instead of 3. The extracted colour coordinates were L*a*b*.

3.3 Workflow protein enriched soup

The workflow diagram in Figure 3.9 shows the steps of making the protein enriched soup. A list of ingredients used is found in Appendix B. The purpose was to develop enriched products in liquid form that would be suitable for elderly and people with swallowing difficulties. The aim was that the product could be used as both a soup base and a sauce. The products were enriched using whey protein concentrate (WPC), sodium, caseinate (caseinate) and fish protein hydrolysate (FPH). Analysis of colour, texture, rheology and IDDSI testing were used describe the characteristics of the products.

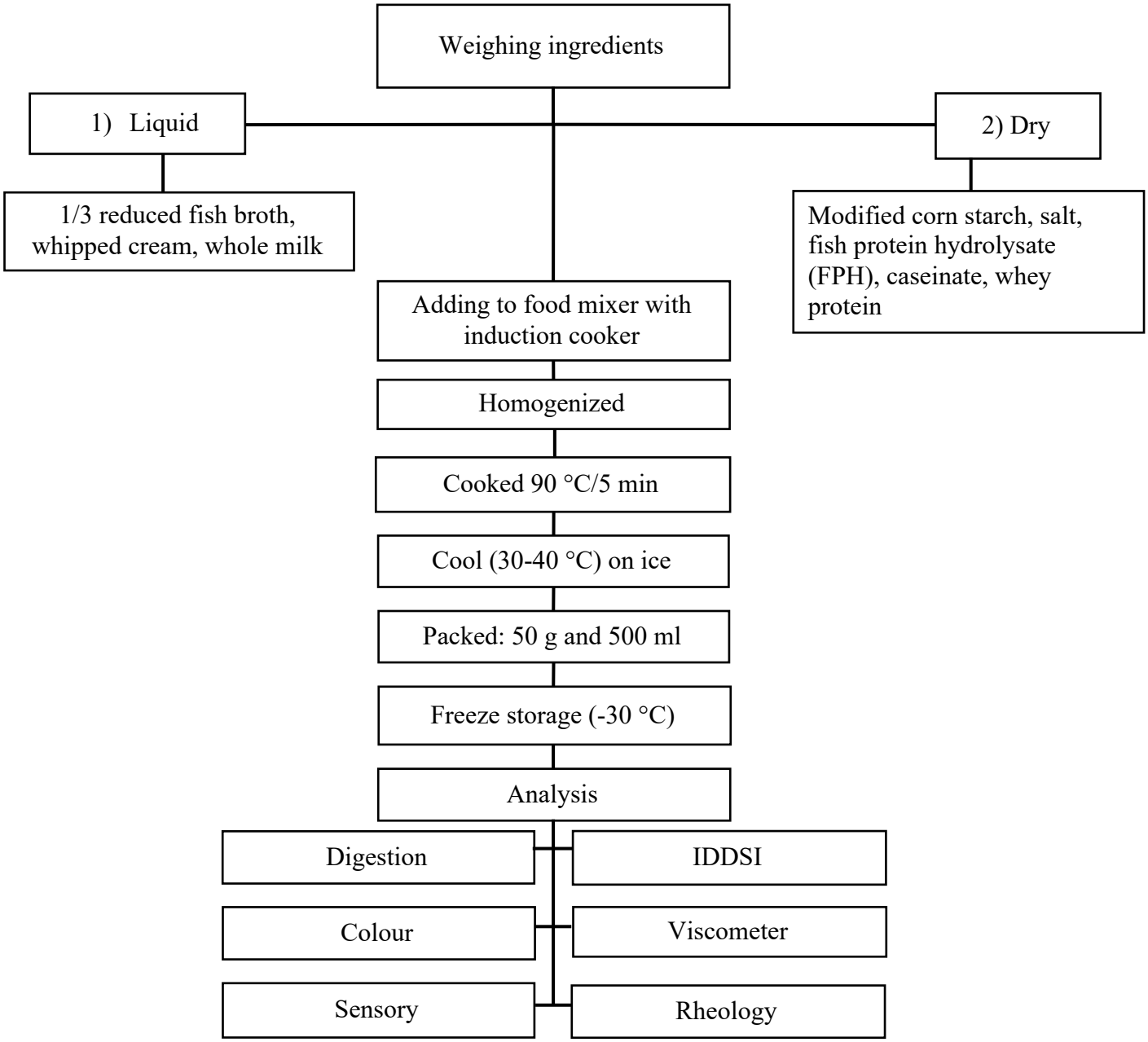

Figure 3.9 Workflow of the production and analysis of texture modified soup.

The work was done in two steps: Preliminary and pilot. In the preliminary step, different recipes of enriched soup were developed, produced and then tested. Analyses used to assess the products in preliminary testing were IDDSI, viscosity, rheology and colour measurements, in addition to tasting. Based on the conclusions from the preliminary step, three of the recipes were continued into the pilot production and testing.

3.3.1 Production of soup

The enriched soup was made using a food mixer with built in induction cooker (Kenwood Cooking Chef, KM096, UK). All the liquid ingredients were first weighed in, and afterwards the dry ingredients (Table 3.5). The mixture was blended with a stick mixer (Robot Coupe MicroMix, France) for about 30 sec or until visibly homogenized, after adding each of the dry ingredients. The mixer was kept on the bottom to prevent a foamy mixture. Also, a spatula was used to crush some of the larger clumps. The bowl was placed back in the food mixer, with a high temperature flexible beater which stirred continuously.

The temperature was initially set to 100 °C, and at speed setting 1. After approximately 8 min the mixture reached 100 °C. To activate the starch the holding time was 5 min at 100 °C (+/- 3 °C). During the cooking procedure the soup got a burn flavour.

To avoid burning of the soup the temperature was reduced to 90 °C (+/- 3 °C). Approximately 2 litres of soup were made in each batch. The soup was then cooled down (approx. 30 °C) in a bowl placed on ice. The soup was then packed in vacuum bags (250 x 300 mm, PA/PEK 20/50, Lietpack, Lithuania) in portions of 500 ml, and sealed in the vacuum machine at 20 % vacuum, and eventually placed in the freezer room (Huurre, Porkka Finland Oy, Finland) at -30 °C. The same vacuum machine and vacuum bag as in chapter 3.1.1 was used.

3.3.2 Recipes in preliminary production of enriched soup

During the preliminary production a total of 7 recipes of soup were developed. The aim was to make a protein enriched soup, while also targeting the texture towards people with dysphagia and elderly. Recipe 1 and 2 was without any protein enrichment, and recipe 2 was used as a starting point when developing the rest of the protein enriched soups in preliminary production. The recipes that were developed in the preliminary production are listed in Table 3.5 and are colour coded according to which recipes were produced together.

Recipe 1 was tasted during production, and it was found to have a distinct dairy taste, most likely due to the amount of whipping cream and milk, since there was not added any dairy enrichment yet. When producing recipe 2, the amount of fish broth was therefore increased (10 %), and the amount of milk was reduced (50 %). Additionally, some salt was added as a taste enhancer. To increase the thickness of the soup the amount of modified corn starch was also slightly increased. Throughout the rest the preliminary producing of soup, the percentage of fish broth, whole milk, whipping cream, salt and modified corn starch was roughly kept the same, and mainly the protein enrichment was adjusted. The relationship between whey protein, FPH and caseinate was kept at 2:1:1, same as for the texture modified products (TMP).

When developing recipe 4 and 5 it was aimed at making a soup that had low-enrichment. For recipe 6, it was aimed at reaching a maximum protein level while the soup still remained a liquid. After some taste and texture evaluation by a semi-trained panel 3 recipe (9 % protein) and 6 (10 % protein) was found to be somewhat thick and unhomogenised. A last recipe with 8 % protein was therefore developed and was used in the pilot production together with recipe 2 (4 % protein, non-enriched) and recipe 5 (6 % protein, low-enriched).

Table 3.5 Ingredients and recipes for the soup. Recipes tested during the preliminary production. The recipes are named by a number representing the order of production. The total nutritional values are calculated, and all values are given in percentage (%).

Ingredient	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1/3 reduced fish broth	50.0	60.8	57.4	58.5	59.4	56.3	57.8
Milk	22.5	11.1	10.5	10.7	10.9	10.3	10.6
Whipping cream	25.0	24.8	23.4	23.9	24.2	23.0	23.6
FPH	0.0	0.0	1.4	1.0	0.6	1.8	1.2
Caseinate	0.0	0.0	1.4	1.0	0.6	1.8	1.2
WPC80	0.0	0.0	2.8	1.9	1.2	3.7	2.5
Salt	0.0	0.3	0.3	0.3	0.3	0.3	0.3
Modified corn starch	2.5	3.0	2.8	2.9	2.9	2.8	2.8
Total protein	4.0	4.1	8.7	7.2	6.1	10.1	8.1
Total fat	10.1	9.6	9.3	9.4	9.5	9.3	9.4
Total salt	0.3	0.6	0.6	0.6	0.6	0.6	0.6

3.3.3 IDDSI testing on enriched soup

IDDSI have developed a framework consisting of 8 texture and viscosity levels (0-7). A main aim has been to use easy testing methods that could be utilized by people with dysphagia, nurses, clinicians, people in the catering industry, to confirm what level a type of food or drink is categorised to (See 2.5).

The IDDSI flow test can be used for liquids within level 0-4. It is a fast, easy test that requires minimal equipment (See 2.5). A 10 ml syringe (BD Luer-Lok™, U.S) was used to perform the IDDSI flow test. The soup to be analysed was thawed at 4 °C for 24 h. The bag of the soup was placed in a laboratory water bath (EcoLine Staredition E 300, LAUDA) to heat up the sample to simulate the serving temperature (55 °C). The water bath temperature was set 2 degrees higher (57 °C) than the desired soup temperature., and also kept warm in the water bath. The syringe (BD Luer-Lok™, U.S, 10 ml) was filled using an additional syringe up to the 10 ml mark, while blocking the nozzle with a finger. As soon as the finger was removed from the nozzle a timer was started, and after 10 sec the nozzle was blocked with a finger again (Figure 3.10). The amount of liquid left in the syringe was used to identify the IDDSI level (Table 3.6).

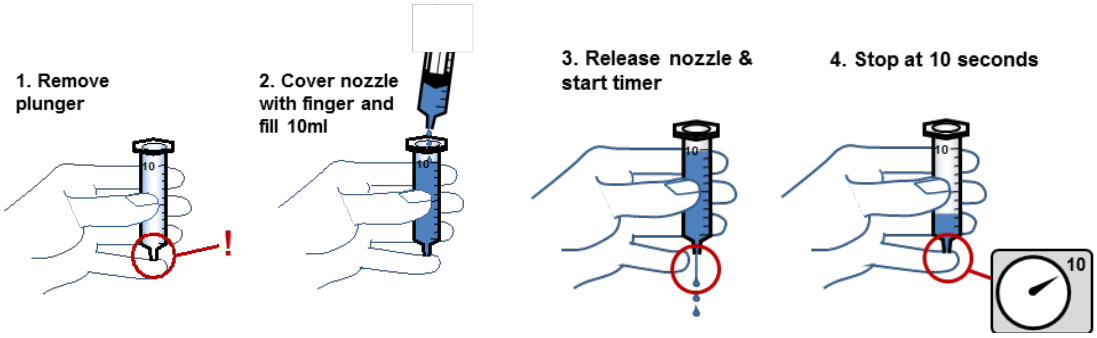

Figure 3.10 The IDDSI flow test in 4 steps.
(https://iddsi.org/IDDSI/media/images/Posters/IDDSI_Poster_Flow_Test_Jan2020.pdf)

Table 3.6 Overview showing how the different IDDSI levels corresponds to the amount of liquid remaining (ml) in the syringe after the IDDSI flow test.
(https://iddsi.org/IDDSI/media/images/Posters/IDDSI_Poster_Flow_Test_Jan2020.pdf)

Liquid remaining in syringe (ml)	Level
1-4 ml	1 – slightly thick
4-8 ml	2 – mildly thick
8-10 ml	3 – moderately thick
10 ml	4 – extremely thick

3.3.4 Viscosity measurements of soup in preliminary step

Viscosity is the measure of a substance´s resistance to motion under applied stress. When measuring the viscosity of enriched soup, it can give and idea about the thickness of the soup and if it will be suitable for people who need texture modified foods.

Brookfield Viscometer DV2T Extra (Brookfield Engineering Laboratories, U.S.A) was used for viscosity measurement (Figure 3.11). The soup was thawed at 4 °C for 24 h and shaken manually 10 times before being poured into a Schott Duran beaker (600 ml, Schott AG, Germany). The beaker was filled up to the 500 ml level mark and placed in a laboratory water bath (EcoLine Staredition E 300, LAUDA) to ensure constant temperature during analysis. The water bath temperature was set 2 degrees higher than the required soup temperature. The soup was measured at 25 °C ± 1.5 and 55 °C ± 1.5, with six repetitions. Analysis temperature was set to 55 °C to simulate the service temperature when eating food and 25 °C was used as room temperature. During the heating, the soups were stirred 4-5 times manually with a spoon to avoid “milk skin” forming on top of the soup.

In order to achieve an accurate zero value, the viscometer level was adjusted using the two feet at the bottom and monitoring the bubble level on the front of the head. The autozero (calibration) was then ready to be carried out. A rotation speed of 100 revolutions per minute (RPM) was used for all soups, but the spindles were switched according to the viscosity of the sample, and which gave torque value within the range (10 %-100 %). The spindles were inserted diagonally to avoid bubbles under the disk. The viscometer was lowered into the soup until the “notch” on the spindle was in the sample (Figure 3.11 B).

The viscosity test was configured by choosing the spindle type and RPM. The sample temperature was recorded for each measurement using the built-in thermometer, and the test was stopped after 30 sec for all the readings. The single point data collection was used to collect the readings from the viscometer.

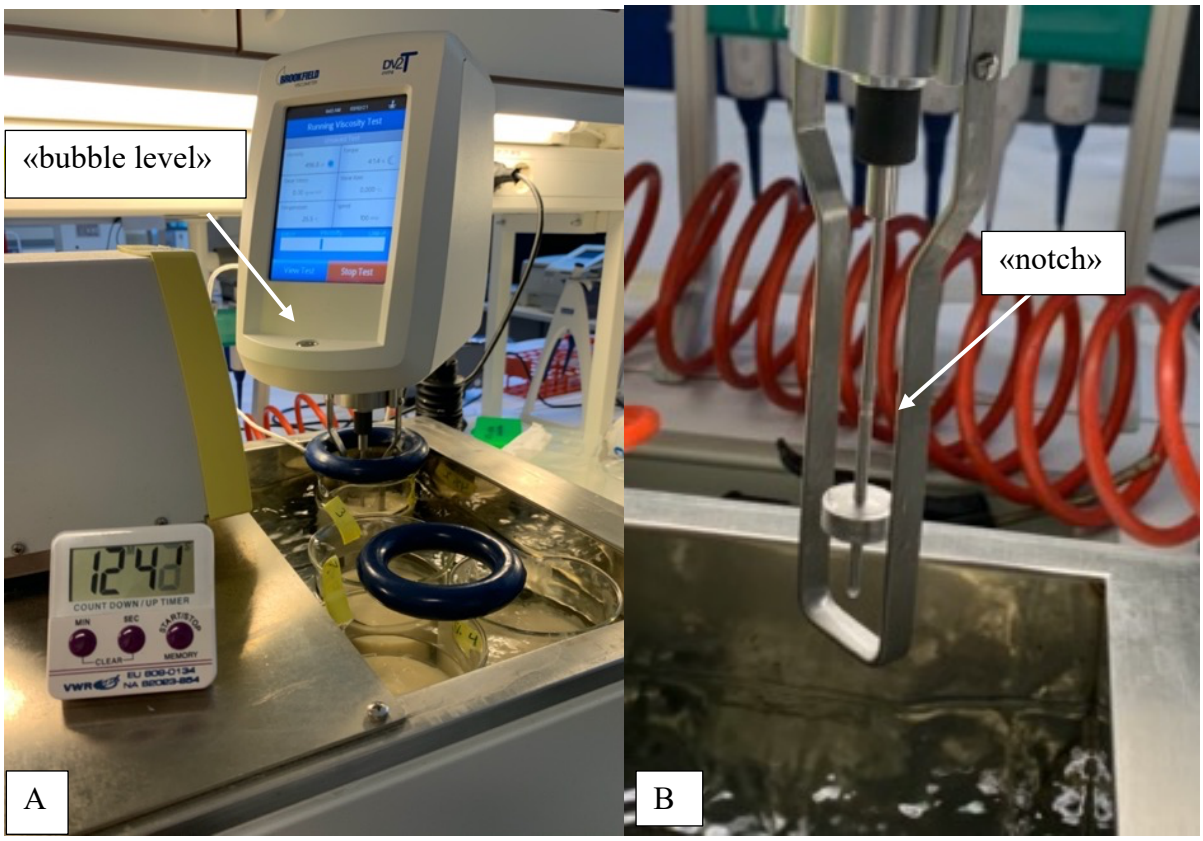

Figure 3.11 A) Viscometer setup with laboratory water bath. The arrow on the figure is pointing to the bubble level. B) The arrow on the figure is pointing to the notch on the spindle. This particular type of spindle is LV-62 (02).

As it was suspected that 30 sec of run time was not enough for the viscosity to stabilise, an additional test was done. The viscometer was run for 30 min, while the parameters where noted each 30 sec. From the data, a graph was made showing the viscosity over a course of 30 min. This was done while the temperature was kept constant at 25 °C (room temperature), 55 °C (service temperature) and at 55 °C while letting it cool outside of the water bath. The viscosity was analysed while letting the food cool for 30 min, similarly to the IDDSI analysis where the soup was tested the at time of service (55 °C), 15 min after serving and 30 min after serving. The temperature, torque value and viscosity were the measured parameters. This method was carried on in the pilot step (See 3.4.2).

3.3.5 Rheological measurements of soup in preliminary step

The rheometer may be seen as a more advanced method to analyse the soup. Rheological measurements would provide more information about the flow properties of the soup than the viscometer. Rheological properties of the enriched soup were analysed by a hybrid rheometer (Discovery HR-2, TA Instruments, US). Air supply and fluid circulation was first turned on due

to the installed temperature system Peltier Plate (New advanced, TA Instruments, US). Next, the cap on the rheometer was removed, and then the equipment was switched on. The rheometer was run on a computer via the software Trios (Trios, v4.3.0.38388, TA Instruments, US). A 2° cone plate geometry (Cone SST ST 40 mm 2deg smart-swap, serial number:105132) was attached to the instrument. It was the preferred geometry for measuring rheological properties of the soup, this decision was based on previous rheological studies done on similar products. The geometry gap was fixated at 52 µm due to the cone shape. Analysis temperature was set to 55 °C to simulate the service temperature when eating food, and flow sweep with controlled shear rate (0.01-1000 s^{-1}) was performed. The same was done at 25 °C to examine how the soup behaves in room temperature.

3.4 Pilot production of soup

A total of seven different recipes were developed in the preliminary production. Three recipes were chosen to move forward with, based on viscosity, rheology and simplified visual and taste testing performed by a semi-trained panel (4 assessors). These were 1) recipe without enrichment of FPH, whey protein or caseinate, 2) recipe with low protein enrichment and 3) recipe with high protein enrichment. The total calculated protein content in these recipes was respectively 4 %, 6 % and 8 %. The procedure of weighing in the ingredients and homogenizing the soup was the same as in the preliminary production (See 3.3.1).

By using an additional thermometer, it was discovered that the Kenwood induction cooker (Kenwood Cooking Chef, KM096, UK) did not give core temperature at 90 °C in the soup, and therefore the modified corn starch was not activated. In the pilot production an optimized method was used. Instead, the soup was mixed and heated in the Kenwood mixer in 2-liter batches for 10 min at 90 °C, before being poured into a large saucepan. In the pilot production 6-7 batches of each recipe was produced to have enough soup for the different analysis. All the batches were mixed together, to avoid minor differences between them. The soup was then heated in the saucepan to core temperature 90 °C, and kept at that temperature for 5 min, using an induction oven (Drop in Base-line 5000, Metos Group, Finland) and by stirring manually. Immediately, the soup appeared thicker than when only the Kenwood mixer was used. As in the preliminary production, the soup was again mixed with a stick mixer (Robot Coupe MicroMix, France) to ensure a homogenized soup and make sure all the batches were merged prior to the heat treatment. The saucepan was then put in ice bath and additionally placed in a

chilled room at 0 °C to cool the soup (30-40 °C). Lastly the soup was packed in vacuum bags (250 x 300 mm, PA/PEK 20/50, Lietpack, Lithuania) in portions of 50 g and 500 ml, and sealed in the vacuum machine at 20 % vacuum, and eventually placed in the freezer room (Huurre, Porkka Finland Oy, Finland) at -30 °C. The same vacuum machine and vacuum bag as in chapter 3.1.1 was used.

3.4.1 IDDSI testing of soup

The method for the IDDSI flow test was described in the preliminary step (See 3.3.3). The method was kept the same in the pilot step, except for the sample preparation. The soup was heated in a convention oven (MSCC61, Metos system Intl., Germany) at 90 °C, 100 % steam. The core temperature was monitored using temperature probes (Testo AG, 176T4, Germany). While analysing, products were kept in a food warming trolley (Termia 950 H, Metos, Finland) with temperature set at 65 °C. Six parallels of each recipe were measured. The recipes that were measured contained different amount of protein, 4,6 and 8 %, respectively. As equally done for the texture modified product (TMP) the soup was measured at time of service (55 °C), 15 min after service and 30 min after service.

3.4.2 Viscosity measurements of soup in pilot step

The method for measuring viscosity of enriched soup was described in chapter 3.3.4. Three recipes with 4, 6 and 8 % calculated protein content was measured. A total of 6 parallels for each recipe weas analysed.

3.4.3 Rheological measurements of soup in pilot step

Rheology measurements of enriched soup was done det same way as in the preliminary step (See 3.3.5). Three recipes with 4, 6 and 8 % calculated protein content was measured. A total of 6 parallels for each recipe weas analysed at 55 °C, additionally the extremes were also measured at 25 °C.

3.4.4 Colour analysis of soup

The remaining soup from the IDDSI flow test was used for the colour analysis. The soup was cooled down to room temperature (approx. 20 °C) and poured in Eddy Jet cups before analysis. A total of 18 parallels of each recipe were measured (Figure 3.12). The method of the DigiEye and DigiPix colour measurement is described in chapter 3.1.6.

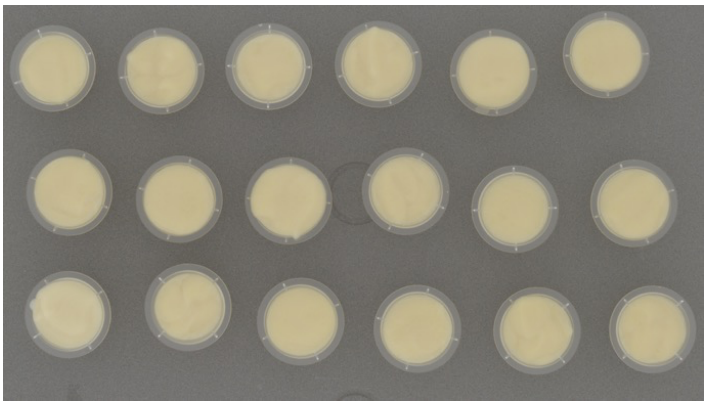

Figure 3.12 Photograph taken of soup in Eddy jet cups by the Nikon camera and Digieye system.

3.5 *In vitro* digestion of TMP and soup

A digestion experiment was carried out in collaboration with the Nofima department at Ås. The following products from the pilot production were sent for analysis: 1) Texture modified products, 2) Enriched soup products, 3) Fish raw material (cod) and 4) Whey protein, caseinate and fish protein hydrolysate mixed with ratio 2:1:1. The products were sent frozen to Ås to perform the digestion experiment (Table 3.7). All the products were examined using both and adult and an elderly *in vitro* model based on the standardised INFOGEST digestion protocol (Brodkorb et al., 2019; Minekus et al., 2014) (Figure 3.13). The procedure for measuring protein digestibility by using size exclusion chromatography (SEC) to determine the amount of digestion breakdown products (peptide size) is described in Rieder et al. 2021.

	Volume /Weight	Contents
Food	1 g	
Simulated salivary fluid (SSF)	1 ml	• Electrolyte solution pH 7 • $CaCl_2(H_2O)_2$(44,1 g/l) (5µl/ml SSF)-1,5 mM • Salivary amylase (**50 Units/ml SSF**) (avhenger av type produkt)
Simulated gastric fluid (SGF)	2 ml	• Electrolyte solution pH 3 • $CaCl_2(H_2O)_2$(44,1 g/l) (0,5µl/ml SGF)-0,15 mM • Pepsin (**4000 Units/ml SGF**)
Simulated intestinal fluid (SIF)	4 ml	• Electrolyte solution pH 7 • Pancreatin (2,5 mg/ml SIF) • Bile (20 mM, 14,9 mg/ml SIF)
Total volume	8 ml	

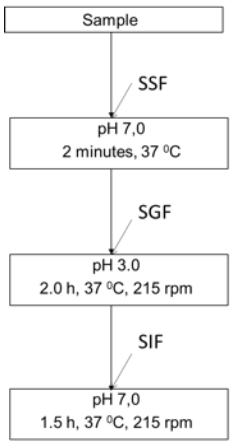

Figure 3.13 The INFOGEST static *in vitro* protocol for the study of gastrointestinal digestion in adults. A similar protocol was used for the simulation of elderly gastrointestinal conditions with higher gastric pH (4.5), and lower levels of pepsin (1000 U/ml SGF), bile (10 mM in SIF) and pancreatin (1.0 mg/SIF).

Table 3.7 Overview of the digestion experiment. *From (*Matvaretabellen*, 2021).

Sample No.	**Product**	**Calculated protein content (%)**	**Adult model**	**Elderly model**
1	Soup	4.13	x	x
2	Soup	8.09	x	x
3	TMP cod	17.4	x	x
13	TMP cod	20.21	x	x
4	Protein mixture	8	x	x
5	Cod fillet, raw	17.9*	x	x
-	Blank	-	x	x

3.6 Statistical analysis

Data from the analysis were tested for possible significant differences using one-way ANOVA in Minitab 19 Statistical Software (Minitab Ltd., UK, 2020). Tukey's Pairwise Comparison test was the main test, followed by a Fisher Pairwise comparison test if the Tukey's would not yield any differences. The level of significance was determined at $p<0.05$.

4 Results and discussion

4.1 Preliminary production of texture modified products

The development of the recipes during preliminary production was performed using primarily nutrient calculations, in addition to texture analysis and semi-trained panel (4 assessors) to evaluate sensory attributes. The objective with these recipes was to obtain products with high amounts of proteins. The texture modified products where mostly aimed for elderly and people with dysphagia, and thus, the nutrient content was important.

Recipes in the preliminary production were developed by changing the amount of added protein enrichment and oil, as well as adjusted with other ingredients. Recipes 1 and 3 did not contain any protein enrichment and was used as starting points for further development. The other recipes were enriched by fish protein hydrolysate (FPH) and the dairy proteins caseinate and whey protein caseinate (WPC80) (Table 4.1). The development of the recipes is described in more detail in chapter 3.1.4.

Table 4.1 Recipes tested during the preliminary production. The recipes are named by a number representing the order of production. The recipes marked with * contains hydrolysate produced by Nofima. The total nutritional values are calculated. All values are given in percentage (%).

Ingredients	**1**	**2**	**3**	**4**	**5**	**6**	**7***	**8**	**9***	**10**	**11**	**12**	**13**
Fish	97.3	93.6	97.3	93.6	93.6	93.6	93.6	91.2	91.2	90.3	88.3	88.3	88.3
Oil	0.0	0.0	1.0	0.9	1.4	1.4	1.4	4.0	4.0	5.0	7.0	10.6	5.4
FPH	0.0	0.9	0.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0	1.3
Caseinate	0.0	0.9	0.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.0	1.3
WPC80	0.0	1.9	0.0	1.9	1.9	1.9	1.9	1.8	1.8	1.8	1.8	0.0	2.6
Total protein	17.4	19.9	17.4	19.9	19.9	19.9	19.9	19.4	19.4	19.2	18.8	15.8	20.2
Total fat	1.1	1.2	2.0	2.1	2.6	2.6	2.6	5.1	5.1	6.1	8.2	11.8	6.6
Total salt	0.9	0.8	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8

Salt (NaCl) was aimed at being as low (<1 %) as possible due to several health issues associated with high intake. However, salt contains functional properties and acts as a taste enhancer and texture modified and cannot be entirely excluded. To obtain a soft but stable texture in the (modified) products, modified corn starch was added for thickening and gelling properties. No remarkable water exudation was observed after final heat treatment of the preliminary production. Previous studies done by Prabhu, 2018 showed that WPC alone gave a prominent

dairy taste when combined with fish protein hydrolysate (FPH). By using both WPC and caseinate enrichment, some bitterness from FPH was masked, while the dairy flavour was experienced less strong. The ratio 2:1:1 between whey protein, caseinate and fish protein hydrolysate (FPH) was continued into the pilot production, since both the taste and texture of the products were satisfying. In addition, earlier work suggested the following ratio 2:1:1 as well with good results (Prabhu, 2018).

4.1.1 Texture analysis of TMP in preliminary step

To document the effect of variation in recipe composition the texture of modified fish products was measured using a TA.XT texture analyser (See 3.1.5). The texture was measured using a puncture test expressed by firmness, or the force Newton (N). There were three parallels for each recipe, and each parallel was measured 3 times at separate places on the product (n=9).

There was initially made two recipes without any protein enrichment, recipe 1 and 3. As shown in Figure 4.1, the texture of recipe 3 had the least firm texture. Compared to recipe 1, the amount of modified corn starch was reduced, and oil was added (Table 4.1). Recipes 2 and 4 had the same amount of protein enrichment. During evaluation of sensory properties by the semi-trained panel, recipe 2 was experienced as drier and firmer, and looked similar to mashed potatoes, while recipe 4 was slightly smoother and softer. The softer texture in 4 was a result of reduced (50 %) amount of modified corn starch and by adding oil. Products 4, 5 and 6 had the same amount of total protein, but there was a higher fat content in recipe 5 and 6 due to added oil, which lead to a smoother mouthfeel. For recipe 6 and 7 the same recipe was used except, a Nofima hydrolysate was used in recipe 7. It was the same case with recipe 8 and 9. The firmness of recipe 10 was higher than recipe 9, but there was also a high standard deviation (± 0.8), so the texture may not differ much from recipe 9. Recipe 11 and 12 contained high amount of oil, which may be why the firmness was lower, but the protein content was also lowered in these recipes. The firmness also appeared to be lower than for the commercially available products. Recipe 13 contained the highest amount of protein (20.2 %) and had similar texture to recipe 2, but since recipe 13 contained oil, it was smoother and more pleasant to eat. To evaluate the different textures, they were also compared to commercially available TMP. The texture measurements of these products were copied from Prabhu, 2018. The commercial salmon product contained a total of 14.7 % protein, and the haddock product contained 13.7 % total protein, which was less than any of the recipes developed. As it appears from the graph,

only a few of the recipes were firmer than the commercial products, which indicated a soft texture, even with high protein content.

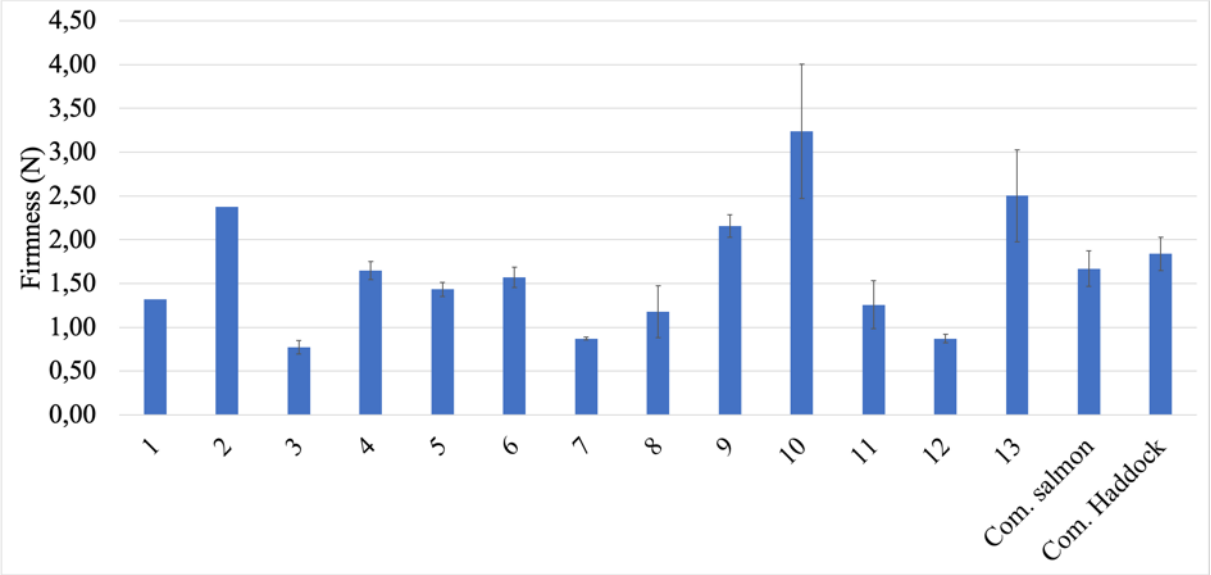

Figure 4.1 Measured firmness (N) in texture modified fish products (Recipe 1-13) compared with two commercial texture modified products. (n=9). Recipes 1 and 3 are standards without protein enrichment.

4.1.2 Colour measurement of TMP in preliminary step

A colour analysis was done on all recipes in the preliminary production. There were three parallels for each recipe, except recipe 1 and 2, which only had one. Measured values of the colour coordinate L* were in the range 84-93. The highest value possible for L* is 100 (white) meaning that all TMP from preliminary production were close to being white (Figure 4.2 A). The a* value indicates red-green colour components, where green is negative and red is positive (Ly et al., 2020). The measured value for the preliminary recipes ranged from -2.6 to 0.9, which would be perceived as a neutral grey with a slight red tint for the positive values and slightly green for the negative values (Figure 4.2 B). The b* values represent yellow (positive) and blue (negative) components. As shown in Figure 4.2 C, the b* value was positive for all the products, ranging from 12-17. This indicates a more yellow product, primarily caused by the added fish protein hydrolysate and WPC80 which has yellow tones.

Recipes 1, 2, 8 and 9 stands somewhat out, in regard to measurements of colour coordinate L*. The colour measurements of these recipes, revealed a slightly less light colour, although this was not very noticeable by eye. Recipes 1 and 2 were the only with negative a* colour coordinates, which indicate some green colour. However, for recipe 1 and 2 only one product

was measured, and standard deviations were lacking, more parallels would perhaps even out the results.

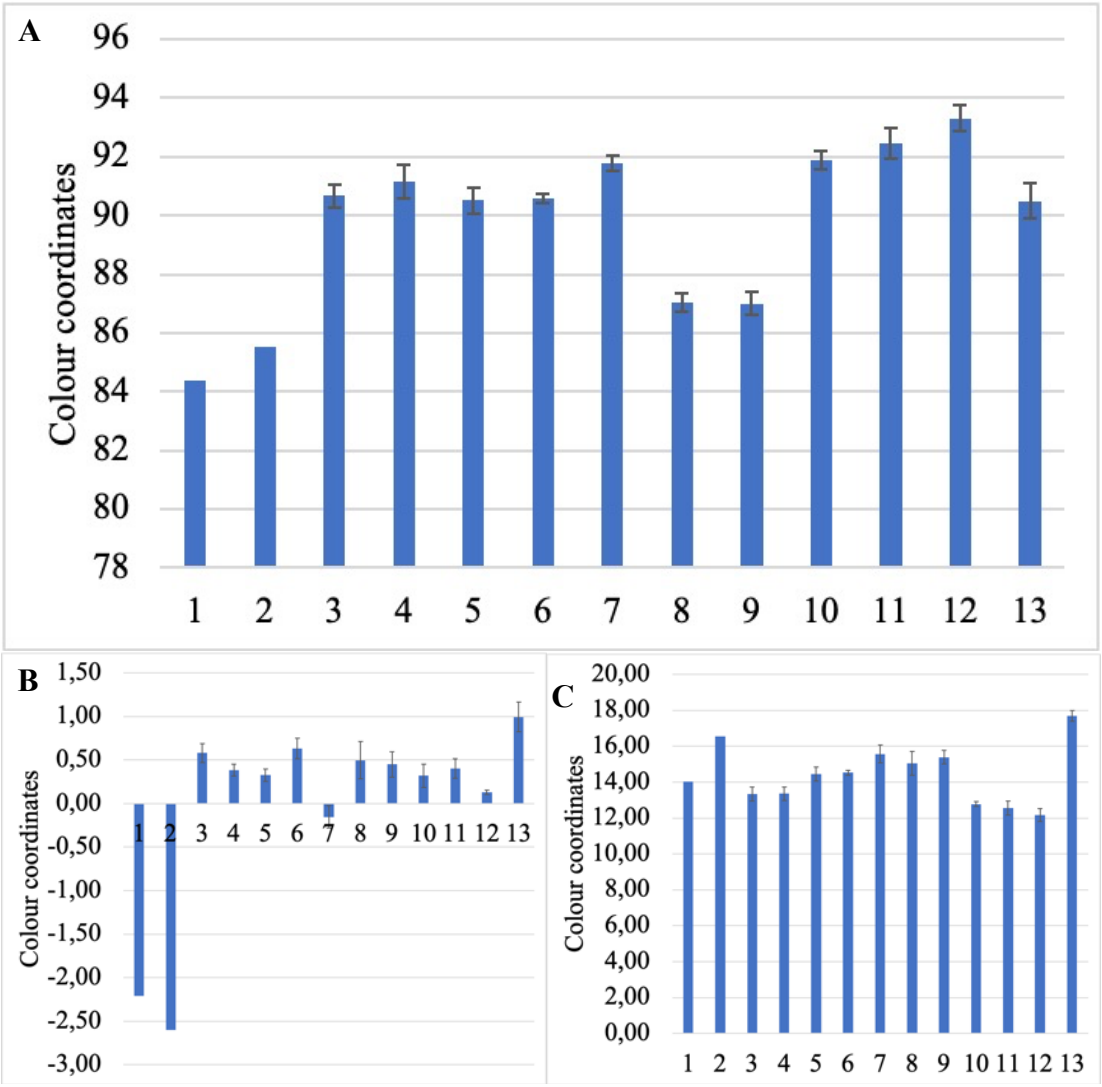

Figure 4.2 Colour measurements of the recipes 1-13 in preliminary production. A) Colour coordinate L*, B) Colour coordinate a*, C) Colour coordinate b*. For recipe 1 and 2 only a single measurement was performed, and standard deviations are therefore lacking. For recipe 3-13 three measurements were done (n=3).

4.1.3 High-pressure processing of TMP

As an alternative to freezer storage of the texture modified products, recipes 6 (19.9 % total protein), 8 (19.4 % total protein) and 11 (18.8 % total protein) were in addition subjected to high-pressure processing (HPP). The aim was to examine if the HPP could give a chilled product with long shelf life and with fresher appearance and better sensory properties. A chilled product with milder processing could be a good alternative to freezing, provided that the same texture was obtained.

4.1.3.1 Texture analysis

The texture analysis was done the same way as the frozen texture modified products described in chapter 3.1.5.1. The product was removed from the container before heated (75 °C) prior to analysis. The process of removing the product from the container may have interfered with the texture results, as it were difficult to get them out of the tray without pressing the sides. The texture of a not HPP-product within each of the three recipes, herby named reference (Ref.) was implemented in the texture measurement. The reference was stored chilled (4 °C), except for recipe 6 which was frozen (-30 °C). Figure 4.3 shows the results from the texture measurements of the high-pressure processed products.

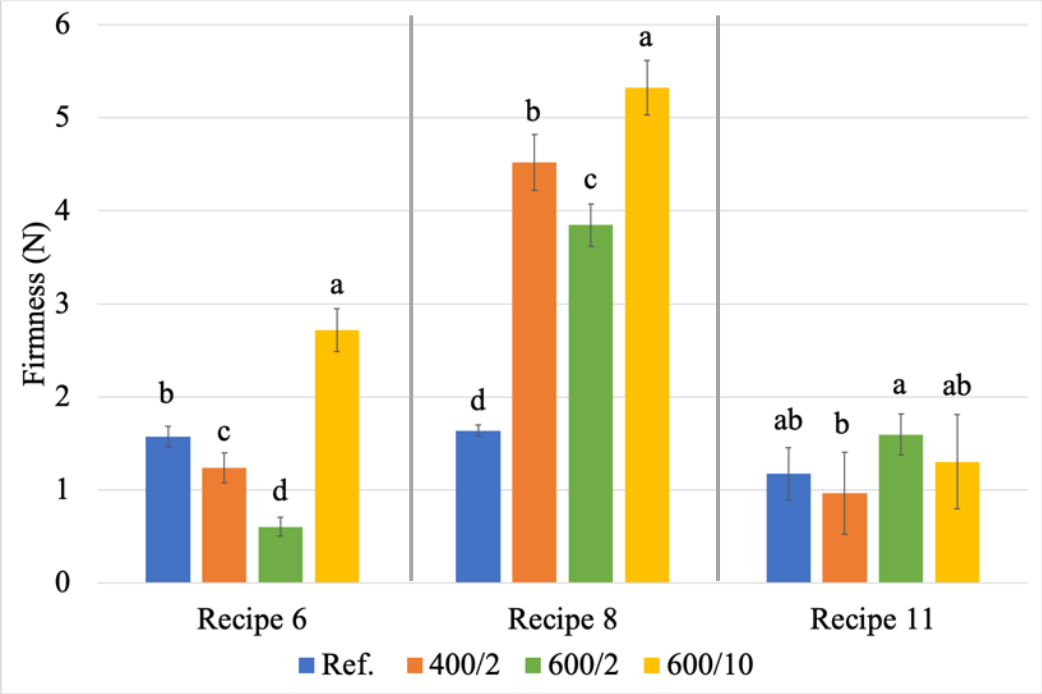

Figure 4.3 Measured firmness (N) in high-pressure processed (HPP) texture modified fish products: Recipe 6,8 and 11. The references has not undergone HPP. The diagram shows how the texture of each recipe was affected by the different HPP treatments. Recipe 6 and 8 (n=6), recipe 11 (n=9).

A one-way ANOVA *($p<0.05$)* analysis was done by comparing the recipe versus HPP-treatment (Figure 4.3). For recipe 6 all the treatments gave significantly different firmness. As expected, the most extreme treatment with 600 MPa for 10 min (600/10) gave the firmest texture. The softest product was the one treated with 600 MPa for 2 min (600/2). For recipe 8 all treatments also gave significantly different firmness. The most extreme treatment with 600 MPa for 10 min (600/10) gave the firmest texture and the not-HPP treatment gave the

softest texture. For both recipe 6 and 8, the 400/2 min treatment yielded a firmer texture than 600/2 min treatment. The statistics revealed that for recipe 11, the 600/2 min treatment gave a significantly firmer texture than 400/2 min, but there was no significant difference towards the other treatments.

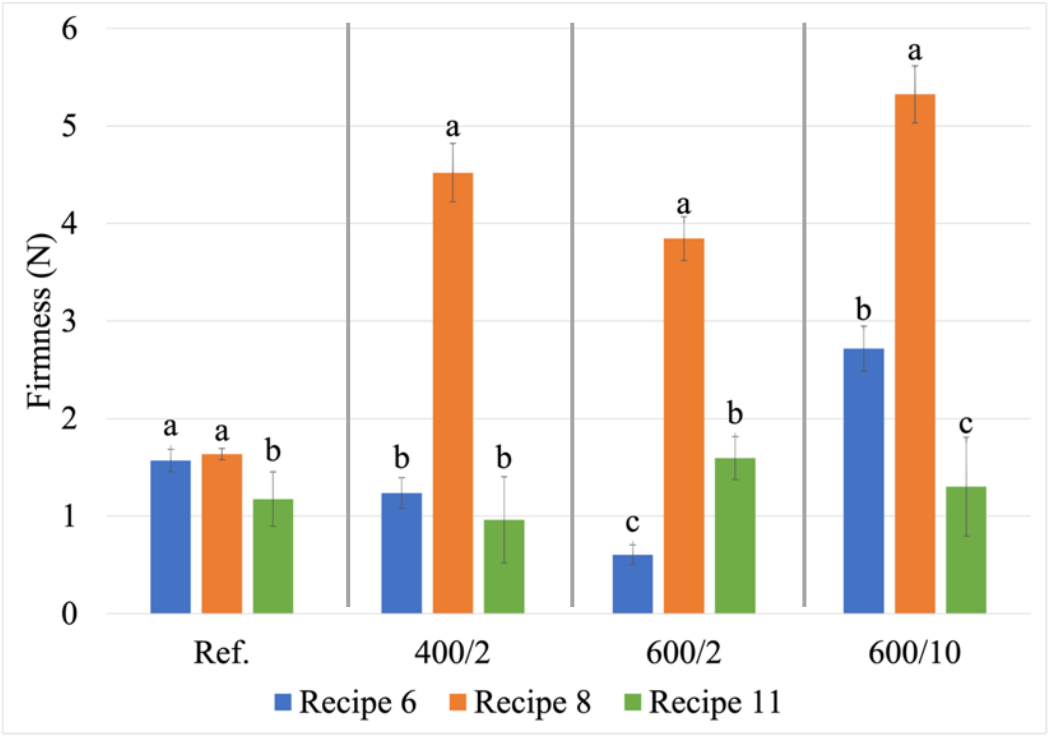

Figure 4.4 Measured firmness (N) in high-pressure processed (HPP) texture modified fish products: Recipe 6,8 and 11. The references has not undergone HPP. The diagram shows how the different HPP treatments affected each of the three recipes. Recipe 6 and 8 (n=6), recipe 11 (n=9).

Additionally, by using one-way ANOVA *($p<0.05$)* each of the different treatments were compared also to each recipe (Figure 4.4). For the products that was not-HPP (Ref.), recipes 6 and 8 were significantly firmer than recipe 11, which may be due to higher protein content. There was no significant difference between the firmness of recipe 6 and 8 for the non-pressurised products. For the products that had been treated with 400 MPa for 2 min (400/2) there was no significant difference between recipe 6 and 11, while recipe 8 was significantly firmer. Recipe 8 was also the firmest of products treated with 600 MPa for 2 min (600/2) and recipe 11 was significantly firmer than recipe 6 at this treatment. For the toughest pressure treatment, 600 MPa/10 min (600/10), there was a significant difference in the firmness of all the recipes. Recipe 8 was the firmest, and recipe 11 was the softest of the three.

The texture measurements of the high-pressure processed products showed that the firmness varied with both protein content and type of treatment, and it was difficult to see a clear pattern after just one experiment on each recipe. Recipe 11 showed least difference between the treatments (Figure 4.3). The reason for this might be due to the randomisation of batches produced prior to HPP, and the differences between the batches were perhaps evened out.

4.1.3.2 Microbiology - Shelf-life test of HPP products

For recipe 11, a shelf-life test with microbiological analyses were carried out on day 0, 17 and 35 after processing. The products were stored at 4 °C until analysis. As shown in Figure 4.5 there was bacterial growth in all four processing parameters. The colonies were examined in a microscope, showing rod shapes spore forming bacteria. The bacteria found was most likely *Bacillus sp.* which could withstand both the used heat treatment (preparation of fish raw material at 90 °C, 10 min) and the HPP treatment (maximum 600 MPa, 10 min). The same number of colonies were found in the reference (not HPP). Even the harsh treatment of 600 MPa/10 min was not sufficient to eliminate the bacteria. Still, after 5 weeks (day 35) there was not considerably more growth than at day 0 or 17 (Figure 4.5). Bacteria were only grown aerobically, which means that the spore forming bacteria had to be *Bacillus sp.* Since the products were stored at 4 °C the growth of *Bacillus cereus* was inhibited. The number of bacteria is therefore not increasing after 17 and 35 days.

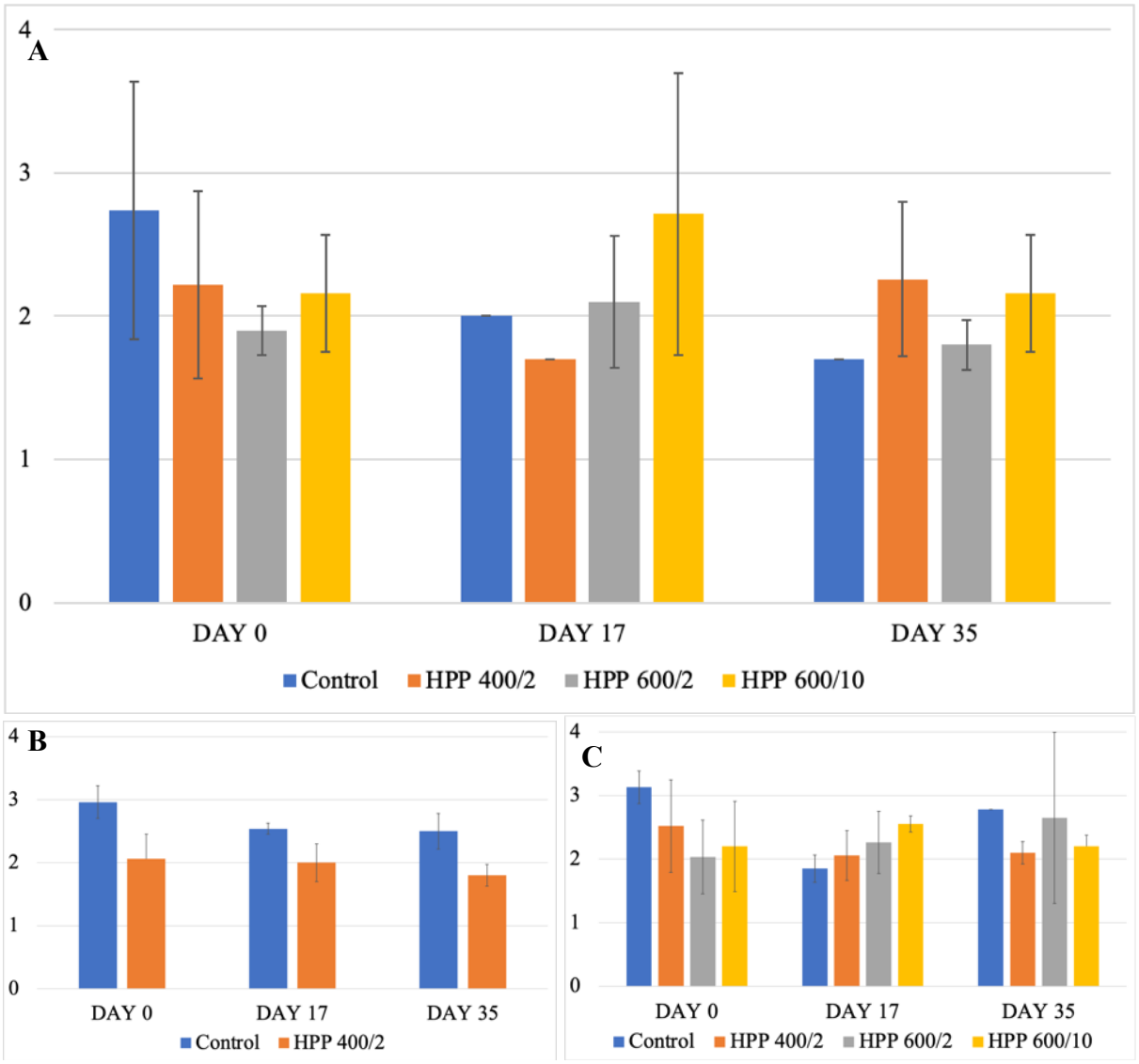

Figure 4.5 Bacterial count of high-pressure processed TMP after stored 0, 17 and 35 days at 4 °C. A) Plated on PCA B) Plated on PCA. (Eddy Jet) C) Plated on TSA with yeast extract.

4.2 Pilot production of texture modified products

After some evaluation recipe 3, 11 and 13 was chosen for the pilot production. Recipe 3 was used as the non-enriched product (17.4 % total protein) , recipe 11 had low enrichment (18.8 % total protein) while recipe 13 had high enrichment (20.2 % total protein). Both the enriched product contained whey protein, Hofseth fish protein hydrolysate and caseinate in the ratio 2:1:1. These three recipes were used for all the analyses done in the pilot step of this thesis and it was investigated how the different protein enrichments influenced the products.

4.2.1 IDDSI – Fork pressure test

The International Dysphagia Diet Standardisation Initiative framework provides simple tests to describe the texture of food and drinks. The tests demand little equipment and can be used by clinicians as well as patients. The IDDSI framework is however not implemented into Norwegian hospitals and nursing homes. The IDDSI test used for the texture modified products was the fork pressure test (See 3.2.1). The test was done in triplicates at time of service, immediately after the product was heated (core temperature 55 °C), and after 15-and 30 min standstill in room temperature to simulate delay during serving. The visual result of the IDDSI fork pressure test shown in Table 4.2. For recipe 3, which was the product with least amount of protein (17.4 %) and no enrichment, there was no blanching observed at the time of service. However, after 15 min a slight blanching of the fingernail was seen, and after 30 min standstill, there was full blanching of the fingernail. Recipe 11 which was enriched and had 18.8 % total calculated protein, had a slight blanching at the time of service. There was also blanching of the fingernail after 15- and 30 min, but more pressure had to be applied after 30 min. Lastly, recipe 13 which had the highest protein enrichment (20.2 %). The blanching was observed at all measuring times, but similarly to recipe 11, more pressure had to be applied to the fork in order to push down on the product as it cooled down. All products were also easily broken apart with the side of fork. The tendency was that the products got firmer when cooling and with increasing protein content, and it was more difficult to press down using the prongs on a fork.

Although the fork pressure test is a simple and fast test, it is strictly a visual test, and test result may vary depending on assessor. The pressure required for the thumb nail to blanch is approximately 17 kilopascals (kPa), which is close to the tongue pressure applied during swallowing or in other words, how much pressure needs to be applied in order masticate food without chewing it. People with swallowing difficulties or elderly may require food that can be easily masticated. According to the IDDSI framework, food that mashes with the application of pressure and does not return to its original shape when pressure is release, can be classified as either level 5-“minced and moist” or level 6-“soft and bite sized” (Cichero et al., 2017).

The temperature of the texture modified products (TMP) was logged during the IDDSI fork pressure test. The temperature at time of service was 55 °C. The average temperature after 15- and 30 min was measured at 45.4 °C and 39.0 °C, respectively.

Table 4.2 Reported visual results from the IDDSI fork pressure test. TOS=Time of service, 15 min AS=15 min after serving and 30 min AS=30 min after serving

	Recipe 3			Recipe 11			Recipe 13		
Parallels	**TOS**	**15 min AS**	**30 min AS**	**TOS**	**15 min AS**	**30 min AS**	**TOS**	**15 min AS**	**30 min AS**
1	No white	Slightly white	white	Slightly white	white	white	white	white	white
2	No white	Slightly white	white	white	white	white	white	white	white
3	No white	Slightly white	white	Slightly white	Slightly white	white	white	white	white

4.2.2 Texture analysis of TMP in pilot step

As mentioned earlier, it has been reported that blanching of the fingernail occurs when 17 kPa of pressure is applied. Still, no literature has validated the fork pressure test with instrumental analysis yet, and it is therefore difficult to correlate to the instrumental values. The IDDSI test was developed to provide a general picture about the texture of a product (Cichero et al., 2017).

To measure the texture of food more precisely, a texture analyser was additionally used. The firmness of the product was reported in Newton. The distance the probe penetrated into the sample, was initially set at 10 mm, but had to be reduced to 7 mm, which was about halfway into the product. This was crucial as when set at 10 mm, the probe sometimes reached the bottom and gave a much higher firmness (N) than anticipated. The products were measured at 55 °C which was defined as the service temperature. Additionally, the texture was measured after 15- and 30 min as in the IDDSI fork test to simulate what happens with the texture when the food cools during standstill after serving. Figure 4.6 shows that all three recipes had a similar pattern in regard to the texture when cooling.

Figure 4.6 shows how the texture of each recipe changed from the time of service to 15 min after serving and to 30 min after serving. The texture was measured to be significantly firmer 15 min after serving for all three recipes. Further, recipe 11 showed significantly increased firmness after 30 min standstill. The one-way ANOVA *($p<0.05$)* analysis also revealed that the texture did not change significantly from 15- to 30 min after service in recipe 3 with no enrichment, and recipe 13 with the highest amount of enrichment.

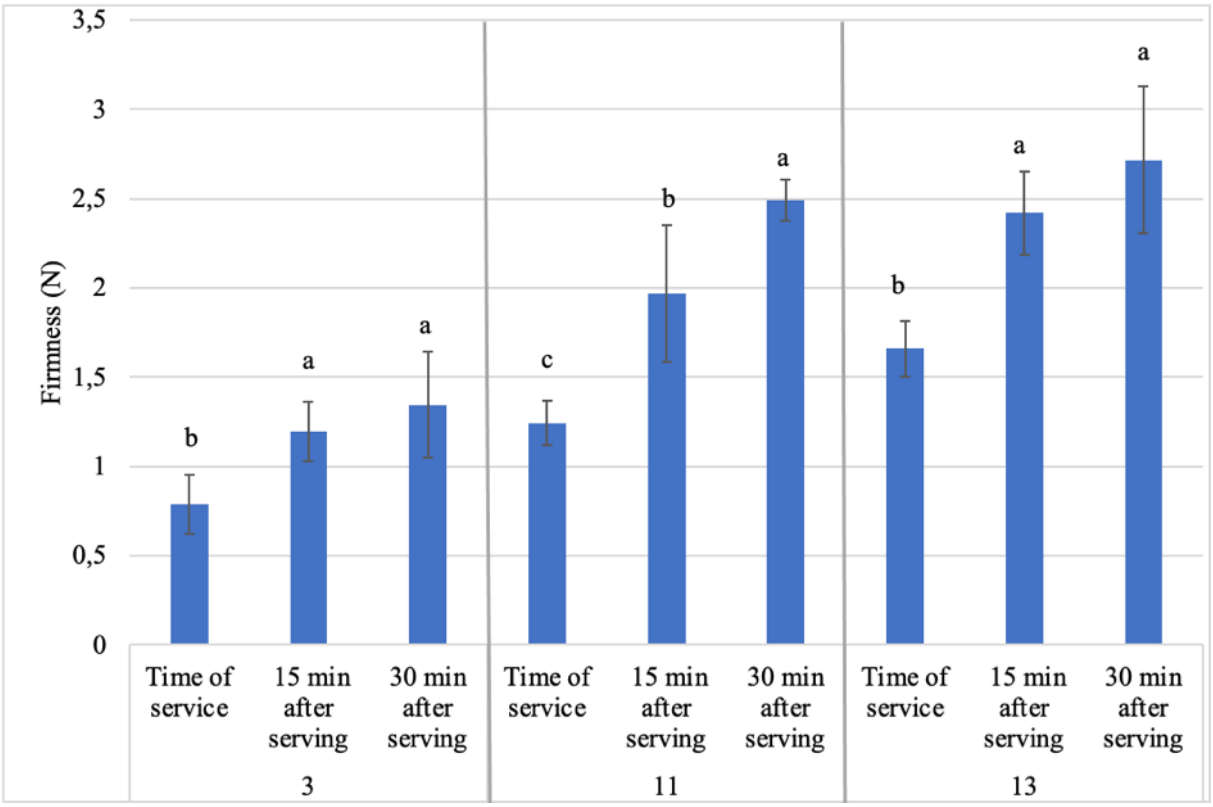

Figure 4.6 Measured firmness (N) of texture modified fish products (3, 11, 13) in pilot production (n=9). Texture was measure at time of service, 15 min after serving and 30 min after serving.

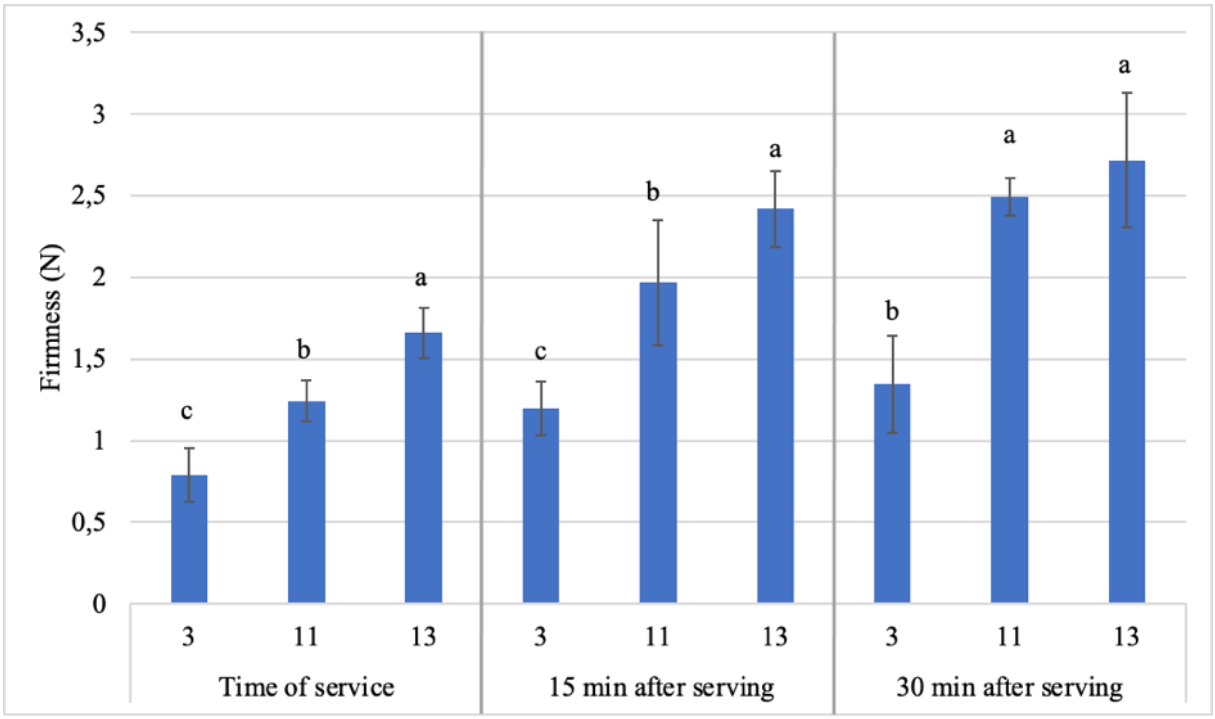

Figure 4.7 Measured firmness (N) of texture modified fish products (3, 11, 13) in pilot production (n=9). Texture was measure at time of service, 15 min after serving and 30 min after serving.

Figure 4.7 compares the texture of each recipe at each measuring time. The one-way ANOVA *($p<0.05$)* analysis revealed that there was a significant difference between the three recipes at the time of service (55 °C) and 15 min after service. As anticipated, recipe 13 with most protein enrichment, had the firmest texture. Recipe 3 with no enrichment was the softest product. There was no significant difference between recipe 11 and 13 after 30 min standstill. The texture analysis showed that the firmness of TMP increased with both amount of protein enrichment and with decreasing temperature.

4.2.3 Rheology on TMP in pilot step

Rheology measurements are most commonly used on fluids, semi-fluids or solids. The method applied to measure the texture modified products was also used in the MSc thesis by Østebrød (Østebrød, 2020). An amplitude sweep was performed on the recipes in pilot production (3, 11 and 13). The output gave storage modulus (Pa) G′ and G′′. The storage modulus G′ was above the G′′ graph which indicated that the sample was closest to being a solid. The aim was to determine the linear viscoelastic region (LVR), and therefore storage modulus G′ (Pa) was plotted against oscillation strain (%). The strain (%) was increased to 1 % which was within the LVR-region (Figure 4.8). The soak time was also increased from 10 sec to 5 min as it was recommended by the Trios software, and also due to the thickness of the sample (14 mm). The gap at which the texture modified product was measured was 500 µm. This could have been increased, as the structures within the sample might have been destroyed due to the compression. However, there was not enough time to optimize this method further. A temperature sweep was carried out in the range from 25 °C to 55 °C, and therefore the amplitude sweep was done at these two temperatures.

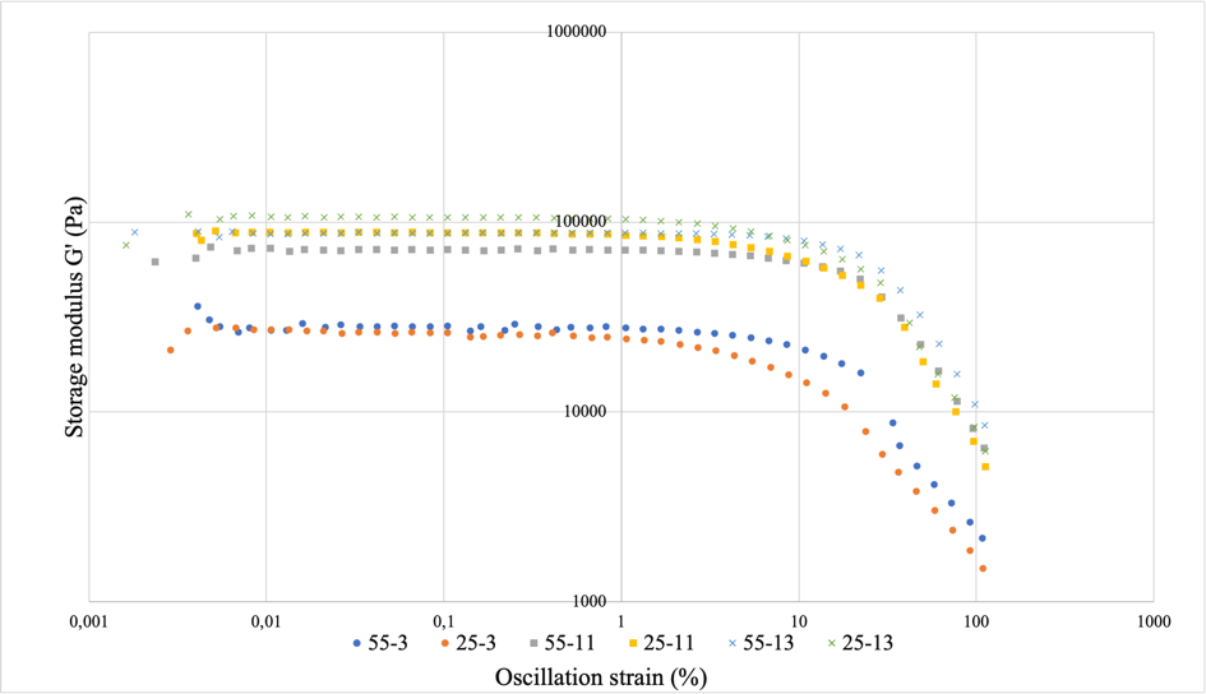

Figure 4.8 An amplitude sweep was performed on recipes 3, 11 and 13. Recipe 3 is indicated by circles, recipe 11 is indicated by squares, and recipe 13 is indicated by crosses. The selected temperatures were 25 and 55 °C. Storage modulus G' (Pa) was plotted against oscillation strain (%) while the frequency (Hz) was kept constant.

The behaviour of product 3, 11 and 13 during heating was investigated with a temperature sweep (Figure 4.9). The sweep started from 25 °C, increased to 55 °C, and then decreased back to 25 °C. Six parallels were measured for each product. Examination of the graph trend revealed a decreasing value of storage modulus when temperature increased. The decreasing trend indicated that the food structure is weakened, due to weaker bonding and less ordered internal structure. Loss modulus G" of all texture modified products (TMP) in pilot production was lower compared to storage modulus G'. The difference between G' and G" indicates the viscoelastic character of the sample. In this case, G'>G" which suggested that the product contains gelled or a solid structure and could be referred to as a viscoelastic solid material (*Basics of Rheology*, n.d.).

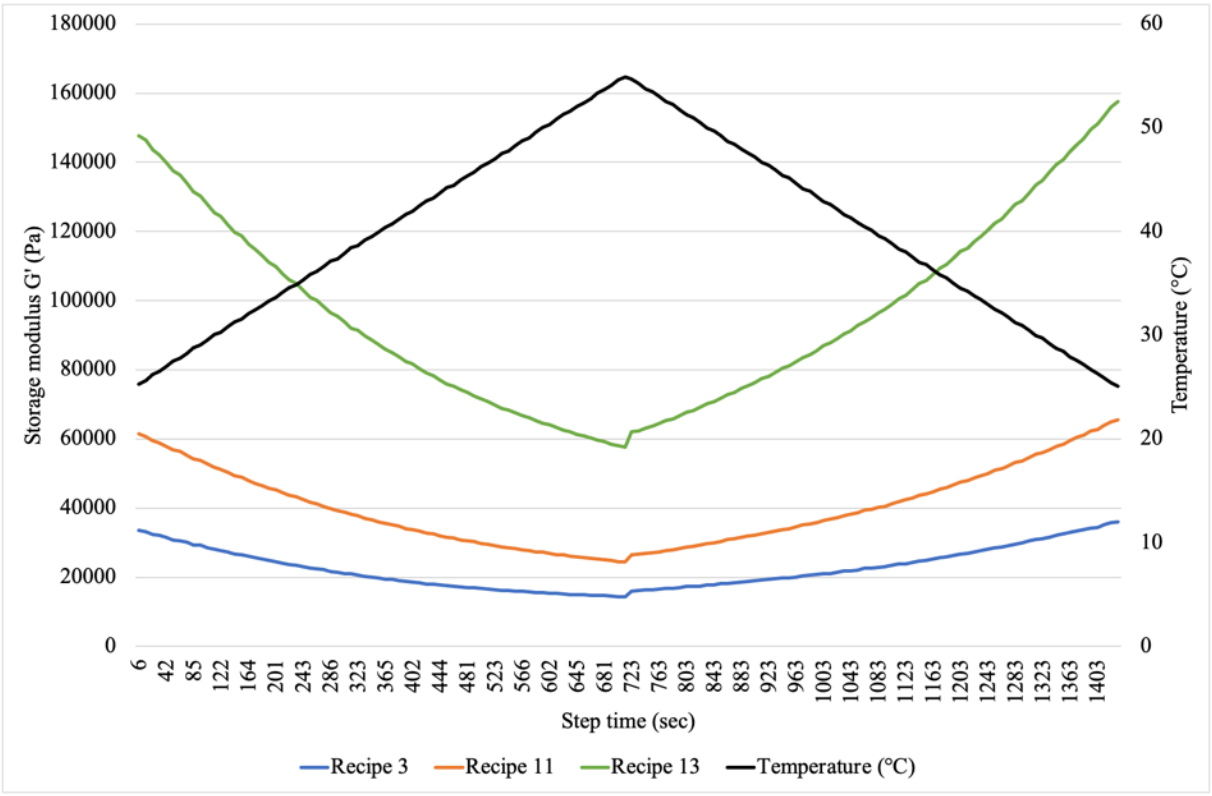

Figure 4.9 A rheological temperature sweep was performed on recipe 3, 11 and 13. The initial temperature was 25 °C, which increased to 55 °C and finally decreased to 25 °C. Storage modulus G' (Pa) was plotted against temperature (°C). Step time (sec) is plotted on the x-axis. (n=6)

One-way ANOVA *($p<0.05$)* showed that there was a significantly lower storage modulus G' (Pa) at 55 °C than at 25 °C. However, there was not a significant difference between 25 °C from the start, and 25 °C after cooling from 55 °C. The same was tendencies were shown for all three recipes. From Figure 4.9 the storage modulus appears to increase with increasing protein enrichment. As mentioned earlier, storage modulus could be associated with elastic behaviour, meaning that the property of maintaining structure in the texture modified product was stronger than its property to flow. This may be why there was no significant difference in modulus G' (Pa) at 25 °C in the start at 25 °C after cooling from 55 °C. The results from the rheological measurements on TMP, might indicate that there is a correlation between storage modulus, protein content and temperature.

4.2.4 Colour analysis of TMP in pilot step

Colour was measured on the same products as from the texture analysis after cooled down to room temperature. A total of 9 parallels where measured. All the products had a light colour as the L* values were between 89-95. According to a Tukey pairwise comparison test, there was

no significant *(p=0.400)* difference between the recipes when measuring the colour coordinates L* and a* (Figure 4.10 A and B). However, when a Fisher pairwise test was applied, recipe 13 was shown to be significantly redder than recipe 3 and 11. For colour coordinate b* there was significant difference between all the recipes. Recipe 13 had the highest b* value which indicate a more yellow product. This may be due to the amount of enrichment added. The value of b* decreased with decreasing protein enrichment (Figure 4.10 C) *(p<0.05)*.

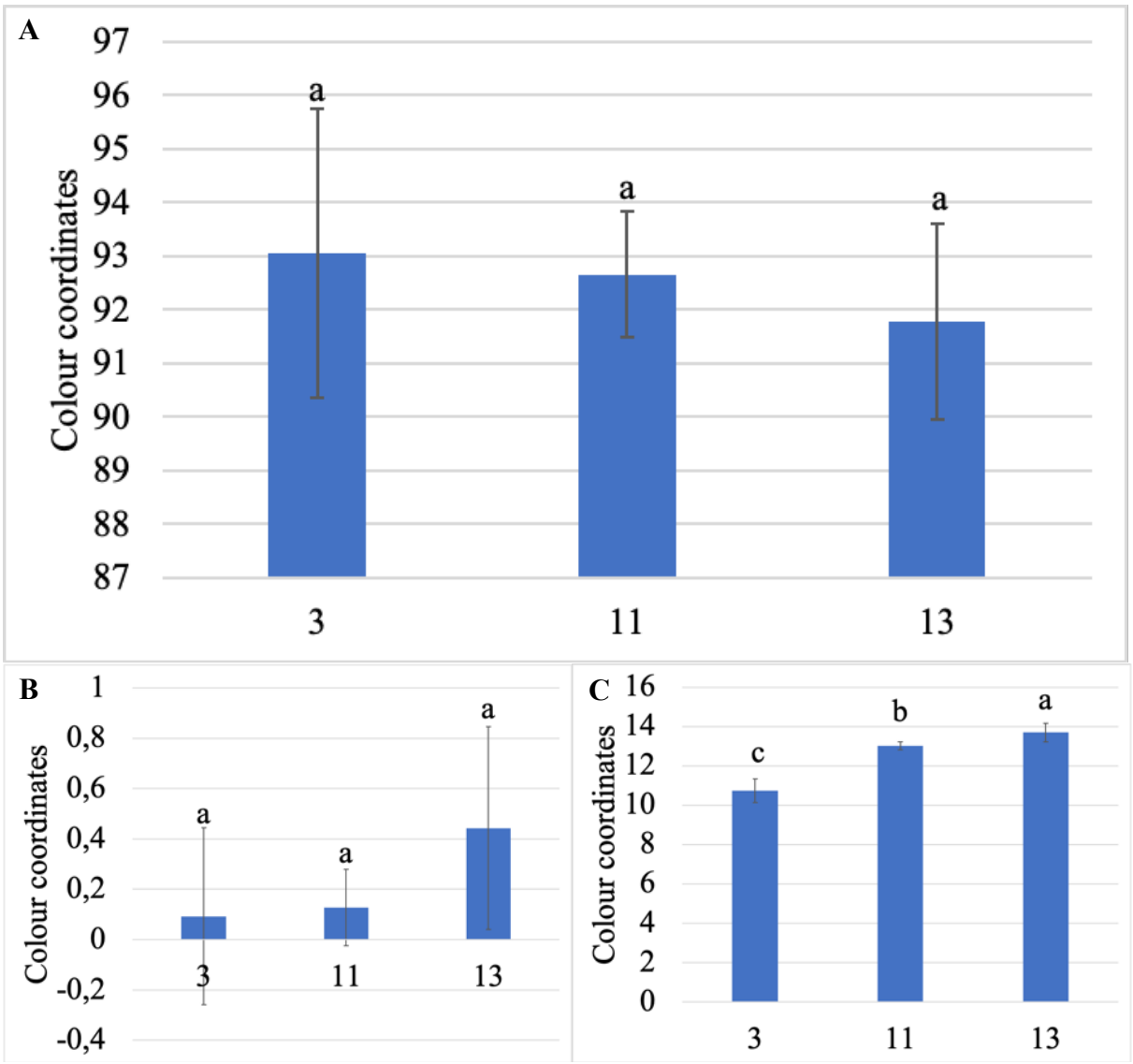

Figure 4.10 Colour measurements of the recipes 3, 11 and 13 in pilot production. A) Colour coordinate L*, B) Colour coordinate a*, C) Colour coordinate b*. Three measurements were done (n=3).

4.3 Preliminary production of enriched soup

The preliminary production of the soup started with a basic recipe. The main ingredients where reduced fish broth, whipped cream and milk. Also modified corn starch and salt was added. The aim with this recipe was to develop an enriched soup that also could be used as a sauce, with the highest possible amount of protein and obtain preferred sensory characteristics that were suitable for elderly and people with dysphagia. As in the production of texture modified products, fish protein hydrolysate (FPH), whey protein concentrate (WPC) and caseinate were used as enrichment. Both non-enriched and enriched recipes were developed. As the protein enrichment of the soup increased, it was more difficult to homogenize, and the heating time seemed to increase slightly. The development of the recipes is described in more detail in chapter 3.3.2.

Table 4.3 Ingredients and recipes for the soup. Recipes tested during the preliminary production. The recipes are named by a number representing the order of production. The total nutritional values are calculated, and all values are given in percentage (%).

Ingredients	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1/3 reduced fish broth	50.0	60.8	57.4	58.5	59.4	56.3	57.8
Milk	22.5	11.1	10.5	10.7	10.9	10.3	10.6
Whipping cream	25.0	24.8	23.4	23.9	24.2	23.0	23.6
FPH	0.0	0.0	1.4	1.0	0.6	1.8	1.2
Caseinate	0.0	0.0	1.4	1.0	0.6	1.8	1.2
WPC80	0.0	0.0	2.8	1.9	1.2	3.7	2.5
Total protein	4.0	4.1	8.7	7.2	6.1	10.1	8.1
Total fat	10.1	9.6	9.3	9.4	9.5	9.3	9.4
Total salt	0.3	0.6	0.6	0.6	0.6	0.6	0.6

4.3.1 Viscosity measurements of enriched soup in preliminary step

To measure the viscosity of the enriched soup a viscometer was used. The same speed was used for all soups (100 RPM), but different spindles had to be used in soups with different texture in order to obtain a torque value within the range (10-100 %). This may have affected the results to some degree. As the run time was only set to 30 sec, and this may not have been long enough for the viscosity to stabilise. The viscosity appeared to be higher at 25 °C that 55 °C, as would

be expected. Figure 4.11 may also indicated that the viscosity increased somewhat with increasing protein content, except for the soup with 10 % protein.

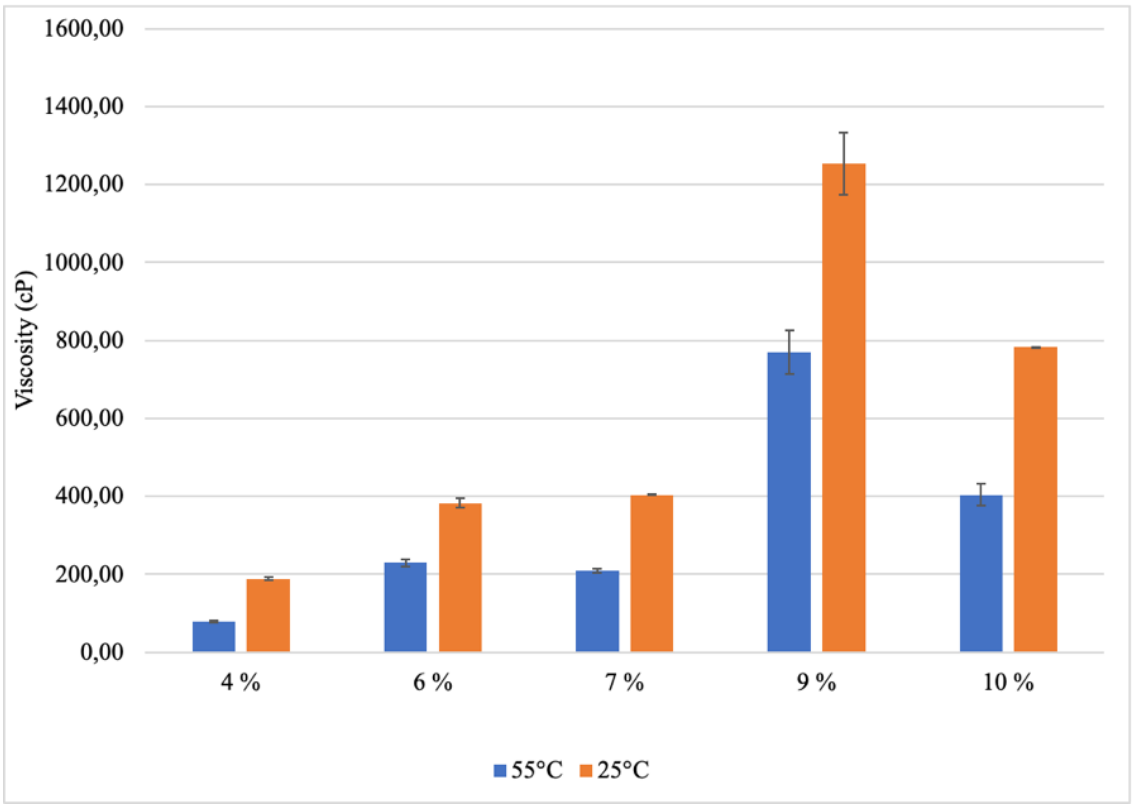

Figure 4.11 Viscosity measurements in preliminary testing of soup with different amount of total protein (%). Run time was 30 sec. (n=6)

Due to the somewhat unexpected results from the first viscosity measurements of the soup. It was decided to repeat the analysis for recipes containing 9- and 10 % protein. The soup was first measured as done previously, and then again aften giving the soup a proper heat treatment at core temperature 90 °C with 5 min holding time. Figure 4.12 shows that the viscosity at 25 °C and 55 °C increased after the soup had been heated to 90 °C with 5 min holding time. This may indicate that the starch has not been sufficiently activated, due to low temperature when producing the soup. The apparent viscosity measured in the other soups, could therefore be higher than shown in Figure 4.11.

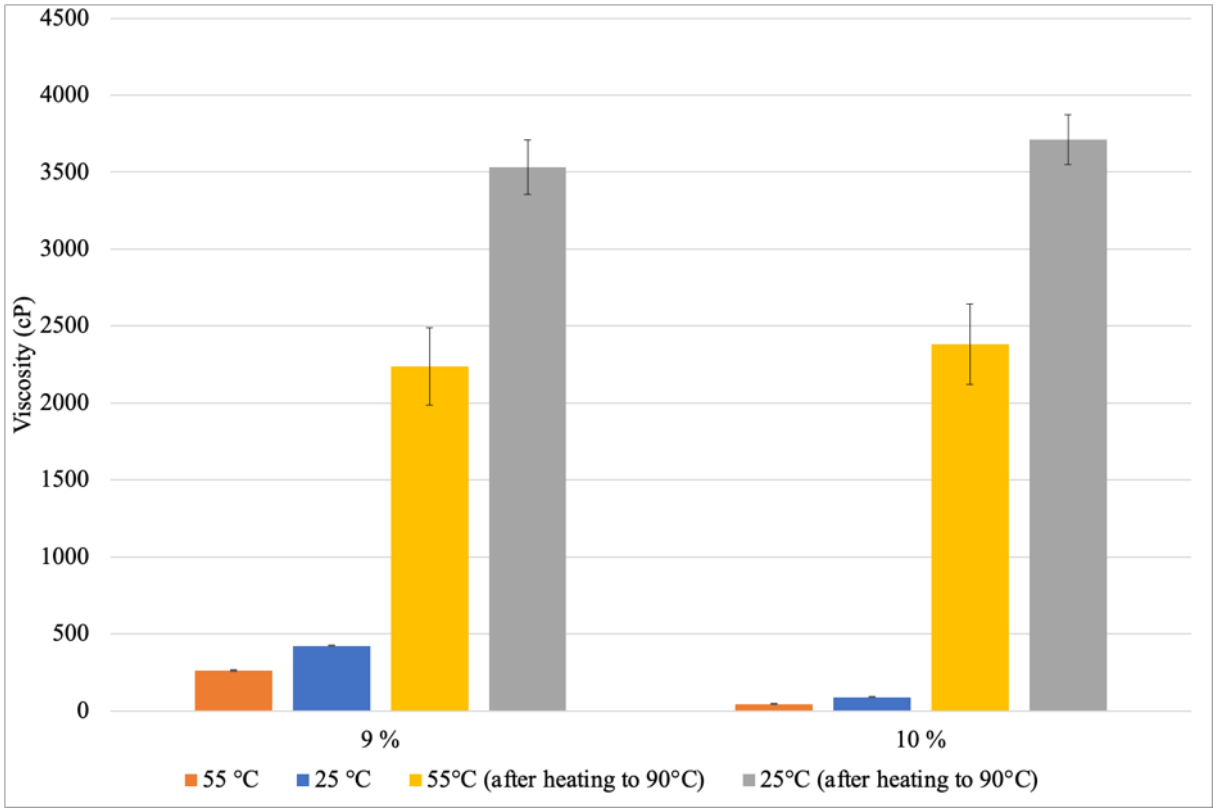

Figure 4.12 Repeated viscosity analysis in soup with 9 and 10 % total protein, with comparison of before and after it being heated to 90 °C. Run time was 30 sec. (n=6)

4.3.2 Rheology measurements of enriched soup in preliminary step

A flow sweep was conducted on the texture modified soup. The geometry used was a cone plate (Cone SST ST 40 mm 2deg smart-swap, serial number:105132) with a 2° angle and fixated gap at 52 µm. As for the measurements done with the viscometer, the flow sweep was done at both 25 °C and 55 °C. All the measurements had the same tendencies as shown in Figure 4.13 where the viscosity decreases with an increase in shear rate. This is commonly known as shear thinning. In the preliminary step only one rheological measurement was done on each recipe, and standard deviations are therefore lacking. Recipes containing 4-, 7-, and 9 % protein were measured at 55 °C. Whereas, recipes containing 6 % and 10 % protein were measured at 25 °C and 55 °C. The rheological measurements were fitted to a best fit graph plotting shear stress vs rate. All of the graphs had a good fit, shown by a R^2 value of 0.99. According to the best-fit model (stress/rate), Herschel-Bulkley equation was a suitable model to describe the flow behaviour of the products. The data from the analysis are shown in Table 4.4

Table 4.4 Data of Herschel-Bulkley Parameters of enriched soup products from preliminary production at different temperatures. The data are from one measurement only, and standard deviations are therefore lacking.

Temp. (°C)	Parameters	4 %	6 %	7 %	9 %	10 %
25	Y. S (Pa)		1.098			1.177
	K (Pa.S^n)		1.063			1.634
	n		0.508			0.514
	R^2		0.995			0.996
55	Y. S (Pa)	0.342	0.743	0.564	1.152	1.353
	K (Pa.Sn)	0.246	0.661	1.311	0.956	1.614
	n	0.696	0.542	0.445	0.538	0.472
	R^2	0.999	0.995	0.999	0.995	0.990

Y. S = Yield stress (Pa)
K = Flow Consistency Constant (Pa.S^n)
n = Flow behaviour index (unitless)
R^2 = Coefficient of determination

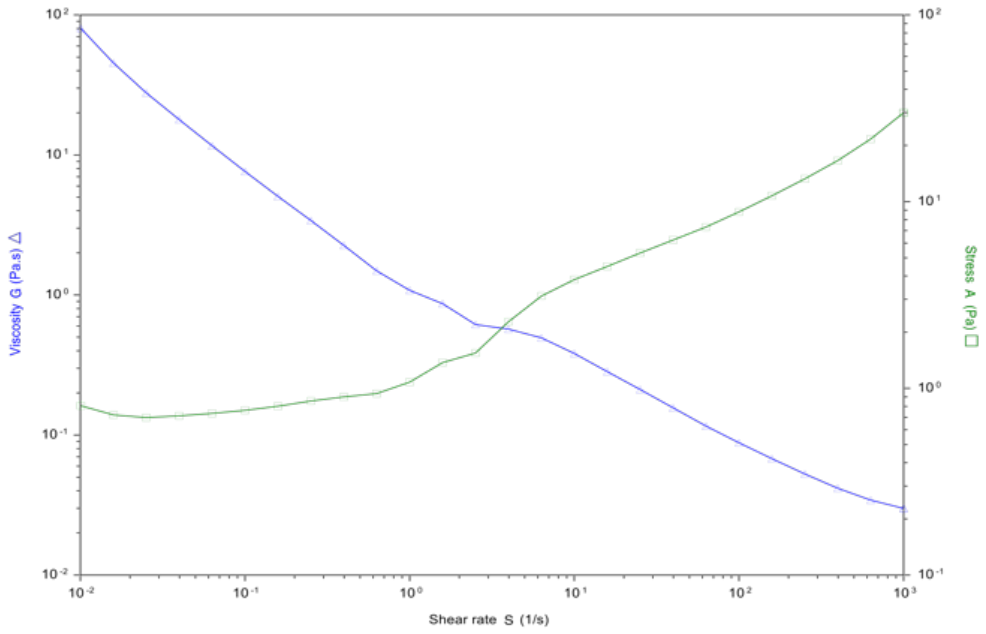

Figure 4.13 Graph from rheological measurment of preliminary production. The soup contained 6 % protein and was measured at 55 °C. Shear rate (1/s) is on the x-axis and viscocity (Pa.s) and stress (Pa) on the y-axis.

4.4 Pilot production of enriched soup

A control thermometer used during heating of the soup in the Kenwood induction cooker, showed that the programmed temperature (90 °C) was insufficient to obtain a core temperature of 90 °C in the soup (Figure 4.12). This could influence both safety and texture properties. Therefore, a new heating procedure was used in the pilot production. The soup was first mixed and heated in the Kenwood mixer for 10 min (at program temperature 90 °C), before being poured in a large saucepan. All the batches were mixed, to even out minor differences. The soup was then heated to 90 °C and kept at that temperature for 5 min, using an induction cooktop and by stirring manually. This heating procedure caused a thicker soup, most likely because of a different activation of the modified corn starch added.

4.4.1 IDDSI – Flow test

The IDDSI flow test was carried out to examine the thickness and flowability of the soup. In general, the enriched soups from the pilot production became significantly *($p<0.05$)* thicker 15- and 30 min after serving, and with increasing protein content. The temperature of the soup was logged during the flow test. The temperature at time of service was 55 °C. The average temperature after 15- and 30 min was measured at 40.1 °C and 32.7 °C, respectively. According to how many ml was left in the syringe after the flow test, the soups were designated an IDDSI level (Table 4.5).

Table 4.5 Results of the IDDSI flow test.

4 % protein		
At service	15 min after service	30 min after service
Level 1	Level 2	Level 2
6 % protein		
At service	15 min after service	30 min after service
Level 3	Level 3	Level 3
8 % protein		
At service	15 min after service	30 min after service
Level 3	Level 4	Level 4

Figure 4.14 shows the results from the IDDSI flow test. Statistical analysis *($p<0.05$)* was done by comparing the time of measurement within each recipe. In the soup with 4 % protein, the ml remaining in the syringe increased significantly from time of service to 30 min after serving. For both the 6- and 8 % soup, the ml remaining was significantly different after 15 min standstill. There was no significant difference in the soup with 6 % protein after 15- and 30 min, and the same went for the soup with 8 % protein. This indicates that as the soup cools down during standstill it thickens.

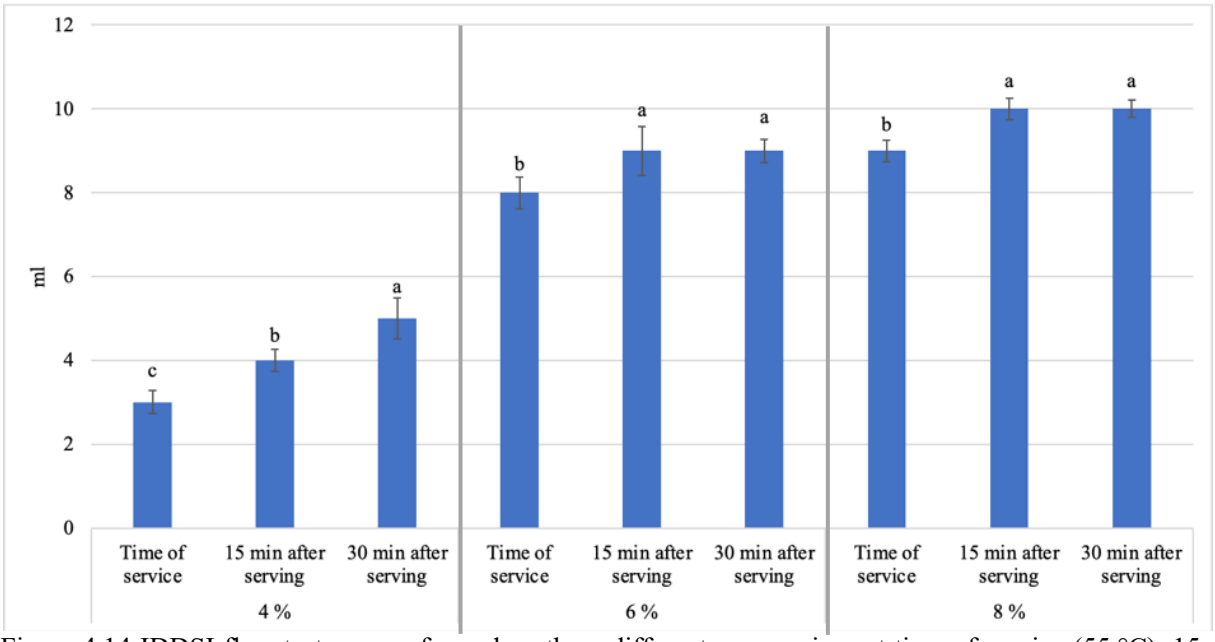

Figure 4.14 IDDSI flow test was performed on three different soup recipes at time of service (55 °C), 15- and 30 min after serving. Comparing the time of measurement within each recipe

In Figure 4.15 the results show the ml remaining for each recipe at time of service, 15 min after serving and 30 min after serving. The three recipes are compared at the different measuring times. The amount of ml left in the syringe increased significantly with increasing protein enrichment of the soup. This indicated that the protein enrichment corresponded to a thicker soup. Even if the soups were significantly different statistically it may not interfere with the designated IDDSI level, as each level is within a range of 4 ml (Table 3.6).

According to the IDDSI framework, the soup with 4 % protein was at level 1–"slightly thick", but as it cooled for 15 min, the level changed to level 2-"mildly thick". After 30 min standstill, the soup (4 %) was still at level 2. The soup with 6 % protein was measured to be at level 3-"moderately thick" at time of service, 15 min after service and 30 min after service. The soup

with 8 % protein also belonged to level 3 at the time of service according to IDDSI. But after 15- and 30 min standstill, the ml remaining in the syringe corresponded to level 4-“extremely thick”. Both level 3 and 4 overlap the food categories of the IDDSI framework, “liquidised” and “pureed”, respectively. The soups at these levels may therefore be too thick to be used as a soup but are perhaps more suitable as a thick sauce.

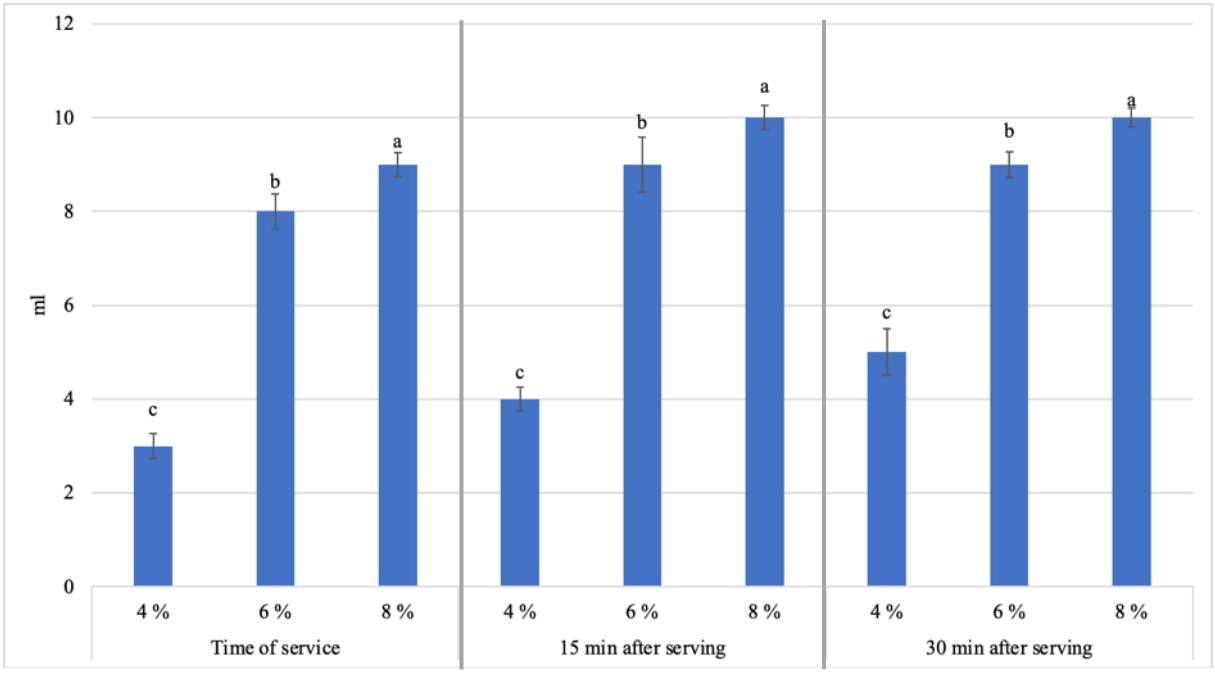

Figure 4.15 IDDSI flow test was performed on three different soup recipes at time of service (55 °C), 15- and 30 min after serving. The three recipes are compared at the different measuring times.

4.4.2 Viscosity measurements of enriched soup in pilot step

From the preliminary step to the pilot step the method for measuring viscosity was changed as described in chapter 3.3.4. Viscosity measurement were done on soup recipes with 4, 6 and 8 % total calculated protein as done in the IDDSI flow test.

After viscosity measurements with a 30 min runtime, there was no significance difference between the viscosity at 25 °C and 55 °C for the soup with 4 % protein. Mean values that do not shear a letter are significantly different (Figure 4.16) *($p<0.05$)*. The statistical analysis revealed that the viscosity did not significantly change with different protein enrichment at 55 °C. The soups containing 6 % and 8 % protein were significantly more viscous than recipe 4 % at 25 °C. Recipe 6- and 8 % were not significantly different at 25 °C.

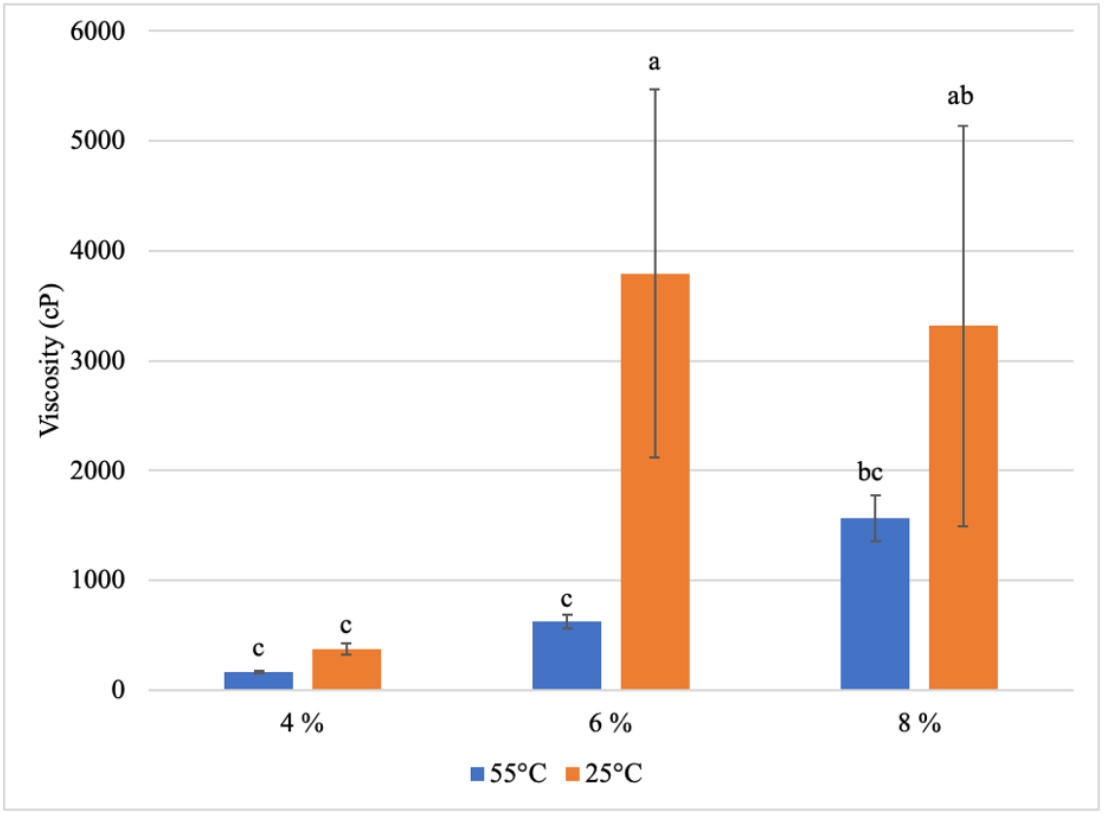

Figure 4.16 Average viscosity measurements (n=6) during a period of 30 min at 25 °C and 55 °C in three soups with a different level of total protein. Viscosity shown in centipoise (cP).

Furthermore, since the viscosity was measured every third minute during a period of 30 min, Figure 4.17 shows how the viscosity changed during 30 min, at 25 °C, 55 °C and when temperature started at 55 °C and no more heat was applied as it cooled. On an average the soups cooled down to approx. 47.6 °C after 15 min and 43.5 °C after 30 min.

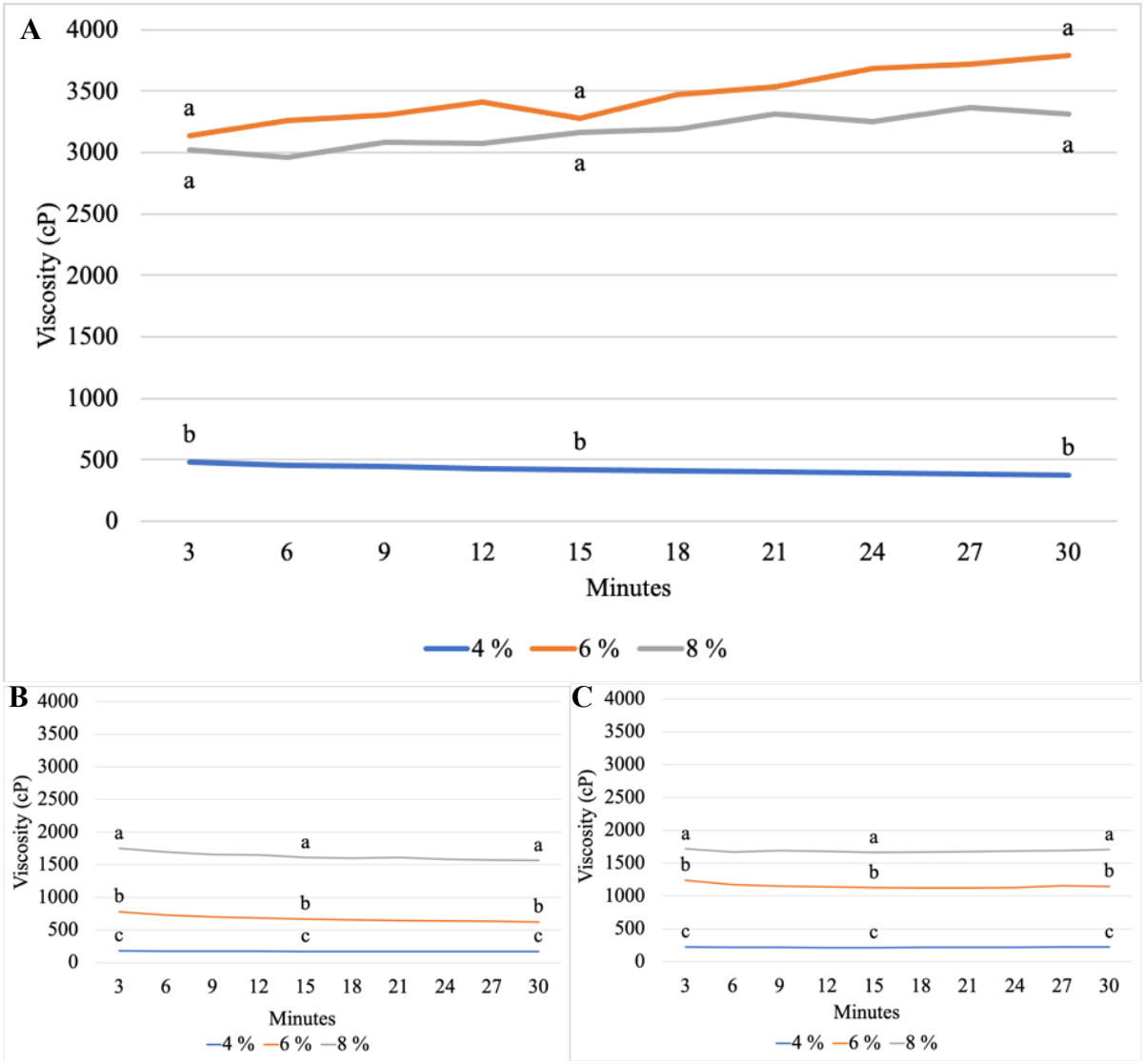

Figure 4.17 Viscosity was measured every 3. min during a total run time of 30 min. A) Temperature was kept constant at 25 °C. B) Temperature was kept constant at 55 °C. C) Temperature started at 55 °C let cooled down, no more heat was applied. (n=6)

The measured viscosity at 25 °C during a total run time of 30 min did not significantly change over time. The measured viscosity of the soup with 4 % protein was significantly lower than for recipes with 6 % and 8 % protein. *($p<0.05$).* The Tukey pairwise comparison showed that the measured viscosity at 55 °C during a total run time of 30 min did not significantly change over time. However, when a Fisher pairwise comparison was applied it showed a significant different viscosity at 3 min and 30 min. The measured viscosity of the soups was significantly different between the three recipes. The soup with 4 % protein was significantly lower than for recipes with 6 % and 8 % protein. And the soup with 8 % had the highest measured viscosity *($p<0.05$).* The measured viscosity when cooling from 55 °C during a total run time of 30 min did not significantly change over time. The measured viscosity of the soup was significantly

different between all the recipes. The soup with 4 % protein was significantly lower than for recipes with 6 % and 8 % protein. And the soup with 8 % had the highest measured viscosity *($p<0.05$)*. The viscosity measurements for 30 min showed that the viscosity increased with increasing protein enrichment.

4.4.3 Rheology measurements of enriched soup in pilot step

The IDDSI framework provide simple tests to determine the flow and thickness of a liquid, but given the complex rheology of texture modified liquids it is an important challenge to identify the IDDSI test result in relation to the various rheological parameters (Hanson et al., 2019). In addition to the IDDSI flow test rheological measurement were carried out on the soup products.

Shear thinning was the main observed factor in all soups. Shear thinning, also known as non-Newtonian behaviour, occurs when the viscosity is decreasing with increased stress. The rheological measurements were fitted to a best fit graph plotting shear stress vs rate. All of the graphs had a good fit with a R^2 value of 0.99. According to the best-fit model (stress/rate), Herschel-Bulkley equation was an adequate model to describe the flow behaviour of the soups. All three soups were measured at 55 °C, and only the extremes 4 % and 8 % were measured at 25 °C.

Table 4.6 Data of Herschel-Bulkley Parameters of enriched soup products at different temperatures. Different uppercase letters express the significant differences *($p<0.05$)*.

Temp. (°C)	**Parameters**	**4 %**	**6 %**	**8 %**
25	Y. S (Pa)	1.866 ± 0.49^{c}		12.003 ± 1.17^{a}
	K (Pa.S^n)	1.222 ± 0.16^{b}		6.823 ± 1.06^{a}
	n	0.558 ± 0.02^{c}		0.461 ± 0.03^{d}
	R^2	0.990		0.993
55	Y. S (Pa)	1.391 ± 0.06^{c}	6.914 ± 1.27^{b}	11.49 ± 0.98^{a}
	K (Pa.S^n)	0.271 ± 0.03^{c}	0.621 ± 0.14^{bc}	1.399 ± 0.19^{b}
	n	0.782 ± 0.01^{a}	0.781 ± 0.01^{a}	0.700 ± 0.01^{b}
	R^2	0.999	0.999	0.999

Y. S = Yield stress (Pa)
K = Flow Consistency Constant (Pa.S^n)
n = Flow behaviour index (unitless)
R^2 = Coefficient of determination

One-way ANOVA *($p<0.05$)* analysis was done for each of the rheological parameters presented in Table 4.6. The analysis of each parameter was compared to all recipes and temperatures. There was a significant difference in yield stress between all three recipes at 55 °C, meaning that the yield stress increased with increasing protein content. The recipes with 4- and 8 % protein, which were also measured at 25 °C, were likewise significantly different. Within each recipe, there was no significant difference in yield stress at temperatures 25- and 55 °C. The pressure gradient created when the bolus is squeezed between the tongue and palate is essential for causing the bolus to flow (Steele et al., 2014). Therefore, the higher the yield stress, the more force is needed to initiate the flow. For individuals with weak swallowing reflux, swallowing food with very high yield stress, may cause post-swallow residues. The high values of yield stress in the Herchel-Bulkley model indicate a high stability of the soup structure (Gibiński et al., 2006). The recipes containing the highest protein content may therefore not be suitable for people with swallowing difficulties.

The flow consistency constant There was a significant difference between recipe 4 % and 8 % at both 25 °C and 55 °C, except there was no difference in the flow consistency constant between the soup with 4 % protein at 25 °C and the 8 % soup at 55 °C. The consistency constant got significantly lower at 55 °C for both 4- and 8 %. A high flow consistency constant (K) is indicative of the viscosity of soup. As a fluid becomes more viscous, the consistency (K) increases (Krystyjan et al., 2012).

The flow behaviour index was significantly different in all soups except between recipe 4 % and 6 % at 55 °C. At this temperature the flow behaviour index was significantly lower in the soup containing 8 % protein. The lowest measured value was in the 8 % soup at 25 °C, while the highest value was the 4 % soup at 55 °C. The flow behaviour index (n) indicates the degree of non-Newtonian characteristics of a fluid. When n=1, it is defined as a Newtonian fluid. If n is between 0 and 1, the fluid is classified as pseudoplastic, exhibiting shear-thinning (Alvarez et al., 2004; Krystyjan et al., 2012).

The blue graph shows viscosity vs shear rate, while the green graph shows shear stress vs shear rate. The figure shows that viscosity is decreasing, while the stress in increasing. This is known as shear thinning (Figure 4.18).

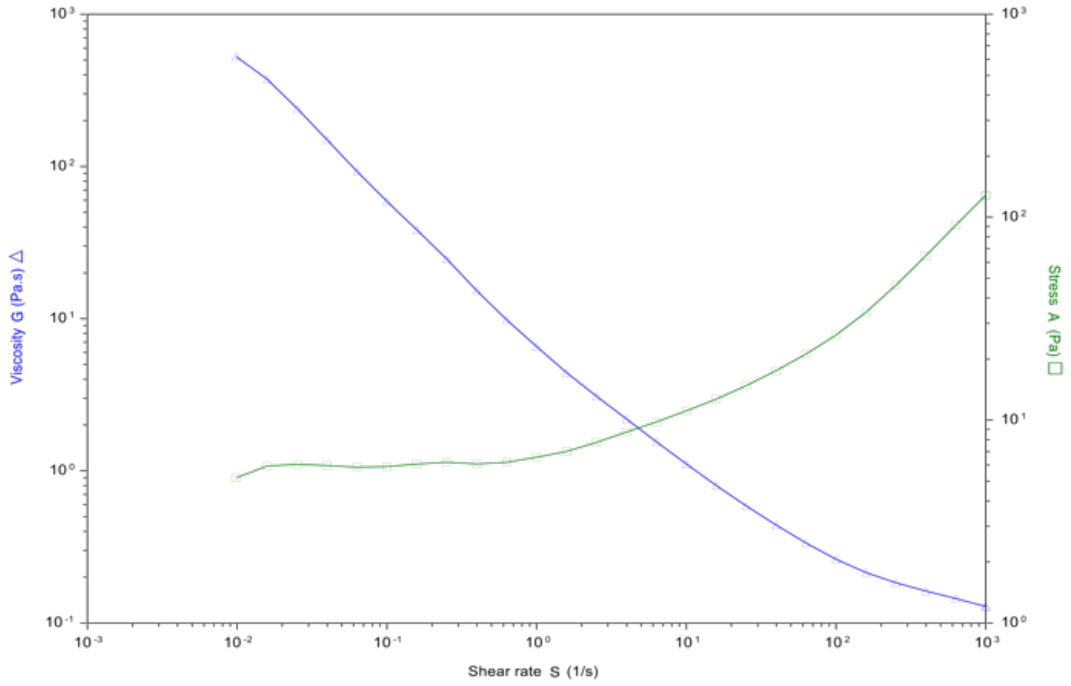

Figure 4.18 Graph from rheological measurment of soup with 6 % protein at 55 °C. Shear rate (1/s) is on the x-axis and viscocity (Pa.s) and stress (Pa) on the y-axis.

4.4.4 Colour measurements of enriched soup in pilot step

Colour was measured on the same products as from the IDDSI flow test when they had cooled down to room temperature. A total of 18 parallels where used. There was a significant difference between the recipes when measuring the colour coordinates L* and a* (Figure 4.19 A and B). Recipe 4 % had the highest L* value which indicate a whiter product, and recipe 8 % was the least white of the three. This may be due to the amount of enrichment added to the recipe with 8- and 6 % respectively. The value of a* was lowest for the recipe with 6 % protein, indicating that this had the least redness. Recipe with 4 % protein had according to the colour measurement more redness. For colour coordinate b* there was no significant difference between the three recipes. Meaning that the recipes had similar yellow tones, even though they had different amount of protein enrichment. The yellow tones of the protein enrichment may be masked by the white colour of ingredients added, such as milk or whipping cream (Figure 4.19 C) *($p<0.05$).*

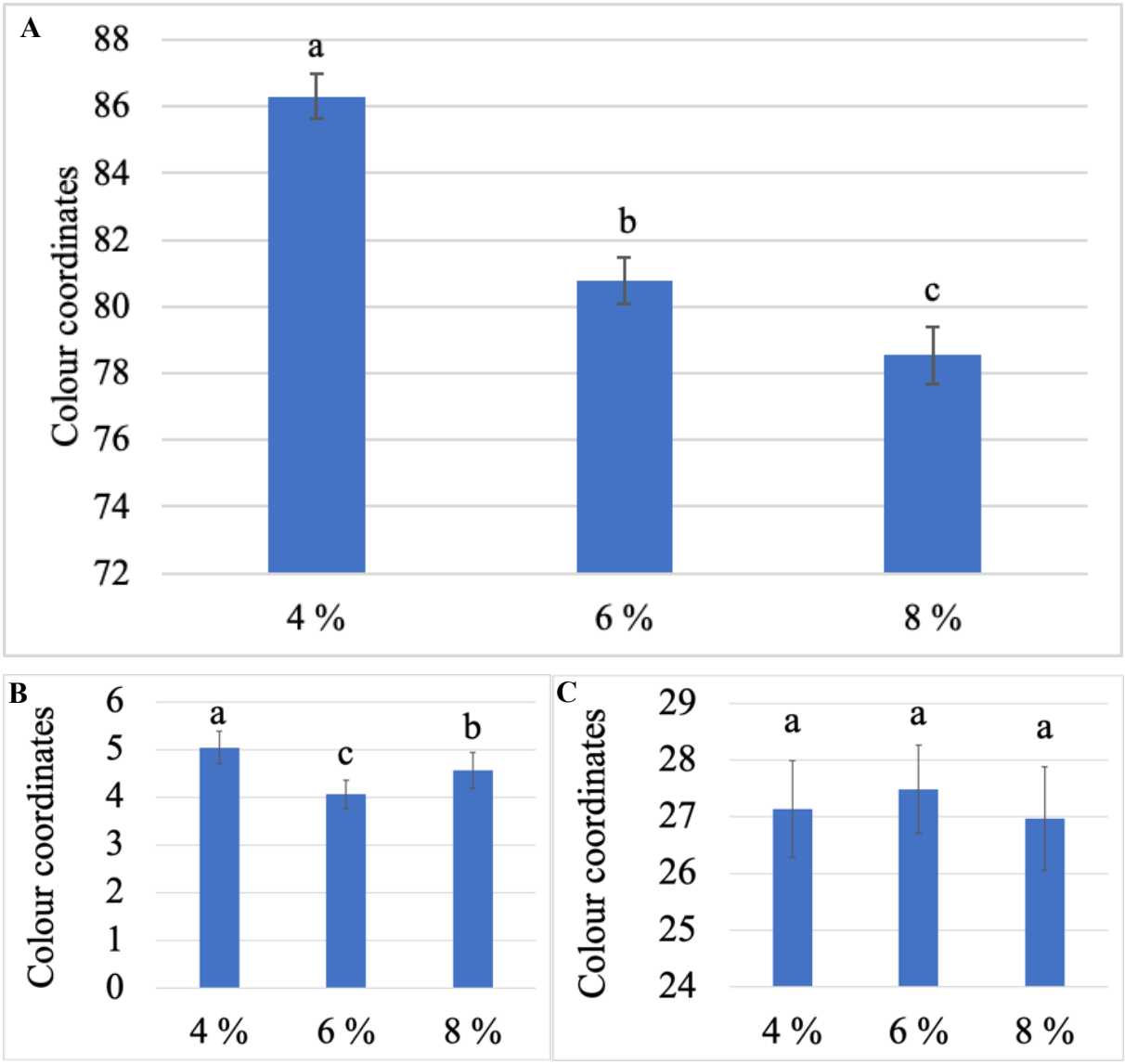

Figure 4.19 Colour measurements of the recipes with total calculate protein 4 %, 6 % and 8 % in pilot production. A) Colour coordinate L*, B) Colour coordinate a*, C) Colour coordinate b*. (n=18)

4.5 Digestion of proteins in INFOGEST models

It has been an interest to develop a mechanistic understanding of the impact of food structure and composition on human health, involving the simulation of digestion in the upper gastrointestinal tract. The standardized protocol that has been used in this work is based on an international consensus developed by the COST INFOGEST network (Brodkorb et al., 2019; Minekus et al., 2014). The method is designed to be used with standard laboratory equipment and requires limited experience to encourage a wide range of researchers to adopt it. It is a static digestion method that uses constant ratios of meal to digestive fluids and a constant pH for each step of digestion. Using this method, food samples are subjected to sequential oral, gastric and

intestinal digestion while parameters such as electrolytes, enzymes, bile, dilution, pH and time of digestion are based on available physiological data. This amended and improved digestion method (INFOGEST 2.0) avoids challenges associated with the original method, such as the inclusion of the oral phase and the use of gastric lipase (Brodkorb et al., 2019).

Size exclusion chromatography (SEC) with UV-detection is a well-recognized analytical tool for measuring molecular weight distributions of protein digests (Wubshet et al., 2017) and has been applied to estimate the proportion of peptides with specific size ranges generated during simulated digestion by partial area integration (Le Roux et al., 2020). In addition to that, SEC can provide an overall qualitative fingerprint of a given digest in the form of chromatograms (Rieder et al., 2021).

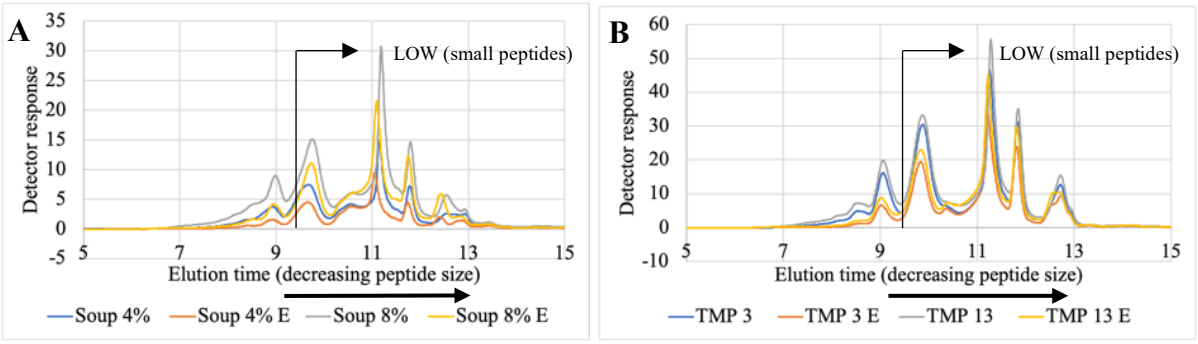

Figure 4.20 SEC chromatogram for A) the liquid product and B) solid product. The analyses were performed on digested samples that were withdrawn after 80 min in the intestinal phase.

Figure 4.20 shows the size exclusion chromatograms for the soups and the texture modified product. “Soup 4 %” was the non-enriched soup, and the sample marked with E indicates that the elderly model was used. “Soup 8 %” was the high-enriched soup, and an elderly and adult model was run on this as well. “TMP 3” was the texture modified product without protein enrichment (17.4 % protein), and the sample marked with E indicates that the elderly model was used. “TMP 13” the texture modified product with the highest protein enrichment (20.2 % protein), and an elderly and adult model was run on this as well.

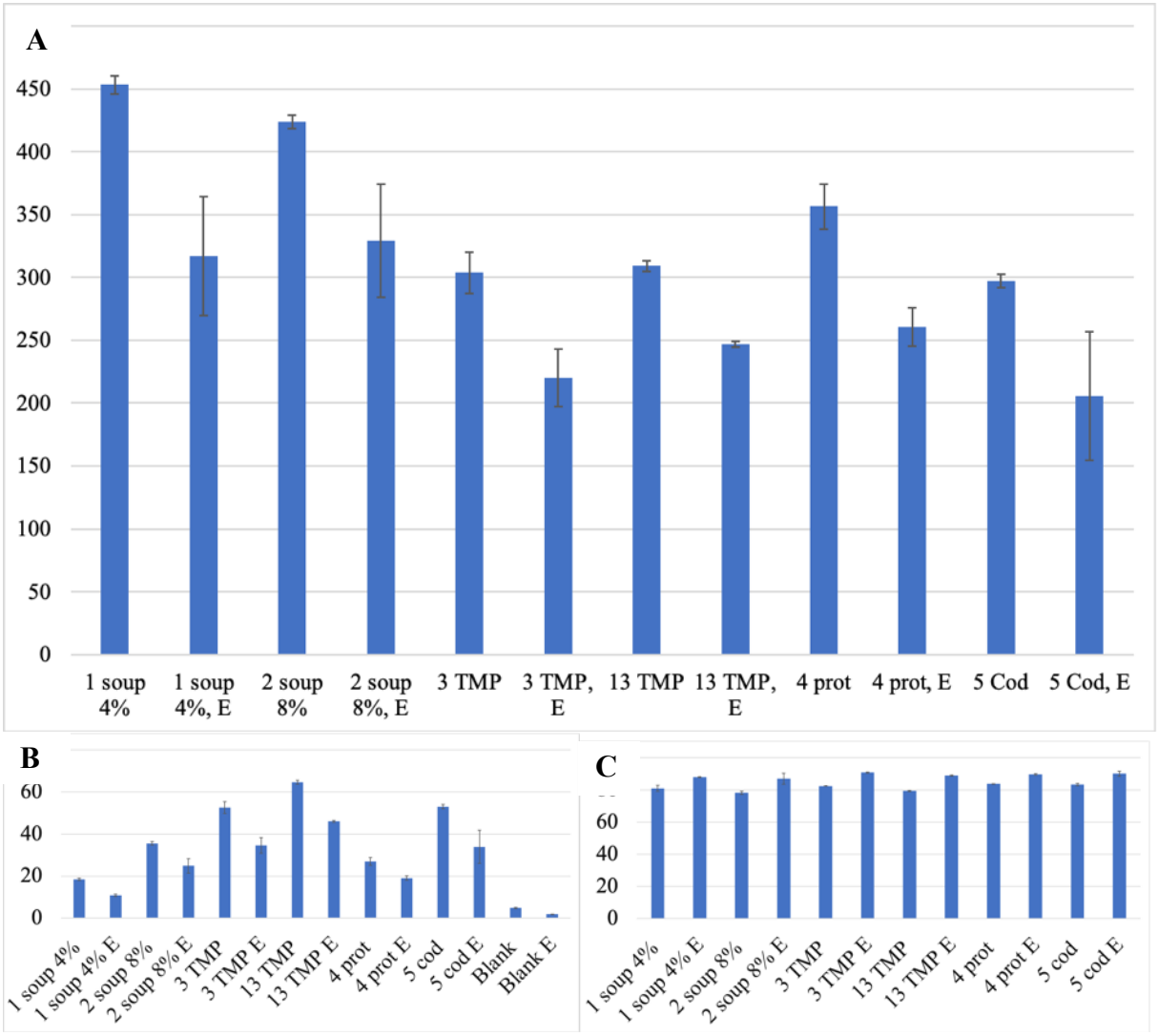

Figure 4.21 A) LOW / total protein (protein digestibility). Small peptides and amino acids (LOW, elution time>9.4 min) per amount of protein in the product (relative units). B) SEC area shows the amount of soluble protein in the intestinal phase (relative units). C) % small peptides (>9.4 min) show the percentage of soluble proteins that are small peptides and amino acids available for uptake in the intestine (degree of hydrolysis).

The protein digestibility is higher in the soup than the TMP product and cod fillet (Figure 4.21 A). This may be due to the lower total protein content in the soup and/or the presence of caseinate (from milk and whipping cream) which is known to be easily digested (Rieder et al., 2021). All products showed slightly reduced protein digestibility in the elderly model, mainly due to lower amounts of soluble protein in the intestinal phase (Figure 4.21 B). Enrichment gives more protein available for absorption in the intestine for both adults and elderly. Protein enriched product have approximately the same digestibility as non-enriched products. The degree of hydrolysis appears to be higher in the elderly model, but the digestion is overall poorer (Figure 4.21 C).

5 Conclusions

Two products were developed based on a request from Stavanger University Hospital for defined products for elderly and people with dysphagia: 1) a texture modified cod product and 2) a protein enriched fish soup. Both products were stored frozen (-30 °C). The products were developed through a series of preliminary productions to achieve high attractiveness and correct texture and finalized through a pilot production where the products were thoroughly analysed. The products are ready for testing in the hospital kitchen.

Texture modified products (TMP)

The texture modified products were enriched with protein from a total of 17.4 % (no added protein) to a maximum of 20.2 % indicating that the user groups can get high amounts of protein in smaller portions. The maximum values were set by highest tolerance levels in texture and sensory properties among the added proteins, whey protein concentrate (WPC), caseinate and fish protein hydrolysate (FPH). The texture was also altered using a modified corn starch and oil was used to increase the smoothness.

The IDDSI fork pressure test of the texture modified product was strictly a visual measurement to determine softness and firmness. All three products from the pilot production were easily broken apart with side of fork, meaning a knife was not required. The products got firmer, i.e., more difficult to press down using the prongs of a fork, when cooling during 15- and 30 min standstill. According to the IDDSI framework, the texture modified products produced in this thesis were measured to be between level 5-“minced and moist” and level 6-“soft and bite sized”, which could be suitable for elderly and for dysphagia patients with less severe degree of swallowing disabilities.

Texture analysis of TMP showed that a higher total protein content gave significantly ($p<0.05$) firmer products compared to non-enriched or less enriched products. The analysis also showed that the temperature of the product affected the texture, getting significantly firmer as it cooled from 55 °C to approx. 40 °C during 30 min standstill. The texture was most affected within the first 15 min of cooling. This shows that it is important to develop products that can maintain texture properties over a broad temperature interval, normally found during serving.

Rheological measurements of TMP showed that there was a significantly *($p<0.05$)* lower storage modulus G' (Pa) at 55 °C than at 25 °C. However, there was not a significant difference between 25 °C from the start, and 25 °C after cooling from 55 °C. The same tendencies were shown for all three recipes. Storage modulus appeared to increase with increasing protein enrichment. This may indicate that there was a correlation between protein content, temperature and storage modulus.

Colour analysis showed that all TMP products had a light colour and there was no significant difference with different amounts of protein enrichment *($p=0.400$)*. However, yellowness of the products increased significantly with increasing protein enrichment *($p<0.05$)*.

High-pressure processing of texture modified products showed that bacteria survived the highest pressure combination at 600 MPa for 10 min. The numbers where <1000 bacteria/g, and no growth was observed when the products were stored for 35 days at 4 °C. This indicate that HPP products could have an acceptable safety for 5 weeks at chilled storage. This means that HPP can be a good alternative to freezing. The texture analyses showed that the firmness changed significantly *($p<0.05$)* with different high-pressure treatments and protein content. For recipe 6 (19.9 %) all the treatments gave significantly different firmness. The most extreme treatment with 600 MPa for 10 min gave the firmest texture. The softest product was the one treated with 600 MPa for 2 min (600/2). For recipe 8 (19.4 %) all treatments also gave significantly different firmness. The most extreme treatment with 600 MPa for 10 min (600/10) gave the firmest texture and the not-HPP treatment gave the softest texture. For both recipe 6 and 8, the 400/2 min treatment yielded a firmer texture than 600/2 min treatment. The statistics revealed that for recipe 11 (18.8 %), the 600/2 min treatment gave a significantly firmer texture than 400/2 min, but there was no significant difference towards the other treatments.

Protein enriched soup

The enriched fish soup products were enriched with protein from a total of 4.0 % (no added protein) to a maximum of 10.1 % indicating that the user groups can get high amounts of protein from a liquid product. The maximum values were set by highest tolerance levels in texture and sensory properties among the added proteins, whey protein concentrate (WPC), caseinate and fish protein hydrolysate (FPH). The soup with high enrichment in the pilot production was

limited to 8 % protein, as the soup with 10 % protein gave a too thick and non-homogenised texture.

IDDSI Flow test revealed that soups with different protein content (4-, 6- and 8 %) were significantly different *($p<0.05$)* but did not belong to different IDDSI levels. This indicate that IDDSI gives relatively broad limits for the different levels and that it is possible to have significant differences in liquid products within the same level. According to the IDDSI framework, the soup with 4 % protein was at level 1–"slightly thick", but as it cooled for 15 min, the level changed to level 2-"mildly thick". After 30 min standstill, the soup (4 %) was still at level 2. The soup with 6 % protein was measured to be at level 3-"moderately thick" at time of service, both 15 min and 30 min after service. The soup with 8 % protein also belonged to level 3 at the time of service according to IDDSI. But after 15- and 30 min standstill, the ml remaining in the syringe corresponded to level 4-"extremely thick". Both level 3 and 4 overlap the food categories of the IDDSI framework, "liquidised" and "pureed", respectively. The soups at these levels may therefore be too thick to be used as a soup and may be more suitable as a thick sauce.

The viscosity measurements of the soups showed that the measured viscosity, during a 30 min runtime, significantly *($p<0.05$)* increased with increasing protein content. The measured viscosity did not significantly change while the soups cooled from 55 °C during 30 min standstill. The non-enriched soup had significantly lower viscosity *($p<0.05$)* than the low- and high-enriched soups. The viscosity was not significantly different at 25 °C and 55 °C for the non-enriched soup and measurements showed increased viscosity with increasing protein enrichment.

Rheology measurements of the soup showed that there was significant difference *($p<0.05$)* in yield stress between all three recipes at 55 °C, meaning that the yield stress increased with increasing protein content, while there was no significant difference in yield stress at the different temperatures within each recipe. The soup with highest protein enrichment at the lowest temperature was revealed to be most viscous.

Colour analysis showed that a higher protein enrichment yielded a significantly less light product *($p<0.05$)*. There was no significant *($p=0.180$)* difference in the yellowness of the soups. Redness of the soups were significantly *($p<0.05$)* different in all the recipes. The non-enriched

soup showed the most redness, while the low-enriched soup showed the lowest redness. This shows that colour differences may appear when adding protein enrichment.

INFOGEST

INFOGEST analysis showed that enrichment gave more protein available for absorption in the intestine for both adults and elderly. Protein enriched product have approximately the same digestibility as non-enriched products. The protein digestibility was higher in the soup than in the TMP product and raw material cod fillet. This may be due to the lower total protein content in the soup and/or the presence of caseinate (from milk and whipping cream) which is known to be easily digested (Rieder et al., 2021). All products showed slightly reduced protein digestibility in the elderly model, mainly due to lower amounts of soluble protein in the intestinal phase.

6 Future work

- The TMP products developed could be suitable for some elderly and people with less severe dysphagia. As different IDDSI level foods are recommended to different patient groups, also with severe dysphagia, the recipes could be further developed to achieve even softer levels.

- The fish protein hydrolysate contributed with a bitter taste when used in high concentrations. Use of a fish protein hydrolysate with less distinct taste, would make it possible to add higher concentrations of sustainable protein, rather than adding dairy proteins as enrichment.

- A quantitative descriptive analysis (QDA) using a sensory panel should be done on both the texture modified cod product and protein enriched soup to evaluate different sensory properties such as odour, flavour and texture that cannot be measured with instruments.

- More research on HPP of texture modified products is needed in order to document the applicability of this technology. Additionally, the HPP experiments of enriched soup should be carried out. Combinations of heat and HPP could be a useful alternative that might yield a softer product and higher level of safety.

- The digestion of protein in enriched and texture modified products should be examined further. And on products that have been through different high-pressure treatments and other heat and processing treatments.

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Appendix A

Datasheet - modified corn starch, produced by Avebe

Avebe

P.O. Box 15
9640 AA Veendam
The Netherlands

E-mail customerservice@avebe.com
www.avebe.com

General product information document

Farinex™ WM 55

Farinex™ WM 55 is an acetylated distarch adipate of waxy maize origin. It is a white to yellowish powder and free from objectionable odours. This product is intended for use in food.

Physical and Chemical Specification

Item		Value	Method complies with
Heavy metals	Arsenic (As)	<= 1.0 mg/kg	Atomic absorption method
	Cadmium (Cd)	<= 0.1 mg/kg	Atomic absorption method
	Mercury (Hg)	<= 0.05 mg/kg	Atomic absorption method
	Lead (Pb)	<= 0.5 mg/kg	Atomic absorption method
Sulphite (as SO_2) on an anhydrous basis		<= 10 mg/kg	ISO 5379
Moisture Content		140 mg/g	ISO 1666

- This product meets the requirements of Regulation (EU) No. 231/2012 on specifications for food additives (E1422).
- This product meets the requirements of the Food Chemical Codex (Food Starch Modified).
- This product meets the requirements of USA 21 CFR § 172.892 (Food Starch Modified).
- This product meets the requirements of the JECFA monograph on modified starch (Codex Alimentarius, INS 1422).

Microbiological Specification

Item	Value	Method complies with
Total aerobic mesophilic count	<= 10000 CFU/g	ISO 4833
Yeasts	<= 250 CFU/g	ISO 21527
Moulds	<= 250 CFU/g	ISO 21527
Enterobacteriaceae (1 g)	Absent	ISO 21528
Salmonella (25 g)	Absent	ISO 6579

Nutritional Properties

Item	Typical value per 100 g[1]	Item	Typical value per 100 g[1]
Energy	345 kcal /1460 kJ	Calcium	0.006 g
Protein	0.4 g	Chloride	0.01 g
Carbohydrates	86 g	Iron	0.001 g
Sugars	—	Magnesium	0.002 g
Fat	< 0.1 g	Phosphorus	0.03 g
Saturates	—	Potassium	0.005 g
Unsaturates (mono- and poly)	—	Sodium	0.03 g
Transfats	—	Vitamins	—

[1] All values are expressed in product as such at maximum specified moisture content (typical values, not a specification).
— Not present in significant amounts.

ref.no. 219106 version 01, issued March 2017, valid till March 2020

Avebe

General product information document

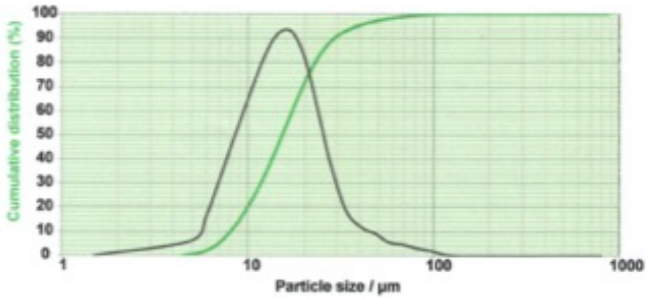
Shelf life and Storage conditions

Farinex™ WM 55 has a best before date of 2 years after the manufacturing date.
Store inside, cool and dry, in sound and well closed packaging. Protect from contamination. Do not store or ship together with odorous or toxic substances. It is advised however, to keep the storage time as short as possible, because the moisture content may change.

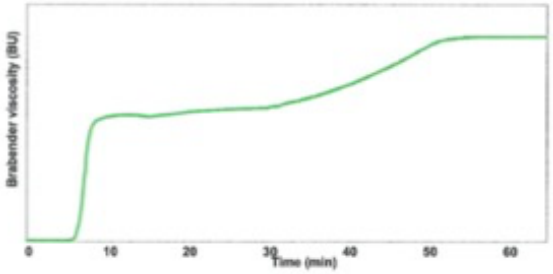
Additional Product Information

Bulk density (typical value, not a specification)
500 kg/m³

Particle size distribution (typical curve, not a specification)
Measured from dry sample (as is) and calculated as spheres



Brabender Viscosity graph



Viscosity
Concentration : 33 g product calculated on dry matter.
Procedure : With demineralised water the sample weight is filled up to a total weight of 500 g.
Brabender E-type; head 350 cmg, n= 75 min⁻¹

Brabender:	Slope	Ramp time (min:sec)	Temperature	Hold time (min:sec)
			50 °C	
	3.0 °C/min	15:00	95 °C	15:00
	-3.0 °C/min	15:00	50 °C	

General product information document

Botanical Origin
- *Zea Mays*; maize

Allergens
Information about allergens is communicated via an allergen statement, which is available on request.

Dietary suitability
- Suitable for vegan, ovo-vegetarian, lacto-vegetarian and ovo-lacto vegetarian diets.
- Kosher and Halal certified.

Intended Use
- Food additive for use in food. It is recommended this product is used in accordance with Regulation (EC) No. 1333/2008 on food additives, or the General Standard of Food Additives (GSFA) of Codex Alimentarius or any other relevant legislation (see disclaimer).
- This product is in its available packaging variants not intended for retail sale.

Labelling advice
Consider the appropriateness of any labelling advice provided by Avebe, having regard to the intended use and local legislations.
- In the EU this product may be designated as modified starch on the consumer label, there is no need to mention an E-number. To mention maize as botanical origin of the starch is optional.
- In the USA this product may be designated as food starch modified on the consumer label.

Safety Data Sheet
The submission of a safety data sheet is not mandatory (Regulation (EC) 1907/2006). Relevant information to enable appropriate handling measures is communicated via a material safety data sheet in English language.

Food Safe Quality
- AVEBE operates in accordance with the general principles, requirements and procedures of food law and of food safety laid down in Regulation (EC) No. 178/2002.
- AVEBE ensures that food hygiene in accordance with Regulation (EC) No. 852/2004 is met during all stages of production, processing and distribution where this falls under her responsibility.
- AVEBE operates a management system accredited under ISO 9001.
- AVEBE conducts HACCP studies and identify relevant agro-chemical (including pesticides and contaminants), microbiological and physical risks to food safety associated with the production, processing and distribution of our products. We deploy adequate measures to mitigate the identified risks. Our employees are trained, our processes are monitored and our procedures are evaluated.

Logistical Information

Harmonised System (HS)
350510 Dextrins and Other Modified Starches.
Importing parties are responsible for customs declaration.

Certificate of Analysis / Certificate of Conformity
Each delivery is accompanied by a Certificate of Analysis/Certificate of Conformity.

Appendix B

List of ingredients used in both preliminary- and pilot production.

Materials	**Information**	**Producer**	**Country**
Cod fillets[a]	Fresh, skinless V-Cut	Domstein AS	Norway
Modified corn starch	Farinex™ WM 55	Royal Avebe U.A.	Norway
Oil[a]	Rapeseed oil	COOP Norge SA	Poland
1/3 reduced fish broth[b]	Fisk – Demi Glace Art. 721004	Salsus AS	Norway
Whipping cream[b]	37 % fat	Tine SA	Norway
Whole milk[b]	3.5 % fat	Tine SA	Norway
Fish protein Hydrolysate (1)	Endurance Salmon protein hydrolysate, 97 % total protein	Hofseth BioCare	Norway
Fish protein Hydrolysate (2)[a]	Salmon backbone protein hydrolysate, 89.8 % total protein	Nofima	Norway
Salt (NaCl)	CAS-no. 7647-14-5	Akzo Nobel salt	Denmark
Whey protein concentrate 80 %	WPC80 Art. 4466 77.4 % total protein	Tine SA	Norway
Sodium Caseinate	KAPA™ JPR 1002 87 % total protein	Armor proteins	France

[a] only used in the texture modified product.
[b] only used in the enriched soup product.

Appendix C

Datasheet - salmon protein hydrolysate, produced by Hofseth BioCare

HOFSETH BioCare®

CERTIFICATE OF ANALYSIS

SALMON PROTEIN HYDROLYSATE

PRODUCT NUMBER: SPH-01-H
COUNTRY OF ORIGIN: Norway

Author: Henriette Heggdal
Version: 002
Approved by: Angelika Florvaag
Approval date: 18.07.2019

DATE OF PRODUCTION	PRODUCTION BATCH	EXPIRY DATE
08.05.17	SPH 17027	05.05.21

PARAMETER	RESULTS	SPECIFICATIONS	METHODS
Color	Light yellow	Light yellow	Visual
pH (2 % solution)	6.4	6.0 – 7.0	MT-002
Moisture	3,23	< 5 %	Journal of AOAC International 93(3), 2010, p 825-832
Total Nitrogen (TN)	16	> 15 %	NMKL 6
Protein (N x 6.25)	100	> 97 %	NMKL 6
Fat	< 0,1	< 0,5 %	NMKL 131
Ash	1,8	< 2.5 %	NMKL 173
Total Aerobic Microbial Count	< 10	< 10 000 CFU/g	AOAC 990.12
Enterobacteriaceae	< 10	< 10 CFU/g	AOAC 2003.01
Listeria Monocytogenes	Absent/25 g	Absent/25 g	AOAC 2016.08
Salmonella	Absent/25 g	Absent/25 g	AOAC 2016.01
Staphylococcus Aureus[1]	< 10	< 10 CFU/g	ISO 16140
Yeasts and Moulds	< 10	< 100 CFU/g	AOAC 997.02
Arsenic (inorganic)[2]	< 0,1	< 0.1 mg/kg	HG-AAS §64 LFGB L 25.06-1 (2008-12), mod.
Cadmium[2]	< 0,01	< 0.05 mg/kg	EN ISO 15763 (2010)
Mercury[2]	0,021	< 0.05 mg/kg	EN ISO 15763 (2010)
Lead[2]	< 0,05	< 0.05 mg/kg	EN ISO 15763 (2010)
WHO-PCDD/F-TEQ[2]	0,0668	< 2 ng WHO-PCDD/F-TEQ/kg	GC-MS/MS
WHO-PCB-TEQ[2]	0,0407	< 3 ng WHO-PCB-TEQ/kg	GC-MS/MS
WHO-PCDD/F-PCB-TEQ[2]	0,107	< 5 ng WHO-PCDD/F-PCB-TEQ/kg	GC-MS/MS

1) Measured and reported every other month.
2) Measured and reported every quarter.

Angelika Florvaag, Quality Manager

17.10.2019
Date

1 of 1 | Hofseth BioCare ASA

Appendix D

Datasheet - salmon backbone protein hydrolysate, produced by Nofima

Peptide size distribution (of % water soluble peptides)		**Total amino acids (g/100 g sample)**	
Mw-peptide > 20000	<0.1	Aspartic acid	7
Mw-peptide 20000-15000	<0.1	Glutamic acid	10.9
Mw-peptide 15000-10000	0.1	Hydroxyproline	3
Mw-peptide 10000-8000	0.2	Serine	3.4
Mw-peptide 8000-6000	0.9	Glycine	8.8
Mw-peptide 6000-4000	3.3	Histidine	1.8
Mw-peptide 4000-2000	12.9	Arginine	5.3
Mw-peptide 2000-1000	18.4	Threonine	3.2
Mw-peptide 1000-500	18.9	Alanine	5.6
Mw-peptide 500-200	20.3	Proline	4.5
Mw-peptide 200-	24.9	Tyrosine	1.7
		Valine	3.2
		Methionine	2.3
		Isoleucine	2.6
		Leucine	5
		Phenylalanine	2.3
		Lysine	6.5

Chemical composition	
Crude protein Kjeldahl (N*6.25) (%)	89.8
Total dry matter (%)	96.3
Ash (%)	9.3
Water-soluble crude protein (g/100 g sample)	88.7
Quality parameters	
Bacterial count (CFU/g)	1500
Putrescine (mg/kg)	110
Cadaverine (mg/kg)	<20
Histamine (mg/kg)	67
Trimethylamin-N (mg N/100 g)	14
Trimethylaminooxide-N (mg N/100 g)	125