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Abstract

The aim of this thesis was to develop two protein enriched products based on haddock and silver smelt, reaching the level of total protein in the utilized fish raw material. Both products were enriched by whey protein concentrate, sodium caseinate and fish protein hydrolysate and further investigated how the proteins affected color, texture, water holding capacity and sensory attributes.

The products were developed as a contribution to the marked of personalized nutrition. Personalized nutrition is aimed for consumer groups with special needs or requirements to obtain a good health status. The first product was a texture modified fish product for elderly and other people with chewing and swallowing problems (dysphagia). The second product was a minced fish product to meet the regular consumer interested in protein enriched products. Many people have found interest in different diets and fitness in later years, which explains the great variation of protein enriched products available today.

Preliminary testing was performed to optimize the processes and to test varying amounts of added protein, liquid, and oil. Rheological analysis, texture analysis including gel-test, texture profile analysis and penetration test, image analysis and water holding capacity were carried out during preliminary production and in pilot production were additional sensory analyses done.

Texture analysis of texture modified products showed that higher total protein with fish protein hydrolysate was significantly firmer than products with less proteins, sensory analysis revealed equal correlation between protein and firmness. Further, it was revealed that higher amount of proteins significantly increased intensity of coarseness and cohesiveness.

Texture profile analysis of minced fish revealed that hardness, force, and gumminess increased significantly with higher level of total protein. Cohesiveness and resilience on the other hand, decreased significantly with more protein enrichment. Sensory analysis showed that minced fish with higher protein enrichment was significantly less intense in sour flavor, while more proteins resulted in a significantly stronger aftertaste. Lower level of protein enrichment significantly increased juiciness and significantly decreased crumbliness.

More added protein gave a significantly yellower tone and darker shade in both products. Water holding capacity was significantly improved by higher level of protein enrichment. Internal

mass was showed through image analysis to significantly increase by higher amounts of proteins in both products.

The pilot products developed in this work show that it is possible to add protein hydrolysates from fish by-products within certain concentrations into new products. It is also possible to enrich minced fish products level of 16-19% total protein, which is comparable to levels in fish fillets. Description of how proteins affect physical, chemical, and sensory characteristics in products after several processing steps is important for further development of attractive protein rich retail products.

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Abbreviations

DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FPC	Fish protein concentrates
FPH	Fish protein hydrolysates
IDDSI	International Dysphagia Diet Standardisation Initiative
ISO	International Organization for Standardization
LBG	Locust bean gum
LES	Lower esophageal sphincter
LVR	Linear viscoelastic region
MPS	Muscle protein synthesis
NIPH	Norwegian Institute of Public Health
PN	Personalized nutrition
QDA	Quantitative descriptive analysis
TMF	Texture modified foods
TMP	Texture modified products
TPA	Texture profile analysis
UES	Upper esophageal sphincter
WPC	Whey protein concentrate
WPI	Whey protein isolate

Table of content

1. Introduction

There is an increasing global trend of developing personalized nutrition (PN), also known as individualized nutrition, to meet the different needs and desires of a consumer (Tartalio 2018; Cavanah and McGroarty 2019). Research and development of PN have already been conducted for several years due to people's interest in nutrition and health, based on the understanding that one size does not fit all. The interest has expanded even further the recent years as more people have received greater knowledge and more preference of quality nutrition and its ability to prevent or delay diseases e.g. the loss of muscles (sarcopenia) in elderly (German et al. 2011; Wilson et al. 2017).

There is even developed scientific areas known as nutrigenomics that are conducting studies at a molecular level of the relationship between PN and the genome of an individual person. In addition to nutrigenomics, other scientific areas like epigenetics and metabolomics are also conducting research regarding PN. The primary aim for them all is to be able to provide PN for individuals and not only for groups of people. Further, PN should desirably be highly customized towards the consumers genetics and metabolism or give specific micronutrients for prevention or reversion of diseases. However, the market today consists of more PN products targeted towards bigger groups (German et al. 2011; Sales, Pelegrini, and Goersch 2014).

Variations of PN aimed towards specific target groups are e.g. sports nutrition, texture modified foods (TMF) for dysphagia patients and finally regular people who simply desire protein rich products. Sports nutrition have obtained a tremendous popularity today among several people who desire to perform workouts from beginners to professional athletes (Henchion et al. 2017; Kim et al. 2019). Further, sports nutrition is subdivided into protein powders for muscle mass or weight gain, as well as bars produced to provide either proteins or energy for intense workouts or prolonged endure training. Even regular products such as baked goods, yoghurts, gelled puddings and candy are protein enriched (Kerksick et al. 2018).

Other PN targeted to groups are nutritional TMF for people suffering from dysphagia as a part of dysphagia management (Sungsinchai et al. 2019). Further, the percentage of the aging population is increasing. This will result in a higher demand of PN meeting the needs of elderly consisting of different texture and nutrients (Affairs 2018). Older age is commonly associated with both loss of appetite, loss of muscle mass and increased muscle weakness. Depending on the individual's health status, PN should consist of energy dense and protein rich nutrition if

the loss of appetite or loss of muscle mass is the area of concern (Volpi, Nazemi, and Fujita 2004).

There has been conducted projects globally in Europe regarding PN e.g. Food4Me, which was founded by EU. The project studied challenges and opportunities of PN through an answered questionnaire received from 1500 participants which showed that PN was more influential on people's diet than conventional, general advices (Food4Me project 2016). There are ongoing projects as well focusing on developing PN such as the project Personalized nutrition for healthy living which is also founded by EU in addition to different countries in Europe. Nestlé Health Science in USA, a subdivision of Nestlé which is researching nutritional science, have started to develop PN as well, customized for individuals (Nestlé 2019).

In Norway, there are ongoing projects as well, such as VårMat and Matlyst (Rosnes, Rognså, and Brierley 2019; Nofima 2018-2021). Matlyst, also called Appetite, is developing foods suitable for elderly. Their preferences and nutritional requirements are taken into account when foods are studied to ensure healthy products suitable for older age. A focal point in Matlyst are TMF made for elderly with swallowing difficulties, known as dysphagia (Rosnes, Rognså, and Brierley 2019). Contrary to Matlyst, VårMat is researching PN more generally including several target groups e.g. elderly, younger, and people with special needs. Further, VårMat investigate consumer insights about nutrition, different processing methods making safe and palatable food and utilization of raw materials and its by-products (Nofima 2018-2021).

Consumers are more concerned with living a sustainable lifestyle in regards of e.g. food consumption and production, and textile purchases. A part of sustainable food production includes utilization of all parts of different raw materials (Cattaneo et al. 2019). Furthermore, the trend of utilizing by-products is increasing in popularity, particularly utilization of fish raw materials within the seafood industry have a growing interest (Lindberg and Ytrestøyl 2018; Petrova, Tolstorebrov, and Eikevik 2018). By-products from the seafood industry consist of discarded residues e.g. heads, bones, and trimmings. A part of by-products are used for processing fish meal and oil, while some amounts are never utilized at all (Aspevik et al. 2017). One way to increase utilization is to recover compounds from the by-products, by e.g. extracting proteins and further hydrolyze them into smaller peptides, referred to as fish proteins hydrolysate (FPH) (Vázquez et al. 2019).

The thesis is a part of Nofima' s strategic project "VårMat" (project no.12234) that focuses on the use and development of personalized nutrition in different stages of human life. It is an interdisciplinary project which involves several other divisions of Nofima AS.

2. Theory

2.1 Specific groups in the society, with needs and effects from personalized food

2.1.1 Fitness and weight loss

There is a trend among fitness enthusiasts, athletes, and even the average consumer about consumption of protein enriched food. Furthermore, studies predict that this trend will increase (Henchion et al. 2017; Kim et al. 2019). The types of commercial protein enriched products available for consumers today vary immensely. Not unexpectedly, the amount of different sports nutrition like protein powders and bars is of considerable size, in addition to less obviously products like e.g., enriched baked goods and sweets. According to Olympiatoppen, an average person in Norway usually gets the recommended daily intake of proteins without the requirements of added supplements, which is 0.75-0.8 g/kg bodyweight. Even knowing that there is a sufficient intake of proteins by the average person, increased protein consumption is still recommended to elderly, growing kids and teens, athletes and simply people with protein deficiencies. Different groups of athletes have been recommended higher protein intake ranging from 1.2 and up to 2.0 g/kg (Olympiatoppen ; Grasso et al. 2019). An adequate intake of proteins is approved to provide several recognized health benefits like e.g., enhancement of muscle synthesis ('Nutrition and Athletic Performance' 2009).

Table 2.1. The table is recreated from Olympiatoppen, based on their recommendations on required protein for varying groups of athletes (Olympiatoppen).

Type of athlete and training regime	Protein (g protein/kg bodyweight)
Amateur exercising occasionally	0.8-1.0
Endurance – moderate amount	1.2
Endurance – great amount	1.6
Strength and high intensity sports	1.2-1.8
Young, growing athletes	2.0

A great percentage of athletes and persons performing strength- and cardio exercises consume a high amount of proteins (Phillips, Moore, and Tang 2007). Different types of athletes require elevated protein intake, depending on the type of sports, as well as biological factors such as sex, height and age ('Nutrition and Athletic Performance' 2009), (Table 2.1). However, the popularity of proteins is not without reason.

Endurance athletes require a higher protein intake due to an increase in oxidation of proteins, which is a method for the body to meet the need for energy. Elevated intake therefore ensure that the amino acids are used for muscle protein synthesis (MPS), a process crucial for skeletal muscle growth, repair, and maintenance. The body synthesizes proteins from amino acids to build skeletal muscles ('Nutrition and Athletic Performance' 2009).

Athletes performing resistance training also require high amounts of proteins to maximize muscle synthesis after exercising, thus increasing muscle growth. The stimulation of muscle synthesis is maximized when a protein rich diet is combined with heavy resistance training thus stimulating the protein synthesis. People doing resistance exercise require an intake higher than the recommended 0.8 g/kg protein, especially essential amino acid ('Nutrition and Athletic Performance' 2009).

Proteins that are demonstrated to influence the MPS positively are whey, casein and soy, with emphasis on whey and casein ('Nutrition and Athletic Performance' 2009). The slow protein casein is digested and absorbed slowly and have a prolonged effect on MPS whilst whey is notably faster. The digestion and absorption rates of whey are due to branched-chain amino acids (BCAA), isoleucine, valine and particularly leucine and its impact on the MPS (Kanda et al. 2016). Further, BCAA stimulate the MPS by activating key enzymes when consumed rapidly after a workout (Blomstrand et al. 2006). Moberg et al. (2014) and Tipton (2009) suggested especially that leucine had a positive influence on the MPS or an effect on inhibition of breakdown or a combination of both. Churchward-Venne, Burd, and Phillis (2012) further proposed that one of leucine's influence on MPS is due to phosphorylation of enzymes and thus activating different pathways needed for translation.

In addition to a reduction energy of intake, exercise is commonly seen as the main way to lose weight (Durrer S.D. et al. 2019). The term weight loss is often used interchangeably with fat loss, although weight loss can consist of e.g. muscle loss, water loss and fat loss. The use of the term weight loss instead of fat loss will be used in this thesis. According to Norwegian Institute of Public Health (NIPH), the prevalence of obesity in 2017 in Norway was 25% of men and 20% of women (Meyer and Vollrath 2017). Globally, 1.9 billion adults were overweight in 2016, and 650 million of them were classified further as obese. The high prevalence of overweight and obesity with their often-following diseases are a major public health issue due to economic costs associated with healthcare. Due to the high percentage of overweight people, extensive research on various diets regarding weight loss have been conducted throughout the

times, including variations in daily intake of fat, carbohydrates, and proteins (Garrow et al. 1978; Mirkin and Shore 1981; Scheer, Codie, and Deuel 1947). Of the many researched diets, protein rich diets have shown promising results. Proteins do not only aid in growth and maintenance of skeletal muscles when exercising, a protein rich diet prevents greater muscle loss during a reduced calorie intake compared to a low protein diet (Bopp et al. 2008).

Even though most people agree on the health benefits provided by proteins, the macronutrient has raised some discussions as well. Some studies have initiated a conversation about a high intake of proteins and kidney disfunction, whereas contrary studies have stated that a high protein intake do not correlate with decreased kidney functions (Institute of Medicine 2005; Phillips, Moore, and Tang 2007). However, patients already suffering from renal disease or disfunction are expected to benefit on a decreased intake of proteins (Ko et al. 2017).

Summarized, protein enriched products should be produced to meet the different preferences consumers possess, whether the aim is to lose weight and/or gain muscles. Products should be specialized to additionally meet the different diet regimes such as low fat, low carbohydrates, or any other possible diet combined with high protein.

2.1.2 Elderly

The average number of elderly people over 65 years age old have been increasing gradually the last years and is predicted to increase further during the next decade. The European Commission predict that the population in Europe will increase from 511 million in 2016 to 520 million in 2070 whilst the population aged between 15 and 64 will decrease from 333 million 292 million (Affairs 2018). The Norwegian population follows an equal trend, as the population in 1918 mostly consisted of younger people, while in 2018 the population consisted of more middle-aged and elderly (Figure 2.1), (Engdahl et al. 2016). The population increase is caused by improved life expectancies and a post- world war II baby boom (Jaul and Barron 2017).

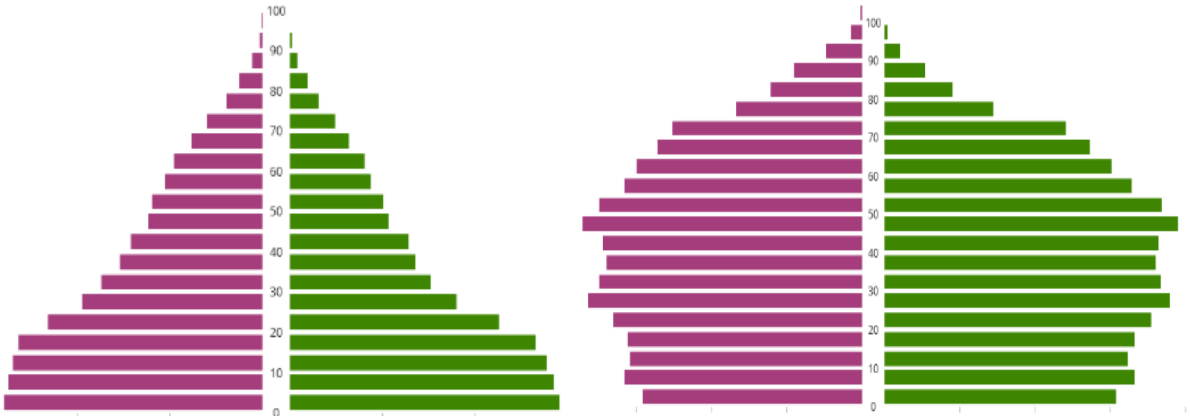


Figure 2.1. The of distribution of age in the Norwegian population ranging from age 0 at the bottom and increasing upwards to 100. The graph on the left is from 1918 and the one at the right is from 2018. The figure is from NIPH (Engdahl et al. 2016).

It is common to experience changes to and within the body when the age is increasing, especially from 65 years and more. A lot of diseases including cardiovascular diseases, cancer, osteoporosis, sarcopenia, and dementia are associated with older age. In addition, elderly commonly experience function loss such as muscle weakness, eyesight, hearing, and loss of appetite. Lastly, many starts to suffer from an overall reduced immune system (Jaul and Barron 2017).

Protein enriched products are one of the focal points in this research, and the emphasis will be on sarcopenia in older people, known as involuntary muscle loss. Sarcopenia is characterized by age-related loss of skeletal muscle mass and strength and can further lead to physical disability, shorter life expectancies and decrease in quality of life (Wilson et al. 2017). The muscle mass in adults commonly starts to decline in the age of 30 and then decreases with 3-8% per decade. When the age of 60 is reached, the rate of muscle loss is even more progressive (Volpi, Nazemi, and Fujita 2004). There is observed a non-linear reduction between muscle strength and muscle mass. Loss of strength is frequently experienced more rapidly than the loss of muscle mass. Wilson et al. (2017) reported that strength decline by 0.5-2% whilst muscle mass on the other decline by 2-4% per year in persons aged 70-79 years.

Fortunately, the progression of sarcopenia can be prevented or even reversed by applying lifestyle changes such as more exercising and a change in diet (Volpi, Nazemi, and Fujita 2004). There is a recognized correlation between decreasing muscle loss and decreasing protein synthesis (Berg et al. 2015; Dangin et al. 2002). Therefore, a protein rich diet consisting of BCAA, especially leucine in addition to other essential amino acids combined with resistance training would aid tremendously in prevention of sarcopenia. In fact, amino acids from digested

proteins are demonstrated to have a similar positive feedback on MPS in elderly as in younger people. The recommended protein intake for elderly is nevertheless higher (1 g/kg body) than the recommended protein intake (0.8 g/kg) (Volpi, Nazemi, and Fujita 2004; Olympiatoppen).

However, a few contrary studies have reported that an increased protein intake by either protein supplements or a high protein diet did not yield the desirable stimulation of muscle protein synthesis (Campbell et al. 1995; Welle and Thornton 1998). These articles were commented by Volpi et al. (2004) which said that the lack of increased MPS in their research is due to the overall low energy intake. The elderly persons have used a great percentage of the proteins as a source of energy instead of muscle growth. Due to the commonly loss of appetite observed in elderly, they often increase their protein intake at the expense of other macronutrients, leaving the overall energy intake unchanged (Volpi, Nazemi, and Fujita 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. Another study found a correlation between vitamin D or polyunsaturated fatty acids like omega-3 and proteins, which increased the MPS even further (Boirie 2019). Protein enriched products based on fish raw materials are therefore suitable for elderly suffering from sarcopenia, due to the vitamin D content in fish muscle.

2.1.3 Dysphagia

Dysphagia is the medical term for chewing and swallowing difficulties, a condition particularly older people suffer from but is also experienced in younger people. Dysphagia is sometimes referred to one defined condition but more frequently referred to as a collection of symptoms regarding difficulties with swallowing. People who suffer from stroke or other neurological disorder often experience dysphagia. In fact, as much as 33% of stroke patients and 50% of patients with other neurological disorders suffers from additionally dysphagia. The prevalence of this condition is shown to be proportional with older age and Sungsinchai et al. (2019) reported that 13% of humans at 65-70 years, 16% of 71-79 years and 33% of 80+ years suffered from dysphagia.

2.1.3.1 Symptoms

Dysphagia can be characterized as a collection of symptoms and swallowing difficulties can be caused from problems with chewing, improper initiation of swallowing reflexes, pulmonary aspiration of food or food that gets stuck in the throat or the sensation of stuck food (Sungsinchai et al. 2019). More symptoms include choking during consumption, coughing or gagging during

swallowing. Other reported symptoms are regurgitation, which is food coming back up, recurrent heartburn, hoarseness, less control of the food in the mouth, recurrent pneumonia and lastly the inability to control saliva. The outcome of these symptoms either combined or on their own result in malnutrition (Oslo universitetssykehus 2017; Newman 2017).

2.1.3.2 Phases of swallowing

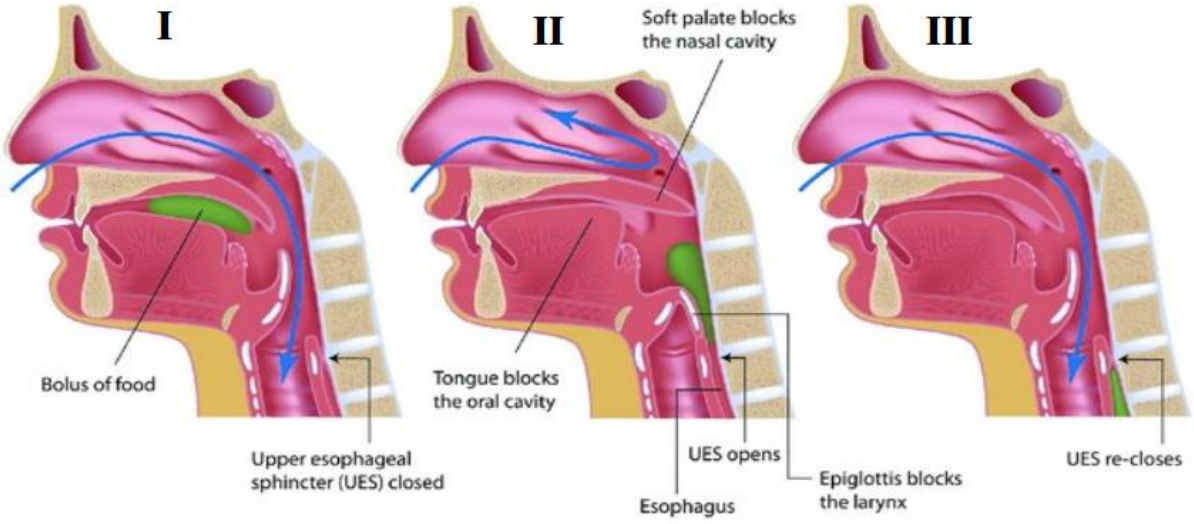


Figure 2.2. The swallowing process consist of three phases. Phase I is the oral phase, phase II is the pharyngeal phase and phase III. The figure is copied from (Fujiso et al. 2018).

The process of swallowing consists of three main phases (Figure 2.2). People can suffer from swallowing difficulties in one or more of the different phases. The first phase is voluntary and referred to as the oral phase where food is processed by the mouth. Food is ingested and placed onto the surface of the back teeth, followed by mastication into smaller particles. Further, the food is softened and formed into a bolus by saliva. A bolus is a semi-solid lump ready for swallowing. After the bolus is suitable for swallowing, it is pushed backwards by the tongue, away from the oral cavity and trough the fauces of the oropharynx (Matsuo and Palmer 2008).

The second, pharyngeal phase is involuntary and only last for 2-10 seconds in a healthy person. The food is transported through the pharynx (throat) and upper esophageal sphincter (UES) towards the esophagus. This movement is initiated by stimulation of receptors in the oropharynx, which further initiate the swallowing reflex. Different mechanisms are simultaneously stopping the food to get into the trachea, preventing pulmonary aspiration (Matsuo and Palmer 2008).

The final, third phase is the esophageal phase and is also involuntary. The movement of the bolus is performed by peristaltic waves, otherwise known as waves of muscular contractions.

The “waves” is regulated by the autonomic nervous system. The phase occurs between UES and the lower esophageal sphincter (LES). LES is tensioned between the passage of boluses to prevent regurgitation. The bolus is transported through a relaxed LES before it reaches the stomach (Matsuo and Palmer 2008).

2.1.3.3 Dysphagia management

A common outcome for persons who are suffering from dysphagia is malnutrition. Malnutrition can further lead to muscle loss due to protein deficiency. Furthermore, a low energy intake is often lacking the adequate amount of proteins because of the overall smaller amount of consumed food. A major part of dysphagia management is therefore to provide a diet consisting of soft food products, easy to ingest and swallow. Such foods are referred to as texture modified foods (TMF). The term TMF is applied when speaking about general texture modified food, while the term texture modified products (TMP) will be applied when speaking about the produced products in this thesis. The TMF should be ready to eat or ready to cook, contain great nutritional value and possess no choking hazard. The texture should preferably be soft, moist, and smooth, but not adhesive and sticky (Sungsinchai et al. 2019).

There is a global initiative increasing attractivity of eating for approximately 590 million people suffering from dysphagia known as International Dysphagia Diet Standardisation Initiative (IDDSI). The organization has suggested standardized terminology and definitions for easier descriptions for texture modified foods through a framework (IDDSI). The framework characterize TMF into eight levels (0-7), depending on the texture or thickness of liquid, level 3-7 is used for food and level 0-4 is used for liquid (Figure 2.3), (Sungsinchai et al. 2019; IDDSI Testing 2019). IDDSI has developed and implemented standardized testing procedures to easy determine the texture of the food or liquid in its intended state of serving. Two examples on such tests are the fork pressure test and spoon tilt test. The fork pressure test is performed by pressing a fork on the ready to serve TMF and assess its behavior simultaneously as the color of the thumb nail blanch is changed to white. The approximately pressure required by the tongue to swallow food is similar to the pressure required to change the color of the thumb to white. The spoon tilt test is used to assess the adhesiveness and cohesiveness of TMF by tilting a spoon containing a small sample. The sample should then be cohesive enough to barely holding its shape and not adhesive enough to be sticking to the tilted spoon (IDDSI Testing 2019).

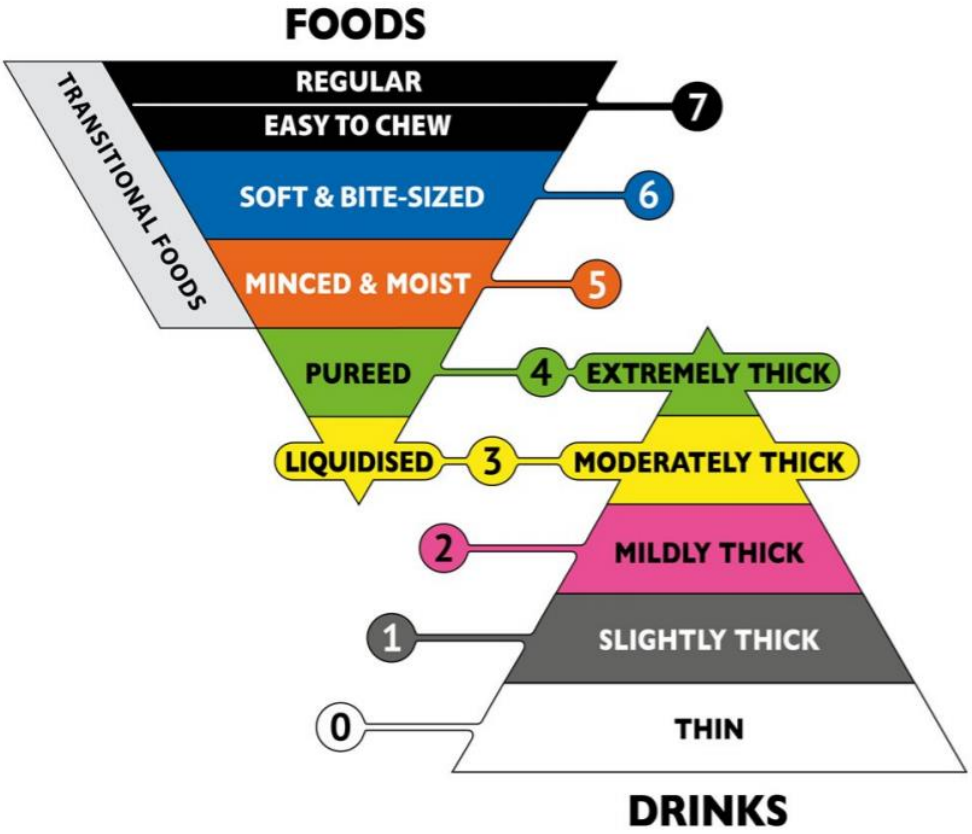


Figure 2.3. Standardized classification of liquid and food based on testing methods from the framework developed by IDDSI (IDDSI Testing 2019).

2.2 Proteins

Proteins are one of the four groups of macromolecules: proteins, lipids, nucleic acids, and carbohydrates. They are the most versatile and abundant of all macromolecules in living systems. Proteins are responsible for taking a part in practically every process in an organism (Berg et al. 2015). Furthermore, a few key processes include peptide hormones responsible for regulation of the metabolism, proteins in the bloodstream such as hemoglobin and plasma albumin functioning as transporters, and structural proteins like collagen (Ferrier 2013).

Proteins are constructed of monomer units named amino acids. More than 300 amino acids in nature have been reported, although 20 amino acids are commonly found in animal proteins (Ferrier 2013). A single amino acid is composed of a protonated amino group, a deprotonated carboxyl group, a hydrogen and finally a side chain or R group as seen in Figure 2.4. R groups are often characterized as four different groups, depending on their chemical structure. Hydrophobic amino acids contain nonpolar R groups, polar amino acids contain neutral R groups with the charge not distributed evenly and the remaining amino acids are either positively charged or negatively charged with a positive or negative charge at pH 7.4,

respectively. The sequence of amino acids refers to the primary structure, otherwise known as a polypeptide. They are linked together by peptide linkages, where the amino groups bond to the carbon of the carboxyl group (Berg et al. 2015).

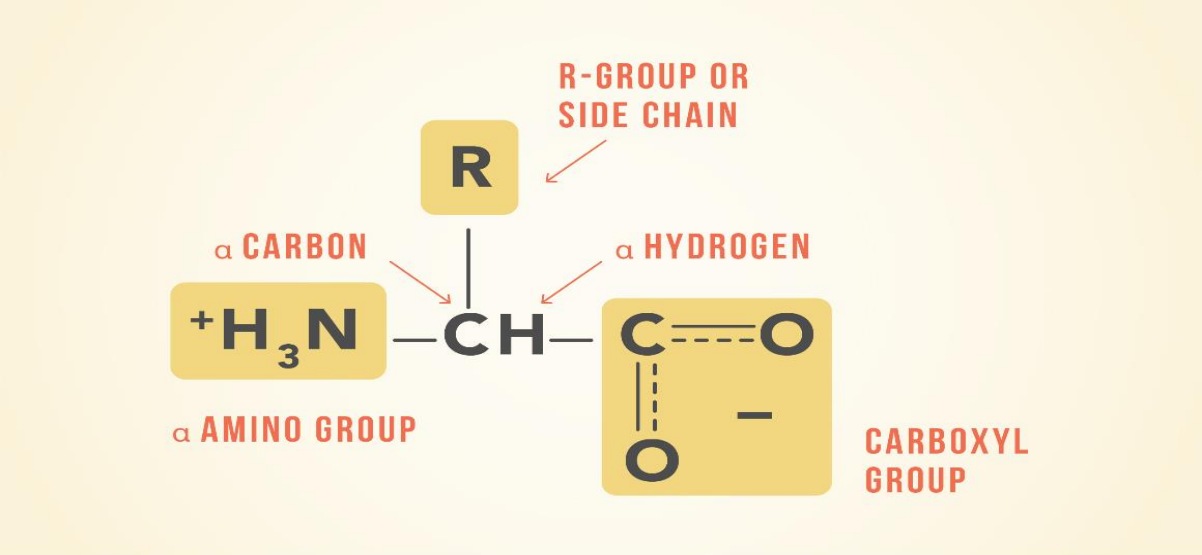


Figure 2.4. The backbone of proteins: amino acids with their attached functional groups carboxyl, amino and R group. The figure is from Technologynetworks (Steward 2019).

The primary structure further determines secondary structure which proteins spontaneously fold into. The secondary structure is stabilized by hydrogen bonds between the amino hydrogen and carboxyl oxygen atoms of the polypeptide chain. The two most regular structures are α helices and β sheets as well as the less known β turns, and Ω loops. The overall three-dimensional structure of the protein is called tertiary structure. This structure is determined by interactions between the R groups of the amino acids. These interactions include hydrophobic bonding, ionic bonds, hydrogen bonds and disulfide bridges. The interior is almost always consisting of nonpolar and hydrophobic interactions by their respective R groups, whilst the charged and polar R groups are located on the surface. Despite all proteins containing primary, secondary, and tertiary structure, not all proteins have quaternary structure. The quaternary structure is an arrangement of two or more polypeptide chains in a protein. A protein with a quaternary structure is hemoglobin, that consist of four subunits or polypeptide chains (Berg et al. 2015).

2.2.1 Fish as raw material

Seafood products contains several important nutrients, such as Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamin D, vitamin B12, iodine and selenium (Aakre et al. 2019). EPA and DHA are two polyunsaturated fatty acids, otherwise known as omega-3 fatty acids (Ghaly et al. 2013). A study even suggests that an increased fish intake with omega-3

fatty acid through dietary compliance gives higher cognitive functioning in preschool children (Øyen et al. 2018). Different products have variation in their nutrient content, although they all contribute to the recommended intake. Both fatty fish and lean fish are good sources of nutrients and highly bioavailable proteins (Aakre et al. 2019). Diets worldwide contains proteins from varying sources such fish, meat, or plants. Proteins derived from animals are often regarded a better source of nutrition compared with plants, due to their content of essential amino acids. The body cannot produce essential proteins and must acquire them through the diet. Fish muscle contains a great amount of these essential proteins (Rasco and Kristinsson 2000). Other benefits are increased bioavailability of minerals like iron by using fish in food e.g. cereal-based foods (Aakre et al. 2019). In summary, fish is a good source of nutrition in a healthy and balanced diet. The nutrient density of fish is especially preferable for elderly with a reduced food intake.

Fish muscles have some differences from terrestrial animals. Their muscle structure is made for swimming in water and therefore have less structural support. Animals on land require a stronger structure because they are more affected by the gravitational force and not surrounded by the mass of water. As a result, the fish muscle typically contains less connective tissue, making the texture more tender. Swimming also requires a different arrangement of the muscle fibers that also have an influence on the tenderness. The tenderness and softer tissue in fish makes the raw material suitable to use in TMF (Rasco and Kristinsson 2000).

The proteins in fish can roughly be characterized as myofibrillar proteins, sarcoplasmic proteins, and structural proteins. The proteins mainly responsible for the binding abilities and water holding capacity are the myofibrillar protein complexes. Furthermore, myofibrillar is the predominantly group found in fish meat. The protein complex contains myosin and actin (Rasco and Kristinsson 2000). The water holding capacity and binding abilities in fish makes it suitable for varying fish products such as surimi, minced fish, nuggets, Norwegian fish cakes and burgers. Minced fish is mainly a paste of stabilized myofibrillar proteins (Careche et al. 2011). When minced fish or other fish containing products are processed e.g. blended, salt is commonly added simultaneously with the fish raw materials. Salt extracts and solubilize myofibrillar proteins from fish muscle which then reveals the protein structure. As a result, the overall water holding capacity is now enhanced. The extracted proteins will additionally function as an emulsifier when oil or another source of fat is added. The effect of salt is most influential when the ionic strength is maximized, which is prior to addition of remaining ingredients. The extracted myofibrillar proteins also increase the binding between the fish meat (Devine, Dikeman, and Jensen 2004; Rotabakk and Iversen 2018; Ramírez et al. 2002).

The haddock fillets used for texture modified products (TMP) in this work were frozen, to eliminate seasonal variations of the raw material. Some of the fish species, and especially wild fish, have varying nutritional status during the seasons (Khitouni et al. 2015). A major fish lot was therefore purchased and fish for all experiments were taken from this single lot of fish. Frozen haddock and frozen silver smelt were used in the TMP while fresh haddock was used in the production of fish mince in addition to frozen silver smelt. More information about the frozen and fresh use of haddock and silver smelt are found in Chapter 2.2.1.2 and 2.2.1.3 respectively.

2.2.1.1 Sustainable use of fish as raw material

The fisheries and aquaculture are major industries established all around the world, and still increasing. The global demand of fishery products is increasing due to a growing population (Blanco et al. 2017). Better ways of handling fish in the processing industry through improved preservation, transportation and refrigeration also influence the increasing amount of consumed fish, in addition to greater variety of fishery products (FAO 2018). According to an annual analysis performed by Sintef, 3,57 million tonnes of fish and shellfish were derived from the Norwegian fishing industry in 2018 alone (Richardsen et al. 2019). A small increase compared with 2017 where the reported amount was 3.5 million tonnes (Richardsen et al. 2018). The utilization of by-products was reported to be 82% in 2018, the highest reported number in Norway (Richardsen et al. 2019). While on a global scale, fish production and aquaculture exceeded 171 million tonnes of fish in 2016 and the utilization of seafood products for direct human consumption was 88% (FAO 2018).

Utilizing by-products from the fish industry can obtain great economic, nutritional, and environmental values by increasing the yield from raw materials. By-products are secondary products produced in addition to the main product. Due to a fishing quota system, there are different limits on harvest volumes. Some raw materials are more expensive and often more challenging to get, caused by the allowed limits. Higher demand and lower supply often increase the price of the products. An increasing interest in using products that normally are regarded as waste is therefore developed to meet the demand of seafood products (Arason et al. 2009). The processes of utilizing by-products should be optimized, to keep it profitable. All parts of the production chain have to be assessed and as a result, the value of by-products will hopefully increase and be classified as raw material and not rest raw material or by-products (Aspevik et al. 2017). The yield of desirable products should also be maximized to highest level

possible (Arason et al. 2009). Plausible nutritious compounds in the by-products must be identified as well, to be used in functional foods (Aspevik et al. 2017).

By-products from the seafood industry consist of large volumes of heads, blood, bones, trimmings, viscera, and skin. The parts are residues from the main products like fillets for human consumption. A part of the by-products is further utilized into processing of fishmeal and fish oil. The remining by-products are discarded without any attend of nutrient recovery, such as fat, proteins, and minerals (Aspevik et al. 2017). The proteins in these residuals also possess possible bioactive properties, in addition to already well-studied effects such as gelling abilities, water holding capacity and emulsion abilities (Aspevik et al. 2017). By-products contain lower commercial value compared to the main products, hence the residuals are discarded and not further processed (Akhtar 2012).

One possible utilization of the by-products is extraction of proteins. Proteins from fish are commonly extracted as fish protein hydrolysates (FPH), fish protein concentrates (FPC) or fish meal. FPC share similarities with fish meal, except in FCP the protein content is higher, the percentage of oil is decreased, and the appearance is more homogenous. Other products that can be produced from by-products are fish oil rich in omega-3 and fish gelatin (Vázquez et al. 2019). The latter is obtained from skin and bones, as gelatin originate from the connective tissue collagen. The filleting industry produces large volumes of fish skin after the filleting process, and optimized use of these by-products would be highly beneficial. Fish gelatin can be applied as cover on medicine and vitamins in the pharmaceutical sector, due to a lower melting point than bovine gelatin, which is the major source of gelatin (Arason et al. 2009).

2.2.1.2 Haddock (Melanogrammus aeglefinus)

Haddock (*Melanogrammus aeglefinus*) is a saltwater fish within the cod family *Gadidae* (Britannica 2008). The fish is popular to add in processed products as well as being consumed on its own as cooked. Norway have been utilizing haddock in different products for several decades in varying minced fish products like “fish cakes” and “fish pudding”. United Kingdom is another country that are utilizing haddock in the popularized products fish and chips. The popularity is due to its good water holding capacity and binding abilities. However, the degree of these properties is diminished by freezing due to protein denaturation. The functionality of the proteins in haddock decreases in a frozen state, fresh fish is therefore often used in minced fish processing (Andreetta-Gorelkina et al. 2016). If the preferred texture of a product is extra soft, less binding ability due to freezing would be eminently suitable.

2.2.1.3 Silver smelt (*Argentina silus*)

Silver smelt (*Argentina silus*) is a bony fish within the *Argentinidae* family (Store norske leksikon 2013). The Silver smelt was not utilized for human consumption in previous years, according to older studies. It was used in the production of fishmeal, and not until later utilized in human food (Mackie and Hardy 1969). The popularity of this fish has risen in later years, especially in Norway, due to its exceptional gelling abilities, binding abilities and water holding capacity (Batista 2006; Hellevik, Synnes, and Stoknes 2005; Sivertsvik 2015). The fish is consumed both as unprocessed fish and in processed products like minced fish and fish nuggets (Hellevik, Synnes, and Stoknes 2005). Silver smelt is commonly stored in a frozen state prior to food production. Furthermore, it is often frozen directly after it is newly caught, still out on sea. This practice obtains the quality of the fish (Gundersen and Dahl 2008). The proteins in silver smelt is less susceptible to frozen denaturation compared to multiple other fish species. As a result, high water holding capacity, binding and gelling abilities are maintained to a high degree after thawing, which makes the specie suitable to store frozen (Hellevik, Synnes, and Stoknes 2005).

2.2.1.4 Fish protein hydrolysates

Various industrial food products have extracted parts of seafood, especially components from fish. One example suggested by Careche et al. (2011) is addition of omega-3 polyunsaturated fatty acids into baked goods and different dairy products. Other components like fish protein hydrolysates (FHP) are added to food in the food processing industry. The company Sprekere Liv AS is an example, they produce the protein powder "Hydroprot" of hydrolyzed fish proteins (Hydroprot). Another example is Pharmapolar with the product "Polarin" (Polarin).

Proteins can be recovered and extracted, e.g. as FPH, FPC and fish meal. The proteins to be hydrolyzed into FPH are commonly purified from pure fish muscular proteins with less than 1% oil and above 90% protein in dry weight (Arason et al. 2009). Non-hydrolyzed fish proteins have poorer accessibility to the peptide sequences of interest and therefore do not contain the same properties. The hydrolysis process aims to access these sequences while the properties are maintained (Ghaly et al. 2013). A selection of reported properties of FPH includes their bioactivity and positive health effects like antioxidant properties, water holding capacity and water solubility (Arason et al. 2009; Onodenalore and Shahidi 1996). Despite the attractive properties of FPH, there is unfortunately a hurdle to overcome when utilizing hydrolysates.

Protein hydrolysates in general are known to possess a certain bitter taste due to the size of peptides as well as the composition. Smaller peptides are commonly perceived as less bitter compared to longer. Enhanced methods of FPH processing can aid in improving the sensory properties. When hydrolysates are added to food products, other ingredients can mask the prominent bitterness (Rasco and Kristinsson 2000).

One way to extract proteins from fish involves protein hydrolysis, which is a commonly used method. The method consists of breaking down proteins into free amino acids and smaller peptides of varying sizes, which are now called fish protein hydrolysates (FPH) (Aspevik et al. 2017). Literally speaking, hydrolysis of a molecule is a reaction where water is used to cleave a molecule into two smaller parts (Hellvåg 2018). This reaction is a useful tool to utilize more of the by-products from the seafood processing industry. These proteins have a long history in the food industry with a variety of uses like milk replacers, stabilizers in beverages and as protein supplements. Hydrolysis of proteins from food; mainly milk and vegetables, have been performed for quite some time now, although FPH have been studied more recently (Rasco and Kristinsson 2000; Olsman 1979; Raikos and Dassios 2014; Vázquez et al. 2020). Studies of using FPH in animal feed production and as protein supplement in food for undeveloped countries were conducted from the 60s onwards, while FPH was already made commercial in the late 40s (Rasco and Kristinsson 2000).

Biological and chemical processing are the most used methods of protein hydrolysis. Previously, chemical hydrolysis was often performed more frequently in the industry, although biological hydrolysis is on the rise. In other words, biological hydrolysis with addition of enzymes is increasing and one example is the use of proteases (Rasco and Kristinsson 2000). The resulting yield from an enzymatic processing is quite high, as the amino acids are well preserved. The utilized enzymes can either occur naturally in the substrate or they can origin from external sources. Internal enzymes normally require long hydrolyzation time in addition to less yield specific yield. External enzymes on the contrary, is highly specific. Therefore, the use of external enzymes is often favorized over internal sources from the substrate itself, although the cost of external enzymatic processing is higher (Aspevik et al. 2017).

Chemical hydrolysis consists of cleaving the peptide bonds of the proteins by using an acid or base, although acid hydrolysis is often preferred over base. The previous popularity of this method in the industry was due to its simplicity and low-cost. However, chemical hydrolysis possesses a few limitations because of variations in the chemical composition (Rasco and

Kristinsson 2000; Aspevik et al. 2017). Some changes and even terminations can occur regarding the amino acids, e.g. tryptophan and cysteine can be destroyed in addition to glutamine and asparagine can be converted to their respective acids (Aspevik et al. 2017). Acid hydrolysis of fish proteins is often performed with hydrochloric acid or sulfuric acid at high temperatures and high pressures. Alkali hydrolysis can be carried out by utilizing sodium hydroxide, even though it can yield poorer products with decreased functionality and a smaller loss in nutritional value. Despite the plausible poorer yield, it is still employed in the food industry to recover and solubilize great amounts of proteins (Rasco and Kristinsson 2000).

2.2.2 Dairy proteins

Dairy proteins are a widely used source of proteins, found in different dairy products like milk and yoghurts. The grouping consists of a variety of different proteins, whey protein concentrates (WPC), whey protein isolates (WPI) and different forms of casein being some of them. The application of powdered dairy proteins used as a source of protein enrichment in varying products is increasing due to their great functionality. The proteins offer a wide array of properties like gelling abilities and water holding capacity, including other factors as varying flavor profiles and nutrition (Wright, Miracle, and Drake 2014). In addition to yield flavors, a study suggested that diary proteins were able to interact with other flavors to enhance or mask them. The study only conducted instrumental analyses and not sensory, they nonetheless revealed interactions (Kühn, Considine, and Singh 2006).

A combination of the diary proteins whey and casein is often recommended due to their varying absorption rates. Casein is known as the “slow” protein because it forms clots in the stomach that decrease the absorption rates and overall digestion time (Boirie 2019). In contrast, whey is known as the “fast” protein mainly due to its higher content of BCAA, primarily leucine, which is rapidly digested. A high content of leucine is favorable when addressing the muscle protein synthesis (MPS). Casein is demonstrated to obtain good effects on the MPS as well, as it yields a prolonged increase of amino acids in the blood. However, when whey and casein are consumed simultaneously, their positive influence on MPS is superior than to the proteins alone (Kanda et al. 2016).

2.2.2.1 Whey

Whey has been applied to an array of different products as infant formula, sports drinks and products, and in regular products like dairy, baked goods and (Buggy et al. 2018). It is originally

in a liquid state, separated from milk or cheese. The liquid consists of proteins, fat, and carbohydrates. Further, whey represents approximately 20% of total protein in milk. Liquid whey is processed thoroughly and dried into protein powders (Xiong 2009). Whey proteins can be categorized as the three main groups WPC, WPI and whey protein hydrolysate. WPI have the least amounts of other components and contains the greatest total protein, whilst WPC is slightly lower in total protein due to higher content of fat and carbohydrates. WPC is further subdivided into powders containing differing total protein, e.g. WPC80 or WPC60 (Henchion et al. 2017). WPC80 containing 80% protein was the selected whey protein to add into the TMP and minced fish products. Whey protein hydrolysate is a hydrolyzed protein powder consisting of smaller peptides and can therefore obtain a certain bitterness (Henchion et al. 2017).

Whey is a globular and hydrophilic protein mainly composed of β-lactoglobulin and α-lactalbumin, in addition to immunoglobulins, serum albumin and Proteose-peptone. The proteins within whey responsible for the gelling abilities whey are known for are α-lactalbumin, serum albumin and immunoglobulins. Only the single protein proteose-peptone obtains good water holding capacity, hence whey is not utilized for its water holding capacity but rather for its gelling abilities (Xiong 2009). The gelling properties often rely on the concentration, heating temperature, and pH, among others. Most gels are heat activated, including gelation of whey. Heat activated gelling of whey is a two-step mechanism. First-step consists of denaturation and unfolding of whey molecules, and then the molecules rearrange and aggregate, which expose functional groups. The new molecular structures can then be influenced by intermolecular interactions, which will consequently yield a three-dimensional network (Rimac et al. 2009).

2.2.2.2 Casein

Casein is a protein that is found in many forms. The protein can be added to a product either as casein or as caseinate bonded to an ion, depending on the desired functionalities (Wright, Miracle, and Drake 2014). Casein can make ionic bonds to sodium, potassium or calcium among others and is then referred to as caseinate. Furthermore, casein that is processed into sodium caseinate is the most common used form in different food products (Lagrange, Whitsett, and Burris 2015). Contrary to caseins, caseinates are considerably more water-soluble (Sarode et al. 2016).

Casein, which is 80% of total protein in bovine milk, consist of α_{S1}, α_{S2}, β, and κ-caseins. The three polypeptide chains α_{S1}, α_{S2}, and β are bonded by noncovalent interactions, forming a micelle. Due to hydrophobic and polar residues that are not distributed well on the amino acid

chain, they are prone to form clusters. As a result, casein possesses extra amphiphilic structures, appropriate for emulsion (Xiong 2009). Their popularity is almost solely due to their properties, as some off flavors as cardboard, animal and musty have been reported. Nevertheless, the majority obtains good and mild flavors (Wright, Miracle, and Drake 2014). Casein and caseinates can bind a great volume of water molecules. Some water gets entrapped within the micelles of bovine casein, in addition to the hydrophilic k-casein bonding to water. Relative to each other, caseinate have been referred to as proteins with terrific water holding capacity, while casein contains lower water holding capacity (Kneifel and Seiler 1993).

2.3 Development of functional protein enriched products

A food product consists of several ingredients which may possess different properties such as water holding capacity, gelling, and thickening abilities. The functional ingredients offer variations in texture in terms of a firmer or softer, dry, or juicy product, or other more desirable attributes. A few ingredients only offer flavors to a product, like garlic powder or pepper. One commonly utilized group of texture modifiers is hydrocolloids.

2.3.1 Hydrocolloids

Hydrocolloids are used in food as additives to change different attributes like texture and viscosity. They are mainly used as thickening and gelling agents. They are a heterogenous group of long chain polymers (polysaccharides and proteins) characterized by their property of forming viscous dispersions or gels when dispersed in water. They consist of hydroxyl (-OH) groups that increase their affinity towards water, making them hydrophilic. They also make a dispersion which is an intermediate between a solution and a suspension (Saha and Bhattacharya 2010).

Most hydrocolloids thicken liquids and many also form gels. Gel formation is the formation of cross-linked polymer chains that form a three-dimensional network which traps water within it to form a structure more resistant to flow, hence the similarities to a solid (Saha and Bhattacharya 2010). The process involves non-specific entanglement of conformationally disordered polymer chains, also called polymer-solvent interaction. Thickening occurs about a critical concentration known as an overlap concentration. Different hydrocolloids that have been used as thickening agents in different foods include starch, xanthan, locust bean gum (LBG), acacia gum and carboxymethyl cellulose. The level of thickening depends on different

factors like the hydrocolloid and its concentration, temperature and what the hydrocolloid is added to (Saha and Bhattacharya 2010).

Cornstarch obtains great water holding capacity, thickening abilities, and gelling abilities when it is added to a liquid mixture that is heated, in other words, thermal activated. Starches consist of the repeating molecules amylose and amylopectin. These are the structures which swell up and break down. When the mixture starts to cool down after heating, leaked amylose links together to generate a three-dimensional network, trapping molecules like water. The trapping of water is synonymous with increased water holding capacity in the finished product. According to Potter (2010), the gelatinization temperature of starch can range up to approximately 95^{o}C, while silva et al. (2016) reported lower temperatures around 65^{o}C. Wüstenberg (2015) suggested gelatinization at temperatures from 60^{o}C and up to 87^{o}C. Regardless, the selected cooking temperature of TMP in this study was 95^{o}C, which ensured maximum gelatinization. The gel will be stable during reheating and obtain its form and water holding capacity and not loose notable amounts of water. The heat stable properties are suitable to apply to TMP, since it is desirable to keep the shape of the soft product intact when served. The required amount of starch, LBG and many other hydrocolloids is smaller when compared to flours, which is beneficial in a protein enriched product, already containing powders of protein (Wüstenberg 2015).

In addition to cornstarch, LBG was used in the TMP in this thesis. The hydrocolloid otherwise known as carob bean gum is a polysaccharide composed of galactose and mannose units, primarily applied in food due to its thickening abilities. Many hydrocolloids can be added in different combinations to intensify the functionality and desired texture of a product. One suggested combination are starch and LBG in milk products and dessert, to enhance gelling, creaminess and thickening (Van Nieuwenhuyzen et al. 2006). The gum is also known to prevent syneresis when combined with starches. Syneresis is a process a gel often undergoes during storage over time, which is loss of water thus gaining a stiffened texture (Loth 1993).

The last hydrocolloid, which was used in the minced fish product, is potato flour. Compared to different extracted starches like potato and cornstarch, flour is basically the original product dried and then pulverized e.g. potato flour. Most of the literature provide information about starches, but not particularly about flours. Nonetheless, it obtains characteristics like corn- and potatostarch. The terms potato flour and potato starch are often used interchangeably as well, although the origin of the products is quite different. The mix of terms makes it more

troublesome to find specific data on potato flour since publications include it in the texts about potato starch.

2.4 Food safety

Consumers desire safe food with absence of pathogenic microorganisms and hazardous chemicals. Food safety is therefore required in food production. Foods that are aimed specifically for vulnerable groups as young, old, pregnant, and immune compromised people, are highly critical and should be free from pathogens (Jay, Loessner, and Golden 2005). Pathogenic microorganisms such as bacteria, parasites, fungi and viruses may either be active and produce toxins in the food or they can be infectious if they appear in specific high doses (Godfree 2003). One of the major challenges is food borne pathogens such as bacteria, parasites, fungi, and viruses contaminating the product (Jay, Loessner, and Golden 2005). Bacteria and viruses are the leading cause for most hospitalizations and deaths associated with food (Fung, Wang, and Menon 2018; Jay, Loessner, and Golden 2005; Bintsis 2017).

TMP and minced fish products produced in this thesis used fish as raw material, which is associated with different bacteria e.g. *Aeromonas, Pseudomonas, Shewanella, Listeria*, and *Vibrio*, which originates from the water the fish is caught from. Although the outer side contains bacteria, the inner muscles are sterile, nevertheless, this does not ensure safe consumption as muscles can be contaminated by the outer microbes during processing and filleting. Heat treatment of products containing fish is therefore important, to ensure a safe product, especially products for vulnerable groups (Jay, Loessner, and Golden 2005).

Several critical production procedures are needed to ensure safe products like good manufacturing hygiene, sufficient heat treatment to inactivate target organisms, rapid chilling and chilled storage temperatures in addition to proper packaging (Jay, Loessner, and Golden 2005). The whole process from handling of the raw materials to final consumption must be controlled to obtain safety. Since TMP have elderly people as a target group, the safety of these finished products is therefore essential.

Sufficient heat treatment is a common practice to pasteurize a product to ensure safety throughout the production chain until final storage and consumption. Bacteria often associated with food poisoning is the genus *Clostridium*, especially the non-proteolytic strains. The spore-forming bacteria are anaerobic that favors growth in extended shelf life products containing an anaerobic packaging (Lindström, Kiviniemi, and Korkeala 2006). Furthermore, some strains

produce neurotoxins responsible for human diseases such as gas gangrene, botulism, and infant botulism (Jay, Loessner, and Golden 2005). Non-proteolytic *Clostridium botulinum*, a part of serogroup II, can form neurotoxins in chilled conditions down to approximately 3ºC (Lindström, Kiviniemi, and Korkeala 2006). Heat treatment for 10 minutes at 90ºC will normally provide a sufficient reduction of potential non-proteolytic *C. botulinum* spores, which is a 6-log cycle reduction. Proteolytic *C. botulinum* on the other hand, require higher temperatures for inactivation. Regarding the temperature of the proteolytic strains to form toxins, the temperature is higher compared to non-proteolytic. Toxins can be produced of Proteolytic *C. botulinum* at minimum 10-12ºC (Chilled Food Association Ltd 2018; Maier et al. 2018). By storing the finished heat-treated product in a refrigerator at 4ºC, preventive measures against proteolytic *C. botulinum* is not needed for food safety. Therefore, several agencies like the U.S. Department of Agriculture (USDA) and Norwegian food safety authorities recommends using a refrigerator, among other methods, for thawing and storage to keep the cold chain unbroken (Agriculture 2010; Mattilsynet 2007). There are in fact mandatory regulations about processed food that concern chilling times and temperatures. E.g. ready meals must be rapidly cooled to 4ºC within two hours after final heat treatment to prevent growth of toxin producing bacteria (Næringsmiddelhygieneforskriften 2009).

2.5 Development of attractive products using physical, chemical, and sensory analysis

Development of food products often require extensive testing, consisting of either physical, chemical, or sensory analyses or a combination. Different attributes such as texture, taste, odor, and appearance can be studied in addition to properties like water holding capacity and shelf life. Analyses can be applied to ensure that the quality is maintained during production and is therefore an important part of food product development.

2.5.1 Sensory Analysis

Sensory attributes of food products like taste, smell and texture will dictate the consumer's interest. The nutritious value in a product is of high importance simultaneously as the sensory properties play a key role. However, nutrition alone will not lead to an accepted product among consumers. Thus, an evaluation of the attributes is crucial in the food industry (Sirangelo 2019). Especially the flavors in food produced for elderly are highly critical, due to the loss of appetite

often observed in aging people (Robinson et al. 2015). The flavors should therefore be appetizing and flavorsome.

Contrary to taste and aroma, simple texture profiles can be provided by different instruments like rheometers and texture analyses. However, in most cases a combination of both sensory and instrumental analysis would be appropriate. Texture variations associated with consumption is often more complex than the results an instrumental analysis can provide. The data from an objective sensory evaluation is therefore combined with data from physical instruments to get a deeper understanding of its texture (Sirangelo 2019). These texture changes in food are results of mastication followed by formation of a bolus lubricated with saliva.

Quantitative Descriptive Analysis (QDA) provides quantitative and objective results, comparable to other physical and chemical analyses (Sirangelo 2019). A descriptive sensory analysis is performed by a trained panel according to selected ISO (International Organization for Standardization). The panelists then derive numbers corresponding to the perceived intensity of each selected attribute, which ranges from no intensity to high intensity (Yang and Boyle 2016).

2.5.2 Texture measurements of food

A numerous of textural parameters regarding different products can be assessed, depending on the primary goal. Some products like chips desire a crunchiness, whilst meat or any other soft food would optimally obtain a certain tenderness. Texture measurements are therefore widely used in food development and research (Bourne 2002). Several people within different groups such as elderly or smaller children requires food which is easy to consume. People suffering from dysphagia and elderly generally requires products with a modified, softer texture. The products must contain great nutritional value in addition to be easy to masticate and swallow (Sungsinchai et al. 2019).

A rapid and reproducible method to determine the firm or soft texture can be performed via a penetration test, additionally known as a puncture test as they are commonly combined. A probe penetrates the surface of a product with a given distance under constant load. The pre-test speed, test speed and post-test speed are also determined in a penetration test. The pre-test speed is the speed the probe holds before it connects with the surface, test speed is the speed it holds during penetration, and the post-speed is the speed of the probe when it retracts to its original height. The increasing force needed to achieve puncture of the surface will eventually reach a peak, the

peak often referred to as a yield-point (Figure 2.5). The measured force derived from the yield-point is the force required to puncture the surface. Due to textural differences in TMP and minced fish, the force (N) is defined as firmness in TMP and hardness in minced fish (Bourne 2002).

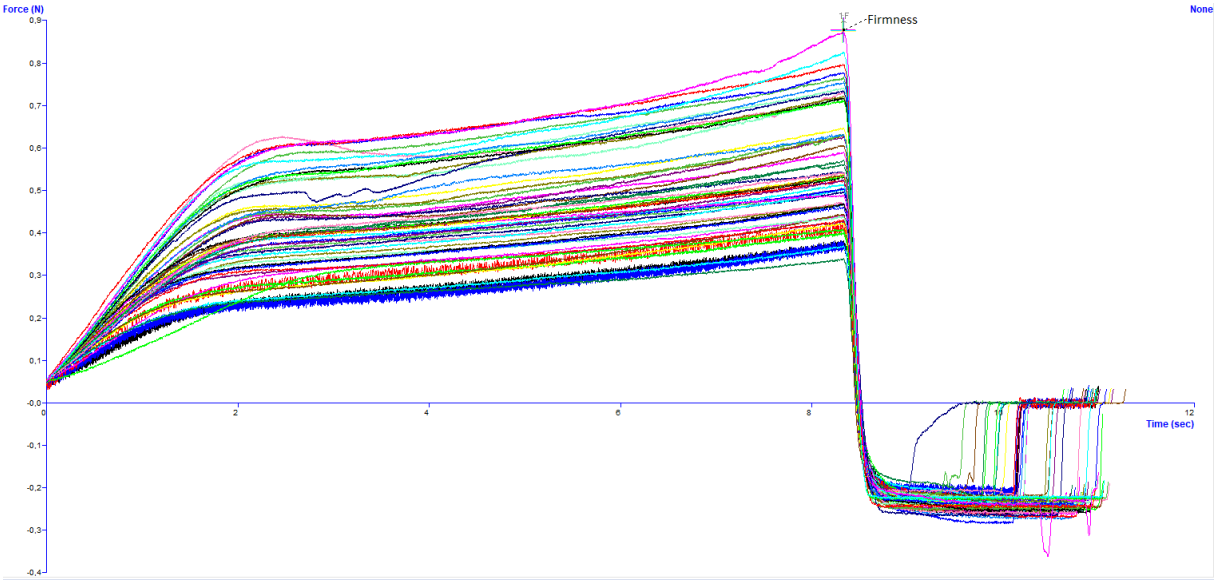


Figure 2.5. A selection of graphs obtained from a puncture test performed on texture modified products. The force (N) needed for penetration is plotted against time resulting in a yield-point, which is identified by the text Firmness.

Other textural parameters as stiffness, hardness, and gel strength are commonly examined regarding gelled products like minced fish and surimi. A method commonly referred to as a gel-test which derive the mentioned parameters is described by Øines (Øines 2019). The force (N) necessary to penetrate the surface is determined, as well as the distance (mm) required for the penetration to occur. Immediately after surface puncture is achieved, the selected probe ascends to its original position. The determined values can be further applied in calculations to derive stiffness (N/mm) and gel strength (N*mm). Furthermore, stiffness is defined by the force exerted per. mm of penetration distance (Øines 2019).

Another commonly applied method on an immensely wide array of food products is Texture Profile Analysis (TPA). TPA is supposedly simulating the way humans consume food by performing a double compression test, otherwise known as a “two bite test” (Bourne 2002). The texture in food do never consist of one attribute, due to its complexity. The overall texture profile is composed of several textural attributes, i.e. a product is never perceived as just hard, cohesive, springy etc.

The basic TPA characteristics include hardness, cohesiveness, springiness, and resilience. The remaining attributes include adhesiveness, gumminess, and chewiness; however, gumminess and chewiness are mutually exclusive due to the calculations they are derived from and which product they are used for. Gumminess is commonly applied on semi-solids, while chewiness is applied on more solid products (Bourne 2002; Texture Technologies). Hardness is the maximum force applied in the first compression, referred to as a point-yield where a sudden change in slope occur. Cohesiveness is defined as the ratio of the area below the second and first peak (A_2/A_1) and tell how the product withstands deformation (Figure 2.6). TMP should be cohesive enough to not fall apart when plated, but on the other hand not too cohesive either, which could make swallowing more problematic. Springiness educate about the sample's ability to physically spring back after deformation. Resilience is how well the product manage to recover to its original height. The remaining parameter adhesiveness is the negative work between the two cycles and tell about the ability of adhering (Bourne 2002; Texture Technologies).

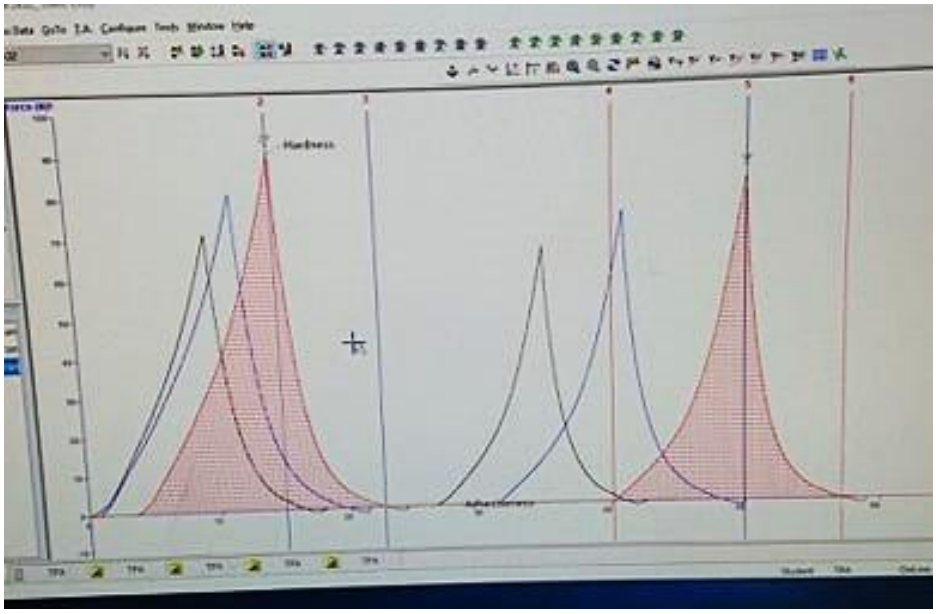


Figure 2.6. The graph is generated from an TPA with force plotted against time. The two peaks with an area colored in red, show the first and second compression cycle from a test.

The compression is generally performed with a compression plate which is greater in size than the product. The plate will then compress the sample a selected distance or strain of sample height. Several suggestions of strain % have been proposed and demonstrated over the decades. Bourne conducted analyses with strain up to 90%, whilst other have conducted TPA with lower strain down to 20-25% (Bourne 2002; Romero de Ávila et al. 2014). Analyses conducted with 66% and up to 90 have been demonstrated as quite destructive, and therefore lower strain from

65% and less are often a more suitable approach. Furthermore, lower strain will easier detect smaller, subtle differences between different variants of a product (Texture Technologies).

2.5.3 Color measurements

One of the first impressions a person receive about a food product is the appearance, followed by the odor. Except for external texture, color is an important part of the appearance. The color can yield an indication of quality before further examinations are conducted (Pathare, Opara, and Al-Said 2012). For example, if the color of red strawberries is overly bright with a tone of green they may not be ready for consumption or on the other hand an undoubtedly dark red color, they are most likely soon to be rotten and stale. Hence, color analysis is a widely used tool in food development, industry, and research, regarding quality assessment and to gain overall knowledge associated with the food products.

Two commonly applied color spaces are CIEL*a*b*, which is described in Cartesian coordinates and CIEL*C*h which is described in cylindrical coordinates. The coordinates in a CIEL*a*b* space is laying opposite to each other. With other words, the color can only exist as either dark or bright (L* black→white), green or red (-a*→+a*) and blue or yellow (-b*→+b). L* ranges from 0 to 100, a* and b* commonly exist in the range -128→+127. Since CIEL*C*h is based on cylindrical coordinates, each color coordinate describes a color characteristic. L* provide information about the brightness, C* (chroma) tell about the color's saturation whilst the remaining coordinate h (hue) provide information about the specific color of the analyzed product (Mokrzycki and Tatol 2011).

2.5.4 Rheology

Rheology is the science of deformation/displacement and flow of matter and can be applied to almost any product. Rheological analyses associated with the food industry study the behavior of either complex products or individual ingredients. A commonly used term in rheology is viscosity: its resistance of flow due to the internal friction caused by interactions between molecules in a fluid or a solid. Even though food is usually a solid or semisolid and behaves accordingly, they can behave as a fluid when sufficient stress is applied. Furthermore, stress is defined as the force per unit area. The result of applied stress can be shear thinning or shear thickening, which in both cases happens due to the product resembling non-Newtonian behavior. Non-Newtonian behavior is the change of a liquid or solid during stress, when the viscosity decreases irreversibly (shear thinning), increase irreversibly (shear thickening), or any

reversible change (thixotropic). Texture analysis is studying the macrostructure while rheology focuses on the microstructure; how the interactions between molecules changes during applied stress (Bourne 2002).

A common method used to apply stress on a sample is direct compression, although there are other, less frequently applied methods as well. Another term regularly used in rheology is strain (%), which is referred to as the change in shape or size of a sample during stress. An amplitude sweep investigates the deformation of a sample by applying increasing strain, otherwise known as oscillatory strain. The region in which the structure is maintained and not deformed, is called the linear viscoelastic region (LVR). A selected oscillation strain (%) within LVR of a sample ensures that the sample behaves viscoelastic and does not get deformed during further analysis. Graphs obtained from such analyses often contain a constant slope prior to a decrease or increase in slope. The graphs visualize the LVR when strain is plotted against the storage modulus G' (Pa). The storage modulus provides information about the ability to store deformation energy. Higher storage modulus normally means a firmer and more ordered structure. Another rheological analysis often applied is a temperature sweep, which investigates the behavior during varying temperatures (Bourne 2002).

2.5.5 Water holding capacity

A common method used to assess quality of foods e.g. meat is water holding capacity. Attributes such as firmness and juiciness can be related to water holding capacity. Texture is influenced by myofibrillar proteins and collagen in meat, as they reduce in size by denaturation during heating. The denatured proteins cause shrinkage of myofibrils in muscles and further lose water which results in a firmer and tougher texture. Less water in meat can additionally be perceived as a less juicy product (Skipnes et al. 2011).

Water holding capacity is defined as the ability of reconstructed meat products and meat to bind and hold water during different processing methods e.g. cooking and mincing, and during storage. Water loss in food is referred to as several different terms like exudate, cook loss, weep, or drip, depending on how the water is lost, e.g. cook loss during heat treatment. Some researchers define water holding capacity as the capacity of meat to hold water, while water binding capacity are used when addressing bound water through processing. Some people use the definitions interchangeably. Others on the other hand, define water holding capacity to include both waters already held by the meat in addition to added water that meat have bound (Warner 2017). The latter definition including both holding and binding will be further applied in this thesis.

3. Materials and Methods

3.1 Workflow diagram

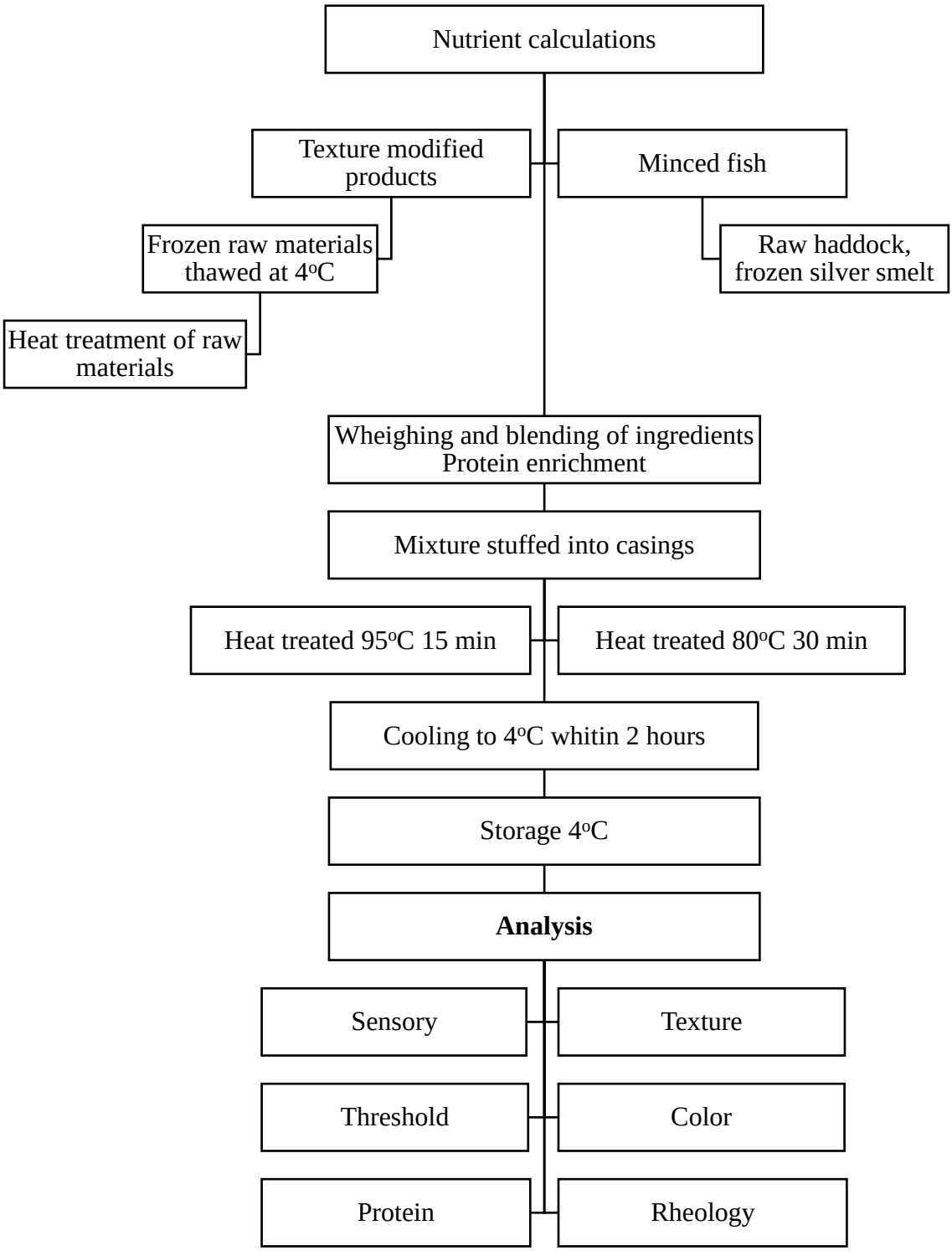


Figure 3.1. A workflow diagram of the production of protein enriched fish products: texture modified products and minced fish, from the initial phase to final analyses.

The workflow diagram in Figure 3.1 show the prosses of making protein enriched minced fish products and texture modified products (TMP). The fish raw material used in TMP is heat treated prior to blending while the raw material is added untreated into the blending of minced fish. The products are heat treated at different temperatures, but the process and analyses after heat treatment is equal. The purpose was to develop two protein enriched products, reaching the level of total protein in the utilized fish raw material haddock and silver smelt. Both products were enriched by whey protein concentrate (WPC), sodium caseinate (caseinate) and fish protein hydrolysates (FPH) and further investigated how the proteins affected color, texture, water holding capacity and sensory attributes. Information about the utilized FPH can be found in Appendix A and B.

3.2 Nutrient facts

Nutrient information is one of the crucial factors in developing recipes, especially towards elderly and people with dysphagia. They require nutrient rich food containing great amounts of proteins. Due to the loss of appetite often observed in elderly, the products should preferably be additionally energy dense (Chapter 2.1.2 and 2.1.3). Another group of people who are highly interested in protein enriched products are athletes, who consumes a great deal of sports nutrition. Enriched products have also gained popularity among regular people (Chapter 2.1.1). Therefore, it is important to develop varying products whether the primary goal is to build muscle mass, lose weight, dysphagia management or as a part of treatment of other diseases.

The texture modified products (TMP) are diluted by a liquid to get a softer texture for easier swallowing. The dilution will additionally affect the total protein in the products. Hence, TMP are added fish protein hydrolysates (FPH) to increase the overall amount of protein. It was desired to maximize the total protein content without the expense of the soft consistency, but nevertheless to contain more proteins than the raw material silver smelt (19.6% protein) and haddock (16.6% protein) (Table 3.1). The same reasoning was applied in developing the recipes of minced fish, as the desired total protein content should be closest possible to the original protein content of the raw material (Table 3.1).

Table 3.1. The nutrient content of haddock fillet and silver smelt (Matvaretabellen 2020).

Materials	**Protein (%)**	**Salt (%)**	**Fat (%)**	**Carb (%)**	**Kj/Kcal**
Haddock fillet	16.6	0.2	0.2	0	290/68
Silver smelt	19.6	0.1	1.2	0.1	378/89

The nutrient content of each recipe was calculated in Excel (v16.0, 1909, Microsoft Corporation, US). Most of the ingredients were already labeled with the required nutritional information. The Norwegian online database known as Matvaretabellen was used to obtain the lacking nutrient facts on the ingredients (Matvaretabellen 2020). The database is a collaboration between Norwegian Food Composition Database and the Norwegian Food Safety Authority.

3.3 Preparation of fish

Haddock used in texture modified products (TMP) were frozen prior to the production, while in the production of minced fish fresh haddock was used. In both productions frozen silver smelt were an additional fish ingredient. The frozen fish raw material was thawed for 24 h at 0°C before the production of minced fish, easier to keep the temperature low during blending. The frozen fish material for TMP on the other hand, was thawed for 24 h at 4°C. The blending process of TMP was based on time and not temperature as the products were not required to be kept cold as the mixture of minced fish products. Thawing at 4°C or below is the recommended temperature due to food safety (Chapter 2.4).

3.3.1 Haddock

Fresh haddock fillets (skinless V-Cut, Nordic Group AS, Trondheim, Norway) which were used in minced fish production arrived one day prior to production. The fillets were first removed of excess bones and skin and then diced into smaller pieces. The pieces of fish were stored cold at 4°C overnight.

Haddock fillets for the modified texture products were cut into 500 g portions and vacuum packed (99.9% vacuum) individually in vacuum bags (220x600mm, PA/PE 70my, LietPak, Lithuania) using a vacuum packing machine (Supermax C, Webomatic, Germany) and stored in the freezer at temperature -20°C until further use.

3.3.2 Silver smelt

The 7 kg blocks of frozen silver smelt (product number: 3184917, Tavan, Lerwick, Faroe Islands) intended for all productions were cut by a Dadaux band saw (SX350, Dadaux, France) into approximately 500 g pieces and vacuum-packed (99.9% vacuum) in identical bags like the haddock fillets. The silver smelt were stored in the freezer at -20°C until further use.

3.4 Texture modified products

3.4.1 Ingredients

Table 3.2. List of ingredients used in both preliminary- and pilot production of texture modified products.

Materials	Information	Producer	Country
Haddock fillet	Fresh, skinless V-Cut, product nr: 1119105	Nordic Group AS	Norway
Silver smelt	7 kg frozen block of silver smelt Product nr: 3184917	Tavan	Faroe Islands
Cornstarch	EAN: 8718114782591 (Maizena)	Unilever AS	Norway
Fish broth	Klar fiskebuljong, Maggi	Nestlé Norge AS	Slovakia
Sunflower oil	EAN: 5020514460058	Olympic Food	U.K.
Ground mustard seed	EAN: 7053260209518	SAGA, L.K. Haaland AS	Norway
Ginger ground	EAN: 7053260203714	SAGA, L.K. Haaland AS	Norway
Ground fennel seed	EAN: 7053260202113	SAGA, L.K. Haaland AS	Norway
White pepper powder	EAN: 7053260187458	SAGA, L.K. Haaland AS	Norway
Whipping cream	37% fat	Tine SA	Norway
Hydrolysate[a]	Endurance Salmon protein hydrolysate, 95% total protein	Hofseth Biocare	Norway
Hydrolysate[b]	Endurance Salmon protein hydrolysate, 97% total protein	Hofseth Biocare	Norway
Hydrolysate[b]	Salmon backbone protein hydrolysate, 89.9% 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^{tm} JPR 1002 87% total protein	Armor proteins	France
Locust bean gum	Art. 40460	Bohlsener Mühle, Gmbh & Co. KG	Germany

a: Was utilized in preliminary production
b: Was utilized in pilot production

3.4.2 Recipes in preliminary testing

The procedure and recipes are based on Prabhus` work (2018) during her master thesis. The optimization process during preliminary testing consisted of the following parameters: 1) the ratio of haddock to silver smelt, 2) cooking of raw materials prior to blending and 3) the blending times. Preliminary testing was used to obtain an improved understanding of the

blending process and how different blending times influenced the final product. Recipes that consisted exclusively of haddock (1A) or silver smelt (1B) or ratio 1:1 haddock and silver smelt (1C) were tested. Precooked silver smelt (1C[a]) and non-precooked silver smelt prior to blending were additionally investigated to see how the binding abilities was influenced by non- and precooking. The recipes in preliminary testing are listed Table 3.3 and further information about the ingredients are listed in Table 3.2. After the optimization phase in preliminary testing was completed, the next phase, preliminary production which consisted of protein enrichment, could be initiated. The whole procedure of making texture modified products (TMP) during preliminary testing is described in Chapter 3.4.4.

Table 3.3. Ingredients in preliminary testing of texture modified products (%). Ingredients with variation are listed first. Calculated total content of proteins, fat and salt are listed below the thick line. Detailed information about each ingredient can be seen in Table 3.2.

Ingredient	**1A**	**1B**	**1C[a]**
Haddock fillet	-	59.0	29.5
Silver smelt	59.0	-	29.5
Salt	0.4	0.4	0.4
Cornstarch	0.6	0.6	0.6
Spice mix[b]	0.2	0.2	0.2
Fish broth	1.2	1.2	1.2
Locust bean gum	0.4	0.4	0.4
Whipping cream	23.5	23.5	23.5
Oil	9.3	9.3	9.3
Hydrolysate	-	-	-
Sodium caseinate	1.8	1.8	1.8
WPC80	3.6	3.6	3.6
Total protein	16.5	14.8	15.6
Total fat	19.2	18.6	18.9
Total salt	0.9	1.0	1.0

a: Both precooked silver smelt, and non-precooked silver smelt was tested in this recipe.
b: Spice mix consist of equal quantity of ground mustard seed, ground ginger, white pepper powder and ground fennel seed.

3.4.3 Recipes in preliminary production

The preliminary production was used to obtain an improved understanding of how different ingredients like whipping cream, oil or protein enrichment influenced the texture of the product. The main goal with the preliminary production was nevertheless to maximize the total protein content in texture modified products TMP, reaching or better exceeding the total protein of the fish raw materials. Different adjustments of whipping cream and oil were tested in addition to fish protein hydrolysates (FPH), whey protein concentrate (WPC) and caseinate. As these products were targeted toward people with dysphagia and elderly, the oil and whipping cream

were adjusted accordingly after the calculated total protein reached a satisfying level. The final product would be both energy dense and have a high total protein content. With other words, which adjustments were required to be able to reach the main goal of energy and protein without losing the desired, soft texture. The recipes used in the protein enrichment process has been listed in Table 3.4. The whole procedure of making TMP in preliminary production is described in Chapter 3.4.4.

Table 3.4. The ingredients used in the preliminary production of texture modified products. Detailed information about each ingredient are listed in Table 3.2. Whipping cream, oil, and protein enrichment are the only variables and therefore located below the thick line. Calculated total protein, fat and salt are listed at the bottom. All values are given in percentage.

Ingredient	**2A**	**2B**	**2C**	**2D**	**2E**	**2F**	**2G**
Haddock fillet	30	30	30	30	30	30	30
Silver smelt	30	30	30	30	30	30	30
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cornstarch	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Spice mix[a]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Fish broth	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Locust bean gum	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Whipping cream	23.5	22	23.5	20	20.7	23.5	20
Oil	8.3	5.2	3.7	5.6	5.7	1.7	5.2
Hydrolysate	-	2.5	2.5	2.9	2.7	3	3
Sodium caseinate	1.8	2.5	2.5	2.9	2.7	3	3
WPC80	3.6	5	5	5.8	5.4	6	6
Total protein	15.8	19.9	19.9	21.2	20.5	21.6	21.5
Total fat	17.9	14.3	13.4	14.0	14.3	11.4	13.6
Total salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1

a: Spice mix consist of equal quantity of ground mustard seed, ground ginger, white pepper powder and ground fennel seed.

3.4.4 Procedure of preliminary testing and preliminary production

The procedures of making TMP in preliminary testing and preliminary production were equal except recipe 1C[a] was processed with non-precooked silver smelt.

The fish raw materials were prepared for the first heat treatment by placing the sealed vacuum bags with thawed fish in perforated aluminum trays (1/1 GN). The silver smelt was lightly compressed by hand to resemble the thickness of the haddock fillet to undergo equal cooking time. The fish raw materials were then cooked in a preheated, convention oven (MSCC61, Metos system Intl., Germany) set on 100^{o}C, 100% steam (Figure 3.2). The core temperature was monitored throughout the cooking by temperature probes (E-Val flex, Ellab, Denmark) placed into the center of the fish. The probe was placed in the thickest part to ensure a

satisfactory core temperature of 95°C for 15 min. After cooking, the fish were submerged in an ice slurry for rapid cooling below 20°C to stop the cooking process before being further processed. The raw materials were now ready to be blended.

Figure 3.2. Heat treatment of vacuum sealed silver smelt and haddock fillets.

Silver smelt, haddock, salt and eventual cooking loss were transferred to a blender (Thermomix tm5, Vorwerk, France). Cooking loss was included to prevent loss of water-soluble proteins (Berg et al. 2015). The salt was added to the fish to enhance its water holding capacity (Puolanne, Ruusunen, and Vainionpää 2001). The rest of the ingredients were added to the blender, followed by fixed blending times and speeds (Table 3.5). The mixture was blended between each added ingredient to ensure a homogenous product (Figure 3.3A).

Table 3.5: Sequence of added ingredients, mixing speed, and times used during addition of different ingredients for preliminary testing and production of texture modified product.

Sequence of addition	**Ingredient**	**Mixing time (min:sec)**	**Mixing speed**
1	Silver smelt	00:30	5
2	Haddock fillet		
3	Salt	02:00	5
4	Whipping cream	01:30	10
5	Oil	01:00	10
6	Cornstarch	00:15	10
7	Hydrolysate	00:30	10
8	Sodium caseinate	01:00	10
9	WPC80	01:00	10
10	Spice mix	00:40	10
11	Fish broth		
12	Locust bean gum		

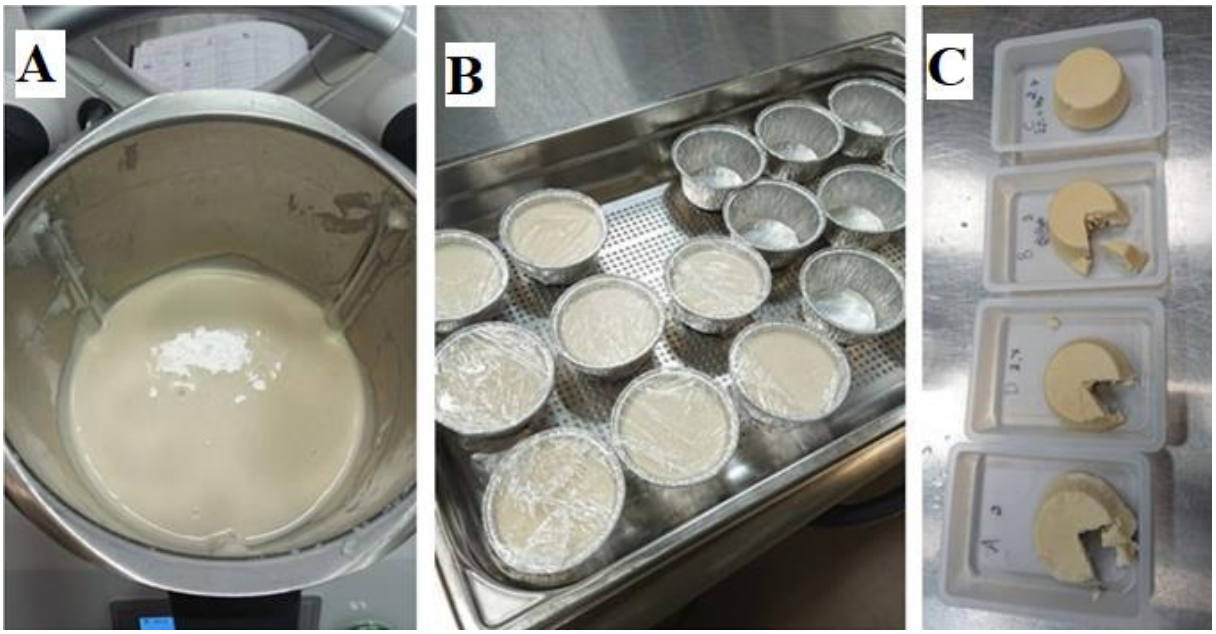


Figure 3.3. Some steps of the preliminary production of texture modified products. A: The homogeneous mixture after blending, B: mixture transferred into aluminum containers and wrapped with plastic cling film, C: the finished product during tasting.

Portion sized aluminum containers (Round, 106ml Ø80, Plus Pack, Denmark) were greased with vegetable fat (Melange formFett, Mills DA, Norway). A portion of 80±2 g of the mixture was then poured into each container, before individually wrapped with plastic cling film (Figure 3.3B). A temperature probe was placed in the center of a selected sample, by penetrating from the side of the aluminum container before the second cooking. The products were cooked with a core temperature of 95ºC for 15 min in the same convention oven as the first cooking (100ºC 100% steam). Work done by Prabhu (2018) suggested that using lower temperatures during cooking could cause water or oil loss during reheating before serving. In addition, the higher temperature ensured a safe product (Chapter 2.4). The products were placed immediately on ice for 2 h after cooking, both to stop the cooking process and to start a rapid chilling. They were stored in a chilled room at 4ºC until further analysis, except one sample from each batch, which were evaluated by a simplified taste and visual test performed in-house in order to contribute to further development of the recipes (Figure 3.3C).

3.4.5 Recipes in pilot production

The recipes in pilot production were further developed using acquired knowledge from the preliminary production. The amount of whipping cream was determined to be constant while oil and protein enrichment were adjusted, including either Hofseth or Nofima hydrolysate, WPC and caseinate. One recipe was used as a standard (ST) and did not contain any FPH. The following recipes was enriched with FPH in following order: high Hofseth texture (HHT), low

Hofseth texture (LHT), high Nofima texture (HNT) and low Nofima texture (LNT). The "high" samples had a calculated total protein of approximately 21.5%, the "low" samples had 19.5% and standard (ST) had 16.2% protein. Detailed information about the recipes are listed in Table 3.6.

Table 3.6. Ingredients used in the pilot production for texture modified products. Oil and protein enrichment are the only variables and therefore located below the thick lines. Calculated total protein, fat and salt are listed at the bottom. The values are given in percentage.

Ingredient	**ST**	**LHT**	**HHT**	**LNT**	**HNT**
Silver smelt	30	30	30	30	30
Haddock fillet	30	30	30	30	30
Salt	0.4	0.4	0.4	0.4	0.4
Locust bean gum	0.4	0.4	0.4	0.4	0.4
Cornstarch	0.6	0.6	0.6	0.6	0.6
Spice mix[a]	0.2	0.2	0.2	0.2	0.2
Fish broth	1.2	1.2	1.2	1.2	1.2
Whipping cream	20	20	20	20	20
Oil	11.2	7.6	5.2	7.4	5
Hofseth hydrolysate	-	2.4	3	-	-
Nofima hydrolysate	-	-	-	2.45	3.05
Sodium caseinate	1.8	2.4	3	2.45	3.05
WPC80	3.6	4.8	6	4.9	6.1
Total protein	16.2	19.5	21.6	19.5	21.5
Total fat	19.5	15.9	13.6	15.7	13.4
Total salt	1.0	1.0	1.0	1.0	1.0

a: Spice mix consist of equal quantity of ground mustard seed, ground ginger, white pepper powder and ground fennel seed.

3.4.6 Procedure of pilot production

Nearly the entire procedure explained in Chapter 3.4.4 of preliminary production was repeated during the pilot production, except the steps which followed the blending.

In preliminary production the mixture was transferred into small aluminum containers after blending. However, in the pilot production the mixture was filled into a Betan casing (Betan, ART: 4210002500, Ø30mm transparent, Viscofan, Czech Republic), (Appendix C) using a motorized sausage filler (H15, Talsa, Talsabell a.s., Spain) (Figure 3.4A). The sausage-like shape of the samples made a suitable uniform product for various analysis which were easier and safer to handle and store than the prior used aluminum containers. The applied casing was soaked for 30 minutes in water prior to filling to increase its elasticity. Before filling the casing, one end was sealed with clips (S 632, poly-clip System, Germany) using the single clip machine (SCH 120, poly-clip System, Germany). Each sample was shaped to approximately 20±1 cm in

length and sealed with clips in both ends to make individual samples. The products final appearance can be seen in Figure 3.4B.

Figure 3.4. Two of the steps during pilot production of texture modified products. A: The utilized sausage filler for the making of the samples, B: the filled samples prior to heat treatment.

One sample was equipped with a temperature probe (Testo 176T4, Testo SE & Co. KGaA, Germany) during sealing. To keep the probe in the center of the sample during heat processing, a piece of a round aluminum container was cut off, folded, and pinned with a hole in the center in which the probe was inserted (Figure 3.5A and B). The heating process was equal to the procedure mentioned in Chapter 3.4.4, except two, bigger convention ovens (MSCC201, Metos system Intl., Germany) were utilized (Figure 3.6A) to be able to cook all samples simultaneously. The products were placed immediately on ice for 2 h after heat processing due to food safety (Figure 3.6B), (Chapter 2.4). The cooling was monitored by the temperature probe Testo 176T4. The samples were stored in a chilled room at 4^{o}C until further analysis.

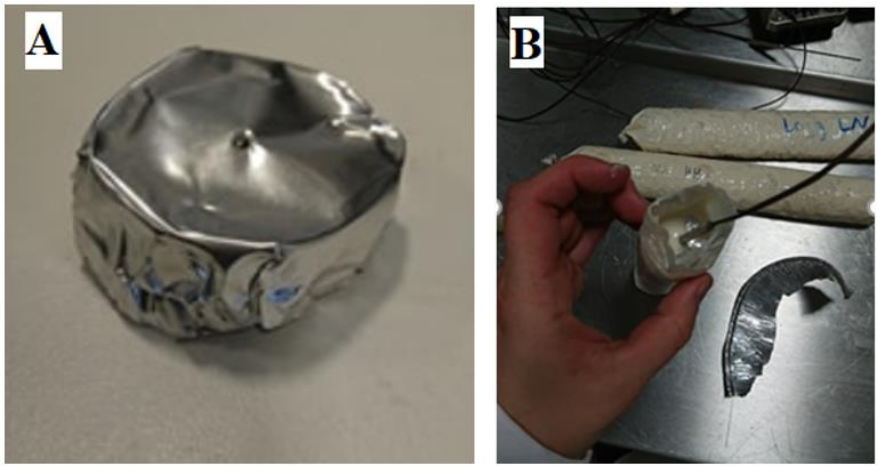


Figure 3.5. A: The bottom of an aluminum container was folded to fit inside the Betan case, B: the folded aluminum piece was placed inside the casing with a probe penetrated through.

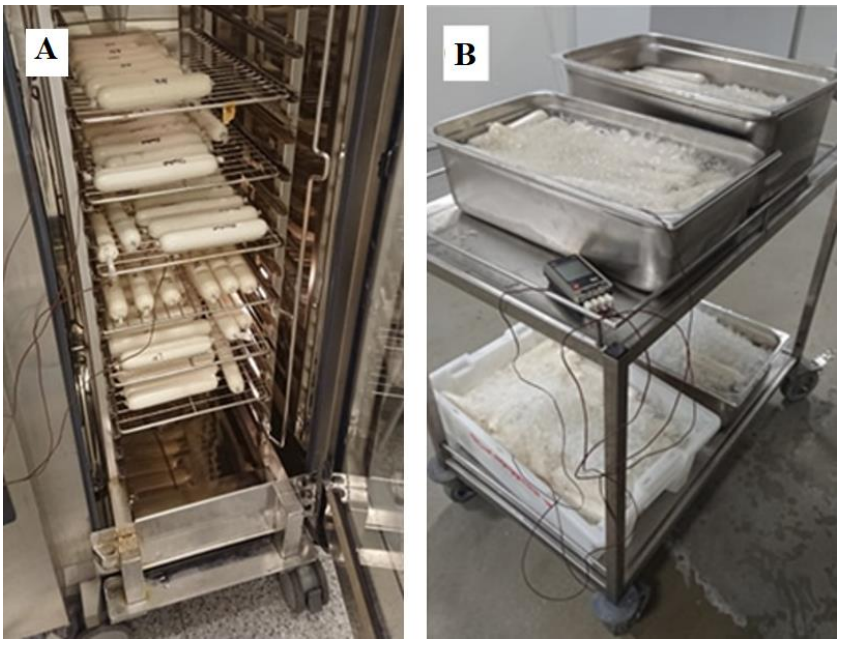


Figure 3.6. A: The heat treatment of the pilot production samples inside the convention oven, B: cooling of samples in an ice slurry after heat treatment with temperature probes.

3.5 Minced fish

3.5.1 Ingredients

Table 3.7. List of ingredients used in both preliminary- and pilot production of minced fish.

Materials	Information	Producer	Country
Haddock fillet	Fresh, skinless V-Cut, product nr: 1119105	Nordic Group AS	Norway
Silver smelt	7 kgs block of frozen silver smelt Product nr: 3184917	Tavan	Faroe Islands
Potato flour	EAN: 7044710081006	Hoff SA	Norway
Sunflower oil	EAN: 5020514460058	Olympic Food	U.K.
White pepper powder	EAN: 7053260187458	SAGA, L.K. Haaland AS	Norway
Nutmeg	EAN: 7053260206418	SAGA, L.K. Haaland AS	Norway
Milk	3.5% fat	Tine SA	Norway
Hydrolysate[a]	Endurance Salmon protein hydrolysate, 95% total protein	Hofseth Biocare	Norway
Hydrolysate[b]	Endurance Salmon protein hydrolysate, 97% total protein	Hofseth Biocare	Norway
Hydrolysate[b]	Salmon backbone protein hydrolysate, 89.9% total protein	Nofima	Norway
Whey protein concentrate 80%	WPC80 Art. 4466 77.4% total protein	Tine SA	Norway
Sodium Caseinate	KAPA[tm] JPR 1002 87% total protein	Armor proteins	France
Salt (NaCL)	CAS-no. 7647-14-5	Akzo Nobel salt	Denmark

a: Was utilized in preliminary production
b: Was utilized in pilot production

3.5.2 Recipes in preliminary testing

The procedure was obtained from earlier work done with minced fish products at Norconserv and the recipes were optimized further (Vatland et al. 1991). Preliminary testing consisted of standardizing the blending times and investigating the effect on texture analysis of increasing amounts of added fish protein hydrolysate (FPH). The first recipe (3A) did not contain any protein enrichment while the remaining recipes were enriched with FPH (3B, 3C and 3D) varying from 15.3 to 19.9% total protein. A detailed collection of the ingredients from both preliminary testing and production and pilot production can be found in Table 3.7. The recipes used to study FPH in preliminary testing of minced products can be observed in Table 3.8.

Table 3.8. Recipes applied in preliminary testing of minced products, given in percentages. Calculated total protein, fat and salt content are included below the ingredients.

Ingredient	**3A**	**3B**	**3C**	**3D**
Silver smelt	30	29	27.9	27.4
Haddock fillet	30	29	27.9	27.4
Salt	1	1	0.9	0.9
Whole milk	30.6	29	27.9	27.4
Oil	3	2.8	2.8	2.6
Potato flour	5	4.9	4.7	4.6
White pepper	0.2	0.2	0.2	0.2
Nutmeg	0.2	0.2	0.2	0.2
Hydrolysate	-	3.9	7.5	9.3
Sodium caseinate	-	-	-	-
WPC80	-	-	-	-
Total protein	11.9	15.3	18.4	19.9
Total fat	4.6	4.3	4.3	4.1
Total salt	1.1	1.1	1.0	1.0

3.5.3 Recipes in preliminary production

After preliminary testing was completed, the next phase could initiate, which consisted of protein enrichment by whey protein concentrate (WPC), caseinate and FPH. White pepper and nutmeg were excluded in the following production due to an overpowering flavor. Several ingredients were adjusted in preliminary production except the fish raw material and salt. Different variations of milk, oil, potato flour and protein enrichment were investigated based on the sole purpose of obtaining highest possible total protein. Because minced fish was targeted towards regular persons and not specific groups, production was concentrated on protein content and not fat. The final recipes of minced fish in preliminary production are listed in Table 3.9.

Table 3.9. Recipes used in preliminary production of minced fish products containing ingredients given in percentage. Calculated total protein, fat and salt content are included below the ingredients.

Ingredient	**4A**	**4B**	**4C**	**4D**	**4E**	**4F**
Silver smelt	30	30	30	30	30	30
Haddock fillet	30	30	30	30	30	30
Salt	1	1	1	1	1	1
Whole milk	30.5	30.5	24.5	24.5	30.5	24.5
Oil	4.5	2.5	4.5	2.5	3	3.5
Potato flour	4	4	4	4	4	3
Hydrolysate	-	0.5	1.5	2	1.5	2
Sodium caseinate	-	0.5	1.5	2	-	2
WPC80	-	1	3	4	-	4
Total protein	11.9	13.6	16.8	18.5	13.4	18.5
Total fat	6.0	4.0	5.9	4.0	4.5	5.0
Total salt	1.1	1.1	1.1	1.1	1.1	1.1

3.5.4 Procedure of preliminary testing and production

Preparation of the raw fish materials were described in Chapter 3.3. The procedure of preliminary testing and production was equal and was therefore described as one procedure in this chapter.

At the day of production, the haddock fillets were deboned and cut to approximately 2x2 cm and stored at 4^{o}C during preparation of the other ingredients. All remaining ingredients, except milk and silver smelt, were weighed according to the recipes listed in Table 3.9. The temperature increase during the blending was kept at a minimum until the final rounds of blending. Hence, the fish and milk were weighed last, to keep the temperature low. If the temperature is getting closer to 20^{o}C and above, the fat in the mixture can liquidize (Vatland et al. 1991).

The fish, both haddock and silver smelt, were added into the food processor (R5V.V., France) and minced with the times and speed shown in Table 3.10, followed by addition of salt before further blending. Milk was poured into the mixture while the food processor was operating at low speed, and then speed was increased. The remaining ingredients were added in the sequence listed in Table 3.10, with the written times and speed.

Table 3.10. Sequence of addition, mixing speed and times performed after addition of the different ingredients.

Sequence	Ingredient	Mixing time (min:sec)	Mixing speed
1	Silver smelt	00:15	12
2	Haddock fillet		
3	Salt	00:30	30
4	Whole milk	00:40[a] 00:20[b]	6[a] 30[b]
5	Oil	00:20	30
6	Potato flour	00:10[c] 00:40[d]	6[c] 30[d]
7	Hydrolysate		
8	Sodium caseinate		
9	WPC80		
10	White pepper		
11	Nutmeg		

a: The milk was poured carefully into the mixture at low speed
b: The speed was adjusted after the milk was added.
c: Mixing time and speed during first round after addition of dry ingredients.
d: Mixing time and speed for second round after the mixture have been stirred by a spatula, due to getting all ingredients down from the wall of the processor.

Betan casings which were soaked for 30 min in water prior filling, were used in the production of the samples. The mixture was transferred into a piping bag (90my 275x550mm, NorEngros AS, Norway) and filled into the casing. The ends were sealed with clips by the single clips machine giving samples at 20±1 cm as described in production of texture modified products (Chapter 3.4.6). One sample was equipped with a temperature probe (Testo 735-2, Testo SE & Co. KGaA, Germany) used to monitor the core temperature during heat processing.

The samples were cooked with a core temperature of 80^{o}C for 30 min (Vatland et al. 1991) in a preheated convention oven (MSCC6, Metos, system Intl,. Germany) set to 100^{o}C with 100% steam. When the core temperature in the samples came close to 80^{o}C, the oven temperature was lowered to 80^{o}C for 30 min. Immediately after cooking, the samples were submerged into an ice slurry for 2 h until 4^{o}C core temperature was reached and then stored in a chilled room at 4^{o}C until further analysis.

3.5.5 Recipes in pilot production

The aim of pilot production was to produce minced fish products with high total protein content, reaching the protein level of the fish raw material. The amount of potato flour was determined to be equal to recipe 4F from preliminary production, for enabling higher addition of protein enrichment. Fish raw materials, salt, milk, and potato flour were kept constant. One recipe was used as a standard (SM), which excluded FPH, for comparison with recipes enriched with either

FPH from Nofima or Hofseth BioCare. The following recipes are referred to as low Hofseth mince (LHM), high Hofseth mince (HHM), low Nofima mince (LNM) and high Nofima mince (HNM) and are listed in Table 3.11. The "low" value refers to a total protein content of approximately 15.8% and "high" 18.8% total protein. The standard (SM) had calculated total protein of 14.1%.

Table 3.11: The recipes used in pilot production of minced fish product with ingredients. Calculated total protein, fat and salt content are included below the ingredients. The values are given in percentage.

Ingredient	**SM**	**LHM**	**HHM**	**LNM**	**HNM**
Silver smelt	30	30	30	30	30
Haddock fillet	30	30	30	30	30
Salt	1	1	1	1	1
Whole milk	25	25	25	25	25
Potato flour	3	3	3	3	3
Oil	8	6.2	2.6	6.1	2.5
Hydrolysate Hofseth	-	1.2	2.1	-	-
Hydrolysate Nofima	-	-	-	1.23	2.13
Sodium caseinate	1	1.2	2.1	1.23	2.13
WPC80	2	2.4	4.2	2.46	4.26
Total protein	14.1	15.8	18.8	15.8	18.8
Total Fat	9.4	7.6	4.1	7.5	4.0
Total salt	1.1	1.1	1.1	1.1	1.1

3.5.6 Procedure for pilot production

The procedures of thawing, deboning, weighing and final heat treatment were equal to the procedures found in Chapter 3.5.3. The ingredients were weighed according the recipes listed in Table 3.11. The method for producing the minced fish products was retrieved from Norconserv (Vatland et al. 1991) and was based on temperature, rather than blending times for the mixing. It is important to maintain low temperatures to prevent liquidizing of fat and liquid separation, which could happen if minced fish exceeds 20^{o}C (Devine, Dikeman, and Jensen 2004).

Figure 3.7. All ingredients of the minced fish were blended in an industrial cutter during pilot production.

The fish were added to a bowl cutter (MTK 662, MADO GmbH, Germany) and run on low speed (Figure 3.7). Salt was evenly distributed while the cutter was running, until the temperature had risen to 2°C. See Chapter 2.2.1 for further information about the influence of salt on fish raw materials. The temperature was measured throughout the mixing with a temperature probe (Testo 176T4, Testo SE & Co. KGaA, Germany). Milk was thereafter poured carefully into the mixture while the bowl cutter was operating at highest speed, followed by addition of oil. The cutter was operated at highest speed until the temperature reached 12°C. The added liquid simplified following blending by creating a sticky surface for dry ingredients to adhere to. The dry ingredients, potato flour and enriched proteins, were added simultaneously and blended until the temperature raised to 17°C. They created friction which increased the temperature and therefore added last. Finally, the cutter ran at low speed for a few rounds to dispose air bubbles.

The procedure of filling the products into casings and making the individual samples were described in Chapter 3.4.6. The samples were then stored in chilled conditions at 4°C for 24 h. After storage, two temperature probes (Testo 176T4, Testo SE & Co, KGaA, Germany) were placed in the center of two samples prior to heat treatment. The heat treatment was equal to the procedure explained in Chapter 3.5.4, except the convention ovens (MSCC201, Metos system

Intl,. Germany) were used. The temperature was monitored during rapidly cooling submerged in an ice slurry for 2 h. Finally, the samples were stored in a chilled room for 4°C.

3.6 Texture analysis

3.6.1 Texture modified product

The texture of modified texture products was measured by using TA. XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) with a 5 kg loading cell and a cylinder probe (Delrin cylinder P/0.5R, Stable Micro Systems, Godalming, UK). All data was collected by the software Exponent (Version 6.1.13.0, Stable Micro Systems, Godalming, UK). The test project which was used was developed from the work of Prabhu (2018), originally made for pâté samples. A test project consists of all the parameters and components needed to run a complete analysis of a specified product. The TA.XT instrument was operated through this software and the selected project of specific settings. The results from this analysis were the textural parameter firmness expressed in N (newton). The sample was penetrated using test mode compression. The firmness was measured as the required force (N) needed to penetrate the sample for a specified distance 10 mm. The pre-test and test speed were 1.50 mm/sec while the post-test speed was 10.00 mm/sec. The analysis initiated when trigger force of 5 g was reached. Further information about the settings can be obtained in Chapter 2.5.2.

A calibration of force and probe height was performed before analysis. The force was calibrated for 2000 g by using a calibration weight. In addition, the probe height was calibrated by placing a heat block (AccuBlock^{tm}, D1200, Labnet International, USA) and a round aluminum container beneath the penetration probe and zeroing the height against the base of the heat block and container.

All measured data was presented in a graph and further analyzed by a selected *macro*; a list of instructions performed automatically. The resulting values given by the *macro* were copied into Excel (v16.0, 1909, Microsoft Corporation, US) for later interpretation.

3.6.1.1 Sample preparation and analysis

Prior to analysis, samples from each production batch were retrieved from the chilled room 4°C. Each sample was sliced by a knife into three pieces with 20 mm thickness. The samples were placed in round aluminum containers and covered completely by plastic cling wrap. They were then transferred to a perforated aluminum tray (GN 1/1) as shown in Figure 3.8A, to get an even

re-heating. The convention oven (MSCC61, Metos System Intl., Germany) had the temperature set to 100ºC with 100% steam. The core temperature during re-heating of the samples was monitored by using a Testo 735-2 temperature probe (Testo Se & Co. KGaA, Germany). The temperature probe was placed in the center of the product, by penetrating the probe through the side of the round aluminum container. The products were heated until the core temperature reached 63ºC. The food and beverage industry in Norway are obliged to keep temperature of food at 60ºC or warmer until serving (Næringsmiddelhygieneforskriften 2009). Since the products were meant to be consumed warm, texture analysis had to be performed at the equivalent temperature as served.

After heating, the samples were transferred directly to a food warming trolley (Termia 950 H, Metos, Finland) with temperature set at 75ºC, to keep the temperature of the products constant until they were analyzed.

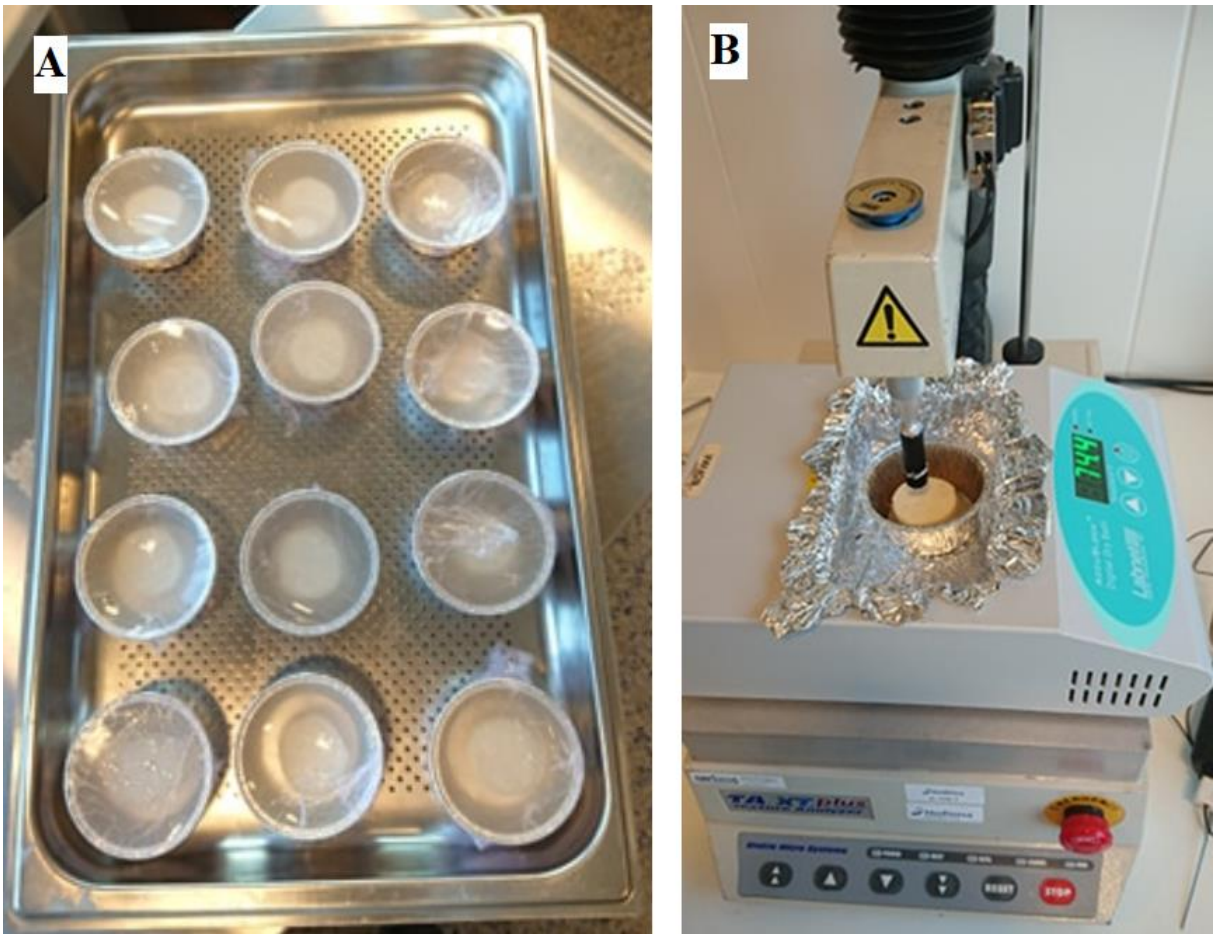


Figure 3.8. A: The sliced samples of texture modified products after heating, B: texture analysis of a sample placed inside the heat block.

The heat block set on 75ºC was placed at the TA.XT instrument to keep the core temperature of the product around 60±2ºC during analysis. All sides on the inside were covered by

aluminum foil (Caterwrap, 450mm*150m, United Kingdom), for easier cleaning and maintaining temperature.

Before analysis, plastic cling film around the aluminum container was removed and the sample was placed into the heat block. A digital thermometer (Exxent 77099, MerxTeam, Sweden) recorded core temperature of one slice per sample before penetration. Three slices from three samples within same recipe were analyzed (n=3*3). Once the probe touched the surface of the slice, it penetrated 10 mm into the sample. The selected penetration distance (10 mm) was half the height of the sample. The samples were measured inside the aluminum containers, to prevent deformation before analysis (Figure 3.8B).

3.6.2 Minced fish

The sample preparation of minced fish followed same procedure as texture modified products (TMP) used in Chapter 3.6.1.1. Size dimensions of the samples, heating and process of analysis were equal, except that the minced fish was analyzed without the small aluminum container. The calibration was similar to the method of texture modified product, but without aluminum container during calibration of the probe height.

3.6.2.1 TPA of minced fish

Several texture parameters of minced fish were measured by using TA. XT Plus Texture Analyzer with a 50 kg loading cell and a compression plate (p/75, Stable Micro Systems, Godalming, UK). All data were collected by the software Exponent (Version 6.1.13.0, Stable Micro Systems, Godalming, UK). The test project was developed from a sample project named TPA (texture profile analysis). The method is based on previous studies (Wu, Sun, and He 2014; Aguirre et al. 2018).

Pre-test and post-test speed were 3.00 mm/sec, test speed was 1 mm/sec, trigger force 5 g, target mode was 60% strain. Trigger force is the force required to initiate the analysis. Other parameters are explained in Chapter 2.5.2. The data obtained from this analysis was the textural properties` hardness (g), adhesiveness (g.sec), springiness, cohesiveness, gumminess, chewiness, and resilience (Chapter 2.5.2). The compression plate performed two compressions with 10 sec in-between. Hardness was measured as the required force g needed to compress the sample with strain 60%. The applied test mode was compression. All the measured data was presented in a graph and further analyzed by a selected *macro*. The resulting values given by

the *macro* were copied into Excel (v16.0, 1909, Microsoft Corporation, US) for later interpretation.

Figure 3.9. Texture profile analysis of minced fish product by using a compression plate and a heat block.

3.6.2.2 Gel-test

The test project gel-test was developed from a project originally made for gel samples (Øines 2019). The textural properties hardness (N), stiffness (N/mm) and gel strength (mm*N) was obtained by TA.XT Plus Texture Analyze. The instrument was equipped with a 5 kg loading cell and a spherical probe (p/5s, Stable Micro Systems, Godalming, UK). The spherical probe punctured the surface by a measured force (N) which yielded hardness and distance in mm, with a trigger force of 5 g and a specified speed of 1 mm/s. The analysis is completed when the probe has punctured the surface of the sample and is returned to its original position. The samples had to be penetrated until the surface structure was ruptured, hence the applied test mode was compression.

All data were collected by the software Exponent, presented in a graph, and further analyzed by a selected macro. The values given by the macro were copied into Excel.

3.7 Rheology

Rheological properties of texture modified products (TMP) were analyzed by a hybrid rheometer (Discovery HR-2, TA Instruments, US). First the air supply, followed by the fluid circulation, had to be turned on due to the installed temperature system Peltier Plate (New advanced, TA Instruments, US). The electronic box used by the temperature system was then switched on before the rheometer could be initiated. The Peltier Plate enables the user to control the temperature with high precision, with ranges from -40°C to 200°C. Finally, the rheometer was connected to the computer via the software Trios (Trios, v4.3.0.38388, TA Instruments, US). The selected geometry was attached to the instrument. Before any analysis could be executed, the instrument was calibrated. The gap, which was the distance between the bottom part of the geometry and Peltier Plate, had to be calibrated as well. This was performed by lowering the geometry approximately 5 mm above the surface and initiate the calibration. A new calibration was required whenever the geometry was detached from the instrument after an analysis.

3.7.1 Sample preparation and measurement of texture modified products

The method is based on the master thesis of Vaka (Vaka 2018). The utilized geometry was a parallel plate (XHATCH, 20 mm, Serial number 111670, TA Instruments). Sample preparation begun after the apparatus and software was initiated and calibrated as mentioned in Chapter 3.7.

A sample was retrieved from the chilled room (4^{o}C) immediately before analysis, to keep the temperature of the samples as equal as possible before each analysis. The Betan casing was removed before the sample was cut to 8 mm thick. An amplitude sweep was first performed on each batch to find a suitable oscillation strain (%) within the linear viscoelastic region (LVR) (Figure 3.10A). Different temperatures were used during the amplitude test, to ensure that all measurements were linear at the same region. The selected temperatures were kept constant at 25^{o}C, 50^{o}C and 80^{o}C, as well as frequency at 1 Hz. Two samples with triplicate were measured at each temperature. The oscillation strain varied from $3.33*10^{-3}$ to 10%. Points per decade were set to ten and the axial force was set on compression mode with force 0.25 N and sensitivity 0.1 N.

The results were copied into Excel (v16.0, 1909, Microsoft Corporation, US) for further processing. After analysis, the storage modulus G` (Pa) was plotted as a function to oscillation strain. The resulting graphs should be linear in the selected region.

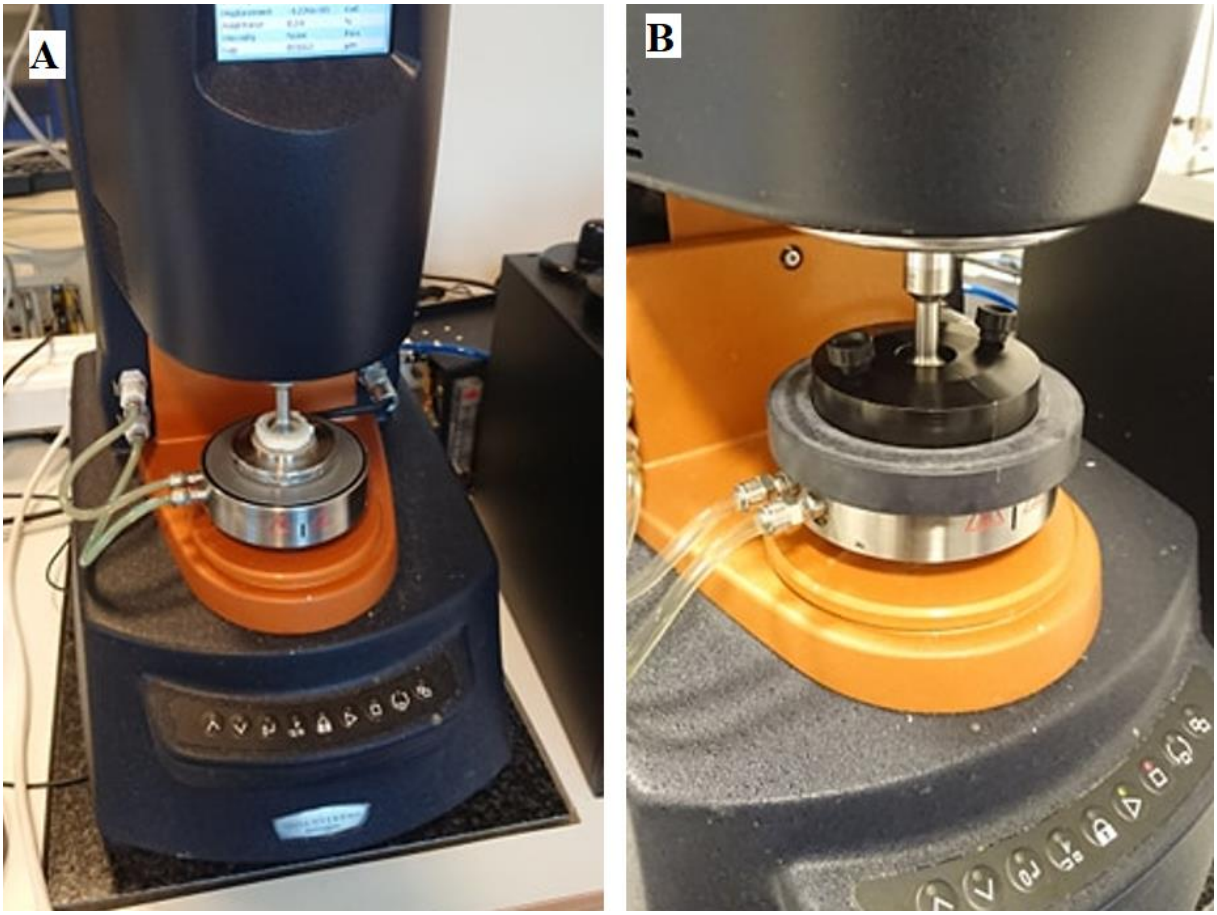


Figure 3.10. The utilized rheometer during the amplitude sweep and temperature sweep. A: the sample placed below the selected geometry during an amplitude sweep, B: the sample beneath a solvent trap during a temperature sweep.

After the strain (%) was selected from the amplitude sweep, a temperature sweep was performed. The temperature started at 20^{o}C, up to 80^{o}C and back down to 40^{o}C. Two samples with triplicate were measured at each temperature. The frequency was kept constant at 1 Hz. The selected parameters points per decade, the axial force, sensitivity, and force were equal amplitude sweep. A solvent trap was used due to the high temperature (Figure 3.10B). The results were copied into into Excel after analysis. The storage modulus G` (Pa) was plotted as a function to temperature.

3.8 Image analysis

3.8.1 Cryosectioning and staining

The procedure regarding cryosectioning, staining and image editing were based on information obtained from the master thesis of Vaka (Vaka 2018). The structure in texture modified products (TMP) and minced fish was investigated by images taken by a camera through a stereoscope. The images were further processed to analyze threshold limited to area.

The products were stored in the refrigerator at 4^{o}C prior to the cryosectioning. The lower temperature eases the handling of the products when samples are cut and placed on the specimen discs. Before analysis, a small sample of 5 mm height and 7 mm width was cut and placed on a specimen disc. Some small drops of freezing medium (Tissue freezing medium, Ref: 14020108926, Leica Biosystems, US) were placed beneath the sample as well. The sample was transferred to the cryostat (Leica CM1860UV, Leica Biosystems, US) with the temperature set at -23^{o}C until all samples were fixed and placed on the object plate holder as seen in Figure 3.11A. Rapid freezing for 10 min was initiated. Though the samples were taken out after approximately one minute to ensure that the samples were completely encased by the freezing medium. This was done after a color change of the medium from clear to white was observed, due to the cold temperature. The samples were quickly placed back into the cryostat until the rapid freezing was completed.

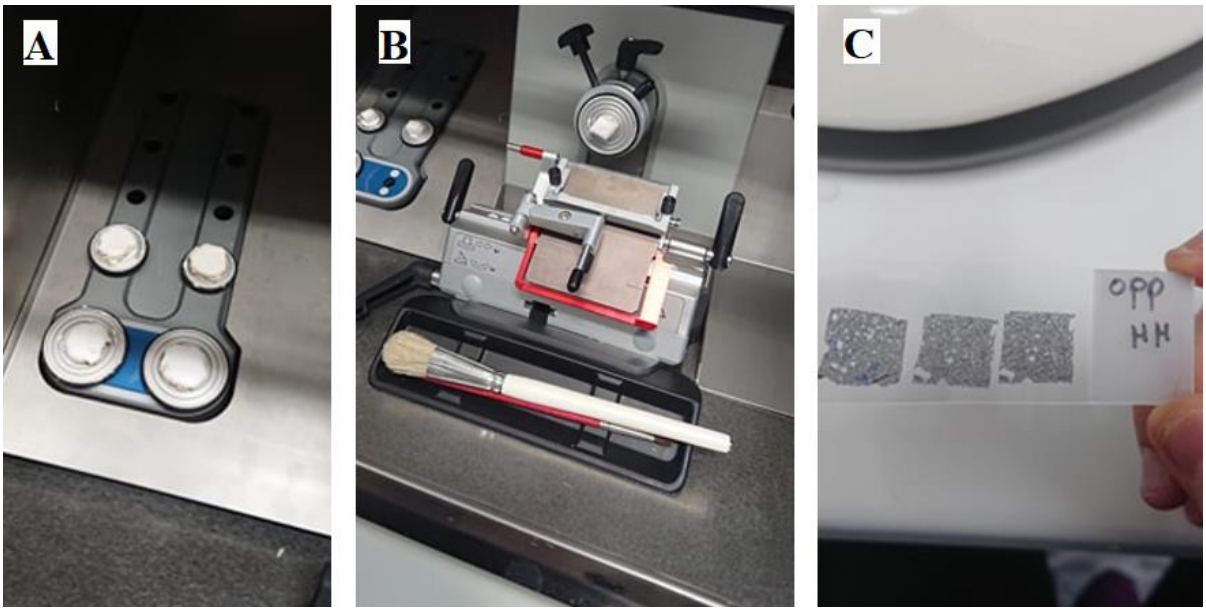


Figure 3.11. The procedure of fixing and staining samples on object glass. A: The samples were placed on the object plate holder during a rapid freezing; B: the microtome inside the cryostat used to cut the sections; C: an example of three sections fixed onto an object glass after staining.

Sections of 100 µm thickness were cut by the microtome until the top layer of medium was removed entirely. The microtome can be observed in Figure 3.11B. A few sections of 10 µm were thereafter cut before the main section was made and transferred onto an object glass (VWR® Microscope Slides, REF 631-1553, VWR®, US). The object glasses were placed on a clean surface to airdry until all sections were finished, which made them less susceptible to detachment during the following staining.

The glass objects were placed in a microscope slide staining rack with the sections turned away from the wall. They were first stained by Orange G (O7252, Sigma-Aldrich, US), which was made with 0.5 g Orange G and 1 ml acetic acid dissolved in 99 ml distilled water, for 5 min. The staining solution was poured carefully into the staining rack while gently tilting it. After the initial 5 min, they were rinsed one time with distilled water. The final staining procedure was performed with methyl blue (M6900, Sigma-Aldrich, US) for 5 min followed by washing with distilled water for further 5 min. Methyl blue solution was made of 0.014 g methyl blue and 1 ml acetic acid dissolved in 99 ml distilled water. After staining was completed, the sections were left to dry for approximately 5 min (Figure 3.11C).

3.8.2 Image analysis

The internal microstructure was observed through a 5x objective lens of a stereo microscope (Leica MZ8, Leica Biosystems, US). A WiFi camera (TC20 Plus, VWR International AS, 30 countries) and its C-mount adapter was placed on the trinocular port of the microscope, and further connected to a tablet (ACER Iconia One 10, model A6003, ACER inc., Taiwan) for imaging and quick viewing. Operation of the camera was carried out through the software Tabkam view (V1.1). The application, camera and tablet are all parts of a system called VisiCam TC20 Plus from VWR International AS. Fiji (Fiji Is Just ImageJ) was used for the image analysis. The images were converted into 8-bit, and then the threshold was adjusted to black and white. The area of dry matter was measured in percentage, by measuring the area limited to threshold.

3.9 Color analysis

The differences in colors of products with variations in protein enrichment were analyzed. Products were retrieved from the refrigerator at 4^{o}C. First the casing was peeled off, before the samples were cut into slices of 20 mm. A DigiEye system (VeriVide Ltd., UK) consisting of a camera, imaging cube and applications was used to perform the imaging and color

measurements. The DigiEye system was calibrated prior to analysis by using a white- and colored calibration board (DigiTizer Calibration Pack, VeriVide Ltd., UK). The samples were transferred into the light box, which was a part of the imaging cube, on an aluminum board. They were photographed by a digital camera (Nikon D90, AF Nikkor 35mm f/2D, Nikon, Japan) still placed on the aluminum tray (Figure 3.12A and 3.12B). Settings of the camera were aperture: 10 and shutter: 1/5. The samples were measured in triplicate from three samples within each recipe. DigiView (VeriVide Ltd., UK) was used to take the photographs while DigiPix (VeriVide Ltd., UK) was used for final color measurements. The extracted color coordinates were CIEL*a*b* and CIEL*C*h (Chapter 2.5.3).

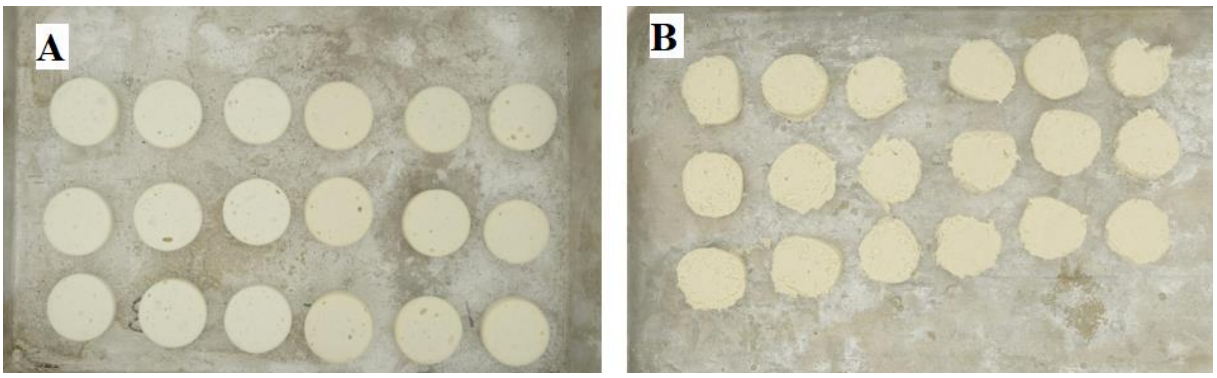


Figure 3.12. Sample photographs taken by the nikon camera and digieye system. A: Minced fish products photographed, B: texture modified products photographed.

3.10 Water holding capacity

3.10.1 Texture modified products

The water holding capacity in texture modified products (TMP) was determined to study and compare the influence of variations in protein enrichments. The process of determining water holding capacity is based upon a method from Veiseth-Kent et al. (2010) with alterations. In this subchapter water holding capacity was investigated using the terms liquid loss (Eq. 1), in addition to water loss (Eq. 2) and mass loss (Eq. 3). Due to the soft consistency and adhesiveness of TMP, the original term fat loss was replaced with mass loss. These factors were calculated by the following equations and expressed as percentage of initial sample weight:

$$Liquid\ loss\ (\%) = \frac{W_3 - W_2}{W_1} * 100 \qquad \text{(Eq. 1)}$$

$$Water\ loss\ (\%) = \frac{W_3 - W_4}{W_1} * 100 \qquad \text{(Eq. 2)}$$

$$Mass\ loss\ (\%) = \frac{W_4 - W_2}{W_1} * 100 \qquad \text{(Eq. 3)}$$

The following parameters W_1-W_4 were determined by weighing and then used to calculate liquid loss, water loss, and mass loss.

$$W_1\ (g) = Sample\ before\ centrifugation$$

$$W_2\ (g) = Filter\ paper\ before\ centrifugation$$

$$W_3\ (g) = Filter\ paper\ after\ centrifugation$$

$$W_4\ (g) = Dried\ filter\ paper$$

Labeled Filter papers (Whatman, Nr. 589[1], 70mm, GE Healthcare, US) was folded into a 1.5*1.5 cm square and placed in a desiccator for 24 h (Figure 3.13C). Sample preparations were carried out with samples retrieved from cold storage (4°C). The casing around the TMP was removed and the samples were cut in slices with a thickness of 7 mm. Each sample weighed 3 g (W_1) and was transferred to a 50 ml falcon tube. Because all remaining traces of moisture were removed from the Whatman filters by the desiccator, they were weighed immediately (W_2) when removed from the desiccator. Thereafter, they were placed in the tube on the top of the sample. The samples were centrifuged (Rotina 420R, Andreas Hettich GmbH & Co. KG, Germany) at 10°C for 10 min and at 1562 rpm (Figure 3.13A and 3.13B). A tweezer was used to remove the wet filter papers before they were weighed once more (W_3). The filter papers were dried for 24 h at 50°C overnight until constant weight was achieved (W_4). The analysis was performed in triplets per sample (n=3x2 samples per recipe).

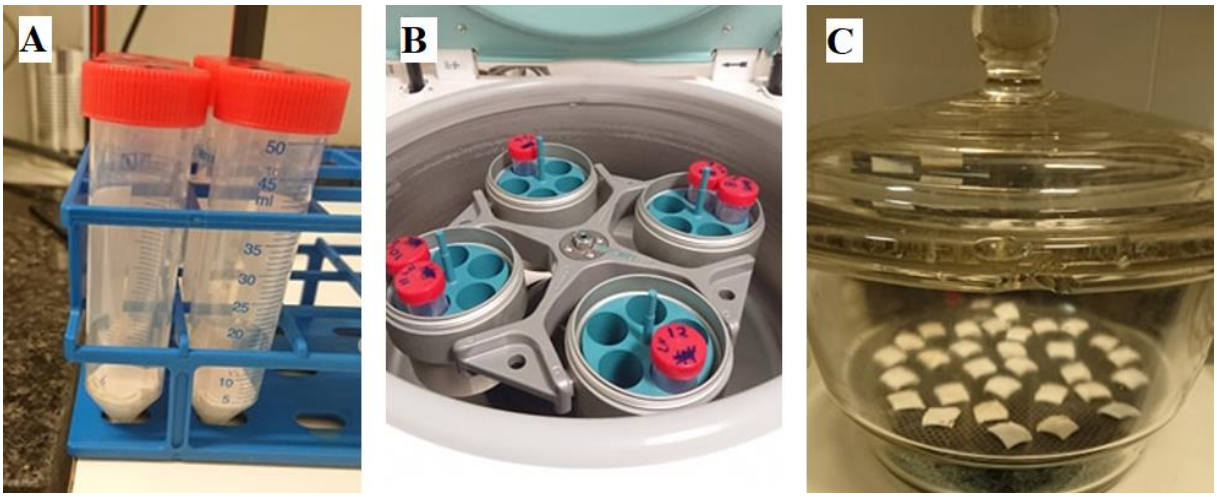


Figure 3.13. A few steps from the procedure of determining water holding capacity. A: Falcon tubes containing the centrifuged samples, B: the falcon tubes placed in the centrifuge, C: folded filter paper drying in the desiccator, removing all moisture.

3.10.2 Minced fish

The water holding capacity for minced fish products were determined by using a method from Skipnes, Østby and Hendricx (2007) with alterations. The method determined water holding capacity (Eq 4), liquid loss (LL) (Eq 5), and dry matter (DM) (Eq 6), which were calculated by the following equations and expressed as percentage of initial sample weight:

$$Water\ holding\ capacity\ (\%) = \frac{100-DM-LL}{100-DM} * 100 \qquad \text{(Eq. 4)}$$

$$Liquid\ loss\ (\%) = \frac{V_1-D_1}{V_1} * 100 \qquad \text{(Eq. 5)}$$

$$Dry\ matter\ (\%) = \frac{D_3}{D_2} * 100 \qquad \text{(Eq. 6)}$$

The following parameters V_1-V_6 were determined by weighing and then used to calculate D_1-D_3.

$$V_1\ (g) = Sample\ before\ centrifugation$$

$$V_2\ (g) = Sample\ and\ cup\ before\ centrifugation$$

$$V_3\ (g) = Sample\ and\ cup\ after\ centrifugation$$

$$V_4\ (g) = Aluminium\ bowl$$

$$V_5\ (g) = Aluminium\ bowl\ and\ sample\ before\ drying$$

$$V_6\ (g) = Aluminium\ bowl\ and\ sample\ after\ drying$$

$$D_1\ (g) = V_3 - (V_2 - V_1)$$

$$D_2\ (g) = V_5 - V_4$$

$$D_3\ (g) = V_6 - V_4$$

The sample cups (Patent No. 321375 B1) used to measure water holding capacity in the minced fish products had to be assembled with a filter (Norconserv, Stavanger, Norway) before they could be applied. They were assembled with a minor drop of oil for ease of handling. A Styrofoam box was filled with ice and a plastic wrap sheet covered the ice, to prevent any moisture interfering with the sample cups. Then the sample containers were transferred to the Styrofoam box (Figure 3.14).

The samples of minced fish were retrieved from the refrigerator (4^{o}C). The casing was removed, before the sample was sliced into 15 mm thick cylinders. A stainless-steel core sampler (28

mm) was used to reduce the diameter of the sample down to 28 mm. The weight of the sample (5 g) was measured (V_1) and then both sample and the sample cups were weighed together (V_2). The samples were centrifuged (Rotina 420R, Andreas Hettich GmbH & Co. KG, Germany) at 4^{o}C for 15 min and at 1800 rpm. The sample cups were placed in the Styrofoam box between each procedure, e.g. weighing and centrifuging (Figure 3.14).

Figure 3.14. Minced fish samples placed in the Styrofoam box above the ice and plastic wrap sheet.

The bottom of each sample cup was taken apart, blow-dried with compressed air, and assembled loosely. Finally, the weighing of the sample inside the sample cup was performed (V_3).

The percentage of dry matter was calculated by subtracting the weight of an empty aluminum bowl (Round, 106ml Ø80, Plus Pack, Denmark) (V_4) from the weight of the sample within the aluminum bowl (V_5). The samples were dried for 24 h at 50^{o}C until constant weight was achieved. After drying, the aluminum bowl with the sample were weighed one last time (V_6).

3.11 Sensory analysis

A quantitative descriptive analysis (QDA) (ISO 13299: 2016) was performed on both minced fish and texture modified products (TMP) to assess their sensory attributes due to variations in protein enrichment. The analysis was performed by a sensory panel of ten assessors at the sensory laboratory at Nofima, Ås. The assessors were selected and trained according to (ISO 8586-1: 2012).

Prior to the analysis, the panel made a proposal of suitable attributes within texture, odor, and taste. A total of 23 different attributes were selected for both the minced fish and TMP (ISO 5492: 2008). A few attributes were replaced during the preliminary testing, to better describe the specific sensory properties. The attributes used in the QDA are listed in Appendix D.

Before shipping the samples to Ås, they were vacuum packaged (95% vacuum) in vacuum bags (220x600mm, PA/PE 70my, LietPak, Lithuania) and placed with freezing packs in a Styrofoam box to maintain chilled temperature (4^{o}C) during transportation. The samples arrived Ås the next day and were stored cold (4^{o}C) prior to the sensory evaluation.

A preliminary testing was performed one day after arrival, with the selected variants. Samples included in the testing were 1) Texture modified products; Standard (ST), and High Hofseth (HHT), 2) minced fish products; Standard (SM) and High Hofseth (HHM). Sample ST and SM were without FPH, while HHT and HHM had the highest protein enrichment with Hofseth FPH. The pretesting was executed to calibrate the panel in the chosen attributes and their intensities in the various samples. The results were analyzed through Profile plot in PanelCheck (PanelCheck V1.4.2).

The QDA was performed 2 weeks after production in two separate tests, one for minced fish and one for TMP. Two replicates from each recipe of minced fish and TMP, a total of 2*10 samples, were included in the testing. Each sample was given a 3-digit code. The samples were served in triplets (n=3+3+3+2) whereas the first sample was a warm-up sample. The products were prepared for sensory evaluation by removing the casing, cutting each sample into 2 cm thick slices, before transferring to a metal baking tray GN 1/1 (Figure 3.15A and 3.15B), and wrapped with two layers of cling film (Wrapmaster 1000 LMF, France). The samples were then re-heated in a convention oven (Electrolux air-o-steam, 260462, Italy) at 100^{o}C, 100% steam for 7 min until the samples reached a core temperature of 60^{o}C. The samples were then transferred to white plastic cups (PS, white, Ø72 mm, Haval Disposables BV, Netherlands) which were placed inside preheated porcelain bowls and covered with aluminum lids (Figure 3.15C and 3.15D). Heating blocks set at 60^{o}C were provided inside the evaluation boots during serving. The assessors removed the plastic cup with the sample from the porcelain bowl during the QDA. Evaluation of odors was executed by cutting the sample in half, followed by evaluation of taste and texture. Data from the QDA was collected by the software program EyeQuestion (Logic8 BV, Nederland).

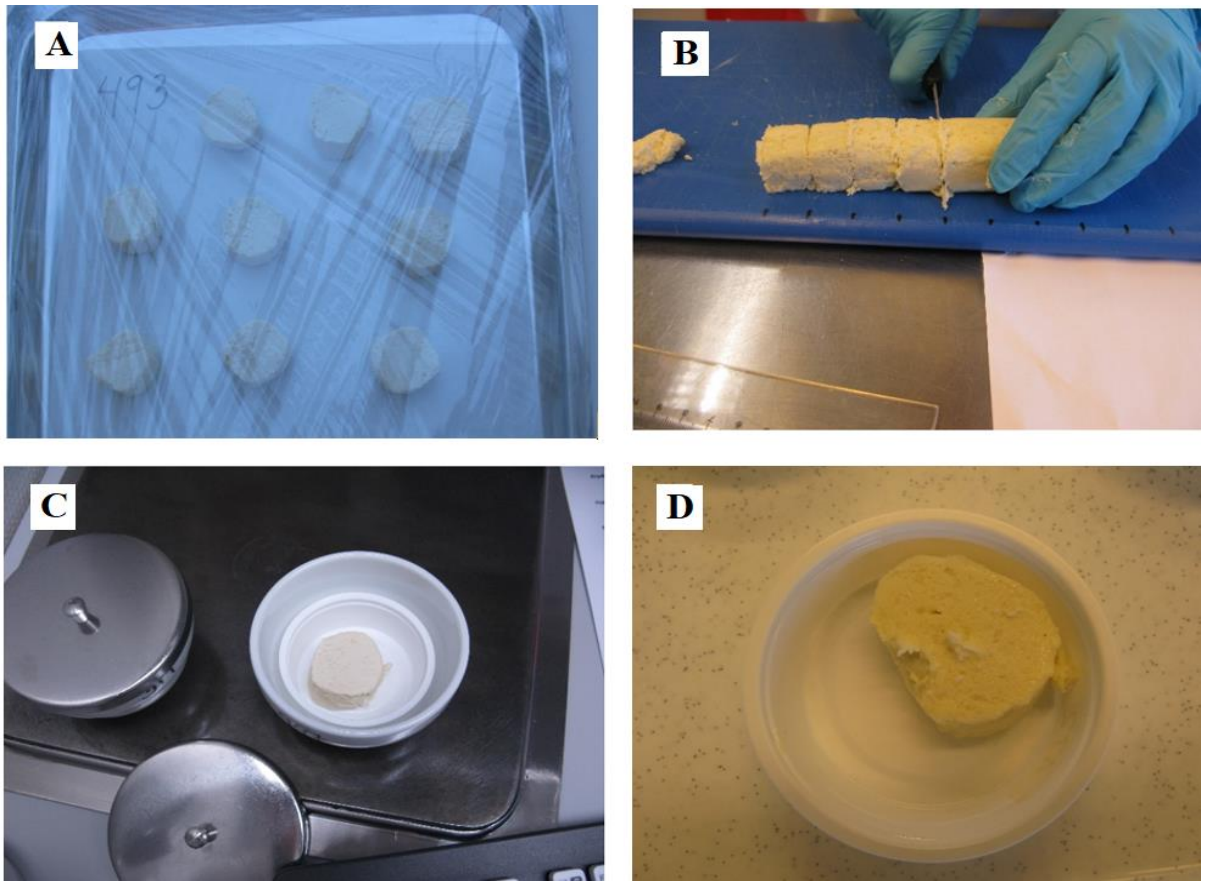


Figure 3.15. Sample preparations of the products prior the QDA. A: Samples placed on a tray and topped with two layers of plastic cling wrap prior heat treatment, B: the samples during slicing, giving an idea of how the texture is, C: a sample placed into the porcelain bowl with lids, D: TMP cut into 2 cm piece and placed into a plastic tray

3.12 Total protein with Kjeldahl method

The crude protein content in texture modified products (TMP) and minced fish from pilot production was determined by Kjeldahl method for further comparison with calculated total protein. Information about calculations can be found in Chapter 3.2. The samples were shipped to Nofima BioLab, Bergen for analysis of crude protein by using the Kjeldahl method. The method determined crude protein by analyzing nitrogen according to ISO 5983-2 and then multiplied the yield with factor 6.25 (ISO 5983-2: 2009).

3.13 Statistical analysis

Data from the analyses were tested for possible significant differences using one-way ANOVA (analysis of variance) 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 testing and production of texture modified products

This thesis is a continuation of previous work by Prabhu (2018) on texture modified products (TMP) for the elderly and people with dysphagia. Prabhu used salmon (*Salmo salar*) and haddock fillets in the initial development of the recipes. The aim for this study was to use previous results and experience to develop protein enriched TMP consisting of fillets from haddock and silver smelt. Recipes with variations of ingredients as whipping cream, oil and protein enrichment were investigated based on the goal of making a soft product with high protein and fat content.

Due to good water holding capacity, gelation abilities and binding abilities, silver smelt is often used for production of processed fish products like Norwegian fish burgers, small patties, and gelled fish mince. Haddock on the other hand, does not obtain the same level of gelation abilities and binding abilities, and is therefore often combined with silver smelt (Sivertsen 2012). Silver smelt is frozen directly after catch, still out on sea. This practice secure high quality of fish, making this species highly utilized in Norway (Gundersen and Dahl 2008). Other countries utilize small volumes of silver smelt and the fish is still regarded as less known (Hellevik, Synnes, and Stoknes 2005).

The fish for TMP was frozen in advance at temperature -20^{o}C in order to have an equal quality throughout the year, this is likely a procedure that would be used by the industry, and it influenced the experimental production by always having fish available at hand. Proteins in frozen fish are more susceptible to changes in structure and functionalities due to protein denaturation (Shenouda 1998). The TMP are soft products and therefore, a plausible loss of structure would aid the final texture. The proteins in silver smelt, on the other hand, are less susceptible to frozen storage and is therefore a good specie to use in combination with frozen haddock. Water holding capacity and binding abilities do not change as much as in other species. Hence, silver smelt is often used from a frozen state in the Norwegian food industry (Hellevik, Synnes, and Stoknes 2005).

The development of the recipes during preliminary testing and production was performed using primarily nutrient calculators, in addition to texture analysis, water holding capacity, color analysis, image analysis and rheology. Since TMP were aimed at elderly and people with dysphagia, the nutrient content was important. The recipes were therefore developed towards

products containing high quantities of proteins and fat. Elderly often have a lower appetite, which presumably lead to nutrient deficiencies and lower intake of food (Robinson et al. 2015). Hence, energy dense products are more desirable to yield a higher level of energy and proteins. The nutrient content of the recipes was in addition compared to both fish raw material (Table 4.1) and nutrients in a commercial product named Sooft Meals manufactured by Vitaelnaering (Appendix E). The selected Sooft Meals used for comparison were reconstructed pollock fillets.

Table 4.1. The nutrient content of haddock fillet and silver smelt (Matvaretabellen 2020).

Materials	Protein (%)	Salt (%)	Fat (%)	Carb (%)	Kj/Kcal
Haddock fillet	16.6	0.2	0.2	0	290/68
Silver smelt	19.6	0.1	1.2	0.1	378/89

4.1.1 Preliminary testing

Preliminary testing was done prior to preliminary production to establish the ratio of silver smelt to haddock and if precooked or raw silver smelt were more suitable for TMP. The influence of binding abilities in final product with precooked compared to non-precooked silver smelt was additionally investigated. The calculated nutrient content of recipes used in preliminary testing in addition to nutrient content of the pollock product from Sooft Meals are listed in Table 4.2.

Table 4.2. Calculated total content of protein, fat, and salt in different recipes during preliminary testing of texture modified products. Ingredients which differed are included. The remaining ingredients are shown in Table 3.3.

Recipe	1A	1B	1C	Sooft Meals
Haddock fillet (%)	-	59.0	29.5	-
Silver smelt (%)	59.0	-	29.5	-
Total protein (%)	16.5	14.8	15.6	13.7
Total fat (%)	19.2	18.6	18.9	10.0
Total salt (%)	0.9	1.0	1.0	1.2

4.1.1.1 Ratio of fish raw material

The ratio of silver smelt to haddock had to be established before protein enrichment could be studied. Recipe 1A consisted of 59% silver smelt while recipe 1B consisted of 59% haddock. The texture measurements of 59% silver smelt (1A) and 59% haddock (1B) indicated that 1A had firmer texture, compared to 1B (Figure 4.1). This may be due to the higher protein content in silver smelt. During production of 1A, the silver smelt gave minimal exudation of water during precooking, due to its water holding capacity (Gundersen and Dahl 2008). Blending of

the product was difficult, probably caused by the lack of free liquid. A simplified evaluation of taste and appearance done by three experienced researchers at Nofima, characterized product 1A as quite adhesive and hard to crush with the tongue in order to swallow the product. In addition to an adhesive texture, the product was evaluated as grainy. This could be improved by increasing the blending times. Recipe 1B with haddock was easier to blend during mixing. The batter could be poured directly into the small aluminum containers, without aid from any additional equipment. The blending times of 1B were increased, which gave a less grainy texture.

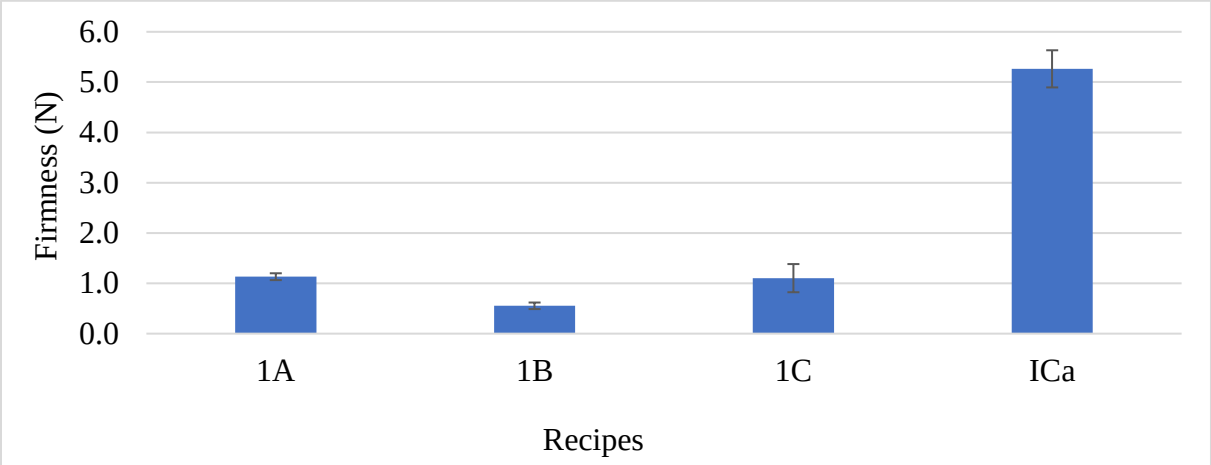


Figure 4.1. Texture measurements of the recipes 1A, 1B and 1C, listed in Table 4.2. Recipe 1A consisted of silver smelt, while 1B consisted of Haddock. The ratio is 1:1 between haddock and silver smelt in recipe 1C. Recipe 1C[a] is made with non-precooked silver smelt (Appendix G).

4.1.1.2 Precooked and raw fish material prior to blending

Recipe 1C contained ratio 1:1 of haddock to silver smelt. Mixture with precooked silver smelt (1C) was more liquified than 1A, and blending was considerably easier to perform. It was not as liquified as product 1B with 59% haddock, hence a ladle was needed to fill the product into the aluminum containers. The texture of product 1C was like 1A, both firmer than 1B (Figure 4.1) and could be explained by the gelation and binding abilities of the proteins in silver smelt. The protein content in recipe 1A and 1C was also higher than 1B, due to silver smelt (Table 4.2), which further strengthens the texture (Hellevik, Synnes, and Stoknes 2005).

Recipe 1C[a] was equal to 1C, except the silver smelt was not precooked before mixing. The difference in binding abilities by adding either non-precooked and precooked silver smelt had not been tested previously and were therefore tested. The blending of the product with raw silver smelt (1C[a]) was difficult and gave a solid and dense product. The taste was more comparable to minced fish than TMP and with a texture that resembled a fine-grained minced

fish. The texture analysis of 1C[a] revealed a hard and firm product (Figure 4.1). Reasonable causes for the firm texture are both a three-dimensional gel formed by the proteins upon heating and exceptional binding abilities, with emphasis on the binding abilities (Bertak and Karahdian 1995). Similar results were obtained by Prabhu (2018) who studied how heat treated compared to raw haddock fillets influenced the texture of the finished product.

A TMP adjusted for elderly and people with dysphagia had to have a soft texture and therefore, it was decided to further develop the recipes based on precooked silver smelt and haddock. The final product would additionally be a safer product with double pasteurization, suitable for elderly and others in the vulnerable groups. Different ratios of haddock to silver smelt were tested, nevertheless, 1:1 ratio was selected for further recipe development. The other examined ratios did not yield any remarkable changes in texture and were therefore excluded from the thesis. The Norwegian food industry use both higher and lower ratios of silver smelt in different fish products, as well as 1:1 ratio with other species (Appendix F) (Amundsen 2017).

4.1.2 Preliminary production

The aim of preliminary production was to maximize total protein and fat content by adjusting oil, whipping cream and protein enrichment, but nevertheless maintaining a soft texture in final TMP. The nutrient content in TMP was additionally developed to exceed the content in fish raw materials, which is listed in Table 4.1. Parts of the recipes used in preliminary production can be seen in Table 4.3, while remaining ingredients can be found in Table 3.4. Energy and carbohydrates were excluded from all the tables containing nutrient calculations, due to that the protein used in this thesis lacked this information.

Table 4.3. Calculated total content of protein, fat, and salt in different recipes during preliminary production. Added ingredients which differed from each other are included. The remaining ingredients are shown in Table 3.4.

Recipe	**2A**	**2B**	**2C**	**2D**	**2E**	**2F**	**2G**	**Sooft Meals**
Whipping cream (%)	23.5	22	23.5	20	20.7	23.5	20	
Oil (%)	8.3	5.2	3.7	5.6	5.7	1.7	5.2	
Hydrolysate (%)	-	2.5	2.5	2.9	2.7	3	3	
Sodium caseinate (%)	1.8	2.5	2.5	2.9	2.7	3	3	
WPC80 (%)	3.6	5	5	5.8	5.4	6	6	
Total protein (%)	15.8	19.9	19.9	21.2	20.5	21.6	21.5	13.7
Total fat (%)	17.9	14.3	13.4	14.0	14.3	11.4	13.6	10.0
Total salt (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.2

The quantity of fish in the recipes used during preliminary production were slightly increased to 60%, as consumers prefer products with high percentage of fish (Table 3.4) (Skogly 2016; Forbrukerrådet 2016). Salt (NaCl) on the contrary, should be low as possible due to several health issues associated with overconsumption however, salt obtains several physical properties as a taste enhancer and texture modifier, and cannot be entirely excluded (Delahaye 2013). The aim was therefore to develop the recipes with approximately 1% salt. Salt was added simultaneously with the fish to activate its proteins during blending, resulting in an increase of water holding capacity (Aursand et al. 2007).

Consider the importance of the soft but stable texture in TMP, texture modifiers were added for thickening and gelling properties. The texture modifiers were locust bean gum (LBG) and cornstarch. One of the great advantages of these hydrocolloids are the low amounts required, while they still increase water holding, gelling, and thickening properties (Van Nieuwenhuyzen et al. 2006). The gelling abilities and water holding capacity are activated by heat treatment, due to formation of a three-dimensional polymer network. Gelled food containing hydrocolloids can exudate water over time through a process called syneresis, which is when the gel stiffens and loses water. This issue is solved by adding the perfect ratio of cornstarch and LBG (Van Nieuwenhuyzen et al. 2006). Starch makes the gel heat stable upon reheating which maintains the form and prevents cooking loss. This ability is crucial in the making of TMP, which requires the products to be soft while keeping the form stable (Wüstenberg 2015). No remarkable water exudation was observed after final heat treatment of the preliminary production and the products maintained its form during reheating.

TMP was made by diluting the product with oil and whipping cream for a less firm texture, which results in lower level of total protein. Whipping cream contains proteins as well as energy and therefore suits more in TMP, compared to milk. Due to the dilution, products were enriched using whey protein concentrate (WPC) and sodium caseinate (caseinate).

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 flavor was experienced less strong (Prabhu 2018). Caseinate additionally offer great water holding capacity and textural improvements to the products (Kneifel and Seiler 1993). Proteins often function as gelling agents as well, often in combination with different gums and starches (Zayas 1997). In context of fitness, WPC is often referred to as the “fast” protein while caseinate is called “slow”, which

means they are digested and used in the muscle protein synthesis (MPS) at different times. A study on dairy proteins towards MPS suggested that a combination of WPC and caseinate had higher impact, than they would separately (Kanda et al. 2016). People often experience muscle loss and decrease in MPS proportional with aging, thus a combination of WPC and caseinate could be appropriate in TMP (Siparsky, Kirkendall, and Garret 2014). Except for the bitterness often associated with FPH, they obtain physical properties desirable for food production like emulsifying ability and water holding capacity (Rasco and Kristinsson 2000). The capacity of holding water is essential in the process of making a soft and juicy product.

4.1.2.1 Water holding capacity

Two methods of determining water holding capacity were tested during preliminary production. They were tested on the standard recipe (2A). The difference between the methods was the placement of filter paper (Figure 4.2). Standard deviations from on results from the method with paper located at the bottom were quite high, compared to the top placement. Small quantities of the sample did attach to the filter paper during centrifugation when placed below the sample, which contributed to the high standard deviations. It was therefore determined to place the filter paper on top of the sample during future measurements. Several methods regarding water holding capacity are based on meat, poultry and fish muscle and not Pâté-like food (Skipnes, Østby, and Hendrickx 2007; Zayas and Lin 1989).

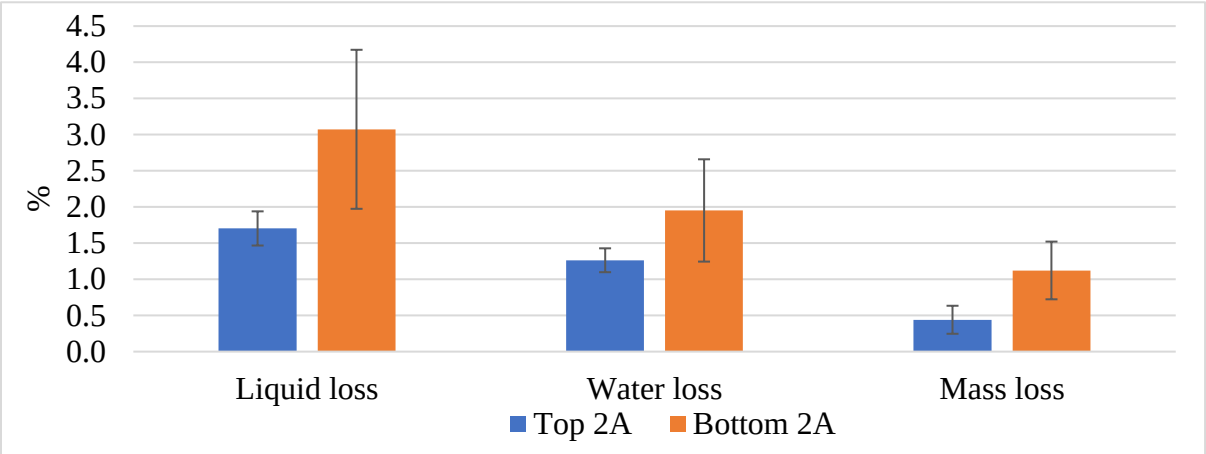


Figure 4.2. Water holding capacity was determined of recipe 2A (%) in preliminary production. Two different methods were applied on the sample for comparison. The filter paper was either placed on the bottom or the top of the sample during centrifugation (Appendix G).

water holding capacity was analyzed by using centrifugation and filter papers. Standard recipe without FPH (2A), low protein enriched recipe (2B) and high protein enriched recipe (2F) were analyzed to determine percentage of water loss (Figure 4.3). Fat loss was redefined as mass loss

due to small amounts of the products remaining on the filter papers after weighing. The results from the analysis did not yield big differences as the values of water loss were between 1.7% and 1.9%. Water loss of TMP was low which is synonymous with high water holding capacity.

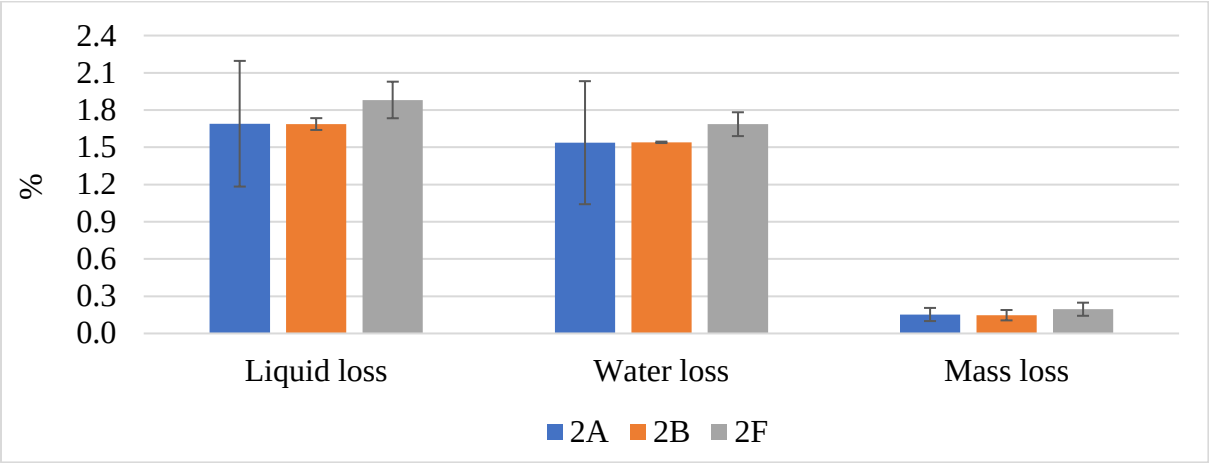


Figure 4.3. Water holding capacity determined of recipe 2A, 2B and 2F in preliminary production (%). The filter paper was placed on the top during centrifugation, dried and weighed (Appendix G).

4.1.2.2 Texture analysis

Recipes in the preliminary production (2A-2G) were developed by changing the amount of added whipping cream, oil, and protein enrichment. Standard recipe (2A) was only protein enriched by the dairy proteins caseinate and WPC (Table 4.3). It was used as a standard in further development, considering FHP was omitted from the recipe, only WPC and caseinate were added. Remaining recipes were enriched by FHP in addition to WPC and caseinate.

Table 4.3. Calculated total content of protein, fat, and salt in different recipes during preliminary production. Added ingredients which differed from each other are included. The remaining ingredients are shown in Table 3.4.

Recipe	**2A**	**2B**	**2C**	**2D**	**2E**	**2F**	**2G**	**Sooft Meals**
Whipping cream (%)	23.5	22	23.5	20	20.7	23.5	20	
Oil (%)	8.3	5.2	3.7	5.6	5.7	1.7	5.2	
Hydrolysate (%)	-	2.5	2.5	2.9	2.7	3	3	
Sodium caseinate (%)	1.8	2.5	2.5	2.9	2.7	3	3	
WPC80 (%)	3.6	5	5	5.8	5.4	6	6	
Total protein (%)	15.8	19.9	19.9	21.2	20.5	21.6	21.5	13.7
Total fat (%)	17.9	14.3	13.4	14.0	14.3	11.4	13.6	10.0
Total salt (%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.2

The texture of standard product (2A) had the least firm texture of all recipes in preliminary production, due to more liquid and less protein enrichment, particularly WPC (Figure 4.4)

(Zayas 1997). Compared to 2A, product 2B had less whipping cream and oil but more added protein. Texture analysis indicated a firmer product than the standard (2A). Product 2C contained equal percentage of whipping cream but less oil as the standard (2A). The protein enrichment in 2B was equal to 2C. Product 2C was experienced as grainier and drier compared to the standard, during a simplified evaluation of taste and appearance. It was similar as 2B in firmness, both firmer than the standard. Varying firmness and sensory texture were results of the decrease in oil and increase in protein enrichment.

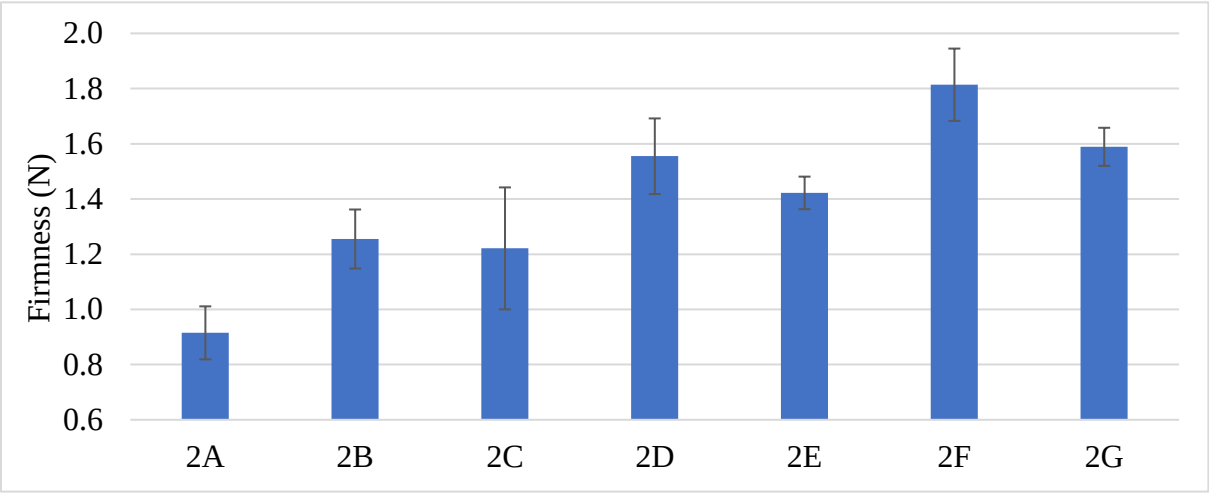


Figure 4.4. Texture measurements of recipe 2A-2G in preliminary production given in newton (Table 4.3). Recipe 2A does not contain hydrolysates, while the remaining ones have added hydrolysates, as well as variations in whey caseinate, whipping cream and oil (Appendix G).

The product 2D contained slightly more whipping cream and oil, but less protein enrichment than 2C. The texture was firmer than the products 2A-2C and most likely 2E, which were all lower in total protein content. This gave an indication that increasing firmness is proportional to increasing total protein content. Firmness is caused by increasing level of protein enrichment, with emphasis on whey due to gelling abilities (Zayas 1997).

The remaining products 2F and 2G was developed based on obtaining highest possible level of total protein content, without compromising the taste and texture. Product 2F was added equal percentage of whipping cream as standard 2A, while it had the lowest amount of oil of the recipes in the preliminary production. Further, 2F was the firmest of all. Evaluation of the taste revealed an adhesive structure. Prabhu reported caseinate as highly adhesive when used exclusively as protein enrichment (Prabhu 2018). Thus, it is possible that water holding capacity of caseinate is plausible for the adhesive texture, in addition to water holding capacity of FPH (Kneifel and Seiler 1993). Adding higher volume of whipping cream and lower volume of oil

could reasonably favor a more adhesive texture as well since the proteins have more accessible water to bind to and hold.

Since TMP are developed based on high energy and protein requirements in the target groups elderly and people with dysphagia (Chapter 2.1.2 and 2.1.3), the whipping cream in 2G was reduced in order to be able to increase oil. By doing so, the level of added protein could remain equal to 2F. The increased oil and decreased whipping cream of 2G compared to 2F seemingly lowered the firmness, while maintaining total protein (Table 4.4).

4.1.2.3 Color analysis

A color analysis was conducted on all preliminary products except 2C by DigiEye. The coordinates L*, a* and b* was measured and analyzed. The difference between the recipes was not examined thoroughly, due to lack of standard deviations. However, the values were included in this study to get an enhanced understanding of the colors. The first impression a person obtains when consuming food are the appearance and colors. Hence, color analysis is a widely conducted method in the food processing industry (Pathare, Opara, and Al-Said 2012).

Measured values of the color coordinate L* were 88-89 for all products, except in 2B which obtained a lower value (Figure 4.5). The difference in 2B is most likely an error seeing that the product is similar to both the standard 2A and 2C in ingredients (Table 4.3). The highest value possible for L* is 100 (white) meaning that all TMP from preliminary production were light, almost white products. The brightness can be explained by the pale haddock, silver smelt and whipping cream (Figure 4.5). The next color coordinate a* states whether the product was perceived as green (negative) or red (positive) (Pathare, Opara, and Al-Said 2012). The measured value ranged from 0.36 to 2.01, which would be perceived as neutral grey with a marginally red tint (Figure 4.6). Parameter b* determines whether the product is blue (negative) or yellow (positive). The obtained values averaged 16-17 revealing a more yellow product (Figure 4.6). The yellow color is caused by the added protein powders and the sunflower oil, all possessing varying yellow tones.

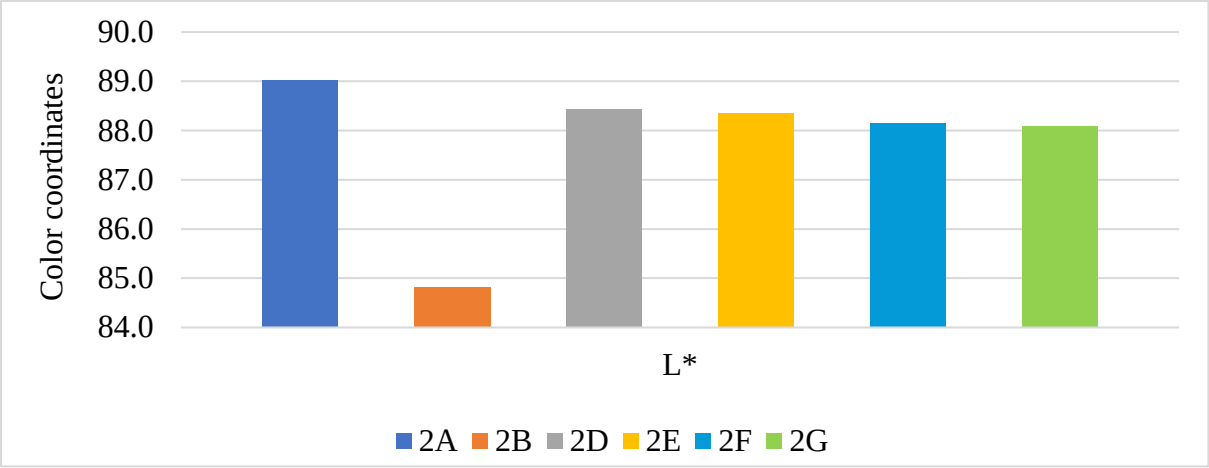


Figure 4.5. Color coordinate L* of the recipes 2A-2G except 2C in preliminary production (Table 4.3). A single measurement was performed of each recipe; standard deviations are therefore lacking (Appendix G).

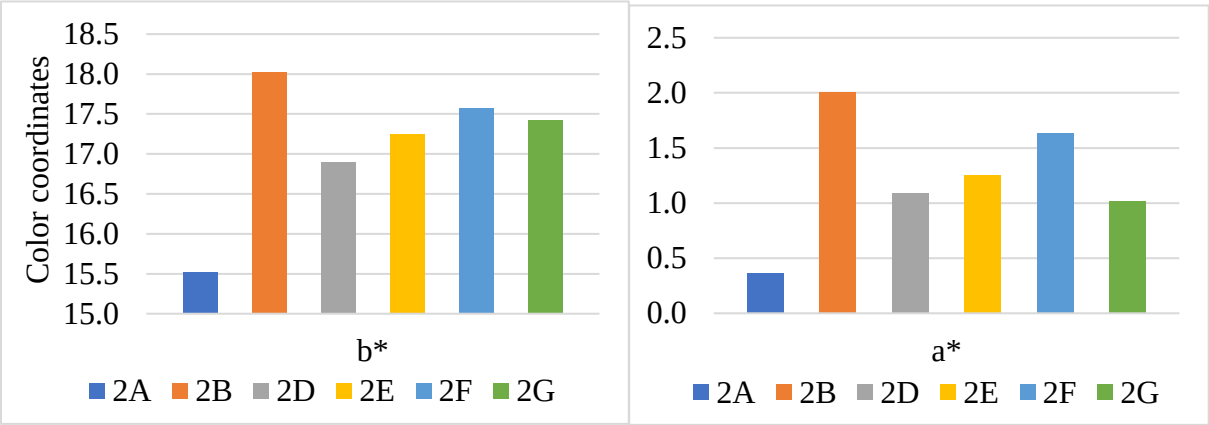


Figure 4.6. Color coordinates a* and b* of recipe 2A-2G except 2C during preliminary production (Table 4.3). A single measurement was performed of each recipe; standard deviations are therefore lacking (Appendix G).

4.1.2.4 Rheology

An amplitude sweep was conducted to investigate the following products 2A, 2E and 2F (Figure 4.7). The primary aim was to determine the linear viscoelastic region (LVR) and therefore, storage modulus G´ (Pa) was plotted against oscillation strain (%). The recipes were selected based on their total protein, to ensure that all products were within LVR. The standard 2A contained the lowest percent of protein, 2E was in between, while 2F on the other hand, contained highest total protein content. The selected oscillation strain 0.086% was further used in the following analysis: temperature sweep. Oscillation strain was determined by using a straight line on the horizontal region of the graphs to select a value when the slope still was zero. A temperature sweep would by conducted over a temperature range associated with reheating and serving of food which is above 60ºC (Næringsmiddelhygieneforskriften 2009).

Therefore, an amplitude sweep would be performed over the same degrees, which were 25°C, 50°C and 80°C.

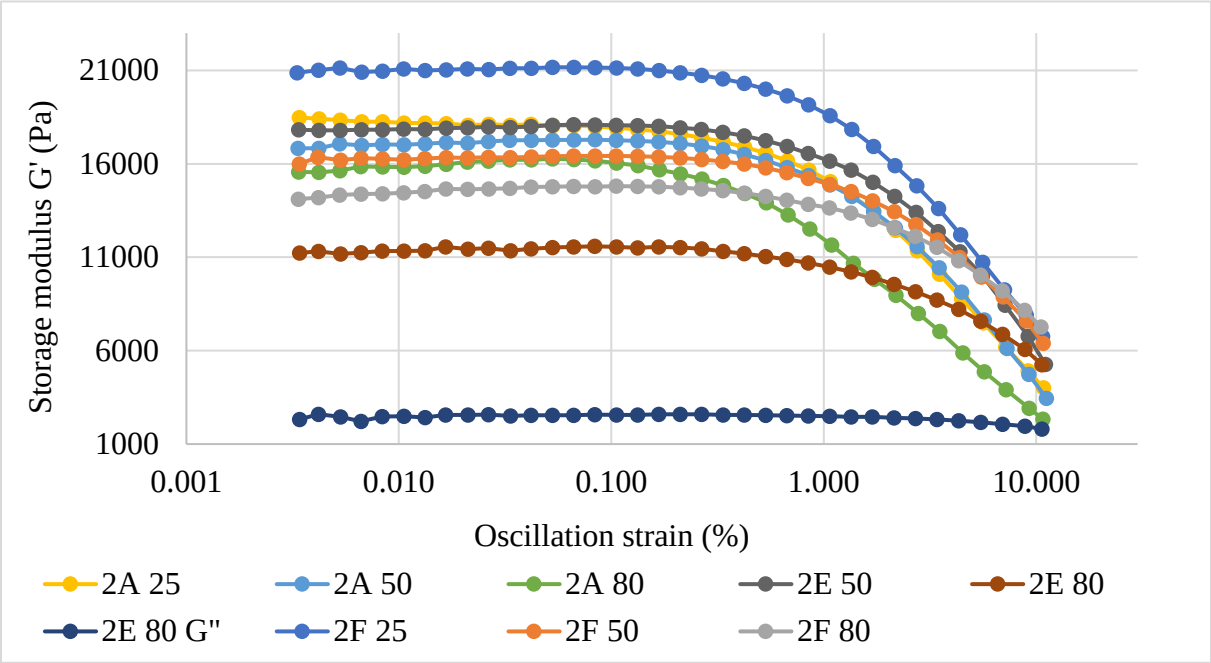


Figure 4.7. An amplitude sweep was performed on standard (2A), middle total protein (2E) and highest total protein content (2F). The selected temperatures were 25, 50 and 80°C. Storage modulus G' (Pa) was plotted against oscillation strain (%) while the frequency (Hz) was kept constant. 2E 80 G" are shown with loss modulus instead of storage modulus.

The behavior of product 2B, 2E and 2G during heating were investigated with a temperature sweep. The sweep initiated from 20°C, raised to 80°C, and then finally decreased to 50°C. The product with lowest percentage of FPH (2B) obtained the highest storage modulus. Product 2E and the highest protein enriched product (2G) on the other hand, obtained lower values of storage modulus (Figure 4.8).

A singular measurement was conducted on each product, to achieve knowledge about the general behavior of TMP. Because parallels were not taken, the graphs were not compared to each other. However, examination of the graphs trend revealed a decreasing value of storage modulus when temperature increased. The decreasing trend suggest that the food structure is weakened, due to weaker bonding and less ordered internal structure (Tunick, Onwulata, and Cooke 2013; Bourne 2002). Considering a higher content of proteins resulted in a firmer texture (Figure 4.4), product 2B should logically show lower storage modulus, compared to 2E and 2G (Figure 4.8). Examinations of plausible differences between the products were conducted during the pilot production.

Loss modulus G” of all TMP in preliminary production was revealed to be lower compared to storage modulus. This difference could be observed in Figure 4.7 between graph 2E 80 G” loss modulus and “2E 80” storage modulus and in Figure 4.8 between product “2E 80 G” of loss modulus and “2E 80” of storage modulus. Difference between G’ and G” indicates the viscoelastic character of the sample. In this case, G’>G” which suggested that the sample contains gelled or a solid structure and could be referred to as a viscoelastic solid material (Anton Paar 2020).

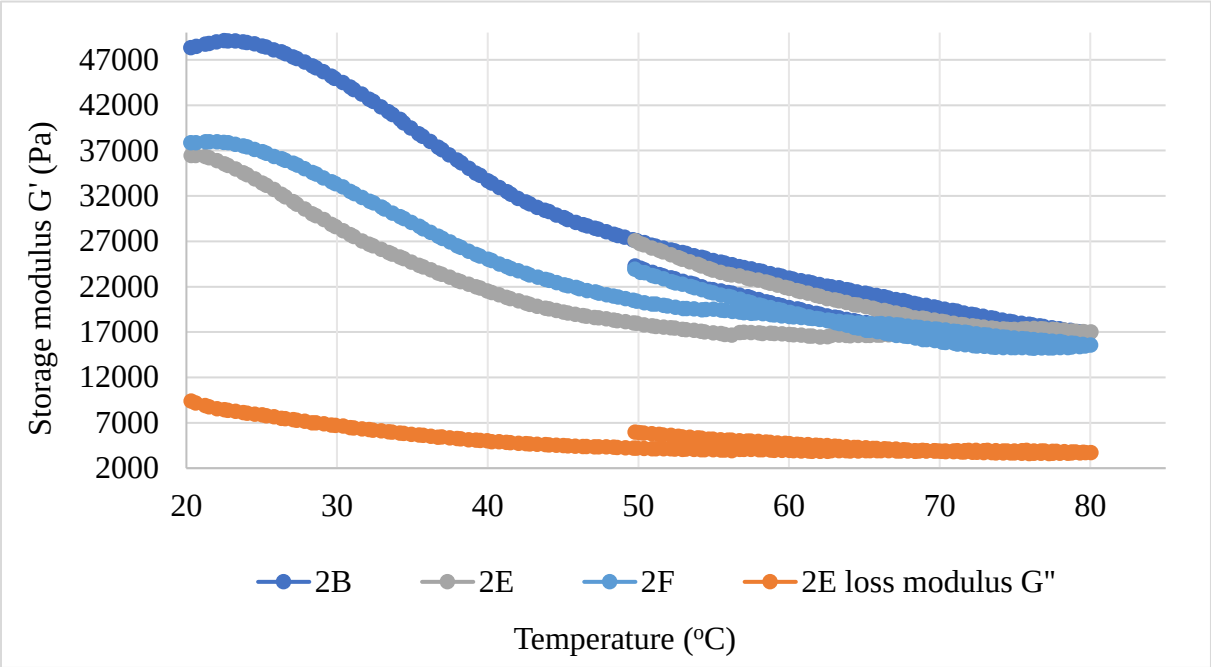


Figure 4.8. A temperature sweep of recipe 2B, 2E and 2G in preliminary production. 2E loss modulus G” show loss modulus and not storage modulus, both with unit Pa. The initial temperature was 20°C, which increased to 80°C and finally decreased to 50°C. Storage modulus G’ (Pa) was plotted against temperature (°C).

4.2 Pilot production of texture modified products

The recipes applied in pilot production were a result of gained knowledge from the preliminary production. The amount of precooked fish per specie continued as 30% in the following recipes. After several recipes with variations of whipping cream were studied in the preliminary production, the final recipes would contain 20%. Sunflower oil would function as one of the variables, together with protein enrichment. The ratio 2:1:1 between whey protein caseinate (WPC), caseinate and fish protein hydrolysate (FPH) would extend into the pilot production, seeing that both 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).

After thorough consideration, filling the products in aluminum containers as the preliminary production was excluded in the pilot production. Betan casings were applied instead, resulting in a more uniform and easier to handle product. A uniform product was a necessity when performing different analyses. The aluminum containers required substantially amounts of handling during the final heat treatment, due to the coating of oil, wrapping with cling film, and cooking. The products, which were in contact with the walls of the containers, would receive a thin and hardened outer layer. Furthermore, browning occurred at the surface of the products during cooking in the aluminum containers. The process is known as a Maillard reaction, a common reaction in the food industry. Amino acids and sugars react by an external force like heating (Tamanna and Mahmood 2015). By applying Betan casings instead, the products would not receive the otherwise common variations in the outer texture.

4.2.1 Recipe development

Five recipes were developed, one used as a standard while the remaining ones contained higher amounts of protein enrichment. The standard recipe was referred to as ST, in which FPH were omitted (Table 4.4). The following recipe Low Hofseth Texture (LHT) contained calculated 19.5% total protein and added Hofseth FPH, nevertheless higher in total protein than ST (16.2% calculated total protein). The third recipe was called High Hofseth Texture (HHT) with 21.6% total protein. The first recipe enriched with FPH produced by Nofima was Low Nofima Texture (LNT) with 19.5% total protein. The hydrolysate from Nofima contained less proteins than Hofseth and therefore, the amount of added protein powders required an adjustment to reach the same level of total protein content. This adjustment made product LNT containing approximately the same total protein content as product LHT. The last recipe was called High Nofima Texture (HNT) with 21.5% total protein. One of the main purposes was to study the effect of adding FPH in texture modified products (TMP). The recipes that contained the same FPH although in varying amounts, were compared to each other and the standard. The total protein in LNT and LHT was developed based on them being comparable. The same process was repeated with HHT and HNT. Different analyses were conducted to investigate how the protein enrichment influenced the final product.

Table 4.4. Ingredients with variations between the recipes in pilot production are listed first, followed by calculated total content of proteins, fat, and salt. Analyzed crude protein by Kjeldahl are listed at the bottom.

Recipe	**ST**	**LHT**	**HHT**	**LNT**	**HNT**
Oil (%)	11.2	7.6	5.2	7.4	5
Hydrolysate Hofseth (%)	-	2.4	3	-	-

Hydrolysate Nofima (%)	-	-	-	2.45	3.05
Sodium caseinate (%)	1.8	2.4	3	2.45	3.05
WPC80 (%)	3.6	4.8	6	4.9	6.1
Total protein (%)	16.2	19.5	21.6	19.5	21.5
Total fat (%)	19.5	15.9	13.6	15.7	13.4
Total salt (%)	1.0	1.0	1.0	1.0	1.0
Total crude protein (%)	17.1	19.4	22.2	19.9	22.1

4.2.2 Kjeldahl method

Total protein of all five pilot products (ST, LHT, HHT, LNT and HNT) were derived from the Kjeldahl method. Nofima Biolab, Bergen conducted the analysis that yielded the data seen in Table 4.4. The products were analyzed to gain knowledge about the factual numbers, furthermore, to examine any resemblance with the calculated values shown in Table 4.4. Standard product (ST) was calculated to 16.2% total protein while the analysis yielded 17.1% total crude protein, a somewhat higher number, nevertheless similar. LHT on the other hand, received almost equal values from the analyzed content which was 19.4% compared to the calculated content of 19.5%. HHT, LNT and HNT got hardly any noticeable difference between the numbers from the analysis. HHT had 21.6% derived from calculations, while the analysis yielded 22.2%. HNT received similar results as HHT with 22.2% from the Kjeldahl method and 21.5% derived from calculations. The remaining product LNT got 19.9% from the Kjeldahl method, and 19.50% from the calculations. Summarized, all products received analyzed total protein content almost similar to the calculations. Small variations could occur since Kjeldahl measured all nitrogen in the sample, which further were used in calculations to indirectly determine total crude protein.

4.2.3 Texture analysis

Texture analysis was performed by measuring the force (N) required to penetrate a certain distance. All products were revealed to be significantly different from each other by one-way ANOVA ($p<0.001$). As predicted, ST was the significantly softest regarding texture from the pilot production due to low total protein content (Figure 4.9). It contained the lowest percent of total protein and highest amount of oil, in addition to not be enriched using FPH, only caseinate and WPC. LHT was significantly firmer than ST. The higher firmness is caused by protein gelation, with emphasis on WPC. On the contrary to caseinate, WPC possesses great gelling abilities through thermal activation (Rimac et al. 2009). The product LNT was significantly firmer than LHT due to more WPC. The FPH from Nofima could affect the firmness a little

more than FPH from Hofseth as well. Even more significantly firmer than the already mentioned was HHT although the significantly firmest product was revealed to be HNT. HNT contained the highest percentage of WPC as well, causing the firm texture. The increase in WPC correlated positively with an increase in firmness. Less liquid and generally more protein enrichment have most likely influenced the texture as well, making the texture firmer.

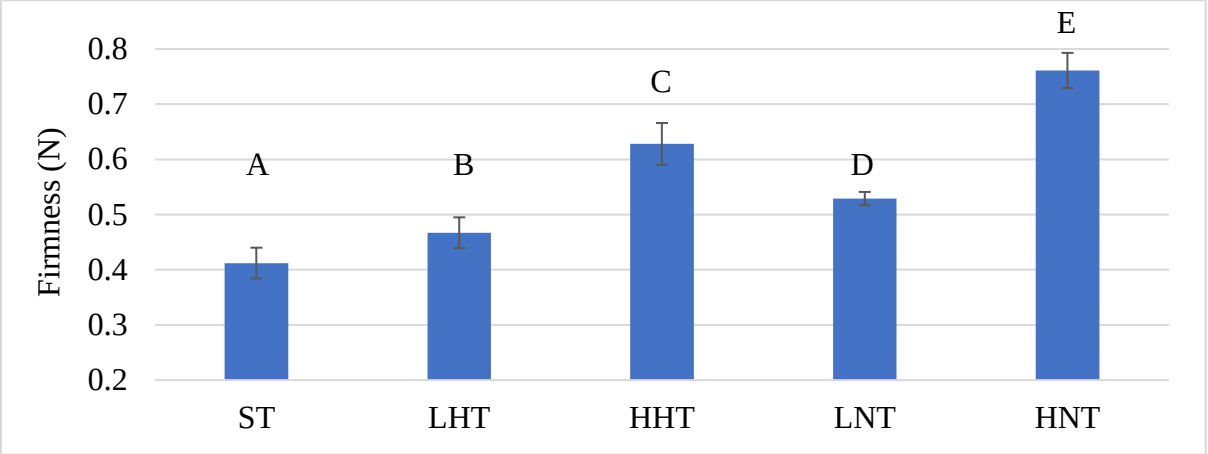


Figure 4.9. Texture analysis of firmness (N) in texture modified products ST, LHT, HHT, LNT and HNT from pilot production (Appendix H).

4.2.4 Color measurement

Color analysis was conducted on the pilot production by measuring the following color coordinates a*, b*, C*, L* and h. The values of C* and h were added to L*, a* and b* in pilot production. Regardless, this study emphasized on L*, a* and b*. The color coordinate a* from ST is almost zero, which is considered as a neutral grey color, nevertheless with a slightly positive value (red) (Figure 4.10). The measurements of HNT increased significantly to more redness, compared to ST. The remaining products LHT, HHT and LNT had similar values, all of them had significantly more redness than ST and HNT ($p<0.001$).

The p value obtained from the L* data was $p=0.013$. A Tukey pairwise Comparison test showed no differences between the products, a Fisher pairwise test was therefore applied instead. L* values of HNT and LNT showed a significantly darker shade than LHT and ST. ST was measured as significantly brighter than HHT, LNT and HNT. The results are caused by added hydrolysates, WPC and caseinate, influencing the brightness. Furthermore, FPH from Nofima contained a stronger yellow color compared to Hofseth, which made LNT and HNT darker. Regardless, all products are still considered as bright products. Even though L* yielded some

significantly differences, nevertheless, the different shades were hard to distinguish from each other visually (Figure 4.11).

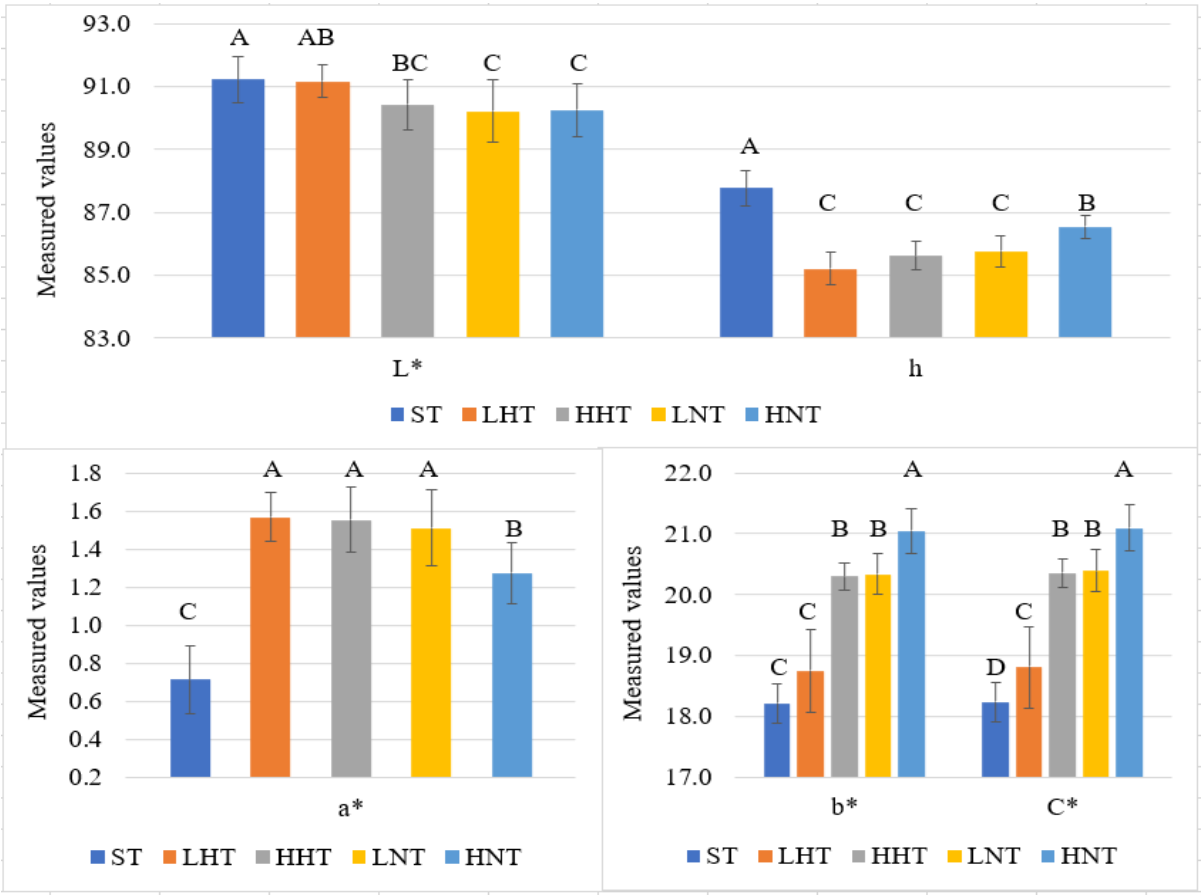


Figure 4.10. The color coordinates L*, a*, b* C* and h were measured of the products ST, LHT, HHT, LNT and HNT in pilot production (Appendix H).'

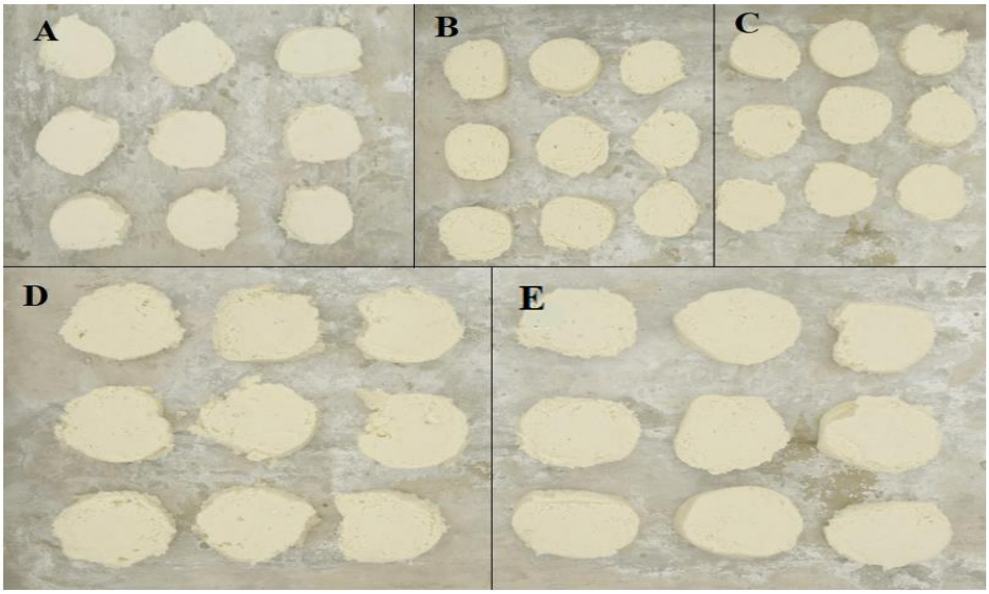


Figure 4.11. Photographs obtained from from color analysis. A:photograph of ST, B: photograph of HNT, C:photograph of LNT, D: photograph of HHT and E: photograph of LHT.

The following color coordinate b* corroborated the suspicions about colorization of the hydrolysate from Nofima ($p<0.001$). The significantly least yellow products are ST and LHT, obtaining similar values. Product HHT and LNT are significantly more yellow than ST and LHT. The most yellow product HNT differed significantly from the other. WPC and Hofseth FPH also contained some yellow colors which influenced the measurements.

Color coordinate C* ($p<0.001$) showed that ST contained significantly least saturation. Product LHT was significantly more saturated, followed significantly by HHT and LNT. Product HNT contained the highest saturation, which significantly differed from the other products. The last color coordinate to be analyzed was h ($p<0.001$). Product ST obtained the significantly highest h, while the value of h from HNT decreased significantly. The remaining products LNT, HHT and LHT obtained the significantly lowest values of h.

4.2.5 Water holding capacity

Water holding capacity was analyzed in TMP from pilot production by centrifugation of sample and filter paper. Liquid loss, water loss and mass loss were derived from the analysis. Investigation of results emphasized on water loss, due to water loss being the parameter used to assess water holding capacity.

Water loss showed some significantly differences ($p=0.022$) when analyzed. The product LHT obtained significantly higher water loss than LNT and HNT (Figure 4.12). The difference can be explained by the higher addition of caseinate and FPH in HNT and HHT, which both proteins obtains great water holding capacity (Zayas 1997; Rasco and Kristinsson 2000). Water loss in ST should be higher compared to the other more protein enriched products, due to less caseinate and no FPH holding and binding water. The results from ST were reasonably being influenced by some error, interfering with the yield. Water holding capacity was nevertheless very good in the TMP.

The next analyzed parameter was liquid loss ($p=0.005$). The product LHT obtained significantly higher liquid loss than ST, HHT and HNT, which all three were similar. The reasoning mentioned above about caseinate and FPH can be applied here as well. The same trend was observed in mass loss as LHT was significantly higher in mass loss compared to ST, HHT, and HNT ($p=0.001$).

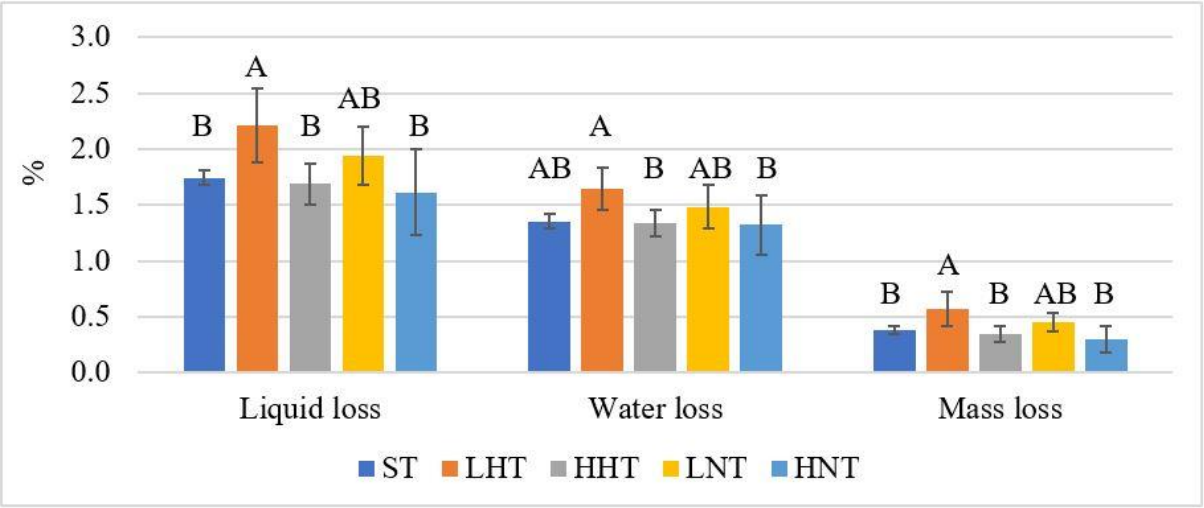


Figure 4.12. Water holding capacity (%) of texture modified products: ST, LHT, HHT, LNT and HNT was analyzed by determining water loss (Appendix H).

4.2.6 Image analysis

Photographs of TMP were analyzed to determine the area limited to threshold (Figure 4.13). ST was the only product which differed significantly from the others, since the size of the colored area was noticeably smaller ($p<0.001$). The remaining products obtained similar values. The black area within the edited photograph seen in Figure 4.14A is made up of matter such as enriched proteins, collagen and muscle proteins, remaining tissue from the fish raw material and other solid ingredients. The white area in Figure 4.14A and B is probably caused by either water, air, or a combination of both.

A more throughout study would be required to find more information about what the different particles consist of. It would be necessary to investigate the ingredients separately and in varying combinations, since TMP are tremendously complex food. The products were constructed of gels, emulsifiers, and foams, which were made by hydrocolloids, whipping cream and, fish raw material and added protein enrichment. Regardless of the complexity of properties and ingredients, the photographs did acknowledge a fine structure made by small particles.

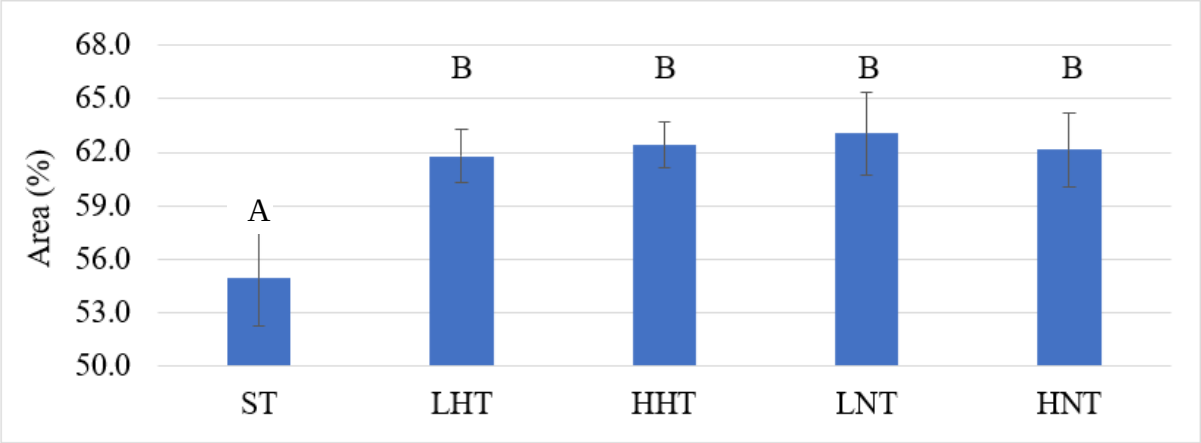


Figure 4.13. Texture modified products; ST, LHT, HHT, LNT and HNT from pilot production were analyzed to derive the area limited to threshold (%). Higher percentage equaled more mass (Appendix H).

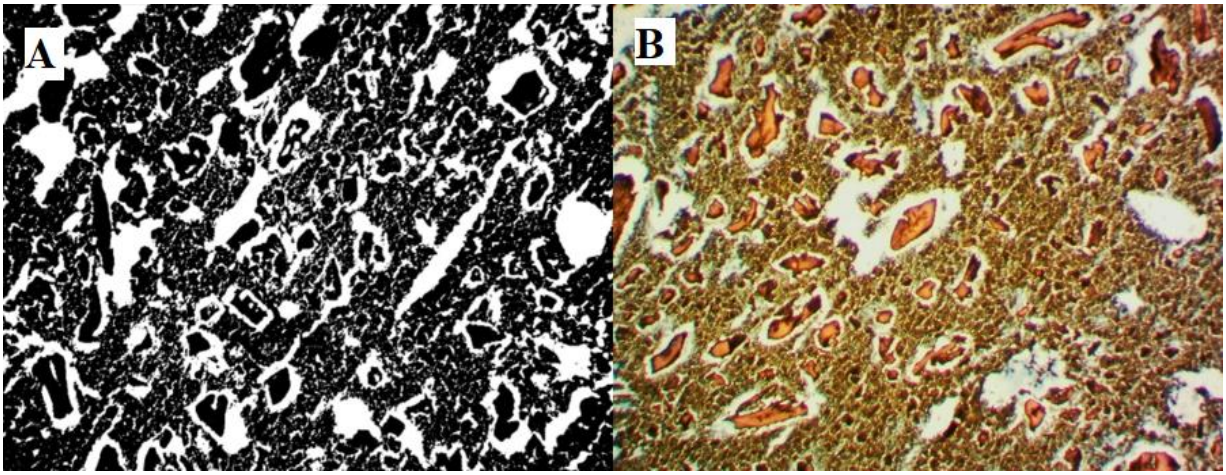


Figure 4.14. A: The appearance of the final image of LHT, after threshold was rendered in Fiji, B: the image of LNT before image editing, immediately after the photographs were shot through the stereoscope.

4.2.7 Sensory analysis

The sensory analysis was performed using QDA on the products ST, LHT, HHT, LNT and HNT. The attributes listed in Figure 4.15 all show significantly differences between two or more. Whilst odor and texture revealed several significant differences, the evaluation of flavors did not.

The sour odor in products ST and HHT was significantly more intense than LHT ($p=0.007$). The sour odor is caused by organic acids, resulting in a fresh and balanced smell. The overall intensity was between 3 and 5 on a scale from 1 to 9. Cloying odor did receive opposite results, as the judges perceived ST and HHT containing significantly less cloying odor compared to

LHT (*p=0.005*). The results could indicate a reasonable correlation between the two odors. Some judges had a few supplemental comments on the taste in several products, saying that the products tasted like sour cream or sour milk (Appendix J). The only addition of liquid except oil was whipping cream. Perhaps the whipping cream had a slightly sour taste, and therefore the smell would appear slightly sour as well. Owing to dairy proteins being processed from milk, caseinate and WPC contains a milky taste as well, which could additionally influence the reported dairy taste. Summarized; when the sour odor is experienced as more prominent, the cloying odor is less intense.

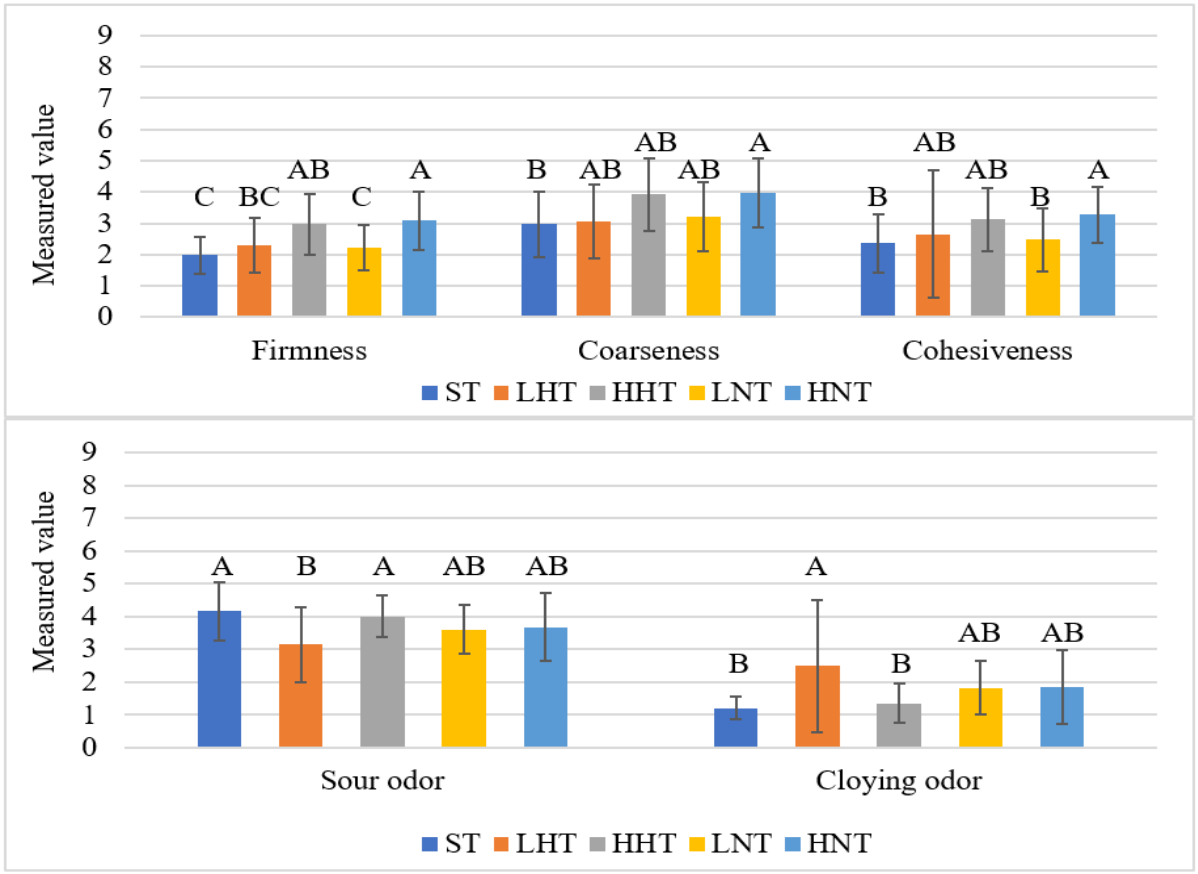


Figure 4.15. Sensory analysis of the attribute's odor, flavor, and texture. The selected attributes showed significantly difference between the products, the remaining attributes can be found in Appendix H.

In relation to texture, HNT was significantly coarser than ST (*p=0.008*). HNT was significantly more cohesive compared to ST in addition to LNT (*P=0.011*). The coarseness is reasonably caused by increased addition of FPH, caseinate and WPC. As mentioned previously, WPC obtains great gelling abilities and with increased amounts of protein and decreased amount of liquid, the product would be experienced as coarser. Few judges had some comments about the texture, as they experience the texture like butter (Appendix I) while other reported a more gritty and drier texture. The intensity of the coarseness was nevertheless not extremely high.

Figure 4.12 from image analysis (Chapter 4.2.6) also revealed several bigger particles among the finer structures, which could substantiate the experience of coarseness. Cohesiveness is also associated with the gelling abilities of WPC; increased addition would lead to a cohesive product. Less amounts would result in the opposite behavior, less cohesiveness.

The remaining textural parameter with significantly differences between the products was firmness ($p<0.001$). The same trend as previous attributes were observed once more in firmness. The products ST and LNT had significantly softer texture than HHT and HNT. HNT was significantly firmer compared to ST, LHT and LNT. The firmer texture in HNT could be explained by the gelling abilities of WPC and less oil (Zayas 1997). Texture analysis (Chapter 4.1.2.2) yielded somewhat the same results with the most protein enriched TMP being the firmest, whilst less enrichment resulted in a softer texture (Figure 4.9). The intensity of firmness from sensory evaluation indicated that LHT and LNT is closer to ST than HHT and HNT (Figure 4.15). This could be a consequence applied by caseinate and FPH. Both obtain great water holding capacity thus making the product retain more water. The firmness would consequently be decreased, which is demonstrated in other studies (Prabhu 2018).

A few additionally texture attributes describing the products were grittiness and adhesiveness but did not differ significantly (Figure 4.16). Adhesiveness is a result of water holding capacity of caseinate and FPH and was quite prominent. An assessor reported that the tongue was required to work immensely to remove all remaining traces of the food, which was not desirable in TMP associated with dysphagia management (Appendix I). The amounts of enriched protein were most likely the reason for the high grittiness.

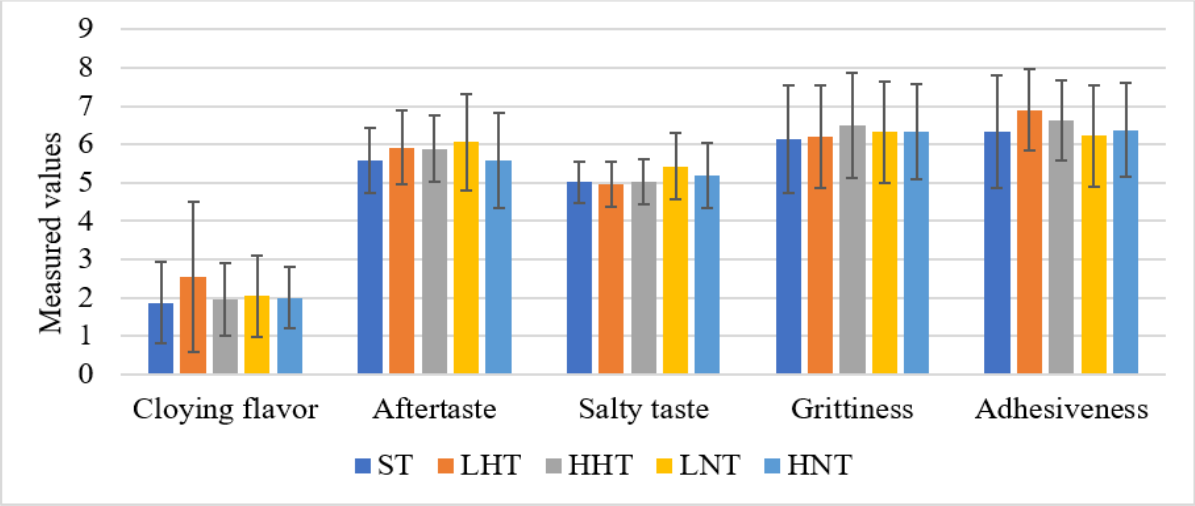
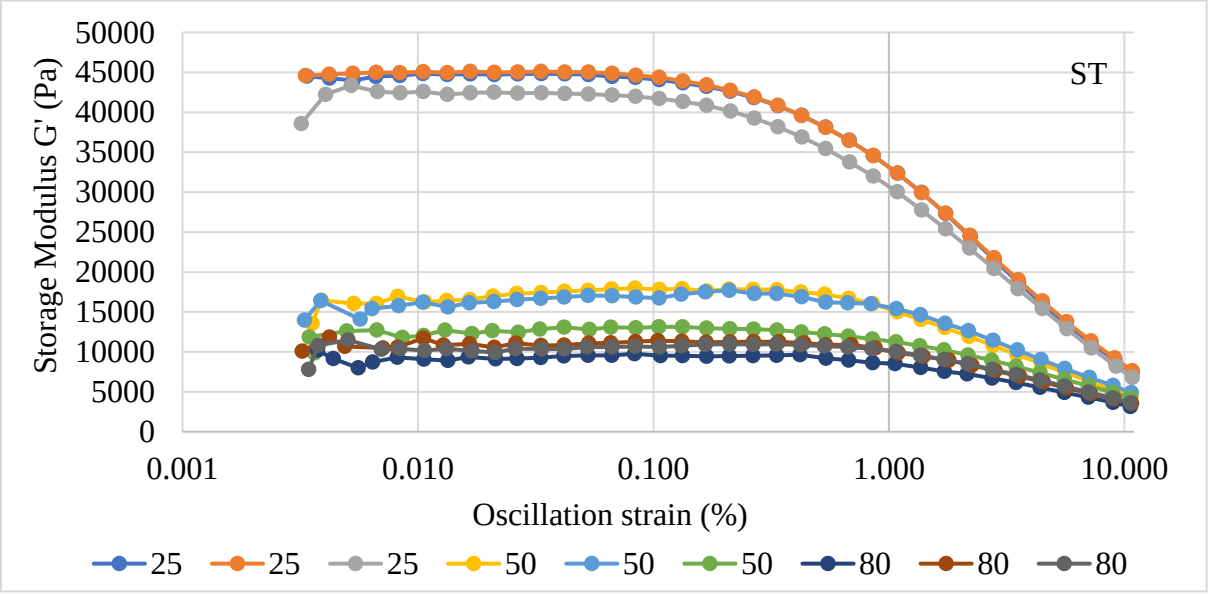


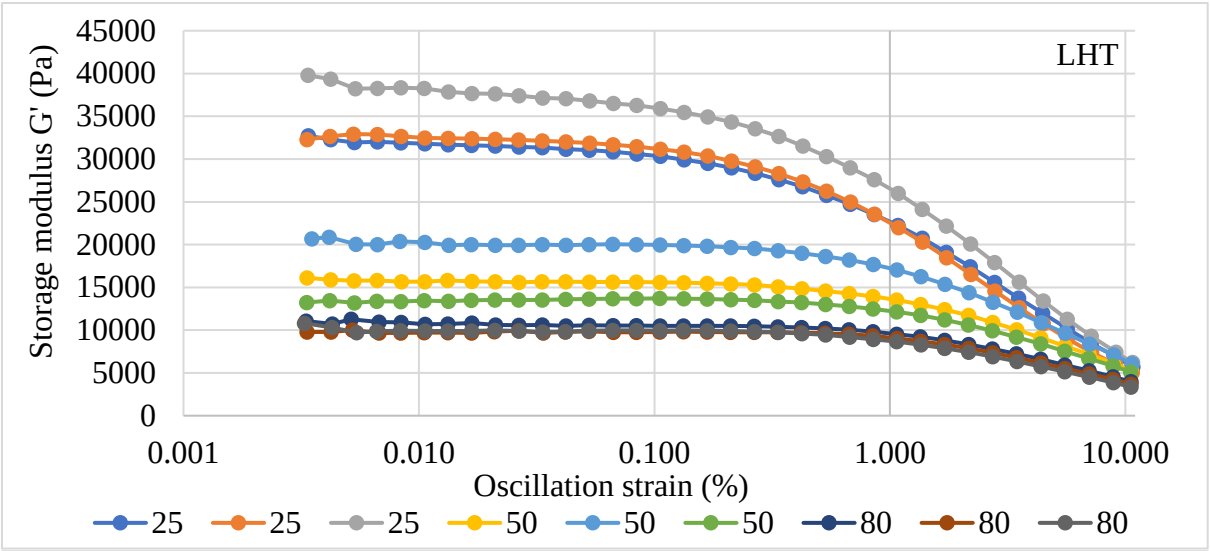
Figure 4.16. Sensory analysis of the attributes cloying flavor, aftertaste, salty taste, grittiness, and adhesiveness. These attributes did not contain significant difference (Appendix H).

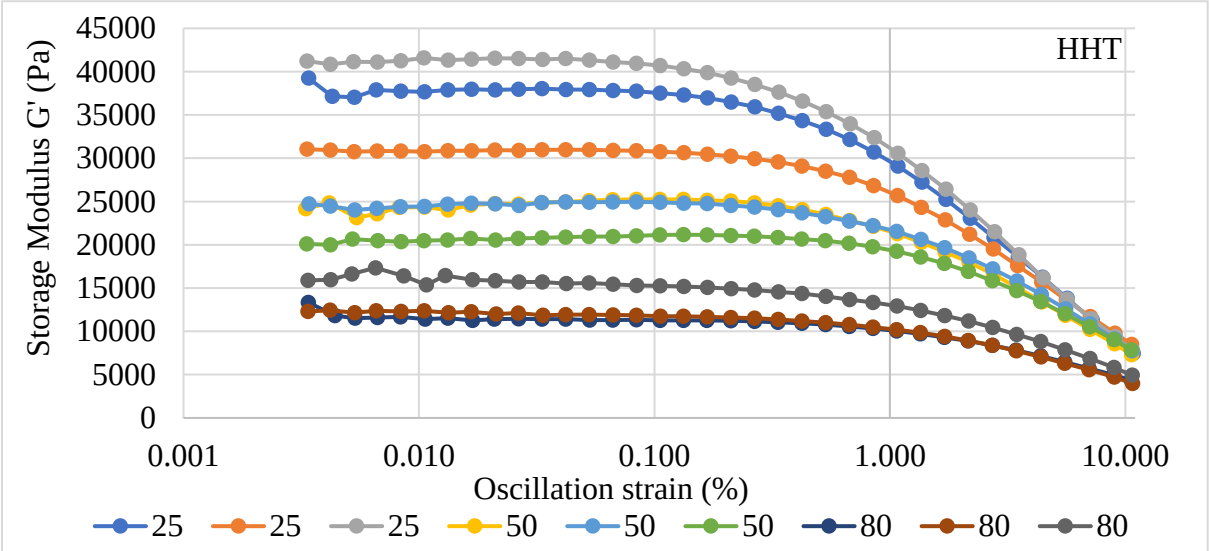
Most numbers regarding flavor intensity were approximately between the values 3-4.3 in all products, except aftertaste, salt, and cloying. Aftertaste and salty taste were perceived with higher intensity, while cloying flavor was experienced as low (Figure 4.15). The salty taste was probably caused by the fish raw material but more likely FPH from Nofima and Hofseth. Fish from saltwater tends to have a slight salty or brine taste.

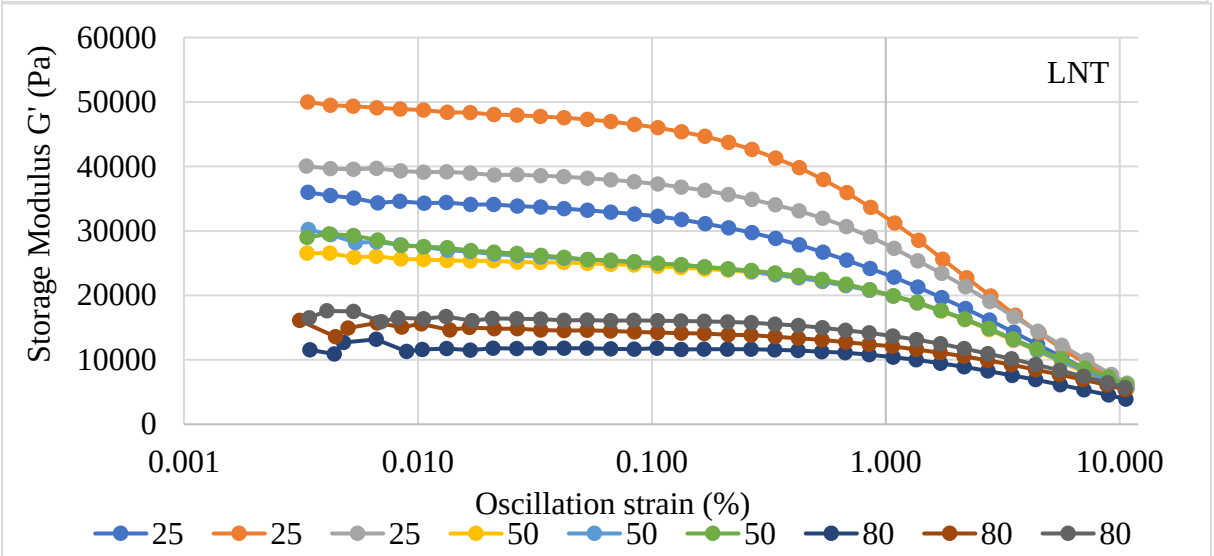
4.2.8 Rheology

An amplitude sweep was performed on the products ST, LHT, HHT, LNT and HNT to determine the linear viscoelastic region (LVR). Storage modulus (Pa) was plotted against oscillation strain (%). The selected strain within LVR was 0.067%, which prevents deformation in the following analysis temperature sweep. Examination of the graphs in Figure 4.17 and 4.18 revealed that increasing temperature and higher percent of total protein consequently decreased the storage modulus. Put differently, a plausible correlation between temperature, total protein, and storage modulus was observed. Further knowledge was gained by temperature sweeps, as the primary aim of an amplitude sweep was to determine the strain (%) for further analysis.




LHT
Storage modulus G' (Pa)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0.001
0.010
0.100
1.000
10.000
Oscillation strain (%)
25 25 25 50 50 50 80 80 80


HHT
Storage Modulus G' (Pa)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0.001
0.010
0.100
1.000
10.000
Oscillation strain (%)
25 25 25 50 50 50 80 80 80


LNT
Storage Modulus G' (Pa)
60000
50000
40000
30000
20000
10000
0
0.001
0.010
0.100
1.000
10.000
Oscillation strain (%)
25 25 25 50 50 50 80 80 80

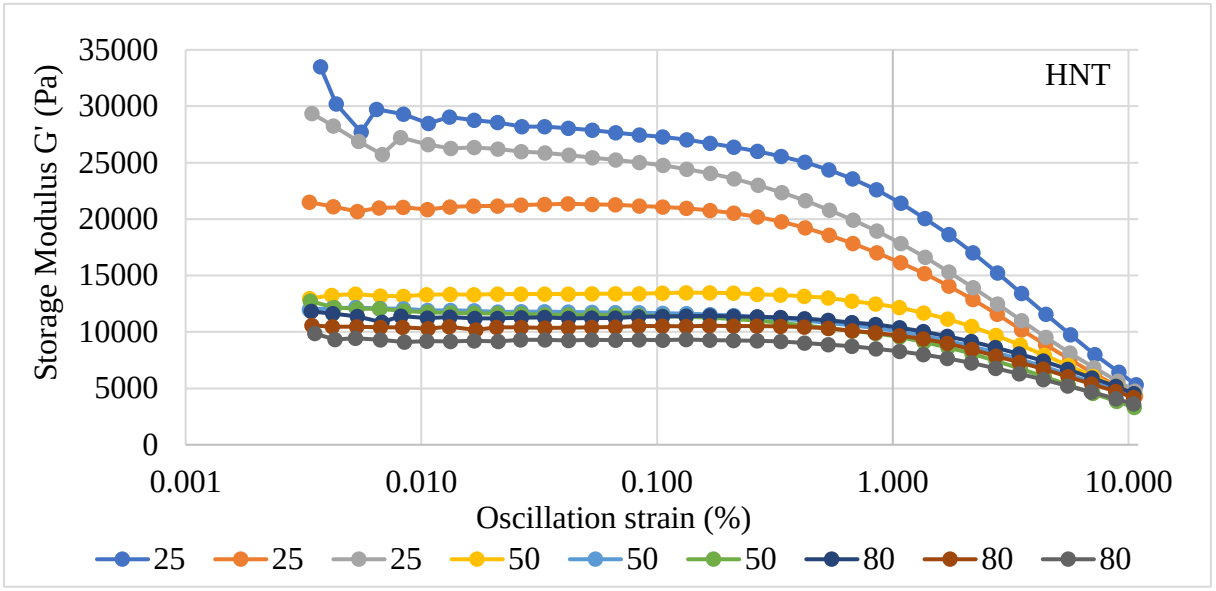
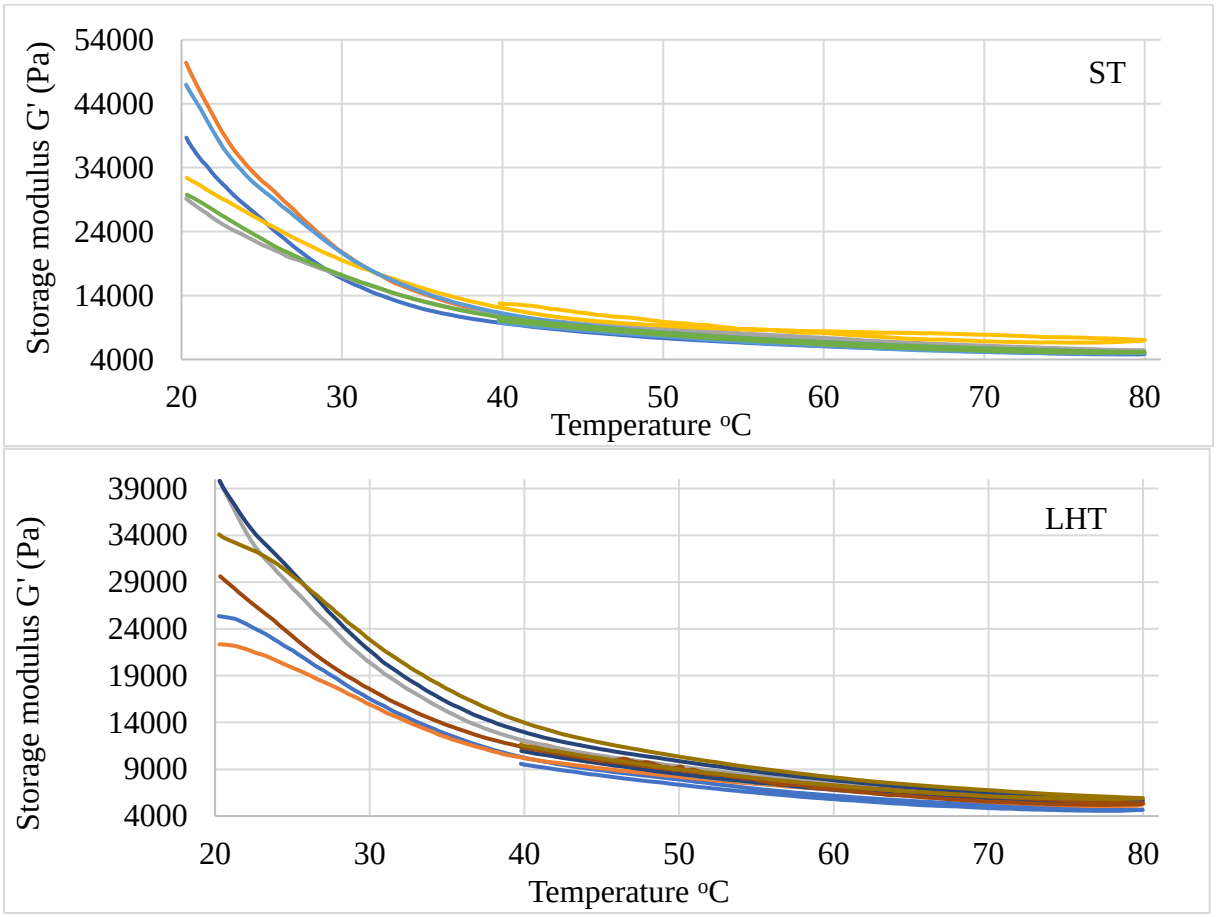


Figure 4.17 An amplitude sweep was performed of sample A: ST, B: LHT, C: HHT, D: LNT, and E: HNT. The selected temperatures were 25, 50 and 80°C. Storage modulus G' (Pa) was plotted against oscillation strain (%) while the frequency (Hz) was kept constant.



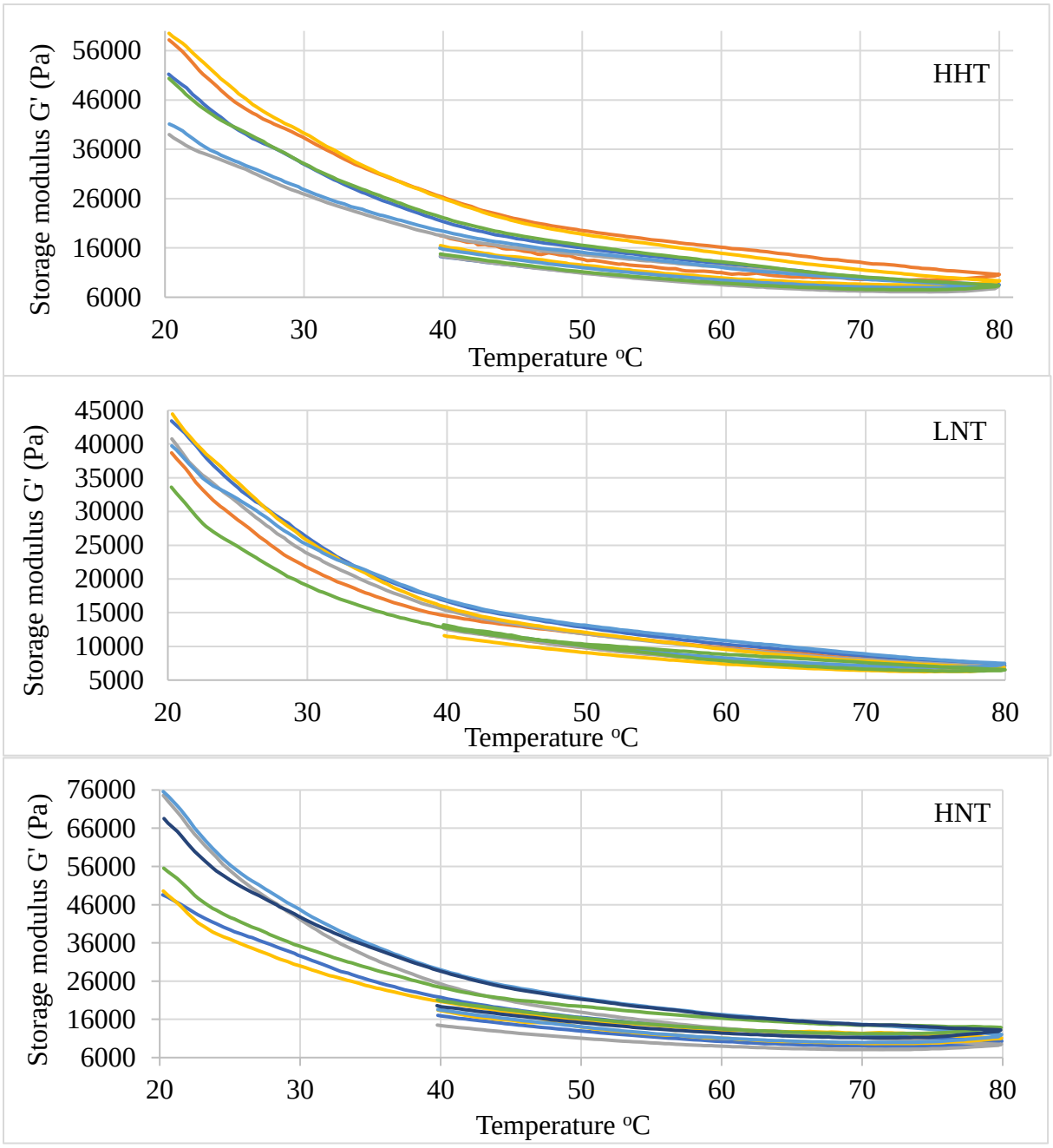


Figure 4.18. A rheological temperature sweep was conducted of A: ST, B: LHT, C: HHT, D: LNT, and E: HNT. The initial temperature was 20°C, which increased to 80°C and finally decreased to 50°C. Storage modulus G' (Pa) was plotted against temperature (°C).

Further analysis of rheological properties was conducted by a temperature sweep (Figure 4.18). The initial temperature was 20°C, increased to 80°C and then decreased to 40°C. Compared to the final temperature applied in preliminary production, the temperature during pilot production was decreased even further from 50°C to 40°C. The initial lower temperature would give better information about the behavior from throughout the heating process of the food during serving. The following cooling after temperature peak at 80°C would additionally yield information about how the molecular internal structure behaves during cool down.

As mentioned previously, a correlation between protein, temperature and storage modulus was observed. ST and LHT yielded the lowest average value of storage modulus, then a slightly increase was observed in LNT, whilst HHT and HNT measured the highest storage modulus. LNT were marginally higher in storage modulus than LHT. Same result was observed in HNT and HHT, which HNT had the highest storage modulus. The decreasing trend in storage modulus suggest that molecular bonds are weakening during the heating and the internal structure is less ordered. The food structure is mainly altered by gelling of proteins like WPC, cornstarch and locust bean gum (LBG) (Tunick, Onwulata, and Cooke 2013). However, there is a plausible difference between the hydrolysates from Nofima and Hofseth, hence HNT and LNT obtained higher values than HHT and LHT. On the other hand, the same difference could also arise because the liquid was decreased, and the amount of WPC was increased in HNT and LNT. The results were equal to the results from texture analysis and sensory analysis of texture.

The results also revealed that the products deformed because of applied stress. The viscosity decreased during increasing temperature and was partially restored when the temperature was decreased. This could be observed in Figure 4.17-4.18 as the graphs slope decreased and then increased, but only partially. In other words, higher temperatures deform the sample, and if the temperature is thereafter decreased, the sample will partially restore its original structure.

4.3 Preliminary testing and production of minced fish

The aim for this part of the study was to develop recipes of protein enriched minced fish that could be transferred to the industry in the future. Additionally, the study would gain knowledge associated with the texture and sensory attributes of minced fish. The recipes were based on minced fish, which could be constructed into small patties, fish burgers or Norwegian “fish pudding”. Several industries are producing protein enriched food, due to high demand in the market. More and more people acknowledge the important key role proteins play in a diet. Athletes, young, sedentary adults, and elderly will all benefit from a sufficient intake of proteins (Chapter 2.1.1)

The ratio of whey protein caseinate (WPC), caseinate and fish protein hydrolysates (FPH) applied in processing texture modified products (TMP) was applied in the recipes of minced fish. The recipes were developed consisting of thawed silver smelt and raw haddock. The good binding abilities, as well as great water holding capacity in silver smelt are highly suitable for minced fish (Sivertsen 2012). The haddock was incorporated into the mixture fresh, without any freezing. Freezing influence the proteins within the haddock in a negative way, whilst silver

smelt is less prone to frozen damage. Haddock obtains better binding abilities when used raw (Chapter 2.2.1.2 and 2.2.1.3) (Shenouda 1998). Silver smelt is often used frozen, as it's normally frozen directly at sea (Gundersen and Dahl 2008). The industry also receives benefits by using frozen silver smelt, due to having the fish available at most times and not depending on delivery from a producer.

In the preliminary testing and production, the mixture of minced fish was filled into Betan casings by a piping bag for simplicity prior to heat treatment. Due to gained knowledge about the benefits and simplicity from using casings for the texture modified pilot production (Chapter 4.2), it was further applied to the minced fish. The Betan casings assures a uniform product, that made analyses easier to conduct. The selected temperature for heat treatment of the minced product was 80^{o}C in core for 30 min. The temperature was extracted from a study regarding minced fish by Greiff et al. (2015) in addition to be recommended in earlier studies at Norconserv (Greiff et al. 2015; Vatland et al. 1991).

4.3.1 Preliminary testing

The main purpose of preliminary testing was to get better knowledge of the process of making minced fish. Four recipes were developed for this purpose. A standard recipe (3A) which was basically a regular minced fish, to use for comparison with FPH enriched recipes. Following recipes enriched with hydrolysates were lower addition (3B), middle addition (3C) and high addition of FHP (3D), in order to analyze how different levels of enrichment influenced the final product (Table 4.5). Other ingredients in 3B-3C remained the same in terms of weight (g) even though percentage of the ingredients varies. The ratio between ingredients in the recipes nevertheless remained the same. Full version of the recipes can be seen in Chapter 3.5.3, Table 3.9. Blending times and speed were optimized as well during preliminary testing. Compared to TMP, the blending times were considerably shorter to keep the temperature in the mince low. Shorter times required maximum speed to blend the mixture properly.

Table 4.5. The calculated content of the following nutrients proteins, fat, and salt in different recipes during preliminary testing of minced fish. The ingredient Hofseth FPH is the only changing variable.

Recipe	**3A**	**3B**	**3C**	**3D**
Hydrolysate Hofseth (%)	-	3.9	7.5	9.3
Total protein (%)	11.9	15.3	18.4	19.9
Total fat (%)	4.6	4.3	4.3	4.1
Total salt (%)	1.1	1.1	1.0	1.0

4.3.1.1 Texture analysis

Prabhu (2018) demonstrated a possible relationship between FPH and texture, showing that increased amounts of FPH would decrease the firmness. Equal relationship could be observed in products 3A-3D in Figure 4.19, which substantiate Prabhu's findings (Prabhu 2018). The textural parameters stiffness, gel strength and hardness all showed the same tendency. Water holding capacity of hydrolysates derived from fish protein makes the mince to retain more water, hence there will be a reduction of the structure (Rasco and Kristinsson 2000). Firmness was applied in Chapter 4.1 and 4.2, due being more suitable to soft TMP. The term hardness was therefore used instead when conducting texture analysis on the minced fish products. All measurements from the analyses are found in Appendix C.

A simplified evaluation of taste and texture was performed by three experienced researchers on the products 3A-3D. Flavor attributes were hard to distinguish due to a prominent taste of white pepper and nutmeg. One of the known sensory characteristics of FPH is occurrence of bitterness and therefore minced fish could show some noticeable bitter taste in product 3D with highest protein enrichment (Rasco and Kristinsson 2000). Though, the bitterness did not occur, most likely masked by the spices, which made a desirable minced product targeted for consumers. However, the spices were excluded in further recipe development, for easier assessment of sensory attributes using FPH as enrichment. Furthermore, spices as flavors can be included in a future processing if desired.

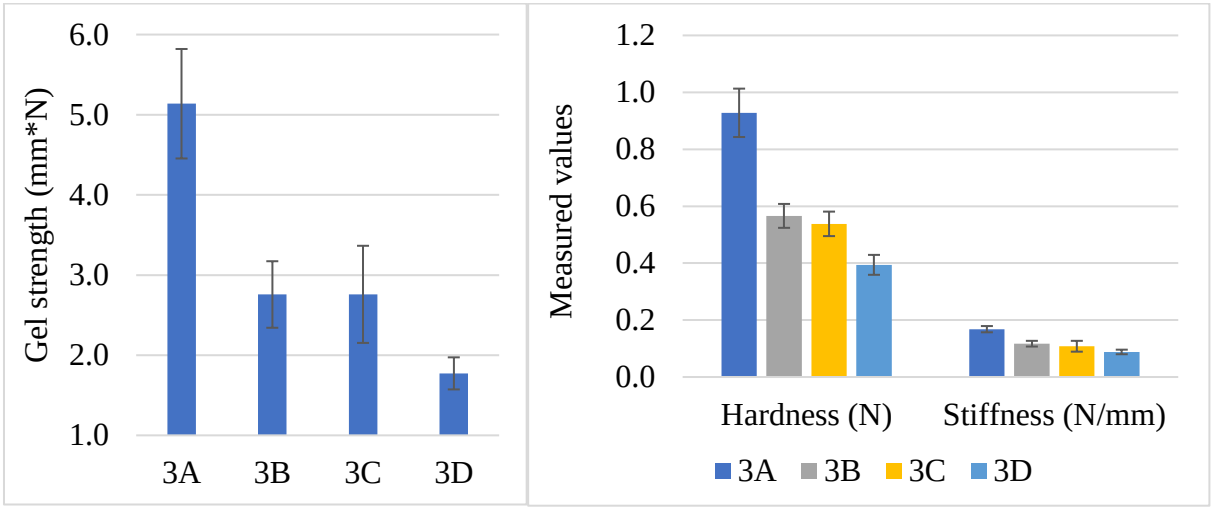


Figure 4.19. Texture analysis of product 3A, 3B, 3C and 3D in preliminary testing of minced fish products. The parameters gel strength (mm*N), hardness (N) and stiffness (N/mm) was analyzed (Appendix J).

4.3.2 Preliminary production

The aim of preliminary production was to investigate which level of adjustments were required in order to produce a minced fish product with total protein like or higher than the natural fish raw materials, which are 16.6% in haddock fillets and 19.6% in silver smelt (Table 4.1). Nutritional content in fish burgers produced by the Norwegian fish food manufacturer Lofotprodukt AS was also used for comparison (Appendix E). Products with variations in milk, oil, potato flour and the protein enrichments WPC, FPH, and caseinate were tested to see in what extent protein enrichment could be done. Furthermore, the spices nutmeg and white pepper was omitted due to overpowering flavor, as explained in Chapter 4.3.1.

Table 4.6. Calculated content of the nutrient's proteins, fat, and salt in different recipes during preliminary production of minced fish. Ingredients with variations between the recipes and nutritional information about the commercial Lofoten fish burgers are listed as well.

Recipe	**4A**	**4B**	**4C**	**4D**	**4E**	**4F**	**Lofoten**
Whole milk (%)	30.5	30.5	24.5	24.5	30.5	24.5	
Oil (%)	4.5	2.5	4.5	2.5	3	3.5	
Potato flour (%)	4	4	4	4	4	3	
Hydrolysate Hofseth (%)	-	0.5	1.5	2	1.5	2	
Sodium caseinate (%)	-	0.5	1.5	2	-	2	
WPC80 (%)	-	1	3	4	-	4	
Total protein (%)	11.9	13.6	16.8	18.5	13.4	18.5	18.2
Total fat (%)	6.0	4.0	5.9	4.0	4.5	5.0	7.5
Total salt (%)	1.1	1.1	1.1	1.1	1.1	1.1	1.1

4.3.2.1 Texture analysis

The standard product produced in preliminary production of minced fish was referred to as 4A. The recipe is similar to standard (3A) from preliminary testing, except for the amount of potato flour which was decreased while oil was increased (Table 4.6). The purpose of decreasing potato flour was to increase added protein enrichment in the remaining recipes 4B-4F. Potato flour starts to gel upon heating at approximately 70^{o}C, which was another reason why to cook the products at 80^{o}C. Additionally, the potato flour have great water holding capacity and thickening abilities (Wüstenberg 2015). A combination of WPC, caseinate and FPH contained the same abilities as well and could therefore be increased whilst potato flour was decreased.

Product 4B contained less oil than the standard but was added protein enrichment instead. The texture analysis showed that the product was marginally harder than standard 4A (Figure 4.20).

The reason for similarity in measurements of texture in 4A and 4B is the small amount of added WPC. The induced gelling by WPC was not high enough to make a noticeable change in hardness, nor the other parameters gel strength or stiffness.

The product 4C was made with less milk instead of less oil when compared to the standard 4A. The amount of proteins was adjusted up further, resulting in a harder product, with higher gel strength and stiffness, as seen in Figure 4.20. The product with both less oil and less milk (4D) obtained the hardest texture due to the increase in added protein enrichment and a decrease in the liquids oil and milk.

4F was like 4D with equal total protein content, except potato flour was decreased and oil was increased. This was done due to enhance flavor and get a softer texture. Furthermore, by elevating the level of oil instead of milk, determination of water holding capacity would not be influenced, in addition to be able to replace some flour with added protein. When 4F was heat treated, cook loss was not observed. The lower percentage of potato flour could therefore be applied later in pilot production.

The product 4E differed from the standard (4A) by replacing a fraction of the oil with FPH instead. The primary purpose of 4E was to deduct the taste through a simplified taste evaluation, as FPH are known for their possible bitterness. The previously discussed products 3B-3D contained spices overwhelming any other flavors. Therefore, product 4E was produced where the spices were omitted. The tasting of 4E did not give any resembles to bitterness. Due to excluding WPC, the texture was more similar to 4B with less oil than the standard and the standard itself.

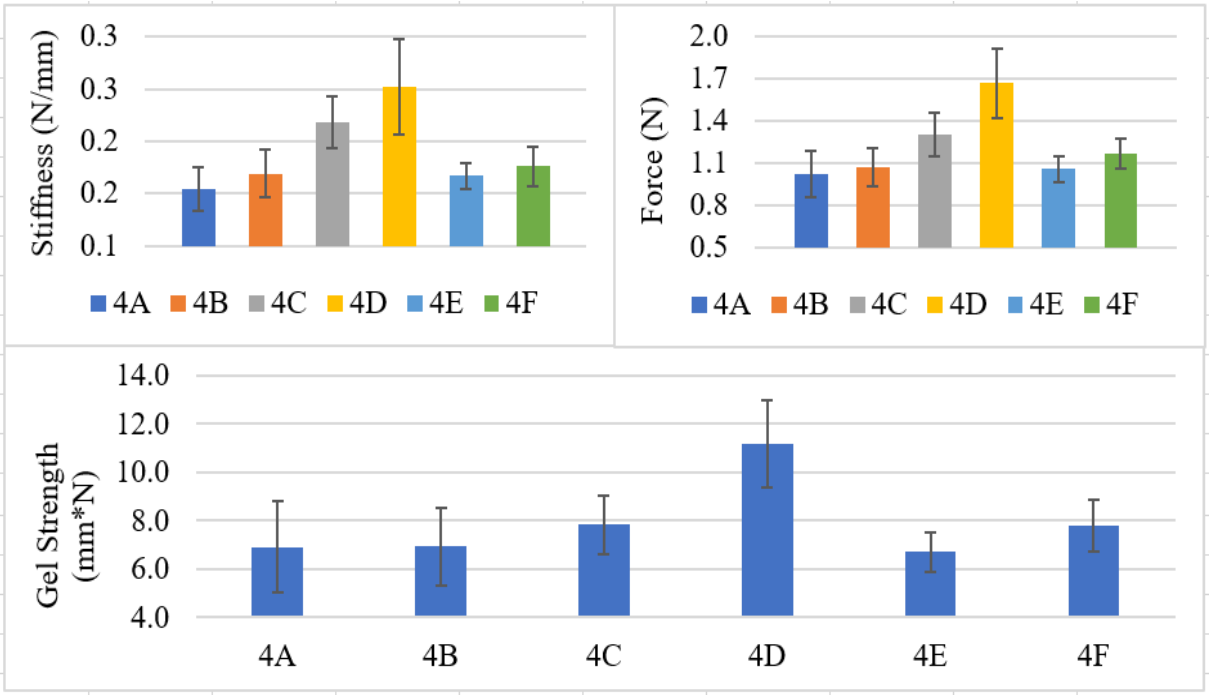


Figure 4.20. Geltest was conducted of the products 4A-4F in preliminary production of minced fish. The measured textural parameters: force needed for penetration (Hardness), gel strength and stiffness (Appendix J).

4.3.2.2 Color analysis

A color analysis was conducted on the minced fish products 4A-4F. 4A, 4B and 4E lacked standard deviations. This was discovered too late, but the measurements did nevertheless provide great information about the colors. All products obtained negative values just below zero of the color coordinate a* (Figure 4.21). A negative value of a* corresponds to green color, however the small value measured in the products would be perceived as a neutral grey color by the human eye. The values of b* on the other hand, revealed positive measurements, meaning that the values of b* corresponds to yellow color. Standard 4A was the least yellow product, followed by 4E and 4B respectively. The FPH seemed to be responsible for the yellow color tone in 4E, whilst the color in 4B could be caused by both the dairy proteins WPC and caseinate, and FPH. Both products 4D and 4F showed more saturated yellow color, due to their amount of added protein.

The color coordinate C* followed the same tendency as b*, with the least saturation in 4A, 4B and 4E and most saturation in 4C, 4D and 4F. The remining color coordinate h measured values too similar for comparison. The last color coordinate L* had the lowest values obtained from the products 4B, 4D and 4E whilst the products 4A, 4C and 4F were the brightest. Since not all

measurements contained standard deviations, there could be errors. This could explain why e.g. 4F with highest total protein content seemed brighter than 4E which only contained FPH and no other added protein enrichment. Air bubbles appearing as dark spots on the surface could be one possible error causing a lower measured value of L*.

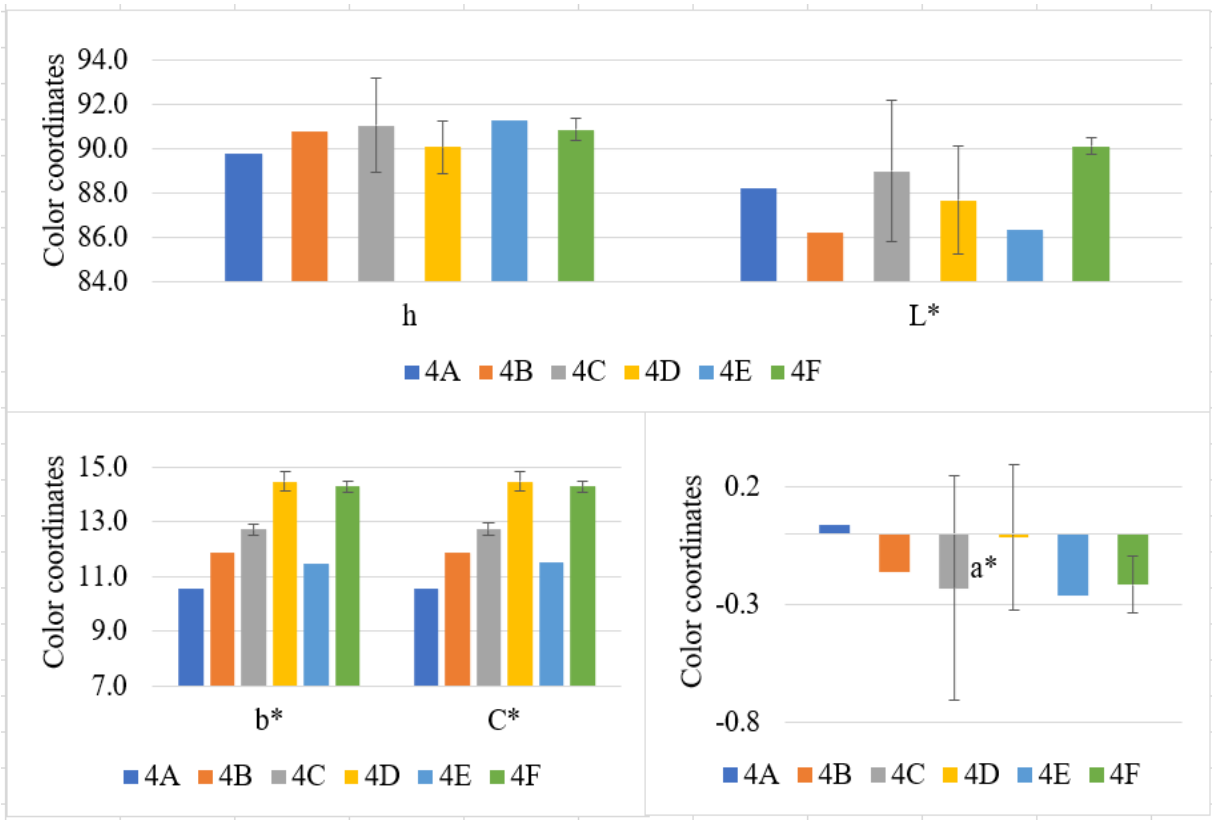


Figure 4.21. The color coordinates L*, a*, b*, C* and h of the products 4A-4F in preliminary production of minced fish. Only product 4C, 4D and 4F had standard deviations (Appendix J).

4.3.2.3 Water holding capacity

The water holding capacity was determined by centrifugation and filter paper, same method as applied on TMP. The selected products were the standard (4A) without any added protein and the highest protein enriched product 4D with FPH, caseinate and WPC. The products were selected to deduce the method and its suitability for the pilot production. As expected, water holding capacity of product 4D was noticeably higher compared to 4A (Figure 4.22). The difference was primarily generated by caseinate and FPH due to their exceptional water holding capacity (Rasco and Kristinsson 2000; Zayas 1997). There was not observed any mass loss, this parameter was therefore excluded from Figure 4.22.

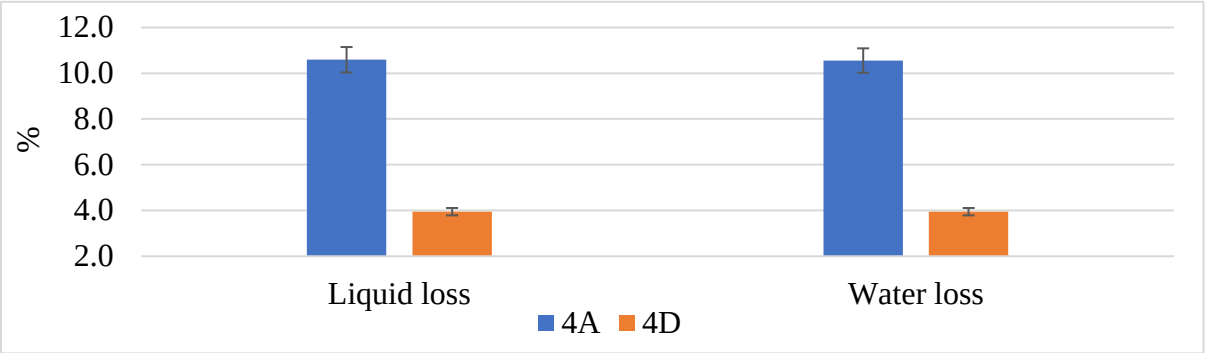


Figure 4.22. The water holding capacity (%) determined by water loss and liquid loss of product 4A and 4D was measured in preliminary production of minced fish products (Appendix J).

4.4 Pilot production of minced fish

The primary aim with pilot production was to produce minced fish products with high total protein. Different amounts of added Fish protein hydrolysate (FPH) were investigated in addition to differences between the two FPH produced by Nofima and Hofseth BioCare. The proteins whey protein concentrate (WPC) and caseinate were also adjusted.

A few practically changes were applied in pilot production due to preliminary experiments. As the finished mixtures from preliminary production were filled into Betan casings by a piping bag, some possible errors could arise. Inhomogeneous filling could cause air bubbles in the mince which in turn could give errors in texture analysis. An electronic sausage stuffer was filling the casings throughout the pilot production instead, with no variation in pressure. Additionally, the robot coupe was replaced by an industrial cutter instead. Bigger batches during the production could be made at once.

4.4.1 Recipe development

Recipes from the preliminary production were used as a base when five new recipes for pilot production were developed. The standard recipe was standard mince (SM), in which FPH was omitted, and had a calculated total protein of approximately 14.1%. The following recipe low Hofseth mince (LHM) had 15.8% total protein. The third recipe was referred to as high Hofseth mince (HHM) and had 18.8% total protein. The first recipe enriched by hydrolysates produced by Nofima was low Nofima mince (LNM) and had 15.8% total protein and finally high Nofima mince (HNM) with 18.8% total protein (Table 4.7).

The percent of potato flour was set at 3%, which was tested in preliminary production of minced fish (Chapter 4.3.2). The percent of milk was set to 25%, as several recipes contained 24.5% with good results. The variables were the protein enrichment; WPC, caseinate and FPH, and sunflower oil. The selected liquid was oil as it would not influence the yield from an analysis of water holding capacity. All recipes could be compared to the standard and from low to high enrichment, as well as LHM against LNM and HHM against HNM.

Table 4.7. Calculated total content of protein, salt, and fat in different recipes during pilot production of minced fish, varied ingredients, and the yield of crude protein from Kjeldahl method at the bottom are listed.

Recipe	**SM**	**LHM**	**HHM**	**LNM**	**HNM**
Sunflower oil (%)	8.0	6.2	2.6	6.1	2.5
Hydrolysate Hofseth (%)	-	1.2	2.1	-	-
Hydrolysate Nofima (%)	-	-	-	1.23	2.13
Sodium caseinate (%)	1.0	1.2	2.1	1.23	2.13
WPC80 (%)	2.0	2.4	4.2	2.46	4.26
Total protein (%)	14.1	15.8	18.8	15.8	18.8
Total fat (%)	9.4	7.6	4.1	7.5	4.0
Total salt (%)	1.1	1.1	1.1	1.1	1.1
Total protein (%)	14.3	16.2	18.6	16.0	19.0

4.4.2 Kjeldahl method

The Kjeldahl method was conducted on the products ST, LHM, HHM, LNM and HNM from pilot production to determine the protein nitrogen content. The yield obtained from Kjeldahl was compared to calculated numbers of total protein (Table 4.7). The analyzed total protein did not deviate from the calculations, as they were approximately equal.

4.4.3 Texture profile analysis

Texture profile analysis (TPA) was conducted on the minced fish products from pilot production. TPA replaced the method gel-test which was applied in preliminary production, due to great diversity of textural parameters from TPA. TPA is an imitation of the way humans eat food. Furthermore, the method was conducted by double repeating motions using compression, simulating the work a jaw performs during consumption and could therefore be suitable to apply the minced fish products. Additionally, the parameters correlate appropriately with sensory analysis (Bourne 2002). There are many contradictory suggestions about the percent of compression. Some studies have applied compression of total height to 33.3%-50% strain (Romero de Ávila et al. 2014; Sharma et al. 2017), whilst other have used from 60% and

up to 90% (Wu, Sun, and He 2014; Rios-Mera et al. 2020; Bourne 2002; Aguirre et al. 2018). After deduction of the referenced studies, 60% compression was selected. The study using 60% compression researched the texture profile of salmon (Wu, Sun, and He 2014). Another study which influenced the determination of parameters, was a research on chicken breasts (Aguirre et al. 2018).

Product HNM was significantly harder than LNM and SM, whilst SM on the other hand were significantly softer than all except LNM (Figure 4.23) ($p<0.001$). Similar same trends could be observed in force. SM was significantly lower in force than LHM and HNM ($p=0.001$). HNM was significantly higher in force than LNM and SM. Minced fish products were produced by utilizing the binding and gelling abilities of hydrocolloids, added proteins, and fish raw materials. However, regarding the pilot production, the only variably factor which contained very good gelling abilities was WPC. The differences in hardness and force were therefore caused by a combination of increased addition of protein enrichment, primarily WPC, and decreased volumes of oil.

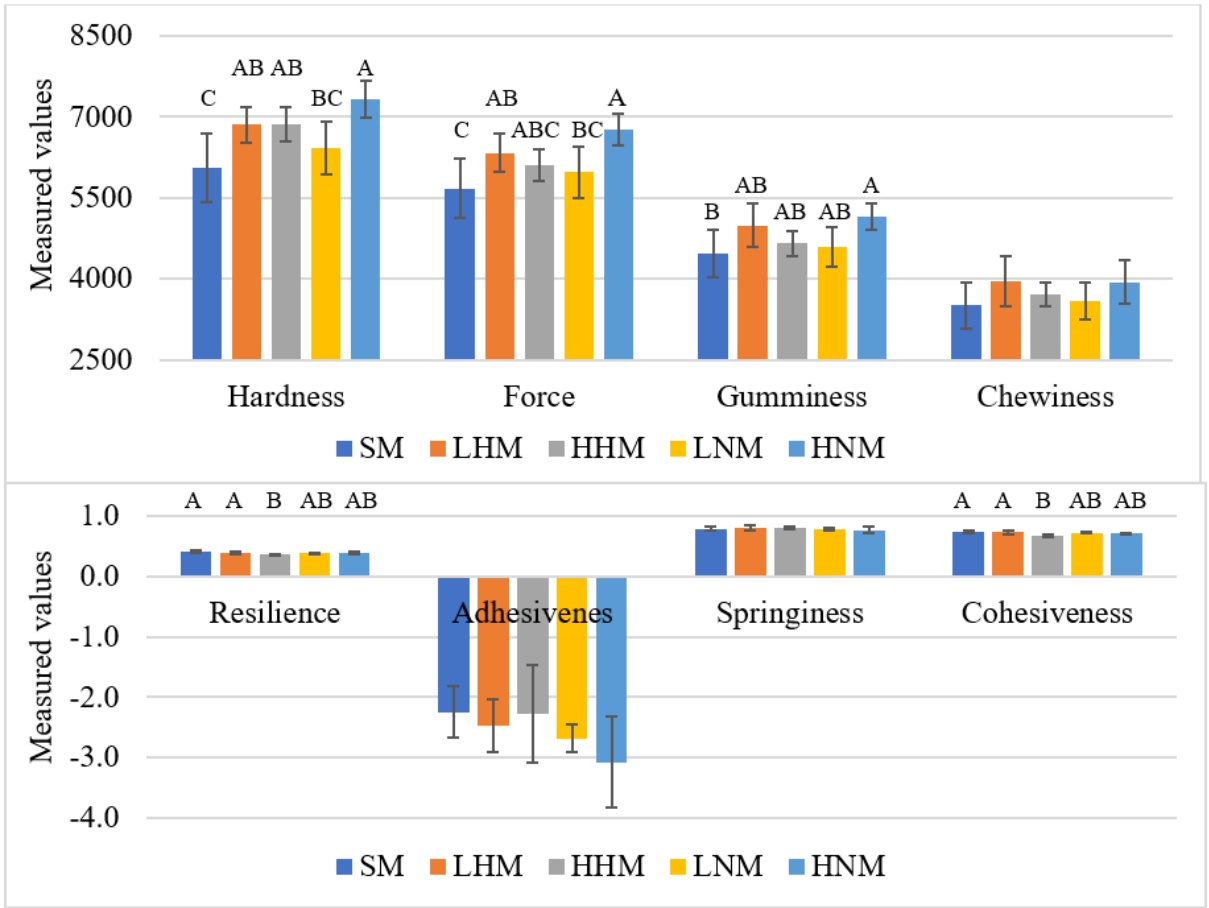


Figure 4.23. Texture profile analysis of minced fish products SM, LHM, HHM, LNM and

HNM. The textural parameters hardness, force, gumminess, resilence, and cohesiveness were revealed to be statistically significant (Appendix K).

Gumminess and chewiness could arise some issues when discussed on the grounds of them being mutually exclusive. The parameters are exclusive due to the calculations they are derived from (Bourne 2002). Gumminess was the exclusively significant parameter of the two ($p=0.012$). Product SM obtained significantly less gumminess than HNM, which was most likely caused by the gelling abilities of WPC (Rimac et al. 2009).

Cohesiveness is defined as the ratio of the area below the two peaks. The products SM and LHM were significantly more cohesive than HHM ($p=0.002$). The remaining textural parameter with significantly differences was resilience ($p<0.001$). Resilience is a measurement of how much the product resist deformation (Singh et al. 2015). The trend observed in the measurements of cohesiveness was repeated by resilience. Because of the equal trend, there was likely a correlation between cohesiveness and resilience.

4.4.4 Color analysis

Color analysis was performed on the minced fish products from pilot production (Figure 4.24 and 4.25). Mostly negative values were derived from the color coordinate a*, except the value obtained from HNM. The negative values of HHM, LNM, SM and LHM differed significantly from the positive value of HNM ($p<0.001$). Further, all the values were just below or above zero and would nevertheless be perceived as grey color.

The color coordinate b* yielded significant difference between each product ($p<0.001$). Most yellow colored product to the least were HNM, HHM, LHM, LNM and SM, respectively. FPH produced by Nofima contained a more prominent yellow color compared to FPH from Hofseth, which most likely was responsible for the significantly stronger yellow color in HNM. Other ingredients responsible for the yellow color were sunflower oil, WPC and caseinate. In summary, the values of b* correlated well with the protein enrichment.

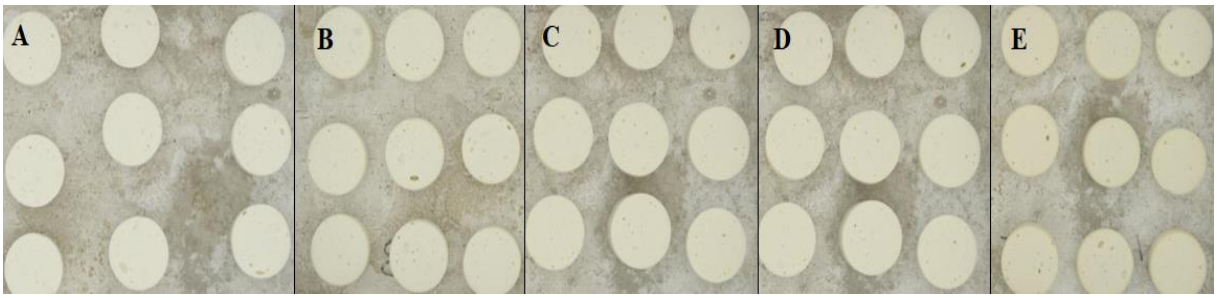


Figure 4.24. Photographs that were used to analyze the colors. A: SM, B: LHM, C: HHM, D: LNM, E: HNM.

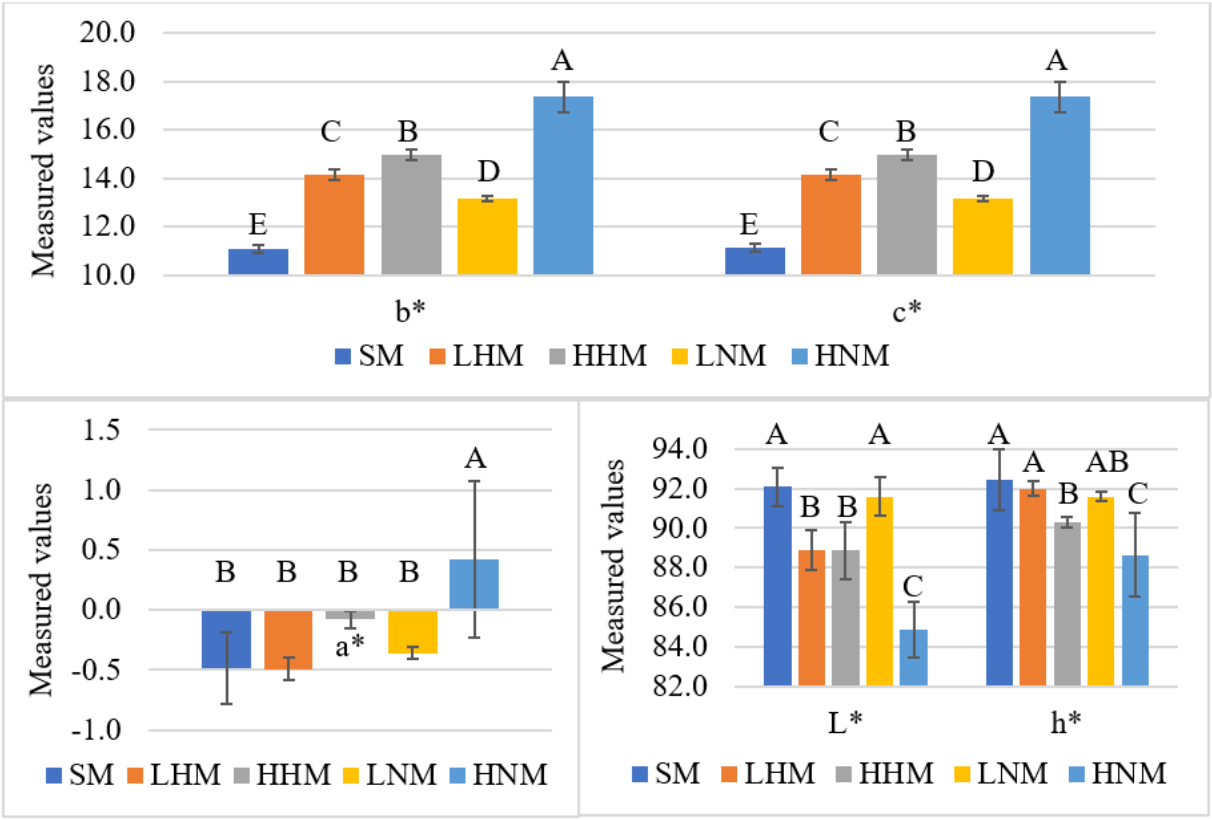


Figure 4.25. The color coordinates L*, a*, b*, C* and h of the products SM, LHM, HHM, LNM and HNM were measured by DigiEye system (Appendix K).

Values derived from the color coordinate L* on the other hand, were more peculiar ($p<0.001$). The products SM and LNM were significantly brighter than LHN, HHM, and HNM. HNM was additionally significantly darker than all other products. There should be a higher difference in brightness between LHM and HHM due higher added protein enrichment in HHM. The minced fish products contained small holes filled with air which appeared as dark spots and could potentially have influenced the results. All products would nevertheless be perceived as bright, almost white colored with varying yellow color tones.

The value of color coordinate h was significantly lower in HNM, compared to the remaining products ($p<0.001$). HHM were significantly lower than SM and LHM. The coordinate C* followed the same trend as b*, as all products were significantly different from each other. HNM contained the highest saturation, followed by HHM, LHM, LNM and SM, respectively.

4.4.5 Water holding capacity

Water holding capacity was determined in pilot production by using sample cups and centrifugation, in addition to measuring dried mass from sample ($p<0.001$). The least water holding capacity was observed in SM and LNM, containing significantly lowest capacity of all (Figure 4.26). LHM had significantly better water holding capacity than SM, LNH, and HNM. The increase in water holding capacity from SM and LNM to HNM, LHM and HHM harmonized with the increase in added protein enrichment. A contradictory result occurred in LHM as water holding capacity decreased when the content of caseinate and FPH increased (Zayas 1997; Rasco and Kristinsson 2000). All products were nonetheless within a desirable range of water holding capacity, although it is challenging to perform comparison with different food products. Water holding capacity can be highly variable, depending on the applied method. Simply put, a small change in centrifugation speed and time would then derive another yield.

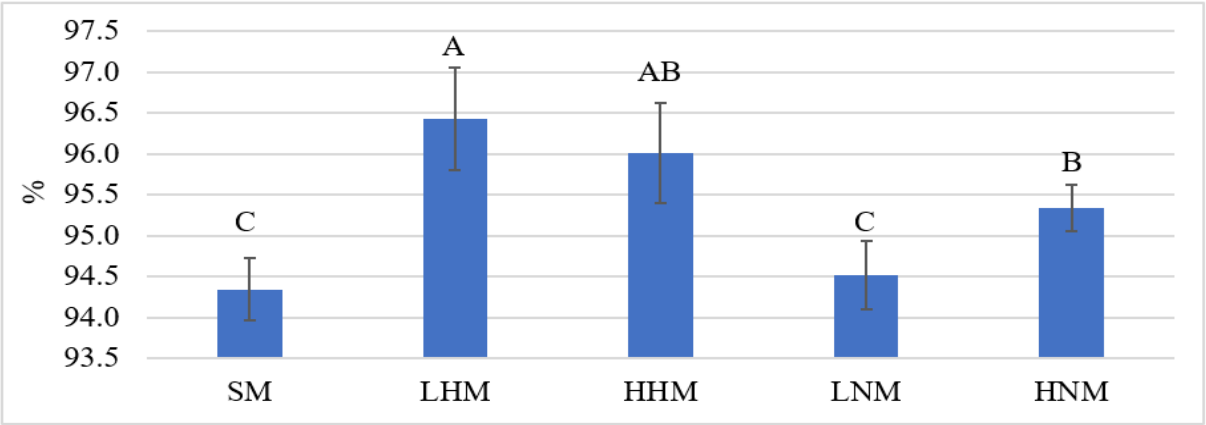


Figure 4.26. The water holding capacity of SM, LHM, HHM, LNM and HNM derived by centrifugation of sample cups and determination of dry matter (Appendix K).

4.4.6 Image analysis

Photographs of minced fish products from pilot production were analyzed to determine the area limited to threshold (%) (Figure 4.27). The gelation of the minced fish was most likely the observable structure in Figure 4.28A and B. Compared to Figure 4.14B from Chapter 4.2.6, the structure was a little airier than the texture from texture modified products (TMP). The overall values of threshold derived from TMP were also slightly higher than that of minced fish, due

to the three-dimensional matrix in the gelled products (Saha and Bhattacharya 2010). The percentage of area limited to threshold of product HHM was significantly higher than SM, LHM, and LNM. HNM was significantly higher than LNM and SM. HHM and HNM was significantly higher than SM and LNM $p<0.001$.

A possible reason for the increased mass was increased addition of protein, especially WPC, which produced a denser gel (Zayas 1997). More solid mass and less liquid could additionally influence the threshold. The differing density of the gelled fish mince were observed in TPA as well, whereas lower protein content yielded a softer texture.

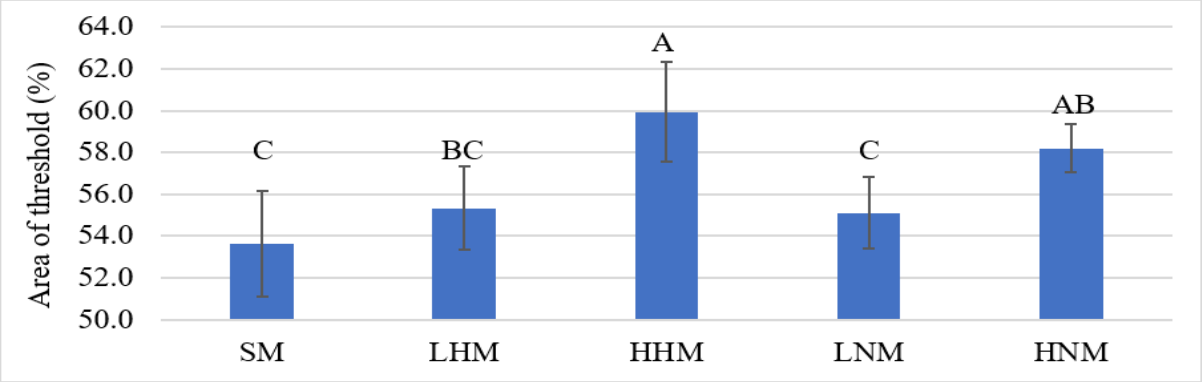


Figure 4.27. Area of threshold (%) in the minced fish products SM, LHM, HHM, LNM and HNM from pilot production (Appendix K).

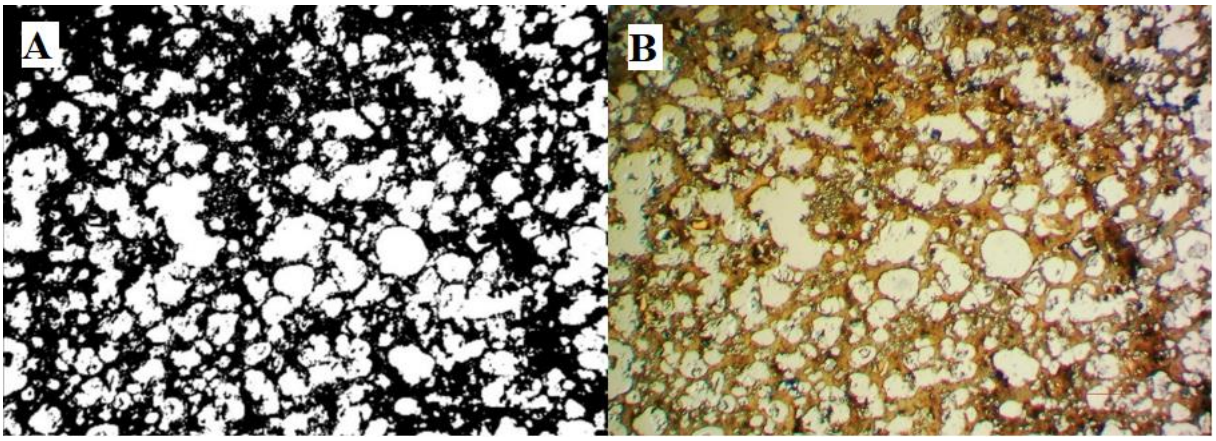


Figure 4.28. A: The appearance of the final image of SM, after threshold are rendered in Fiji, B: image of SM before analysis, immediately after the photographs were shot through the stereoscope.

4.4.7 Sensory analysis

A Sensory analysis was performed using QDA on the minced fish products SM, LHM, HHM, LNM and HNM from pilot production. The attributes listed in Figure 4.29 show significantly differences between two or more, except bitter taste and fish odor.

All minced fish products contained a relatively high intensity of aftertaste, when compared to the intensity of other flavors and tastes. A Fisher pairwise comparison test had to be done to observe differing grouping between the products. HHM contained a significantly stronger aftertaste than LHM, LNM, and SM (*p=0.019*). HNM contained a significantly stronger aftertaste compared to SM and LHM. Several judges from the sensory evaluating panel reported that the aftertaste reminisced of blood (Appendix J). Further explanation by the judges revealed that the taste of blood in fact was a distinguishable metallic flavor and not blood itself (Appendix J). The intensity of metallic flavor was not remarkably higher than any other attributes, plausible due to it being stronger a certain time after consumption and not during. Hence, the metallic taste was characterized as an intense aftertaste instead. The source of the taste was uncertain, as no metallic flavor was present during preliminary production. The products have most likely been influenced by a metal container during storage or production. If the metallic flavor was caused by FPH or other added protein, the taste should be experienced in TMP as well. There was nevertheless a correlation between higher total protein and aftertaste.

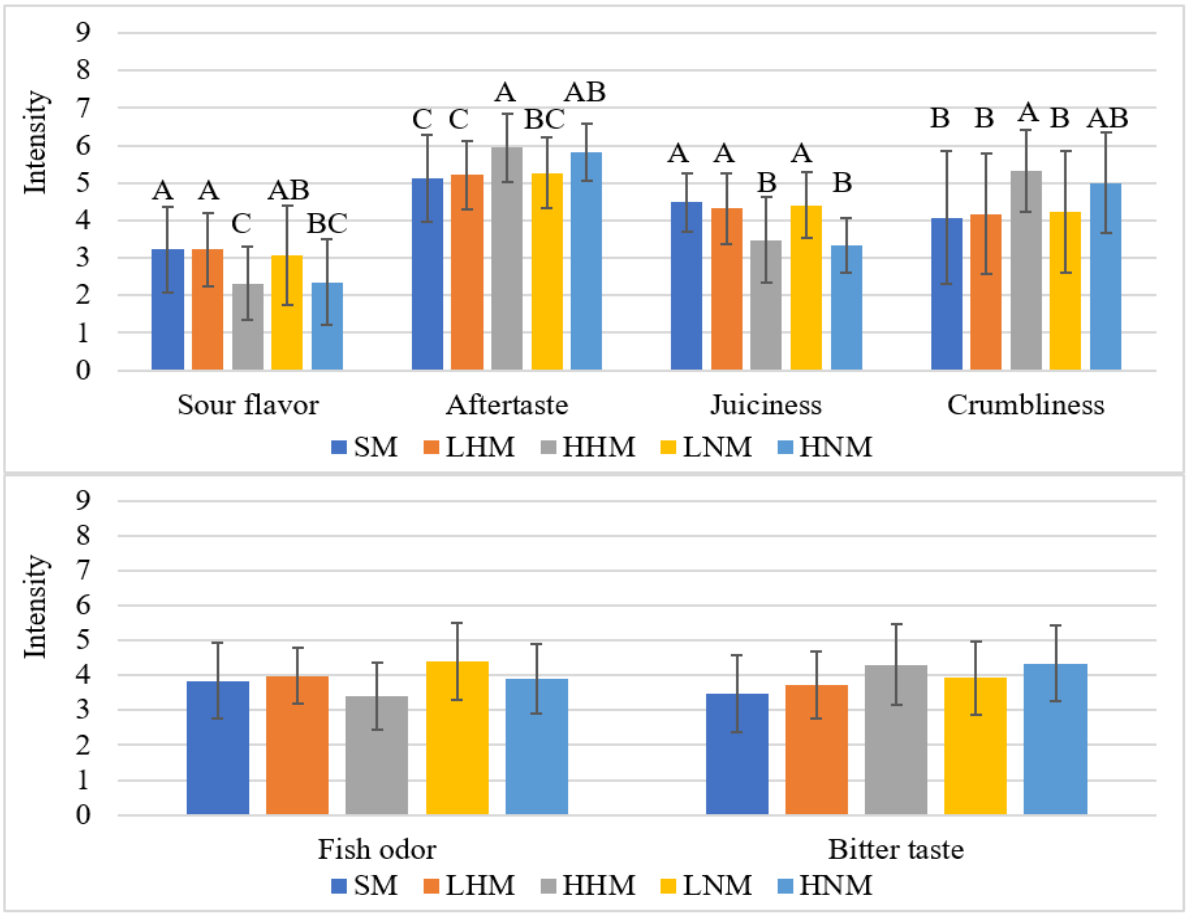


Figure 4.29. Sensory analysis of the attributes in terms of odor (fish), flavor (sour, bitter, aftertaste), and texture (juiciness, crumbliness). The selected attributes except Bitter taste and fish odor have *p<0.05*, the remaining attributes can be found in Appendix K.

The products SM, LHM and LNM were revealed to be significantly juicier compared the more protein enriched HHM and HNM (*p<0.001*). An explanation for the difference in juiciness could be due to increasing level of dry protein enrichments and decrease of oil in HHM and HNM. The product could therefore be experienced as drier and less juicy. The water holding capacity associated with caseinate and FPH would naturally hold a great ratio of water within the product, thus making it juicier than if only WPC was the single added protein. However, with less liquid and addition of enough powders of any kind, the product would eventually be perceived as dry.

A Fisher pairwise comparison was conducted on the attribute crumbliness because a Tukey test would only derive one grouping of all minced fish products, despite obtaining *p=0.031*. The intensity of crumbliness was perceived as relatively high, depending on the amount of added proteins. The texture in HHM was significantly crumblier than that of SM, LNM and LHM. The crumbly texture was indubitably a result of higher percentage of protein enrichment. The results fitted well with the tendency observed of juiciness. Increased addition of added protein made a less juicy but crumblier product.

Although the sour flavor was statistically significant *p=0.013*, a Tukey pairwise comparison test did not yield any other grouping than A. Instead, a Fisher pairwise comparison test was applied on the attribute. The products SM and LHM were significantly sourer than HHM and HNM. HHM contained significantly less intensity in sour flavor compared to SM, LHM, and LNM. Less added protein enrichment in the product was possibly experienced as a sourer taste. The bitter taste on the other hand, indicated the opposite, as the products containing most FPH were seemingly perceived as more bitter, although not statistically significant (*p=0.060*). If the intensity of bitterness is less prominent, there is a possibility that the product would taste sourer instead. Either way, the bitterness is most likely caused by the increase of FPH, due to the bitter taste they often contains (Aspevik et al. 2017)

The p value of fish odor was *p=0.051* and not significant, although contained a Tukey pairwise Comparison test with differing grouping of the products and was therefore mentioned. The product HHM contained the lowest intensity of fish odor, whilst LNM contained the strongest. All the products were produced with the same percentage of fish raw material, the only remaining ingredient possible of increasing fish odor was FPH.

5. Conclusion

Two protein enriched fish products with haddock and silver smelt were developed. The first product was a texture modified product (TMP) for elderly and people with dysphagia. The second product was a minced fish product for the average consumer. The products were enriched with whey protein concentrate (WPC), caseinate and Fish protein hydrolysate (FPH) from either Nofima or Hofseth. The variables in the recipes were added protein enrichments and liquid (sunflower oil).

Texture analysis of TMP showed that a higher total protein content, with emphasis on more whey, was significantly firmer ($p<0.001$) than products with less protein enrichment. Sensory analysis also showed that higher level of protein enrichment obtained significantly firmer texture than less enriched products ($p<0.001$). The products standard (ST) and high Hofseth texture (HHT) had significantly less intensity in sour odor than low Hofseth texture (LHT) ($p=0.007$) while ST and HHT were significantly stronger in cloying odor than LHT ($p=0.005$). There is clearly a correlation between the attributes cloying odor and sour odor. Remaining attributes showed that products with higher total protein gives a significantly coarser (p=0.008) and significantly more cohesive ($P=0.011$) texture.

Color analysis showed that TMP with higher level of protein enrichment were significantly darker ($p<0.001$) and had more yellow color ($p=0.013$) than products with less proteins. High Nofima texture (HNT) with FPH from Nofima had the significantly strongest yellow color ($p<0.001$). Water holding capacity of TMP were measured, which showed that higher total protein content, particularly caseinate and FPH, had significantly better water holding capacity ($p=0.022$). Threshold obtained by image analysis showed that less total protein content and no FPH yielded less mass ($p<0.001$).

Texture profile analysis of minced fish revealed that hardness ($p<0.001$), force ($p<0.001$), and gumminess ($p=0.012$) all increased significantly with higher level of total protein. On the contrary, cohesiveness ($p=0.002$) and resilience ($p<0.001$) were reduced significantly with more protein enrichment by FPH from Hofseth. Sensory analysis showed that minced fish with higher protein enrichment was significantly less intense in sour flavor ($P=0.013$), while more proteins resulted in a significantly stronger aftertaste ($P=0.019$). Lower level of protein enrichment significantly increased juiciness ($p<0.001$) and significantly decreased crumbliness ($p<0.031$).

Color analysis of minced fish revealed that higher level of protein enrichment yielded a significantly more yellow color ($p<0.001$) and significantly darker ($p<0.001$). High Nofima mince (HNM) enriched by FPH from Nofima was measured to obtain the significantly strongest yellow color ($p<0.001$). Threshold obtained by image analysis showed that less total protein content and no FPH yielded less mass ($p<0.001$). Water holding capacity in minced fish products was significantly better in three higher protein enriched products, compared to the standard SM ($p<0.001$) due to FPH and caseinate.

The focus on personalized nutrition for groups in the society with special needs is increasing and still few protein enriched products is available for chilled retail sale. The pilot products developed in this work show that it is possible, both to use protein hydrolysates from fish by-products and to enrich the products to a level of 19-21.5% proteins. Description of how proteins affect physical, chemical, and sensory characteristics in products after several processing steps is important for further development of protein enriched products to specific user groups.

6. Future work

The texture of texture modified products were modified by hydrocolloids and the protein powders whey protein concentrate, sodium caseinate and fish protein hydrolysates to become soft but still cohesive. Future development should focus on taste, appearance, and shape to make palatable and appetizing products, with the soft consistency intact.

IDDSI (International Dysphagia Diet Standardization Initiative) has developed a framework containing standardized testing methods e.g. spoon tilt and fork pressure test. The methods use human power to characterize texture and thickness, and then divides food and liquids into levels from 0 to 7. Instrumental methods with regards of standardized settings should be developed to categorize food or liquids according to a framework such as the one containing testing methods. Instrumental methods are easier to perform without interference from possible human errors and are more reproducible and comparable for the food industry.

Methods of analyzing water holding capacity of foods containing pâté-like texture are needed, as most methods are associated with firmer products such as muscle meat.

A basic recipe for minced fish was developed without any spices. Future processing can include different flavor profiles e.g. nutmeg or mace and white pepper, for consumers who desire products with flavor of fish. More exotic flavors as chili, bacon, or cheese could additionally be investigated to meet consumers needing to increase intake of fish but are not particularly fond of the pure fish flavor.

Minced fish was processed by filling Betan casing with the blended mixture and heat treated into uniform “fish pudding” to make analysis easier. Other shapes should be tested such as fish patties or Norwegian fish cakes and burgers..

Image analysis was used to see the inner structure of the products. The method should be further developed to get an even bigger understanding of the microstructure. Different ingredients should be examined individually to derive their appearance and structure. The gained knowledge could then be related to rheological analysis of the internal structure.

The products from pilot productions are still at a stage for experimental analysis. A next step should be to link the product development to consumer insight in order to meet the attractiveness expected by the user groups and to successfully enter retail chains.

References

Aakre, I., A.R. Alvheim, L. Dahl, J.Ø. Dalane, E. Kielland, M. Kjellevold, M.W. Markhus, and S. Nøss. 2019. "New data on nutrient composition in large selection of commercially available seafood products and its impact on micronutrient intake." In *Food Nutr Res*.

Affairs, Economic and Financial. 2018. "2018 Ageing Report: Policy challenges for ageing societies." In *Economy, finance and the euro*. European Commission.

Agriculture, United States Department of. 2010. 'The Big Thaw- Safe Defrosting methods', Accessed 24 September 2019. https://www.fsis.usda.gov/shared/PDF/Big_Thaw.pdf.

Aguirre, M.E., C.M. Owens, R.K. Miller, and C.Z. Alvarado. 2018. 'Descriptive sensory and instrumental texture profile analysis of woody breast in marinated chicken', *Poultry Science*, 97: 1456-61.

Akhtar, Jawad. 2012. 'How a Co-Product Differs from a By-Product', Accessed 29 August 2019. https://sapexperts.wispubs.com/scm/articles/how-a-co-product-differs-from-a-by-product?id=7a92e6eddf1d4ee0896052e6631c7c18#.XWfFvOgzY2w.

Amundsen, B. 2017. 'Denne fisken spiser du mye av uten å vite det', Forskning.no, Accessed 5 June 2020. https://forskning.no/oppdrett-fisk-sprak/denne-fisken-spiser-du-mye-av-uten-a-vite-det/373771.

Andreetta-Gorelkina, I.V., K. Greiff, T. Rustad, and I.G. Aursand. 2016. 'Reduction of Salt in Haddock Mince: Effect of Different Salts on the Solubility of Proteins', *Journal of Aquatic Food Product Technology*, 25: 2-5.

Anton Paar. 2020. 'Amplitude sweeps', Anton Paar, Accessed 13 June 2020. https://wiki.anton-paar.com/en/amplitude-sweeps/.

Arason, S., M. Karlsdottir, T. valsdottir, R. Slizyte, T. Rustad, E. Falch, J. Eysturskard, and G. Jakobsen. 2009. "Maximum resource utilisation -Value added fish by-products." In *Nordic innovation centre*, 1-20. SINTEF.

Aspevik, T., Å. Oterhals, S.B. Rønning, T. Altintzoglou, S.G. Wubshet, A. Gildberg, N.K. Afseth, T.D. Whitaker, and D. Lindberg. 2017. "Valorization of Proteins from Co- and By-products from the Fish and Meat Industry." In *Topics in Current Chemistry*, 1. Springer.

Aursand, I.G., K. Greiff, U. Erikson, L. Gallart-Jornet, N. Perisic, A. Kohler, N.K. Afseth, R. Ofstad, I. Storrø, and K. Josefsen. 2007. "Low salt products." In, 4. The Norwegian Research Counsil.

Batista, I. 2006. 'By-catch, underutilized species and underutilized fish parts as food ingredients.' in F. Shahidi (ed.), *Maximising the Value of Marine By-Products* (Woodhead Publishing).

Berg, J.M., J.L. Tymoczko, G.J. Gatto Jr, and L. Stryer. 2015. 'Protein Composition and Structure.' in, *Biochemistry* (W.H.Freeman & Co Ltd,).

Bertak, J.A., and C. Karahdian. 1995. "Surimi-based imitation crab characteristics affected by heating method and end point temperature." In *Journal of Food science*, 292.

Bintsis, Thomas. 2017. "Foodborne pathogens." In *AIMS Microbiology*, 530. AIMS Microbiology.

Blanco, M., J.A. Vázquez, R.I. Pérez-Martín, and C.G. Sotelo. 2017. "Hydrolysates of Fish Skin Collagen: An Oppertunity for Valorizing Fish Industry Byproducts." In *Marine Drugs*. MDPI.

Blomstrand, E., J. Eliasson, H.K. Karlsson, and R. Köhnke. 2006. 'Branched-chain amino acids activate key enzymes in protein synthesis after physical exercise', *J Nutr*, 136: 269s-73s.

Boirie, Y. 2019. 'Chapter 16 - Whey Protein and Muscle Protection.' in Stéphane Walrand (ed.), *Nutrition and Skeletal Muscle* (Academic Press).
Bopp, M.J., D.K. Houston, L. Lenchik, L. Easter, S.B. Kritchevsky, and B.J. Nicklas. 2008. 'Lean mass loss is associated with low protein intake during dietary-induced weight loss in postmenopausal women', *Journal of the American Dietetic Association*, 108: 1216-20.
Bourne, M.C. 2002. *Food Texture and Viscoxity* (Elsevier Science & Technology Books).
Britannica. 2008. "Haddock." In *Britannica*, edited by The Editors of Encyclopaedia Britannica.
Buggy, Aoife K., Jennifer J. McManus, André Brodkorb, Sean A. Hogan, and Mark A. Fenelon. 2018. 'Pilot-scale formation of whey protein aggregates determine the stability of heat-treated whey protein solutions—Effect of pH and protein concentration', *Journal of Dairy Science*, 101: 10819-30.
Campbell, W.W., M.C. Crim, V.R. Young, L.J. Joseph, and W.J. Evans. 1995. 'Effects of resistance training and dietary protein intake on protein metabolism in older adults', *Am J Physiol*, 268: 1143-53.
Careche, M., A.J. Borderias, I. Sánchez-Alonso, and E.K. Lund. 2011. 'Functional foods: concept to product.' in M. Saarela (ed.), *Functional seafood products* (Woodhead Publishing Ltd).
Cattaneo, C., V. Lavelli, C. Proserpio, M. Laureati, and E. Pagliarini. 2019. 'Consumers' attitude towards food by-products: the influence of food technology neophobia, education and information', *International Journal of Food Science & Technology*, 54: 679-87.
Cavanah, C., and B. McGroarty. 2019. "Nutrition Gets Very Personalized." In *2019 Global wellness Trends*. Global Wellness Summit.
Chilled Food Association Ltd. 2018. "Guidelines for setting shelf life of chilled foods in relation to non-proteolytic *Clostridium botulinum*." In. UK: Chilledgood.
Churchward-Venne, T.A., N.A. Burd, and S.M. Phillips. 2012. 'Nutritional regulation of muscle protein synthesis with resistance exercise: strategies to enhance anabolism', *Nutrition & Metabolism*, 9: 40.
Dangin, M., Y. Boirie, C. Guillet, and B. Beaufrère. 2002. 'Influence of the Protein Digestion Rate on Protein Turnover in Young and Elderly Subjects', *The Journal of Nutrition*, 132: 3228S-33S.
Delahaye, F. 2013. "Should we eat less salt?" In *Archives of cardiovascular diseases*, 324-26. Elsevier.
Devine, C., M. Dikeman, and W. Jensen. 2004. *Encyclopedia of Meat Sciences* (Academic Press).
Durrer S.D., L. Busetto, D. Dicker, N. Farpour-Lambert, R. Pryke, H. Toplak, D. Widmer, V. Yumuk, and Y. Schutz. 2019. 'European Practical and Patient-Centred Guidelines for Adult Obesity Management in Primary Care', *Obesity Facts*, 12: 40-66.
Engdahl, B.L., C.S. Nielsen, V. Hjellvik, A. Reneflot, R.B. Nes, C.L. Berg, F. Gjertsen, B. Grinde, A.H. Ranhoff, K.J. Husabø, O.J. Steingrimsdottir, H.S. Blix, M.E.M.T. Vollrath, and B.H. Strand. 2016. "Health among the elderly in Norway." In *Public Health Report Editorial Group,*. Norwegian Institute of Public Health (NIPH).
FAO. 2018. 'The State of World Fisheries and Aquaculture ', FAO- Food and Agriculture Organization of the United Nations, Accessed 2 September 2019. http://www.fao.org/3/i9540en/i9540en.pdf.
Ferrier, D.R. 2013. *Biochemistry* (Wolters Kluwer Health).
Food4Me project. 2016. 'EU-funded Food4Me project paves way for personalised nutrition to better public health', Accessed 8 June 2020.

https://www.eufic.org/en/collaboration/article/eu-funded-food4me-project-paves-way-for-personalised-nutrition-to-better-pu.
Forbrukerrådet. 2016. "Andel fisk i fiskeprodukter." In.
Fujiso, Y., N. Perrin, J. Giessen, N.E. Vrana, F. Neveu, and V. Woisard. 2018. 'Swall-E: A robotic in-vitro simulation of human swallowing', *PLoS ONE*, 13: e0208193.
Fung, F., H.S. Wang, and S. Menon. 2018. "Food safety in the 21st century." In *Biomedical journal*, 89-94. Elsevier B.V.
Garrow, J.S., M.L. Durrant, S. Mann, S.F. Stalley, and P.M. Warwick. 1978. 'Factors determining weight loss in obese patients in a metabolic ward', *Int J Obes*, 2: 441-7.
German, J.B., A.M. Zivkovic, D.C. Dallas, and J.T. Smilowitz. 2011. 'Nutrigenomics and Personalized Diets: What Will They Mean for Food?', *Annual Review of Food Science and Technology*, 2: 97-123.
Ghaly, A.E. , V.V. Ramakrishnan, M.S. Brooks, S.M. Budge, and D Dave. 2013. "Fish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review." In *J Microb Biochem Technol*, 112-18.
Godfree, Alan. 2003. *Handbook of Water and Wastewater Microbiology* (Elsevier).
Grasso, A.C., Y. Hung, M.R. Olthof, W. Verbeke, and I.A. Brouwer. 2019. "Older Consumers' Readiness to Accept Alternative, More Sustainable Protein Sources in the European Union." In *Nutrients*.
Greiff, K., I.G. Aursand, U. Erikson, G. Jakobsen, and T. Rustad. 2015. "Effects of type and concentration of salts on physicochemical properties in fish mince." In *Food Science and Technology*, 221. Elsevier.
Gundersen, B., and R.W. Dahl. 2008. "Optimalisering av kvaliteten på fryst hvitlaksmasse." In.: Nofima.
Hellevik, A.H., M. Synnes, and I. Stoknes. 2005. "Underutilised fish species. Raw material properties of muscle fraction from Greater Argentine, Northern Wolffish and Polar Cod." In *Consumerproducts of the EU-project SEAFOOD plus*. Ålesund: Møre Research.
Hellvåg, Helmer. 2018. 'Hydrolyse', Accessed 29 August 2019. https://snl.no/hydrolyse.
Henchion, M., M. Hayes, A.M. Mullen, M. Fenelon, and B. Tiwari. 2017. "Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium." In, 1-13. Foods.
Hydroprot. 'Hydroprot', Sprekere Liv As, Accessed 2 June 2020. https://hydroprot.no/.
IDDSI. 'International Dysphagia Diet Standardisation Initiative', Accessed 1 June. https://iddsi.org/.
IDDSI Testing. 2019. 'IDDSI Framework Testing Methods 2.0', IDDSI, Accessed 1 June 2020. https://ftp.iddsi.org/Documents/Testing_Methods_IDDSI_Framework_Final_31_July2019.pdf.
Institute of Medicine. 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids* (The National Academies Press: Washington, DC).
ISO 5492:. 2008. "Sensory analysis - Vocabulary, 2. edition." In.: International Organization for Standardization.
ISO 5983-2:. 2009. "Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content - Part 2: Block digestion and steam distillation method." In.: International Organization for Standardization.
ISO 13299:. 2016. "Sensory analysis - Methodology - General guidance for establishing a sensory profile. 2. edition." In.: International Organization for Standardization.

ISO 8586-1:. 2012. "Sensory analysis - General guidance for the selection, training and monitoring of assessors - Selected assessors. 2. Edition." In.: International Organization for Standardization.
Jaul, E., and J. Barron. 2017. 'Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population', *Front Public Health*, 5: 335.
Jay, J.M., M.J. Loessner, and D.A. Golden. 2005. *Modern food microbiology* (Springer).
Kanda, A., K. Nakayama, C. Sanbongi, M. Nagata, S. Ikegami, and H. Itoh. 2016. "Effects of Whey, Caseinate, or Milk Protein Ingestion on Muscle Protein Synthesis after Exercise." In *Nutriens*, 9. MDPI.
Kerksick, C.M., C.D. Wilborn, M.D. Roberts, A. Smith-Ryan, S.M. Kleiner, R. Jäger, R. Collins, M. Cooke, J.N. Davis, E. Galvan, M. Greenwood, L.M. Lowery, R. Wildman, J. Antonio, and R.B. Kreider. 2018. 'ISSN exercise & sports nutrition review update: research & recommendations', *J Int Soc Sports Nutr*, 15: 1-38.
Khitouni, I.K., N.B. Mihoubi, A. Bouain, and F.B. Rebah. 2015. "Seasonal variations in proximate and fatty acid composition of golden grey mullet Liza aurata (R, 1810) from the Tunisian coast." In *International Journal of Agricultural Policy and Research*, 273-74. Journal Issues.
Kim, S.W., J.F. Less, L. Wang, T. Yan, V. Kiron, S.J. Kaushik, and X.G. Lei. 2019. 'Meeting Global Feed Protein Demand: Challenge, Opportunity, and Strategy', *Annual Review of Animal Biosciences*, 7: 221-43.
Kneifel, W., and A. Seiler. 1993. "Water-holding Properties of Milk Protein Products - A Review." In *Food structure*, 297-98. Food structure.
Ko, G. J., Y. Obi, A. R. Tortorici, and K. Kalantar-Zadeh. 2017. 'Dietary protein intake and chronic kidney disease', *Curr Opin Clin Nutr Metab Care*, 20: 77-85.
Kühn, J., T. Considine, and H. Singh. 2006. 'Interactions of Milk Proteins and Volatile Flavor Compounds: Implications in the Development of Protein Foods', *Journal of Food science*, 71: 72-82.
Lagrange, V., D. Whitsett, and C. Burris. 2015. 'Global Market for Dairy Proteins', *Journal of Food science*, 80: 16-22.
Lindberg, D., and T. Ytrestøyl. 2018. "Ja til mer av laks." In *Tekfisk*. Fiskeribladet AS.
Lindström, M., K. Kiviniemi, and H. Korkeala. 2006. "Hazard and control of group II (non-proteolytic) Clostridium botulinum in modern food processing." In *International Journal of Food Microbiology*, 92-99. Elsevier.
Loth, F. 1993. 'Guar, Locust Bean, Tara and Fenugreek Gums.' in, *Industrial gums: Polysaccharides and their derivatives* (John Wiley & Sons, Ltd).
Mackie, P.R., and R. Hardy. 1969. "A lipid analysis of the Greater Silver Smelt [Argentina silus (Ascanius)] and an evaluation of its potential for food and fish meal production." In *International Journal of Food Science & Technology*, 241-42.
Maier, M.B., T. Schweiger, C.A. Lenz, and R.F. Vogel. 2018. "Inactivation of non-proteolytic Clostridium botulinum type E in low-acid foods and phosphate buffer by heat and pressure." In.: PLoS ONE.
Matsuo, K., and J.B. Palmer. 2008. 'Anatomy and physiology of feeding and swallowing: normal and abnormal', *Phys Med Rehabil Clin N Am*, 19: 691-707.
Mattilsynet. 2007. 'Stemmer det at varm mat ikke skal settes i kjøleskapet?', Mattilsynet, Accessed 30 January 2020. https://www.matportalen.no/matsmitte_og_hygiene/tema/smittestoffer/stemmer_det_at_varm_mat_ikke_skal_settes_i_kjoleskapet.
Matvaretabellen. 2020. "Matvaretabellen." In.: Mattilsynet.
Meyer, H.E., and M.E.M.T. Vollrath. 2017. "Overvekt og fedme." In *Folkehelserapporten*. Norwegian Institute of Public Health,.

Mirkin, G.B., and R.N. Shore. 1981. 'The Beverly Hills diet. Dangers of the newest weight loss fad', *Jama*, 246: 2235-37.
Moberg, M., W. Apró, I. Ohlsson, M. Pontén, A. Villanueva, B. Ekblom, and E. Blomstrand. 2014. 'Absence of leucine in an essential amino acid supplement reduces activation of mTORC1 signalling following resistance exercise in young females', *Appl Physiol Nutr Metab*, 39: 183-94.
Mokrzycki, Wojciech, and Maciej Tatol. 2011. 'Color difference Delta E - A survey', *Machine Graphics and Vision*, 20: 4.
Næringsmiddelhygieneforskriften. 2009. "Kapittel V. Utfyllende nasjonale bestemmelser om temperaturkrav (Næringsmiddelhygieneforskriften)." In, edited by Landbruks- og matdepartementet Nærings- og fiskeridepartementet, Helse- og omsorgsdepartementet,. Norway.
Nestlé. 2019. 'NESTLÉ HEALTH SCIENCE EXPANDS INTO PERSONALIZED NUTRITION WITH ACQUISITION OF PERSONA™', Nestlé Health Science, Accessed 10 June 2020. https://www.nestle.com/sites/default/files/2019-08/nestle-health-science-persona-press-release-22-08-2019.pdf.
Newman, T. 2017. "What causes difficulty swallowing (dysphagia)?" In.: Medical News Today,.
Nofima. 2018-2021. 'VårMat', Nofima, Accessed 10 June 2020. https://nofima.no/prosjekt/varmat/.
'Nutrition and Athletic Performance'. 2009. *Medicine & Science in Sports & Exercise*, 41: 709-31.
Øines, S. 2019. "Lab-S Geltest." In *Metode- og arbeodsbeskrivelser*, edited by Nofima, 1-5.
Olsman, H. 1979. 'Hydrolyzed and autolyzed vegetable proteins as functional food ingredients', *Journal of the American Oil Chemists' Society*, 56: 375-76.
Olympiatoppen, Ernæringsavdelingen. 'Fakta om protein og idrett', Olympiatoppen, Accessed 25 May 2020. https://www.olympiatoppen.no/fagstoff/idrettsernaering/faktaark/page467.html.
Onodenalore, A.C., and F. Shahidi. 1996. 'Protein Dispersions and Hydrolysates from Shark (Isurus oxyrinchus)', *Journal of Aquatic Food Product Technology*, 5: 43, 49-56.
Oslo universitetssykehus. 2017. 'Spise- og svelgevansker (dysfagi)', Oslo universitetssykehus, Accessed 12 May 2020. https://oslo-universitetssykehus.no/behandlinger/spise-og-svelgvansker-dysfagi.
Øyen, J., I. Kvestad, L.K. Midtbø, I.E. Graff, M. Hysing, and K.M. Stormark. 2018. "Fatty fish intake and cognitive function: FINS-KIDS, a randomized controlled trial in preschool children." In *BMC Medicine*, 13. BMC medicine.
Pathare, P.B., U.L. Opara, and F.A.J. Al-Said. 2012. "Colour Measurement and Analysis in Fresh and Processed Foods: A Review." In *Food Bioprocess Technol*. Springer.
Petrova, I., I. Tolstorebrov, and T.M. Eikevik. 2018. 'Production of fish protein hydrolysates step by step: technological aspects, equipment used, major energy costs and methods of their minimizing', *International Aquatic Research*, 10: 223-41.
Phillips, S.M., D.R. Moore, and J.E. Tang. 2007. 'A critical examination of dietary protein requirements, benefits, and excesses in athletes', *Int J Sport Nutr Exerc Metab*, 17: 58-76.
Polarin. 'Polarin', Pharmapolar, Accessed 10 June 2020. https://pharmapolar.no/derfor-bor-du-ta-det-nye-kosttilskuddet-polarin/.
Potter, J. 2010. *Cooking for geeks* (O'Reilly Media Inc).
Prabhu, L.A. 2018. 'Texture and sensory properties of modified fish products for dysphagia patients', Master's Thesis Universitet i Stavanger.

Puolanne, E.J., M.H. Ruusunen, and J.I. Vainionpää. 2001. "Combined effects of NaCl and raw meat pH on water-holding in cooked sausage with and without added phosphate." In *Meat Science*, 2. Elsevier.
Raikos, V., and T. Dassios. 2014. 'Health-promoting properties of bioactive peptides derived from milk proteins in infant food: a review', *Dairy Science & Technology*, 94: 91-101.
Ramírez, J., R. Uresti, S. Téllez, and M. Vázquez. 2002. 'Using Salt and Microbial Transglutaminase as Binding Agents in Restructured Fish Products Resembling Hams', *Journal of Food science*, 67: 1778-84.
Rasco, B.A., and H.G. Kristinsson. 2000. "Fish Protein Hydrolysates: Production, Biochemical, and Functional Properties." In *Critical Reviews in Food Science and Nutrition*, 43-44, 46-59. Taylor & Francis.
Richardsen, R., M.S. Myhre, R. Nystøyl, G. Strandheim, and A. Martinussen. 2018. "Analyse marint restråstoff 2017 " In.: Sintef Ocean AS Kontali
Richardsen, R., Magnus S. Myhre, R. Nystøyl, A. Martinussen, and G. Strandheim. 2019. "Analyse marint restråstoff 2018." In.: SINTEF Ocean AS Kontali.
Rimac, S., V. Lelas, T. Bosiljkov, and M.B. Sabolović. 2009. "Thermal gelation of whey protein at different pH values." In *Chemical Engineering Transactions*. Faculty of Food Technology and Biotechnology.
Rios-Mera, J.D., E. Saldaña, M.L.M. Cruzado-Bravo, M.M. Martins, I. Patinho, M.M. Selani, D. Valentin, and C.J. Contreras-Castillo. 2020. "Impact of the content and size of NaCl on dynamic sensory profile and instrumental texture of beef burgers." In *Meat Science*, 1-8. Elsevier.
Robinson, S., A. Pilgrim, H. Roberts, and A.A. Sayer. 2015. "An overview of appetite decline in older people." In, 1-3. Nurs Older People.
Romero de Ávila, M.D., M.I. Cambero, J.A. Ordóñez, L. de la Hoz, and A.M. Herrero. 2014. "Rheological behaviour of commercial cooked meat products evaluated by tensile test and texture profile analysis (TPA)." In *Meat Science*, 310-15.
Rosnes, J.T., G.H. Rognså, and M. Brierley. 2019. 'Ny internasjonal standard for konsistenttilpasset mat', Accessed 10 June 2020. https://www.matlyst.org/single-post/2019/11/29/Ny-internasjonal-standard-for-konsistenstilpasset-mat.
Rotabakk, B.T., and S.G. Iversen. 2018. 'Fiskekakenes innerste hemmeligheter', Forskning.no, Accessed 3 June 2020. https://blogg.forskning.no/fra-fjord-til-bord/fiskekakenes-innerste-hemmeligheter/1093410.
Saha, Dipjyoti, and Sevendu Bhattacharya. 2010. "Hydrocolloids as thickening and gelling agents in food: a critical review." In *J Food Sci Technol*, 587-88, 90-92, 94. Springer.
Sales, N.M.R., P.B. Pelegrini, and M.C. Goersch. 2014. 'Nutrigenomics: definitions and advances of this new science', *Journal of nutrition and metabolism*, 2014: 202759-59.
Sarode, A., P. Sawale, C. Khedkar, S. Kalyankar, and P. Rd. 2016. 'Casein and Caseinate: Methods of Manufacture.' in.
Scheer, B.T., J.F. Codie, and H.J.Jr. Deuel. 1947. 'The effect of fat level of the diet on general nutrition; weight loss, mortality and recovery in young adult rats maintained on restricted calories', *J Nutr*, 33: 641-48.
Sharma, M., E. Kristo, M. Corredig, and L. Duizer. 2017. "Effect of hydrocolloid type on texture of pureed carrots: Rheological and sensory measures." In *Food Hydrocolloids*, 479-83. Elsevier.
Shenouda, S.Y.K. 1998. "Theories of Protein Denaturation During Frozen Storage of Fish Flesh." In *Advances in Food Research*, 275-76. Elsevier.
Silva, A., D. Sousa, E. Silva, T. Fontes, R. Tomaz, and M. Barros. 2016. 'Temperature Influence in Cornstarch Gelatinization for Froth Flotation', *International Proceedings of Chemical, Biological and Environmental Engineering*, 96: 7-11.

Singh, P., R. Singh, A. Jha, P. Rasane, and A.K. Gautam. 2015. "Optimization of a process for high fibre and high protein biscuit." In *Journal of Food Science and Technology*, 1394-403. Springer.
Siparsky, P.N., D.T. Kirkendall, and W.E. Garret. 2014. "Muscle Changes in Aging: Understanding Sarcopenia." In *Athletic Training*, 36. Sports Health.
Sirangelo, T.M. 2019. "Sensory Descriptive Evaluation of Food Products: A Review." In, 354-59. Journal of Food Science and Nutrition Research.
Sivertsen, K.I. 2012. 'Lavsaltprodukter av fisk', Norges teknisk-naturvitenskapelige universitet.
Sivertsvik, M. 2015. "Vassil eller kvitlaks?" In.: Nofima.
Skipnes, D., S. Johnsen, T. Skåra, M. Sivertsvik, and O. Lekang. 2011. 'Optimization of Heat Processing of Farmed Atlantic Cod (Gadus morhua) Muscle with Respect to Cook Loss, Water Holding Capacity, Color, and Texture', *Journal of Aquatic Food Product Technology*, 20: 331-40.
Skipnes, D., M.L. Østby, and M.E. Hendrickx. 2007. 'A method for characterising cook loss and water holding capacity in heat treated cod (Gadus morhua) muscle', *Journal of Food Engineering*, 80: 1078-85.
Skogly, A.H. 2016. 'Ikke nok fisk i fiskemat', Forbrukerrådet, Accessed 6 June 2020. https://www.forbrukerradet.no/vi-mener/2015/fpa-mat-og-handel-2015/ikke-nok-fisk-i-fiskemat/.
Steward, K. 2019. 'Amino acid Chart', Accessed 25 May 2020. https://www.technologynetworks.com/applied-sciences/articles/essential-amino-acids-chart-abbreviations-and-structure-324357.
Store norske leksikon. 2013. 'Vassild', Universitetet i Oslo, Accessed 25 May 2020. https://snl.no/vassild.
Sungsinchai, S., C. Niamnuy, P. Wattanapan, M. Charoenchaitrakool, and S. Devahastin. 2019. "Texture Modification Technologies and Their Opportunities for the Production of Dysphagia Foods: A Review." In *Comprehensive Reviews in Food Science and Food Safety*, 1898-912. John Wiley & Sons, Ltd.
Tamanna, Nahid, and Niaz Mahmood. 2015. "Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition." In *International Journal of Food Science*, edited by Mitsuru Yoshida, 1. Hindawi Publishing Corporation.
Tartalio, M. 2018. 'Consumer Food Trends: Tapping into Personalized Foods', Accessed 1 May 2020. https://clarkstonconsulting.com/insights/consumer-food-trends/.
Texture Technologies. 'Overview of Texture Profile Analysis', Texture technologies, Accessed 19 May 2020. https://texturetechnologies.com/resources/texture-profile-analysis.
Tipton, K.D., T.A. Elliott, A.A. Ferrando, A.A. Aarsland, and R.R. Wolfe. 2009. 'Stimulation of muscle anabolism by resistance exercise and ingestion of leucine plus protein', *Applied Physiology, Nutrition, and Metabolism*, 34: 151-61.
Tunick, M.H., C.I. Onwulata, and P.H. Cooke. 2013. "Using Temperature Sweeps To Investigate Rheology of Bioplastics." In *Physical Methods in Food Analysis*, 90-91 and 102-03. ACS Symposiom Series.
Vaka, Mette Risa. 2018. 'Varme- og saltinduserte kvalitetsendringer i Atlantisk torsk (Gadus morhua)', Universitetet i Stavanger.
Van Nieuwenhuyzen, W., V. Budnik, T. Meier, and L. Popper. 2006. "Functional use of hydrocollois in food systems." In *Functional peptides*, 39-42. Agro Food Industry Hi Teck.
Vatland, A.I., R. Ragård, R. Øystad, and T. Jubal. 1991. "Produksjon av fiskefarse." In.: Produktteknisk avdeling.

Vázquez, J.A. , A. Meduíña, A.I. Durán, M. Nogueira, A. Fernández-Compás, R.I. Pérez-Martín, and I. Rodríguez-Amado. 2019. "Production of Valuable Compounds and Bioactive Metabolites from By-Products of Fish Discards Using Chemical Processing, Enzymatic Hydrolysis, and Bacterial Fermentation." In *Mar. Drugs*.

Vázquez, J.A., I. Rodríguez-Amado, Carmen G. Sotelo, N. Sanz, Ricardo I. Pérez-Martín, and J. Valcárcel. 2020. 'Production, Characterization, and Bioactivity of Fish Protein Hydrolysates from Aquaculture Turbot (Scophthalmus maximus) Wastes', *Biomolecules*, 10: 310.

Veiseth-Kent, E., K. Hildrum, R. Ofstad, M. Rørå, P. Lea, and M. Rødbotten. 2010. 'The effect of postmortem processing treatments on quality attributes of raw Atlantic salmon (Salmo salar) measured by sensory and instrumental methods', *Food chemistry*, 121: 275-81.

Volpi, E., R. Nazemi, and S. Fujita. 2004. 'Muscle tissue changes with aging', *Current opinion in clinical nutrition and metabolic care*, 7: 405-10.

Warner, R.D. 2017. 'The Eating Quality of Meat, IV Water-Holding Capacity and Juiciness.' in Fidel Toldra´ (ed.), *Lawrie´s Meat Science* (Woodhead Publishing).

Welle, S., and C.A. Thornton. 1998. 'High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women', *Am J Physiol*, 274: 677-83.

Wilson, D., T. Jackson, E. Sapey, and J.M. Lord. 2017. 'Frailty and sarcopenia: The potential role of an aged immune system', *Ageing Research Reviews*, 36: 1-10.

Wright, J.M., R.E. Miracle, and M.A. Drake. 2014. "Sensory Properties of Dairy Proteins." In *Milk Proteins*, 473-88. Elsevier.

Wu, D., D.W. Sun, and Y. He. 2014. "Novel non-invasive distribution measurement of texture profile analysis (TPA) in salmon fillet by using visible and near infrared hyperspectral imaging." In *Food chemistry*, 417-25. Elsevier.

Wüstenberg, T. 2015. *Cellulose and Cellulose Derivatives in the food industry: Fundamentals and Applications* (Wiley-VCH Verlag GmbH & Co. KGaA).

Xiong, Y.L. 2009. "Dairy proteins." In *Ingredients in Meat Products: Properties, Functionality and Applications* Springer Science and Business Media.

Yang, X., and R.A. Boyle. 2016. 'Chapter 3 - Sensory Evaluation of Oils/Fats and Oil/Fat–Based Foods.' in M. Hu and C. Jacobsen (eds.), *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats* (AOCS Press).

Zayas, J.F. 1997. 'Gelling Properties of Proteins.' in, *Functionality of Proteins in Food* (Springer: Germany).

Zayas, J.F., and C.S. Lin. 1989. 'Effect of the Pretreatment of Corn Germ Protein on the Quality Characteristics of Frankfurters', *Journal of Food science*, 54: 1452-56.

Appendix A

Information of salmon protein hydrolysate produced by Hofseth

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 B

Information of 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

Quality parameters	
Bacterial count (CFU/g)	110
Putrescine (mg/kg)	<20
Cadaverine (mg/kg)	67
Histamine (mg/kg)	14
Trimethylamin-N (mg N/100 g)	125
Trimethylaminooxide-N (mg N/100 g)	1500
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

Appendix C

Information about Betan casing which was used in production of minced fish and in pilot production of texture modified products.

ABC
Arne & Cornelius AS

tqm 9000

50 PRODUKTSPESIFIKASJONER/00 Tarmer/02 Plasttarmer

P-00.02. TQM
00 rev: Godkjente
04 bøker 1.94
NY BASE
(Notes1)

Dokument tittel: **Betan**

PRODUKTBESKRIVELSE

Produktgruppe
Plasttarm

Produsent og opprinnelsesland
Naturin GmbH & Co., Tyskland

Pakningsstørrelse
Avhengig av kaliber. Leveres i ruller à 500, 1.000 eller 1.500 m, raffet/rynket sticks à 20, 30, 40 eller 50 m, eller i snitt à 18 -130 cm.

TEKNISKE DATA

Kjemiske data
Ett-lags tarm basert på en blanding av polyamid (nylon) og modifisert polyetylen. Farget tarm er tilsatt pigment eller pigmentblandinger.

Vanndamp barriere	17 g/d qm
Oksygen barriere	17 ccm/d qm bar
Veggtykkelse	35 - 45 µ
Krymping (1 min. i vann ved 80°C)	14%

Bakteriologiske data
Ingen bakteriologisk aktivitet

Allergener

Type allergen	Inneholder	Kommentar
Glutenholdig korn og produkter fremstilt av glutenholdige korn	N	
Skalldyr og produkter fremstilt av skalldyr	N	
Egg og produkter fremstilt av egg	N	
Fisk og produkter fremstilt av fisk	N	
Peanøtter og produkter fremstilt av peanøtter	N	
Soya og produkter fremstilt av soya	N	
Melk og produkter fremstilt av melk (herunder laktose)	N	
Nøtter og produkter fremstilt av nøtter	N	
Selleri og produkter fremstilt av selleri	N	
Sennep og produkter fremstilt av sennep	N	
Sesamfrø og produkter fremstilt sesamfrø	N	
Sulfitt i konsentrasjon på 10 mg/kg eller mer	N	

GMO-status
GMO-fritt iht. EU forordning 1829/2003 (produkt) og 1830/2003 (merking).

Andre data

Farge	Klar, hvit, rød, orange, sort, gul, brun, honning-gul, klar gul, klar rød, messing, lys brun, rødbrun, røyk-brun, gul-orange, gull, sølv, hasselnøtt
Trykk	Mulig inntil 6 farger

Kaliber 20 - 120 mm

PRODUKTEGENSKAPER

Betan er en selvkrympende allround plasttarm, helt fri for PVDC, og totalt problemfri ved kjøring. Produktet har høy mekanisk styrke, er motstandsdyktig mot mugg og varme helt opp til 121°C. Tarmens gode barriere egenskaper mot oksygen og ultrafiolett lys hindrer at produktet mister farge. Betan er lett å skrelle, har et appetittvekkende utseende, holdbarhetsbevarende egenskaper ved kald lagring, gir ikke kokesvinn og den revner ikke ved klipsing og kutting.
Følgende parameter er kaliberavhengig: flatbredde, veggtykkelse, krymping, anbefalt fyllekaliber, anbefalt diameter på fyllerør, lengde for og lengde på raffet/rynket sticks.

BRUK AV PRODUKTET

Område

Meget godt egnet til kokte farse- og skinke produkter av kjøtt, fisk og fjørfe.

Stopping

Tarmen må bløtlegges i kaldt vann i 30 minutter før bruk. Tarmen skal overstoppes til angitt stoppekaliber, og kan klipses eller knytes. Kokes hengende eller liggende. Avkjøles i dusj eller på kjølerom. For maksimal etterkrymping, kort avkjøling i vann/dusj i 2-5 minutter før videre avkjøling på kjølerom. Betan trenger ingen ytterligere etterkrymping.

LAGRING

Lagringstemperatur

Temperatur under +23 °C.

Holdbarhet

24 måneder ved lagring i uskadet original-emballasje, i mørkt rom med under 50% relativ fuktighet.

REGISTRERING

Materialet er godkjent i overensstemmelse med Norsk lovgivning - EK-sertifikat nr. 1510.

HELSE/MILJØ/SIKKERHET

Forhåndsregler

Må ikke spises. Tarmene avgir ingen skadelige fraksjoner til miljøet eller produktet.

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Appendix D

Detailed information about attributes applied in sensory analysis in pilot productions of both minced fish and texture modified products, obtained from Nofima Ås. Some attributes were applied in analysis of both products, some have been used on one type of product.

Odor

Sour odor	Relateres til en frisk, balansert lukt som skyldes organiske syrer
Syrlig lukt	Ingen intensitet = ingen syrlig lukt
	Tydelig intensitet = tydelig syrlig lukt
Metallic odor	Lukt av metall (ferrosulfat)
Metallukt	Ingen intensitet = ingen metallukt
	Tydelig intensitet = tydelig metallukt
Dairy odor	Lukt av meieriprodukter som varm melk og smør
Meierilukt	Ingen intensitet = ingen meierilukt
	Tydelig intensitet = tydelig meierilukt
Spicy odor	Lukt av krydder, f.eks. pepper, muskat
Krydderlukt	Ingen intensitet = ingen krydderlukt
	Tydelig intensitet = tydelig krydderlukt
Fish odor	Lukt av prosessert fiskeprodukt som fiskekaker, graten etc.
Fiskelukt	Ingen intensitet = ingen fiskelukt
	Tydelig intensitet = tydelig fiskelukt
Cloying odor	En ufrisk/kvalmende lukt
Emmen lukt	Ingen intensitet = ingen emmen lukt
	Tydelig intensitet = tydelig emmen lukt

Smak

Sour flavor	Relateres til en frisk, balansert smak som skyldes organiske syrer
Syrligsmak	Ingen intensitet = ingen syrlig smak
	Tydelig intensitet = tydelig syrlig smak

Sweet taste	Relateres til grunnsmaken søt (sukrose)
Søtsmak	Ingen intensitet = ingen søtsmak
	Tydelig intensitet = tydelig søtsmak
Salty taste	Relateres til grunnsmaken salt (NaCl)
Saltsmak	Ingen intensitet = ingen saltsmak
	Tydelig intensitet = tydelig saltsmak
Bitter taste	Relateres til grunnsmaken bitter (kinin, koffein)
Bittersmak	Ingen intensitet = ingen bittersmak
	Tydelig intensitet = tydelig bittersmak
Umami taste	Relateres til grunnsmaken umami
Umamismak	Ingen intensitet = ingen umami smak
	Tydelig intensitet = tydelig umami smak
Metallic flavor	Smak av metall (ferrosulfat)
Metallsmak	Ingen intensitet = ingen metallsmak
	Tydelig intensitet = tydelig metallsmak
Dairy flavor	Smak av meieriprodukter som varm melk og smør
Meierismak	Ingen intensitet = ingen meierismak
	Tydelig intensitet = tydelig meierismak
Spicy flavor	Smak av krydder, f.eks. pepper, muskat
Kryddersmak	Ingen intensitet = ingen kryddersmak
	Tydelig intensitet = tydelig kryddersmak
Fish flavor	Smak av prosessert fiskeprodukt som fiskekaker, graten etc.
Fiskesmak	Ingen intensitet = ingen fiskesmak
	Tydelig intensitet = tydelig fiskesmak
Cloying flavor	En ufrisk/kvalmende smak
Emmen smak	Ingen intensitet = ingen emmen smak
	Tydelig intensitet = tydelig emmen smak

Aftertaste	Ettersmak etter 15 sekunder uten skylling
Ettersmak	Ingen intensitet = ingen ettersmak
	Tydelig intensitet = tydelig ettersmak

Texture

Hardness	Mekanisk teksturegenskap relatert til kraften som må til for å bite
Hardhet	gjennom prøven med jekslene ved 1. bitt
	Ingen intensitet = ingen hardhet, myk (kremost)
	Tydelig intensitet = tydelig hardhet
Firmness	Mekanisk teksturegenskap relatert til kraften som må til for å bite
Hardhet	gjennom prøven. Bedømmes med jekslene ved 1. bitt
Fasthet	Ingen intensitet = ingen fasthet, myk
	Tydelig intensitet = tydelig fasthet, hard
Juiciness	Væske som skilles ut i prøven, munnfølelse etter flere tygg
Saftighet	Ingen intensitet = tørr prøve
	Tydelig intensitet = høy grad av væskeutskillelse ved tygging
Crumbliness	Mekanisk teksturegenskap relatert til kohesjon og den nødvendige kraft
Smuldrethet	som skal til for å bryte opp et produkt til smuler eller biter
	Ingen intensitet = ingen smuldrethet (glatt)
	Tydelig intensitet = tydelig smuldrethet
Gumminess	Mekanisk teksturegenskap relatert til kohesjonen til et mørt produkt.
Gummiaktighet	I munnen er det relatert til den anstrengelse som kreves for å finfordele produktet til en tilstand klar for svelging.
	Ingen intensitet = ingen gummiaktighet
	Tydelig intensitet = tydelig gummiaktighet (eks. vingummi)
Adhesiveness	Mekanisk teksturegenskap relatert til kraften som skal til for å fjerne et
Klebrighet	stoff som kleber seg fast i munnen. Bedømmelse etter at prøven er spyttet ut.
	Ingen intensitet = ingen klebrighet (lite sitter igjen i munnen)
	Tydelig intensitet = tydelig klebrighet (vanskelig å fjerne prøven)

Astringency Astringent	Beskriver en kompleks følelse, fulgt av sammentrekninger, tørrfølelse, snurping av huden eller slimhinner i munnen, produsert av stoffer som tanniner (garvestoffer) fra slåpetornbær. Ingen intensitet = ingen astringens tydelig intensitet = tydelig astringens
Coarseness Grovhet	Geometrisk teksturegenskap relatert til partikkelstørrelse og partikkelform i et produkt. Ingen intensitet = ingen grovhet Tydelig intensitet = tydelig grovhet
Grittiness Sandete	Teksturegenskap knyttet til partikkelstørrelse og partikkelform i et produkt. Ingen intensitet = ikke sandete (glatt) Tydelig intensitet = tydelig sandete (grov)
Cohesiveness Sammenhengbarhet	Mekanisk strukturell egenskap relatert til den tid eller antall tygg som kreves for å tygge produktet til en tilstand klar for svelging. Ingen intensitet = ingen sammenhengbarhet Tydelig intensitet = tydelig sammenhengbarhet
Chalky Tråhet/krittaktig	En kompleks følelse (tørrhetsfølelse eller tråhet) av slimhinner i munnen, krittaktig. Ingen intensitet = ingen tråhet Tydelig intensitet = tydelig tråhet

Appendix E

The nutrient content of sooft meals obtained from www.vitaelnaering.no.

https://vitalernaering.no/wp-content/uploads/2017/11/N%C3%86RINGSDEKLARASJON-SOOFT-MEALS-1.pdf assessed 3 May 2020

Fiskefilet

Ingredienser:
fisk (pollock) 48 % **fløte** 31 % (fløte av 15 fett og 1% storfegelatin), **kremost** 17 %, salt, stabilisator: johannesbrødkj.mel og guarkj.mel, **eggehvite**, tapiokastivelse, storfegelatin, gjærekstrakt, løk, maltodekstrin, krydder.

Næringsinnhold pr.100 gram ferdig vare:
Energi: 616 kJ /147 kcal, Fett: 10 g, hvorav mettet fett: 5,9 g, Karbohydrater: 3,2 g, hvorav sukkerarter: 0 g, Protein: 13,7 g, Salt: 1,2 g.

Næringsinnhold pr. porsjon:
Energi: 1817 kJ / 434 kcal, Fett: 29,5 g, hvorav mettet fett: 17,4 g, Karbohydrater: 9,4 g, hvorav sukkerarter: 0 g, Protein: 40,4 g, Salt: 3,5 g.

The nutrient content of Lofoten Proburger obtained from Lofoten.no

https://www.lofoten.no/produkt/lofoten-proburger-spicy-chili Assessed 7 May 2020

Ingredienser

Fisk 66 % (hvitlaks, torsk, hyse), melk, Norvegia-ost (melk), potetmel, fiskeprotein, sjalottløk, rapsolje, chili, løk, hvitløk, krydder (inkl. sort pepper, koriander, sitronekstrakt, muskatblomme), salt, konsistensmiddel (karragenan).
Stekt i rapsolje.

Næringsinnhold

Energi 662 kJ/165 kcal
Fett 7,5 g
- hvorav mettet fett 1,6 g
- hvorav enumettet fett 4,1 g
- hvorav flerumettet fett´ 1,8 g
Karbohydrater 4,6 g
- hvorav sukkerarter 1,1 g
Protein 18,2 g
Salt 1,1 g

Appendix F

A selection of Norwegian processed fish products with ingredient list.

https://www.lofoten.no/produkt/familiens-fiskekaker Assessed 13 June 2020
Lofoten Familiens fiskekaker
Fisk 60 % (hvitlaks 32 %, hyse 22 %, torsk 6 %), rismelk (vann, rismelkpulver (ris, olje, salt)), potetmel, rapsolje, løk, muskatblomme, salt, hvit pepper, sellerisalt.

https://www.lunsj.no/926-fiskermannen-fiskekaker.html Assessed 13 June 2020
Fiskemannen 65%
Hvitfisk (65 %) [kvitlaks (Argentina silus), sei (Pollachius virens), hyse (Melanogrammus aeglefinus)], vann, kassavastivelse, surhetsregulerende middel (E326, E261), mysepulver, rapsolje, salt, krydder (inkl. muskatnøtt, sort pepper, løkpulver).

https://norges.online/produkt/fiskekaker-nordnorske-460-g Assessed 13 June 2020
Fiskekaker Nordnorske, Sjøfrisk NORGE
Fisk 70 % (hvitlaks (Argentina silus)49 %, hyse (Melanogrammus aeglefinus) 21 %) melk (25 %), tapiokastivelse (4 %), rapsolje (3 %), salt, løk, fløtepulver, muskatblomme, hvit pepper.

https://kolonial.no/produkter/29713-fiskeriet-burger-m-purre-paprika-80/ Assessed 13 June 2020
Fiskeburger Purre & Paprika 80% Fiskeriet
Kvitlaks (Argentina silus) (50,5%), hyse (Melanogrammus aeglefinus) (29,9%), helmelk, tapiokastivelse, rapsolje, tørket purreløk (0,3%), gressløk, paprika, chili, svart, pepper, salt, løkpulver, gjærekstrakt, krydder, muskatnøttekstrakt, muskatblommeekstrakt, svart pepperekstrakt.

https://kolonial.no/produkter/29714-fiskeriet-burger-m-steinbit-gresslok-80/ Assessed 13 June 2020
Fiskeburger steinbit og gressløk, 80% fiskeriet
Kvitlaks (Argentina silus) (52%) hyse (Melanogrammus aeglefinus) (19%), helmelk, steinbit (Anarhichas lupus) (9%), rapsolje, tapiokastivelse, tørket gressløk (0,5%), tørket purreløk, salt, hvitløkspulver, dekstrose, gjærekstrakt, krydder, muskatblomme, tomatpulver.

https://www.lofoten.no/produkt/80-grov-fiskeburger-med-torsk-og-hyse Assessed 14 June
86% Lofotburger med torsk og hys
Fisk 86 % (hyse 43 %, torsk 22,5 %, hvitlaks 20,5 %), melk, smør (melk), potetmel, potetflakes, løk, dijonsennep, pepper, salt, sellerisalt, hvitløk, muskatblomme. Stekt i rapsolje.

Appendix G

Detailed information about conducted analyses from preliminary production of texture modified products

Recipe	**1A**	**1B**	**1C**	**ICa**
Firmness	1.132±0.068	0.553±0.065	1.103±0.28	5.264±0.369

Recipe	**2A**	**2B**	**2C**	**2D**	**2E**	**2F**	**2G**
Firmness	0.915	1.255	1.221	1.555	1.422	1.814	1.589
SD	0.096	0.107	0.221	0.137	0.059	0.131	0.069

Recipe	**2A**	**2B**	**2D**	**2E**	**2F**	**2G**
L*	89.03	84.83	88.44	88.35	88.15	88.09
a*	0.36	2.01	1.09	1.25	1.63	1.02
b*	15.52	18.02	16.90	17.25	17.58	17.42

Recipe	**2A Bottom**	**2A Top**	**2A**	**2B**	**2F**
Liquid loss	1.70±0.24	3.07±1.10	1.69±0.51	1.69±0.05	1.88±0.15
Water loss	1.26±0.17	1.95±0.71	1.54±0.50	1.54±0.01	0.15±0.10
Mass loss	0.44±0.19	1.12±0.40	1.88±0.05	1.69±0.04	0.20±0.05

Appendix H

Detailed information about conducted analyses from pilot production for texture modified products.

Recipe	ST	LHT	HHT	LNT	HNT	P
Firmness	0.412±0.028	0.467±0.028	0.628±0.038	0.529±0.012	0.761±0.032	0.000

Recipe	ST	LHT	HHT	LNT	HNT	P
a*	0.72±0.18	1.57±0.13	1.56±0.17	1.51±0.20	1.28±0.16	0.000
b*	18.21±0.32	18.74±0.68	20.29±0.22	20.33±0.33	21.05±0.37	0.000
C*	18.23±0.33	18.81±0.67	20.35±0.23	20.39±0.34	21.09±0.38	0.000
L*	91.23±0.73	91.16±0.51	90.42±0.80	90.21±0.99	90.24±0.86	0.013
h	87.76±0.55	85.20±0.52	85.61±0.45	85.76±0.50	86.53±0.38	0.000

Recipe	ST	LHT	HHT	LNT	HNT	P
Liquid loss	1.7409	2.2159	1.6882	1.9380	1.6154	0.005
SD	0.0643	0.3305	0.1834	0.2611	0.3832	
Water loss	1.3550	1.6459	1.3407	1.4846	1.3206	0.022
SD	0.0655	0.1849	0.1191	0.1959	0.2663	
Mass loss	0.3859	0.5700	0.3475	0.4534	0.2947	0.001
SD	0.0355	0.1558	0.0726	0.0807	0.1187	

Recipe	ST	LHT	HHT	LNT	HNT	P
Threshold	54.94±2.66	61.78±1.52	62.40±1.30	63.08±2.33	62.15±2.09	0.000

Recipe	ST	LHT	HHT	LNT	HNT	p
Smell						
Sour odor	4.165±0.888	3.150±1.156	4.010±0.644	3.600±0.743	3.685±1.039	0.007
Metallic odor	3.675±1.096	3.550±1.229	3.160±0.836	3.585±1.147	3.425±1.055	0.606
Dairy odor	4.320±0.710	4.100±1.019	4.760±1.087	4.415±0.840	4.180±0.972	0.205
Spicy odor	3.905±0.833	3.585±0.939	3.680±0.871	3.720±0.699	3.780±0.750	0.795
Fish odor	4.100±0.699	3.695±0.876	4.185±0.709	3.800±0.720	3.880±1.078	0.305
Cloying odor	1.215±0.360	2.500±2.013	1.360±0.602	1.820±0.821	1.855±1.115	0.005
Taste						
Sour flavor	3.915±0.957	3.020±1.155	3.865±0.861	3.885±1.184	3.760±1.293	0.059
Sweet taste	3.280±0.887	3.425±0.963	3.495±0.739	3.340±0.849	3.550±0.919	0.866
Salty taste	5.015±0.544	4.975±0.584	5.035±0.594	5.435±0.864	5.180±0.850	0.226
Bitter taste	3.790±0.890	4.065±1.111	4.500±1.029	4.405±0.922	4.350±0.899	0.139
Umami taste	3.945±1.021	4.210±1.235	4.090±1.214	4.205±1.191	4.315±1.386	0.897
Metallic flavor	3.980±1.338	4.105±1.507	3.920±1.395	4.095±1.690	3.870±1.626	0.984
Dairy flavor	4.055±0.644	3.915±0.949	4.070±0.731	4.090±0.522	3.890±0.658	0.847
Spicy flavor	4.270±0.709	4.205±0.889	4.575±0.859	4.545±0.614	4.765±0.855	0.156
Fish flavor	4.370±0.650	3.880±0.914	4.465±0.731	4.450±0.631	4.055±1.046	0.086
Cloying flavor	1.875±1.051	2.550±1.954	1.945±0.947	2.045±1.065	2.005±0.790	0.439
Aftertaste	5.580±0.836	5.920±0.958	5.880±0.866	6.060±1.266	5.580±1.239	0.694
Texture						
Firmness	1.980±0.601	2.295±0.875	2.975±0.986	2.205±0.723	3.080±0.917	0.000
Coarseness	2.965±1.053	3.060±1.167	3.925±1.167	3.210±1.120	3.965±1.103	0.008
Grittiness	6.125±1.404	6.205±1.338	6.505±1.370	6.330±1.324	6.345±1.240	0.917
Cohesiveness	2.360±0.924	2.660±2.036	3.130±1.009	2.470±1.001	3.275±0.905	0.011
Adhesiveness	6.335±1.464	6.895±1.063	6.630±1.033	6.220±1.316	6.380±1.222	0.429
Chalky	4.740±1.235	4.800±1.470	5.105±1.471	5.060±1.717	4.955±1.134	0.909

Appendix I

A selection of reported comments from the sensory panel from Nofima, Ås, which conducted a sensory evaluation on texture modified products and minced fish.

Comment	**1**	**2**	**3**	**4**	**5**	**6**
Texture modified products						
Odor	Butter	Curry	Burned	Cooked milk	Sour cream	Sour milk
Taste	Butter	Dried bread	Pepper	Burning	Flour	Sour milk
Texture	Butter	Cream	Adhesive	Curry	Crumbly	Used tounge
Minced fish						
Odor	Butter	Feed	Cooked milk	Wool	Spice	TMA
Taste	Butter	TMA	Nauseating	Spice	Sour cream	Milk
Texture	Blood	Metal	Fishbone			

Appendix J

Detailed information about conducted analyses from preliminary production of minced fish.

Recipe	4A	4B	4C	4D
Force	0.928±0.085	0.566±0.042	0.538±0.043	0.394±0.035
Gel strength	5.137±0.683	2.757±0.415	2.759±0.606	1.773±0.200
Stiffness	0.168±0.011	0.117±0.010	0.108±0.019	0.088±0.008
Distance	5.512±0.305	4.916±0.480	4.983±1.119	1.773±0.209

Recipe	5A	5B	5C	5D	5E	5F
Force	1.023	1.072	1.305	1.669	1.057	1.167
SD	0.162	0.138	0.155	0.251	0.093	0.109
Gel strength	6.926	6.935	7.852	11.186	6.708	7.803
SD	1.904	1.592	1.216	1.826	0.824	1.065
Stiffness	0.154	0.169	0.218	0.252	0.167	0.176
SD	0.021	0.023	0.025	0.046	0.012	0.019
Distance	6.700	6.419	6.007	6.738	6.708	6.230
SD	0.895	0.923	0.403	0.883	0.824	0.623

Recipe	5A	5B	5C	5D	5E	5F
a*	0.04	-0.16	-0.23±0.48	-0.01±0.31	-0.26	-0.22±0.12
b*	10.56	11.89	12.71±0.23	14.47±0.35	11.49	14.29±0.21
C*	10.56	11.89	12.72±0.22	14.47±0.35	11.50	14.29±0.21
L*	88.21	86.24	89.00±3.21	87.69±2.43	86.35	90.14±0.35
h	89.77	90.78	91.06±2.15	90.07±1.22	91.28	90.87±0.51

Recipe	5A	5D
Liquid loss	10.59±0.56	3.95±0.16
Water loss	10.55±0.534	3.99±0.16
Mass loss	0.14	

Appendix K

Detailed information derived from analyses in the pilot production of minced fish.

Recipe	SM	LHM	HHM	LNM	HNM	P
Hardness	6054.385	6854.860	6872.840	6424.899	7334.142	0.000
SD	630.911	333.791	318.697	485.312	338.585	
Force	5675.805	6337.888	6102.464	5976.495	6776.303	0.001
SD	550.976	357.470	298.341	472.596	293.417	
Adhesiveness	-2.240	-2.470	-2.281	-2.680	-3.070	0.248
SD	0.939	0.431	0.808	0.235	0.751	
Springiness	0.785	0.792	0.799	0.781	0.765	0.585
SD	0.030	0.041	0.029	0.018	0.050	
Cohesiveness	0.739	0.727	0.678	0.716	0.702	0.002
SD	0.027	0.036	0.020	0.014	0.018	
Gumminess	4468.135	4988.587	4656.341	4596.394	5145.623	0.012
SD	436.158	404.841	233.574	357.727	245.080	
Chewiness	3513.274	3957.134	3717.309	3595.404	3943.072	0.157
SD	422.524	472.456	215.133	345.665	404.586	
Resilience	0.412	0.387	0.357	0.384	0.383	0.000
SD	0.022	0.019	0.013	0.011	0.020	

Recipe	SM	LHM	HHM	LNM	HNM	P
a*	-0.48±0.30	-0.49±0.09	-0.08±0.07	-0.36±0.05	0.42±0.65	0.000
b*	11.11±0.16	14.14±0.22	14.98±0.22	13.17±0.12	17.36±0.61	0.000
C*	11.13±0.16	14.15±0.22	14.98±0.22	13.17±0.12	17.38±0.63	0.000
L*	92.09±0.98	88.90±1.01	88.88±1.46	91.57±0.97	84.88±1.40	0.000
h	92.46±1.53	91.98±0.36	90.31±0.28	91.58±0.28	88.66±2.08	0.000

Recipe	SM	LHM	HHM	LNM	HNM	P
Water Holding Capacity	94.34±0.37	96.43±0.63	96.01±0.61	94.51±0.41	95.38±0.28	0.000

Recipe	SM	LHM	HHM	LNM	HNM	P
Threshold	53.64±2.53	55.33±2.00	59.93±2.37	55.10±1.72	58.20±1.16	0.000

Recipe	SM	LHM	HHM	LNM	HNM	p
Smell						
Sour odor	3.055±1.075	2.835±0.790	2.355±0.993	3.115±1.255	2.540±1.245	0.131
Metallic odor	3.315±1.034	3.435±1.186	3.405±1.405	3.475±1.110	3.570±1.113	0.973
Dairy odor	3.955±0.964	3.830±1.100	3.775±1.370	3.970±0.964	3.850±1.429	0.982
Spicy odor	4.020±1.358	3.500±0.693	3.515±0.705	3.430±0.969	3.865±0.704	0.183
Fish odor	3.840±1.084	3.980±0.796	3.420±0.961	4.395±1.104	3.905±1.001	0.051
Cloying odor	2.540±1.426	2.960±1.914	3.515±1.936	2.840±1.665	3.505±1.788	0.321
Taste						
Sour flavor	3.225±1.144	3.225±0.985	2.325±0.990	3.060±1.321	2.355±1.144	0.013
Sweet taste	3.960±1.501	3.685±1.427	3.900±1.132	3.670±1.425	3.815±1.176	0.947
Salty taste	4.030±0.918	4.165±0.955	4.425±1.034	4.470±0.840	4.625±0.922	0.266
Bitter taste	3.480±1.110	3.730±0.959	4.305±1.153	3.925±1.048	4.330±1.085	0.060
Umami taste	3.240±1.110	3.595±1.054	3.635±1.109	3.690±1.031	3.605±0.954	0.680
Metallic flavor	3.550±1.210	3.805±1.259	4.000±1.605	3.800±1.265	4.015±1.291	0.807
Dairy flavor	3.775±1.050	3.740±1.052	3.535±1.293	3.565±0.948	3.495±1.318	0.911
Spicy flavor	3.945±1.566	3.580±0.924	4.025±1.063	3.645±1.065	3.810±0.814	0.679
Fish flavor	4.010±1.299	4.760±0.479	4.030±1.148	4.720±0.769	4.400±1.447	0.075
Cloying flavor	3.280±2.145	3.360±1.842	4.610±1.878	3.370±1.928	4.275±1.660	0.084
Aftertaste	5.135±1.165	5.210±0.926	5.940±0.906	5.275±0.959	5.825±0.773	0.019
Texture						
Hardness	3.885±0.815	3.750±0.861	3.740±0.784	3.785±0.768	4.275±0.660	0.168
Juiciness	4.490±0.774	4.330±0.945	3.480±1.142	4.410±0.868	3.330±0.727	0.000
Crumbliness	4.080±1.767	4.180±1.609	5.325±1.082	4.230±1.627	5.000±1.348	0.031
Gumminess	4.475±1.135	3.770±1.272	3.380±1.191	3.930±1.164	3.995±1.499	0.104
Adhesiveness	2.570±1.245	2.775±1.299	3.480±1.494	2.515±1.013	3.190±1.232	0.079
Astringency	2.465±1.330	2.665±1.388	3.065±1.386	2.595±1.218	2.745±1.169	0.665