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Abstract

Starch is one of the major ingredients that is incorporated into minced fish products due to its ability to modify the texture and improve the stability during refrigerated storage. Fish mince products were developed that contained different starches. Potato starch is a commonly used starch ingredient in fish mince products. The aim of this thesis was to produce fish mince products with specific textural and structural attributes and with a long shelf-life (up to 30 days), using an alternative and sustainable starch ingredient. It was also analysed what effect two different packaging methods (casings and modified atmosphere packaging (MAP)) had on the properties and on the shelf-life of the products.

Preliminary analyses of fish mince with different starches were performed to find suitable starches to use further in the pilot production being the shelf-life study. Based on the results found from the preliminary productions, two variants of fish mince were further analysed in the shelf-life study: one containing potato starch ("Potetmel", Hoff, Norway) and one containing native pea starch (AMN Pea Flour Concentrate Ground Pellet, Food Grade, Norway). Texture analyses of the potato starch variant (MAP), the potato starch variant (stored in casings) and the native pea starch variant showed that some of the samples from the pea starch variant showed lower measurements in hardness ($p < 0.001$), chewiness ($p= 0.001$), gumminess ($p < 0.001$), springiness ($p= 0.324$), cohesiveness ($p= 0.043$), and resilience ($p= 0.027$) than some samples from the other variants. The resulting parameters obtained from the texture profile analyses (TPA) suggest that the storage time and packaging have little impact on the texture of the products. All three variants had had a significant ($p < 0.001$) decrease in the water holding capacity (WHC) from day 17 to 28. The results from the colour measurements in the colour coordinate b* in the shelf-life study showed that the native pea starch variant had a more yellow colour than the potato starch variant. From the sensory evaluation a trend was observed within the taste of the samples correlating with the storage time, where the fish taste became more indistinct and less fresh/pure. The results from the microbiological analyses of the fish mince products correlated with the sensory evaluation of the samples on the last day of analyses (day 29), where there was an increase in the bacterial count of spoilage organisms found on the samples. Based on the microbiological analyses and the sensory evaluation of the variants the products were thought to have a shelf-life of at least 28 days. The textural and sensory properties of the fish mince variants suggests that native

pea starch could be a good and sustainable alternative starch ingredient in fish mince products.

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1. Introduction

Food consists of three major compounds: carbohydrates, proteins, and lipids which form a network of particles and molecules. Food properties such as texture, structure and stability are influenced by interactions between all the components of the product. Starch is one of the major ingredients belonging to the carbohydrates that is incorporated into minced fish products due to its ability to modify the texture and improve the stability during refrigerated storage (Tee & Siow, 2017). The textural properties of starch-based foods are mostly controlled by the interaction of starch polymers and water. The macromolecules of starch are amylose and amylopectin, found in foods such as potatoes, wheat, rice, cassava, peas, and other raw materials. It is widely used in food systems for thickening, gelling and stabilizing properties. The type and amount of starch is crucial to obtain an optimal recipe developed to meet the requirements of the consumer. Water-soluble proteins such as dairy proteins have thickening and gelling properties. Starches have thickening properties and absorbs water that enable a smooth surface of the product and stabilizes the product to enable a long shelf-life. Selection of the optimal product combination, in this case a blend with protein and starch, will give thickening and gelling properties desired in a fish mince product (Nieuwenhuyzen et al., 2006). When producing food products, testing is required to ensure that these requirements are met. Analyses of the appearance, taste and colour of foods are important to ensure a desirable product for consumers. The three main acceptability factors of foods being appearance, flavour, and texture (Bourne, 2002). Additional testing of properties like water holding capacity and shelf life are necessary to produce a safe food product of high quality.

The aim of this thesis was to produce fish mince products with specific textural and structural attributes and with a long shelf-life (up to 30 days), using an alternative and sustainable starch ingredient. In terms of sustainability, starch reduces food waste by extending the shelf-life of food products. Waste minimisation is an acute issue in today´s resource scarcity (Starch Europe, 2015). By producing starches in an economically friendly and sustainable way, it contributes to further minimization of food waste. Hence, it was desirable to use starches in the fish mince recipe that were produced in this manner. Fish mince products were produced using some of the most widely used starches for fish mince products, like potato, tapioca, and modified corn starch, as well as two variants of pea starches: native pea starch

and modified pea starch. The pea starches used in this thesis were produced by the Norwegian company AM Nutrition. AM Nutrition focuses on sustainable production of pea products (information provided by AM Nutrition, 2021). There is a lack of studies that have been examined on the use of pea starch in fish mince products and its effects on the textural and sensory properties of the products as well as properties like water holding capacity and colour measurements. Hence, specific research of the properties of pea starch in fish mince was the aim of this bachelor´s thesis. An overview of the experimental design and analyses is given in figure 1.

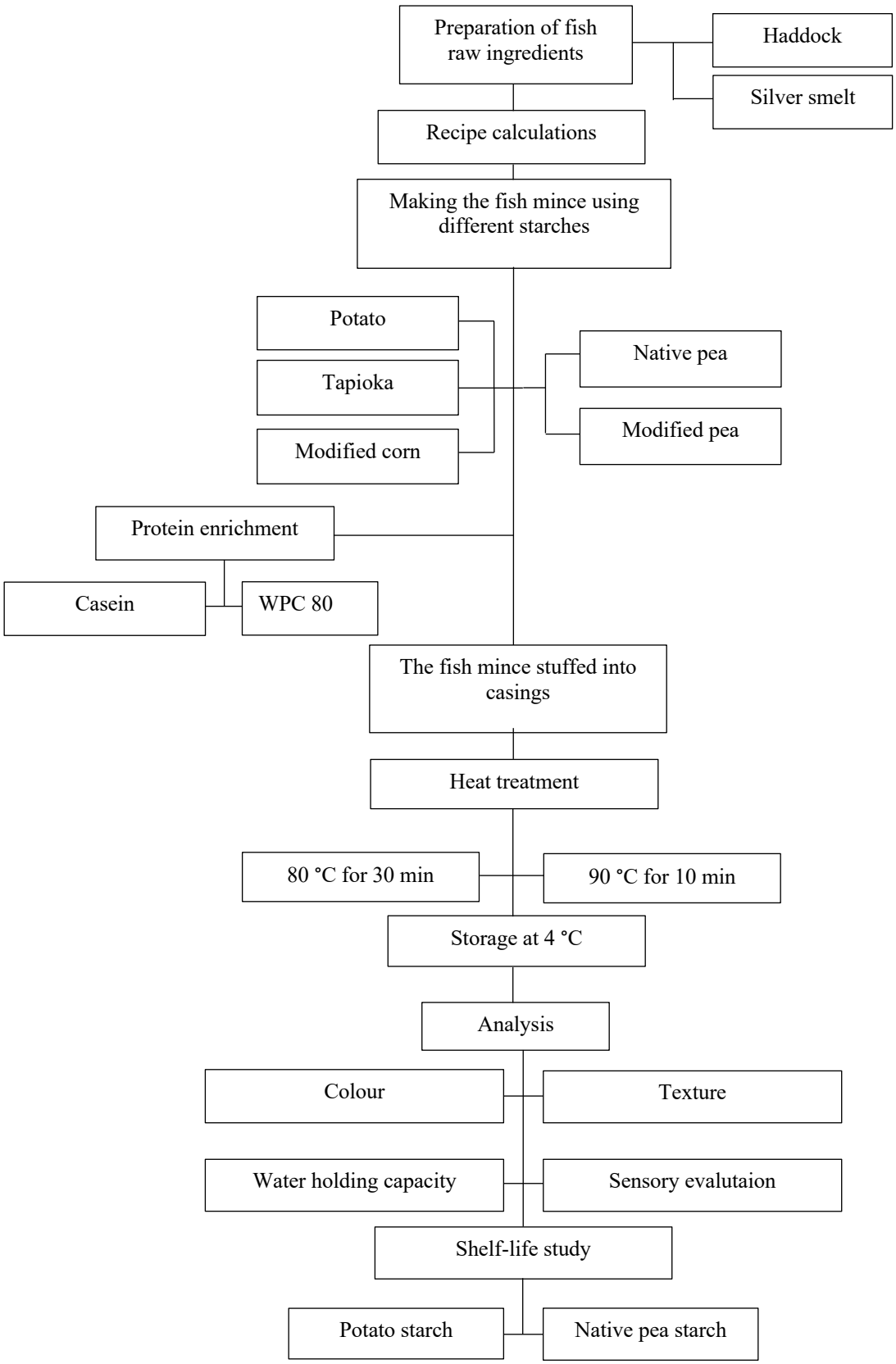

Figure 1. A workflow diagram of the production of minced fish products with different starch content.

2. Theory

The products and methods used in this thesis are described in the following subchapters. The basic elements of the fish mince are described first, followed by the analyses performed on the produced fish mince products.

2.1 Functional properties of starch

Hydrocolloids are a structurally diverse class of food polysaccharides found throughout nature. Most natural hydrocolloids are plant derived. Plant sources of hydrocolloids are the least expensive among other groups of hydrocolloids since they require less processing. The prime reason for use of hydrocolloids in food systems are thickening (Manzoor et al., 2020). The gelation properties of hydrocolloids are a result of hydrogen bonding, hydrophobic interactions, and cross-links between the polymer chains. Gelation involves the aggregation of polymer chains which provide the 3-dimensional network structure of gels. The more molecules involved in the aggregation, the more rigid the gel will be. Other parameters such as temperature and the structure of the hydrocolloid in question can influence the arrangement of the gel network, hence influence the rigidity of the gel (Manzoor et al., 2020).

Starch comprises many glucose units linked together through glycosidic bonds. The viscosity of starch gels depends on the particle size. A reduced particle size increases the viscosity and gel-like behaviour of the starch (Manzoor et al., 2020; Obadi & Xu, 2021). The physicochemical properties of native starch include gelatinization, swelling power, and solubility. Heating of starch in the presence of water promotes a process where the starch molecules swell and leads to an increase in viscosity. This process is known as gelatinization (Conde-Petit, 2003). The gelatinization temperature of starch is claimed to increase with the presence of additional ingredients (Tee and Siow, 2006). Gelatinized starch bind water as they are released from protein during protein denaturation. The swelling power represents the amounts of water a starch can absorb per gram of starch at a given temperature. As starch absorbs water and expands, it makes the gel network more compact and firmer. Solubility corresponds to the percentages of leached amylose and amylopectin (Obadi & Xu, 2021; Pietrasik & Soladoye, 2021). High percentage of amylopectin in starch, have been reported to allow better hydration which enhances swelling power, viscosity, gelatinization capacity and water binding. High amylose starches are more prone to water loss compared to starches with higher amylopectin (Pietrasik & Soladoye, 2021).

There are issues related to sustainability when it comes to all foods being produced, including starch. According to Starch Europe (2015) the three pillars of sustainability include economic pillar, environmental pillar, and social pillar. Starches are versatile food products used as ingredients and functional supplements in food. The demand for carbohydrates will increase in line with the increasing population of the world. Farmers in the EU must comply with some of the strictest rules and regulations in the world in regards of biodiversity, preservation and development of natural farming systems, water management, and climate change management. These regulations are provided by the Common Agricultural Policy. In addition, rural development programmes exist that promote environmentally sustainable farming practices. One of the core concepts in the European starch industry is waste minimization. The starch industry processes every part of the plant, less than 1 % is not valorised (Starch Europe, 2015).

In this thesis different starches were used in the production of fish mince products, including native starches and modified starches. Bourne (2002) defines native foods as those where the original structure of the agricultural goods remains essentially intact. Starches can be modified in many ways, but the texture of native foods can only be changed by heating, cooling and size reduction (Bourne, 2002). One way of modifying starches is using a heat-moisture treatment. This method is typically used to control the molecular mobility of the starch functional groups. These molecular alterations influence the physicochemical and structural attributes of the modified starch system. Typically, the thermal applications used in heat-moisture treatment of starches are mainly from dry-heat sources from convection ovens, microwave systems and steam-heat from autoclave systems (Dudu et al., 2019). In fish mince products starch at 3-12 % is commonly added and the most frequently used starches include potato, corn, and tapioca (Tee and Siow, 2006). Yoon et al. (1997) reported that starch added at lower concentrations (<3 %) is more effective in increasing the gel strength of fish mince products than higher concentrations (6-9 %). Other studies have also shown that potato starch and corn starch added at 0-6 % yield the best properties in gel strength (Tee and Siow, 2006). In this thesis 3 % starch was used in the production of fish mince. This ratio of starch was used to bring out the desired gelatinization properties of starch. The ratio was kept relatively low to prevent the fish mince products to become too firm and compact.

2.2.1 Potato, Tapioca, and modified corn starch

Potato and tapioca starch are characterized by low gelatinization, high viscosity, and quick swelling (Obadi & Xu, 2021). Potato starch has a low gelatinization temperature and is preferable to use in products where a high gel strength is desired. The low gelatinization temperature allows for better starch granule swelling and results in high gel strength of the product (Tee and Siow, 2006).

Cassava (*Manihot esculenta*) also known as tapioca, is a tuberous root shrub widely cultivated in Africa, Latin America and Asia. It is valued for its ability to grow under harsh climatic conditions, and it is a cheap source of flour and starch. Tapioca is a starch with a bland flavour and have excellent thickening and gelling attributes (Dudu et.al., 2019).

Modified corn starch (Farinex™ WM 55, Arne B. Corneliussen AS, Netherlands) was used in the preliminary production of this thesis. Farinex is an acetylated distarch adipate of waxy maize, meaning it is a modified starch. This type of modified starch is obtained by esterification of food starch with acetic anhydride and esterification/cross-linking with adipic anhydride. Acetylation results in substitution of hydroxyl groups on the starch molecule with acetyl esters (FAO, 2017). Waxy maize starch, also known as waxy corn starch, consists of almost only amylopectin molecules (Schwartz & Whistler, 2009). Hence, waxy corn starch has great swelling power and gelatinization capacity (Chapter 2.1).

2.2.2 Pea starch

Pulses, including peas, have been important components of the human diet due among others to their content of starch and protein. Pulses are defined as legumes harvested solely for their seed which is consumed directly (Dahl et al., 2012). Pulses play a key role in improving food security and in creating more sustainable and climate-resilient food systems. The nutritional and environmental value of pulses are still underestimated, and consumption remains low in Western Europe. However, consumers are interested in a healthy and sustainable diet and understanding their behaviours could help grow the pulse industry. Few studies have examined consumer´s preferences for pulse products (Paffarini et al., 2021). Peas, specifically the yellow or green peas known as dry, smooth, or field peas, are naturally dried seeds of *Pisum sativum*. These peas are grown around the world for human and animal consumption. Peas have long been recognised as an inexpensive and readily available source

of protein and carbohydrates as well as vitamins and minerals. The high nutrient density if peas make them a valuable food commodity. *Pisum sativum* consists of 46 % starch. Pea starch contains an intermediate level of amylose. Specifically smooth peas contain 27,8 % amylose (Dahl et al., 2012).

In this thesis pea starch manufactured by a Norwegian company called AM Nutrition was used. AM Nutrition is owned by the Agro-Cooperative Felleskjøpet Rogaland Agder which focuses on sustainable production of pea fractions. They produce pea protein, pea starch and pea fiber concentrates by air-classification of yellow peas (*Pisum sativum* L.). Air classification is a technological method of separating particles according to their size. The production process is a "dry" process with no addition of water, and with a minimal use of energy (information provided by AM Nutrition, 2021).

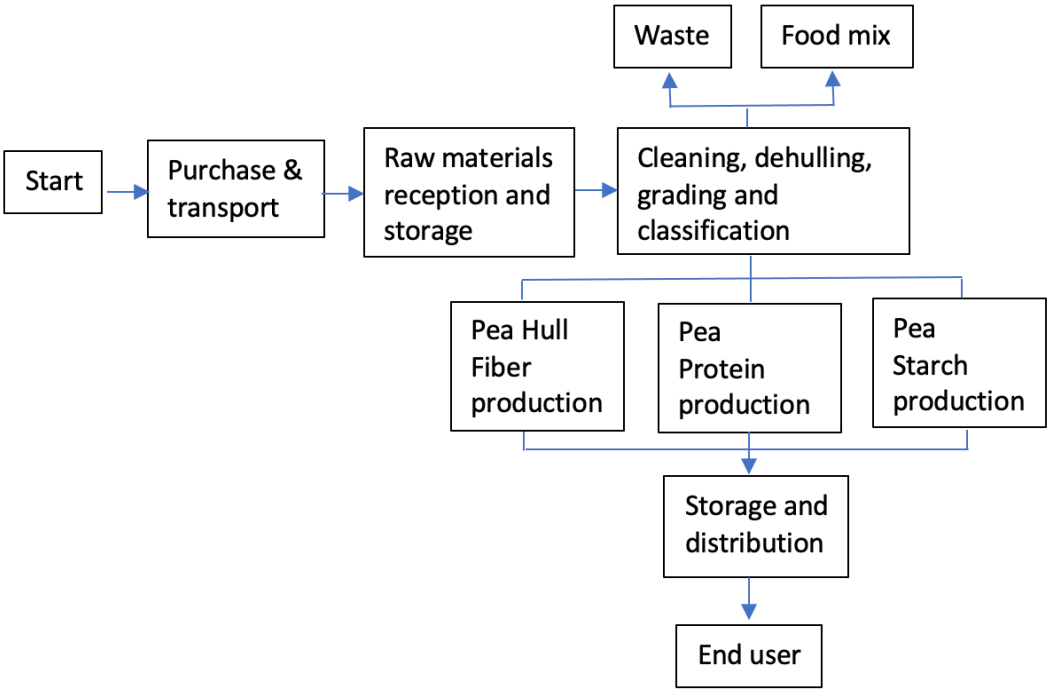

Figure 2.2.2. Process diagram for pea flour concentrate (information provided by AM Nutrition, 2021).

Studies have demonstrated the potential of both potato and tapioca starch in various fish- and meat-based products (Tee and Siow, 2006). Thus, the aim of this thesis was to evaluate the quality of fish mince products containing different starches, one of them being pea starch. Also considering the economic part, pea starch is a useful ingredient to study further for use

in fish mince products. In this thesis two variants of pea starch were used, one native pea starch and one modified pea starch called.

2.3 Proteins

Haddock (*Melanogrammus aeglefinus)*

A variety of fish species can be used as ingredients in fish mince. Cleaned and clean-cut fish fillets of ordinary whitefish species can be used. Haddock is a common fish species used in fish mince. The ability of fish proteins to bind water is the property that has the greatest significance for the consistency in the finished product. The binding ability is affected amongst other factors by the salt and temperature of which the fish mince is produced with. The salt causes the fish proteins to absorb water and the water-binding ability increases. (Vatland A.I., 1991).

Silver smelt (*Argentina silus*)

Since the binding capacity of fish is greatly reduced by freezing, freeze storage and thawing, the addition of frozen raw material should be limited in the production of fish mince. Frozen silver smelt has been proven to be an exception to the rule that the binding capacity is reduced by freezing. Silver smelt that is properly processed retains its water-binding ability better than other fish species (Vatland A.I., 1991). In 1974, the Canning Industry´s Laboratory in Norway made the first major experiments using silver smelt in fish mince. Silver smelt was found to have unusually good binding properties. In addition, it showed that it can withstand both freezing and mechanical treatment without losing its binding properties. Fish mince using silver smelt mixed with other fish species made it possible to start year-round production of fish mince based on frozen raw materials (Sivertsvik, 2021).

2.3.1 Dairy proteins

Milk contains two main types of proteins: whey proteins and caseins. Casein and whey proteins are dairy proteins that have an emulsifying effect and can increase the water-binding ability in fish mince (Vatland A.I., 1991).

Dairy proteins may interact and form chemical complexes during heat treatment. Whey proteins aggregate with each other and bind to casein and form whey protein/casein

complexes. Chemical complexes between milk proteins are known as coaggregates of milk proteins. The degree of chemical complex formation is influenced by the degree of heat treatment. The interaction between whey proteins and caseins increases with increasing temperature from 75 °C to 90 °C (Jovanovic et al., 2005). The fish mince products produced in this thesis were enriched with whey protein concentrate (2 %) and casein (1 %). This ratio of protein enrichment was chosen based on previous work derived from Therese N. Østebrød (2020). Based on her work the given ratio of WPC and casein proved to provide good gelling properties in fish mince products.

Whey proteins

Some of the most important products based on whey proteins are whey protein concentrate (WPC) and whey protein isolate (WPI). WPC and WPI have excellent functional and nutritive properties and are therefore widely used in the food industry. Whey protein concentrates have protein content varying from 35-80 %. Ultrafiltration is the most widely used method to produce WPC. WPI have higher levels of protein and lower levels of lipids, lactose and salts and are therefore functionally better than WPC, but due to the higher cost of production their production is limited (Jovanović et al., 2005;(Nicolai & Chassenieux, 2021)

Whey proteins are heat-labile proteins and thermal treatments cause change of their physicochemical properties including water holding capacity and gelling properties. Whey proteins are completely denatured after heating at 90 °C for 5 minutes. The ability of whey proteins to form gels capable of holding water and other components while providing textural properties is very important to the consumer acceptability of many foods. WPC´s have different gelling capacities and the gelling process is influenced by temperature, duration of heating, pH, ionic strength, concentration of salt, protein sugar and lipids (Jovanović et al., 2005).

Casein

Casein is organized as micelles, spherical complexes with different diameters. (Nicolai & Chassenieux, 2021;(Ptiček Siročić, 2017). The micelles are stable at temperatures up to 90 °C but coagulate at higher temperatures. The presence of whey proteins influences the heat-induced gelation of casein micelles, due to the whey protein/casein complexes formed during

heat treatment (Nicolai & Chassenieux, 2021). The presence of both whey proteins and casein in a fish mince product will provide better gelling abilities.

2.4.1 Texture analysis

The definition of texture states as follows: “texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics” (Szczesniak, 2002).Several tests have been developed to measure the texture of a food product. The tests performed in this thesis are gel-test and texture profile analysis (TPA). During texture analyses force is applied to the food samples. Force is often expressed in N (newton). The force is measured at the surface of the sample and is considered an external variable (Lu & Abbott, 2004).

The firmness (N) of a food product can be measured in a penetration test. In this thesis the puncture test is referred to as a gel-test. The gel-test measure the force (N) required to puncture a probe through the sample as well as the distance (mm) required before penetration occurs. The depth of the probe into the sample is held constant (Bourne, 2002). The force applied when penetration occurs express the resistance of the sample to compression. The penetration distance at the point of penetration is an expression of the elasticity of the sample. The penetration distance reveals the quality of the protein in the fish mince product. If a product has a longer penetration distance it indicates that the quality of the raw material is higher (Lu and Abbott, 2004; Øines, 2020).

TPA is a test where a bite-size piece of food is compressed two times imitating the action of the jaw. The sample is placed on a baseplate and compressed and decompressed by a selected probe (compression plate) attached to the texturometer. The compression plate must be of greater size than the sample to be tested. The textural properties obtained by the TPA test are hardness (N), adhesiveness (N.sec), springiness, cohesiveness, gumminess, chewiness, and resilience. Hardness is defined as the height of the force peak on the first compression cycle. Adhesiveness is defined as the negative force area of the first compression and represents the work necessary to pull the compression plate away from the sample. Springiness is defined as the distance that the food sample recover its height during the time that elapsed between the end of the first compression and the start of the second compression. Cohesiveness is defined as the ratio of the positive force areas under the first and second compressions. Gumminess is derived by calculating the product of hardness x cohesiveness. Chewiness is defined as the

product of gumminess x springiness, which is derived from hardness x cohesiveness x springiness. Resilience is defined as the degree to which the sample returns to its original shape in terms of speed and forces derived (Bourne, 2002).

2.4.2 Water holding capacity

The water holding capacity (WHC) of food products depend among other on heat-induced structural changes. The food industry and consumers WHC is of importance in relation to juiciness and firmness of the food product, although it is not known how much the loss of WHC affect the juiciness of the food product. Sensorial analysis has shown that juiciness is increasingly lost with increasing heat load and both WHC and juiciness is related to the heat load applied to the food product (Skipnes et al., 2007).

The WHC describe the ability of the heat-treated food product to withhold water during centrifugation. A sample is centrifuged at constant speed for a desired time and the liquid loss is measured as the amount of liquid that passes through the filter of the sample cup. The WHC is determined by the weight loss of the sample in percent of the initial weight of the sample. In addition, dry matter was determined and used for calculation of the WHC of the sample (Skipnes et al., 2011; Skipnes, 2011).

2.4.3 Colour measurements

The colour of food products is a sensory attribute. The presence of one stimulus may influence the judgement of another stimulus. Hence, the overall impression of a food product defines whether a product is desirable to consumers (Szczesniak, 2002). Colour measurements was performed during analyses in this thesis to compare the effect of different starch content on the colour of the fish mince products.

The colour of food products acts as an important impression to consumers. Mathematical models help to describe colours by fixed values. The most common model is three-dimensional and creates a colour with parameters. The International Commission on Illumination (CIE, Commission Internationale de lÉclairage) calculated the colour space L*a*b*. The colour coordinates in the CIEL*a*b* space lies opposite to each other, and every colour is described by the three components L*, a* and b*. L* describes the lightness from black to white, where 0 means black and 100 is the maximum light intensity possible without causing eye damage. a* describes colour in the green-red field and ranges from -128

(green) to +127 (red). b* describes colour in the blue-yellow field and ranges from -128 (blue) to +127 (yellow). In the middle of the ranges (a*= 0, b*= 0) only grey values exist. The colours lying on opposite sides of the L* plane and on the a*b* plane can´t be seen simultaneously, meaning that either dark or bright is seen, either red or green and either yellow or blue is seen (Mokrzycki and Tatol, 2011).

2.4.4 Sensory evaluation

Consumers wants to eat attractive, nutritional, and balanced foods with a good flavour. Food properties are influenced by interactions between the components of the food product (Nieuwenhuyzen et.al., 2006). Hence, sensory analyses were performed on the fish mince samples containing different starches to detect differences in the overall sensory attributes. Heat-treatment and storage conditions are also factors that affect the sensory attributes of food products.

The four principal quality factors of food are appearance, flavour, texture, and nutrition. Appearance comprises factors such as colour, shape, and size. Flavour comprises taste and odour, the so-called “chemical senses”. Texture is primarily defined as the response of the tactile senses to contact between the body and the food product. The tactile sense is the primary method to sense texture. Sensory evaluation is defined as the measurement of a product´s quality based on information from the five senses. Correlating measurements of physical properties with sensory analyses performed by people is important to provide a better basis for the overall quality of a product (Bourne, 2002).

2.5 Shelf-life: Food safety

The shelf-life of a food product is influenced by many aspects of manufacturing and storage conditions, and these influence the microbial growth on the food product (Betts, 2021). The properties of the raw materials and the ingredients, the processing treatments, and the technology- and packaging materials set limits on the safe shelf-life. If the shelf-life is assigned too short, then the manufacturing costs may by high and the profit low. If it is too long, then there is potential for food spoilage or growth of pathogens and the product will not meet the requirements of food safety.

Sources that may contribute to the microbial content of products are the raw materials used, ingredients, equipment, and production personnel. Fish mince is exposed to higher risk of infection throughout the whole product than whole fish fillets, due to the mixing and broader handling process and addition of ingredients that may contain microorganisms. The shelf-life of fish mince products must therefore be ensured by an adequate heat treatment that is designed to the desired shelf life.

When choosing time-temperature combinations for heat-treatment, one must choose a target organism for the heat-treatment and then select the level of deactivation of the target organism. Recommended inactivation of some pathogens that are common in raw materials and ingredients are given in Table 1.

Table 1. Application of heat and hurdle principles of mild heat-treated foods (Modified by Rosnes et.al. 2011).

Target organism	Heat resistance (min) [a]	Recommended heat-treatment	Storage conditions to inhibit surviving organisms
Psychrotrophic *C.botulinum* type E	D_{90}= 1,5	90 °C/ 10 min 6 D non-proteolytic *C.botulinum* type B and E	< 10 °C [b]
L.monocytogenes and other non-spore forming pathogens	D_{70}= 0,3	70 °C/ 2 min 6 D *L.monocytogenes*	< 3 °C [c]
Bacillus cereus	D_{100}= 1,36	100 °C/ 48 min	< 4 °C

[a] Most heat resistant species in the target group (ECFF 1996)
[b] 10 °C- lowest growth temperature for proteolytic *C.botulinum*. If food is likely to support *B.cereus* growth, the limit should be lowered to 4 °C, which is the lowest growth threshold for psychrotrophic *B.cereus*.
[c] 3,0 °C- lowest growth threshold for non-proteolytic *C.botulinum* (Graham et.al. 1997).

The microbiological flora changes during heat-treatment and vegetative pathogens like *Listeria monocytogenes* are inactivated at temperature higher than milk pasteurization (70 C for 2 min). Spore forming bacteria like *Clostridium botulinum* and *Bacillus cereus*, are left in the product. Non-proteolytic *Clostridium botulinum* produces a powerful toxin in food that causes botulism. It is important to control spores from *C. botulinum* that may still be present in the food after heat treatment because they may germinate leading to toxin formation in a favourable environment (Callaghan, 2008). A 6-log reduction of *Bacillus cereus* require 100 °C for 48 min (Table 1). This is a treatment that is higher than acceptable for minced products, based on sensory properties. In the shelf-life study of this thesis, Bacillus cereus specific media was used to detect surviving spores. The criteria for heat tolerance from *Clostridium* are often used as a basis when choosing time-temperature combinations for heat-treatment. It is desirable to heat-treat the product as little as possible to maintain a high

quality of the product. For pasteurization non-proteolytic *Clostridium botulinum* type E is used as target organism. Pasteurization is a heat treatment that inactivates a lower number of bacteria than sterilization. During heat treatment the food product will undergo changes that can be quantified as a pasteurization value. The pasteurization value was calculated based on the heat treatment during the shelf-life production and was used in the safety evaluation for determination of microbial inactivation of *Clostridium botulinum.* An internationally accepted reduction value for pasteurization is a lethality by 6 log units (Grönqvist et al., 2014).

A common time and temperature combination applied to achieve pasteurization is 90 °C for 10 min (Table 1) (Callaghan, 2008). Pasteurization at 80 °C for 30 min was previously used in Norway for fish mince products to be sold in grocery stores, stored chilled (Vatland et.al. 1991). Some of the ingredients are not activated in the mince until it is heated to a certain temperature, such as heat-swelling starches (Chapter 2.2.). Thus, when choosing time-temperature combinations, it is necessary to evaluate both food safety aspects and functionality of the ingredients. In the preliminary production in this thesis both time-temperature combinations, 90 °C for 10 min and 80 °C for 30 min, were applied. In addition to heat treatment packaging can be used to inhibit bacterial growth. A commonly used packaging method is modified atmosphere packaging (MAP) where the use of CO_2 inhibits bacterial growth (Sivertsvik et.al., 2002). CO_2 dissolves into moist food products during storage and the inhibitory effect of bacterial growth is proportional to the amount of dissolved CO_2. A high gas volume to product volume ratio increases the dissolution of CO_2 into the food product. Some concentrations of CO_2 commonly used in the headspace of the package are 60 % and 40,2 % (Hansen et al., 2016). For the remaining ratio of gas in the package, N_2 is used as a fill gas. Normally, the food in the package fills up about 2/3 of the container and the remaining 1/3 is filled with gas. The food products produced in the shelf-life production in this thesis filled up a smaller portion of the package. Therefore, to obtain an equal amount of CO_2 dissolution, the package was filled up with a lower gas volume to product volume ratio.

3 Materials and Methods

3.1 Preliminary production

Preliminary productions of fish mince with different starches were performed to find suitable starches to use further in the pilot production being the shelf-life study. Two different pasteurization temperatures and times were tested for the preliminary production of the potato starch (Appendix B) variant. Pasteurization of 90 °C for 10 min and 80 °C for 30 min were tested. In the production of the other variants being tapioca (Appendix C), modified corn (Appendix D), native pea (Appendix E) and modified pea (Appendix F), pasteurization of 90 °C for 10 min was applied.

Preparation of fish raw material

Prior to production of fish mince, the fish raw ingredients were prepared. Fresh haddock fillets were delivered to Nofima by fish distributor Domstein AS. The haddock originated from the Northeast Atlantic (FAO 27). Shortly after arrival excess skin and bones were removed from the fillets, and were then cut into smaller pieces of 600 g. The fillet pieces were packed in plastic bags (220x600mm, PA/PE 70my, LietPak, Lithuania) and vacuumed at 99,9 % vacuum using a vacuum machine (Supermax C, Webomatic, Germany). The packages were stored in a freezer room (-30 °C) until further use. The silver smelt processed by Tavan, Faroe Island was delivered to Nofima in frozen blocks of 7 kg each which were cut into 600 g pieces using a bandsaw (SX350, Dadaux, France). The silver smelt pieces were vacuum packed (99,9 % vacuum) in the same plastic bags as the haddock and stored at -30 °C. Large quantities of fish were prepared and frozen to avoid unnecessary variables in the fish mince batches. The results, especially from microbiological tests, could have been affected if the fish used in the different fish mince productions came from different batches.

3.1.1 Ingredients

Table 3.1. The ingredients used in the fish mince in preliminary productions and in the shelf-life study.

Ingredient	%
Haddock fillet	30,0
Silver smelt	30,0
Salt	1,0
Milk 3,5 % fat	25,0
Starch	3,0
Sunflower oil	8,0
WPC 80	2,0
Casein	1,0
Total	100,0

For more information about the ingredients used in the production of fish mince products see Table A in Appendix A.

3.1.2 Production procedure

Prior to production the frozen fish raw materials were thawed at 0 °C for 15-18 hours. The rest of the ingredients were weighed in accordance with the recipe for production of 2 kg batches of minced fish. The liquid ingredients and the fish were placed on a tray (GN 1/1) filled with ice slurry (figure 3.2) to keep the temperature of the ingredients as low as possible before mixing.

Figure 3.2. Weighed ingredients and raw materials placed on ice before mixing.

The ingredients were mixed using a cutter (Robot Coupe R 5 V.V.) (figure 3.3) following the blending times and speeds in Table 3.2. The fish ingredients and salt were added first to

utilize the binding ability of the fish. The salt acts on the fish proteins and causes them to swell. Starch was added at the end and used as a friction source to raise the temperature of the fish mince. At a temperature of around 14 °C the starch grains rupture and begin to form a gel (Vatland et.al., 1991).

Table 3.2. Mixing times and speeds used in the preliminary production.

Order of additions	Ingredients	Mixing time (min:sec)	Mixing speed
1	Fish raw materials (Haddock and Silver smelt)	00:15	12
2	Salt	00:30	30
3	Milk, 3,5 %	00:40[a] 00:20[b]	6[a] 30[b]
4	Oil	00:20	30
5	Starch	00:10[c] 00:40[d]	6[c] 30[d]
6	WPC		
7	Casein		

a: The milk was poured slowly into the mixture at low speed.

b: Mixing time and speed after addition of milk.

c: Initial mixing time and speed after addition of the dry ingredients.

d: Second mixing time and speed after addition of the dry ingredients, and after the ingredients stuck on the sides in the mixer were detached with a spatula.

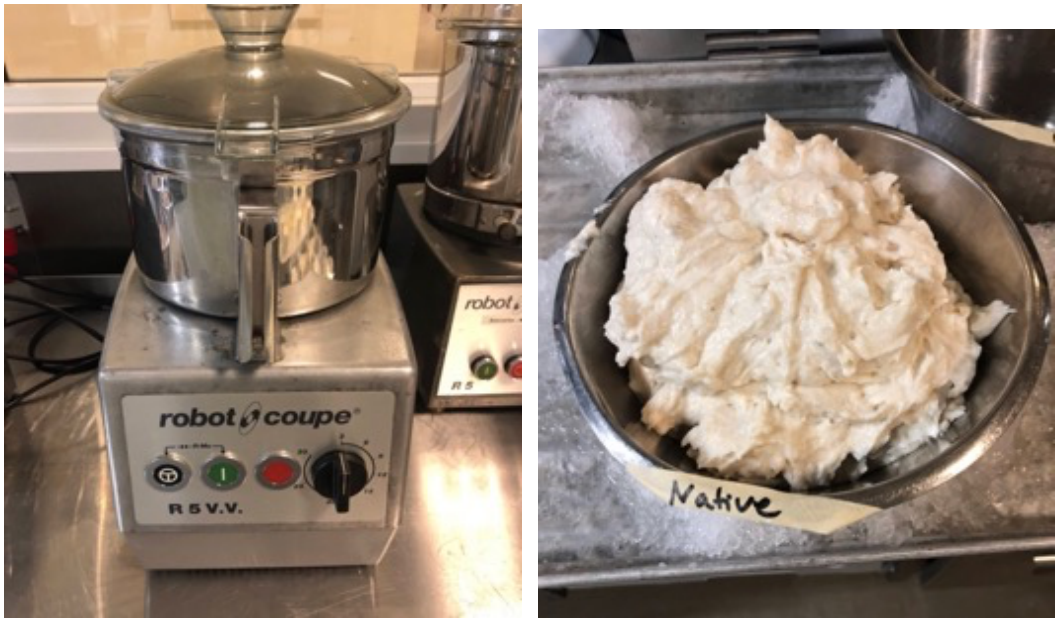

Figure 3.3. Left: Robot Coupe mixer used to blend all ingredients and raw materials. Right: One batch of freshly mixed fish mince with native pea starch.

After mixing the fish mince was filled into casings (Betan, ART: 4210002500, Ø30 mm white, Viscofan, Czech Republic) using a manual sausage stuffer (Model 5 Litre De Luxe, Tre Spade, Italy) (Figure 3.4). The fish mince began to form gel after mixing and it was therefore stuffed into casings immediately after mixing. Samples of 20±5 cm were made. The samples were sealed with clips (S 632, poly-clip System, Germany) by using a single clip machine (SCH 120, poly-clips System, Germany). Prior to heating three casings with minced fish (the potato starch variant) were equipped with a temperature probe (Testo 176T4, Testo SE & Co, KGaA, Germany) before they were sealed. Pre-cut pieces of plastic polystyrene were put in at the end of the samples to fix the temperature probe in a central position of the sample (Figure 3.5). It was important to place the probes as close to the centre of the samples as possible to measure correct core temperature and to ensure sufficient heat-treatment of the samples. If the probes were placed closer to the sides inside the samples, the temperature probes would show too high temperature compared to the core of the samples and the heat-treatment would be insufficient. A fourth probe was used to measure the temperature in the oven during heat-treatment.

Figure 3.4. The manual sausage stuffer (Model 5 Litre De Luxe, Tre Spade, Italy) used to stuff the fish mince into casings.

Figure 3.5. Left: A temperature probe cut out from a heat-treated sample. The hole in the sample shows where the probe was positions during heat treatment. Right: Temperature probes inside three different samples with cords attached to the data storage device (Testo 176T4, Testo SE & Co, KGaA, Germany).

Two temperature and time combinations were applied in the production of the potato starch variant, 90 °C for 10 min and 80 °C for 30 min (Chapter 2.5). Both temperature and time combinations were applied to test the effect it had on the properties of the fish mince product. The samples were placed into a preheated convention oven (MSCC61, Metos system Intl., Germany) set to 100 °C and 100 % steam. The core temperature was monitored during cooking (Figure 3.5). A timer was started once the samples were placed in the oven. When the core temperature of the sample with the lowest temperature reached 80 °C, the come-up time was noted. The come-up time to reach 80 °C was applied when cooking the second batch, instead of using temperature probes to monitor the core temperature during cooking. Then the temperature in the oven was lowered to 80 °C and the samples were cooked further for 30 min. The same procedure was used when cooking samples at 90 °C. When the sample with the lowest core temperature reached 90 °C, the oven temperature was reduced to 90 °C and a timer was used to monitor the cooking for 10 min. After cooking, the samples were

placed in a bucket with iced water for cooling. The temperature probes were still inside the samples during cooling. When the samples reached a core temperature of 4±2 °C, they were stored in a chilled room set to 4 °C.

In the production of the other variants (tapioca, modified corn, native pea, and modified pea) the same come-up time for a core temperature of 90 °C was applied during heat-treatment. The same cooling time (approximately 30 minutes) was applied for the cooling of the samples before storage.

3.1.3 Texture analyses

The texture of samples from the different fish mince variants (potato starch, tapioca starch, modified corn starch, native pea starch and modified pea starch) was measured to reveal which variants were suitable to be analysed further in the shelf-life study. Gel tests were performed on the samples to measure the firmness (N) of the different fish mince variants. In addition, a texture profile analysis (TPA) was performed to gain a broader understanding of the overall texture of the samples.

Samples from each of the six fish mince variants were retrieved from the chilled room (4 °C). Each sample was sliced into 2 cm thick pieces using a sharp knife after the casings were removed. The samples were placed in round aluminium containers (Round, 106 ml Ø80, Plus Pack, Denmark) and covered with plastic cling wrap. The representative selection consisted of a triplicate from three samples (n= 3x3) from each variant. An equal number of parallels (n= 3x3) was also measured on a commercial fish pudding ("Fiskepudding", produced by Maritim Food AS for Rema 1000, Norway) for comparison. All the following texture analyses were performed on room tempered samples (21±2 °C).

Gel test

A texturometer was used to measure the texture of the fish mince samples, TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). The project file contained the parameters and components needed to run an analysis of a specific product. To measure the firmness of the samples, the TA.XT instrument was equipped with a 5 kg loading cell and a cylinder probe (Derlin cylinder P/5S, Stable Micro Systems, Godalming, UK) (Figure 3.6). The firmness was measured as the required force (N) needed to penetrate the sample. The

force and probe height were calibrated before analysis. The force was calibrated using a calibration weight of 2 kg. The probe height was calibrated for 35 mm (Table 3.3). The TA.XT instrument was set to apply a trigger force of 5 g, and penetrate the samples to a depth of approximately half of the total height of the product, 12 mm (Table 3.3).

The TA.XT instrument was adjusted with the following settings:

Table 3.3. Setting for gel-test.

T.A. Settings		Probe height	
Test mode	Compression	Return distance	35 mm
Pre-test speed	1,00 mm/sec	Return speed	10 mm/sec
Test speed	1,50 mm/sec	Contact force	5 g
Post-test speed	8,00 mm/sec		
Target mode	Distance		
Distance	12,00 mm		
Trigger Force	5,0 g		

The data was collected by the software exponent (Version 6.1.18.0, Stable Micro Systems, Godalming, UK). A selected *Macro* analysed the measured data, and the resulting values were copied into Microsoft ® Excel ® 2021 (Version 2110) for further interpretation. The macro contained a list of instructions that was performed on the measured data automatically.

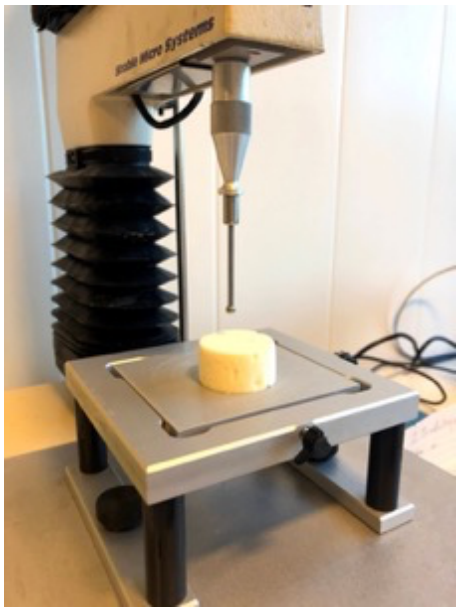

Figure 3.6. TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with the cylinder probe P/5S, ready to perform a penetration test on a sample.

Texture Profile Analysis (TPA)

A TPA is a so called “two-bite test” that simulates chewing of the samples (Chapter 2.4.1). TPA was performed on the samples to gain information about how the samples are affected in an eating situation. TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) was used to perform the TPA on the samples. The TPA test obtained the textural properties: hardness (N), adhesiveness (N.sec), springiness, cohesiveness, gumminess, chewiness, and resilience (Chapter 2.4.1). The instrument was equipped with a 50 kg loading cell and a compression plate (P/75, Stable Micro Systems, Godalming, UK). The force was calibrated using a calibration weight of 10 kg, and the probe height was calibrated for 30 mm (Table 3.4). The TA.XT instrument was adjusted with the settings listed in Table 3.4.

Table 3.4. Settings for TPA..

T.A. Settings		Probe height	
Pre-test speed	2,00 mm/sec	Return distance	30 mm
Test speed	3,00 mm/sec	Return speed	10 mm/sec
Post-test speed	5,00 mm/sec	Contact force	5 g
Target mode	Strain		
Strain	60 %		
Time	5 sec		
Trigger Force	5,0 g		

The compression plate performed two compressions with a 10 sec pause in-between each compression as shown in figure 3.7. All the data was collected by the software exponent (Version 6.1.18.0, Stable Micro Systems, Godalming, UK), and further analysed by a selected *Macro*. The analysed values were copied into Microsoft ® Excel ® 2021 (Version 2110) for further interpretation and presented in bar charts.

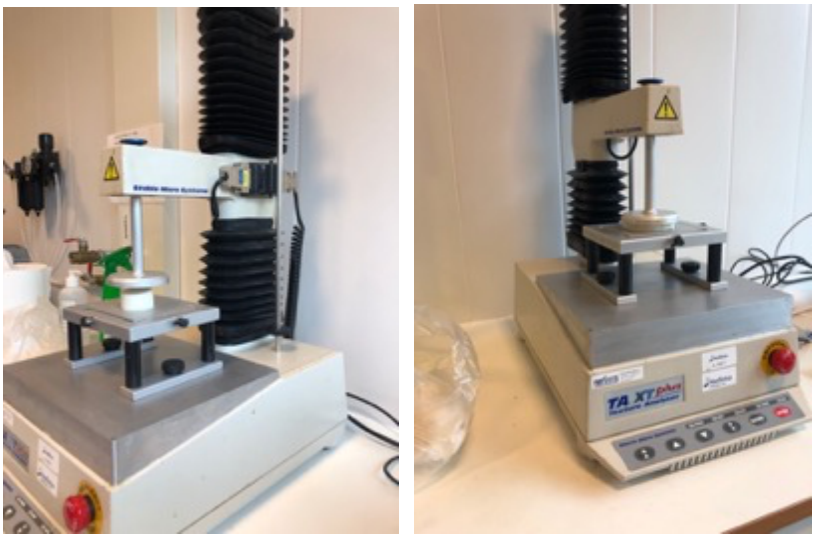

Figure 3.7. TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with the compression plate P/75. Left: The instrument is ready to perform a compression test on a sample. Right: The instrument is performing a compression on a sample, about to return to the initial height.

3.1.4 Water holding capacity

The water holding capacity (WHC) of the samples from each variant of fish mince was determined to compare the ability of the variants to hold water. The WHC was determined based on a method from Skipnes, Østby and Hendricx (2007) with alterations. The method determined water holding capacity, liquid loss, and dry matter. The parameters were calculated using the following equations and were expressed as percentage of the initial sample weight:

$$Water\ holding\ capacity\ (\%) = \frac{100 - dry\ matter - liquid\ loss}{100 - dry\ matter} * 100 \quad \text{(Equation 1)}$$

$$Liquid\ loss\ (\%) = \frac{V1 - D1}{V1} * 100 \quad \text{(Equation 2)}$$

$$Dry\ matter\ (\%) = \frac{D3}{D2} * 100 \quad \text{(Equation 3)}$$

The parameters V1-V6 were determined by weighing.

V1 (g)= Sample before centrifugation

V2 (g)= Sample and cup before centrifugation

V3 (g)= Sample and cup after centrifugation

V4 (g)= Aluminum bowl

V5 (g)= Aluminum bowl and sample before drying

V6 (g)= Aluminum bowl and sample after drying

The parameters V1-V6 were used to calculate the parameters D1-D3.

D1 (g)= V3- (V2-V1)

D2 (g)= V5- V4

D3 (g)= V6-V4

The sample cups (Patent No. 321375 B1) used to measure water holding capacity were assembled according to figure 3.8. A Styrofoam box was filled with ice slurry and a sheet of aluminium foil covering the ice, which the sample cups were placed on top of (Figure 3.9). The fish mince samples were cut to fit the diameter of the sample cups using a stainless-steel core sampler (28 mm). The samples were cut into pieces weighing 5±2 g. The empty sample cups were weighed, before the cups and samples were weighed together respectively. The samples were centrifuged (Rotina 420R, Andreas Hettich GmbH & Co. KG, Germany) at 1800 rpm for 15 minutes at 4 °C.

Figure 3.8. Sample cups used to measure the water holding capacity of the fish mince. From left: filter, two bottom pieces, the cup itself, already assembled sample cup.

Figure 3.9. Samples of fish mince in the sample cups prepared for WHC analysis.

After centrifugation, the sample cups were placed back into the Styrofoam box. One by one, the bottom of the sample cup was removed, blow-dried using compressed air, before assembled back and placed back into the Styrofoam box. The sample cups with the centrifuged samples were then weighed again.

To calculate the percentage of dry matter, aluminium containers (Round, 106 ml Ø80, Plus Pack, Denmark) were used. Empty aluminium containers were weighed, before samples of 5±2 g was crumbled and placed into the bowls as shown in figure 3.10. The containers and samples were weighed together respectively. They were then dried for 18-22 h at 105 °C before they were weighed in dry state.

Figure 3.10. Aluminium containers with fish mince samples before (left) and after (right) drying for 18-22 h at 105 °C.

3.1.5 Colour measurements

To detect any differences in colour and appearance among the different starches used in the preliminary testing colour measurements were performed. Samples were prepared as described in chapter 3.1.3. The same samples were used for colour measurements before they were used in TPA. Colour measurements were performed using a DigiEye system (VeriVide Ltd., UK) consisting of a digital camera (Nikon D90, AF Nikkon 35 mm f/2D, Nikon, Japan) and an imaging cube. DigiView (VeriVide Ltd., UK) was used to take the photos, while DigiPix (VeriVide, Ltd., UK) was used to perform the colour measurements. Before analysis the DigiEye system was calibrated using a white- and colour calibration boars (DigiTizer Calibration Pack, VeriVide Ltd., UK). The samples were put on a black board as shown in figure 3.11 and placed inside the imaging cube. The extracted colour coordinates, CIEL*a*b*, were copied into Microsoft ® Excel ® 2021 (Version 2110) and presented in a bar chart.

Figure 3.11. Sample photograph taken by the Nikon camera (Nikon D90, AF Nikkor 35 mm f/2D, Nikon, Japan) and DigiEye system (VeriVide Ltd., UK). The samples are taken from the production batch made with modified corn starch (Farinex).

3.1.6 Sensory evaluation

Sensory evaluation was performed on the fish mince preliminary variants to gather information and detect differences in appearance, odour, taste, and texture. Fish mince containing tapioca starch, modified corn starch, native pea starch, modified pea starch and potato starch (heat-treated at both 80 °C and 90 °C) were retrieved from the chilled room. They were sliced into approximately 0,5 cm thick pieces, placed on cardboard plates, and covered in plastic cling wrap. The evaluation was performed on room tempered samples

(18±2 °C), by a semi-trained panel. Brief descriptions within the sensory properties were provided and collected in tables (Table 4.1.9). The commercial fish pudding was left out in the sensory analysis.

3.2 Shelf-life study

One aim was to develop a product with a shelf life of up to 30 days. A 28-day shelf-life study was conducted for the fish mince products stored at 4 °C. Based on the results found from the preliminary productions, two variants of fish mince were further analysed in the shelf-life study: one containing potato starch ("Potetmel", Hoff, Norway) and one containing native pea starch (AMN Pea Flour Concentrate Ground Pellet, Food Grade, Norway). The fish mince samples containing potato starch heat-treated at 80 °C for 30 min and at 90 °C for 10 min achieved approximately similar results in texture. Due to food safety, the pasteurization temperature of 90 °C for 10 min was applied for the shelf-life study. The fish mince samples containing pea starches showed results that were the most different from the samples containing potato starch. The samples containing native pea starch showed more desirable results and was therefore chosen to be further analysed and compared with fish mince containing potato starch, in the shelf-life study.

Table 3.2. Timetable of the various analyses performed on following days after production.

Day nr.	Analyses performed:
0	Production of fish mince, heat-treatment, and packing (MAP).
1	Microbiological analyses, TPA, colour measurements, WHC.
2	TPA of heated samples.
7	Microbiological analyses, TPA, colour measurements, WHC.
17	Microbiological analyses, TPA, colour measurements, WHC.
28	Microbiological analyses , TPA, colour measurements, WHC.
29	TPA of heated samples.

The differences the packaging made on the storage condition during the first few days in the shelf-life study were assumed to be insignificant. The potato starch samples stored in MAP and casings were assumed to be equal the first days in the shelf-life study. Therefore, the

analyses conducted of the potato starch variant were performed only on samples stored in MAP on day 1, 2 and 3 after production.

3.2.1 Production procedure

The raw fish ingredients and the dry ingredients were prepared as described in chapter 3.1. The ingredients used for the production are listed in Table 3.1. Due to a larger volume of the production batches, the ingredients were mixed using an industrial cutter (MTK 662, MADO GmbH, Germany) (Figure 3.12). Plastic bags filled with ice were placed inside the cutter to cool it, prior to mixing. The fish was added first, and the cutter was run on low speed. Salt was added and the cutter was running at low speed until the mixture reached a temperature of 2 °C. Milk and oil was added while mixing on low speed, then on high speed until the mixture reached a temperature of 12 °C. The dry ingredients were added while mixing on low speed and then on high speed until the mixture increased to a temperature of 17 °C to ensure a high enough temperature for the starch to begin to form gel. Starch increased the friction and caused the temperature of the mixture to rise. Therefore, starch was added last in the mixing process. After the mixing was completed, the cutter was set to run at low speed for a few minutes to remove air bubbles accumulated during mixing. The mixture was filled into the same casings used in the preliminary production, but with a bigger motorized sausage stuffer (H15, Talsa, Talsabell a.s., Spain). Three temperature probes (Testo 176T4, Testo SE & Co, KGaA, Germany) were installed in three casings to measure the core temperature, one casing from the mixture containing potato starch and two casings from the mixture containing native pea starch. A fourth temperature probe was used as a control for the oven temperature. The samples were sealed using the same clips and single clips machine described in chapter 3.1.2. The samples were heat-treated in two convention ovens (MSCC201, Metos system Intl., Germany) set to 100 ℃ and 100 % steam. Once the temperature probe inside the sample with the lowest core temperature reached 90 ℃, the oven temperature was reduced to 90 ℃ and a timer was set to 10 min. After heat-treatment the samples were immediately placed in buckets with ice slurry water. When the samples had been in ice water for about 50 minutes, they had cooled to 4 ℃.

Figure 3.12. Mixed fish mince inside the industrial cutter (MTK 662, MADO GmbH, Germany) used in the production for the shelf-life study.

To simulate a repacking procedure often performed by the industry after heat treatment, two different packaging methods were used in the shelf-life study: Modified atmosphere packaging (MAP) and casings (Betan, ART: 4210002500, Ø30 mm white, Viscofan, Czech Republic). After cooling one half of the potato starch samples were left in the casings used during heat treatment, while the other half and all the samples from the pea starch variant were unwrapped from the casings. These samples were cut into 2 cm pieces, where three and three pieces were placed in CPET trays (C 2187-1F Black CPET- Faerch Group, Denmark) before they were flushed with 47 % CO_2 and 53 % N_2 and sealed using a lidding film (Cryovac OSF33ZA, PET sealant, thickness 33 µm, oxygen permeability 60 $cm^3/m^2/24$ h/bar (23 °C, 0 % RH), Sealed Air, Norway) by a tray sealer (Multivac T200, Multivac Group, Germany) (Figure 3.13). Random samples were analysed for gas mixture during packing to ensure the correct ratio of gases were filled into the packages. A gas analyser (PBI Dansensor Checkmate 9900, Ringsted, Denmark) was used to measure the atmosphere inside the packages. The O_2 level should be around 0,05 % and the CO_2 level should be around 47 % inside the packages after packing. The O_2 level was expected to rise during storage, and the CO_2 level was expected to sink during storage. Before each microbiological analysis, the gas level inside the packages to be tested, was checked. The packages and the casings were stored in a chilled room at 4 °C.

Figure 3.13. Left: Modified atmosphere packing machine with finished sealed packages containing three sample pieces each. Right: A random sample check of the atmosphere inside the packages.

3.2.2 Texture profile analysis (TPA)

Due to a more complex spectre of texture parameters obtained from TPA in contrast to gel-test, only TPA was performed on the samples in the shelf-life study. The TPA of the fish mince samples from the shelf-life test followed the same procedure and the same settings as the preliminary samples described in chapter 3.1.3. On day 1 after production, three packages from each variant, potato starch and pea starch, were retrieved from the chilled room (4 °C). Two pieces from each package (n= 2x3) were measured using TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) with the same settings as described in chapter 3.1.3.2. On day 7, 17 and 28 in the shelf-life study TPA was measured in the three pieces from two packages (n= 3x2) from each variant, in addition to two samples from three casings (n= 2x3) from the potato starch variant. The total number of parallels for each variant analysed was n=6.

TPA of heated samples

The previous texture profile analyses were all performed on room tempered samples. In addition, TPA was performed on heated samples from the shelf-life study. Samples were

taken from the start (day 2) and the end (day 30) of the shelf-life test to detect possible changes in texture during storage. This procedure was performed in such a way where the samples were first heated to a specific temperature (63 °C) and analysed, and then put in room temperature (21±2 °C) and analysed again after 30 minutes. The procedure was performed this way to simulate an eating situation.

On day 2 after production, five packages from each mixture (potato and pea) were retrieved from the chilled room. Three samples were used to monitor the temperature during the whole process. One sample from the potato starch variant, and two samples from the pea starch variant were put in separate round aluminium containers (Round, 106 ml Ø80, Plus Pack, Denmark). Temperature probes (Testo 176T4, Testo SE & Co, KGaA, Germany) were placed in the centre of each sample by penetrating the probe through the side of the aluminium container as shown in figure 3.14. Each container was wrapped in plastic cling wrap. Six samples from both variants were placed in two aluminium trays (GN ½) with associated lids, one tray per variant. The samples including the ones equipped with temperature probes, were placed in a preheated convention oven (MSCC201, Metos system Intl., Germany) set to 100 °C and 100 % steam. The samples were heated to a core temperature of 63 °C due to the regulation from the food and beverage industry in Norway to keep the temperature of food at 60 °C or warmer until serving ("Næringsmiddelhygieneforskriften", 2009, Chapter 5). After re-heating, the samples were transferred to a food warming trolley (Termia 950 H, Metos, Finland) (Figure 3.14), preheated to 75 °C, to keep the temperature of the samples above 60 °C until analysis.

Figure 3.14. Left: Temperature probe inside a sample in an aluminium container wrapped in plastic wrap. Middle: A temperature probe penetrated through an aluminium container into a sample. Right: The food warming trolley used to keep the samples warm until analysis (Termia 950 H, Metos, Finland).

TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) was used to perform the TPA on the heated samples. The TA.XT instrument was calibrated and set with the same settings as described in chapter 3.1.3. The samples were retrieved from the food warming trolley and analysed one by one to ensure a constant core temperature. First, three samples from two packages (MAP) (n= 3x2) of each variant were analysed while the core temperature was still around 60±3 °C. Then the same number of samples (n= 3x2) including the samples equipped with temperature probes were placed in room temperature. After 30 min in room temperature, the samples were analysed. The core temperature of the samples was between 30-33 °C during the last analyses. TPA on heated samples was performed again on day 30 after production, following the same procedure as described above except:

3.2.3 Water holding capacity

The water holding capacity was determined following the same procedures as described in chapter 3.1.4. On day 1 after packing, three packages from each variant were retrieved, and one sample from each package was analysed (n=1x3). On day 7, 17 and 28 one sample from three packages from each variant in addition to one sample from three casings were analysed (n= 1x3).

3.2.4 Colour measurements

The colour of the fish mince samples from the shelf-life test was measured in the same way as described in the preliminary production in chapter 3.1.5.

3.2.5 Food safety: Microbiological analyses

The aerobic count and specific spoilage organisms in the fish mince products were determined using the NMKL 184 (2006) method. Agar solutions and solid plates were prepared prior to analyses. Iron agar, 0,85% peptone salt diluent and Long & Hammer-agar (L&H-agar) was prepared following the NMKL 184 (2006) method. The iron agar was used to determine total viable counts and black colonies, often determined as hydrogen sulphide producing bacteria, and the pour plate technique was applied. L&H-agar was used to determine the psychrotrophic aerobic plate counts using spread plating. Commercial plates with *Bacillus cereus* selective agar were bought in advance (Oxoid CM0617). The plates contained PEMBA medium developed by Holbrook and Anderson (1980). Typical *Bacillus cereus* colonies give peacock blue colonies with precipitate and peacock blue medium, while other *Bacillus* strains give straw-coloured colonies.

Preparation of samples

On day 1, 7, 17 and 28 after production, microbiological analyses were performed of samples. On day 1, three samples of 25 g each (n= 3) were retrieved from each of the two different fish mince variants, both raw samples and heat-treated. The samples were transferred into sterile blender bags (Separator 400, Grade packaging Ltd, UK) and the bags were filled with 0,85 % peptone salt diluent until a total weight of 250 g. A blender SMASHER® (AES blueline, bioMérieux, France) was used to homogenize the samples (Figure 3.15) The machine was set to homogenize for 120 sec at 560 strokes/min. After homogenization, each sample was diluted by ten-fold dilution series. Homogenized sample water was poured into sterile tubes (Falcon tubes, 50 ml, Sarstedt, Germany), which was the first dilution (10^{-1}) in the ten-fold dilution series. 400 µl homogenized sample water was pipetted into sterile tubes (Falcon tubes, 15 ml, Sarstedt, Germany) and diluted in 3600 µl 0,85 % peptone salt diluent. The dilution series were made up to 10^{-3}-dilution.

Figure 3.15. Left: Blender bag containing sample and 0,85 % peptone salt diluent, ready to be homogenized. Middle: Open blender SMASHER® (AES blueline, bioMérieux, France). The picture shows how the blender bag is placed inside the blender, and the plates used to beat the bag homogenizing the content. Right: Blender bag with sample already homogenized.

1 ml from dilution 10^{-1} and 10^{-2} from each dilution series was plated in iron agar via the pour plating technique. The plates were incubated upside down at 25 °C for 72 hours. Dilution 10^{-1} and 10^{-3} was spread on L&H-agar using Eddy Jet (Eddy Jet v.123, Nerliens Mezansky, Norway) (Figure 3.16) and incubated at 15 °C for 7 days. 0,1 ml from dilution 10^{-1} from each series was spread on Pemba plates using a sterile L-shaped rod. The plates were incubated at

30 °C for 7 days. After the incubation period, the colony forming units (cfu) were counted on all media.

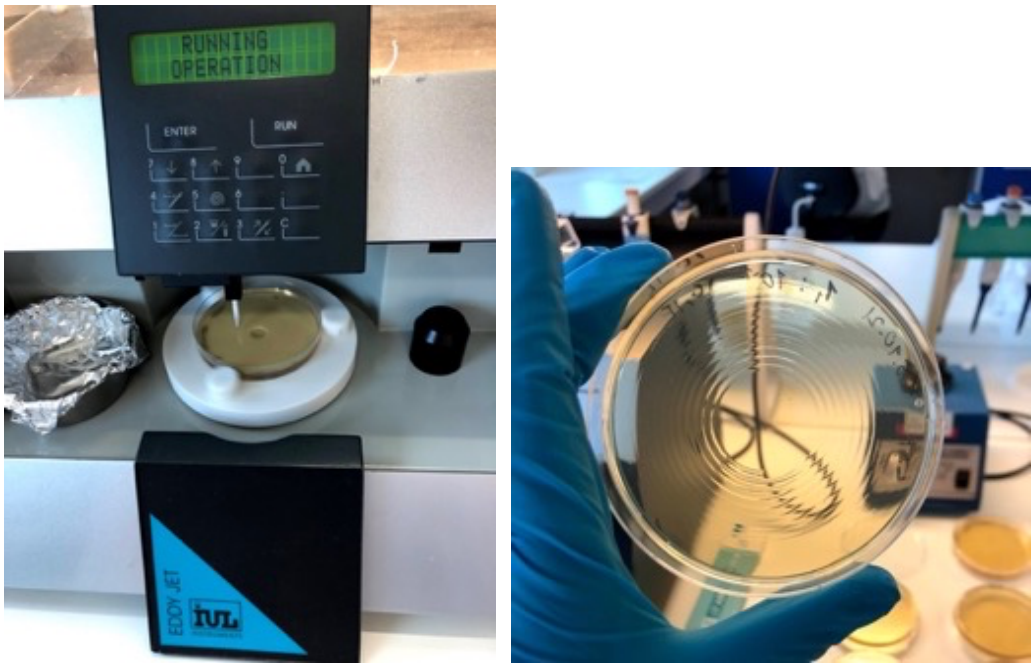

Figure 3.16. Left: Eddy Jet (Eddy Jet v.123, Nerliens Mezansky, Norway) spreading sample dilution on a plate with L&H-agar. Right: L&H-agar with sample spread on it by Eddy Jet.

3.7 Sensory evaluation

Two packages from each MAP-variant were retrieved from the chilled room on day 3 and 29. One package from each variant was served at room temperature (18±2 °C), while the other was served warm (58±1 °C). All samples were put into a porcelain bowl with lid (Figure 3.7). The warm samples were heated at 80 °C (100 % steam) for 10 minutes in a convection oven and kept warm at 60 °C until serving. The room tempered samples were evaluated first, then the tempered samples. A semi-trained panel evaluated the samples based on appearance, odour, taste, and texture blindly in an open discussion. Brief descriptions within each category were provided and collected in Table 4.2.8.

Figure 3.7 Samples in porcelain bowls with associated lids prepared for heating in the sensory evaluation of the shelf-life study.

3.7 Statistical analysis

Data from the analyses from the preliminary production and the shelf-life study were tested for significant differences using one-way ANOVA in Minitab 19 Statistical Software (Minitab Ltd., UK, 2020). ANOVA is an analysis of variance where the level of significance was determined at $p<0.05$. The test used to find possible significant differences was Tukey´s Pairwise Comparison test. All results are given as mean± SD unless stated otherwise.

4 Results and Discussion

4.1 Preliminary production

Fish mince products with different starches were produced in the preliminary production to find suitable starches. The starches used were potato starch, one of the most common starches used in fish mince products, tapioca, modified corn, native pea, and modified pea starch. All measurements and analyses performed on the preliminary products were conducted on three samples from three packages, n=3x3.

4.1.5 Recipe development

Two different pasteurization time and temperature combinations were tested for the potato starch variant to find the most suitable combination to use for the other variants. The

pasteurization time and temperature combinations tested were 90 °C for 10 min and 80 °C for 30 min.

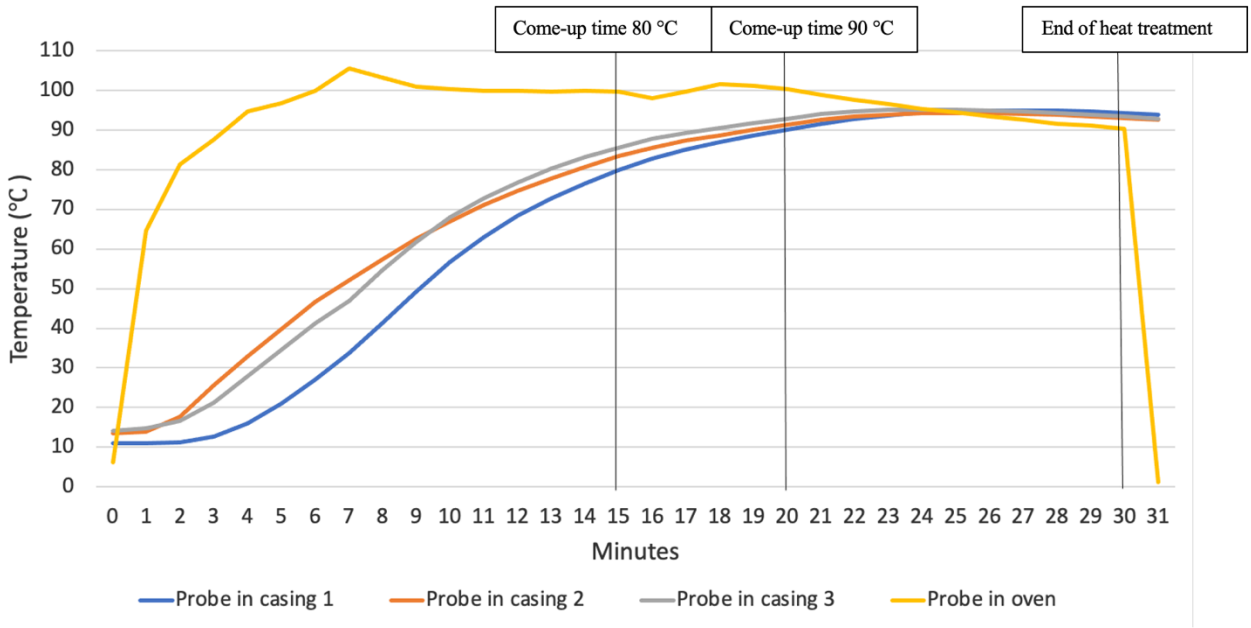

Figure 4.1.5 The figure shows the monitored core temperature of the samples during heat treatment in the preliminary production of the potato starch variant. The samples were heat treated at 90 °C for 10 min. Probes 1, 2 and 3 were placed inside three separate samples. Probe 4 was placed inside the oven.

Figure 4.1.5 shows the temperature monitoring during the heat-treatment of the potato starch variant. Temperature probes were placed in three separate casings to monitor the core temperature of the samples during heat-treatment. As describe in Chapter 3.1.2 the heat-treatment at 90 °C for 10 min was used to find the come-up time for the core temperature to reach 80 °C, that was later used in the heat-treatment at 80 °C for 30 min instead of using temperature monitoring with probes. In figure 4.1.5 the vertical line furthest to the left shows the come-up time for the core temperature in the samples to reach 80 °C (15 minutes). The vertical line in the middle of the figure shows the come-up time for the core temperature to reach 90 °C. A timer was set to 10 minutes at this point (after 20 minutes in the oven). The vertical line to the right in the figure represents the end of the heat-treatment, when the samples had been cooked at 90 °C for 10 minutes, and the samples were placed in ice slurry water for cooling.

4.1.6 Texture analysis

Recipes in the preliminary production were developed by comparing different starch variants. The texture of the fish mince variants produced in the preliminary production was measured in a gel-test and a texture profile analysis (TPA) (Chapter 3.1.3).

Gel-test

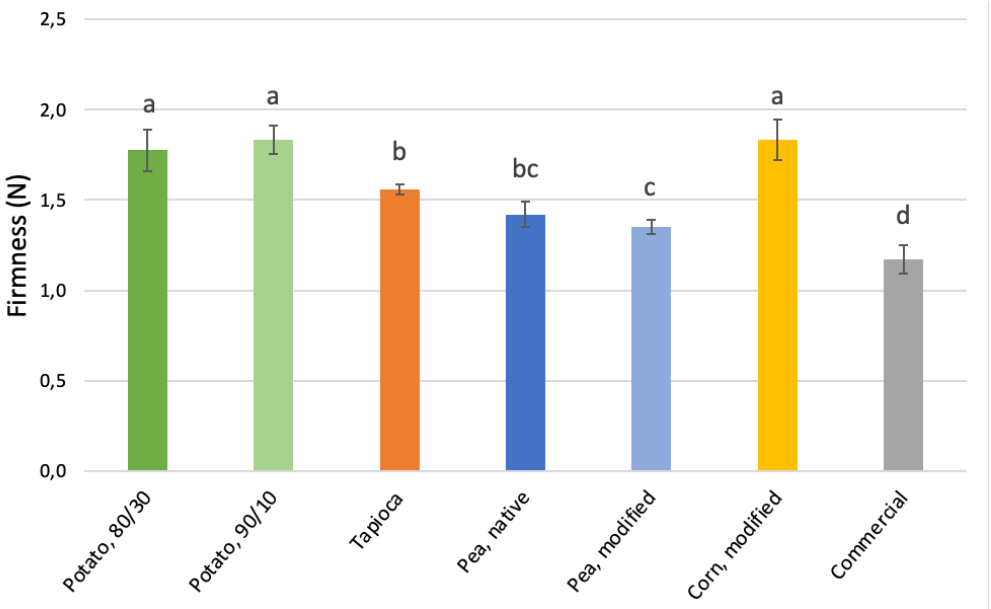

Figure 4.1.6.a The figure shows the measured values for firmness (N) in the gel-test of the fish mince variants produced in the preliminary production.

Figure 4.1.6 shows the measured values from the gel-test which provided measurements on the firmness (N) of the samples. The fish mince variants containing potato starch and modified corn starch revealed the highest values of firmness, i.e., they were the samples that required the highest force (N) over distance (mm) before penetration occurred (Bourne, 2002). The fish mince variants containing tapioca and native pea starch were significantly ($p < 0.001$) less firm than the potato starch and modified corn starch variants, but the tapioca variant was firmer than the modified pea starch variant and the commercial fish pudding. The commercial fish pudding was the least firm. This was not expected as it contained the same starch type as the tapioca variant produced in the preliminary production. However, the amount of starch in the commercial fish pudding was unknown. Hence, the significant difference in firmness between the produced tapioca variant and the commercial fish pudding may be explained by a lower amount of starch in the commercial fish pudding. Although further examination is needed to support this explanation.

Texture profile analysis

Texture profile analysis (TPA) was performed on the samples produced in the preliminary production to detect differences in texture based on different starches used in the recipes. The parameters obtained in the TPA correlates with sensory analysis (Bourne, 2002).

Figure 4.1.6 Fish mince samples from the potato starch variants heat-treated using the two different time and temperature combinations in the preliminary production after TPA was performed. Samples to the left was from the variant heat-treated at 90 °C for 10 min, samples to the right were from the variant heat-treated at 80 °C for 30 min.

Figure 4.1.6 shows a picture of samples from the potato starch variant in the preliminary production after TPA was performed on them. The picture is included as an example of how the samples appearance changed after TPA. Some samples had visible cracks and were more destroyed while other samples looked the same after TPA was performed.

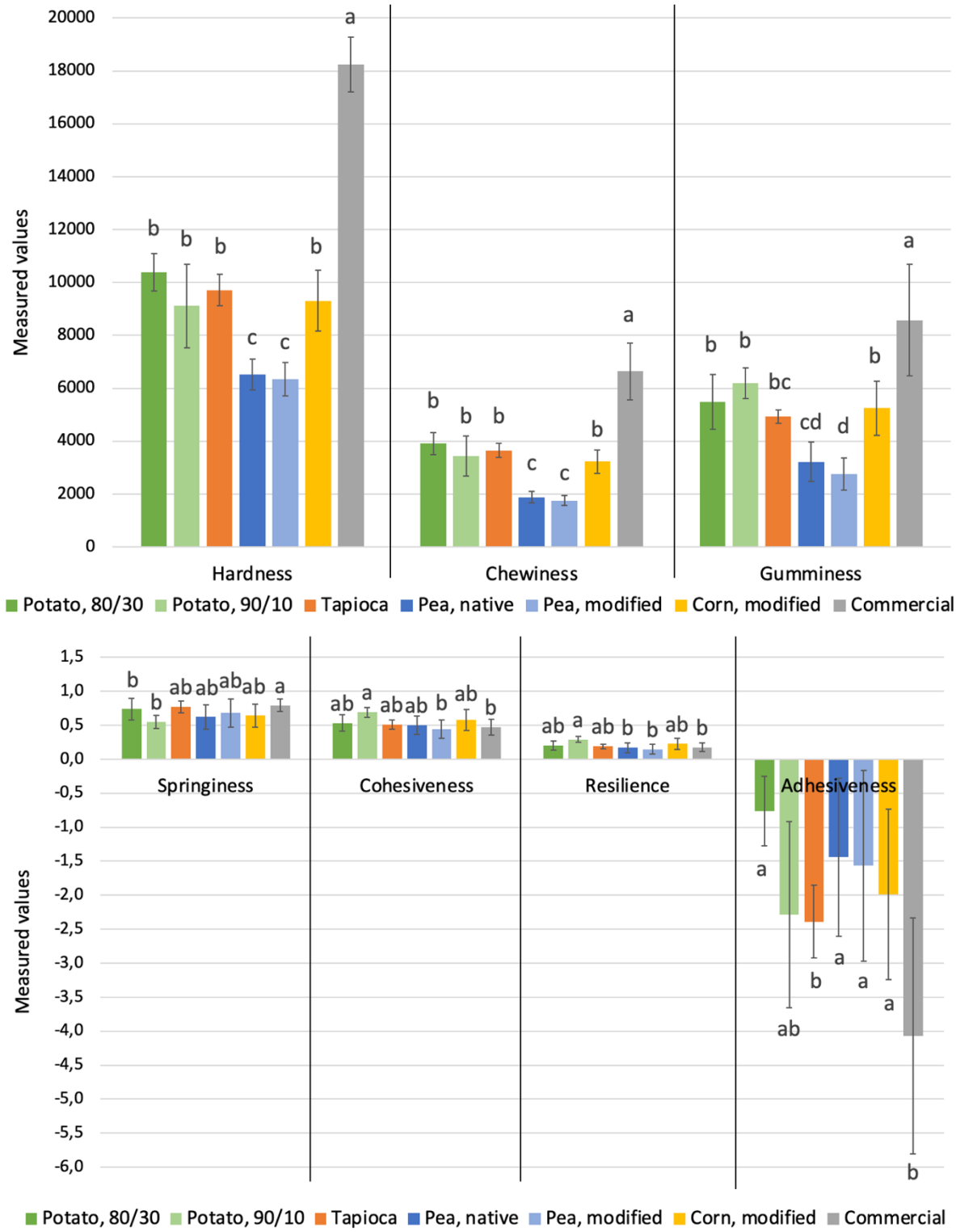

Figure 4.1.6.b The figure shows the measured values for TPA of the fish mince variants produced in the preliminary production.

The commercial fish pudding was significantly ($p < 0.001$) the hardest of the samples. The potato starch variants, the tapioca and the modified corn starch variant were significantly harder than the native and modified pea starch variants. Similar results were observed in the

chewiness ($p < 0.001$) and gumminess ($p < 0.001$) of the samples. The potato starch variants were significantly less springy ($p= 0.032$) than the commercial samples. Springiness is defined as the distance the food sample recover its height between the two compressions (Chapter 2.4.1). There were significant differences ($p= 0.009$) in cohesiveness between the potato starch (90/10) variant and modified pea starch variant and commercial fish pudding. Significant differences ($p= 0.003$) were also found in resilience between the potato starch (90/10) variant and the native pea starch, modified pea starch and commercial fish pudding. Resilience is a measurement of the products resistance to deformation (Chapter 2.4.1). The potato starch variant (90/10), the commercial fish pudding, and the tapioca variant showed significant difference in adhesiveness from the rest of the variants, being the most adhesive of the samples (the potato starch (80/30) variant, the pea starch variants, and the modified corn starch variant). The potato starch variants and the pea starch variants were among the fish mince products produced in the preliminary production that had the most significant differences in TPA measurements. In a report from Peitrasik and Soladoye (2021), where the properties of low-fat bologna were compared with the use of native pea starches as an alternative to modified corn starch, the bologna made with pea starches had a higher cohesiveness and chewiness compared to those formulated with modified corn starch. This was the opposite of what was measured for the cohesiveness and chewiness of the fish mince products in this thesis, where the pea starch variants revealed both lower cohesiveness and chewiness compared with the modified corn starch variant. The bologna produced in the report from Peitrasik and Soladoye (2021) was formulated with the same percentage of starch as in the production in this thesis (3 %). However, the bologna was not enriched with WPC and casein like the fish mince products in this thesis, and that may have had an impact on the textural properties.

4.1.7 Water holding capacity

The water holding capacity of the fish mince samples was determined by centrifugation.

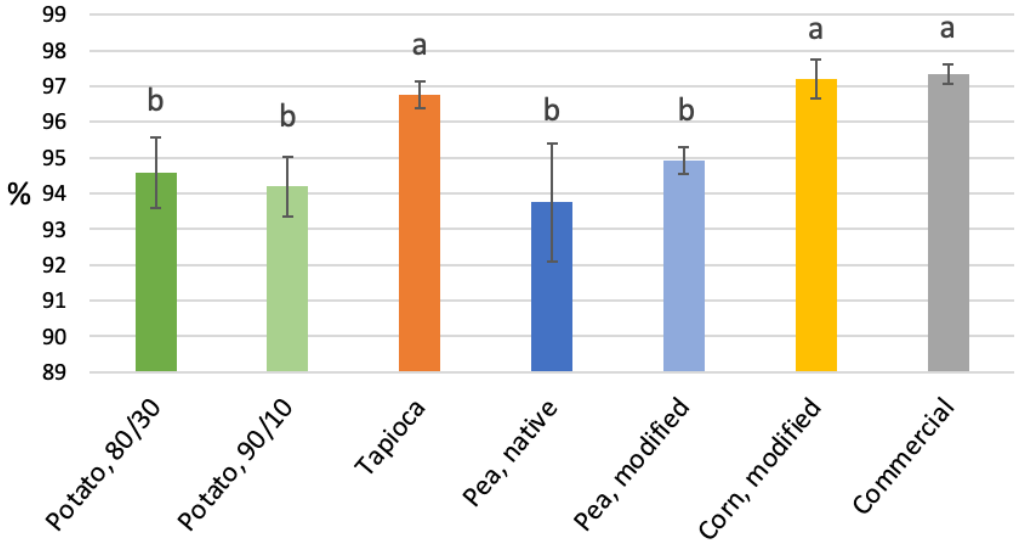

Figure 4.1.7.a The figure shows the water holding capacity (WHC) in percent of the fish mince variants produced in the preliminary production.

The commercial fish pudding, the tapioca and modified corn starch variant had the significantly ($p < 0.001$) highest WHC. It was expected that the commercial fish pudding and the tapioca starch variant would have similar water holding capacities as the commercial fish pudding also contained tapioca starch. No significant differences were detected for the WHC of the potato starch variants and the pea starch variants. Compared with the results for WHC found in a report from Tee and Siow (2017), that compared the effects of tapioca and potato starch on the properties of frozen fish balls, the WHC of the potato starch and tapioca starch variants produced in this thesis was higher. The WHC of fish balls with tapioca starch (3 %) was below 92 %, and the WHC of fish balls with potato starch (3 %) was approximately 90 % in the report from Tee and Siow (2017). However, the fish balls produced in the report was not enriched with WPC and casein like the fish mince products produced in this thesis. The heat-treatment was also different for the fish balls, where they were cooked in 100 °C water for 5 minutes. These differences may have an impact on the properties of the products, including the WHC.

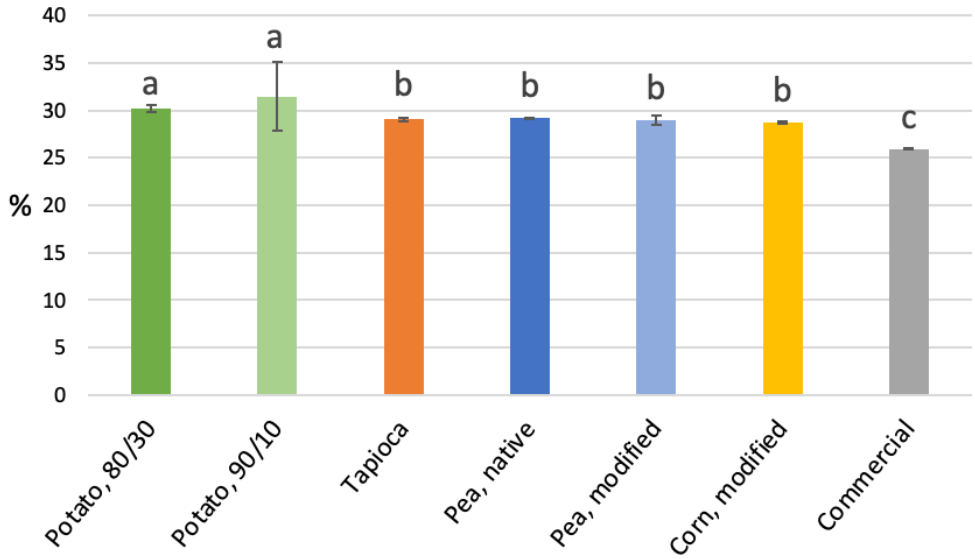

Figure 4.1.7.b The figure shows the dry matter in percent of each fish mince variant produced in the preliminary production.

The potato starch variants consisted of the significantly ($p < 0.001$) highest percentage of dry matter relative to the total weight of the samples. This was reflected in the low water holding capacity of the variants. The commercial fish pudding was significantly different from all the other variants, consisting of the least percent of dry matter. Based on the results from the WHC and the dry matter of the samples it indicates that there may be a negative correlation between the two.

4.1.8 Colour measurements

Colour analysis was conducted on the preliminary products by measuring the colour coordinates L*, a* and b* in the CIEL*a*b* space (Chapter 2.4.3).

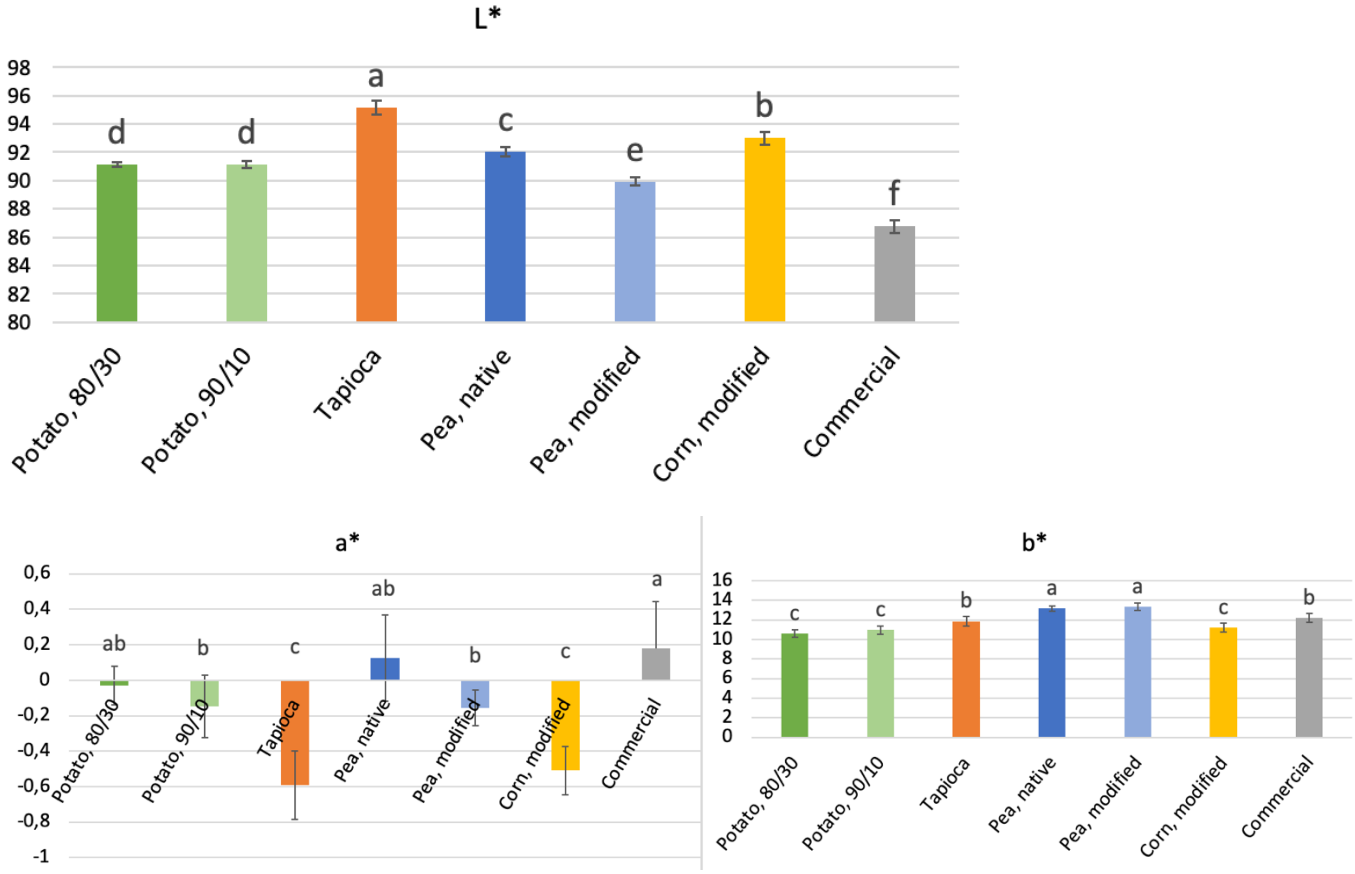

Figure 4.1.8 Colour coordinates L*, a* and b* measured of samples from the six fish mince variants produced in the preliminary production in addition to the commercial fish pudding.

The tapioca starch variant was significantly ($p < 0.001$) different in the colour coordinate L* with the highest value, meaning it was the lightest of the samples. This was not expected as the commercial fish pudding, which is also made with tapioca starch, had the significantly lowest value in the colour coordinate L*. This means that the commercial fish pudding was the darkest of the sample. The colour coordinate a* of the potato starch (80/30) and the native pea starch variant were significantly different ($p < 0.001$) from the tapioca and the modified corn starch variants. The values for the colour coordinate a* of the potato starch (80/30) and the native pea starch variant were close to zero, which is considered as a grey colour. The potato starch (90/10) variant and the modified pea starch variant showed slightly negative values for the colour coordinate a*, and were significantly different from the tapioca, modified corn starch variant and the commercial fish pudding. The tapioca and modified corn starch variant showed the most negative values for the colour coordinate a*, which correlates to a slightly greener colour. However, the green colour is very subtle due to range of the colour field that ranges from -128 to +127 (Chapter 2.4.3), and the values of the variants were more positive than -1. The values of the pea starch variants were significantly ($p < 0.001$)

higher in the colour coordinate b* than the rest of the variants. The tapioca starch variant and the commercial fish pudding were significantly different from the potato starch variants, the pea, and the modified corn starch variants, in the colour coordinate b*. Positive values in the colour coordinate b* are considered slightly yellow colours (Chapter 2.4.3).

4.1.9 Sensory evaluation

Below are the results from sensory evaluation of fish mince samples from the preliminary productions presented in table 4.1.9 based on starch content.

Table 4.1.9. Sensory evaluations of fish mince variants from the preliminary production.

Variant:	Appearance:	Odour:	Taste:	Texture:
Potato starch (heat-treated at 80 °C for 30 min)	White surface. A few air bubbles. Some pieces of fish fillet (not homogenous)	Faint smell of fish.	Weak taste of fish. Mild taste of flour. Taste of old fish.	Very firm. Dry. Grainy.
Potato starch (heat-treated at 90 °C for 10 min)	White surface. A few air bubbles. Some pieces of fish fillet in a few samples.	Faint smell of fish mince.	Fish taste of low intensity. Sweet. Astringent (dry feeling).	Firm. Juicy. Less grainy than potato starch heat-treated at 80 °C.
Tapioca starch	White and smooth surface. More air bubbles than the potato starch variants. Homogenous.	Smell of fish. Mild odour.	Pure and clear taste of fish. Mild taste of flour.	Firm. Juicy. Less grainy than the potato starch variants.
Modified corn starch	White surface. Some air bubbles.	Sweet odour. Faint smell of fish.	Sweet. Pure fish taste. Some sweet aftertastes.	Moderate firmness. Juicy. Sticky.
Native pea starch	Grey and grainy surface. Air bubbles. Very few pieces of fish fillet.	Fish odour. Smell of peas.	Low intensity of fish taste. Aftertaste of peas. Taste of legumes.	Very grainy. Low firmness. Juicy and low chewing resistance.
Modified pea starch	Darker grey surface than the native pea starch variant. Rough surface. Juicy looking surface.	Clearer smell of peas than native pea starch variant Faint fish odour.	Clearer taste of peas than native pea starch variant. The taste of peas drowns out the taste of fish. Aftertaste of peas.	Soft and grainy. Crumbly. Juicy and sticky.

The semi trained panel evaluated the potato starch variants to be the firmest of the samples. They also had the least clear taste of fish. The modified corn starch variant had a sweet odour and taste, unlike the other variants. The pea starch variants were the grainiest of the samples. Based on appearance, the modified pea starch variant was greyer and had a rougher looking surface than the native pea starch variant. The modified pea starch had the clearest taste and the strongest aftertaste of peas of the two. It also smelled more of peas than the native pea starch variant. Based on the sensory evaluation the native pea starch was the pea starch variant most suitable to be further assessed in the shelf-life study. The tapioca starch variant,

the potato (90/10) starch variant, and the native pea starch variant were among the preferred variants in the overall sensory evaluation.

4.2 Shelf-life study

It was desirable to include two starch variants in the shelf-life study which had opposite properties from each other. Based on the TPA results in the preliminary analyses the potato starch variants and the native pea starch variants were on the opposite ends of the TPA parameters. Based on the colour measurements conducted on the fish mince products produced in the preliminary production, there were significant differences observed in all the colour coordinates (L*, a* and b*) between the potato starch variant (90/10) and the native pea starch variant. Based on the sensory evaluation of the preliminary products the tapioca starch variant, the potato (90/10) starch variant, and the native pea starch variant were among the most interesting. In addition to the analyses mentioned, due to potato starch being a commonly used starch ingredient in fish mince products (Chapter 2.1) and due to the desire to find a sustainable alternative starch ingredient, it was desirable to test potato starch and native pea starch further in the shelf-life study. Due to the minimal number of studies that have been conducted on the consumer´s preferences for pulse products and on the use of pea starch in fish mince products (Chapter 2.2.2), it was decided to choose the native pea starch for further analyses in the shelf-life study over the modified pea starch. The primary aim with the shelf-life study was to produce fish mince products with a specific texture, good sensory properties, and a shelf life of up to 30 days. All measurements and analyses performed on the products in the shelf-life study was conducted on six parallels, n=6, unless stated otherwise.

4.2.1 Production procedure

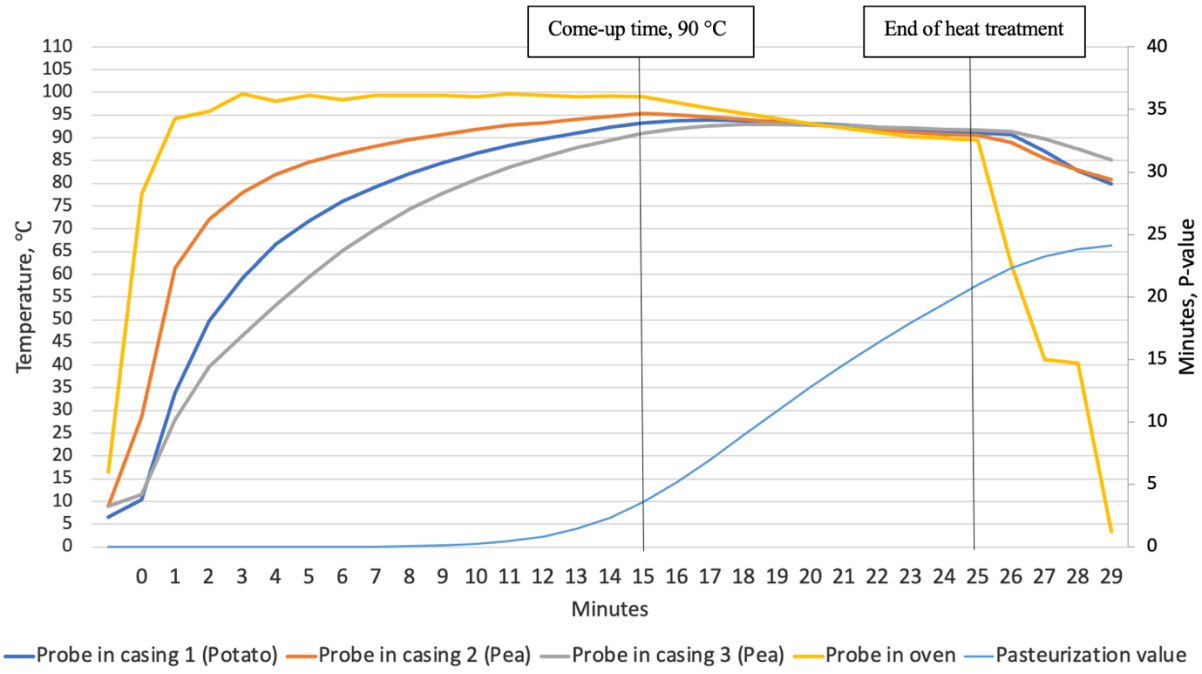

Figure 4.2. The figure shows the monitored core temperature of the samples during heat treatment in the production of fish mince products for the shelf-life study. Probe 1 was placed inside a sample from the potato starch variant. Probes 2 and 3 were placed inside samples from the native pea starch variant. Probe 4 was placed inside the oven. The bottom line (light blue) in the graph shows the calculated pasteurization value from the data measured with probe 3, which had the lowest pasteurization value of the samples monitored.

The monitored core temperature of the samples during heat-treatment in the production of fish mince products for the shelf-life study, are shown in Figure 4.2. The line at the bottom in the figure represents the pasteurization value for the heat-treatment. The vertical lines in the figure represents the start and the end of the heat-treatment where the fish mince products were heat-treated at 90 °C for 10 min. At the end of the heat-treatment (vertical line to the right) the pasteurization value was 21 minutes. However, the heat-treatment of the products continued for a while after they were taken out of the oven and put in ice slurry water for cooling, due to the time of temperature reduction in the core of the samples. This adds to the pasteurization value and results in a total pasteurization value of approximately 24 minutes. This is a higher heat-treatment than what is necessary to achieve a 6 log reduction of non-proteolytic *C.botulinum* type B and E (Chapter 2.5). The heat-treatment of D_{90}= 1,6 (Table 1) gives a 15 log reduction of the target organism *C.botulinum.* The intense heat-treatment may lead to reduced quality in terms of the sensory properties of the fish mince products and may

have an influence on the texture of the products due to the thickening and gelling properties of starch and added protein (Chapter1).

4.2.2 Texture analysis

TPA replaced the method gel-test in the shelf-life study due to the greater diversity of textural parameters obtained from TPA. The TPA measurements was conducted on n=6 samples from each day except the pea variant on day 1 where n=4 due to some deviating values obtained from TPA that was excluded from the results. All measurements from the potato MAP variant on day 7 were also excluded due to abnormal measurements that were incomparable to the other variants.

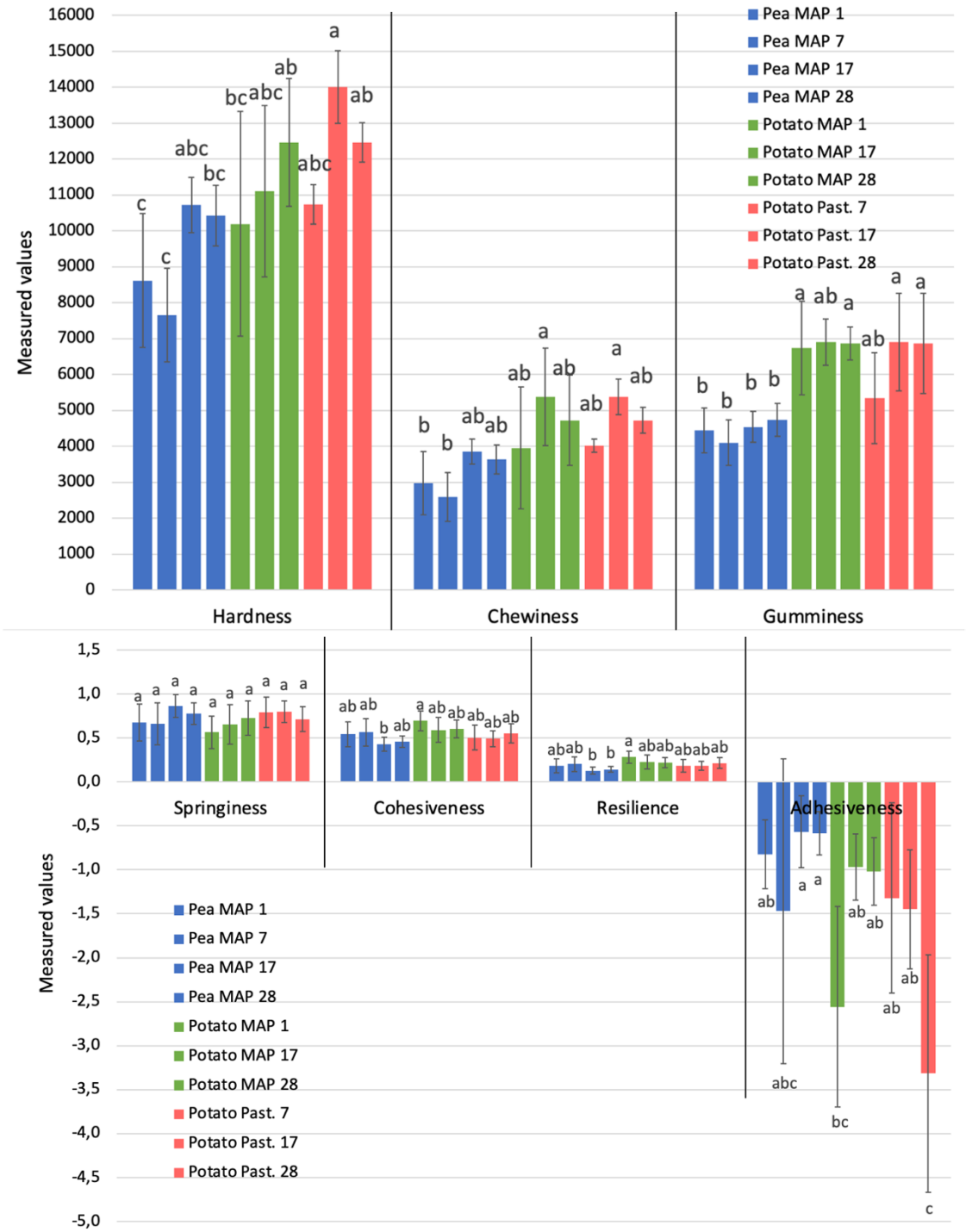

Figure 4.2.2 The figure shows measured values for TPA of fish mince samples taken out on day 1, 7, 17 and 28 in the shelf-life study.

Figure 4.2.2 shows the measured parameters from TPA of fish mince samples in the shelf-life study. There were significant differences in hardness ($p < 0.001$), chewiness ($p = 0.001$), gumminess ($p < 0.001$), springiness ($p = 0.324$), cohesiveness ($p = 0.043$), and resilience ($p = 0.027$) between the variants, but no significant differences within each variant against the storage time. The potato starch stored in casings (Potato past.) from day 28 in the shelf-life

study was significantly ($p < 0.001$) more adhesive than the samples from the same variant from day 17 and 7. However, there were significant differences in measurements between the variants. The pea starch variant from day 1 and 7 were significantly softer than the potato starch variant (MAP) from day 28 and the potato starch variant (past.) from day 17 and 28. The pea starch variant from day 1 and 7 also showed significantly lower measurements in chewiness than the potato starch variant (MAP) from day 17 and the potato starch variant (past.) from day 17. The pea starch variants from day 1, 7, 17 and 28 showed significantly lower measurements in gumminess than the potato variant (MAP) from day 1 and 28 and the potato variant (past.) from day 17 and 28. There were no differences in springiness between the variants. The pea variant from day 17 had lower cohesiveness than the potato variant (MAP) from day 1. The pea variant from day 17 and 28 had lower values in resilience than the potato starch variant (MAP) from day 1. The pea variant from day 17 and 28 was less adhesive than the potato variant (MAP) from day 1 and the potato (past.) variant from day 28. The resulting parameters obtained from the TPA suggest that the storage time has little impact on the texture of the products. The TPA results also indicates that the packaging has little effect on the texture of the products.

4.2.3 TPA of heated samples

To simulate a realistic eating situation the samples were heated before TPA was conducted on the samples in the shelf-life study. After TPA was performed of warm samples (60±3 °C) the samples were put in room temperature for 30 minutes and TPA was performed again. The core temperature of the samples after 30 minutes in room temperature was between 29-35 °C. TPA was conducted this way to detect changes in the texture of the fish mince products during cooling after they are heated and prepared for eating.

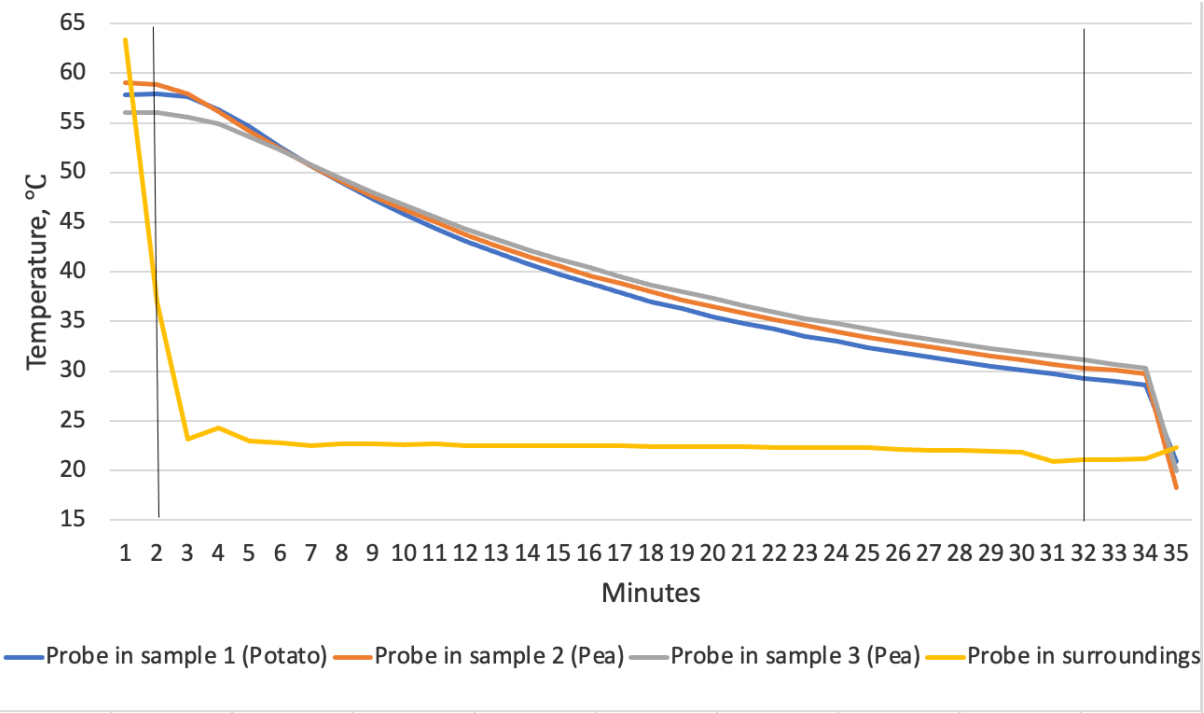

Figure 4.2.3.a The chart shows the monitored core temperature of the samples on day 2, after they were heated to a core temperature of 63 °C and then placed in room temperature (21±2 °C). The chart shows how the temperature inside the samples sinks during 30 min. Probes 1, 2 and 3 were placed inside three separate samples. Probe 4 was not placed in any sample, monitoring the surrounding temperature.

Figure 4.2.3.a shows the monitored core temperature of the fish mince samples during the 30 minutes they were placed in room temperature on day 2 after production. The vertical lines in the figure represents the start and the end of the 30 minutes the samples were placed in room temperature. The vertical line to the right represents the core temperature of the samples at which the second TPA measurements were performed. During the time that the samples were placed in room temperature the core temperature dropped 30±3 °C.

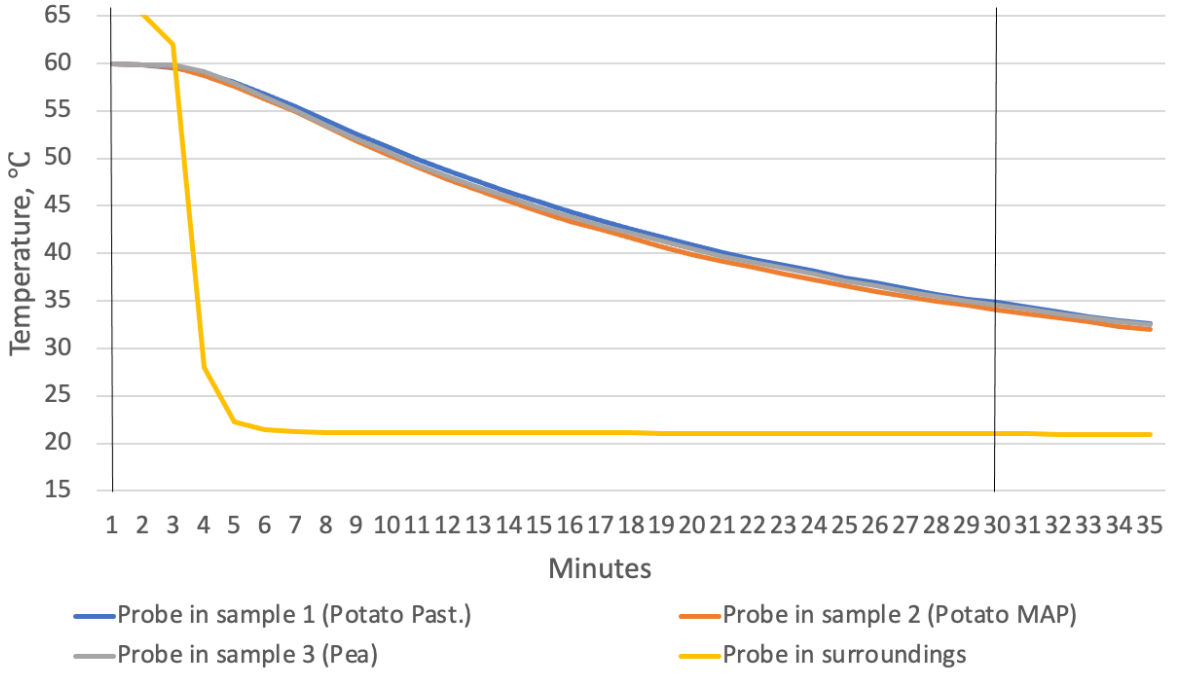

Figure 4.2.3.b A chart showing the monitored core temperature of the samples on day 30, after they were heated to a core temperature of 63 °C and then placed in room temperature (21±2 °C). The chart shows how the temperature inside the samples sinks during 30 min. Probes 1, 2 and 3 were placed inside three separate samples. Probe 4 was not placed in any sample, monitoring the surrounding temperature.

The monitored core temperature of the fish mince samples during the 30 min they were placed in room temperature on day 30 after production (Figure 4.2.3.b). The vertical lines in the figure represents the start and the end of the 30 minutes where the samples were placed in room temperature. The core temperature of the samples on day 30 was higher compared to the temperature of the samples on day 2, after being in room temperature for 30 min. This is due to the higher core temperature of the samples on day 30 before they were removed from the food warming trolley. On day 2 it was noted that the temperature inside the food warming trolley did not reach the temperature it was set to have (75 °C). Therefore, there were some differences in the core temperature of the samples on the different days, but overall the core temperature of the samples dropped 25±3 °C during the 30 minutes in room temperature.

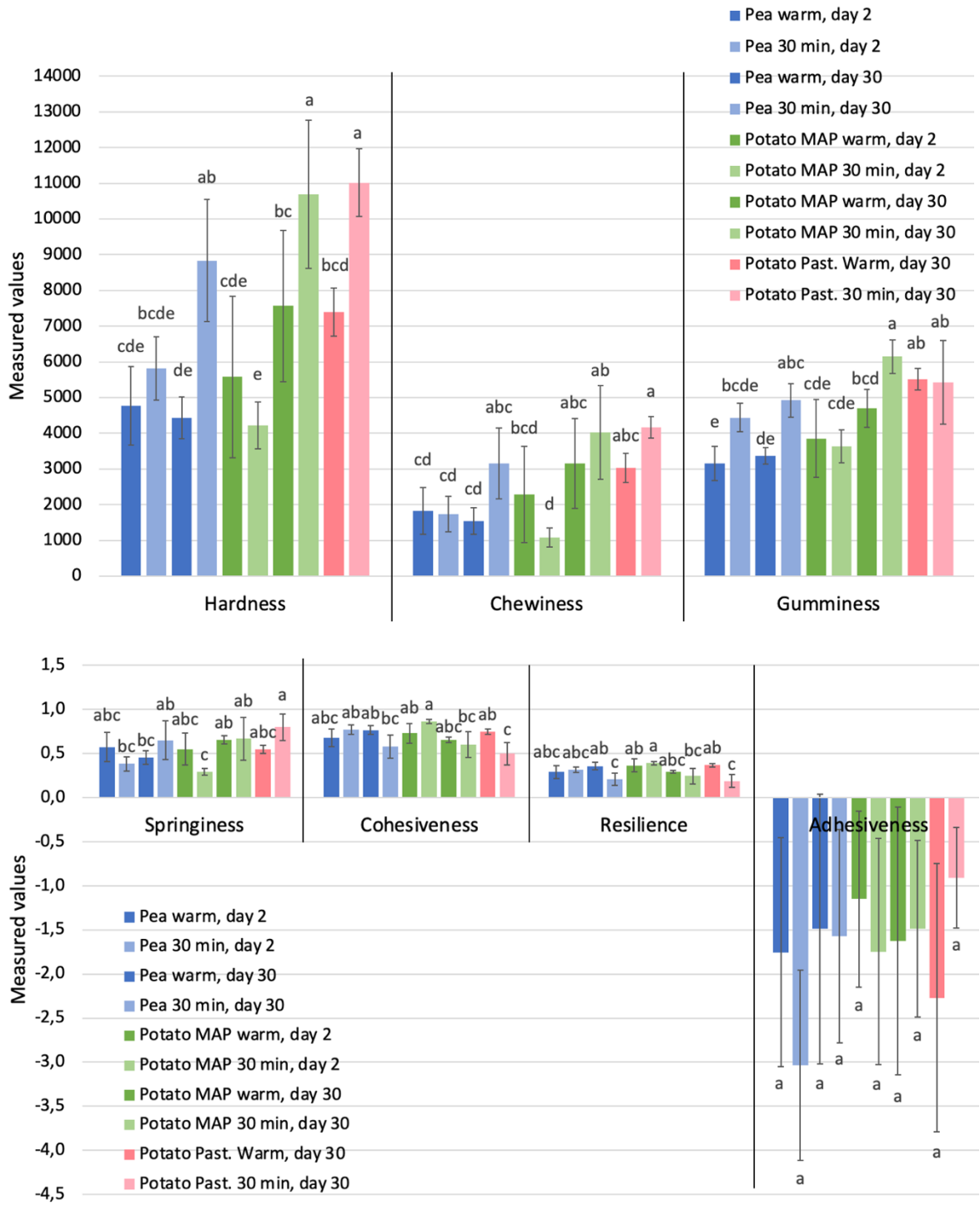

Figure 4.2.3 The figure shows the measured values for the textural parameters obtained from TPA of heated (60±3 °C) samples and samples left in room temperature for 30 min, on day 2 and day 30 in the shelf-life study.

Figure 4.2.3 represents the measure values for the textural parameters obtained from TPA on day 2 and 30 in the shelf-life study. The figure includes measurements performed on heated samples (60±3 °C) and samples left in room temperature for 30 minutes, where the core temperature of the samples was 30±3 °C. There were significant differences in a few

measurements obtained from TPA within each variant between heated (60±3 °C) samples and samples after 30 min (30±3 °C). The heated samples of the pea starch variant from day 30 were significantly softer ($p < 0.001$) than the same samples after 30 min in room temp. The same difference in hardness were found on samples from the potato starch (MAP) variant and the potato starch (past.) variant from day 30. There were significant differences found in chewiness ($p < 0.001$), springiness ($p < 0.001$) and adhesiveness ($p= 0.297$) between samples from different variants, but no significant differences from the same variant heated compared to 30 min in room temperature. The heated pea variant from day 30 was significantly ($p < 0.001$) less gummy than the same variant after 30 min in room temperature. The same difference in gumminess was found between the heated and 30 min in room temperature for the potato (MAP) variant from day 30. The heated potato (past.) variant from day 30 was significantly more cohesive ($p < 0.001$) than the same samples after 30 min. The same difference was found in resilience ($p < 0.001$) of the same samples. The heated samples of the pea variant from day 30 was also more resilient than the same samples after 30 min. Compared to the TPA measurements performed on room tempered samples (Chapter 4.2.2) there were no noticeable differences between the variants when they were heated.

4.2.3 Water holding capacity

The measurements of WHC and dry matter on the samples from the shelf-life study was conducted on three samples from each variant, n=3. This was done due to relatively similar results among the same variant in the preliminary analysis and the number of parallels was therefore reduced for the analyses in the shelf-life study.

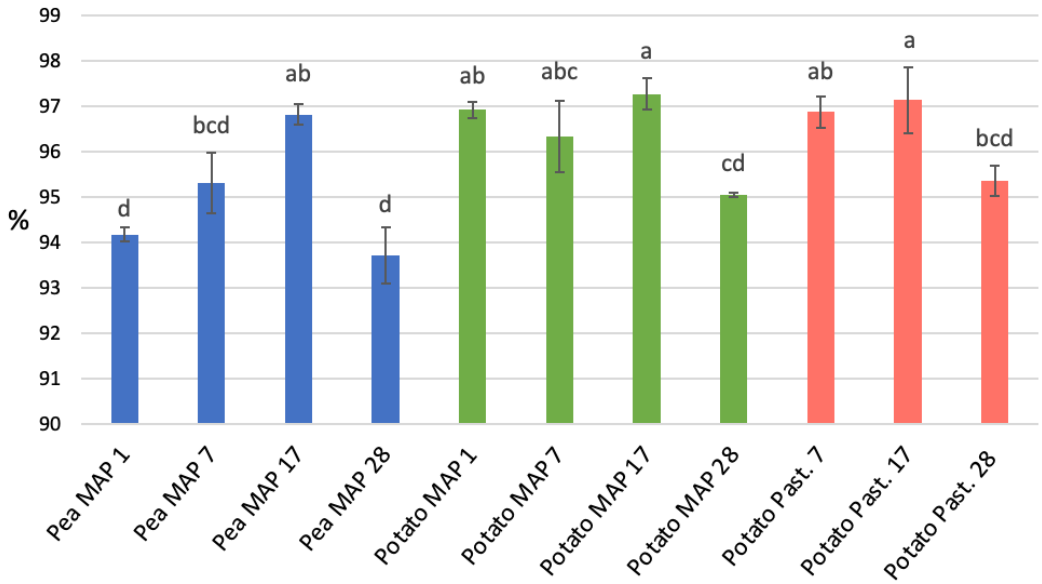

Figure 4.2.6.b The figure represents the calculated values for WHC of samples from each variant on day 1, 7, 17 and 28 in the shelf-life study.

There were significant differences ($p < 0.001$) in the water holding capacity of the variants from day 17 to 28. All three variants had a decrease in the WHC on day 28. There is no obvious explanation for this drop in WHC on day 28. The pea starch variant had a significant increase in WHC from day 1 to 17. Significant differences were found between the pea variant from day 1 and 28, and the potato (MAP) variant from day 1, 7 and 17, and the potato (past.) variant from day 7 and 17. The pea starch variant had a lower WHC.

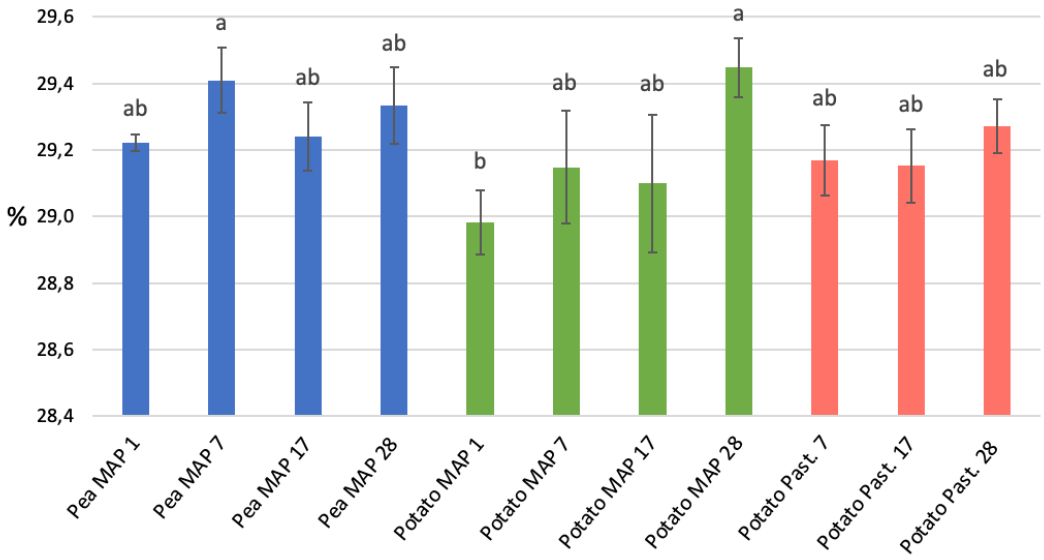

Figure 4.2.6.b The figure shows the results calculated for dry matter on the fish mince variants on day 1, 7, 17 and 28 in the shelf-life study.

There was only a significant difference ($p= 0.026$) in dry matter within the potato (MAP) variant from day 1 to 28. The percentage of dry matter relative to the total wight of the samples increased from day 1 to 28. No significant differences in dry matter were found within the other variants. The only significant difference in dry matter between the variants was between the pea starch variant from day 7 and the potato (MAP) variant from day 1. The pea starch variant had a higher percentage of dry matter compared to the potato (MAP) variant. This difference in dry matter between pea starch fish mince products and potato starch fish mince products was different than what was found in the preliminary analysis of dry matter (Chapter 4.1.7). In the preliminary analysis the potato starch variant had a higher percentage of dry matter than the pea starch variant. However, in the shelf-life analysis an

extra variable being the storage time must be taken into consideration, and the results cannot be compared directly.

4.2.4 Colour measurements

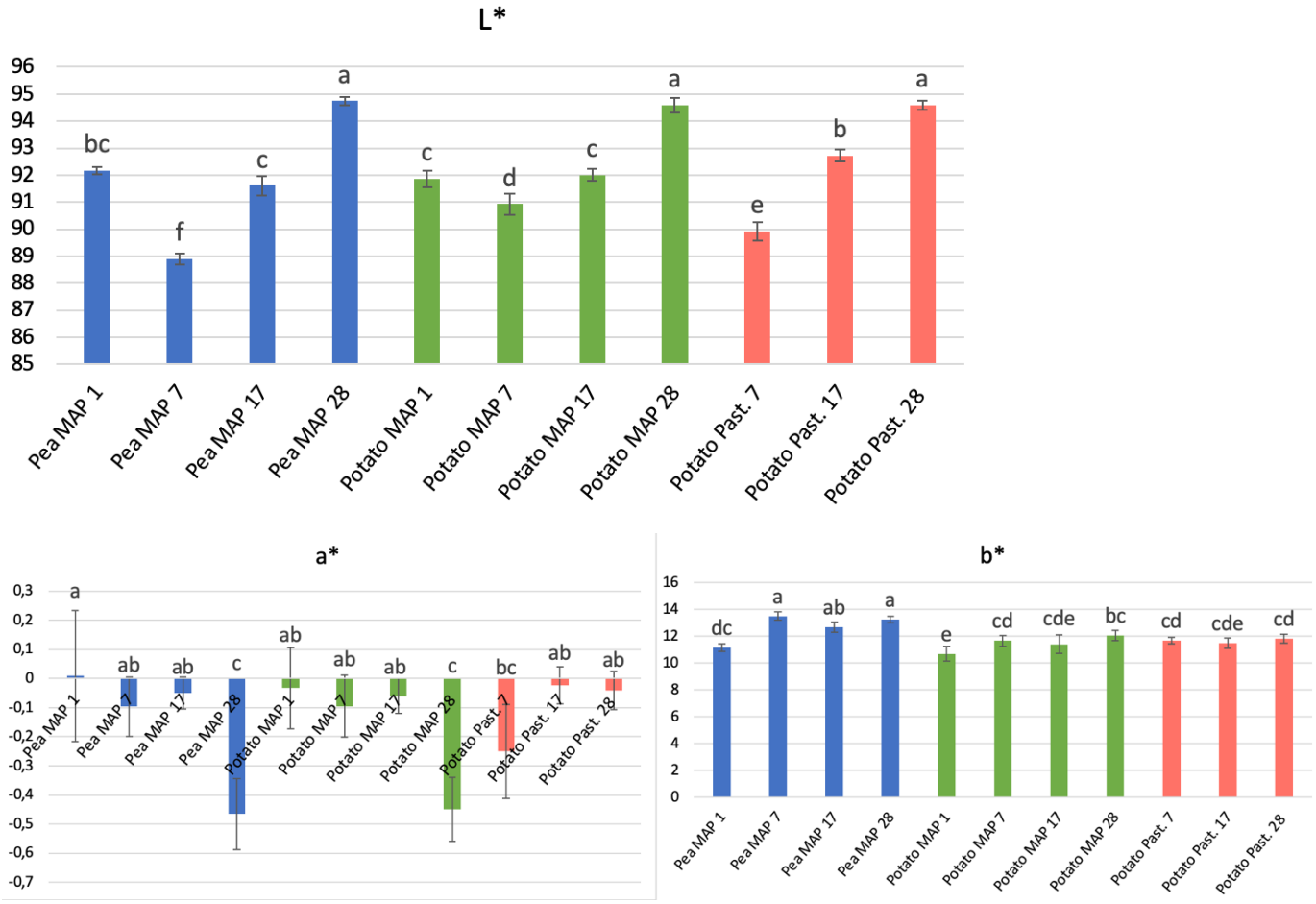

Figure 4.2.7 The figure shows the measured values from the colour analysis in the shelf-life study for each fish mince variant.

Significant differences in the colour coordinate L* ($p < 0.001$) were found within each variant on the different days in the shelf-life and between the variants. There was an increase in the lightness of the pea starch variant and the potato starch (MAP) variant from day 7 to 28. The lightness of the potato (past.) variant increased from each day in the shelf-life. The potato (MAP) variant from day 7 was lighter than the potato (past.) variant from day 7, which in turn was lighter than the pea variant from day 7. The potato (past.) variant from day 17 was lighter than the other two variants form the same day. The pea variant and the potato (MAP) variant from day 28 had significantly lower values in the colour coordinate a* ($p < 0.001$) than the previous days within the same variant. The potato (past.) variant from day 7 had lower values in the colour coordinate a* than the pea variant from day 1. There was a

significant ($p < 0.001$) increase in the colour coordinate b* within the pea variant and the potato (MAP) variant from day 1 to day 7. The pea variant from day 7, 17 and 28 had higher values in the colour coordinate b* than the potato (MAP) variant from day 7 and 17, and the potato (past.) variant from day 7, 17 and 28. Higher values in the colour coordinate b* correlates with a more yellow colour (Chapter 2.4.3). The results from the colour measurements in the colour coordinate b* in the shelf-life study corresponds with the results from the preliminary production (Chapter 4.1.8), where the native pea starch variant showed a more yellow colour than the potato starch variant.

4.2.8 Microbiological analysis

The microbial analysis of the fish mince samples produced in the shelf-life study were performed on three parallels (n= 3) from each variant on day 1, 7, 17 and 28 after production (Figure 4.2.8 a-c). In these figures, it has been chosen to keep different scaling on the y-axis to highlight the differences between the different starches. The fish mince products were not tested for growth of *Clostridium botulinum.* A reason for this was because the laboratory was not certified to work with *C.botulinum*. The general method called "Anaerobic sulphite-reducing bacteria, NMKL 56" can be used as a method for determination of the number of anaerobic, sulphite-reducing bacteria present in foods. But still further identification of *C.botulinum* is needed. Another reason why the fish mince products were not tested for growth of *C.botulinum* was because the pasteurization time-temperature combination (90 °C for 10 min) was sufficient to achieve the requirement of 6 log reduction of *C.botulinum* (Chapter 2.5). This is sufficient documentation that the survival of this bacteria in the products is under control. Due to changes in legislation in Europe, there are currently no guidelines given from the Food Safety Authorities for spoilage bacteria present in the products. This provides a greater flexibility for food companies to create their own levels for what is an acceptable limit of certain bacteria in food products. The raw samples in the microbiological analyses were taken from mixed fish mince before it was heat-treated. The gas level inside each modified atmosphere package was checked before microbiological analyses. The O_2 level was expected to rise, and the CO_2 level was expected to sink during storage (Chapter 3.2.1). On day 7, the gas level inside one MAP with the pea starch variant was: 0,241 % O_2 and 39,5 % CO_2. The gas level inside one MAP with the potato starch variant was: 0,246 % O_2 and 41,3 % CO_2. On day 17, the gas level inside one MAP with the pea starch variant was: 0,420 % O_2 and 37,1 % CO_2. The gas level inside one MAP with the potato starch variant was: 0,481 % O_2 and 39,0 % CO_2. On day 28, the gas level inside one

MAP with the pea starch variant was: 0,614 % O_2 and 34,3 % CO_2. The gas level inside one MAP with the potato starch variant was: 0,782 % O_2 and 35,3 % CO_2. The gas measurements are in line with the expectations for the changes in the gas ratio up to day 28. The gas levels of O_2 are low, and the CO_2 levels are relatively high through the entire shelf-life. Between day 17 and 28 the CO_2 levels decreased. This change can be reflected in the results below. The gas levels inside the MAP have an impact on the shelf-life of the products i.e., the inhibitory effect of bacterial growth is proportional to the amount of dissolve CO_2 into the food product (Chapter 2.5) (Sivertsvik et.al., 2002).

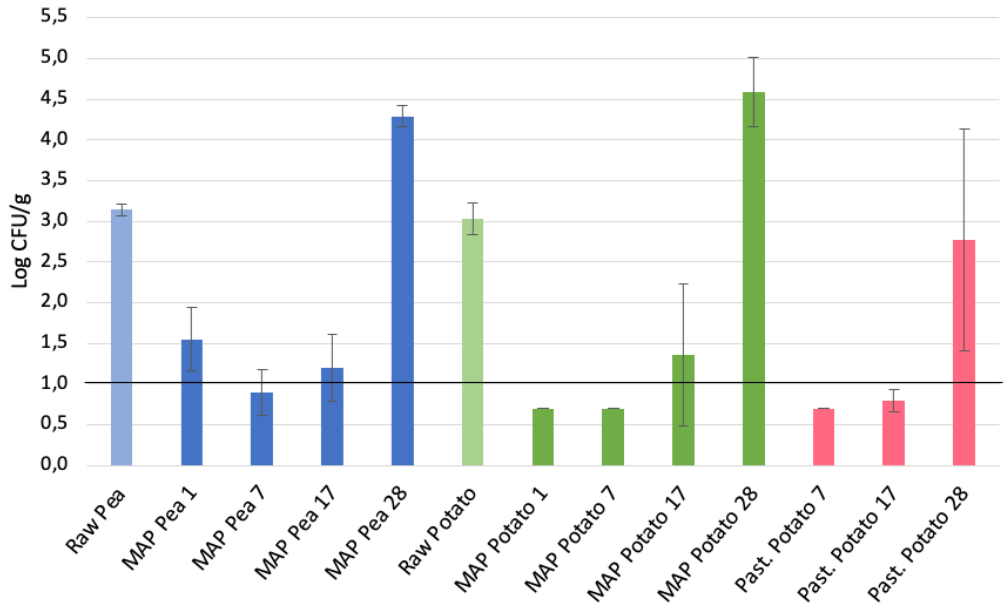

Figure 4.2.8.a The figure shows the CFU/g calculated from the counted number of CFU on the iron agar plates in the shelf-life study. The horizontal line across the figure represents the detection limit for the specific method based on the dilution and volume of sample solution spread on the plate.

The aerobic counts on Iron Agar were used to determine hydrogen sulphide producing bacteria found as specific spoilage organisms in chilled stored fresh fish (NordVal International, 2006). The NMKL 184 (2006) method is used for fish products that are fresh or lightly preserved before they are served. In this thesis, the method was used on the products that were both raw and pasteurized to examine the bacterial content in the products. From figure 4.2.8.a the colony numbers on the iron agar plates from the raw samples are log 3,14 CFU/g for raw native pea products and log 3,03 CFU/g for raw potato starch products. According to microbiological guidelines given by the Norwegian Food Safety Authority from 2002 (presented online, not available anymore due to EU regulations), bacterial counts with a

lower limit (m) of log 4 CFU/g and an upper limit (M) of log 5 CFU/g are set for CFU in raw shellfish and fish products. The level is for products to be eaten without heat-treatment and the fish mince products in this thesis can belong to this category. The fish mince products are pasteurized, and this inactivates specific spoilage organisms, and it may be concluded that these numbers of CFU are below an acceptable limit of specific spoilage organisms. The iron agar was used to determine total viable counts and black colonies determined as hydrogen sulphide producing bacteria (Chapter 3.2.5). The numbers of hydrogen sulphide producing bacteria, counted as black colonies on iron agar, in the fish mince products were for raw fish mince from the pea starch variant: mean= log 2,78 CFU/g, raw fish mince from the potato starch variant: mean= log 2,64 CFU/g, and for heat-treated fish mince products: mean< log 1. Because of the low numbers the results were excluded from the figure (Figure 4.2.8.a). The numbers of hydrogen sulphide producing bacteria found in the raw fish mince samples are thought to come from the raw fish materials (haddock fillet and silver smelt). However, no samples were taken from the raw fish materials, so this assumption is not supported by analysis. In a report by Rao (2009) the number of hydrogen sulphide bacteria found in fresh fish (the freshwater fish *Labeo rohita*) was 2,45 log CFU/g. In his report Rao stated that a hydrogen sulphide producing bacteria count of more than 3 log CFU/g appeared to be the limit between freshness and loss of freshness of the fish. Based on this, it can be concluded that the numbers of hydrogen sulphide producing bacteria found on the iron agar from the raw samples is lower than the limit for fresh fish. On day 1 the numbers of CFU/g from the pea starch variant exceeded the detection limit (log 1 CFU/g). According to the microbiological guidelines given by the Norwegian Food Safety Authority from 2002, bacterial counts of a lower limit (m) of log 2 CFU and an upper limit (M) of log 3 CFU is set for heat-treated fish mince products stored in vacuum or MAP on the day of production. The counts from the pea starch variant on day 1 does not exceed the upper limit and is considered acceptable. Between day 17 and 28 of all three variants, it was found an increase in the aerobic counts on the iron agar. This indicates that the growth of the spoilage organisms of the stored products was slow and increase of spoilage organisms above the detection limit was seen between day 17 and day 28. However, according to the microbiological guidelines given by the Norwegian Food Safety Authority from 2002, bacterial counts of a lower limit (m) of log 5 CFU and an upper limit (M) of log 6 CFU is set for heat-treated fish mince products stored in vacuum or MAP on the day of expiration. The results of aerobic count on day 28 is below the lower limit in the previous guidelines for all three variants. Because the pasteurized potato starch variant was untouched after heat-treatment, lower aerobic counts

were expected for this variant. The variants stored in MAP was reopened after heat-treatment and cut into pieces before they were packed again. This may have an impact on the microbial growth on the products and on the shelf-life of the products since they are exposed to a greater risk of contamination (Chapter 2.5). From figure 4.2.8.a the results show that the aerobic count on the pasteurized potato variant is lower than on the MAP variants, which was consistent with the assumption that this variant had lower recontamination. However, the standard deviation was higher for the pasteurized potato variant compared to the other variants. Some counts deviated a lot from the average, and the uncertainty of the counts was higher.

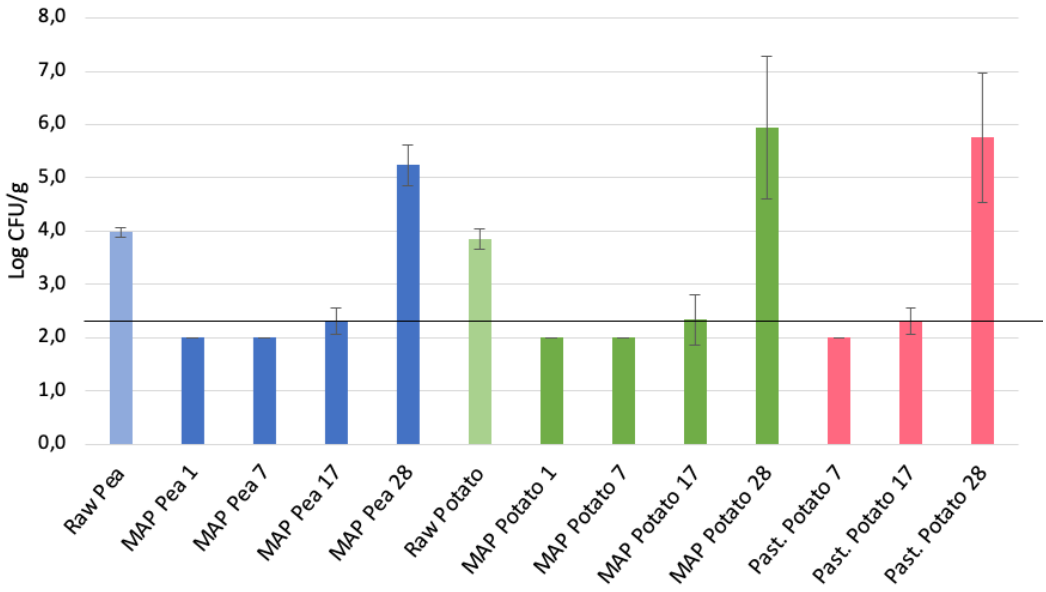

Figure 4.2.8.b The figure shows the number of log CFU/g calculated from the counted number of CFU on the L&H agar plates in the shelf-life study. The horizontal line across the figure represents the detection limit for the specific agar based on the dilution and volume of sample solution spread on the plate.

The L&H-agar was used to determine the psychrotrophic aerobic count in the fish mince products produced in the shelf-life study (Chapter 3.2.5). The L&H agar is used for detection of psychrotolerant bacteria, incubated at 15 °C, in products stored in vacuum or MAP (NordVal International, 2006). The raw fish mince samples had a bacterial count of log 3,88 CFU/g of products with native pea starch and 3,85 CFU/g for products with potato starch. Based on the earlier microbiological guidelines given by the Norwegian Food Safety Authority from 2002, for bacterial counts in raw shellfish and fish products meant to eat without heat-treatment, the results from the raw fish mince variants were below the lower limit for bacterial counts. As observed in Figure 4.2.8.a, there was an increase in the bacterial

growth on the products between day 17 and 28. The bacterial count on the variants from day 28 are below the upper limit (M= log 6) in the guidelines given by the Norwegian Food Safety Authority from 2002 for what is acceptable in fish mince products stored in vacuum or MAP on the day of expiration. The bacterial count on the L&H agar for the pasteurized potato variant deviates from the assumption that the pasteurized potato starch variant would reveal lower aerobic counts than the variants stored in MAP.

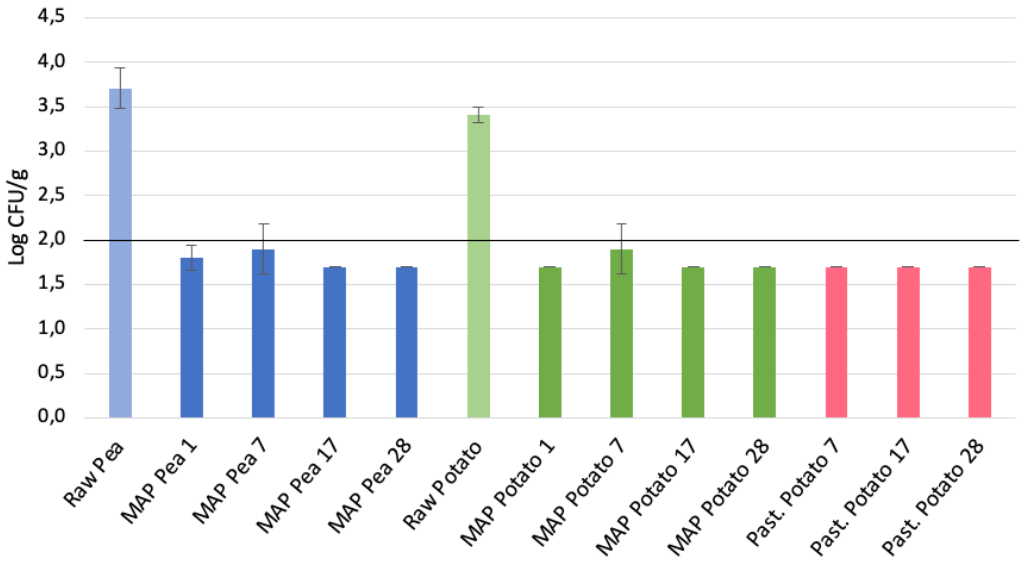

Figure 4.2.8.c The figure shows the number of log CFU/g calculated from the counted number of CFU on the Pemba plates in the shelf-life study. The highlighted line across the figure represents the detection limit for the specific agar based on the dilution and volume of sample solution spread on the plate.

Figure 4.2.8.c shows that the number of bacteria grown on the Pemba medium only exceed the detection level on the raw fish mince. The bacteria that grew on the Pemba plates did not have characteristic colours for *Bacillus cereus*. Since the samples with raw products had no heat treatment, most likely the bacteria grown on the plates were other members of the bacteria species, and not *Bacillus cereus*. These results showed that *Bacillus* species were not a health hazard in the products, and either low numbers of *Bacillus* were present in the ingredients and/or the heat-treatment (90 °C for 10 min) was sufficient to control the survival and growth of the pathogenic *Bacillus* species. Due to the dilution series used for plating and the volume used on the different media, the three microbiological analyses have different detection limits. Iron agar, with a detection limit of log 1, show that the pea variant, potato (MAP) variant and the potato (pasteurized) variant have numbers below or close to the detection level on day 17.

Based on the bacterial counts on all three media, the results show that the fish mince products got higher spoilage bacterial numbers between day 17 and 28. The bacterial counts do not exceed the upper limits for what is acceptable in fish products from an earlier microbiological guideline given by the Norwegian Food Safety Authority from 2002. Therefore, the result from the microbiological analyses show that the fish mince products can be given a shelf-life of at least 28 days. It is possible that the shelf-life of the products could be set to longer than 28 days. This must, however, be documented in an extended shelf-life study.

4.2.9 Sensory evaluation

In table 4.2.8 the results from sensory evaluation of fish mince samples stored in MAP on day 3 and 29 after production are presented. The two different starch variants, potato starch and native pea starch and two serving temperatures, room temperature (18±1 °C) and re-heated (58±1 °C).

Table 4.2.9. Sensory analysis of fish mince variants from the shelf-life study.

Variant:	Day	Appearance:	Odour:	Taste:	Texture:
Potato starch, served room tempered (A).	3	White and smooth surface. A few air bubbles. Some jelly-like lumps in one sample.	Faint smell of fish mince. Stronger odour after the samples were divided into pieces	Pure fish taste of low intensity. Moderate milky taste. Metallic aftertaste.	Very firm. Juicy. Grainy.
Potato starch, served room tempered (B).	29	White surface. Smooth colour. Air bubbles.	Hint of fish odour. Smell of cardboard.	Mild and faint taste of fish. Fresh taste.	Firm/hard. A little grainy. A bit dry.
Potato starch, served warm (A1).	3	White. A little bloated. Looks like A.	Little fishy smell. Hint of crab odour.	Weak and tame fishy taste. Salty and some sweet taste. Distinct milky taste. Slight metallic aftertaste.	Slightly grainy. A little slippery. Softer and more porous than A.
Potato starch, served warm(B1).	29	White and smooth surface. Bloated.	Smell of fabric and warm milk. Little fish odour.	Hint of fish. Mild and little taste.	Smoother than B. Less grainy than B. Spongy and porous. Juicy and tender.
Native pea starch, served room tempered (C).	3	White and slightly yellowish. Smooth surface. Some small holes.	Fishy smell that disappears quickly. More fishy smell than A.	Sweeter than A. Clear fish taste. Taste of shellfish. Slightly less milky taste than A.	Solid. Somewhat dry. Grainier than A. A little agile.
Native pea starch, served room tempered (D).	29	Yellow/white. Smooth surface. Some air bubbles.	Mild smell of shellfish. Fresh odour.	Sour and salty. Aftertaste of shellfish. Impure taste.	Loose and agile. Grainy and dry.
Native pea starch, served warm (C1)	3	White and yellowish surface. Inflated. Some air bubbles.	Slight cardboard-like smell. Stinging crab smell. More odour than A1.	Clear fish taste. Clear salty. Weak taste of shellfish.	Grainy and porous. Less fixed than C. A little slippery and agile.
Native pea starch, served warm(D1)	29	Yellow/white. Smooth surface. Air bubbles. Bloated. Brown/yellow spots.	Burnt odour. Hint of shellfish. Mild odour.	Salty. Hint of shellfish. Mild taste.	Finely grained. Elastic. Smoother than D. Some more juicy than D.

Based on the sensory evaluation of the samples stored in MAP in the shelf-life study the appearance of the samples seems to be mostly affected by heating and not the storage time. The samples become inflated when heated. A trend was observed within the taste of the samples correlating with the storage time. The fish taste becomes more indistinct and less fresh/pure. The native pea variant served room tempered even had a sour taste, which does not belong in fish mince products. The sour taste is a sign of spoilage. The results from the microbiological analyses (Chapter 4.2.8) of the fish mince products correlates with the sensory evaluation of the samples on day 29. The microbiological analyses revealed an increase in the bacterial count of spoilage organisms found on the samples on day 28. This supports the assumption that the products had started to spoil on day 29. Although there were observed some changes in the taste of the fish mince products on day 29, they were not considered inedible. This further supports the assumption that the products have a shelf-life of at least 28 days. It was not performed a sensory evaluation of the pasteurized potato starch variant, therefore a comparison between sensory properties and microbiological results cannot be drawn for this variant. A trend was also observed within the odour of the samples stored in MAP where the odour gets less distinct when the samples are heated. Strange odours appear within the warm samples like smell of fabric, cardboard, and burnt smell. The samples that were served warm seem to have a more desired texture than the samples served at room temperature. The warm samples were smoother and juicier than the correlating room tempered samples. Also, less graininess was observed within the potato starch variant served warm compared to the room tempered.

5 Conclusion

Fish mince products were developed that contained different starches. It was desired to find a sustainable alternative starch ingredient in fish mince products. From the preliminary production fish mince variants containing potato starch, tapioca starch, modified corn starch, native pea starch and modified pea starch were analysed to detect differences in texture, WHC, colour and sensory properties. Based on the results in the preliminary analyses the potato starch variants and the native pea starch variants were on the opposite ends of the properties, and it was chosen to analyse these two starch variants further in the shelf-life study. The primary aim with the shelf-life study was to produce fish mince products with a specific texture, good sensory properties, and a shelf life of up to 30 days. It was also

analysed what effect two different packaging methods (casings and MAP) had on the properties and on the shelf-life of the products.

Texture analyses of the potato starch variant and the native pea starch variant showed that there were significant differences in hardness ($p < 0.001$), chewiness ($p= 0.001$), gumminess ($p < 0.001$), springiness ($p= 0.324$), cohesiveness ($p= 0.043$), and resilience ($p= 0.027$) between the variants, but no significant differences within each variant against the storage time. Some of the samples from the pea starch variant showed lower measurements in hardness, cohesiveness, chewiness, gumminess, resilience, and adhesiveness than some of the samples from the potato starch variant (MAP) and the potato starch variant (stored in casings). The resulting parameters obtained from the TPA suggest that the storage time and packaging have little impact on the texture of the products. The textural properties of the products were affected by the starch ingredient.

TPA of heated (60±3 °C) samples and samples after 30 min (30±3 °C) showed that immediately after heating, samples of the native pea starch variant, the potato starch (MAP) variant and the potato starch (stored in casings) variant were significantly softer ($p < 0.001$) than after 30 min in room temp. There were significant differences found in chewiness ($p < 0.001$), springiness ($p < 0.001$) and adhesiveness ($p= 0.297$) between samples from different variants, but no significant differences from the same variant heated compared to 30 min in room temperature. All three variants had a significant ($p < 0.001$) decrease in the WHC from day 17 to 28. There was a significant ($p < 0.001$) increase in the lightness (L*) of the pea starch variant and the potato starch (MAP) variant from day 7 to 28, and the lightness of the potato (past.) variant increased from each day in the shelf-life. The results from the colour measurements in the colour coordinate b* in the shelf-life study showed that the native pea starch variant had a significantly ($p < 0.001$) more yellow colour than the potato starch variant. Based on the sensory evaluation of the samples stored in MAP the appearance of the samples seemed to be mostly affected by heating and not the storage time. A trend was observed within the taste of the samples correlating with the storage time, where the fish taste became more indistinct and less fresh/pure. The results from the microbiological analyses of the fish mince products correlated with the sensory evaluation of the samples on day 29, where there was an increase in the bacterial count of spoilage organisms found on the samples on day 28. Based on the microbiological analyses and the sensory evaluation of the variants the products were evaluated to have a shelf-life of at least 28 days.

The focus on waste minimisation is increasing in today´s resource scarcity (Starch Europe, 2015). The textural and sensory properties of the fish mince variants suggests that native pea starch could be a good and sustainable alternative starch ingredient in fish mince products.

6 Future work

The fish mince products in this thesis were given a high heat load and the pasteurization value was 24 minutes at 90 °C compared to the target P_{90}= 10 min, which is the necessary heat-treatment for inactivation of 6 log of non-proteolytic *C.botulinum* type B and E (Chapter 4.2.1). Future development should focus on optimizing the heat-treatment of the products to obtain possibly better textural and sensory properties.

The aim of future development of fish mince products containing pea starch should be to optimize the sensory texture of the products to achieve a smoother and less grainy texture. It may also be beneficial to focus on optimizing the taste of the products containing pea starches to obtain less aftertaste of peas. To assess whether pea starch is to replace potato starch in fish mince products in the future, more measurements and assessments must be conducted.

In a future industrial production, the fish mince products could be fried on the surface as a burger before packaging in sealed trays with modified atmosphere. Such frying could result in inactivation of a contamination flora and thus give the products an even longer shelf-life.

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Appendix A

Table A. List of ingredients used in the recipe in the preliminary production of fish mince and the production for the shelf-life study.

Ingredients	Producer	Country
Haddock fillet	Domstein AS	Northeast Atlantic (FAO 27), Norway
Silver smelt	Tavan	Faroe Island
Salt	Akzo Nobel salt	Denmark
Milk 3,5 % fat	Tine SA	Norway
Sunflower oil	Rema Koin AS	Netherlands
WPC 80%	Tine SA	Norway
Casein	Armor proteins	France
Potato starch	Hoff SA	Norway
Tapioca starch	Manna	Cambodia
Modified corn starch	Arne B. Corneliussen AS	Netherland
Native pea starch	AM Nutrition AS	Norway
Modified pea starch	AM Nutrition AS	Netherlands

Appendix B

Information about the potato starch used in the preliminary production of fish mince products and in the production for the shelf-life study.

Ingredienser

Glutenfritt naturprodukt uten tilsetningsstoffer. 80% stivelse, 20% vann

HOFF POTETMEL

500 GRM

HOFF Potetmel er et glutenfritt produkt med stort bruksområde! Perfekt til kakebaking, jevning av sauser og gryter, men også til en hel del kjerringråd. Har du prøvd potetmel mot maur, som flekkfjerner eller som tørrsjampo? Les mer på www.potetmel.hoff.no

Varenummer: 00008100 | EPD-nummer: 643320 | GTIN: 07044710081006

Næringsinnhold

Pr. 100 gram.

Energi	1 367 kJ/ 326 kcal
Fett	0,30 g
hvorav	
- Mettede fettsyrer	0,20 g
Karbohydrat	81,00 g
hvorav	
- Sukkerarter	0,00 g
Protein	0,10 g
Salt	0,00 g
* av daglig referanse inntak	

Oppbevaring

Temperatur, min	0 grader (C)
Temperatur, max.	25 grader (C)
Holdbarhet, total	1460 dager
Holdbarhet, min. antall dager fra levering til sluttbruker/kjøkken	50 dager
Lagringsgrad	
Holdbarhet etter åpning (kjøl og frysevarer)	*Ikke oppgitt*
Holdbarhet etter tining (fryste varer)	*Ikke oppgitt*

Produktinformasjon

Opprinnelsesland	Norge
Opphavsland	Norge
Varegruppe	Matmel
Varenummer	00008100
EPD-nummer	643320
GTIN	07044710081006
GTIN2	07044710881002
GTIN3	07044710881002
Opprettet dato	03.10.2014
Oppdatert dato	15.04.2021

Appendix C

Information about the tapioca starch used in the preliminary production of fish mince products.

Tapiokamel er laget av tørkede røtter fra cassava planten. Det er naturlig glutenfritt, men kan inneholde spor av gluten.

Melet har ingen merkbar lukt eller smak og koagulerer eller separeres ikke når det blir avkjølt. Det bidrar til å binde glutenfrie oppskrifter og forbedre tekstur og sprø skorpe til glutenfrie bakverk. Gir en jevn og kremaktig tekstur.

Tapiokamel* *økologisk

Kan inneholde spor av: Gluten, Soya, Nøtter og Sesamfrø

per 100 g:

Energi 1498 kJ/358 kcal
Fett 0,02 g
> hvorav mettet 0,01 g
Karbohydrater 88,69 g
> hvorav sukkerarter 3,35 g
Protein 0,19 g
Salt 0,003 g

Produsert: Kambodsja

Varenummer: 1179

VEGAN

VEGETAR

ØKOLOGISK

Appendix D

Information about the modified corn starch used in the preliminary production of fish mince products.

Avebe

Arne B. Corneliussen AS
Kabelgaten 39
0580 OSLO
NORWAY

Certificate of conformity and analysis

Product	: FARINEX WM 55
Delivery number	: 81179535 / 20
Customer reference	: 8059742
Customer material	: 4720005970
Container / Truck id	: 60BJN5 / OR49PN
Quantity of order	: 6000 KG
Sampling procedure	: At Random
Shipping date	: 22 Aug 2019
Production date	: 16 Feb 2019
Best before date	: 16 Feb 2021

Batch 19B91631 6000 KG 6 PAL

Characteristic	Value	Minimum	Maximum	Unit	Remarks
Moisture content	127		140	mg/g	Analysis
Sulphite content (as S02)	<= 9,9		9,9	mg/kg	Conform
Arsenic	<= 1,0		1,0	mg/kg	Conform
Cadmium	<= 0,1		0,1	mg/kg	Conform
Mercury	<= 0,05		0,05	mg/kg	Conform
Lead	<= 0,5		0,5	mg/kg	Conform
Total aerobic mes. count	<= 10000		10000	CFU/g	Conform
Yeasts	<= 250		250	CFU/g	Conform
Moulds	<= 250		250	CFU/g	Conform
Enterobact.in 1 g	<= 100		100	CFU/g	Conform
Salmonella in 25 g	Absent				Conform

Controlled by : Quality Control Department
Ter Apelkanaal, the Netherlands

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22 Aug 2019

Appendix E

Information about the native pea starch used in the preliminary production of fish mince products and in the production for the shelf-life study.

Product specification

AMN Pea Flour Concentrate

Product name: AMN Pea Flour Concentrate, ground pellet
Grade: Food grade
Type: Yellow Pea, *Pisum sativum* L.
Description: Pea Flour Concentrate. Rich in starch, obtained after dry fractionation of pea seed
Color: Creamy light yellow
Odor and taste: Typical of pea
Other aspects: The product must be free from infestation by insects and contamination by rodents and foreign matter

Packaging/transport: 25 kg, big bag
Storage conditions: Store in a dark, cool and dry place
Shelf life: 24 months if stored under proper conditions
Application: Food and feed applications
Country of origin: Norway
Legislations: LOV 2003-12-19 nr 124 (Matloven)
FOR 2008-12-22 nr 1623 (Næringsmiddelhygieneforskriften). (EU) No 852/2004

Chemical profile:	**Result, %** «dry matter basis»
Starch	70±3
Protein	12±1,2
Moisture	Max 12
Fibre	1,5±0,8

Physical data:	
Bulk density, kg/m³	650 – 700
Particle size, µm	App. D(0,9): 590, d(0,5): 150, d(0,1): 20
Viscosity, cP	App. 4000

Microbiology:	
Salmonella	Negative/25g
Total Plate Count	< 10000 cfu/g
Mould	< 500 cfu/g
Yeast	< 500 cfu/g

Undesirable substances:	
Heavy metals	According to EU legislation
Pesticides	According to EU legislation
GMO	Non GMO
Allergens	Traces of gluten

Revision history:

Version	**Date**	**Responsible**	**Comments**
1	15.05.2018	K. Kvernberg	
2	20.09.2018	K. Kvernberg	Updated chemical profile. Revision.
3	13.09.2021	K. Kvernberg	Change in chemical profile and photo

AM Nutrition

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4016 STAVANGER
NORWAY

Phone: +47 51 88 70 00

Appendix F

Information about the modified pea starch used in the preliminary production of fish mince products.

Product specification

AMN CarryMoist™

Product name: AMN CarryMoist™, powder or kibbles
Type: Yellow Pea, *Pisum sativum* L.
Description: Pre geletinized pea starch concentrate (PSC). PSC is the starch fraction, obtained after dry fractionation of pea seed
Color: Creamy light yellow
Odor and taste: Typical of pea
Other aspects: The product must be free from infestation by insects and contamination by rodents and foreign matter

Packaging/transport: Bag, Big bag
Storage conditions: Store in a dark, cool and dry place
Shelf life: 12 months if stored under proper conditions
Application: Technical applications
Country of origin: Netherland

Chemical profile:	**Result, %** Dry matter basis
Starch	73 ± 3
Moisture	Max 12
Fiber	1,5 ± 0,8

Physical data:	
Bulk density	400 – 700 kg/m^3
Water binding/absorption	> 4 ml/g
Oil binding/ absorption	> 0,8 ml/g
Particle size distribution	D(90): 500 µm, D(60): 250 µm, D(20): 100 µm

Revision history:

Version	**Date**	**Responsible**	**Comments**
1	03.03.2021	K. Kvernberg	Draft
2	22.07.2021	K. Kvernberg	Revision

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