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Writer: Sunniva Moldstad Stormark	……Sunniva M. Stormark…… (Writer's signature)
Faculty supervisor: Cathrine Lillo External supervisor(s): Jan Thomas Rosnes and Bjørn Tore Rotabakk	
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Content

Abstract

Red king crab (RKC) is a gourmet product of economic importance, and increased distribution and sales are expected. RKC has a relatively short shelf-life after cooking and new processing and packaging solutions are requested to reach new markets. The aim of this thesis was therefore to examine processing technologies to extended shelf-life of the RKC muscle (merus) and evaluate quality and safety aspects for the chosen shelf-lives. This was done by following the sous-vide method using three different heat treatments (95 °C for 11 min, 80 °C for 4 min and 15 sec and 60 °C for 10 min and 30 sec). The heat treatments were chosen in order to inactivate 6-log of non-proteolytic *Clostridium botulinum*, *Listeria monocytogenes* and *Vibrio parahaemolyticus*. It was expected that treatment at 95 °C gave shelf-life of 30 days, 80 °C shelf-life of 21 days and 60 °C shelf-life of 7 days when stored at 4 °C.

The microbiological quality of merus were analyzed using total viable counts (TVC) on Iron agar and Long & Hammer agar, and the presence of *Bacillus* colonies was detected on Pemba agar. Other quality analyses were water holding capacity (WHC), dry matter, color, drip loss and cook loss.

The results showed differences in analyzed quality parameters between the treatments. Heat treatments at 60 °C and 95 °C led to a significantly ($p= 0.002$) higher drip. There was not detected a significantly difference in and cook loss between the different heat treatments. Drip loss did not change ($p > 0.05$) with time of storage. The result from WHC showed that treatment at 60 °C and 80 °C led to a significantly ($p = 0.002$) higher WHC than at 95 °C, and WHC increased significantly ($p = 0.001$) with time of storage equivalently for all treatments. Treatment at 95 °C led to a significantly ($p < 0.001$) higher dry matter content than treatment at 60 °C and 80 °C. Dry matter also increased significantly ($p = 0.045$) with time of storage equivalently for all treatments. Treatment at 95 °C led to a significantly ($p = 0.002$) lighter color than in merus treated at 60 °C, while merus treated at 80 °C were not significantly lighter or darker than merus treated at 60 °C or 95 °C.

Prior to the present thesis, it was not known if it was possible to remove the muscles from the exoskeleton in raw, frozen, and thawed clusters. This was examined in a preliminary experiment by using high pressure process (HPP) technology (250 MPa) to shuck the muscles from the exoskeleton. The results showed that the muscle could be removed in one piece, although it was more difficult than removal from fresh crab legs. It was therefor decided to use frozen legs in the experiments, to show that frozen legs can be used in industrial production independent of harvesting seasons of RKC.

The analyses from the storage experiment indicate that all heat treatments extended the shelf-life to the expected storage periods. The products were evaluated to be of satisfactory quality, and with further optimization of processing, this can become useful methods for industrial use.

Acknowledgements

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1 Introduction

Red king crab (*Paralithodes camtchaticus*) (RKC) is an alien species that originally originated from the north part Pacific, and was introduced to the Kola fjord in North-West Russia as an attempt to make a new commercial fishery in 1960s (Orlov & Ivanov, 1978). RKC caused problems in local gillnet fisheries and because of this, came to Norwegian management and research institutions attention in 1992. At this time, RKC was mainly seen in the areas around Varangerfjord, but expanded further west, which led to challenges for fisherman in the expanded area (Sundet & Hoel, 2016).
In 2004 Norwegian authorities opened fishery of the species to control the expansion. Clusters are bought either fresh, fresh and frozen or processed and frozen (Lorentzen et al., 2014). RKC was the same year divided the area of RKC into two different zones at 26 ° east meridian as
Figure 1.1 show. West to this, is free harvesting area to anyone. While east to the meridian is the quota regulated area. This is where only fisheries can work (Lorentzen et al., 2018a).

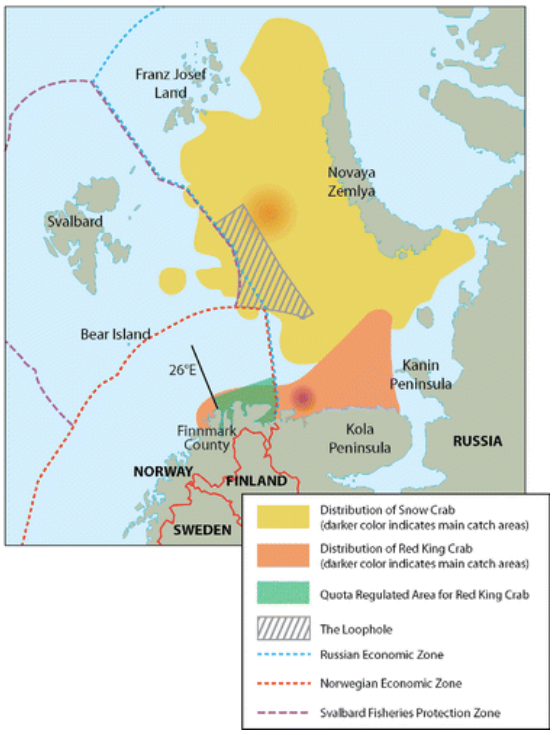

Figure 1.1 Distribution of RKC and Snow crab in the Barents Sea. From (Lorentzen et al., 2018b).

The expansion was still considered to be a problem by the Joint Norway-Russia Fisheries Commission for several years until it was decided in 2007 that each country would manage the crab fisheries separately (Sundet & Hoel, 2016).
The Norwegian industry of RKC became an all year industry in 2008. There are both challenges and advantages with this. This give an economical advantage over those who have a seasonal industry, but the quality of RKC is worse around spring than fall. This is due to physiological changes occurring at that time (Lian et al., 2021).

The first step of processing is slaughtering. This includes splitting the crab into two clusters and removing carapace, stomach and hepatopancreas. Then draining, cleaning and cooking the clusters. Heat treatment of clusters include boiling or steaming, followed by cooling by freezing, either in a tunnel freezer or in a brine with NaCl at – 18 °C (Lorentzen et al., 2019; Lorentzen et al., 2018a).

RKC is an exclusive food product with sensory properties that are appealing to consumers and thus of high demand. RKC is therefore of high economic value to northern Norwegian coastal region. In 2017 as much as 2131 metric tons of RKC were exported from Norway, with a value of 509 million Norwegian kroner (NOK) (Lorentzen et al., 2019). In 2018 and 2019 the first sale value of RKC was 391 and 302 million NOK (Lian et al., 2021). The male crabs are generally of a higher price than female crabs. As clusters there are however not a big difference in regard to the gender (Lorentzen et al., 2014). Main parts are exported to Asia and North America. Mostly alive but cooked-frozen clusters (of cheliped and walking legs joined into a shoulder) are also exported. Heat treated RKC have been documented to have as short shelf-life as 5-8 days (Lorentzen et al., 2014). Therefore, different kinds of preservation have been evaluated in order to prolong the time for distribution and marketing to the costumer.

Sous-vide treatment has proven to extend shelf-life of food products up to several weeks. The treatment includes heating vacuum packaged food at mild temperatures, and the food remains in the pouch until it is getting reheated and then opened and consumed. The risk of recontamination is eliminated in the packaged and microorganisms of concern are anaerobic or facultative anaerobic because of the vacuum packaging (which reduces the access to oxygen drastically). The treatment also makes it is easier to maintain vitamins, minerals, moisture and other properties in the food as it stays in the pouch.
Little to no preservatives are used as extra hurdles, and sous-vide processed food therefore rely on heat and changes in atmosphere to control pathogenic and spoilage organism present in the food (Nissen et al., 2002; Zavadlav et al., 2020).

Expanding shelf-life of RKC by implementing sous-vide, can make it easier for stores to sell RKC, which will make RKC more available for consumers. E-commerce make products more available for consumers. Online grocery shopping first became a trend in the United States in late 1980s. It has then expanded into a worldwide trend. E-commerce is beneficial for both producers and consumers. It expands the geographical areas (of where) the products are sold, and consumers can therefore access more products. Consumers are especially skeptical to buy perishable food online.
There is a shorter time frame for food due to the shelf-life, as compared to clothes and electronics. This has been a limiting factor for the online shopping of groceries as a trend, it is however still expected to grow (Voldnes et al., 2021). More perishable food products are now available to buy online, but still very few seafood products. The main objective of this thesis is therefore to examine and document production, processing, packaging and storage

condition, that is needed to obtain safe e-commerce of RKC, see Figure 1.2, below. Sous-vide was used at treatment method and result from three different heat regimes are compared.

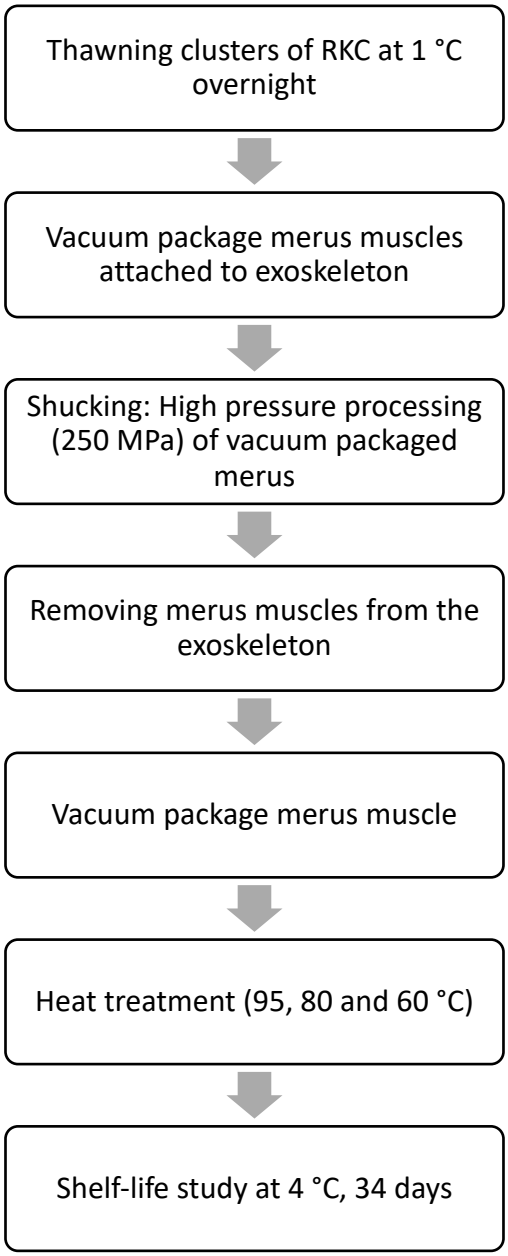

Figure 1.2: Workflow diagram of the production of packaged red king crab (RKC) products for E-commers.

2 Theory

Red king crab (*Paralithodes camtchaticus*) (RKC) is a delicacy and of high demand. Norwegian fisheries industry has benefited financially by selling RKC, making RKC an important income product. Shelf-life of freshly cooked RKC is 5-8 days according to Lorentzen et al. (2014). Sous-vide is a well-known treatment method commonly used on a variety of different food products. Sous-vide food products normally have a shelf-life of several weeks (Zavadlav et al., 2020). As RKC is a gourmet product, the consumer therefore expects a product of high quality. It is desired with an appealing look on the product, juicy, firm and elastic texture and a high whiteness of the cooked muscle fibers (Lian et al., 2021). It is also beneficial for consumers with RKC with prolonged shelf-life, as this will make the product more accessible.

2.1 E-commerce

High-volume and low-margin characteristic of online grocery sales with net cost savings gave high hopes to online grocery sales to grow as a trend. However, many online grocery businesses went bankrupt in the early 2000s. The trend is on the rise today, and consumers are getting more comfortable buying groceries online (Voldnes et al., 2021). However, according to a study from Nofima, French consumers are specially sceptic to buying fresh seafood products online from French stores. (Alm, 2021). The study showed that it is important to gain the costumers trust, as a consumer is more likely to try out an online store after a good experience with the physical store, the same goes for products from brands. Participants in the study wanted information about how the producent and online store maintain freshness of the product. How the fish is produced, packaged, temperature in transportation and storage, and conditions and time of storage. Alm advised to include others' reviews and information about how costumers are compensated if the product is of poor quality as well as the other information the participants wanted. Working on a better collaboration between the producents and store can help in giving the consumers all the information they need to trust that the products are of good enough quality to buy online (Alm, 2021).

Over the past year, there has been an increase in demand for ordering food via the internet, especially during COVID-19 pandemic. For chilled products, it is especially important (and of bigger challenge) to keep the product at the correct temperature. There has been done a study by Everis (2020) examining the effect of temperature abuse during storage. The highest temperature for storing chilled products allowed in the UK is 8 °C. Food was placed in cardboard box with ice box. All boxes were exposed to temperature above 8 °C for different times. 4 Boxes were packaged with 8-12 different food products. The result showed that it can be difficult to keep the core temperature of the food below 8 °C, if they are stored at 20 °C for several hours. This study emphasized how important correct temperature is in regard to food safety (Everis, 2020). Different countries have different regulation for temperature during storage. The Norwegian Food Safety Authority demands that food perishable food are stored at 4 °C or lower (Forskrift om næringsmiddelhygiene, 2009).

2.2 Thermal processing

Thermal processing is used to preserve food by inactivating microorganisms and enzymes that might contribute to spoilage and quality reduction.
The temperature and time in the heating process are adjusted to inactivate the set target organism while maintaining as much as possible of the sensory properties.

When deciding thermal processing required for the specific food, equations describing the death rate for the target organism are (indirectly) used. Equation 1 below describe number of survival target organism at a given time.

Equation 1

$$N = N_0 * 10^{-\left(\frac{t}{D}\right)}$$

N is the number of survival target organism, N_0 is target organism before treatment, t is time and D-value refers to decimal reduction time which is the time required to reduce target organism with 1 log.

The temperature and D-value relation are given by z-value. z-value refers to the temperature raise necessary to decrease the D-value with 1 log. Equation for z-value is given in Equation 2.

Equation 2

$$D = D_{ref} 10^{\frac{T_{ref}-T}{z}}$$

D_{ref} is the decimal reduction time at reference temperature (°C), T_{ref} is the reference temperature (°C) and z is the z-value.

In heat treatments, such as pasteurization and sterilization, Equation 2 is used for calculation of temperature and time. An international level of a pasteurization to obtain safe products is given by a reduction of 6-log of the bacterial numbers of a chosen target organism, like *Listeria monocytogenes* (*L.* monocytogenes) or non-proteolytic *Clostridium botulinum* (*C. botulinum*) type E. Sterilization is given by a reduction of 12-log of spores from proteolytic *C. botulinum* type A (Skipnes, 2011).

D-value is decimal reduction time and is defined as the time it takes to kill 90 % (equivalent to 1 $\log_{10}$) of a target organism at a given temperature. D-value can be used to predict the required heat treatment to obtain a safe product, e.g. to calculate time necessary to kill 6-log of bacteria (equivalent to 6 D). Time and temperature required depend on target organisms.

The temperature in such heat treatments, always refer to the coldest spot in most products, which in most cases is the core temperature.

P-value is calculated accordingly to Equation 3 below.

Equation 3

$$P = \int 1\,0^{T-T_{ref}/z}\; x\; t$$

P-value is defined as the pasteurization value and is time (in minutes) it takes to reduce 6-log of a chosen target organism at a given temperature.
T is core temperature, T_{ref} is reference temperature, z is z-value of the target organism and t is time. In this study, the target organisms were *Vibrio parahaemolyticus*, *L. monocytogenes* and non-proteolytic *C. botulinum*.

2.3 Microbiology

2.3.1 Pathogenic bacteria

Since crab has not previously been made for sale through e-commerce, it has been necessary to examine different shelf-lives and associated heat loads. Specific pathogenic target organisms are then used for evaluation of the shelf-lives. The heat resistance of the target organisms used in this study and recommended heat treatment are shown in Table 2.1 below.

Table 2.1: Inactivation parameters, recommended heat treatment and hurdle principles of mild heat treated foods (Modified from Rosnes et al. (2011))

Target organism	Heat resistance (min)[a]	Recommended heat treatment for 6-log reductions (6D)	Storage condition to inhibit surviving organisms
Psychotropic *C. botulinum* type E	D_{90} = 1.5	90 °C / 10 minutes	< 10 °C[b]
L. monocytogenes and other non-spore forming pathogens	D_{70} = 0.3	70 °C / 2 minutes	< 3 °C[c]
L. monocytogenes	D_{75} = 0.04	70 °C / 2 minutes	< 3 °C
V. parahaemolyticus	D_{70} = 0.001	70 °C / 2 minutes	< 3 °C
V. parahaemolyticus	D_{55} = 3.5[d]	55 °C / 15 minutes	< 3 °C
Bacillus cereus	D_{100} = 1-36	100 °C / 48 minutes	< 4 °C

[a] Most heat resistance species in the target group (ECFF, 2005)
[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 psychotropic *B. cereus*
[c] 3 °C lowest growth threshold for *C. botulinum* (Graham et al., 1997)
[d] (Roberts & Tompkin, 1996)

C. botulinum is a spore forming bacteria growing under anaerobic conditions. There are two groups: Group I is mesophilic proteolytic *Clostridia* (type A, B and F) that are used as target organism for sterilization, and psychotropic non-proteolytic *Clostridia* (type E, B and F), used as target organisms for pasteurization products. The spores of proteolytic *Clostridia* can survive normal cooking temperatures, but will not grow below 10 °C. Thus, non-proteolytic *C. botulinum* type E or B are used as target organism for pasteurized products. Their spores can be inactivated at 90 °C for specific chosen treatments. *C. botulinum* produces a neurotoxin that causes botulism. Botulism is a food intoxication that attacks humans' central nervous system, it can cause death within a couple of days if not properly treated. *C. botulinum* type E is the type that is most found in seafood. These bacteria do not spoil the food and will therefore not give the consumer a warning. Other than proper thermal processing, heavy salting or drying are considered to be effective means to prevent growth of the bacteria (Feldhusen, 2000).

L. monocytogenes is a facultative pathogen and can multiply at refrigerated temperatures in vacuum packaged food in temperatures between 0-45 °C. The psychotropic growth at 0-4 °C causes a specific health hazard, as surviving cell will grow at chilled temperatures during the shelf-life (Huss et al., 2000; Ricci et al., 2018). *L. monocytogenes* cause the disease listeriosis with 1 to 9 cases per 1,000,000 people yearly (Tompkin, 2002). The illness covers only 0,02% of all foodborne diseases while it covers about 28% of all deaths by foodborne diseases. Healthy humans can tolerate a high doses of *Listeria* (e.g. 10^6 CFU/g), while vulnerable and immunocompromised people may be infected by doses higher than 100 CFU/g (Tompkin, 2002). This level is therefore set as legislation in EU (European Union, 2005). In controlled environment, growth within a niche is the major concern. *Listeria* contamination is caused by a poor hygiene or a hygiene that is not well adjusted to preventing growth of *Listeria* (Tompkin, 2002). *L. monocytogenes* is found in fresh and coastal water and in fish from these places. Contamination or recontamination of seafood can occur even with low levels of (<100 CFU/g) of *L. monocytogenes*. Heat treatment is an efficient process to control the survival of the pathogen, other preventive procedures include sanitizing programs designed for inactivation of *L. monocytogenes* (Huss et al., 2000). The pathogen is therefore of more concern in ready-to-eat (RTE) products that are not reheated than in RTE that are reheated.

V. parahaemolyticus is a Gram-negative, halophilic, facultative anaerobic non-spore forming rod. The bacterium is commonly found in the marine environments and different seafoods, including crab (Su & Liu, 2007). *V. parahaemolyticus* is a human pathogen, the prevalence of the pathogen favors higher temperatures in water. *Vibrios* are indigenous in aquatic environment, and there has not been documented a correlation between occurrence of *Vibrio* and commonly applied indicator bacteria of fecal contamination. Indicator organism including coliforms, do therefore not give information about possible presence of *Vibrio spp. Vibrios* are typically sensitive to heat treatment (Løvdal et al., 2021). *V. parahaemolyticus* is not commonly used as target organisms for heat treatment in the same manner as *L. monocytogenes* and *C. botulinum*. Their presence in marine raw material nevertheless means

that it can serve as a target bacterium for food safety during short shelf-lives based on very light heat treatments.

During treatment of merus at 80 °C and 95 °C, vegetative pathogens are killed if the treatment is successful. It is therefore expected very low numbers of normal seafood spoilage organism present in merus immediately after treatment, compared to merus treated at 60 °C. In general, non-spore forming, facultative, psychotropic pathogens of concern in regard to sous-vide treated foods are *L. monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophila.* These pathogens grow under refrigerated temperatures and under anaerobic conditions. In cases with temperature abuse during storage, some non-spore forming, mesophilic, facultative anaerobes are of concern. *Salmonella* and *Staphylococcus aureus* are not typically of marin origin but can be contaminants from handling and processing. All of the vegetative pathogens mentioned above, will be eliminated by heat treatment step in sous-vide at 80 °C and 95 °C. They are thus not expected to be present in treated RKC at these temperatures.

C. botulinum and *Bacillus cereus* are spore-forming pathogens of specially concern in regard to sous-vide treated food. The spores will survive mild heat treatment and can start to germinate during storage. To inactive *B. cereus* with 6-log units, it is required with a heat treatment of 100 °C for 48 minutes (Table 2.1), and spores present in the merus products will therefore survive the chosen sous-vide treatments.
The figure below illustrates possible contamination routes of raw merus in this study. Properly sealing of the sous-vide plastic bags is a critical control point to avoid leakage and secondary contamination after heat treatment.

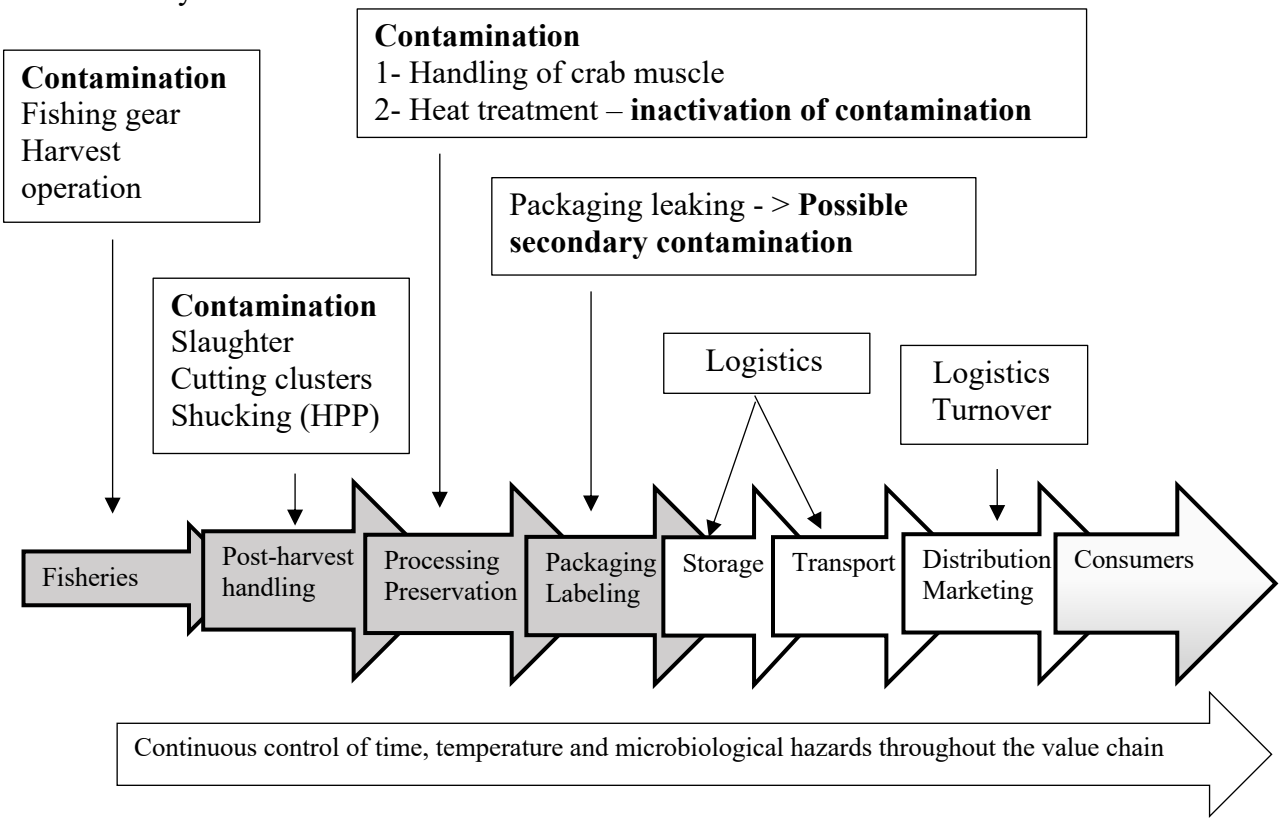

Figure 2.1:Flow diagram over possible contamination routes in the production and distribution chain of RKC products.

2.3.2 Spoilage

Different groups of specific spoilage organism participate in the breakdown and deterioration of raw materials of food. For seafood products, hydrogen sulfide producing bacteria is an example of such a spoilage organism and, the amount of hydrogen sulfide producing bacteria has been shown to correlate with the shelf-life of seafoods (Lorentzen et al., 2014).
An autolytic breakdown process starts rapidly after death of RKC. This process includes a loss in freshness and rapid growth of bacteria. Biochemical and microbiological changes lead to different changes including changes in odor and flavor. Degradation of proteins occur caused by endogenous and bacterial enzymes. The muscle meat of RKC is prone to spoilage also due to a high content of free amino acids and nitrogenous compounds (Lorentzen et al., 2018a). The heat treatment used in the different pasteurization will also inactivate many of the autolytic enzymes, in addition to increase the safety and shelf-life.

Iron agar was used to measure hydrogen sulfide producing bacteria, Shewanella, and some types of Aeromonaceae, Enterobacteriaceae, Vibrionaceae and lactic acid bacteria are examples of bacteria that grow on the agar. Hydrogen sulfide producing bacteria appear as black colonies on the agar.

Even though hydrogen sulfide producing bacteria is considered to be the main specific spoilage organism of fresh seafood from temperature waters, it was only detected sporadically in a study by Lorentzen et al. (2014) examining the shelf-life of cooked RKC, and therefore indicating that it is not an appropriate spoilage organism for RKC, however, there was detected a rapid growth of *Pseudomonas fragi* for the first days, and this organism was believed to have a significant impact on shelf-life of the crab. It was even thought that the absence of hydrogen sulfide producing bacteria might be explained by *Pseudomonas fragi* outgrowing hydrogen sulfide producing bacteria Lorentzen et al. (2014).

Level of microorganisms vary within the leg on RKC. Based on the study done by Lorentzen et al. (2014) part of merus closest to shoulder contain highest concentration of spoilage microorganisms. The gills are closer to the shoulder and, Lorentzen has discussed that as an explanation of the higher numbers.

Table 2.2: Modified from the guidelines from Norwegian Food Safety Authority (Statens næringsmiddeltilsyn, 2002).

Product	Parameter	Limit of acceptable value on last day before expired		No of tests (n), and no of tests with acceptable value (c)	
Fish mince product; heat treated, vacuum or gas-packed	TVC	m pr. g	M pr. g	n	c
		10^5	10^6	5	2

The Norwegian Food Safety Authority (Mattilsynet) published guidelines in 2002, for shelf-life for vacuum packaged seafood, modified version is illustrated in Table 2.2, above. It was advised to test 5 (n) parallels, where 2 (c) of them should be below the limit of 6-log (M).

In the study by Lorentzen et al. (2014) examining shelf-life of clusters of RKC, there was detected a correlation between high level of microorganisms on TVC and shelf-life. When the crab meat was considered to be expired, the level of microorganisms was between log 4.5 and log 5. It is therefore expected to be about the same level of microorganisms on TVC when merus examined in present study expire.

2.4 Raw material

RKC is typically found in waters with the temperature range of 2-7 °C but can tolerate wider temperature range during seasonal migration. The edible part of RKC mainly consist of the meat in the appendages (i.e., muscle around the chelipeds and walking legs) (Lian et al., 2022).

RKC is a good source of proteins. The proteins contain higher levels of tyrosine, histidine, arginine, tryptophan and cysteine compared with fish species. The crab meat contains saturated, unsaturated fatty acids like long chain omega-3 fatty acids, essential and free amino acids and minerals. RKC also contains vitamins and minerals including calcium, iron, zinc, potassium and phosphorus. It is desired with a treatment that does not lead to a high loss of these properties. RKC contains volatile compound, which is believed to explain the special taste (Nanda et al., 2021). Muscle meat from RKC is of high quality as it contains low proportion of cholesterol (Dvoretsky et al., 2021). A study examining the fatty acid profile of RKC, found an average of 78.5 ± 0.7 % moisture, 18.0 ± 1.4 % protein, 0.79 ± 0.07 % fat, and 1.7 ± 0.2 % ash in RKC leg meat that had been cooked earlier that same day (Lian et al., 2022).

2.4.1 Quality of the raw materials

General points in regard to quality of cod include the three following factors, how and for how long the fish lived, how the fish died, and processing *ante mortem.* How and for how long the fish lived include feed/starvation, exercise, age, maturation and season, How the fish died include capture method, method of sacrifice and microbial status. Processing *ante mortem* include handling, cleanliness and temperature (Blikra, 2019). It can be assumed that these factors also are of importance in regard to quality of raw RKC. Killing before cooking versus after cooking has been proven to have an impact of shelf-life of *Cancer pagurus* (Anacleto et al., 2011). Starvation during live holding at temperatures of 5 °C and 10 °C for three months have been proven to affect the level of fatty acid (Lian et al., 2022). The wanted quality of cooked RKC include a marine-fresh smell, fresh color, firm and juicy texture, fresh and sweet flavor (Lorentzen et al., 2014).

A study looking at difference in the quality of RKC between autumn and spring, found a significantly higher values of different quality parameters including hepatosomatic and cheliped index. The hepatosomatic index can be used as a biological and nutritional status of RKC. This is believed to be explained by the fact that it is the organ mainly responsible for

the digestion and absorption of nutrients and storage of reserves. The poorer quality during molting in the period from March to May, is causing difficulties for fisheries industries. The study speculated whether or not it could be a solution to feed postmolt (after molting) RKC caught in the spring during post-capture to improve their quality, as the poor quality is believed to be caused by the fact that RKC does not eat for about a month and in this period, different reserves are mobilized from the hepatopancreas. (Lian et al., 2021).There has also been done observations on movements of RKC, which has indicated that the third leg is less stimulated for muscle growth than the other two (Lian et al., 2021). This indicate that season is of importance in regard to the quality of the raw product.

Blue discoloration (melanosis) is a known problem in producing RCK for the industry. The discoloration is considered to be unacceptable to consumers.
The discoloration is assumed to be caused by a reaction of oxygen with copper in the hemocyanin (Lorentzen et al., 2014).
Melanosis is defined as appearance of dark pigments on joints and parts of RKC that are injured. It occurs with RKC when it is in contact with the atmosphere for a few hours. It is assumed that this alone does not indicate spoilage. Refrigeration or storage on ice does not stop the process, but occur at slower pace (Lorentzen et al., 2018a).

Heating changes the form and texture of the jelly-like raw muscles in fish. It changes from a jelly-like muscle to firm and springy. Temperatures above 45 °C cause protein denaturation, which reduce dimension of myofibrils and collagen. This result in a shrinkage of the muscle fibers and sarcomere length. This further lead to muscles squeezing out liquid, as Ofstad et al. (1996) documented in cod and salmon. Myosin and action are myofibrillar proteins involved in contraction and relaxion of muscles (Skipnes et al., 2011). Heat treatment of RKC will change the structure and shape of muscles in RKC.

2.5 Properties of sous-vide products

Sous-vide is a mild heat treatment method that is used to extend shelf-life of minimally processed food product (Baldwin, 2012). Sous-vide refers to a method of cooking food slowly in a vacuum-sealed pouch at low temperature to retain most of the nutrients, juice and aroma. Consumers request fresh-tasting, high-quality, preservative-free meals with little added salt, that are easy to prepare, i.e. to be microwaved, or rapidly heat treated before serving. This has led to an increase in production of minimally processed products, often grouped as ready meals. Ready-to-eat (RTE) foods or refrigerated processed foods of extended durability (REPFED) products can both be ready meals (Juneja, 2003). Most sous-vide treated food products are reheated before serving and would be defined as ready meals or REPFED products. It is, however, likely that sous-vide treated king crab can be eaten without being reheated, e.g. in a salad, and therefore can belong to the RTE group.

Vacuum-sealing make the heat transfer efficiently from water or steam to the food and eliminate the risk of recontamination during storage. Thus, there will be less aerobic bacterial growth. Sous-vide is often a mild heat treatment or pasteurization treatment and inactivation

of bacteria depends on the chosen heating step (Baldwin, 2012). There will still be expected growth of surviving anaerobic or facultative anaerobic bacteria, making this important to monitor when shelf-life is evaluated. For pasteurized foods it is not possible to make food entirely safe by inactivating all pathogens. It is, however, possible to reduce the chance of foodborne illness drastically with the correct heat treatment and a documented shelf-life.

After heat treatment, the food is quickly cooled to chilled temperatures. This is to reduce growth of surviving bacteria or bacterial spores. In case the RKC products are produced as a REPFED product, and labelled with a reheating advice, this can make the products safer for the consumers (Juneja, 2003). This step is, however, outside the producer's control and cannot be relied upon in a risk assessment.

Sous-vide processes has many benefits in addition to food safety. It helps to preserve volatile compounds and avoid processes of oxidative deterioration. Causing unwanted changes in smell and taste. There are also benefits related to health. By keeping the food in a sealed bag during processing, the consumer get a higher extent of compounds like vitamins from the food since there is a higher retention after processing (Roascio - Albistur & Gámbaro, 2018). This is beneficial as crab meat contain proteins, high level of fatty acids and different minerals. Amino acids are important when it comes to the flavor of the RKC. Lower heat treatment temperature has proven to reduce loss of amino acids, giving better maintenance of taste in product (Lorentzen et al., 2021).

Shelf-life of sous-vide processed food is normally 1-6 weeks. Temperature abuse under storage of sous-vide food can lead to high levels of surviving vegetative or spore-forming food-borne pathogens, especially when preservatives are not added. Most sous-vide treated food have a low-acid and high-moisture, and little to no preservatives are used in the products. Salt can be used to control growth of pathogens, although in healthier food, less salt is added, making it more challenging to control growth of surviving bacteria (Sebastiá et al., 2010).

2.6 High pressure processing

High pressure processing (HPP) has ability to extend shelf-life of products without using heat, with nutrients and quality of the food still maintained. The technology involves added pressure using liquid as a transmitter. This technology can be used to denature enzymes. Uniform pressure is added to the product, and the pressure does not cause damage to the product as long as the food is not hollow or contain empty space within it. The behavior of food under pressure are explained by the three principles, Le Chatelier's principle, Pascal's principle and principle of microscopic ordering. Le Chatlier's state that added pressure will lead to change the equilibrium of reaction towards the reaction leading to a bigger decrease in volume than before the added pressure.
Pascal's principle state that HPP give uniform pressure to the product, and it will return to its original shape afterwards. Principle of microscopic ordering state that the temperature and pressure will work as antagonist to the chemical reaction and molecular structure (Elamin et al., 2015; Yordanov & Angelova, 2010). The technology from HPP has promising result for

removal of meat from shellfish and crustaceans. It even has been documented that it even has beneficial effect on quality of the product. Pressure in HPP can also be adjusted to inactivate microorganisms. HPP hydrate the raw proteins in the meat, while traditional cooking dehydrate the proteins which lead to weight loss (Raghubeer, 2007).

HPP shuck crab meat without heat. Shucking is a process where the muscles get released from the exoskeleton, this make it possible to remove the muscle in one whole piece from the exoskeleton (Raghubeer, 2007). The muscle fibers of RKC lay in sheets or bundles attached to the exoskeleton in one of the sides in the joints (Lorentzen et al., 2019). HPP used for shucking at pressures normally between 250 and 400 MPa for 1 to 3 minutes (Raghubeer, 2007). In a study by Kristoffersen et al. (2009), effect of pressure range from 260 MPa – 280 MPa and temperature between 15 °C - 25 °C for 90 seconds were examined on thawed clusters. The results showed that HPP made removal of muscles easier than without the use of HPP. It was easiest to remove muscles that had been treated at 280 MPa, 15 °C for 90 seconds, however there was not a big difference from cluster treated at 260 MPa, 15 °C for 90 seconds.

2.7 Quality measurements

2.7.1 Digital measurement of color

Color is a central quality indicator for food, it can indicate spoilage. Color is affected by the chemical, biochemical, microbial and physical changes occurring during growth, maturation, postharvest handling and processing. Measurement of color in food is commonly used as an indirect indicator for other quality attributes including flavor and content of pigment because it is simple and the result correlates well with other physicochemical properties (Pathare et al., 2013).

For consumers, the appearance of food is an important quality indicator. A central part of the appearance is the color (Pathare et al., 2013). Food is judged by its color after the processing, and it is therefore useful to analyze color after treatment. Changes in expected colors can indicate problems with processing or packaging (Pathare et al., 2013).

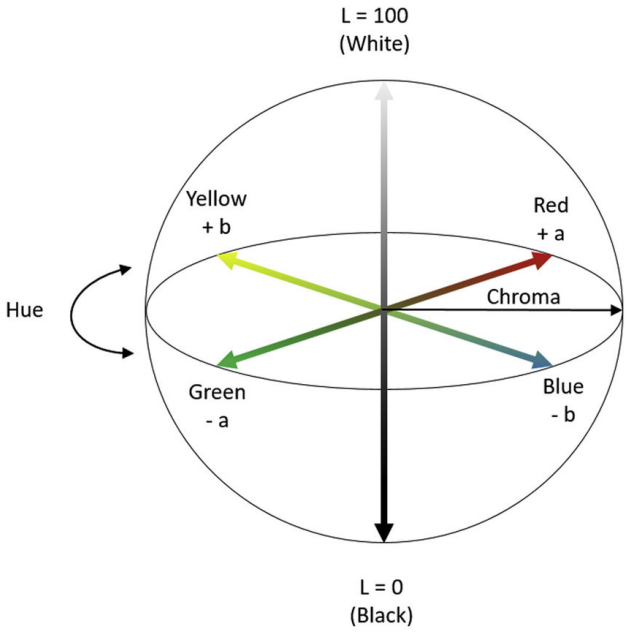

Figure 2.2: From: Ly et al. (2020)

To measure color of the samples, a calibrated digital photo imaging color-measuring system can be used. Lightness, intensity of color on red-green axis and intensity of color on yellow-blue axis. The three measures are complementary to each other. The result will therefore either be defined as light/dark, red/green and yellow/blue. L* measure the lightness, from 0-100. 100 meaning it is white. a* measure intensity of color on the red-green axis. Positive number means it is red, negative number means it is green. b* measure intensity of color on the yellow-blue axis. Positive number means yellow and negative number means it is blue as illustrated in Figure 2.2.

Skipnes et al. (2011) studied color changes of cod muscles as a result to heat treatment at different temperatures from 20 °C – 95 °C and time intervals of 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40 and 60 min. The color change and got whiter due to denaturation of proteins. In measured lightness, there was not detected a big difference for fish heated at 70 °C and above, but there was a significant difference between fish heated at 30 °C and 40 °C, this was due to denaturation of proteins, which occurs at 35 °C.

2.7.2 Drip loss after heat treatment and storage

Drip loss is a process of transfer of water from myofibril to the extracellular space.
The biggest source of drip loss in RKC cluster has proven to be the liquid that is entrapped in the legs in the interstitial space between the cooked meat and exoskeleton, which comes out through the shoulder joint of the cluster (Lian et al., 2021). It has been suggested by Sun et al. (2017) that there can be correlation with drip loss to proteolytic activity in the muscle during storage. It is desired with low drip loss in food products (Lian et al., 2021).

Protein denaturation has been considered as the main reason for water loss and textural changes in fish during thermal processing (Ofstad et al., 1996). Cook loss and WHC of cod

muscle do not correlate to the major protein transition as measured by DSC. Denaturation by heating, lead to expulsion of water (Ofstad et al., 1993). A study by Skipnes et al. (2011), showed that cook loss continued to increase even with samples heated to higher temperatures than temperature required to denature proteins. The fluid transport in fish has been presented as an equation for the total flux of vapor and liquid which is driven by a pressure gradient, gradient of water concentration and temperature gradient in the food. Water expelled out, will surround the fish and this cause a concentration gradient to build up. This goes against the expulsion of water. Depending on the time/temperature history and condition of the fish, proteins will be dissolved in the expelled water and decrease the concentration gradient in the opposite direction. The temperature gradient is given by the heat equation and will be log-linearly decreasing from a maximum when heating is started and approach zero as the temperature in the fish is evening out (Skipnes et al., 2011).
Structural changes and protein denaturation are irreversible (Ofstad et al., 1993; Skipnes et al., 2008). A small amount of the liquid will be absorbed by the fish during cooling, but it is not expected to cause any swelling (Skipnes et al., 2011).

Cook loss is liquid expelled out of the muscles during treatment. In a study by Skipnes et al. (2008) cook loss was measured to examine the relation between cook loss at different temperatures. The result showed that cook loss increase significantly in cod treated at temperatures above 80 °C.

2.7.3 Dry matter

Merus consist of water and dry matter. By weighing sampled before and after drying, it is possible to calculate how much water and dry matter samples consist of. This is further useful to calculate WHC, as they relate to each other (Skipnes, 2011).

2.7.4 Water holding capacity (WHC)

WHC is used as a quality parameter (Traore et al., 2012). For consumers the juiciness is considered to be an important parameter for the food quality. WHC can be used as an indicator for juiciness and firmness in the food (Skipnes, 2011). Denaturation of proteins cause myofibrils to shrink and expel water which result in a product with less juicy and more firm texture.

Level of protein hydration and the viscosity of liquid systems in food are related. WHC is used as a measure of proteins ability to retain water against gravity both physically and physiochemically. The most important role is their ability to interact with water, this further determines proteins functions including water binding and retention, swelling, solubility. Swelling is considered as an important function as many foods are water swollen systems. It can be defined as spontaneous uptake of water by a protein matrix. WHC is further of big importance looking at food texture. Water retention also play an important role in food texture, but also color and sensory properties of food products. Eating quality and storage stability are both affected by water content and water binding. The amount of water held by a protein powder or solid material with presence of excess water characterize WHC (Zayas, 1997, pp. 76-77).

Water in muscle structure can be divided into the three following parts, bound, immobilized and free. Bound water is the part close to hydrophilic non-water components, including proteins. Water inside and the layer surrounding proteins are considered as the bound. Interaction between water and hydrophilic sidechains in the amino acids constituents of water is responsible for keeping the water in place. The immobilized water is considered to be the part next to the bound water. The water with the ability to move freely are considered as the free water (Blikra, 2019).

Cod muscles share similarities with RKC muscles. Cod consist of 95 % immobilized water, which has the ability to move freely throughout the muscle structure. Cod muscles contain 0.3% fat, and the mean liquid loss for non-fatty species is 18.6%. About 0.1% of total tissue water content is chemically bound. 5-15% is immobilized in the myofibrils while the rest is extra-myofibrillar water. This is the part that are the most crucial for WHC. This water is located between myofibrils and cell membrane, between muscle cells and muscle bundles. Immobilized water is trapped within the muscle by capillary action that are generated by small "capillaries". Loss of water occurs due to expulsion of water from the myofibrils as they shrink when filaments approach. Heat-induced structural changes, sarcomere length, pH, ionic strength, osmotic pressure, and state of the *rigor mortis* affect WHC (Skipnes, 2011).

In a study by Skipnes et al. (2011) examining WHC in cod, heated at different temperatures (from 20 °C to 95 °C) at temperature above 30 °C, there was detected a decrease in WHC at this temperature due to denaturation of proteins. The measured WHC at temperatures above 30 °C changed over time. It was detected that it is possible with a 6-log reduction of *L. monocytogenes* (70 °C for 2 minutes) with a relatively high WHC.

Heated cod at different temperatures and time and looked at WHC, gave interesting result, because it showed that heating cod from 55 °C to 75 °C for 20-30 minutes, WHC measured was higher than what was measured at both higher and lower temperatures (Skipnes et al., 2011).

In a study Lorentzen et al. (2019) examining the effect of live holding of RKC at the temperature of 5 °C and 10 °C for up to 92 days, concluded with that the different temperature had an effect on WHC. 10 °C led to a higher value of WHC than at a temperature of 5 °C.

3 Materials and methods

3.1 Raw material

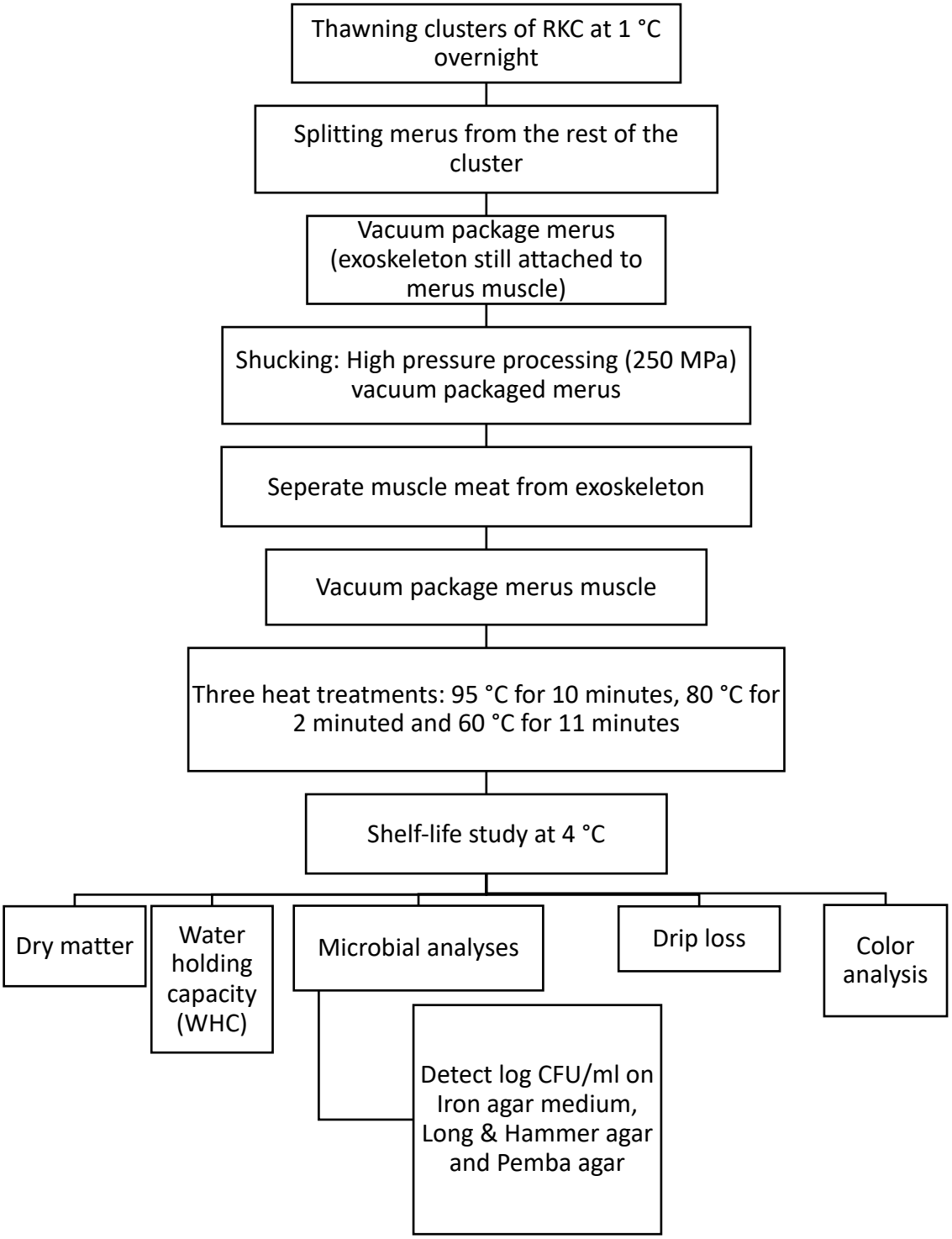

Figure 3.1: Workflow diagram over the process RKC went through from thawing to the analysis performed on RKC to decide its shelf-life.

Overview over the processing raw muscle meat from RKC was exposed to in this shelf-life study is illustrated above in Figure 3.1

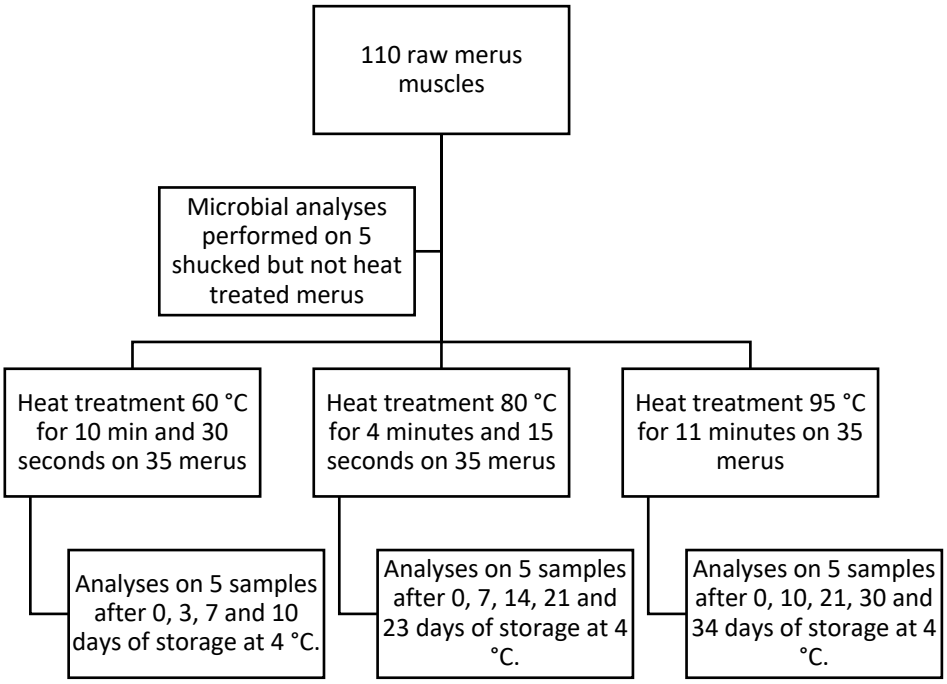

Figure 3.2: Flow diagram over how merus are divided into different treatments and days samples are performed on them.

Figure 3.2 is an overview over how the total 110 merus were divided into different treatments and further analyzed.
Frozen and glazed raw clusters of 500-700 gram were sent frozen from Honningsvåg (Norfra, Honningsvåg, Norway) to Nofima in Stavanger and kept frozen at – 30 °C for 6 days.

3.2 Processing

Figure 3.3: Thawed clusters used in the experiment.

Before treatment, the clusters were thawed at 1°C in the air overnight.
110 merus were removed from clusters and plastic bag (160x200mm, PA/PEK 20/50, LietPak, Lithuania) was wrapped around them and placed inside a bag (250x300mm, PA/PEK 20/50, LietPak, Lithuania) together with 4 other merus and vacuum packaged at 99% (Supermax C, Webomatic, Germany). The bag was then placed inside another and vacuum packaged in the same manner. This was done to prevent holes in the bag.

Figure 3.4: Vacuum packaged merus before shucking,

The bags were then high pressure processed (HPP) (Shucked) by placing the bag in the chamber as done in Figure 3.6, and the lid was then closed. The merus were high pressure processed in a high hydrostatic pressure machine QFP 2L-700 (Avure Technologies Inc., Columbus, USA) at 25 °C at 250 MPa for 2 minutes.

Figure 3.5:A: High pressure machine used to add pressure to merus in order to shuck them. B: Vacuum packaged merus was placed in the chamber with water

Figure 3.6: Vacuum packaged food placed in the chamber to be high pressure processed by the machine.

The muscles were then removed from their exoskeleton, by using scissors, knife and fingers to separate the muscles from the exoskeleton. Figure 3.7 A show how the exoskeleton looked

after muscle removal. The muscles were then placed on ice with a layer of plastic wrap between them as Figure 3.7 B show.

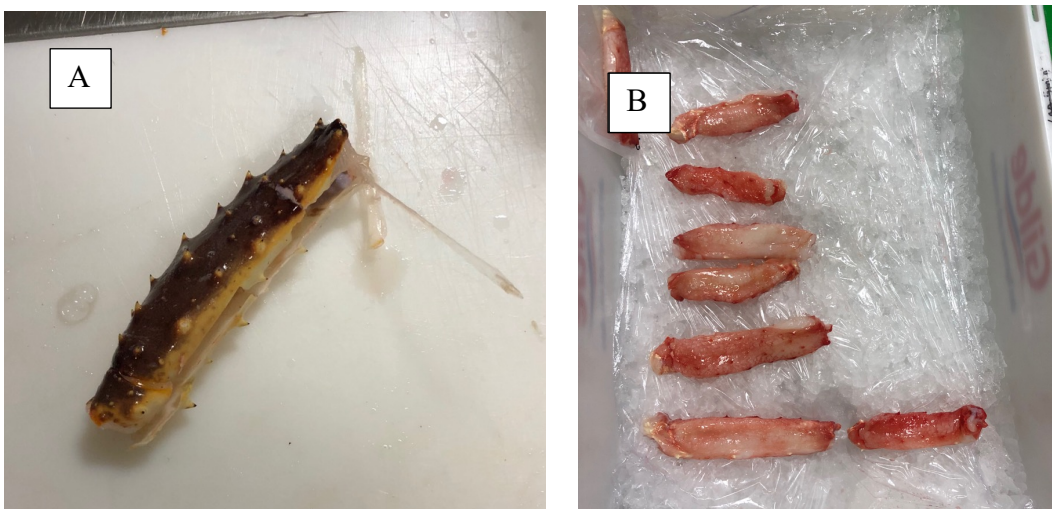

Figure 3.7: A: Exoskeleton and tendons after muscle removal. B: Muscle merus disconnected from exoskeleton, on a layer of plastic wrap on ice.

5 merus were randomly selected out for microbial analyses as Figure 3.2 show.
105 remaining merus were placed in labelled plastic bags and vacuum packaged in the same manner as done before HPP. Figure 3.8 A show merus that had been placed in labelled bags before vacuum packaging, and Figure 3.8 B is of merus after vacuum packaging. Some were connected to a temperature measurer (Eval Flex, ellab, Denmark) by making a hole in the bag and connecting it to the merus before vacuum packaging. This was done to measure the core temperature to some of merus during heat treatment.

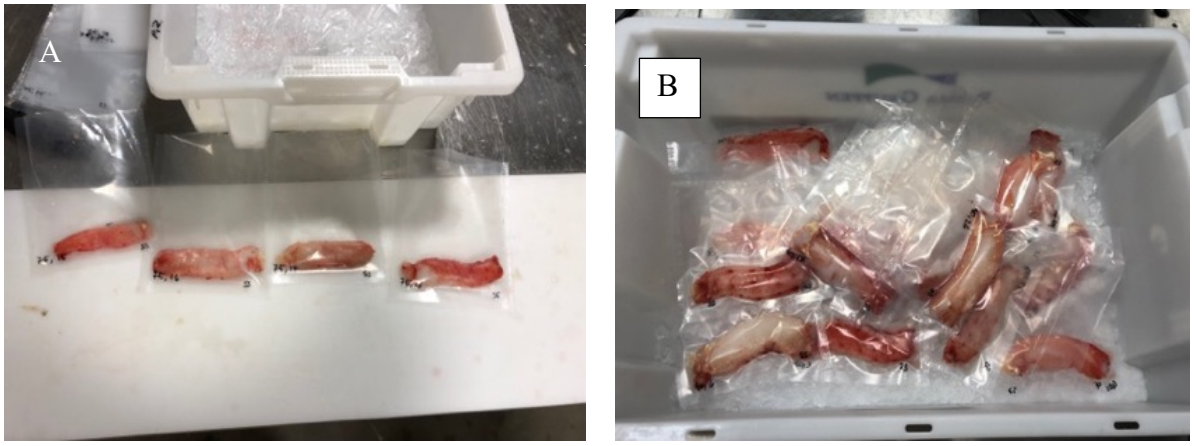

Figure 3.8: A: 4 merus muscles, put in labelled bags prior to vacuum packaging,
B: merus after vacuum packaging and before heat treatment.

Temperature measures (Eval Flex, ellab, Denmark) were calibrated before heat treatment. 105 merus were divided into 3 groups consisting of 35 merus. 35 Merus were heat treated in water bath (Lauda E100, and Lauda 2, Ecoline, Norway) with stirring. They were treated at the different temperatures, 95 °C for 11 minutes, 80 °C for 4 minutes and 15 seconds and 60 °C for 10 minutes and 30 seconds. The selected times and P-value were determined in a preliminary heat treatment, as explained subsequently (3.2.1). Figure 3.9 show merus treated at 95 °C. A temperature measure was placed in the core of the merus and water bath to control water bath to control temperature in the water bath. The P-value was measured by measuring

the core temperature. P-value was measured to verify that aimed P-value was reached during treatment. 5 samples from each heat treatment were randomly selected out as Figure 3.2 show. The remaining merus were stored at 4 °C after heat treatment.

Figure 3.9 Merus during heat treatment at 95 °C.

3.2.1 Preliminary heat treatment

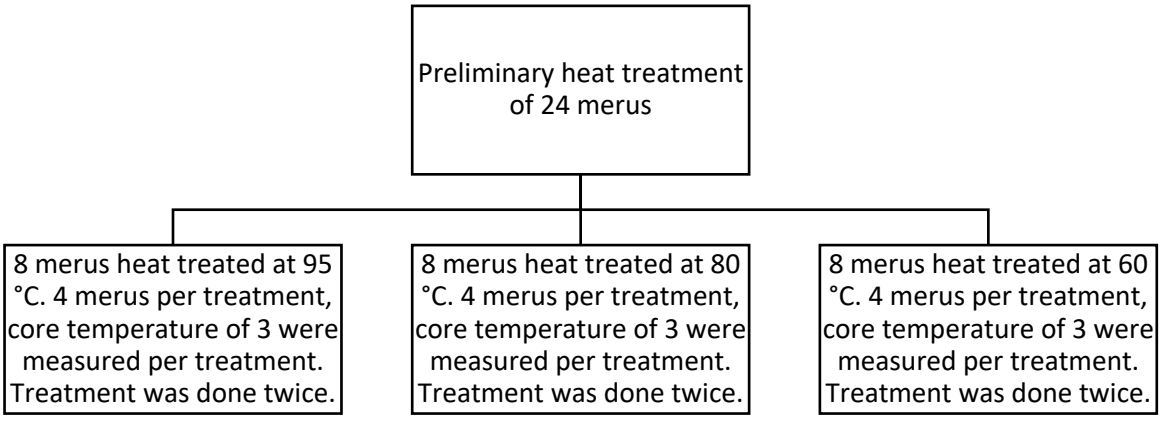

Figure 3.10: Overview over how the 24 merus heat treated in preliminary heat treatment were divided..

Prior to shelf-life study, the time and temperature combinations were tested with temperature measurements in the core of the merus. The aim was to obtain recommended g log reduction of the target organisms non-proteolytic *C. botulinum*, *L. monocytogenes* and *V. parahaemolyticus* as described in Table 2.1. The water bath temperatures were set 5 °C higher than target temperature (95 °C, 80 °C and 60 °C instead of 90 °C, 75 °C and 55 °C) in order to reduce time to obtain the log reduction in the core. A total of 24 merus muscle were heat treated in the same manner as previously explained (in heat treatment of merus in shelf-life study). The process followed the path from thawing to heat treatments as shown in Figure 1.2. They were divided into 3 groups, each consisting of 8 merus for the different heat treatments of 95 °C, 80 °C and 60 °C. 4 merus were heat treated per treatment and core temperature of 3 merus were measured, as Figure 3.10 illustrate. Each heat treatment was repeated twice, giving a total of 6 core measurements from each temperature. Merus treated at 95 °C were treated for 8 minutes, merus treated at 80 °C were treated for 5 mintes and merus treated at 60 °C were treated for 10 and 11 minutes in the preliminary heat treatments.
This gave temperature curves, and curve with the lowest heat measurements used (worst case scenario) to calculate the time to obtain 6-log reduction for the three target organisms, using D- values for the target organisms Table 2.1. These measurements results showed that merus treated at 95 °C must be treated for 11 minutes, merus treated at 80 °C must be treated for 4 minutes and 15 seconds and merus treated at 60 °C must be treated for 10 minutes and 30 seconds. The time and temperature combinations were used in the main shelf-life study.

3.2.2 Preliminary Shucking

Prior to shelf-life study, it was unknown if it would be possible to remove muscles in frozen and thawed clusters. The muscles in thawed clusters are more difficult to remove from the exoskeleton compared to clusters that have not been frozen. However, there are some challenges with handling live RKC. They would have to be killed, and due to microbial growth, the timeframe for handling and processing of them is shorter. The whole process would have to be done in one day, compared to thawed RKC where it is easier to split the process up in case of inefficiency or problems during the processing. Prior to the shelf-life study HPP was tested on part of thawed legs and shoulder from RKC and to further remove the muscles from the exoskeleton. This work was also carried out to obtain skills for removing merus muscles without making holes in the muscles. RKC was high pressure processed in the same manner as in the shelf-life study.

3.3 Analyses

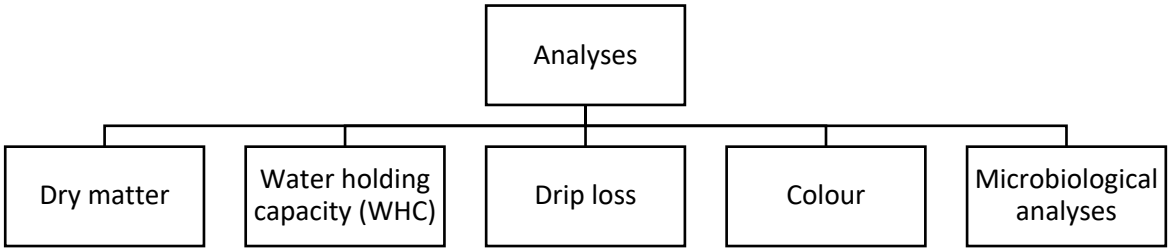

Figure 3.11: Analyses performed on samples of merus.

5 analyses shown in Figure 3.11 above, were performed on 5 parallels of merus from each heat treatment right after heat treatment. 5 Merus were analyzed after being stored for different times at 4 °C, as Table 3.1 show. The last two times of analyzation were set to the expected expiration date and a couple of days after.

Table 3.1: Overview over when 5 merus from the different heat treatments were analyzed. The merus treated at 60 °C were analyzed at four days, and not five as the rest.

Analyses of Merus / Heat regimes	The first analyses	The second analyses	The third analyses	The fourth analyses	The fifth analyses
60 °C	Day 0	Day 3	Day 7	Day 10	-
80 °C	Day 0	Day 7	Day 14	Day 21	Day 23
95 °C	Day 0	Day 10	Day 21	Day 30	Day 34

3.3.1 Drip loss

Drip loss was measured, consisting of liquid released during treatment and storage since the bags were not opened before analyzation.
Vacuum packaged merus were weighed. The total weight consisted of merus, liquid released during treatment and storage and the bag. The merus was then removed using a sterile tweezer, and the remaining bag with liquid was weighed (liquid loss + bag) and weight of bag (4.9 g) was subtracted to calculate liquid loss. The drip loss after heat treatment and storage was then calculated using the equations below. For the merus weighed directly after treatment on day 0, cook loss was calculated and not drip loss.

Equation 4

$$Liquid\ loss\ (g) = T_{tot} - (m_{tot} + bag)$$

T_{tot} = total weight of heat treated (and stored) merus in bag with liquid loss (g), m_{tot} = merus after treatment (and storage) (g).

Equation 5

$$Drip\ loss\ \% = \frac{Liquid\ loss}{T_{tot} - bag} * 100$$

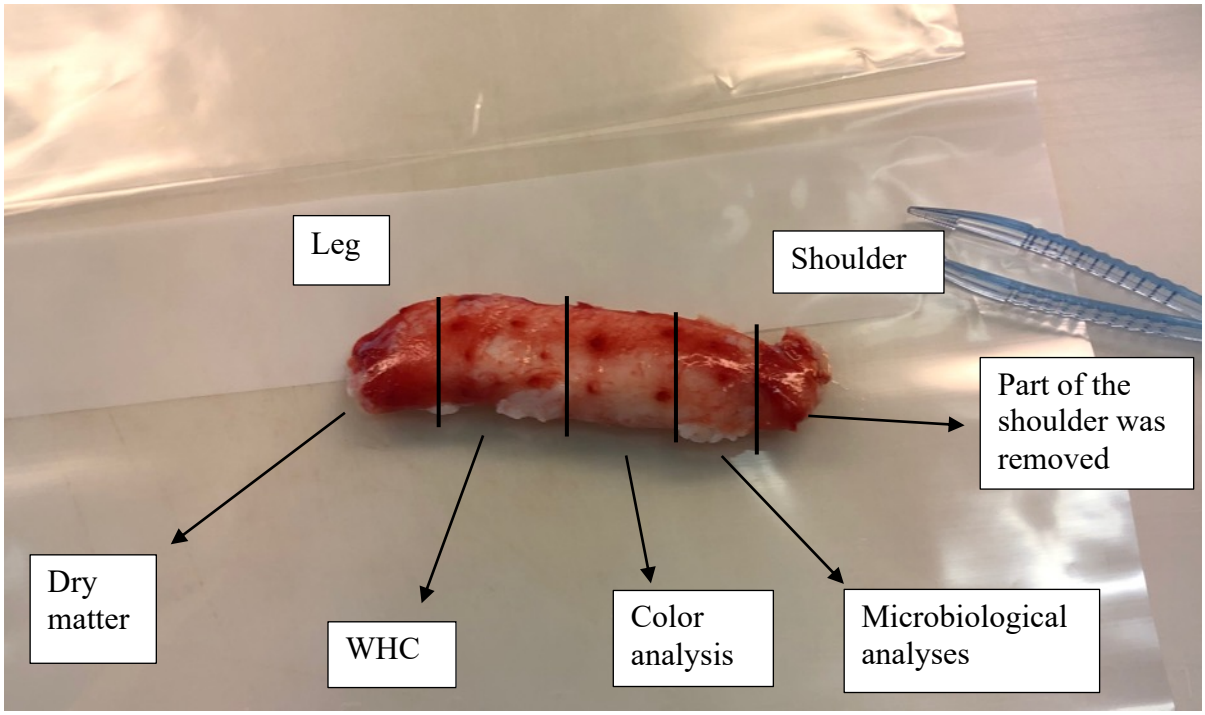

Figure 3.12: Merus were divided into different analyses as shown above. The shoulder part is the right part of the merus, and leg is the left part.

The merus were divided for the different analyses as Figure 3.12 show. The tools including, knife, scissor, cutting board, were cleaned with 70 % ethanol before getting in contact with new merus, a sterile stomacher bag without filter was placed on the cutting board and switched between every merus. Sterile tweezers were only in contact with one merus. About 8.0-10.0 gram of sample part was weighed out for microbiological analyses, the samples for color analyzation were about 2 cm in length. It was attempted to maintain a stable weight of the samples for WHC and dry matter, but as the merus sizes varied, so did these samples. Microbiological analyses were done first and the remaining pieces of merus were placed in labelled bags on ice and stored at chill temperature (4 °C) as Figure 3.13 below show.

Figure 3.13: Samples of merus for color, WHC and dry matter analyses. Picture was taken before they were moved to a room with refrigerated temperature.

3.3.2 Microbiological analyses

Iron and L&H agar were used to measure the spoilage organism hydrogen sulfide producing bacteria, *Shewanella,* some types of Aeromonaceae, Enterobacteriaceae, Vibrionaceae and lactic acid bacteria. Hydrogen sulfide producing bacteria appear black on the agar.

Diluent, Iron agar and L-cysteine mixtures and petri dish with Long & Hammer (L&H) agar were made in advance of the analyses following the procedures in NMKL 184 (Nordic Committee On Food Analysis, 2006). Which is meant for fresh and lightly preserved fish. Iron agar was made in volume of 400 mL in 500 mL bottles. They were stored at refrigerated temperature (2-4 °C).

Before sampling, bottle with Iron agar was placed in water bath at high temperature to let boil while merus were cut for the different analyses, and after it had liquified it was moved to a water bath or drying cabinet with the set temperature of 45 °C.

Samples of 8-10 g were weighed (Mettler Toledo, PB3001, Nr.59, Norway) in sterile blender bags with filter (Separator 400, Grade packaging Ltd, UK) and diluted tenfold by adding diluent to the bag. The mixture was then homogenized in blender SMASHER® (AES blueline, bioMérieux, France) for 120 seconds. The machine used in this study is in Figure 3.14 A, below. A homogenized mixture from this study is in Figure 3.14 B below. The samples were further diluted with two and third tenfold into 10^{-2} and 10^{-3} dilution of the sample in 2 mL sterile tubes by pipetting 150 µL of 10^{-1} sample and 10^{-2} sample with 1350 µL diluent.

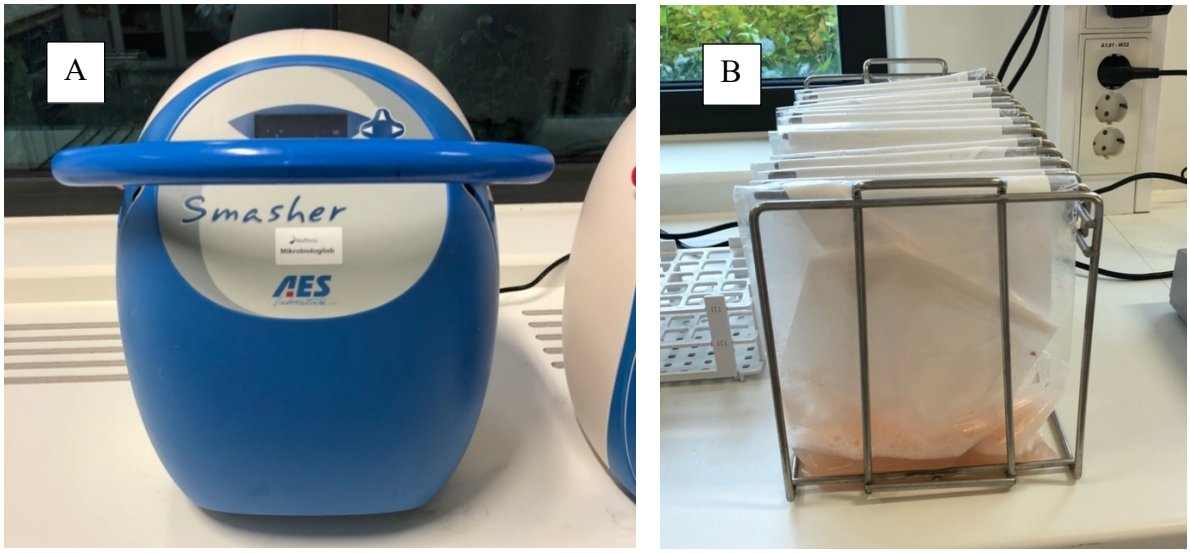

Figure 3.14: A: Smasher used to homogenize merus sample. B: Homogenized mixture in sterile blender bag

The petri dishes were labelled accordingly to the labelled bags and with dilutant and date. 49.2 µL of 10^{-1} and 10^{-3} dilutant were pipetted over to petri dish with L&H agar by using Eddy-jet machine (Eddy Jet v.123, Nerliens Mezansky, Norway), in Figure 3.15, below. Then incubated at 15 °C for 5-7 days.

Figure 3.15: Eddy-jet used to spread 10^{-1} and 10^{-3} dilution of the samples on L&H agar.

After iron agar had stabilized at a temperature around 45 °C, 3.2 mL L-cysteine was added carefully to the mixture to avoid bubbles. 1 mL of 10^{-1} and 10^{-2} dilutions were pipetted to petri dishes on a sterile bench. One layer of iron agar was added following pour plate method, the dishes were stirred by 8 clockwise rotations and 8 anti-clockwise rotations. After the first layer had solidified, another layer was poured over and stirred in the same manner. The plates were then incubated at 25 °C for 72 hours ± 6 hours.

100 µL of 10^{-1} dilution of the samples were spread on Pemba agar, using spread plate method, and incubated at 30 °C for 24 hours. Pemba agar was used to detect growth of *B. cereus*. Other organisms grow on the agar as well, but *Bacillus* have a blue color and surrounded by a blue zone. The plates were placed upside down in the incubator and checked regularly in order to be able to count colonies before swarming appeared or before the numbers became too many to count (TNTC).

3.3.3 Color

White merus muscles were photographed as illustrated in Figure 3.16, below, using DigiEye full system (VeriVide Ltd. Leicester, UK). The DigiEye system was calibrated prior to analyzation, using a white- and colored calibration board (DigiTizer Calibration Pack, VeriVide Ltd., UK). The samples were placed in a lightbox with daylight (6400 K) which was part of the imaging cube, on an aluminum board. The samples were photographed using a digital camera (Nikon D290, AF Nikkon 35 mm f/2D, Nikon, Japan). Digiview (VeriVide Ltd., UK) was used to take the photographs and Digipix software (VeriVide Ltd, Leichester, UK) was used to analyze the color. Areas that did not represent the color of the samples, including holes, were avoided.

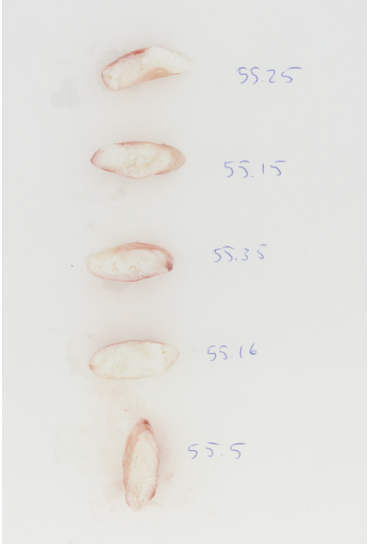

Figure 3.16: Some of the merus that were color analyzed.

3.3.4 Dry matter

Labelled aluminum cups were weighed before and after the sample was placed inside the cups. Samples weighed from 1.472-7.458 gram. The cups were placed on a tray as Figure 3.17 A show, and then dried at 107 °C for 16 – 22 hours. Followed by 30 minutes in a desiccator as Figure 3.17 B below show. The samples were then weighed again. The weights were written down and used to calculate the amount of dry matter and water in sample as Equation 6 and Equation 7 show. Water (%) was used to calculate WHC as Equation 8 show.

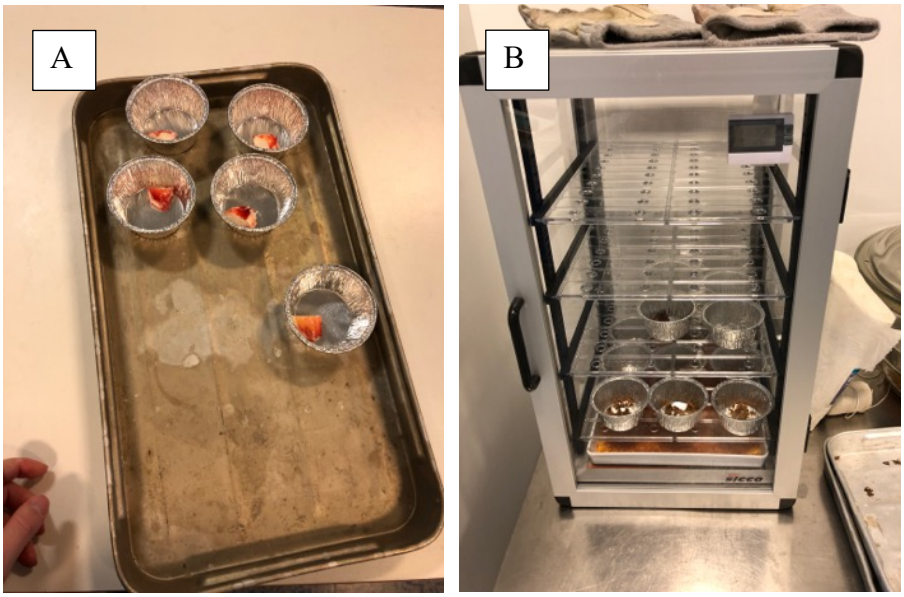

Figure 3.17:A: Samples in aluminum cups before drying. B: Dry samples in desiccator.

Equation 6

$$Water\ (\%) = \frac{(W_1 + W_2) - W_3}{W_2} * 100$$

W_1 = Weight of aluminum cup without sample (g), W_2 = Weight of the sample before drying (g), W_3 = Weight of dry sample in aluminum cup (g)

Equation 7

$$Dry\ matter\ (\%) = 100 - Water(\%)$$

3.3.5 Water holding capacity (WHC)

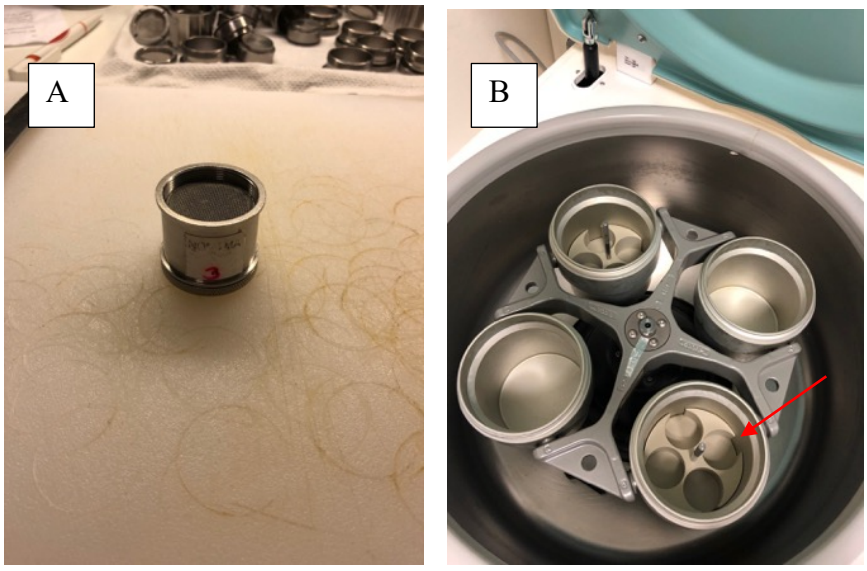

Figure 3.18: A: Cups placed in centrifuge to measure WHC of samples. B: Inside the centrifuge, the cups were placed in the small holes as the arrow points to.

The cups (Hettich, Germany) consist of four major parts, two bottom parts, one filter and one tube. The bottom parts were put together using a few droplets of oil. Both the filter and the bottom were screwed in the tube, into what Figure 3.18 A show.
The cups were then placed on layer of aluminum on ice in a Styrofoam box to cool the cups down. Samples were placed in cups and weighed. Samples weighed from 1.457 – 5.98 gram. The weight of samples with and without the weight of the cup were written down. The samples were placed where the arrow point in Figure 3.18 B, and centrifuged (Rotina 420R, Andreas Hettich GmbH & Co. KG, Germany) at 4 °C for 15 minutes at 1800 RPM. The bottom was opened and blow-dried with compressed air. The dry cups with the samples were then weighed again. WHC was calculated after dry matter and water was measured.

Equation 8

$$WHC\ (\%) = 100 * \frac{Water\ (\%) - weight\ loss\ in\ sentrifugee\ (\%)}{Water\ (\%)}$$

3.4 Statistical analysis

Data was analyzed in Minitab v.19 (Minitab Inc. USA) Statistical software, using GLM and Tukey's HSD test ($p < 0.05$) in factorial analysis of heat treatment. Storage time is continuous variable, and was included as a covariant in all the GLM. All results are shown as mean ± st. dev. unless else is stated.

4 Results

4.1 Preliminary heat treatment

Based on the preliminary heat treatment, time required was calculated as shown in Table 4.1 below. The aimed P-vaues are the theoretical values from guidelines, while the right column in the table show the time required in core of the merus at the water bath temperatures.

Table 4.1 Calculated P-value and time required for the different heat treatments.

Temperature	Aimed P-value	Time required
95 °C	10	11 min
80 °C	2	4 min and 15 seconds
60 °C	15	10 min and 30 seconds

4.2 Preliminary HPP shucking

The preliminary experiments showed that it was possible to remove the muscle in one piece from the exoskeleton. This was done easily enough to use frozen and thawed RKC in this study. The muscles were still more attached to the exoskeleton after HPP, than the case for RKC that have not been frozen.

4.3 Heat treatment

The merus were treated at the different temperatures, 95 °C for 11 minutes, 80 °C for 4 minutes and 15 seconds and 60 °C for 10 minutes and 30 seconds. The selected times were determined in a preliminary heat treatment, as explained subsequently (3.2.1).

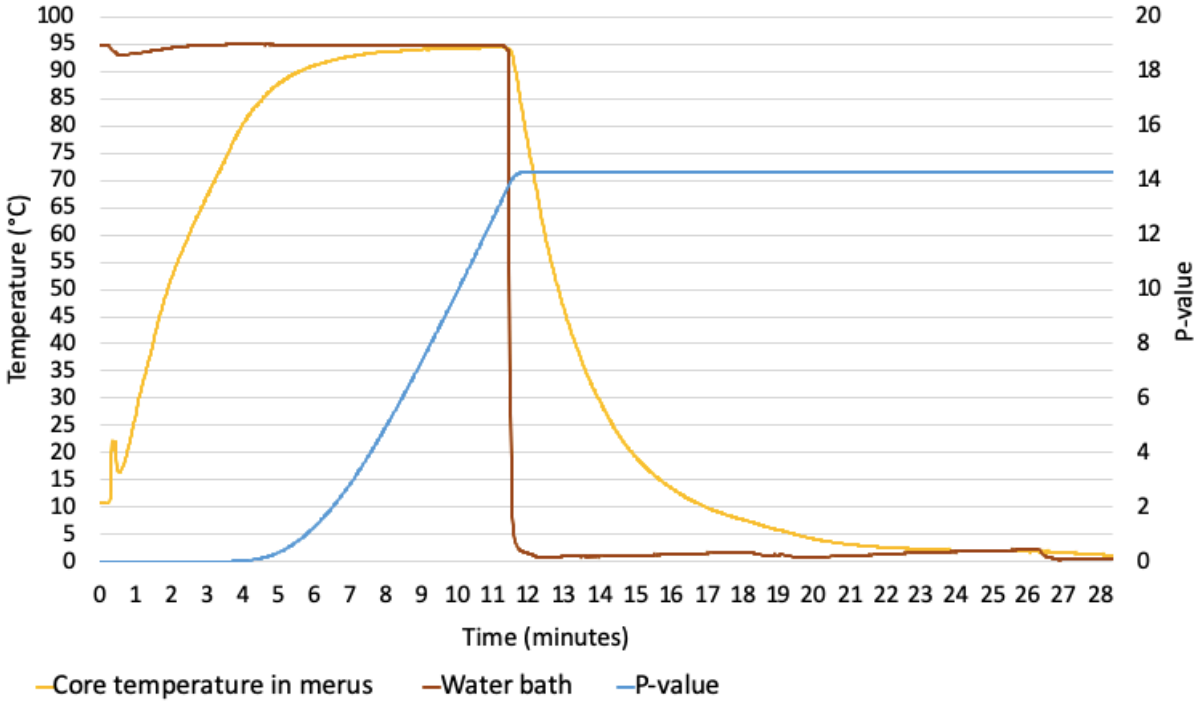

Figure 4.1: Measured temperature in core of merus, water bath and P-value.

In the shelf-life study, temperature probes were only used as documentation for the samples exposed to water bath temperature of 95 °C (Figure 4.1). The core temperature of merus increased to 94 °C at the end of the treatment and P-value accumulated to above 14. (Figure 4.1). Meaning the P-value of merus treated at 95 °C exceeded the aimed P-value.
As the line illustrating the P-value in Figure 4.1, the P-value increased even after the cooling had started. This is because it takes time to decrease the temperature through the center of the merus.

4.4 Microbiological analyses

Time of storage was detected as a significant ($p = 0.001$) factor on all three agars, but there was not detected a significance ($p > 0.05$) difference between the different heat treatments on any agars.

4.4.1 Iron agar

The result from microbial growth on Iron agar after heat treatment at 60 °C, 80 °C and 95 °C, is shown in Figure 4.2, below. High standard deviation for almost all samples were detected, especially for merus treated at 80 °C and 95 °C on day 21. As merus were stored, there was detected a growth of microorganisms on the Iron agar. For merus treated at 80 °C, there was detected a peak in level of microorganisms at day 14, but the lower level afterwards is probably due to the natural variation among the different merus.

For merus treated at 60 °C, there was detected a significantly ($p < 0.001$) lower level of log CFU/g on day 0 and 3 than on the samples from day 7 and 10.

For merus treated at 80 °C, there was detected a significantly ($p = 0.014$) lower level of log CFU/g on day 0 and 7 than on samples from day 14. Samples from day 21 and 23 were not significantly ($p > 0.05$) different from the previous mentioned days.
For merus treated at 95 °C, there was detected a significantly ($p = 0.042$) lower level of log CFU/g on samples from day 0 than on samples from day 34. The samples on the days 10, 21 and 30 were not significantly ($p > 0.05$) different from day 0 nor day 34.

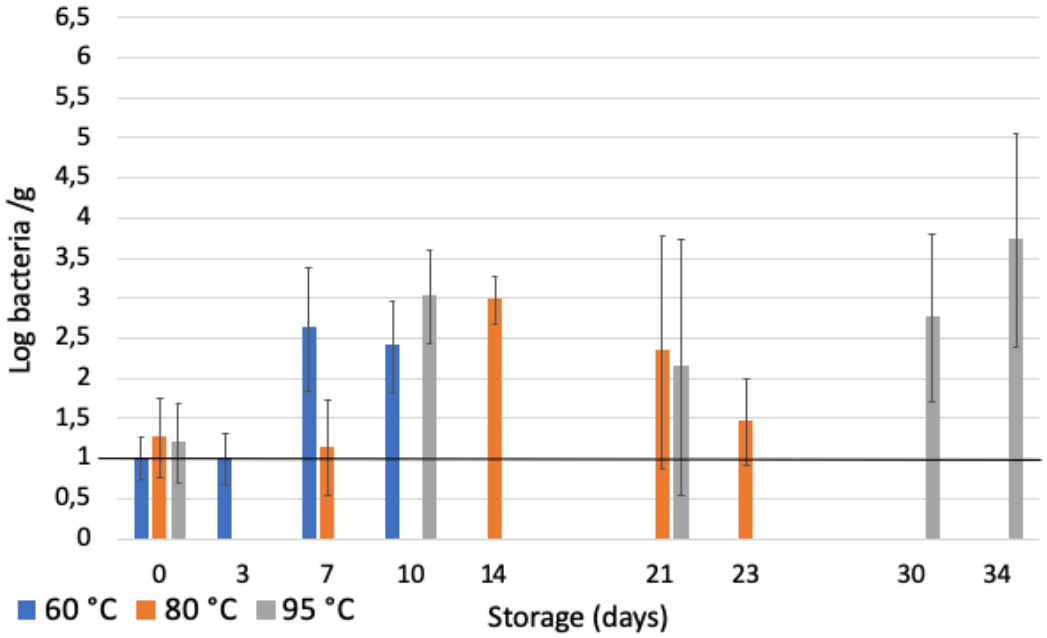

Figure 4.2: Result of microbial growth on Iron agar for merus treated at the different heat regimes and stored at different times at 4 °C. The black line (with y-value of 1) is the detection limit for iron agar counts.

4.4.2 Long & Hammer agar

Time of storage had a significant ($p = 0.001$) effect on level of microbial growth on Long & Hammer agar.
The result from microbial growth on Long & Hammer agar is illustrated in Figure 4.3, below. The standard deviation of samples from day 7 were high. It was detected an increase in growth in this analysis followed by a decrease at the end of the shelf-life as well, but not at noticeable as in Iron agar, with the exception of merus treated at 95 °C, this is also likely due to natural variation.
For merus treated at 60 °C, there was detected a significantly ($p = 0.007$) lower level of log CFU/g on day 0, 3 than on day 7. Level of log CFU/g on day 10 were not significantly ($p > 0.05$) different from the other days.
There was not detected a significant ($p = 0.118$) difference for times of storage in the other heat treatments.

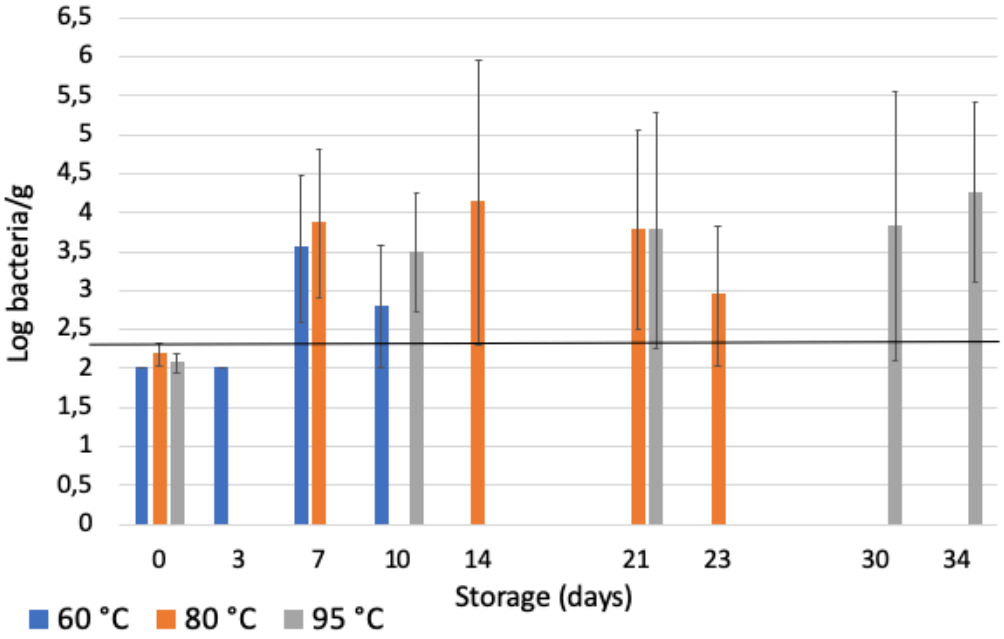

Figure 4.3 Result of microbial growth on L&H agar from merus treated at different heat regimes and stored at different times at 4 °C. The black line (with y-value of 2.3) is the detection limit for Long & Hammer agar counts.

4.4.3 Pemba agar

The result from microbial growth on Pemba agar is illustrated in Figure 4.4, below. The standard deviation for most of the samples, was not noticeably high, with the exception of samples of merus treated at 95 °C on day 21, 30 and 34. For the heat treatments at 60 °C and 80 °C the level of CFU/g did not change so much during the shelf-life, but there was detected a rapid growth between day 30 and 34 on merus treated at 95 °C from 2.42 log CFU/g to 3.59 log CFU/g.

There was detected a significant ($p < 0.05$) difference between times of storage from merus treated at 80 °C and 95 °C. For merus treated at 80 °C, there was detected a significantly ($p = 0.018$) lower level of CFU/g on samples from day 23 than from day 21. Samples from day 0, 7 and 14 were not significantly ($p > 0.05$) different from the samples from day 21 and 23. Samples from merus treated at 95 °C, merus from day 0, 21 and 10 were significantly ($p = 0.002$) lower than samples from 34. Samples from day 30 were not significantly different from the other days.

There was not detected a significant ($p = 0.052$) difference for times of storage and microbial growth on Pemba agar for merus treated at 60 °C.

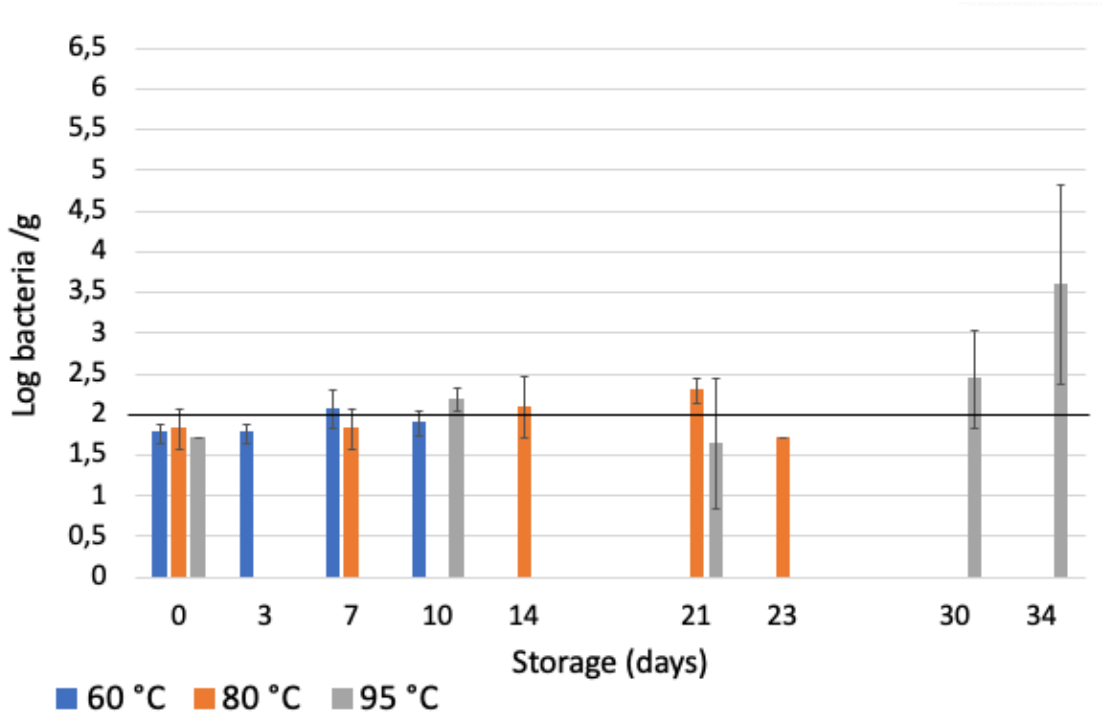

Figure 4.4: Result of microbial growth on Pemba agar from merus treated at different heat regimes and stored at different times at 4 °C. The black line (with y-value of 2) is the detection limit for Pemba agar counts.

Most colonies were yellow in color. Some were blue, with a zone around it, indicating it could be a type of *Bacillus*. They were looked at using a microscope and one of them looked like it could be spores of *B. cereus.* However, it is required with further testing to verify it. The possible *B. cereus* was detected in merus treated at 95 °C and had been stored for 10 days at 4 °C. The rest looked are likely a different strain of *Bacillus (*Figure 4.5 B*)*.

Throughout the study, there were only observed hydrogen sulfide producing bacteria from samples from heat treatment at 95 °C. This was observed in 3 petri dishes from merus stored for 34 days and in 1 petri dish stored for 30 days (Figure 4.5 A).

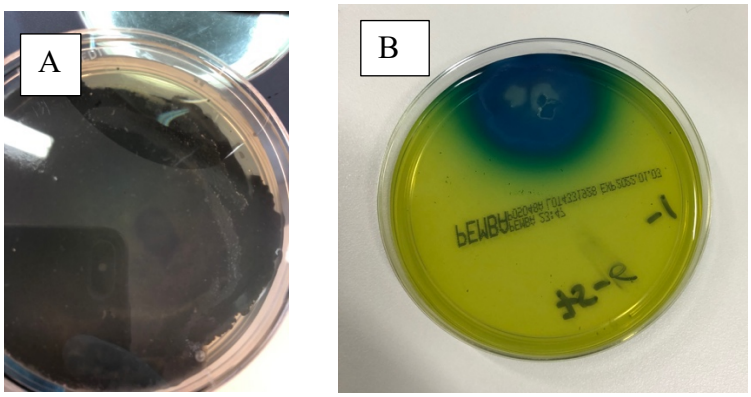

Figure 4.5:A: Hydrogen sulfide producing bacteria on Iron agar with sample of merus heated at 95 °C from day 34. B:Pemba agar with two blue colonies. One of them was looked at in a microscope, and it did not look like B. cereus. The colonies are likely to be Bacillus.

4.5 Cook loss

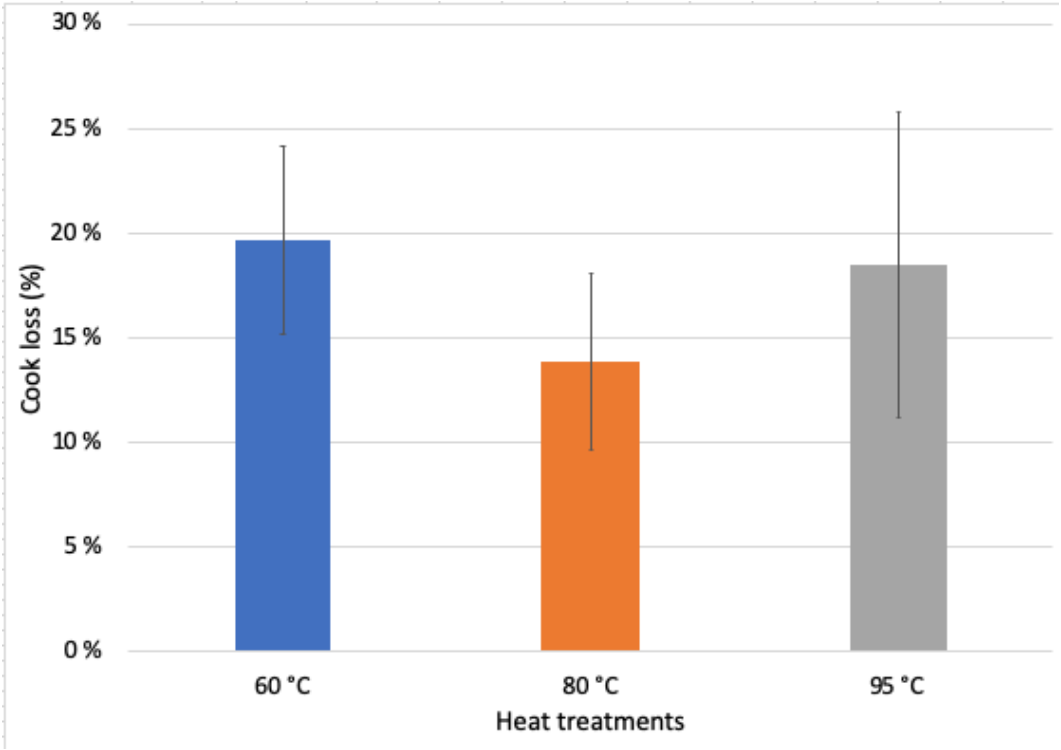

Figure 4.6: Cook loss of merus treated at the different heat regimes.

There was not detected a significant (p = 0.091) difference in cook loss between the different heat regimes (Figure 4.6). As the figure show, there was not detected a significant difference due to the high standard deviation.

4.6 Drip loss after heat treatment and storage

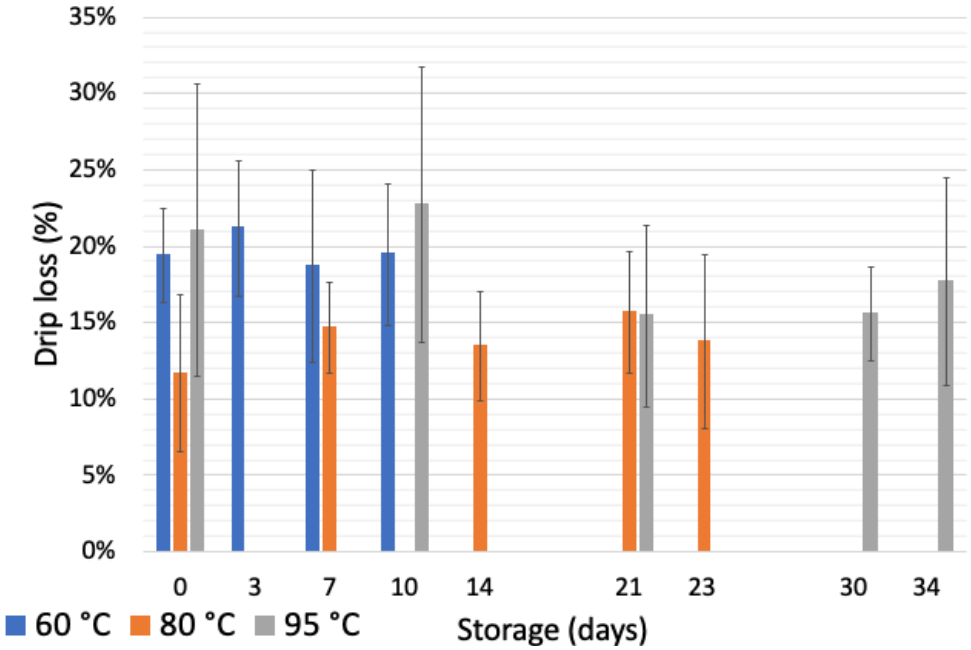

Figure 4.7: Drip loss from merus heat treated at 60 °C, 80 °C and 95 °C, and stored at 4 °C after treatment The result on day 0, is not drip loss but cook loss as the liquid loss was weighed directly after treatment.

There was detected a significant ($p = 0.002$) difference in drip loss between the different heat regimes. Merus heated at 95 °C and 60 °C had a significantly higher drip loss than merus heated at 80 °C (Figure 4.7). There was not detected a significant ($p = 0.228$) difference in drip loss between different times of storage. Figure 4.7 show measured drip loss in merus exposed to different temperature regimes and times of storage.

4.7 Dry matter

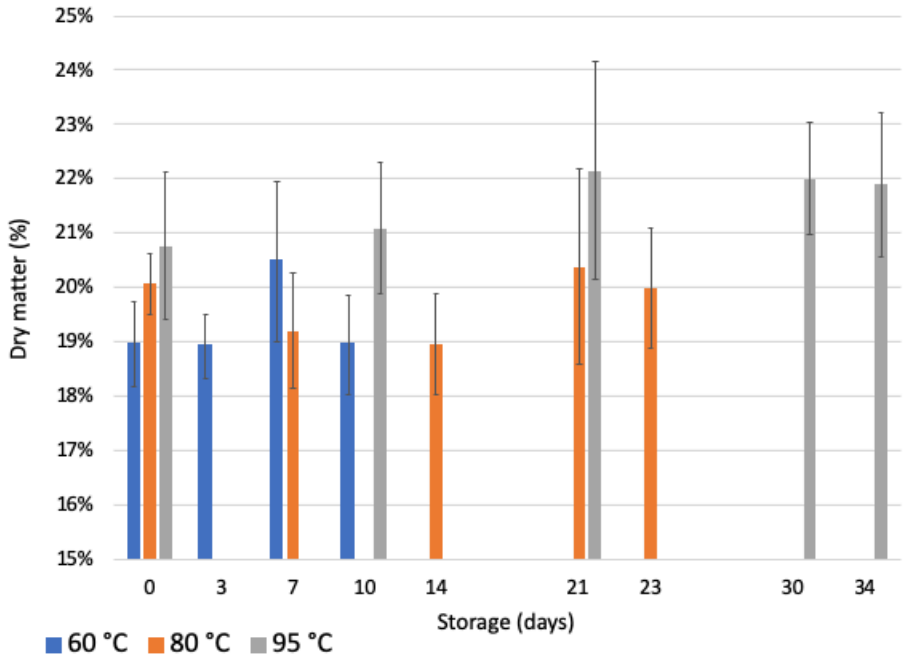

Figure 4.8:Measured dry matter of merus exposed to different heat regimes and times of storage.

There was detected a significant ($p < 0.001$) difference in dry matter value between the different heat regimes. Merus treated at 60 °C and 80 °C had a significantly lower value than merus treated at 95 °C.
There was detected a significant ($p = 0.045$) difference in dry matter value between the different times of storage as well. Dry matter increased slightly with time of storage. The estimated increase is the same for the different heat regimes. Figure 4.8 show measured dry matter values.

4.8 WHC

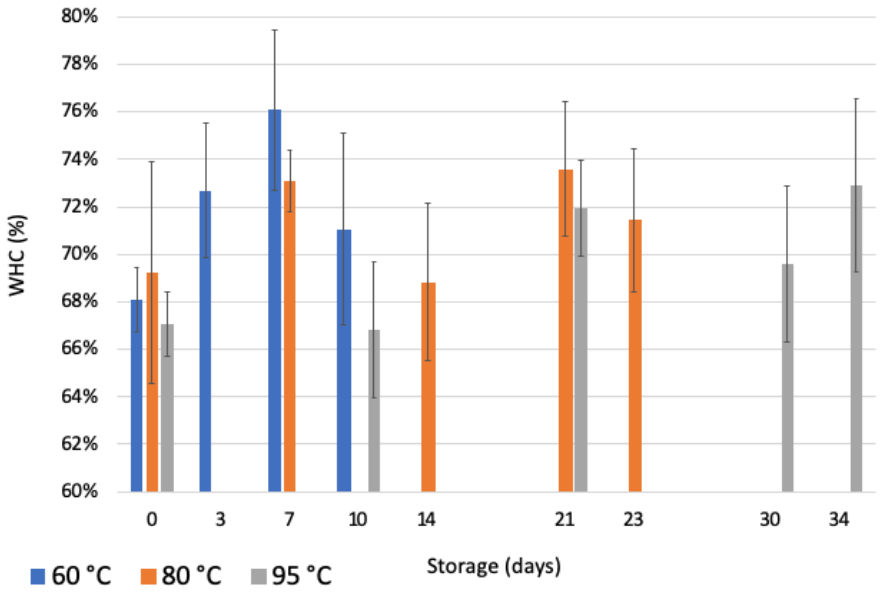

Figure 4.9: Measured WHC values of merus exposed to different heat regimes and times of storage.

The heat regimes at the temperatures of 60 °C, 80 °C gave a significantly ($p = 0.002$) higher WHC values than heat treatment at 95 °C (Figure 4.9). There was also detected a significant ($p = 0.001$) difference in WHC-value between different times of storage, where WHC increased with longer time of storage.

Color

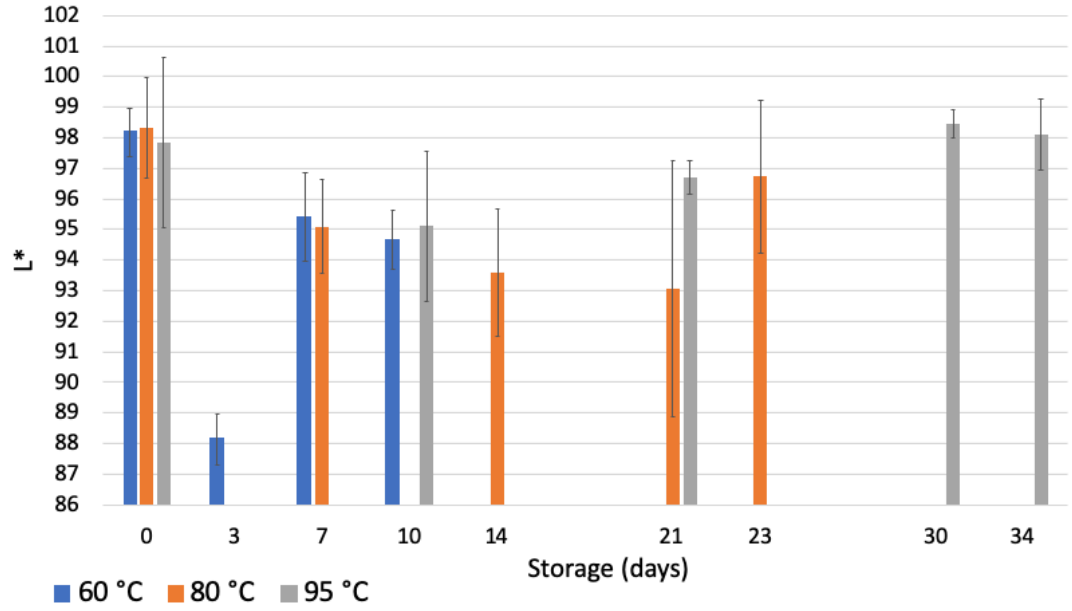

Figure 4.10 Measured L of merus exposed to different heat regimes and times of storage.*

There was detected a significant (p = 0.002) difference in L*-value between the different heat regimes. L*-value of merus heated at 95 °C was significantly lighter compared to 60 °C. 80 °C were not significantly different from 60 °C or 95 °C. There was not detected a significant (p = 0.778) difference in L*-value between the different times of storage. Figure 4.10 show the different L*-values measured in the samples.

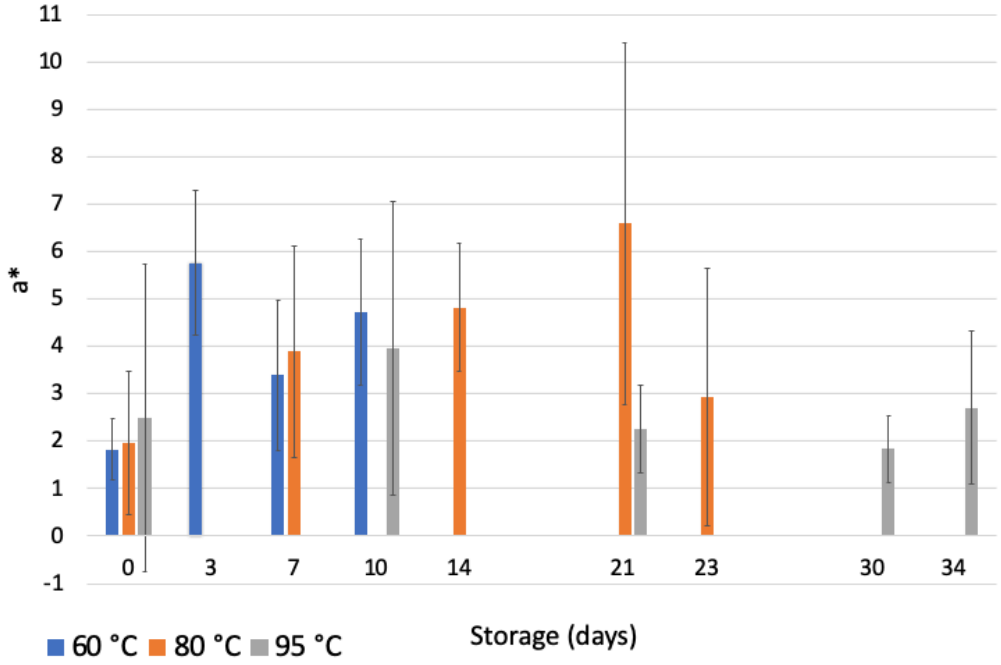

Figure 4.11: Measured a-values of merus exposed to different heat regimes and times of storage.*

No significant differences in neither a*-value nor b*-value were found on any of the design factors (heating regime and storage time) Figure 4.11 show the measured a*-values, and Figure 4.12 show the measured b*-values.

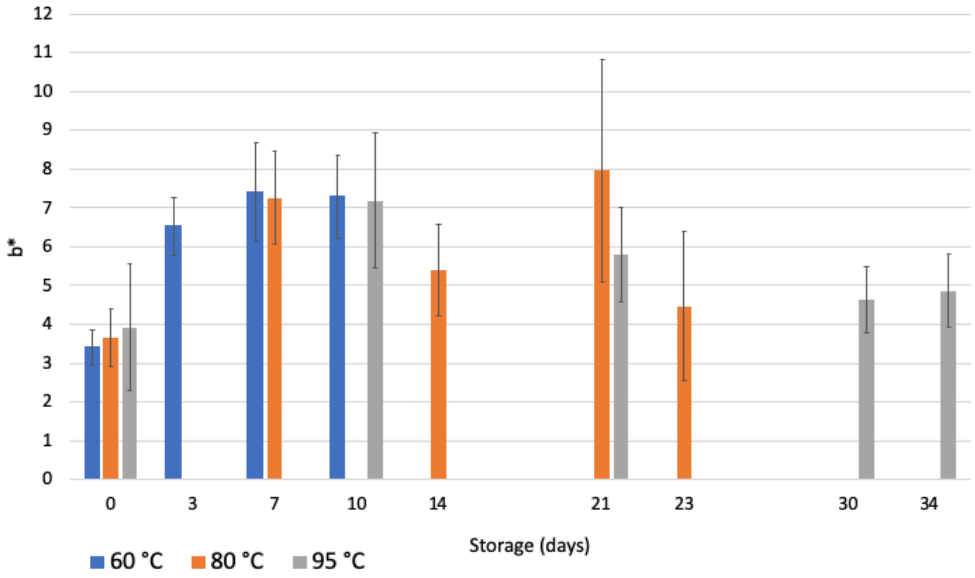

Figure 4.12: Measured b-values of merus exposed to different heat regimes and times of storage.*

5 Discussion

5.1 Treatment used in this shelf-life study

Sous-vide is a mild heat treatment that is used to extend shelf-life of minimally processed food product (Baldwin, 2012). Sous-vide refers to a method of cooking food slowly in a vacuum sealed pouch at low temperature to retain most of the nutrients, taste, juiciness and aroma. Due to these good properties the method has been used by the Hotel-Restaurant-Catering (HoReCa) segment for a long time, but mostly for cook serve systems without any storage and long shelf-life (Mikkelsen). King crab is considered to be a product of high quality and sought after in a gourmet context. Therefore, there was a need to investigate whether this raw material could be produced on an industrial scale and with new technology, so that it could fit into an online store chain with sufficient food safety. The main innovative elements tested in this work were: 1) use of frozen RKC, 2) use of HPP for shucking and 3) use of sous-vide treatment for 3 different shelf-lives in the refrigerated state.

5.2 Pasteurization values

The three heat treatments used in this study, were chosen as examples of possible treatments that can be used in the future e-commerce chain. The requirements can vary from local production to international distribution. A local production can sell products with shorter shelf-life than international distribution can. Target organisms described in Table 2.2 were used to set up time-temperature combinations that provided sufficient food safety throughout the shelf-life. The safety and shelf-life evaluation follow international recommendations (The safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to non-proteolytic *C. botulinum*). The final choices of time and temperature that were calculated from the temperature measurements in the water baths in the preliminary experiment (Table 4.1) have given safe and sufficient inactivation of the target bacteria. The additional measurement of core temperature at 95 °C showed that pasteurization value was 14 minutes (Figure 4.1) which is higher than $P_{90} = 10$ and give 9.3 log reduction of non-proteolytic *C. botulinum*, compared to 6-log reduction. Satisfactory heat treatment was also documented by bacterial counts immediately after processing (Day 0) with low bacterial numbers, close to or below detection level of the method (Figure 4.2, Figure 4.3 and Figure 4.4). High pasteurization value gives safer products, but may also have a negative impact on the quality of the food. For future, applications work should be done to optimize the time-temperature combinations for both safety and quality purpose.

5.3 HPP for shucking

Merus muscles were removed in one piece, but many muscles were teared up. This had a negative impact on the appearance of the merus, making them less appealing. In the study by Kristoffersen et al. (2009) examining effect of HPP on thawed frozen raw RKC, an increase in pressure from 260 to 280 MPa at 15 °C for 90 seconds, made the removal slightly easier. It is

therefore expected that the removal can be easier by increasing the pressure. The removal got easier as it was worked on, so it is likely that the result will get better with practice. This overall indicate that frozen RKC can be used in the industry, instead of seasonal harvesting. This is beneficial as it has been proven that the quality of postmolt RKC is poorer than premolt (Lian et al., 2021).

5.4 Microbiological analyses

At the end of the shelf-life of the merus analyzed in this study, it was expected with about as high levels of TVC as detected in shelf-life study by Lorentzen et al. (2014) on clusters of RKC. In that study, TVC from legs increased to about log 4.5 CFU/g in sample from shoulder on day 5, and log 5 CFU/g in sample from legs on day 8. At this time, the product was considered to of poor quality and therefore setting the shelf-life to 5-8 days for freshly cooked RKC. As sous-vide treatment was not used and they were exposed to air in the study by Lorentzen, it was therefore expected with longer shelf-life in the present study than in that study. The microbiological analyses done in study by (Sun et al., 2017) on swimming crab examined the effect of storing the crab in MAP varying part consisting of CO_2 and oxygen. The result showed that MAP slowed down the microbiological growth in total aerobic count. This support the expectation of a slowed microbial growth by changing the atmosphere around the food. As the conditions were different in that study than in present study, it was not expected with the same levels of microorganisms in present study. The mean values from the result in present shelf-life study did not exceed that level of microorganisms from the study by Lorentzen et al. (2014) nor the limit of good quality in the guidance by Norwegian Food Safety Authority from 2002 (Statens næringsmiddeltilsyn, 2002) (Table 3.1), which was set to an average of 5 log. The result indicates that vacuum packaging merus reduced the microbiological growth even more than expected.

It was detected a high value of standard deviation, meaning that level varied between the samples. This is causing a higher uncertainty associated with the result. Most of the result were at relatively low levels of CFU/g.
Many of the samples with higher or lower levels of CFU/g (than other samples from the same day), had the corresponding high or low levels in samples from the other dilutions and on both Iron agar and Long & Hammer agar. Some merus were exposed to air for a period during handling of samples and contamination can be one cause of the variance, although such contamination is likely to be small. The temperature during storage was logged, and there was no indication of temperature variation during storage.

There occurred a rapid growth of spoilage organism in some samples on Iron and L&H agars, some of the values therefore had to be estimated. For merus treated at 95 °C, samples from day 10, 21,30 and 34 had 1-2 parallels that were TNTC (too numerous to count). For those treated at 80 °C there were 2 parallels with TNTC, and there was 1 on day 21. For merus treated at 60 °C, there was one sample on day 7 on iron agar that was TNTC. This was expected as this was at the end of expected shelf-life.

The high values detected in merus treated at 95 °C and 80 °C could indicate that it had occurred something wrong during processing. It is possible that leakage might have appeared in some of the bags, in cases were not all bags were properly sealed. A small hole in the sealing may lead to penetration of contaminated water from the cooling water. However, in general, all packages kept their original shape, apparently with vacuum. It is therefore not so likely that this caused the high standard deviation.

B. cereus is a pathogen which will survive heat treatment, it was however not expected a high level of them in the samples. As there was only found one colony that can be presumptive *B. cereus* indicating that the pathogen is present at low levels, so the result fits the expected level of the pathogen. It further verifies that this pathogen should be taken into consideration in regard to shelf-life.

The overall results from the microbiological analyses verifies that shelf-lives were extended by using sous-vide method as treatment. As the merus treated at 80 °C and 95 °C then have a shelf-life of 21 and 30 days, this indicates that there is a potential for online stores to sell this as a product safely. As the shelf-life of merus treated at 60 °C have the relatively short shelf-life of 7 days, it might be more difficult for online stores to safely sell this product, but there is a potential for online stores, for instance for stores where shipping does not several days.

5.5 Cook loss

The meat muscles in RKC are similar to muscles in cod. In the study by Skipnes et al. (2011) for cook loss of cod treated at different temperatures for different times, the result followed the trend of higher heat load gave higher cook loss. Cod heat treated at 50 °C for about 20 minutes had a higher cook loss than cod heated at 70 °C for less than 10 minutes. This result is interesting as it indicates that a pasteurization of *L. monocytogenes* can be used and still get a product with lower cook loss than pasteurization of *V. parahaemolyticus*.
The result from the present study seem to follow the same trend, as those treated for the longest times at 60 °C and 95 °C had similarly high cook loss, and merus treated for shorter time at 80 °C had lower cook loss. There was not detected a significant difference. This is due to the high standard deviation (illustrated in Figure 4.6). The high standard variation was not expected. This can partially be explained by the differences in the sizes of merus. The cook loss was measured of merus that weighed from 25.9 g – 51.5 g (treated at 95 °C), 34.2 g - 51.2 g (treated at 80 °C) and from 34.7 g – 49.5 g (treated at 60 °C). It is likely that the smallest merus had a higher core temperature for a longer time than the bigger merus.

5.6 Drip loss

In a study by Lian et al. (2021) examining quality difference between RKC postmolt, from the spring and intermolt from the autumn. Drip loss after storage at refrigerated storage was 2.6 % ± 1.2 % for those stored for 1 day, 6.0 % ± 3.0 % for those stored for 3 days and 7.5 % ± 3.4 % for those stored for 5 days. The heat treatment aimed at a core temperature of 92 °C in the merus for 11.5-12 minutes. Cook loss was lost as they were not vacuum packaged.

In the study by Sun et al. (2017) examining effect of modified atmosphere packaging and superchilling on shelf-life of swimming crab, drip loss was measured. The trend showed an increasing drip loss in this study was well. The results from these studies cannot be directly related to the present study, however, they indicate that it could be expected with increasing drip loss with increased time of storage. It was not explained what time and temperature were used during treatment in the study by Sun et al. (2017), it is therefore unknown what treatment in present study had the most similar temperature and time. In the study by Lian et al. (2021) the result indicates that there is expected with an increase in drip loss in merus treated at 95 °C, however, this can also be expected for the rest as well. This was not detected in this study.

Skipnes et al. (2011) stated that in cases of heat-treated fish stored with liquid expelled during treatment, in a vacuum pouch. This will stop drip loss from increasing as long as it is stored under refrigerated temperature. The dissolved proteins will coagulate on the fish surface, resulting in a stop of more drip loss from being released. If this applies to RKC muscles too, it can explain why the drip loss did not increase with increased time of storage. The merus were kept on ice before weighing them, and therefore had a relative low temperature when drip loss was measured.
As Sun et al. (2017) suggested that there is a correlation between drip loss and the proteolytic activity in muscles, the result can indicate this activity was kept at a slow pace by keeping merus in the vacuum packaging.

It was detected a difference of significance between samples heated at different temperatures ($p = 0.002$), temperature during treatment is of importance when it comes to drip loss. As it is preferred with lower drip loss, merus heated at 80 °C had the most promising result. In Figure 4.7, times of storage was not detected to be of significance ($p > 0.05$). Merus treated at 60 °C and 95 °C had a significantly higher drip loss than merus treated at 80 °C.
The result of WHC and dry matter for merust treated at 95 °C support the high drip loss, as it is expected that more liquid is released when WHC is relatively low and this can cause the dry matter to increase. More proteins in RKC are likely to be denaturized at the treatment at 95 °C leading to more liquid getting expelled out and therefore having a worse ability to retain water. The higher drip loss in merus treated at 95 °C might indicate that those were overcooked, but there was not done enough analyses on the quality of the muscles to draw this conclusion.

5.7 Dry matter

In the study by Lorentzen et al. (2019) RKC that were killed and cooked on day 0, had a water content of 78.5 % ± 0.8 %, meaning the dry matter was about 21.5% ± 0.8%. As the clusters in the study were heat treated in boiling water, it was therefore expected with a similar result in merus treated at 95 °C. This result fit well with the result of merus in this study (Figure 4.8). The drip loss for merus treated at 95 °C were significantly higher than merus treated at 80 °C, it was therefore not surprising that the level of dry matter was significantly higher than in merus treated at 80 °C. It was surprising that level of dry matter for merus treated at 60 °C

was so low, considering the drip loss. This can indicate that cook loss in merus treated at 60 °C consisted of less water than merus treated at 95 °C. However, the result from dry matter in merus treated at 60 °C fit well with result from WHC for merus treated at 60 °C. The result of dry matter in merus treated at 95 °C, indicate that treatment at 95 °C give the least juicy product.

5.8 WHC

In the same study as mentioned in 5.7, above, by Lorentzen et al. (2019), the result on RKC treated at day 0, had measured WHC of 67.8 % ± 1.3% This also fit pretty well with the result on merus treated at 95 °C in present study. Based on results from the study by Skipnes et al. (2011), it would be expected that treatment at 60 °C and 80 °C would lead to a higher WHC than treatment at 95 °C. The estimated values from the study were lower than the results in present study. As the study is examining cod and not RKC, it is not expected that estimated values can be directly used in present study. The results in present study fit however, well with the trend from the study by Skipnes et al. (2011). In cod, a treatment at 90 °C for 10 minutes, lead to denaturation of most proteins, resulting in high cook loss and low WHC (Skipnes, 2011). This result fit pretty well with the result seen in merus treated at 95 °C.

Both dry matter and WHC are affected by the time of storage. As it is a relation between them, it is not surprising that both are affected. Time of storage was of ($p = 0.001$) significance, WHC increased with time of storage. This indicate that the proteins ability to retain water increase. However, time of storage was also of ($p = 0.045$) significance for dry matter, this also increased with time of storage. It is surprising that both are increasing with increased time of storage. Increase in WHC indicate increased ability to retain water in the muscles, increase in dry matter indicate increase in water being released from the muscles. The p-value is noticeably smaller in WHC, indicating time is affecting WHC more than in the case with dry matter. This is overall a surprising result, which should be investigated further. The increase of WHC with time of storage, can explain why drip loss did not increase with time as expected.

It was unexpected that merus treated at 60 °C had a high drip loss combined with high value of WHC, and low value of dry matter. Denaturation of myosin and sarcoplasmic proteins might partially explain the result of merus treated at 60 °C. Denaturation of myosin lead to reduced ability to retain water in the proteins, and denaturation of sarcoplasmic proteins increase WHC as this cause muscles to shrink the capillaries and they close them partially. It is possible that at the treatment at 60 °C led to a denaturation of the myosin as well the process of denaturation of sarcoplasmic proteins had started, leading to high drip loss as well as an increase in WHC possibly after liquid was expelled out as cook loss.
The result of high drip loss and WHC combined with low dry matter in merus treated at 60 °C can indicate that time in the water bath can have played a major role.

The relatively high WHC values in merus treated at 60 °C could be explained by the fact that a lot of liquid was lost during treatment and storage, resulting in that there is less liquid left in

the merus to be lost during centrifugation. However, it is then expected a higher amount of dry matter than detected.

5.9 Color

In the study examining color changes on cod muscles by Skipnes et al. (2011), the whiteness in cod treated at 60 °C was measured to be between 70-80, the cod treated at 80 °C had a measured whiteness of around 80-85 and those treated at 90 °C had about the same result as for those treated at 80 °C. The whiteness measured in that study were not significantly different from each other in cod treated at temperatures above 70 °C. As the white muscle in cod share similarities with meat in RKC, about the same result was expected. In the study by Lorentzen et al. (2014) examining shelf-life of RKC cluster heat treated in boiling water at 22-24 minutes. Storage time had a significant ($p < 0.05$) impact on changes in color. It was observed that the red color from the surface started to mix in the surface cut, causing the samples to get a redder color.
The L* value decreased from 92.7 at day 2 to 90.1 at day 14 in samples shoulders. The a*-value increased significantly ($p < 0.001$) from 1.85 to 4.41 from day 2 to day 14 in samples from legs. L* decreased significantly ($p < 0.001$) from 91.8 at day 2 to 89.4 at day 14 in samples from legs.
Modified atmosphere has been proven to have a positive effect on L* in swimming crab. The result cannot be directly used in this study, the atmosphere was changed in a different way than in present study. CO_2 was added and superchilling was used. It can still indicate that changing the atmosphere by using vacuum packaging, can prevent the L* values to decrease as quickly as it normally does during storage at 4 °C (Sun et al., 2017).

It was expected that the trend in present study would be that an increasing temperature would lead to whiter color. It was therefore expected that merus treated at 95 °C and 80 °C could be significantly lighter than merus treated at 60 °C. However, the result also showed that there was not significant difference between merus treated at 60 °C and 80 °C. Based on the results in study by Lorentzen et al. (2014), it could be expected that time would be of significance for L* and a*-values, however, based on study by Sun et al. (2017), it was not surprising that time of storage did not change L* and a*-values in present study. This indicate that vacuum packaging had a positive effect on the appearance of merus, by maintaining the color of freshly treated merus for a longer time.

6 Conclusion

Removal of the merus muscle from the skeleton of frozen and thawed RKC legs was possible by using high pressure processing at 250 MPa at 15 °C for 2 minutes (to shuck the legs). This means that industrial companies can use frozen raw material which give a simpler logistics, and which is not dependent on seasonal harvesting.

All three pasteurization treatments at 95 °C for 11 minutes, 80 °C for 4 minutes and 15 seconds and 60 °C for 10 minutes and 30 seconds had acceptable microbial levels (CFU/g) during their shelf-lives of 30, 21 and 7 days, respectively.

Merus treated at 95 °C were significantly ($p = 0.002$) lighter than merus treated at 60 °C.

Merus treated at 80 °C had a significantly ($p = 0.002$) lower level of drip loss than merus treated at 60 °C and 95 °C.

Merus treated at 80 °C and 60 °C had significantly ($p = 0.002$) and ($p < 0.001$) higher level of WHC and lower dry matter than merus treated at 95 °C.

Results from drip loss, WHC and dry matter showed that merus treatment at 95 °C give a significantly drier product than the other treatments.

The results indicate that treatment at 80 °C gave product of the highest quality, however, merus treated at 60 °C and 95 °C were not of a noticeably worse quality.

7 Future work

The storage temperature in this shelf-life study was set to 4 °C, in future work it can be of interest to use higher temperatures (4-8 °C) in order to detect effects of temperature abuse and chilled temperature regulations in other European countries. It can be advised for online stores that they are controlling the temperature, and possibly check the quality of all products before they are shipped. This is likely to make people less skeptical to buying fresh seafood.
The shelf-life should further be optimized to find the best combination of time and storage in order to obtain a good food quality and safety.

It was challenging to remove the muscles from the exoskeleton, for future work it could therefore be advised to examine further different pressures, temperatures and time of treatment to possibly make the removal easier. It is likely that a treatment at pressure 280 MPa will make the removal easier. However, it could be interesting to investigate the effect of different pressures looking at a wider range to get the most optimized shucking of merus muscles from the exoskeleton.

It can also be of interest to further examine how long RKC can be frozen before the quality get below acceptable level, considering if it is possible to freeze premolt RKC and sell them in the spring when RKC are molting.

Microbiological analyses should be taken on more parallel samples during storage as there was detected a high standard deviation in this study and this makes the result less accurate.
It could be of interest to measure other possible pathogens present in the merus, and document how these pathogens can be controlled by processing and use of decent shelf-lives.

To better understand the result of drip loss, WHC and dry matter for merus treated at 60 °C, it could be interesting to perform differential scanning calorimetry, to be able to decide what kind of proteins that are denaturized at this low temperature. Might also clarify the role if the proteins denaturized and what role the time in the treatment had.

Since RKC is a gourmet product and the aim of this study was to develop a gourmet product after treatment, it can be of interest to measure other quality parameters such as texture properties and sensory analysis.

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