Master's thesis

NTNU
Norwegian University of Science and Technology
Department of Biotechnology and Food Science

Ramja Jeyakumaran

Rest Raw Material from Atlantic Salmon and Norwegian Chicken

Effects of Thermal Treatment and Different Drying and Storage Methods on Quality and Stability

Master's thesis in Chemical Engineering and Biotechnology
Supervisor: Turid Rustad
June 2019

NTNU
Norwegian University of Science and Technology

Ramja Jeyakumaran

Rest Raw Material from Atlantic Salmon and Norwegian Chicken

Effects of Thermal Treatment and Different Drying and Storage Methods on Quality and Stability

Master's thesis in Chemical Engineering and Biotechnology
Supervisor: Turid Rustad
June 2019

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

NTNU
Norwegian University of
Science and Technology

Preface

This Master's Thesis concludes five years of studying Chemical Engineering and Biotechnology at Norwegian University of Science and Technology (NTNU) in Trondheim. The work was conducted at the Department of Biotechnology and Food Science between January and June, 2019. The thesis was written in collaboration with Kystmiljø AS.

The laboratory work was conducted during spring 2019 at the Food Science Laboratory and at the Thermal Engineering Laboratory at NTNU Gløshaugen. Some of the laboratory work was also conducted at the Food Science Laboratory at NTNU Kalvskinnet.

First and foremost, I would like to thank my supervisor, Professor Turid Rustad, for the advice and support she has provided me during this process. I have been extremely lucky to have had a supervisor who cared so much about my work and patiently guided me throughout the stressful times. I would also like to thank my co-supervisor, Dr. Ignat Tolstorebrov, who provided help beyond what was required. Thanks to Tina Olaussen at Kystmiljø AS who took time to bring the raw materials to the lab during the cold winter. I am especially grateful for the help provided by Dr. Janna Cropotova who was willing to help me during the work with lipid oxidation. Zdenka Bartosova at the Department of Biotechnology and Food Science has been vital when carrying out the MS analysis and I am grateful for her support. A big thanks to Oskar Speilberg for his frequent help with the laboratory work. Also thanks to Siri Stavrum at the Food Science Laboratory for the help and always finding chloroform whenever I was in need. I also wish to thank the other students at the lab who made the long days much more enjoyable.

Thank you to my parents and my sister, Thivja, for the encouragement and unconditional love. They have inspired me to work hard every day and I dedicate this milestone to them. Lastly I would like to thank my great friend, Sajitha, for always being there for me.

Ramja Jeyakumaran

Trondheim, June 2019

Abstract

The consumption of marine products in the world has according to FAO (2018) reached over 120 million tonnes the last ten years. Increasing consumption will lead to increasing amount of marine products which are not consumed. These products are commonly referred to as rest raw material or by-products. Production of rest raw material is also relevant in poultry farming, as chicken is the second most consumed animal meat in Norway. There have recently been great interest in effective utilisation of these materials to produce high-value products, however, these materials are highly perishable and require appropriate preservation. The aim of this thesis was to study the effectiveness of drying as a preservation method, and analyse the quality and stability of the dried products.

This Master's Thesis was conducted in collaboration with Kystmiljø AS, which provided the Atlantic salmon intestines and Norwegian chicken intestines utilised in this study. Characteristics of the materials such as the chemical composition and fatty acid composition were mapped. Thermal treatment was conducted prior to drying where different temperatures were studied for effective separation of fat, stickwater and sediment. Fat-reduced sediment were dried by either vacuum freeze drying, infrared drying or hot air drying. Quality parameters such as the water activity, water content, colour and lipid and protein oxidation were measured of the dried materials. These parameters were also measured for samples stored over thirty days using two different packaging methods (vacuum packing and in plastic bag). The stability of the dried materials were investigated using glass transition temperature.

Seasonal variation of the lipid content was proved for both materials. The salmon intestines contained approximately 25 % omega-3 fatty acids compared to around 3 % in the chicken intestines. The analysis of fatty acid composition showed that the salmon contained more amounts of PUFAs, hence was more susceptible to oxidation. Saturated fatty acids and omega-6 fatty acids were more abundant in the chicken intestines. The difference in the fatty acid composition contributed to the wide range of melting points of the oils from the materials.

Thermal treatment was examined at temperatures from 40-90°C. The amount of fat separated increased as the temperatures increased for both materials. However, the lipid content in the fat-reduced sediment increased as well. Based on the results from thermal treatments, 50°C was chosen and used as extraction temperature in the further analysis.

Drying of the salmon intestines resulted in powder-like properties. The infrared dried salmon had the largest water content (approx. 13 %) and vacuum freeze dried salmon had the lowest (approx. 2 %). The chicken intestines obtained paste-like properties after drying in oven. The oven dried chicken samples contained between 40-50 % moisture, and it was concluded that this type of drying was not sufficient for the chicken intestines. High water content of the chicken intestines resulted in growth of mould during the storage experiment. The results from the lipid oxidation study on stored samples showed decreased levels of peroxide value and conjugated dienes for both materials. However, concentration of TBARS varied for each sample, and could be due to interactions with peptides and amines.

The degree of hydrolysis was larger for the chicken intestines compared to salmon intestines.

Two glass transition temperatures (-104°C and -67°C) were detected in the salmon intestines and these temperatures were associated with lipids and proteins. The DSC analysis showed that high temperature drying separated the fat better than the vacuum freeze drying method. The vacuum freeze drying resulted in absorption of lipid on the surface making these samples more perishable.

It was shown that optimal temperature for thermal treatment and drying was dependent on the type of material and the chemical composition. Oven drying at 50°C and 100°C was not suitable for the chicken intestines and further analysis of other drying methods of this particular material should be conducted. Significant difference in the packaging methods was found for the chicken intestines, which could be due to the high water content. The dried products could be applicable as ingredients in pet food.

Sammendrag

I følge FAO (2018) har forbruket av marine produkter på jorda nådd over 120 millioner tonn de siste ti årene. Det økende forbruket har ført til økende mengde marine produkter som ikke kan bli brukt til menneskelig konsum. Disse produktene blir ofte kalt restråstoff eller bi-produkter. Dette er også et problem i kylling produksjonen ettersom kylling er en av de mest spiste kjøtt-slagene i Norge. Det har nylig vært mye interesse rundt effektiv utnyttelse av disse materialene til å produsere høy-verdi produkter, men disse materialene er svært ustabile og krever gode bevaringsmetoder. Hensikten med denne masteroppgaven har vært å studere tørking som en type konserveringsmetode, og analysere kvalitet og stabilitet av de tørkede produktene.

Oppgaven er skrevet i samarbeid med Kystmiljø AS som også leverte råmaterialene brukt. Innholdet av tørrstoff, aske, protein, lipider og fettsyrer i innvoller fra atlantisk laks og norsk kylling ble undersøkt. Termisk behandling ved ulike temperaturer ble gjennomført før tørking, og effektiviteten av separering av fett, limvann og fett-redusert sediment ble undersøkt. Fett-redusert sediment ble tørket ved bruk av tre ulike tørkemetoder: vakuum frysetørking, infrarød tørking og varmluftstørking. Kvalitetsparametere som vannaktivitet, vanninnhold, farge og lipid- og protein-oksidasjon ble målt av de tørkede produktene. Et lagringsforsøk med to ulike lagringsmetoder (vakuumpakket og i plastpose) ble også gjennomført, og de samme kvalitetsparameterene ble målt. Stabiliteten til tørkede produkter ble undersøkt ved hjelp av glasstemperatur.

Sesongvariasjon i fettinnholdet i innvollene ble funnet. Lakseinnvollene og kyllinginnvollene inneholdt henholdsvis 25 % og 3 % omega-3 fettsyrer. Fettsyreanalysen viste store mengder flerumettede fettsyrer i lakseinnvollene sammenlignet med kyllinginnvollene. Større mengder mettede fettsyrer og omega-6 fettsyrer ble funnet i kyllingen. Disse variasjonene i fettsyreinnhold bidro til et stort smeltepunktsområde for de ulike materialene.

Termisk behandling ble studert ved 40-90°C. Mengden fett separert økte med økende temperatur, men lipid innholdet økte samtidig i det fett-reduserte sedimentet. Basert på resultatene fra de termiske behandlingene ble 50°C valgt som ekstraheringstemperatur i de videre analysene.

Tørkemetodene resulterte i pulver-lignende egenskaper for lakseinnvollene. Infrarød tørking av laks resulterte i høyest vanninnhold (ca. 13 %) og vakuum frysetørking resulterte i lavest vanninnhold (ca. 2 %). Kyllinginnvollene ble seig og klebrig etter tørking i ovn, og disse prøvene hadde et vanninnhold mellom 40-50 %. Det ble konkludert at denne tørkemetoden ikke egnet seg for kyllinginnvollene. Høyt vanninnhold førte også til vekst av mugg under lagringsforsøket. Avtagende nivåer av peroksid verdi og konjugerte diener ble funnet for begge råmaterialene, og kan indikere økende produksjon av sekundære lipidoksidasjonsprodukter. Men konsentrasjonen av TBARS varierte for alle prøvene, noe som kan indikere interaksjoner med peptider og aminer. Hydrolysegraden var større for kyllinginnvollene i forhold til lakseinnvollene.

To glasstemperaturer (-104°C and -67°C) ble detektert i lakseinnvollene, og disse temperaturene ble forbundet med henholdsvis lipider og proteiner. DSC analysen viste at tørking ved høy temperatur separerte fettet bedre enn ved bruk av vakuum frysetørking. Vakuum frysetørking resulterte i absorpsjon av lipider på overflaten, dermed forventes det at disse prøvene er mer utsatt for forverring.

Det ble vist at optimal temperatur for termisk behandling og tørking var avhengig av type materiale og den kjemiske sammensetningen. Tørking i ovn ved 50 og 100°C var ikke tilstrekkelig for kyllinginnvollene og andre metoder bør undersøkes videre for dette råstoffet. Signifikant forskjell i pakkemetode ble funnet for kyllinginnvollene, men dette kan være forårsaket av høyt vanninnhold. De tørkede produktene kan brukes som ingredienser i dyrefôr.

Abbreviations

CD Conjugated dienes

DCM Dichloromethane

DH Degree of hydrolysis

DHA Docosahexaenoic acid

DSC Differential scanning calorimetry

EPA Eicosapentaenoic acid

FA Fatty acid

FFA Free fatty acids

HPLC High-performance liquid chromatography

LOQ Limit of quantification

MDA Malondialdehyde

MS Mass spectrometry

MUFAs Monounsaturated fatty acids

NTNU Norwegian University of Science and Technology

PUFAs Polyunsaturated fatty acids

PV Peroxide value

SD Standard deviation

SFC-MS Supercritical fluid chromotography coupled with mass spectrometry

TAA Total amino acid

TBA 2-thiobarbituric acid

TBARS Thiobarbituric acid reactive substances

TEP 1,1,3,3-tetraethoxypropan

Contents

1 Introduction

This Master's Thesis is a continuation of the specialisation project conducted during fall 2018. The background and theory are based on the project work and are extended in this thesis.

1.1 Background

The consumption of marine products in the world has according to FAO (2018) [1] reached over 120 million tonnes the last ten years. Figure 1 shows the growth and the utilisation of fish in the period between 1950 and 2016. The figure also illustrates the growth in consumption of marine products as food. This increase is due to population growth, revenue growth and urbanisation. The urbanisation seems to drive the demand for marine products. Demand for a more varied diet in developing countries is also influencing this growth [1, 2]. As seen from the figure, about 80 to 90 % of the total marine products are destined for food consumption. An increasing consumption will therefore lead to an unsustainable increase in production of marine by-products which are not consumed. These by-products may contain significant amounts of nutritional resources such as proteins and lipids.

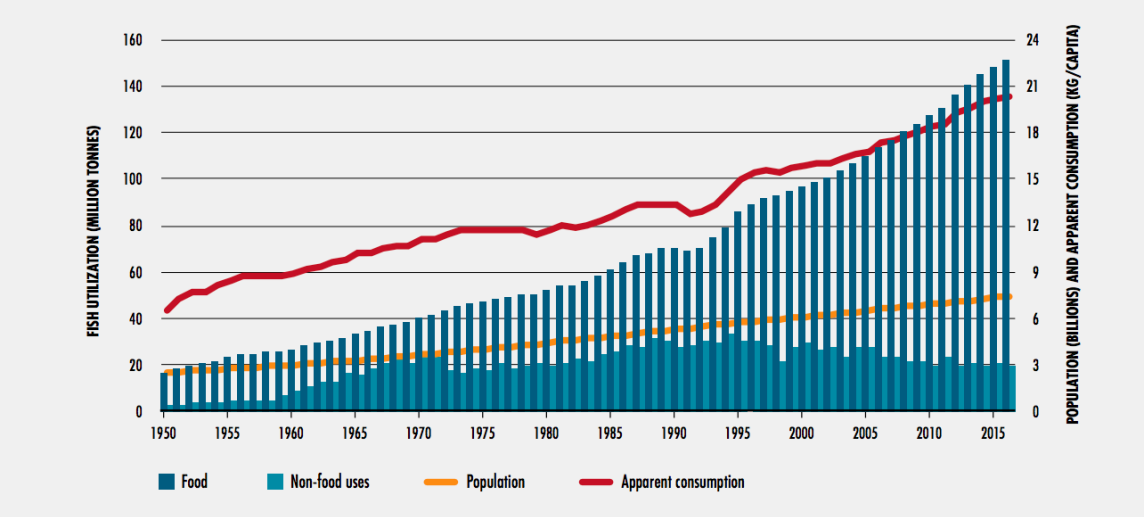


Figure 1: The world utilisation and apparent consumption of fish from 1950 to 2016. The diagram excludes aquatic mammals, crocodiles, alligators, caimans, seaweeds and other aquatic plants. The left axis shows the fish utilisation in million tonnes, and the right axis shows the population in billions. The apparent consumption of fish is given in kg per capita [1].

The remaining of the marine products which are not consumed is referred to as marine by-products or rest raw material [3]. A descriptive definition of what the rest raw material consist of is given in Section 1.3. The rest raw material is mostly reduced to fish-meal and fish oil. Fish-meal is a type of proteinaceous flour which is obtained after milling and drying of the wet by-products. A variant of the processing is described in detail in

Section 1.4. Fish oil is a rich source of long-chain polyunsaturated fatty acids (PUFAs), which are important in the human diet. The oil is obtained by pressing cooked fish and separating the oil from the solid material. In addition to the two main processes used to treat the sidestreams, there is also a possibility to produce fish silage. Fish silage is a rich source of hydrolysed proteins, which is mainly utilised as feed additive in aquaculture and in pet food. The silage is obtained by preserving fish and fish by-products in acid [1, 4]. The acid helps the existing enzymes in the fish to speed up the breakdown of proteins into smaller units. The acid also prevents any microbial spoilage that could occur as the by-products are highly susceptible. However, the use of this method results in products only suitable as feed for animals. It is therefore desirable to find other preservation methods which can ensure preservation of the valuable properties found in the rest raw material, hence increasing the value of these materials on the market.

Products such as protein hydrolysates and oils are dependent on the quality of the rest raw material. The quality must be preserved during storage and transportation. Transportation is relevant, because treatment of by-products are usually done at processing plants [2]. The plants are often located centrally, and transportation from slaughtering plants to the closest reception facilities can require up to 2-3 days of transportation. Since the rest raw material is easily degradable, long transportation time is challenging. Additionally, the material is occasionally exposed to higher temperatures during transportation. Together with the rising temperature, and the activity of microbes and enzymes in the raw material will result in boiling. This can in worst case cause explosions. Boiling in this case is referred to as overflow of material in the transportation tank. Increase in temperature can also be caused by direct sunlight on the tanks and friction inside the tanks. Friction will occur during transportation due to the material being viscous. To minimise this problem cooling or drying of the rest raw material could be used to reduce the perishability. Both cooling and drying can increase shelf life, thus decrease the spoilage of the material. There are only limited research conducted on the quality changes in dried rest raw materials, thus a study on this has been conducted through this thesis. Kystmiljø, a Norwegian company based in Trondheim, is developing solutions for transportation and storing of rest raw material. The company delivers solutions to handle waste and preserve the environment, decreasing the risk of marine pollution. Furthermore they are also specialised in handling rest raw material [5]. This thesis is written in collaboration with Kystmiljø as a study of characteristics and properties of the rest raw material that Kystmiljø are responsible for transporting.

In addition to marine rest raw material, Kystmiljø also transports rest raw material from chicken. Chicken is the second most consumed animal meat in Norway, after pork. The consumption has been steadily increasing in the last 50 years, which has resulted in a significant increase in rest raw material from poultry. According to a report from Nofima on mapping of rest raw material from agriculture, the theoretical amount of rest raw material from chicken in Norway was approximately 56,000 tonnes in 2015 [6]. This is estimated to be about 51 % of the total biomass slaughtered. It is important to consider that this is a theoretical value, as large number of products are sold including bones. All in all Nofima states that it is possible to yield 74 % meat per chicken acceptable to consume. The rest have potential to undergo treatment and be sold as higher quality products [6].

1.2 Aim of thesis

Rest raw material has to be transported from slaughtering plants to processing plants. Thus, long hours of transportation and days of storage are expected, resulting in increasing possibility of deterioration. Change in the materials have on occasions caused boiling of the material during transportation. It is therefore possible to preserve the material by cooling or drying. The effect of cooling was studied in the project work completed during fall 2018. This Master's Thesis is a continuation of the project work, and the main objective of this thesis was to study the quality changes in the materials when utilising different drying methods as a possibility for preservation.

One part of the work in this thesis characterises the rest raw material from Northern Atlantic salmon and from Norwegian chicken. Thermal treatment of the materials before the drying process was also studied using different temperatures. Different drying methods of the materials were also studied and the quality changes of these samples were analysed. The changes in quality attributes of the intestines from salmon and chicken were assessed by determining the water activity, water content, colour change, different lipid oxidation products and degree of hydrolysis of the dried intestines. The aim of the thesis was also to study the stability of the material during storage, hence a storage experiment was conducted where the same quality parameters were examined after 30 days. It is expected to utilise these results to maintain high quality of the rest raw materials during transportation and storage.

1.3 Rest raw material

It is important to differentiate between the terms rest raw material and waste. Waste products are products that cannot be used for feed or other applications and must be discarded. Rest raw material is considered to be by-products obtained after production of main products like fillets. Cut offs, bones, feathers, backbones, heads, livers and intestines are all by-products that are not regarded as ordinary saleable products, however these can be utilised after treatment. The rest raw material is usually a mixture with different types of fractions, and therefore must be treated in conditions required for viscera. Different rest raw material fractions require different conditions because of varying perishability. Viscera is highly perishable and cannot be transported over a longer period of time, contrary to backs, cut-offs and heads which are more stable. The treatment of the raw material is important as it affects the quality of the hydrolysed proteins that are produced from it. Hydrolysed proteins are products obtained from enzymatic hydrolysis of proteins to smaller peptides. The peptides in the protein hydrolysate usually contain 2-20 amino acids [7]. Protein hydrolysates are utilised for human consumption and can be used as nutritional supplements. The rest raw material is highly degradable and must undergo treatment to prevent enzymatic degradation and deterioration. Factors like pH, temperature and salt concentration can be used to control the microbial and enzymatic activity, which can be used to ensure good quality of the product [3].

The rest raw material can be categorised into three categories based on the risk for animals and public health. Materials from category 1 are highly perishable, and are normally incinerated. Category 2 materials, which consist of dead fish, cannot be used to produce feed for animals. Materials in category 3 have the lowest risk and are normally used to produce animal feed after further processing [8]. This thesis will only focus on material classified under category 3. However, the majority of rest raw materials from category 3 are used as fertiliser and as feed for animals, where the profitability is low. However, application in the production of dietetic products, pharmaceuticals, cosmetics and as constituents in the biotechnology industry can increase the profitability. Producing bioactive peptides, enzymes and biopolymers can make exploitation of rest raw material more profitable [1, 3].

1.3.1 Protein fractions

Protein fractions found in marine rest raw materials are used in production of hydrolysed proteins, peptides, amino acids, gelatin, collagen and protamine [9]. The protein fractions in the material are primarily utilised in the production of hydrolysates, which are proteins broken down to smaller peptides [3, 10]. The hydrolysates are rich sources for proteins, and are therefore desirable in the food industry. The hydrolysed proteins can be added into protein supplements and can be used as stabilisers in beverages. They also have functional properties like water-holding capacity, emulsification and foam forming, which can improve quality of food [10].

1.3.2 Marine lipids

Marine lipids are valuable components proven to be beneficial for health. They are particularly known to contain high amounts of long chain PUFAs, which make these lipids distinctive from other lipid sources. Omega-3 acids are types of PUFAs confirmed to reduce cardiovascular diseases, inflammation and are important to maintain a normal metabolism. Fatty fish like salmon is a good source for these lipids. Other lipids like glycolipids and squalene are considered to have beneficial effects on health, and increases the incentive for further utilisation of fish by-products [3].

1.3.2.1 Fatty acids

Many properties of lipids are associated with the fatty acids which lipids consist of. Fatty acids found in food contain even number of carbon atoms, which are unbranched. The conformation of the double bonds in the carbon chains and the abundance have a significance in the physical properties of the lipids. For instance, high content of unsaturated fatty acids or a high proportion of short-fatty acids result in lower melting temperature. The double bonds can also occur in *cis* or *trans* formation, which has an effect on the shape of the molecule, thus will affect the melting temperature as well [11]. Based on the structures and double bonds, fatty acids can be divided into three groups; saturated fatty acids, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids. Saturated fatty acids contain only single bonds and therefore have a higher melting point than unsaturated fatty acids. Palmitic acid (C16:0) is the most common saturated fatty acid found in animal lipids (20-30 %). MUFAs contain only one double bond and oleic acid (C18:1) is one of the MUFAs that occur abundantly in nature. PUFAs contain two or more double bonds and fatty acids in fish usually contain up to six double bonds. These PUFAs are generally liquid in ambient temperature and can be either identified as omega-3 fatty acids. Many PUFAs have to be obtained through diet as these fatty acids cannot be synthesised in humans [12].

Omega-3 PUFAs such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, have beneficial effects on human health. According to Arab-Tehrany et al. (2012) [13] DHA can prevent a number of diseases including inflammation and cancer, and EPA may play an important role in cardiovascular and immunological health in humans. The modern Western diet consists typically of high omega-6 and low omega-3 PUFAs. Research in this area have shown that by increasing the omega-3 consumption, the risk of chronic diseases could reduce. This makes the marine lipids, which consists of long chain omega-3 and omega-6 PUFAs, more favourable to include in our diet [14]. Marine lipids are however highly unsaturated, making these fatty acids very susceptible to oxidation [13, 15].

1.3.2.2 Rancidity

PUFAs are highly susceptible to oxidation and rancidity. Rancidity can occur in two different pathways, hydrolytic rancidity and oxidative rancidity. Rancidity is related to

loss of nutritional value, safety, functional property changes and environmental pollution [16]. Hydrolytic rancidity involves hydrolysis resulting in free fatty acids (FFA) and other products like glycerol. This reaction is catalysed by endogenous lipases found in the material. Amount of free fatty acids in the material can be used to control changes in quality of the material. High quality oils extracted from marine lipids should have a low FFA content, and the FFA content in the oil from the by-products are used to determine if the material can be used in further production of oil without any need for removal of FFA to meet quality demands. Hydrolytic rancidity can be subdued by lowering temperature, moisture content or deactivating the lipases [17].

Oxidative rancidity of lipids involves oxygen attack on glycerides, and high PUFAs content make the lipids more susceptible to oxidation. Oxidation is influenced by the presence of oxygen, temperature and degree of unsaturation of the fatty acids [3, 17]. By-products may contain blood which works as a prooxidant and increases the oxidation. Lipid oxidation may affect the flavour and reduce the nutritional value of the product, and it may give rise to high levels of certain compounds such as aldehydes, hydroperoxides and epoxides. Oxidation can be subdued by reducing exposure to oxygen, light and high temperatures. Addition of antioxidants is also a possible solution [17].

Oxidative rancidity or autoxidation occurs via a free radical chain mechanism, which consists of the basic steps of initiation, propagation and termination [18]. Initiation begins with abstraction of a hydrogen molecule from a fatty acid molecule (RH). This reaction, given in Equation 1, can be catalysed by heat, light or metal ions to form a free radical. The alkyl free radical ($R^\bullet$) will then react with atmospheric oxygen, and form an unstable peroxy free radical ($ROO^\bullet$). The unstable peroxy free radical will in turn abstract a hydrogen atom from another fatty acid, hence forming a hydroperoxide (ROOH) and a new alkyl free radical ($R^\bullet$). The hydroperoxides are called primary products of the oxidation. The new alkyl free radical will initiate further oxidation causing chain reactions (propagation) which is given in Equation 2. The propagation can be terminated by forming non radical products. General equations for terminations in oxidation are given in Equation 3 [18].

$$\mathrm{RH} \longrightarrow \mathrm{R}^\bullet + \mathrm{H}^\bullet \tag{1}$$

$$\begin{aligned} \mathrm{R}^\bullet + \mathrm{O}_2 &\longrightarrow \mathrm{ROO}^\bullet \\ \mathrm{ROO}^\bullet + \mathrm{RH} &\longrightarrow \mathrm{ROOH} + \mathrm{R}^\bullet \end{aligned} \tag{2}$$

$$\begin{aligned} \mathrm{R}^\bullet + \mathrm{R}^\bullet &\longrightarrow \mathrm{RR} \\ \mathrm{R}^\bullet + \mathrm{ROO}^\bullet &\longrightarrow \mathrm{ROOR} \\ \mathrm{ROO}^\bullet + \mathrm{ROO}^\bullet &\longrightarrow \mathrm{ROOR} + \mathrm{O}_2 \end{aligned} \tag{3}$$

The end products formed in the termination step are mainly aldehydes such as hexanal and malondialdehyde (MDA). These are low molecular weight volatile compounds which release distinctive aromas and can also affect the flavour properties [16]. Determination of MDA, for instance using TBA, is generally used together with peroxide value (PV) and

conjugated dienes (CD) to assess lipid oxidation in fish and meat [19]. Further theory on these analytical methods are given in Section 1.8. The main fatty acids found in lipids of animal tissues are oleic, linoleic and arachidonic fatty acids. The different hydroperoxides formed are dependent of these compounds and varies from the animal and the fraction of the animal which the fat is derived from [16].

Hydroperoxides and the secondary oxidation products can interact with the proteins in the material. This interaction can impact the stability of the flavour during storage and processing of the material. This could lead to decrease in nutritional value of the material [16].

1.4 Processing of rest raw material

Processing of rest raw material can be done by various methods such as enzymatic treatment or heat treatment. By heat treatment the rest raw material can be separated into three phases; liquid phase, fat phase and a solid phase. The liquid phase consists mainly of stickwater, which is a solution of protein and water soluble compounds such as dissolved protein. These proteins are valuable and can be utilised to produce antioxidants and vitamins. Oil and fat-soluble components remain in the fat-phase. High quality marine oils, which consist of health beneficial PUFAs, are attracted and there is a large market for this. The solid phase, which is reduced in fat content, can consist of bones and other solid substances that could be used to produce bone meal, gelatin and collagen [20]. However, this is dependent on the type of rest raw material being processed. Intestines, which contain barely any bones cannot be used to produce bone meal and collagen.

The principle of the processing by heat treatment is to separate the three fractions completely as possible, however this has to be done with the least possible expenses and under conditions providing the highest quality as possible [21]. The processing begins with heating of the rest raw material. This is when the fat cells rupture and release oil and physio-chemical bound water. The heating temperature has to be suitable as it could reduce the quality of the material. The oil and water are then strained from the solid material and the solid material is pressed into a press cake to remove even more liquid. This can also be done using a centrifuge. Amount of liquid and solid will vary based on the animal, type and fraction of rest raw material. The liquid, or press water, is separated into oil and stickwater using a decanter. The oil is stored in tanks. Separated stickwater is further evaporated in a multi-effect evaporator to produce a concentrate. This concentrate is added to the press cake formed with the solid phase. Remaining sludge from the press water is separated and also returned to the solid phase. The solid phase will then consist of press cake, sludge and concentrate, and will be mixed, milled and dried [21, 22]. An illustration of the whole process is given in Figure 2.

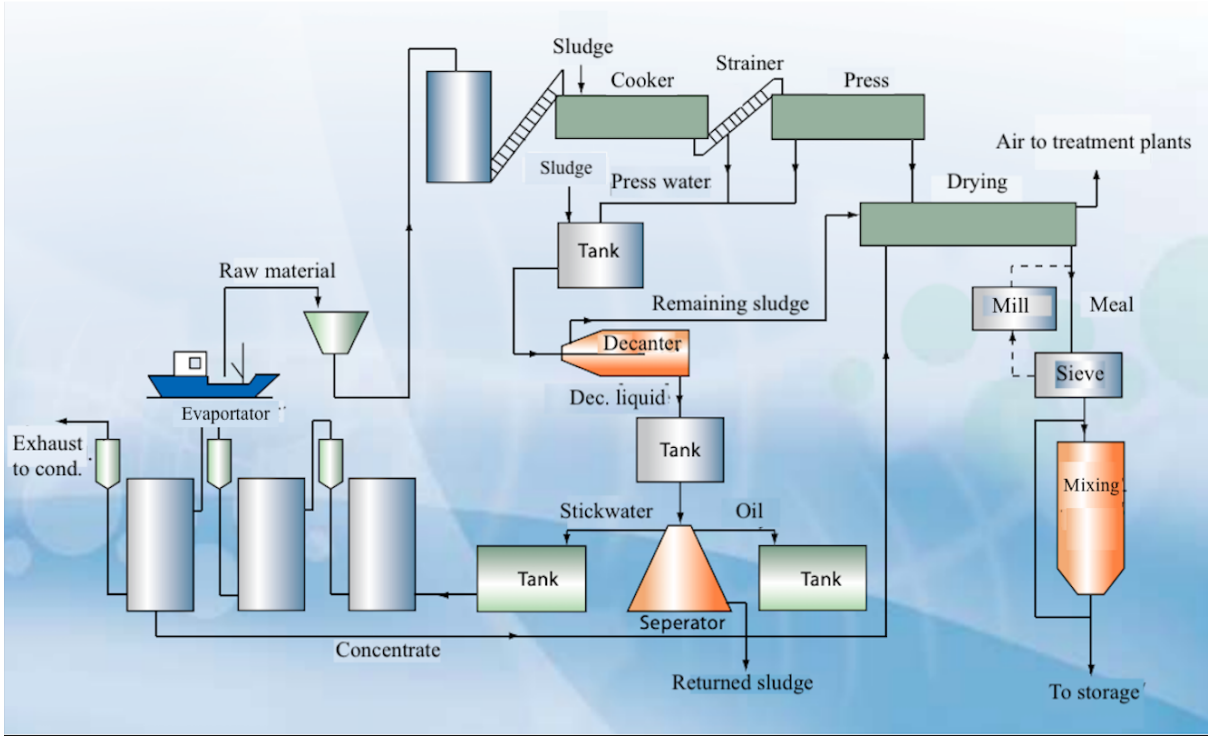


Figure 2: Flow sheet of a general processing plant where rest raw material is converted to oil and meal [22].

According to FAO (1986) [21] the initial heating process of the rest raw material was earlier done at 100°C as it was shown that a high temperature as this would result in optimum performance. It has later been proven that the fat cells are broken down at a lower temperature, below 50°C, and it is therefore possible to reduce the energy requirement, reduce the heating time and preserve the quality as protein coagulation occurs at temperatures above 75°C. However, the determination of an optimal temperature is dependent of the particular raw material and has to be established through initial assessments [21].

Separation of liquid phase (oil and stickwater) from the solid phase can be done by either pressing and filtration or by centrifugation. Centrifugation is beneficial as it is more controllable and is a faster unit operation compared to pressing and filtration. Since centrifugation is quicker, it also allows for less heat requirements. The centrifuge has the ability to produce a more fluid and soft material which is beneficial for the further processing of the solid material [21].

1.4.1 Drying

The drying process is necessary to convert the unstable mixture of sludge, press cake and concentrate into a stable and dry fish meal. The drying of the material will also reduce the water and microbial activity making it more stable and extending the shelf-life. The drying will also reduce some enzymatic processes as well. The temperature for the drying have some limitations as reduction of quality and nutritional value, especially of the

proteins, should be avoided [21]. Drying can be done with different techniques depending on the heat input. The heat could be transferred as convection (eg. air drying, oven drying, spray drying), conduction (eg. vacuum freeze drying) and radiation (eg. infrared drying) or a combination of these. The techniques require that the material is spread into a thin layer to let water vapour escape from below the surface, which will make the drying process more homogeneous [23].

Vacuum freeze drying can be divided into three stages. These are (i) freezing of the food, (ii) subliming the ice and (iii) removal of small amounts of water bound to the product. Stage (ii) and (iii) are also called primary and secondary drying, respectively. The initial freezing process of the product can be done by any conventional method such as blast, plate or liquid gas freezing. At least 95 % of the water in the food material should have been converted to ice to obtain a successful drying. The frozen product will be sealed in a chamber, where pressure is reduced rapidly. The rapid reduction of pressure is necessary to avoid melting of the product. When the vacuum is established, the pressure is kept low throughout the drying process and the temperature is kept below the saturation temperature corresponding to the pressure inside the chamber. The ice will sublime in this stage, and water vapour formed during this sublimation will be removed by the condenser resulting in drying of the food material. The benefits of using freeze drying can be seen in the quality of the dried products. The liquid in the product does not move during the freezing and the product will therefore not shrink. This results in light porous structure, which is useful for re-hydration of the dried product [24].

Oven drying of food is performed by applying heated air, which dries the material by convection. The water vapour will then be removed from the surface of the material by the air stream [24]. As the water is removed from the surface of the material, the water inside the material will move to the surface, and there are possibilities of creating a crust on the surface of the material which happens during fast drying. This is a result of precipitation of extractives such as proteins, salts and sugars. In a slow drying process these extractives are able to diffuse back into the sample evenly, and would make the product more compact. The fast drying of the product makes the surface hard, but the inside becomes porous [25].

Radiation can also be used to dry materials by directing it onto a material, where the radiation is absorbed and converted to heat. The energy from the radiation is usually absorbed on the surface and into a very shallow layer of the material below the surface. For that reason the infrared drying require thin layers to achieve a successful drying process. Drying of food materials with radiation is complex as the components in the food, such as fat and protein, have different absorption patterns. The water in the food material will also influence the overall absorption of the energy. Thus, these components make it difficult to achieve a uniform drying. Infrared drying is not commonly used in the food industry as it is difficult to control the heating rate as well as the removal of large amounts of water [24].

Spray drying is a commonly used method to dry slurries and liquids. The material fed into the spray dryer is converted into a fine mist of small droplets with droplet size ranging from 10 µm to 200 µm. While the spray dryer produces small droplets, heated air will evaporate the moisture from the droplets because of the large surface area formed. The

drying time is short, thus this method can be utilised on heat-sensitive materials as heat damage is limited [24]. However, spray drying is an effective method for drying liquids and is therefore restricted in its use.

Research on drying of by-products has, to the knowledge of the author, not been published. The basis of the work conducted in this area of the thesis is therefore based on literature and research conducted on drying of foodstuff, especially meat from salmon and chicken.

1.4.2 Packaging

If drying is going to be applied to the by-products, it will be necessary to preserve the quality during storage. Packaging is therefore important to prevent the material from pathogenic microorganisms and against moisture and re-hydration. The packaging is incorporated in food processing and preservation. The packaging method depends on the food, costs and the environment. The environmental factors affecting the packaged food includes permeability characteristics, temperature, light, chemical compatibility of the packaging material and the contents of the package and microbial contamination. Permeation of water, vapour and gases into and out of the package can occur, and for dry products the level of moisture can increase. This can increase the water activity, and over a critical level this can induce microbial spoilage. Hence, the packaging material should have low permeability to water vapour and must be sealed properly to achieve an extended shelf life. The shelf life can also be extended by creating an atmosphere in the package with low amounts of oxygen. This can be done by utilising modified atmosphere, which is achieved by replacing the oxygen with CO_2 or nitrogen gas, or by vacuum packaging. Change in temperature, which can be caused by exposure to light or rising temperature in the environment, can also influence the spoilage of the food. Fats are for instance more prone to develop rancidity when exposed to light and higher temperatures. Heat-resistant materials are a possibility to prevent temperature rising in the packaging. The compatibility of the packaging and the by-products is also important as the packaging should not give rise to toxic substances as well as the packaging should keep out microbial contamination which can develop rapid spoilage of the food [24].

1.5 Engineering properties of rest raw materials

Engineering properties are properties related to thermal, physical and rheological properties that are important in food engineering. The parameters give important knowledge for food processing operations. The increasing number of food products that are made today generate a higher demand for knowledge concerning thermal properties of a wide range of products [26].

There are little information about the properties of the rest raw material. There are two major categories commonly examined for dried products: the engineering properties of the drying products and the quality. The rest raw materials are highly susceptible to deterioration, and maintaining quality can therefore be done by drying. Drying of products

causes change in the physical properties producing a chain reaction of various changes in chemical and biological properties. Accordingly by analysing the physical changes that occur, a broader understanding of how the material is prone to deterioration could be relevant when studying the quality changes. Glass transition is a parameter connected to changes occurring during drying, and could be a necessary tool when studying drying of rest raw materials.

1.5.1 Glass transition

Glass transition is a phenomenon important when studying stability of food products. When a material is dried it can enter an amorphous state or a so called glassy state, which happens when the crystallisation is not given sufficient time during the drying process. Thus, the material maintain a matrix with gel like properties. This is however dependent of the composition of the material, the drying process and the drying conditions. The quality of the material will be affected by the glass transition as components like enzymes and microorganisms are encapsulated in matrices, and a change in the physio-chemical properties will influence the quality [27]. When a material has reached the glassy state, the particles inside the material are trapped inside the gel matrices and are held stable. It is therefore desired to reach this state during drying.

When reaching the glass transition temperature (T_g) structural change in the amorphous state of a material can be observed. Physical property of a food material changes with temperature, and especially for temperatures above T_g. The structural change causes change in physical and chemical quality of the material as well as the material begins to enter a solid/rubbery state [27]. Proteins and lipids do not have a significant effect on T_g, however water has a great influence on the decrease of the glass transition temperature. An increasing amount of water in the material results in a decreasing T_g [28].

Crystallisation occurs at temperatures higher than T_g, and is a process which is related to the quality of food. Crystallisation may promote release of substances from encapsulation found in the glass. These substances can for instance be water, oil, enzymes and microorganisms. The liberation of water during crystallisation will increase the moisture content and T_g is likely to reduce. The reduction causes increase in mobility of oxygen and the diffusion rate resulting in increasing oxidation. The liberated water is then also available for microorganisms, thereby increasing the water activity and microbial growth. The liberated oil from the encapsulation will also undergo rapid oxidation. Hence, T_g is an important parameter in food processing and can be utilised to understand the quality change occurring during drying. T_g can be determined using analytical methods such as differential scanning calorimetry (DSC) [29].

1.6 Water content and water activity

Foods with high water content are more prone to rapid deterioration due to biological and chemical changes. It is possible to delay or prevent spoilage by removing the water. Reduced water content will increase the food concentration, making the food more resis-

tant against microbial attack. When the concentration of food has been increased to a point where microbial growth is controlled, the food becomes less susceptible to chemical and physical changes [30]. The major influence on food stability is not the abundance of water, but the availability of water to the microorganisms and enzymes. The availability of water is measured by the concept of water activity [11]. Pure water, without any dissolved substances, is 100 % available for microorganisms to use. In the by-products, which contain substances like protein, will decrease the availability of the water for the microorganisms. The by-products will therefore have less water activity than pure water. Water activity is described as the ratio between vapour pressure of water in the food (p) and the vapour pressure of pure water at the same temperature (p_0). The activity will range from 0 to 1 [11, 30, 31]. The water activity is given in Equation 4.

$$a_w = \frac{p}{p_0} \tag{4}$$

The deterioration rate for foods is dependent on the water activity. The deterioration, such as lipid oxidation and enzyme activity, can emerge from different types of chemical and enzymatic changes. A generalised rate of deterioration for food is given as a function of water activity in Figure 3.

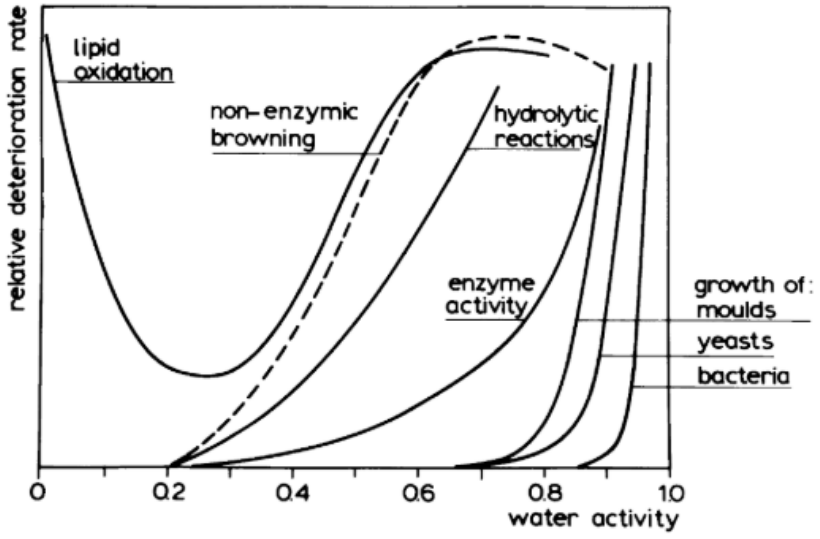


Figure 3: Generalised rate of deterioration for foods as a function of water activity [32].

As seen from Figure 3, the water activity can be used to control the microbial growth, and determine the necessary water content to achieve desirable water activity. The figure also show that even low water activity can cause higher level of lipid oxidation and for this reason a very low a_w is not always desirable. Water activity is related to water content in a non-linear relationship known as the water sorption isotherm, given in Figure 4. The water sorption isotherm can be used as a tool to determine the water content needed for the desirable a_w, and the stability of the product in different storage conditions [30, 33].

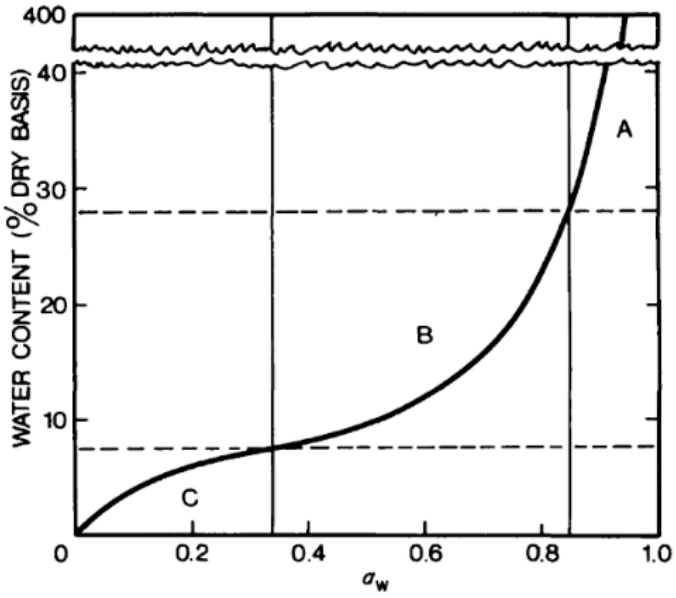


Figure 4: General water sorption isotherm of a typical food, where water activity (a_w) is a function of the water content in the material [30].

The isotherm in Figure 4 is divided into three parts, A, B and C, which makes it possible to analyse the type of bounded moisture that may be dominating in the material. Water in region C is very stable and can in many ways behave like a solid. This water is also believed to be bound by adsorption and chemi-sorption and does not freeze at any temperature. Hence, it is called *unfreezable water*. The water in region B is less firmly bound to the material. The water in region C and B differs remarkably from water in region A of the isotherm, as this water is considered as *free water*. This water is mechanically bound to the system and is only exposed to weak restrictive forces, which is indicated by the steepness of the isotherm in this region [30, 34].

Control of water activity helps limit or slow down certain undesirable reactions, such as lipid oxidation, enzymatic reactions and protein oxidation. This will hence maintain quality and safety of the food and extend the shelf life. The objective is to preserve the food without changing it from its natural state, and the level of preservation achieved will ultimately determine if the preservation method is feasible [30].

Dried products usually have a water activity below 0.6 and water content below 25 %, which is sufficient to avoid microbial growth. The water content in fatty fish such as salmon is expected to be around 65 %, while in meat products it is expected to be around 60 % [25].

1.6.1 Microbial growth

Microorganisms have an effect on changes in quality, and these changes are considered to contribute to food spoilage. Microorganisms have four main growth phases; lag phase, exponential phase, stationary phase and death phase. Water activity can be shown to

influence these phases by affecting length of the lag phase, the growth rate, population size during the stationary phase and the presence or absence of growth. Microorganisms have a minimal a_w which permit growth, however in food technology the desirable a_w level can be higher than the minimal a_w for growth if pathogens are unable to produce a sufficient amount of toxic products [30]. Typical water activity in fresh meat is around 0.98, which is high compared to the minimal required water activity for normal bacteria which is around 0.91 [11].

1.7 Colour as a quality parameter

Colour is an important quality parameter and can be used to govern biochemical, microbial and physical changes in foods. Colour can be correlated with other qualities such as nutritional value, and is an easy method to detect defaults in the products [35].

Colour can be characterised by different colour systems such as the Hunter L, a, b colour scale system. The Hunter L, a, b scale uses a three dimensional coordinate system to place the colours based on a sample's lightness and the intensity of the following colours; red, green, yellow and blue. The coordinate system for the Hunter L, a, b colour scale system is illustrated in Figure 5. The axis from top to bottom describes the lightness of the product and is measured from 0 to 100, where 0 is denoted to the colour black and 100 is denoted to white. The a and b axis do not have any specific value limit. The positive values of the a axis is denoted to the colour red, and the negative values reflects the colour green. For the b axis the positive values indicate the colour yellow and the negative values indicate blue [36].

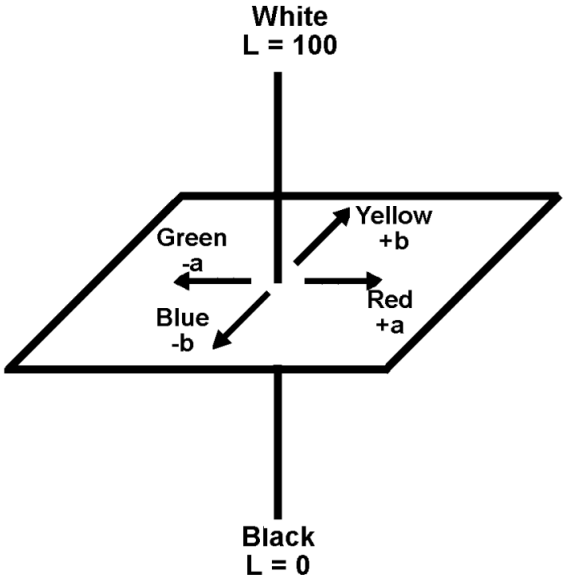


Figure 5: Diagram of the Hunter Lab colour space including the L, a and b colour scale [36].

1.8 Background for analytical methods

1.8.1 Quantitative analysis of dry matter and ash

In fish and chicken, the dry matter is mainly made up of ash, lipid and protein content. The ash content is used to determine the amount of minerals and other inorganic material present in the food [37].

1.8.2 Soluble protein quantification

Determination of soluble protein concentration can be done using the Lowry method. The Lowry method is based on both the Biuret test and the Folin-Ciocalteau test. The Biuret test involves the production of Cu^+ when peptide bonds react with copper under alkaline conditions. The copper-ion catalyses the Folin-Ciocalteau test, involving reduction of the Folin-Ciocalteau reagent and oxidisation of aromatic amino acids like tryptophan and tyrosine. Cysteine will also contribute to the redox reaction [38, 39]. The reduction reaction results in a blue solution caused by the heteropolybdenum blue molecule. The protein concentration can be determined by measuring the absorbance of the blue solution [38].

1.8.3 Total protein quantification

The total nitrogen and protein content of an organic or an inorganic sample can be determined using the Kjeldahl method. The method can be divided into three major steps; digestion, distillation and titration. The digestion step involves converting organic nitrogen into ammonium ions (NH_4^+) [40]. This is shown in Equation 5.

$$\text{Sample(-}\mathbf{N}\text{)} + H_2SO_4 \xrightarrow{\text{catalyst}} (\mathbf{N}H_4)_2SO_4 + CO_2 + H_2O \tag{5}$$

In the next step, the distillation, the ammonium ions are converted into ammonia (NH_3) by addition of NaOH, as expressed in Equation 6. The ammonia gas is absorbed by aqueous boric acid ($B(OH)_3$), which forms solvated ammonium ions as shown in Equation 7 [40].

$$(\mathbf{N}H_4)_2SO_4 + 2\,NaOH \rightleftharpoons 2\,\mathbf{N}H_3(gas) + Na_2SO_4 + 2\,H_2O \tag{6}$$

$$B(OH)_3 + \mathbf{N}H_3 + H_2O \rightleftharpoons \mathbf{N}H_4^+ + B(OH)_4^- \tag{7}$$

The remaining boric acid, which does not absorb the ammonia gas, is titrated with a strong acid such as sulfuric acid (H_2SO_4), as shown in Equation 8. This is a direct titration, and the end point of the titration is determined potentiometrically [40].

$$B(OH)_4^- + H_2SO_4 \rightleftharpoons SO_4^{2-} + B(OH)_3 + H_2O \quad (8)$$

The amount of titrant used in the titration can be used to calculate the amount of nitrogen in the sample. Thus, the total protein amount in the sample can also be calculated [40].

1.8.4 Total amino acid composition

The total amino acid composition can be used to evaluate the total protein content of a sample. The total amino acid composition can be determined using high-performance liquid chromatography (HPLC) which identify and quantify the different amino acids in a sample [41]. Concentrations of each type of amino acids identified by HPLC can be used to calculate the total percentage of amino acids in the sample. Not all types of amino acids are determined from this method as some amino acids are not detected with HPLC and for instance is tryptophan destroyed in the hydrolysis conducted during the analysis.

1.8.5 Quantification of hydroxyproline

Determination of hydroxyproline is usually utilised to determine the amount of collagen in a sample as hydroxyproline is the major component in collagen. It is possible to determine the amount of hydroxyproline based on Leach (1960) [42] by oxidation of hydroxyproline with hydrogen peroxide in the presence of alkaline copper sulphate. Excess of hydrogen peroxides are removed by heat, and *p*-dimethylaminobenzaldehyde is added to react with the oxidation products in the presence of diluted sulphuric acid to produce red/pink colour. The intensity of the colour is compared to standards [42].

1.8.6 Degree of hydrolysis

The degree of hydrolysis is a measure of the free amino groups released, indicating the progress of protein degradation of the material. One method of determining the degree of hydrolysis is by using formol titration, involving addition of formaldehyde. Amount of free amino groups must be calculated with regard to the protein concentration in the product [43].

1.8.7 Lipid extraction

Determination of lipid content was done using the Bligh and Dyer method for lipid extraction and purification. The method is based on the creation of a biphasic solution containing chloroform and the extracted lipids in one phase, and a non-lipid phase containing water and methanol. The biphasic solution is created as a result of difference in density, as chloroform has a higher density than water. Evaporation of chloroform will yield purified lipid extract [44].

1.8.8 Peroxide value

Peroxide value (PV) is a measurement used to quantify hydroproxides in fat. PV is determined by an iodometric method, which is based on the reduction of the hydroperoxide group (ROOH) with iodine ion (I^-). The amount of iodine (I_2) released will be proportional to the concentration of peroxide present in the fat. The released I_2 are evaluated by titration with thiosulfate (NaS_2O_3). The chemical reaction involved in this analysis is given Equation 9 [18].

$$\begin{aligned} ROOH + 2H^+ + 2KI &\longrightarrow I_2 + ROH + H_2O + 2K^+ \\ I_2 + 2Na_2S_2O_3 &\longrightarrow Na_2S_4O_6 + 2NaI \end{aligned} \tag{9}$$

Results may be influenced by potential drawbacks such as iodine being absorbed by unsaturated sites of the fatty acids and releasing of iodine from potassium iodine caused by oxygen being present in the solution which is titrated. Reactivity of the peroxides as well as temperature and time may also affect the results [18].

1.8.9 Conjugated dienes

Conjugated dienes content reflect the formation of primary oxidation products. Lipids containing methylene-interrupted dienes show a shift in the positioning in the double bonds during oxidation. This is due to isomerisation and conjugate formation. The formation of conjugated dienes (CD) is proportional to the oxygen uptake and the formation of peroxides in lipids [18].

1.8.10 Thiobarbituric acid reactive substances (TBARS)

Hydroperoxides are formed during primary lipid oxidation, and are further decomposed to produce secondary oxidation products. Thiobarbituric acid reactive substances (TBARS), such as malondialdehyde (MDA), are secondary oxidation products. TBARS are prone to form colour complexes with 2-thiobarbituric acid (TBA). The reaction with TBA has been widely used to determine oxidative deterioration occurring in fats and oils [45].

1.8.11 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is an analytical method applicable for measuring temperature and heat flow associated with transitions in a material as a function of time or temperature. A sample is either heated, cooled or held at a constant temperature while the energy absorbed or released is measured. The results will give information about the chemical and physical changes that occurs during exothermic, endothermic or heat capacity changes in the sample. The information acquired is given as the difference in change from a reference sample, usually an empty pan [46].

1.8.12 Measurement of water activity

The measurement of water activity can be done by determining the exact temperature at which condensation of water vapour occurs, the dew point temperature. The dew point is determined by cooling air until the water content changes. This occurs when the water content reaches a saturation point, which is achieved when condensation can be observed on a smooth, cooled surface such as a mirror. The dew point temperature is related to the water activity, and a dew point measuring instrument can calculate the water activity based on this principle [30].

1.8.13 Fatty acid composition

The fatty acid composition can be determined by hydrolysis of extracted fat to fatty acids, and using mass spectrometry (MS) to analyse the composition of the different FAs. The hydrolysis is performed by using etanolic KOH which reacts with the fat and forms glycerol and fatty acid potassium. The reaction equation is given in Figure 6. The fatty acid potassium is then converted into fatty acids by adding a concentrated acid, such as sulphuric acid. Hence, the fatty acids are liberated from potassium as potassium forms a salt [47, 48].

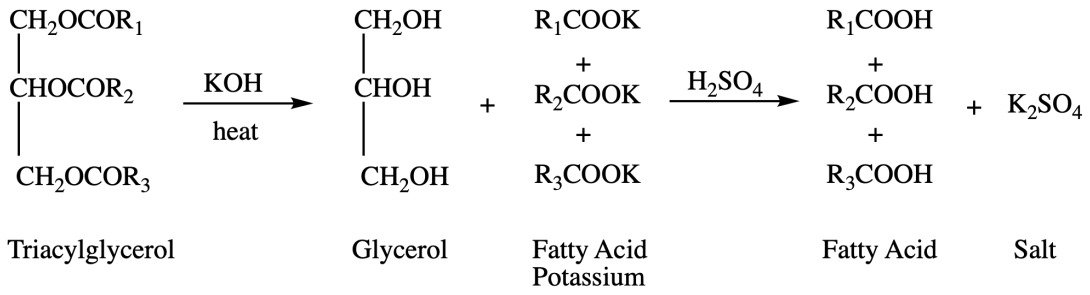


Figure 6: Hydrolysis of triacylglycerols to free fatty acids.

2 Material and methods

2.1 Materials

Rest raw material from chicken and salmon were utilised in this thesis. The rest raw material from chicken intestines included gizzards and gallbladders. The intestines are pictured in Figure 7 **(a)** before mincing in an industrial mincer. The intestines were obtained from freshly slaughtered chicken from the food company Norsk Kylling AS, Norway. The intestines were transported from Støren to NTNU Trondheim on February 21, 2019. The intestines were kept in Styrofoam boxes during transportation. The chicken intestines were immediately sorted into smaller plastic bags and stored in a −40°C freezer after approximately an hour of transportation until further analysis.

The rest raw material from salmon were also intestines, and taken from the slaughtering plant of the company Salmar AS, Norway. The salmon intestines, pictured in Figure 7 **(b)**, were transported January 15, 2019 from Frøya to NTNU Trondheim. The salmon intestines were sorted into buckets and containers before transportation. After five hours of transportation the intestines were immediately stored in a −40°C freezer until further analysis.

The materials were divided into batches, where batches were minced using a food processor after thawing for 24 hours in a −4°C cooler. The mincing was done right before analysis to produce homogeneous mixtures.

(a) Chicken intestines **(b)** Salmon intestines

Figure 7: Mincing of the chicken intestines from Støren **(a)**. The chicken intestines were minced before transportation to the laboratory at NTNU. **(b)** shows the salmon intestines from Frøya.

2.2 Overview of the procedures

The rest raw materials were minced before analysis. The mincing process varied as the food processor in the lab was damaged and was replaced with a commercial blender. The commercial blender was later replaced with a new food processor. Thus, the particle size of the minced material varied from sample to sample. The chemical composition of the raw material (dry matter, ash, protein and fat content) was determined before the thermal treatment. Both materials were thermally treated. The thermal treatment of the materials is described in Section 2.3. The thermally treated materials were separated and the fat-reduced sediment was dried using different drying methods. Stickwater and fat were separated from the materials, and analysed. The amount of soluble proteins and the degree of hydrolysis was analysed for the stickwater. The amount of lipid in the fat fraction and the oxidation of the lipids were also analysed. The fat-reduced fraction was dried using three different methods, and the dried products were analysed both before and after a storage period of 30 days. The storage experiment was carried out using two different storage methods as well. An overview of the procedure is given in Figure 8.

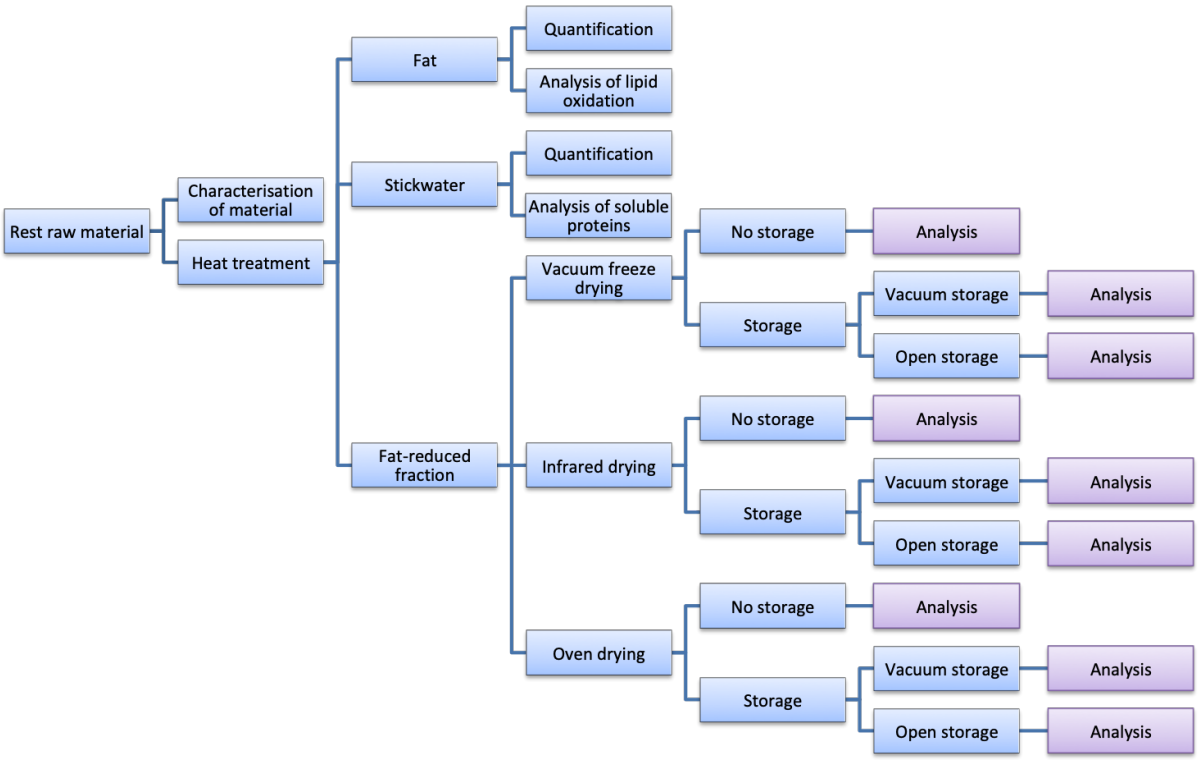


Figure 8: Flow chart of the procedure conducted during this thesis on both chicken and salmon intestines. The purple boxes represent the analysis which are further described in Figure 9.

The analysis carried out on the dried materials is illustrated in Figure 9, and was conducted to analyse the quality changes occuring after drying and changes occurring during the storage experiment. The effect of packaging of the dried materials were also analysed

as two packaging methods were studied. The composition of the dried materials were also analysed as small variations in the different batches were likely.

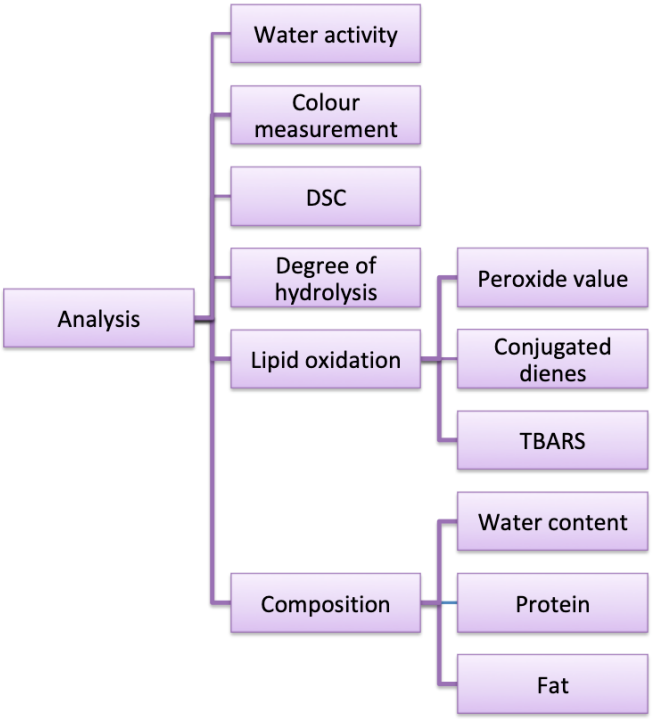


Figure 9: Flow chart of the procedure conducted on the dried materials before and after a storage experiment conducted over a month.

2.3 Thermal treatment of the raw material

Thermal treatment was performed to remove fat from the raw material before the drying process. Treatment at a low temperature was desired since quality of the material was expected to decrease at high temperatures. Increased temperatures would also increase enzymatic activity which induce degradation of proteins and lipids. The temperature used for the thermal treatment was optimised based on the amount of fat separated and visible quality changes, such as colour of the material. Initial temperature of the thermal treatment was set to 40°C based on Skjellegrind's thesis (2013) [49]. Higher temperatures (50°C, 65°C, 80°C and 95°C) were also tested. About 100 grams of minced raw materials were used for these tests. It was decided to use 50°C for the thermal treatment as it gave a high yield of separated fat while at the same time the colour of the material remained the same. This is thoroughly discussed in Section 3.2.

Raw material were first minced for five minutes before being packed into plastic bags. These bags were then placed in a water bath which was heated up to 53°C. The raw materials were then heated to 50°C. The temperature was monitored using a thermometer placed inside the bags. This took 20 minutes. The temperature of the water bath was decreased to 50°C after the temperature of the material had reached 50°C. The material

was held in the water bath for five more minutes after reaching the desired temperature. The heat treated material was then distributed in large centrifuge cups, and centrifuged for 15 minutes at 4,240 G at 4°C. Fat-reduced sediment was decanted from the liquid phase, and the liquid phase was centrifuged again with the same conditions. Oil and water were separated after the second centrifugation. The three different fractions were weighed.

2.4 Mass balance

A mass balance was used to determine the amount of fractions (sediment, stickwater and oil) of the thermally treated rest raw materials. Equation 10 gives the mass balance, where *rrm* expresses the rest raw material, w is the weight and %dw is the dry weight of the fractions in percentage.

$$\mathrm{w}_{rrm} \cdot \%\mathrm{dw}_{rrm} = \mathrm{w}_{sediment} \cdot \%\mathrm{dw}_{sediment} + \mathrm{w}_{stickwater} \cdot \%\mathrm{dw}_{stickwater} + \mathrm{w}_{oil} \cdot \%\mathrm{dw}_{oil} \quad (10)$$

2.5 Drying of the rest raw materials

The materials were dried with three different drying processes; vacuum freeze drying, drying in an oven (hot air) and an infrared dryer. The different drying processes were tested to evaluate how easy it was to dry the materials and at the same time how the quality was affected.

Spray drying of the materials was considered as a fourth drying process. However, this drying method produces droplets with droplet size ranging from 10 µm to 200 µm which required the materials to consist of very small particles [24]. This was concluded to be ineffective for these materials, and the spray drying was disregarded.

2.5.1 Vacuum freeze drying

Fat-reduced fractions which were thermally treated at 50°C were vacuum freeze dried. A thin layer of the fractions were spread on a glass dish, and frozen at −80°C for an hour. The desired thickness of the layer was about 1/2 to 1 cm thick to obtain an even drying throughout the material. Figure 10 shows the fat-reduced salmon intestines in a glass dish.

Figure 10: Fat-reduced sediment from salmon intestines in glass dish before freezing and vacuum drying. Preferable thickness of the layer should be between 1/2 and 1 cm.

The frozen materials were then placed in the vacuum dryer (CHRIST ALPHA 2-4 LSC plus). The vacuum drying process was predetermined based on earlier experience done by Tolstorebrov. The process is given in Table 1. The pressure was set to 0.300 mbar, and the materials were dried in the vacuum chamber for 24 hours.

Table 1: Vacuum freeze drying process with temperature progress and time.

Temperature [°C]	−30	−30	−25	−20	−10	−0	40
Time	5 minutes	3 hours	3 hours	3 hours	2 hours	1 hour	3 hours

2.5.2 Hot air drying (drying in oven)

The oven drying was done at 50°C and 100°C. Drying at 50°C was used since the materials were heat treated at this temperature. This temperature was suitable to maintain the quality of the material. Two types of ovens were used, an oven without humidity control (A) and another with 10 % humidity (B). The oven with 10 % humidity had a fan which caused flow of warm air across the surface during the drying, consequently making the drying process more rapid. Oven B did also have a more precise temperature control. The thickness of the raw materials had to be small in this drying process to get an even drying of the materials. The salmon intestines spread on the plates are pictured in Figure 11. The drying processes were mapped by weighing the drying samples at regular intervals during the processes.

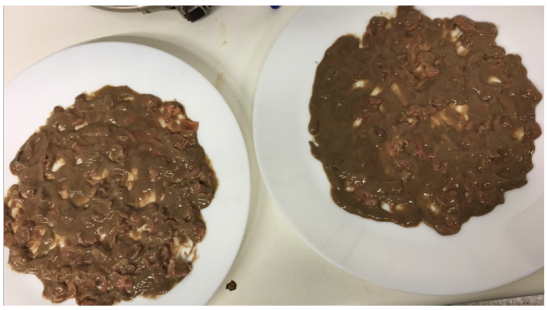


Figure 11: Fat-reduced sediment from salmon intestines spread on plates before drying in oven at 50°C and 100°C.

2.5.3 Infrared drying

The salmon intestines were dried with a 500 W infrared dryer for 45 minutes. The infrared system was installed into an oven, and the drying process was conducted in this oven with only the IR dryer providing radiation to heat up the materials. The same plates used in the oven drying were used for this drying method as well. Samples were weighed before and after drying to determine the water evaporated from the materials.

The IR drying was planned to be conducted on both the chicken and salmon intestines. However, because of the strong odour of the chicken intestines and limited ventilation in the thermal engineering laboratory, the IR drying of the chicken intestines was not conducted.

2.6 Storage experiments

A storage experiment on the dried materials were conducted for 30 days. The dried materials from all the drying methods for both salmon and chicken were vacuum packed and stored in plastic zip-lock bags at ambient temperature. The ambient temperature was estimated to be around 20°C in the laboratory. The packages were stored in a fume hood as odour of the materials stored in zip-lock bags was expected to become stronger during the experiment.

Analysis of the dried materials were conducted after 30 days. Protein quantification, degree of hydrolysis, lipid content and oxidation parameters such as peroxide value, conjugated dienes and TBARS were analysed after the storage experiment. The water content as well as colour were also inspected. The glass transition temperature were measured and compared with the wet raw materials. The dried materials were labeled based on the drying and the storage methods, and is presented in Appendix A.

2.7 Analytical methods

2.7.1 Dry matter and ash content

The dry matter and ash content were determined using the method described in AOAC [50]. Three parallels were made for the determinations. The dry matter content was calculated using Equation 11.

$$\frac{\text{Dry weight of sample [g]}}{\text{Weight of sample [g]}} = \text{Dry matter [\%]} \tag{11}$$

The ash content was calculated using Equation 12.

$$\frac{\text{Weight of ash [g]}}{\text{Weight of sample [g]}} = \text{Ash [\%]} \tag{12}$$

2.7.2 Soluble protein quantification: Lowry method

The content of soluble proteins were determined using the Lowry method described in Lowry et al. (1951) [51]. The test was conducted on salmon and chicken intestines and on the stickwater from both intestines. Three parallels were made for each material. Approximately 1 g of each sample was homogenised for 40 seconds in 10 mL before centrifugation at 4230 G for 10 minutes. The water phases were used in the Lowry method as described in the methodology. Standard curves were also made based on known protein standards. The standard curves are presented in Appendix D.

Equation 13 gives the formula for calculation of protein content in a sample using the standard curve made from the Lowry procedure.

$$\frac{c \cdot d \cdot y}{x} = \%\ \text{protein content} \tag{13}$$

The concentration c in [g/mL] is determined from the standard curve and the absorbance measured. d is the dilution factor, y in [mL] is the amount of water added to the sample before homogenisation and x is the amount of sample weighed out in [g].

2.7.3 Total protein quantification: Kjeldahl method

The Kjeldahl method is a method used to determine the nitrogen content in a material and from this the protein content can be determined. The method was performed with an instrument as described in Buchi manual (2013) for the Kjeldahl method on meat products [52]. The method was conducted on the raw materials from chicken and salmon, and on the dried chicken and salmon intestines.

The instrument calculated the nitrogen content and protein content according to Equation 14, 15, 16 and 17.

$$\mathrm{w}_N = \frac{V_{sample} - V_{blank} \cdot z \cdot c \cdot f \cdot M_N}{m_{sample} \cdot 1000} \tag{14}$$

$$\%\ \mathrm{N} = \mathrm{w}_N \cdot 100 \tag{15}$$

$$\%\ \mathrm{P} = \mathrm{w}_N \cdot \mathrm{PF} \cdot 100 \tag{16}$$

$$\%\ \mathrm{N}_{Gly} = \frac{\%\ \mathrm{N} \cdot 100}{\mathrm{P}} \tag{17}$$

The weight fraction of nitrogen, w_N, is calculated in Equation 14 by finding the difference in amount of titrant used for the sample and for the blank (V_{sample} - V_{blank}) in [mL] and multiplying this by the molar valence factor (z), the titrant concentration (c) in[mol/L], the titrant factor (f) and with the molar mass of nitrogen (M_N). f is given as 1 in the

Buchi manual (2013). The molar mass of nitrogen is 14.007 g/mol. This product is then divided by the weight of the sample (m_{sample}), which is converted to [mL/L] by dividing with 1000.

The percentage of nitrogen, %N, is calculated by multiplying w_N with 100. This is shown in Equation 15. The percentage of protein is calculated by multiplying w_N, the sample-specific protein factor (PF) which is 6.25 in meat products and with 100.

Equation 17 shows how to calculate the percentage of weight of nitrogen corrected for the purity of the reference substance, glycine (% N_{Gly}). This is done by dividing the nitrogen percentage (%N) with the purity of glycine in % (P) and multiply with 100 to obtain the percentage.

2.7.4 Total amino acid composition

This method was performed to verify the total protein content gained from the Kjeldahl method as there were some trouble running the Kjeldahl instrument. Vacuum freeze dried chicken was the **only** sample that was tested for this method. Triplicates were made of this sample. The sample was hydrolysed with HCl and prepared as described by Blackburn (1978) [41] before being injected into HPLC.

Concentrations of different amino acids in the sample was gained from the HPLC, and the total amino acid concentration was calculated based on Equation 18.

$$\frac{C \cdot V \cdot D}{1000 \cdot m} = \text{Total amino acids [mg/g sample]} \tag{18}$$

C is the concentration gained from the instrument in [µg/mL],V is the volume of the extract made in [mL], D is the dilution used and m is the mass of the sample in [g]. 1000 is the correction value used to convert from µg to mg.

2.7.5 Hydroxyproline

The hydroxyproline was determined as described by Leach (1960) [42]. This method was performed on triplicates of vacuum freeze dried chicken intestines by fellow student Papa L. The concentration of hydroxyproline was determined using a standard curve and the amount was determined using Equation 19.

$$\frac{C \cdot V \cdot D}{1000 \cdot m} = \text{Hydroxyproline [mg/g sample]} \tag{19}$$

C is the concentration of the hydroxyproline determined from the standard curve in [µg/mL], V is the volume of the extract in [mL], D is the dilution used, m is the weight of the sample and 1000 is the conversion factor.

2.7.6 Degree of hydrolysis: Formol titration

The degree of hydrolysis was determined by formol titration as described by Taylor W. H. (1957) [43]. An automatic titrator (TitroLine® 7000) was used to titrate NaOH until the pH reached 8.5. The concentration of free amino groups in the samples were calculated using Equation 20.

$$\frac{A \cdot B \cdot 14.007 \cdot 100}{C \cdot 1000} = \%\ \text{of free amino groups} \tag{20}$$

A is the amount of NaOH used in [mL], B is the concentration of the solution used for titration (0,1 M NaOH in this experiment) and C is the amount of sample in [g]. The degree of hydrolysis was calculated using Equation 21, where % of free amino groups was divided by the total number of nitrogen.

$$\frac{D \cdot 100}{E} = \text{degree of hydrolysis [\%]} \tag{21}$$

D is the % of free amino groups calculated using Equation 20, and E is the % of nitrogen in the sample. E is calculated using the soluble protein content of the sample, found from the Lowry method (in %), and dividing it by 6.25 (assuming that 16 % of the protein is nitrogen).

2.7.7 Bligh and Dyer lipid extraction

The Bligh and Dyer method was used to extract lipids. The methodology is described in Bligh & Dyer (1959) [44]. The percentage of lipid was calculated using Equation 22.

$$\frac{a \cdot b \cdot 100}{c \cdot v} = \text{total lipid content [\%]} \tag{22}$$

Equation 22 uses the weight of sample (v) in [g], the added chloroform (b) in [mL], the amount of chloroform-phase evaporated (c) in [mL] and the amount of lipid left after evaporation (a) in [g] to determine the lipid content.

2.7.8 Peroxide value

The peroxide value (PV) was determined for oil samples according to AOCS Official Method Cd 8b-90 [53] using a titration method with 0.01 M $Na_2S_2O_3$. The determination was conducted on lipids from both chicken intestines and salmon intestines, and dried materials before and after storage. The dried materials contained less fat, thus chloroform phases were made during the Bligh & Dyer extraction method and used in this analysis. Since the chloroform phases were used, the titrant concentration was changed to 0.001 M.

Equation 23 was used to calculate PV in [meq O_2/kg oil], where meq O_2 is an abbreviation for milliequivalent of oxygen. 1 milliequivalent O_2 equals 0.5 millimole oxygen.

$$\frac{(V - B) \cdot T \cdot M}{w} = \text{PV [meq } O_2\text{/kg oil]} \tag{23}$$

V is the volume in [mL] of consumed $Na_2S_2O_3$ during titration of oil sample, B is the volume in [mL] of consumed $Na_2S_2O_3$ during titration of blank sample and T is the concentration of the titrant which was 0.001 M for the dried samples. These parameters were all multiplied with the molarity (1000), and divided by the weight of the oil sample (w) in [g].

2.7.9 Conjugated dienes

Spectrophotometric method of conjugated dienes (CD) was performed based on a modified method by Aubourg (1998) [54] and Mozuraityte et al. (2017) [55]. This analysis was performed with oil in chloroform phases extracted from the Bligh & Dyer method. The amount of conjugated dienes (C-value) in each sample was calculated based on Equation 24 and 25.

$$\frac{A_{233}}{\epsilon \cdot l} = c_{CD}[\text{mmol/mL}] \tag{24}$$

$$\frac{c_{CD} \cdot 1000}{C/D} = \text{C-value [mmol/kg lipid]} \tag{25}$$

A_{233} is the absorbance measured at 233 nm, ϵ is the molar absorptivity of linoleic acid hydroperoxide ($2.525 \cdot 10^{-4}$ $M^{-1}cm^{-1}$), l is the path length of the cyvette (1 cm), C is the concentration of the sample in g/mL, D is the dilution factor and 1000 is the conversion factor to convert the results to mmol/kg lipid.

2.7.10 TBARS

Method for determination of thiobarbituric acid reactive substances was performed as described by Ke & Woyewoda (1972) [56]. The method was performed on the chloroform phase with approximately 0.5 grams of oil extract in 10 mL chloroform. 200 µL of the oil-chloroform mixture was then analysed for TBARS. Samples were taken in triplicates, and a standard curve was made for each analyses performed. The standard curve was made based on 1,1,3,3-tetraethoxypropane (TEP) solutions.

The absorbance of the TEP solutions and oil samples were measured at 538 nm by the use of a plate-reader, with distilled water as reference. TBARS in the oil samples were calculated using Equation 26.

$$\frac{Sample_{Abs} - b_{std}}{a_{std} \cdot l \cdot 1000} = \text{µmol TBARS/g lipids} \quad (26)$$

The parameter $Sample_{Abs}$ is the absorbance measured for the oil sample with the plate-reader, b_{std} is the intercept of the standard curve made and a_{std} is the slope of the standard curve. l is the lipid content in the oil sample given in [µL/g lipids] and the whole equation is divided by the conversion factor of 1000 which converts the value to [µmoles/g lipids].

2.7.11 Fatty acid composition

The fatty acid (FA) composition can be determined using mass spectrometry (MS). Fat was extracted from the raw material using the Bligh and Dyer method, and then hydrolysed to free fatty acids (FFA). The method for hydrolysis was based on Christie & Han (1996) [47] and Salimon et al. (2011) [48] with some modifications supervised by Bartosova (2019) [57].

The method performed in the lab is given as followed: Extracted fat was hydrolysed using ethanolic KOH (20 M KOH diluted 10 times in 96 % ethanol). 1 mL of ethanolic KOH was added to kimax-tubes which contained around 3 to 10 mg of oil sample. The mixture was then incubated in a heating block at 70°C for 90 minutes while being flushed with N_2 gas. 1 mL of 4 M sulphuric acid and 2 mL of dichloromethane (DCM) was added to each tube after incubation. The tubes were then whirl-mixed for 60 seconds and centrifuged at 900 G for 10 minutes. Two phases were observed after centrifugation, and the lower phase with DCM and fatty acids were transferred to vials. The vials were flushed for 30 seconds with N_2 before storage at -20°C. The vials were stored for approximately 18 hours before analysis with supercritical fluid chromotography coupled with mass spectrometry (SFC-MS) [47, 48, 57].

SFC-MS was utilised to detect the different types of fatty acids in the salmon and chicken intestines. CO_2 was used as the primary mobile phase with methanol and 0.1 % formic acid solution and methanol and 0.1 % ammonium hydroxide (NH_4OH) as co-solvents. The co-solvents were freshly prepared in the laboratory. The solutions would ionise the fatty acids which would then be detected by the instrument and appear in the spectrum obtained. Negative ionisation mode of SFC-MS, where one hydrogen atom in the molar mass is removed, was used in this analysis. Hence, the molar masses detected from the MS are subtracted with 1 g/mol to adjust for this ionisation mode [47, 48].

A pre-made internal standard was utilised in this analysis. The internal standard is a compound that should be of similar chemical species as the species of interest and should not interfere with the signals of the compounds in the sample. The internal standard used in this chromatography run was tridecylic acid (C13:0) with a concentration of 40 µg/mL.

Chromatograms were made in the software of the instrument and the area of each peak were also obtained. The abundance of each type of fatty acid was calculated based on Equation 27.

$$\frac{\text{Area of peak}}{\text{Total area}} \cdot 100\% = \% \text{ of total area} \tag{27}$$

2.7.12 Differential scanning calorimetry

DSC analysis was done to achieve information about the thermal properties of the rest raw material. The glass transition temperature, melting energy and melting peaks were found for chicken intestines and salmon intestines. The glass transition temperature can be utilised to analyse the structure in the dried materials. The melting energies can be utilised to determine the amount of water bound to the material and can also be utilised to estimate the amount of heat required to remove the water.

The DSC analysis was performed as described by Tolstorebrov et al. (2014) [58] with some modifications. The same DSC instrument (DSC Q2000) equipped with a Liquid Nitrogen Cooling System, as described in the article, was used. An empty hermetically sealed aluminium pan was used as reference. The samples were homogenised to obtain representative results. Around 10 mg of sample of each material were used in this analysis. The samples in this experiment were cooled down from ambient temperature (20°C) to −150°C with a cooling rate set to 10°C per minute. Analysis of the results were also done as described by Tolstorebrov et al. (2014) [58], by using the derivated heat flow curve obtained from the DSC analysis. The derivated heat flow curve shows the temperature when melting is completed on the bottom surface layer of the product [59]. The glass transition of the dried materials and the raw material were determined using the derived heat flow curve from DSC analysis. Structural change, which indicates the glass transition, occurs in a range of temperatures beginning in the onset temperature and ending in the end temperature. The inflection point in this range, where the curvature changes, is the point reported as T_g.

2.7.13 Water activity and water content

The water activity of the dried samples were measured using a water activity measurement instrument (AQUALAB Dew Point Water Meter 4TE), which had a deviation of ± 0.003 [60]. The samples were placed in a plastic cup and placed in the instrument. The water activity and the dew point temperature were displayed on the instrument.

The water content of the dried samples were analysed by placing the samples in an oven for two hours at 130°C. The water content procedure was the same as the determination of the dry matter except for the use of a higher temperature as the samples were dried and contain small amounts of moisture.

2.7.14 Colour measurement

The colour of the dried products were assessed with a colour measurement instrument (ColourFlex EZ Spectrophotometer). The instrument was calibrated with standards that

came with the instrument. The samples tested were placed in a glass cup and covered with an opaque cover, which minimised ambient light from being detected during measurement. The colours were determined based on the Hunter L, a, b colour values.

2.8 Statistical analysis

Standard deviations were computed to determine the uncertainty of the results. Microsoft Excel was used for managing the data and computing the standard deviations.

The data were also subjected to analysis of variance with one-way ANOVA. The means were accepted as significantly different at 95 % confidence level ($p < 0.05$). The calculations were carried out in the software StatPlus.

3 Results and discussion

3.1 Chemical composition of the raw materials

The chemical composition of the salmon and chicken intestines were determined analytically by measuring the dry matter, ash, total protein, soluble protein and lipid content. All raw data are given in Appendix B, C.1, D and E.1.

3.1.1 Salmon intestines

The chemical composition of salmon intestines is presented in Table 2. The values are given as mean ± standard deviation (SD) in %.

Table 2: Chemical composition of salmon intestines. All values are given in % of wet weight. Values are given as mean ± SD with n = 3. Total protein content was not determined fall 2018 for the material.

Salmon intestines	Dry matter	Ash	Total protein	Soluble protein	Lipid
Fall 2018*	73.1 ± 1.5	0.4 ± 0.02	-	3.2 ± 0.1	59.0 ± 10.3
Spring 2019	34.8 ± 2.8	1.0 ± 0.1	12.6 ± 0.5	8.0 ± 0.004	25.8 ± 1.0

*Results from project work by Jeyakumaran (2018) [61].

Dry matter is made up of ash, protein and lipid content. The sum of these components was 39.4 %, which is about 5 % larger than the dry matter content found experimentally. The difference is probably due to deviations in the experiments conducted. Of the three measurements it was expected that extraction of lipids could have had largest impact on the deviation since problems during transfer and evaporation could have occurred. The ash content was determined based on drying in oven at 500°C and the total protein content was determined based on the Kjeldahl method which was conducted instrumentally. The dry matter and fat content obtained spring 2019 was in agreement with the values for visceral tissue from Atlantic salmon reported by Aursand (1994) [62] (approx. 30 % dry matter and 27 % lipids).

Small seasonal variations was expected in salmon [63], and similar variations can therefore be observed in the composition of the by-products. The lipid content increases during fall, and according to Mørkøre & Rørvik (2001) [63] the increase is largest between July and November. The variation from fall to spring was observed for the lipid content of the salmon intestines. Although the lipid content decreased, it made up around 74 % of the dry matter which was similar to the amount from fall 2018 (about 80 %). Hence, decrease in lipid content of the salmon intestines was dependent of the season and should be taken into consideration in industrial oil production.

The total protein content of the salmon intestines was determined to be approximately 12.6 % and as seen in Table 2, the amount of soluble protein made up almost 63 % of

the total protein content. This is a large amount, and there is a possibility of losing these proteins in the stickwater causing great loss of proteins from the material during separation.

3.1.2 Chicken intestines

The chemical composition of chicken intestines is presented in Table 3. The values are given as mean ± standard deviation (SD) in %.

Table 3: Chemical composition of chicken intestines. All values are given in % of wet weight. Values are given as mean ± SD with n = 3. Total protein content was not determined fall 2018 for the material. Total protein content was not determined fall 2018 for the material.

Chicken intestines	Dry matter	Ash	Total protein	Soluble protein	Lipid
Fall 2018*	31.1 ± 0.2	0.9 ± 0.03	-	6.30 ± 0.1	25.4 ± 0.6
Spring 2019	30.6 ± 0.6	0.8 ± 0.2	11.9 ± 0.3	7.5 ± 0.01	17.1 ± 0.3

*Results from project work by Jeyakumaran (2018) [61].

The sum of ash, protein and lipid content was about 30 %, and was in good agreement with the experimental dry matter content. Low levels of ash was found in the chicken intestines, as in the salmon intestines. This was expected as the intestines do not contain bones. Small seasonal variations were found in the chicken intestines, however these variations were smaller compared to the observations of the salmon intestines. As seen from Table 3, the lipid content of the chicken intestines was 8 % larger in fall 2018. The rest of the components were quite similar for the two seasons.

The lipid content only made up about 56 % of the dry matter, unlike the salmon intestines with 74 %. Both the amount of lipids and the composition of fat will influence the properties and the oxidation rate of the materials and was taken into account in the discussion of the thermal treatment (Section 3.2). Seong et al. (2015) [64] reported almost similar protein content (11.8 %) of small intestine from chicken. The moisture and fat content was around 10 % higher and 15 % lower, respectively. This shows that the Norwegian chicken had a higher lipid content, which will presumably lead to shorter shelf life due to oxidation reactions.

The total protein content determined from the Kjeldahl method, was approximately 12 %. This was larger than the soluble protein content determined from the Lowry method. The soluble protein made up approximately 63 % of the total protein content for both the chicken and the salmon intestines.

3.2 Optimisation of temperature for thermal treatment

Thermal treatment of fish is usually conducted at 90-95°C before the phase separation, but high temperatures and long heating time will result in reduction of the quality of the oil [65]. Hence, oil separation was studied for the salmon and chicken intestines to find an optimal temperature. The temperatures for the thermal treatment of the raw materials were chosen based on the amount of fat separated and the colour changes. Initial tests on the salmon and chicken intestines were conducted at 40, 50, 65, 80 and 90°C. Fat-reduced sediment, stickwater and fat were separated after the thermal treatment. The fat-reduced sediment was the dry matter excluding most of the fat, which was separated after the first round of centrifugation. The stickwater and fat were separated from each other after the second round of centrifugation. The initial tests were conducted on approximately 100 grams of material for each thermal treatment. Neither materials had an emulsion phase between the fat phase and the stickwater after the thermal treatments.

3.2.1 Salmon intestines

The percentages of the different fractions separated after the thermal treatments at different temperatures are given in Table 4 for the salmon intestines. Mass balance calculations conducted to obtain the values below are presented in Appendix F.

Table 4: Amount of fat-reduced sediment, stickwater and fat obtained after thermal treatment of salmon intestines at 40, 50, 65, 80 and 90°C. Amount of material lost during the treatments are also given. The values are given as % of the sample weight.

Temperature	**Fat-reduced sediment** [%]	**Stickwater** [%]	**Fat** [%]	**Lost** [%]
40°C	61.2	25.1	0.245	13.4
50°C	47.2	30.5	14.5	7.86
65°C	46.0	32.6	14.9	6.55
80°C	55.9	26.6	1.18	16.7
90°C	53.3	34.3	4.37	8.00

The amount of stickwater increased with increasing temperature, however the amount decreased at 80°C and increased again at 90°C. The same pattern was observed for the amount of fat separated. Whenever the amount of fat and stickwater increased the amount of fat-reduced sediment decreased and vice versa. The amount of lost material was not dependent on the temperature, but on the separation process. Since the material had to be transferred from the bag to centrifugation cups and centrifugation had to be done twice, the loss of material would vary. Most of the fat and stickwater were lost due to the difficulty of removing the fractions completely from centrifuge cups.

The amount of fat was substantially reduced at 80°C and 90°C compared to the lower temperatures. This was also observed at the lab, as the materials lumped together during the treatment at these temperatures making the fat separation less effective. Even if the samples were stirred periodically at intervals, the material still lumped together. This could be attributed by the fat being more bound to the material, which made it more difficult to separate the fat later in the process.

The lipid content of the fat-reduced sediments were also determined to evaluate differences between the different temperatures. The lipid contents in % are given in Table 5. The lipid content of the minced salmon intestines without thermal treatment is also given. The calculations for these values are given in Appendix E.1, E.2, E.3 and E.4.

Table 5: Lipid content in fat-reduced sediment of salmon intestines after thermal treatment at 40, 50, 65, 80 and 90°C. The lipid content for minced salmon intestines without thermal treatment is also given. The values are given as mean ± SD in %.

Material	**Temperature for thermal treatment**	**Lipid content** [%]
Raw material (salmon intestines)	-	25.8 ± 0.98
Fat-reduced sediment	40°C	23.5 ± 1.07
Fat-reduced sediment	50°C	10.2 ± 0.08
Fat-reduced sediment	65°C	22.4 ± 0.63
Fat-reduced sediment	80°C	30.7 ± 0.68
Fat-reduced sediment	90°C	28.7 ± 1.83

It can be seen from Table 5 that the lipid content reduced with 13 % from 40°C to 50°C, however the lipid content increased for the sediment treated at 65°C and 80°C. The increase may have been caused by more stickwater being separated during the thermal treatment. Thus, the concentration of lipids in the sediment increases. Thermal treatment of herring by-products at 60-90°C reported by Carvajal et al. (2015) [66] showed the same trend as seen for the salmon intestines for the same temperatures. The lipid content decreased at 80°C and increased again at 90°C. The relationship between the fat separation and the lipid content in the fat-reduced sediment at different temperatures is presented in Figure 12.

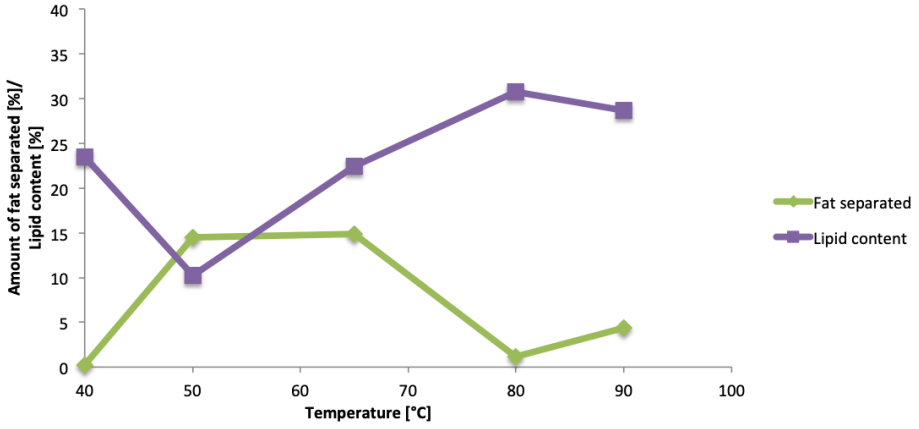


Figure 12: Relation between fat separation and lipid content of fat-reduced sediment after thermal treatment at 40, 50, 65, 80 and 90°C of salmon intestines.

The lipid content of the raw material was used as reference for the concentration of lipids after the thermal treatments. According to Figure 12 there was a reduction in lipid content at 50°C, however the content increases as the temperature increases. This increase was reflected in the amount of fat separated after the thermal treatment. As less fat was separated, lipid concentration increased in the fat-reduced sediment. The stickwater was also separated, which could also induce the increase of lipid content. In the context of fat separation and lipid content, the optimal temperature for the thermal treatment was where the percentage of the lipid content of the fat-reduced sediment was lower than the amount of fat separated. This was, according to the results obtained, at 50°C for the salmon intestines. However, other factors like enzyme activity and oxidation rate of the material may affect the quality of the fractions. The colour observed for the fat-reduced sediment and the stickwater after treatment at 50 and 65°C is given in Figure 13.

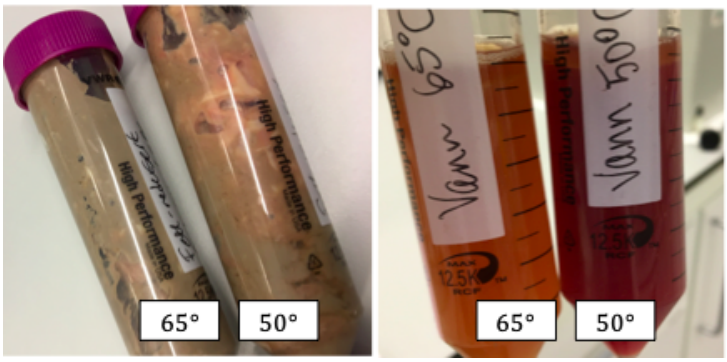


Figure 13: Fat-reduced sediment (left) and stickwater (right) from salmon intestines after 50°C and 65°C thermal treatment.

The original colour of the salmon intestines before mincing was pink with some grey slurry, which is pictured in Figure 7 **(b)**. The salmon intestines became light pink after mincing. The raw material became less pink after the thermal treatment as seen for 50°C and 65°C presented in Figure 13. The heat treatment at 80°C and 90°C turned the sediment less pink. The stickwater was originally red as seen in Figure 13 and could be attributed by some haemoglobin (from blood) left in the material. The colour of the stickwater became lighter for the increasing temperature which could indicate denaturation of heme proteins. The changes in the stickwater composition could be further studied as the stickwater might have a potential to replace fish meal [67].

Thermal treatment at high temperatures such as 80°C and 90°C is not applicable as the fat separation was not optimal for these temperatures. The denaturation increases of the proteins and lipids. Increasing temperature also require more energy which can signify large operational costs if this method is utilised at a large scale.

3.2.2 Chicken intestines

The distribution of fractions of the chicken intestines for different temperatures are given in Table 6. Raw data are given in Appendix F.

Table 6: Amount of fat-reduced sediment, stickwater and fat obtained after thermally treating chicken intestines at 40, 50, 65, 80 and 90°C. Amount of material lost during the treatments are also given. The values are given as % of the sample weight.

Temperature	**Fat-reduced sediment** [%]	**Stickwater** [%]	**Fat** [%]	**Lost** [%]
40°C	83.1	7.47	3.95	5.53
50°C	75.2	13.1	6.01	5.73
65°C	61.5	28.5	7.61	2.41
80°C	73.5	19.9	5.73	0.85
90°C	69.8	17.1	8.62	4.46

The amount of stickwater and fat increased from 40°C to 65°C, and decreased at 80°C. As seen for the salmon intestines, the amount of fat-reduced sediment of the chicken intestines decreased as the amount of stickwater increased and vice versa. The amount of fat did not decrease as expected at 90°C. The reason could be that the chicken intestines did not lump together as much as the salmon intestines at these temperatures, which is reasonable as the chicken intestines come from a warmer environment compared to the intestines from the salmon. Thus, the chicken have a wider range of reasonable fat extraction at higher temperatures.

The lipid content of the fat-reduced sediment for the different temperatures was also analysed and is given in Table 7. The calculations for the lipid content of the raw material and the heat treated chicken intestines are given in Appendix E.1, E.2, E.3 and E.4.

Table 7: Lipid content in fat-reduced sediment of chicken intestines after thermal treatment at 40, 50, 65, 80 and 90°C. The lipid content for minced chicken intestines without thermal treatment is also given. The values are given as mean ± SD in %.

Material	**Temperature for thermal treatment**	**Lipid content** [%]
Raw material (chicken intestines)	-	17.2 ± 0.26
Fat-reduced sediment	40°C	18.2 ± 0.47
Fat-reduced sediment	50°C	7.38 ± 0.04
Fat-reduced sediment	65°C	8.99 ± 0.17
Fat-reduced sediment	80°C	17.0 ± 0.11
Fat-reduced sediment	90°C	15.2 ± 0.22

The lipid content decreased with almost 11 % after treatment at 50°C, but continued to increase for temperatures above 50°C. The same pattern was observed for the salmon intestines, and is most likely due to the increasing removal of stickwater. The lipid content increased with almost 8 % again at 80°C. This is probably due to effective removal of water at this temperature and according to Table 6 was not effective for the separation of fat. It

can therefore be concluded that thermal treatment above 80°C might be unnecessary since drying of the material is expected to remove this water regardless. However, if beneficial use of the stickwater separated from the chicken intestines is established, the thermal treatment above 80°C could be more desirable. The relation between the fat separated and the lipid content of the fat-reduced sediment could give a better understanding of the progress, and is presented in Figure 14.

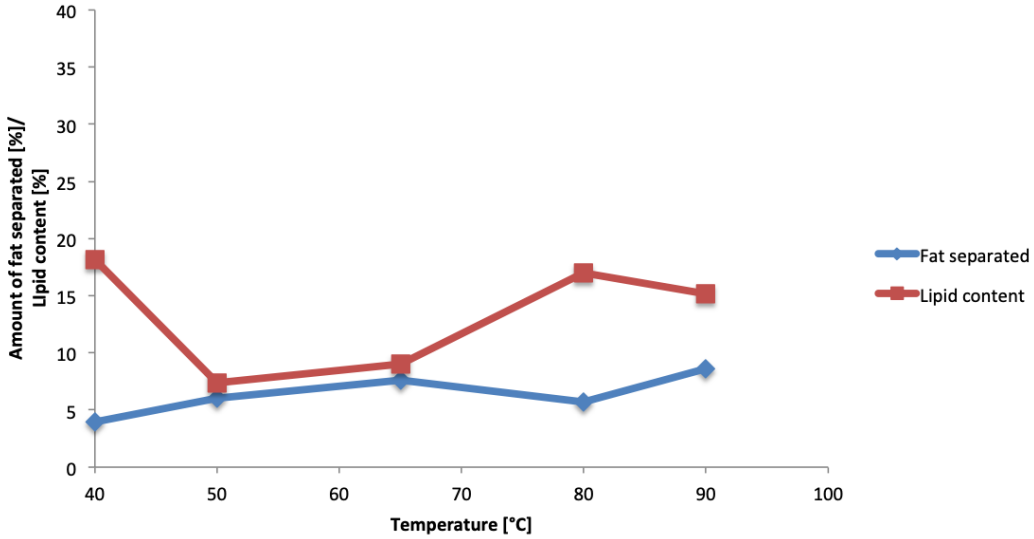


Figure 14: Relation between fat separation and lipid content of fat-reduced sediment after thermal treatment at 40, 50, 65, 80 and 90°C of chicken intestines.

The difference in lipid content and fat separated at 50°C and 65°C were almost similar, as seen in the figure. This area is the closest to the amount of fat separated, however lipid content lower than amount of fat separated was not detected. However, lipid content was found to be lowest at 50°C. Since this was not observed, the temperature for thermal treatment was set to 50°C making it easier to compare with the salmon intestines. Changes in colour was also observed and is presented in Figure 15.

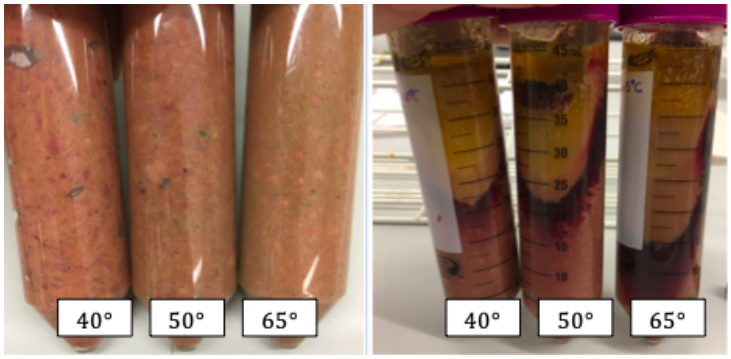


Figure 15: Fat-reduced sediment (left) and centrifuged material with layers of sediment, fat and stickwater (right) from chicken intestines after 40, 50 and 65°C thermal treatment.

The fat-reduced sediment had a slight change in colour for the different temperatures as seen in Figure 15. The fat-reduced sediment was originally pink and changed to a light

brown colour at 65°C. The sediment treated at higher temperature was slightly darker. Difference in separation of the three fractions is also pictured in the figure (Fig. 15). The amount of water and fat separated from the sediment was large for the material treated at 65°C compared to the materials treated at 40°C and 50°C. The colour of the stickwater did not change as it was observed for the salmon intestines.

According to an unpublished research by Janthed P. (2019), the general protolytic activity in the chicken intestines from Støren was highest at 60°C. The activity increased until 60°C and decreased again at 70°C. This showed that the thermal treatment performed at temperatures close to 60°C resulted in high enzymatic activity, thus increasing the protein degradation. Based on this, the thermal treatment could have an optimal effect at temperatures higher than 60°C as the protolytic activity decrease in this range. However, according to Figure 14 the lipid content increased and the amount of fat separated decreased at higher temperatures. Thus, heat treatment below 60°C could be an option as the protolytic activity and the lipid content were lower at 50°C compared to at 70°C. Based on the results from the thermal treatment of salmon and chicken intestines, 50°C was chosen and used as extraction temperature in the further analysis conducted in this thesis.

The thermal treatment of the material was performed to remove the initial fat content in the intestines before drying. The effect of removing parts of the initial fat content can be observed from the analysis done on the FFA content during storage by Ytterås (2019) [68]. It was reported that the FFA content in the chicken intestines increased after 72 hours of storage. This was not observed for the salmon intestines. The results from this study shows that removal of the fat in the chicken intestines was favourable to slow down the deterioration of the material and could increase the shelf life of the material.

The amount of fat separated from the chicken intestines was lower compared to the amount separated from the salmon intestines. The difference may play a role in the importance of the thermal treatment for the two materials as well as fatty acids found in the salmon are more favorable for human consumption. The fatty acid composition for both materials is further discussed in Section 3.3.

3.3 Fatty acid composition

The fatty acid composition was determined using mass spectrometry (MS). The chromatograms are given in Figure 16 for both salmon intestines and chicken intestines. Note that the samples had different dilutions. The salmon intestines were diluted 80 times and the chicken intestines 60 times.

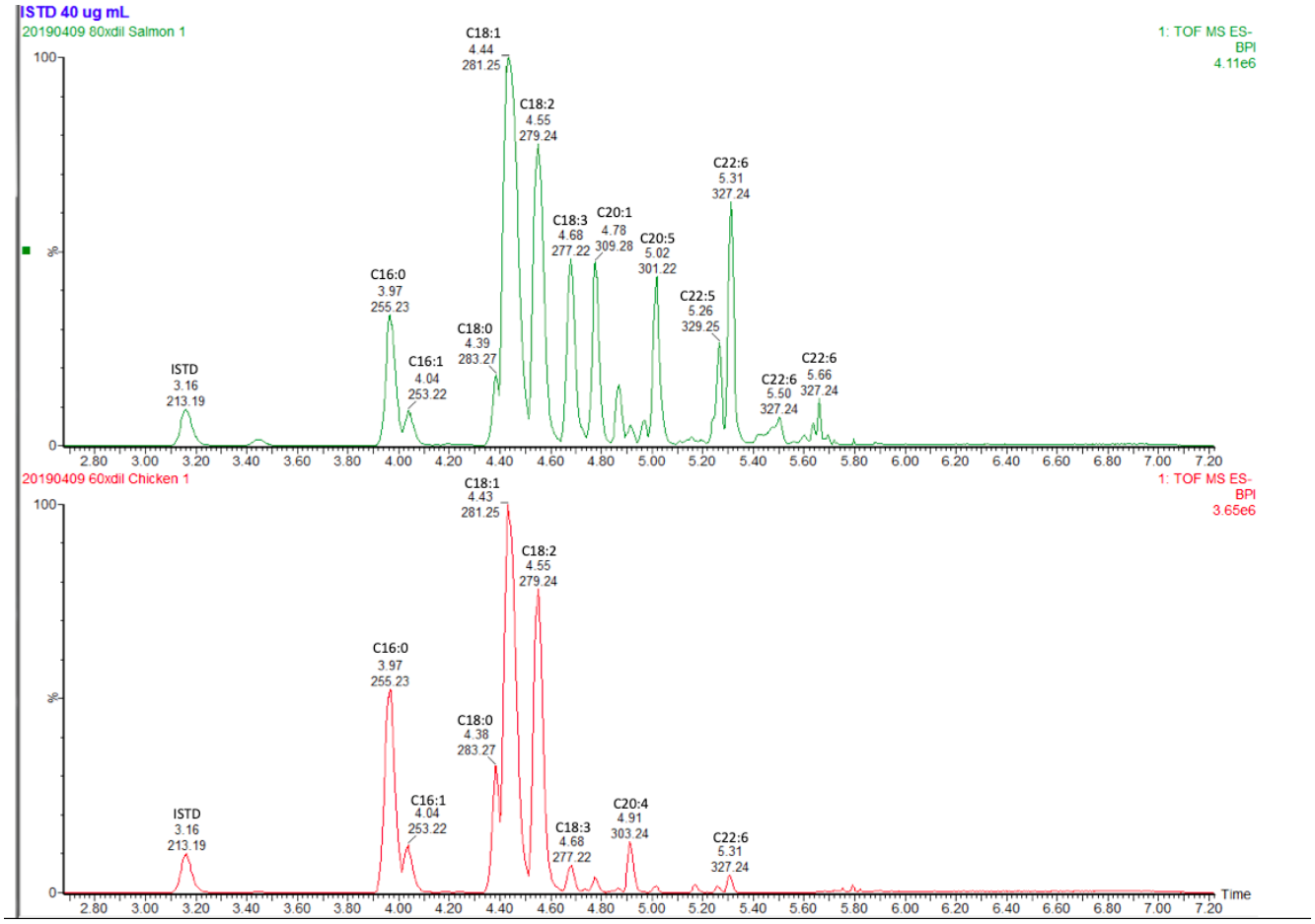


Figure 16: Chromatograms from supercritical fluid chromatography coupled with mass spectrometry (SFC-MS) performed on oil extracted from salmon intestines (green) and chicken intestines (red). Every peak is denoted with the lipid number of the fatty acid, retention time and the mass of the fatty acid. The first peak in both chromatograms shows the tridecylic acid (C13:0) which was the internal standard (ISTD) used. The molar masses given in this figure take the negative ionisation into account, thus 1 g/mol have been subtracted from the original molar mass values.

The peaks in the chromatogram represent different fatty acids detected in the MS. Each peak has been denoted the lipid number, retention time and the mass of the compound which are shown in the figure for both chromatograms and a list of these are also given in Appendix G. The first peak in both chromatograms indicate the internal standard (ISTD) and this was used to calibrate the abundance of the fatty acids. The first axis in

the chromatograms represent the retention time of each compound and the second axis represent the abundance. However, most peaks were difficult to spot in the chromatogram and the chromatograms were therefore zoomed in. Thus, the second axis cannot be used to evaluate the abundance of each fatty acid. Instead the area of each peak obtained from the software was used to calculate the abundance. The abundance of each fatty acid is given in Table 8 for the salmon intestines and Table 9 for the chicken intestines.

Table 8: The abundance of each fatty acid identified with mass spectrometry in oil extracted from the salmon intestines. The table shows the name of the fatty acids, the corresponding lipid number, area of each peak in the chromatogram and the calculated percentage of total area. The sum of saturated fatty acids, PUFAs, omega-3 and omega-6 fatty acids are also given.

Common name	**Lipid number**	**Area of peak**	**% of total area**
Myristic acid	C14:0	6799.06	0.41
Palmitic acid	C16:0	89815.81	5.43
Palmitoleic acid	C16:1	32990.36	1.99
Stearic acid	C18:0	58037.51	3.51
Oleic acid	C18:1	628871.50	38.02
Linoleic acid	C18:2	243662.09	14.73
α-linolenic acid	C18:3	163422.67	9.88
Paullinic acid	C20:1	100535.01	6.08
Eicosadienoic	C20:2	30056.45	1.82
Dihomo-γ-linolenic acid	C20:3	20177.16	1.22
Arachidonic acid	C20:4	24880.28	1.50
EPA[a]	C20:5	95382.09	5.77
Docosatetraenoic acid	C22:4	2330.79	0.14
DPA[b]	C22:5	56539.70	3.42
DHA[c]	C22:6	100535.18	6.08
Σ Saturated[d]			9.35
Σ PUFA[e]			44.42
Σ n-3[f]			25.15
Σ n-6[g]			19.27

[a] Eicosapentaenoic acid, [b] Docosapentaenoic acid, [c] Docosahexaenoic acid
[d] includes C14:0, C16:0 & C18:0
[e] includes C18:3, C20:5, C22:5, C22:6, C18:2, C20:2, C20:3 & C20:4
[f] includes C18:3, C20:5, C22:5 & C22:6
[g] includes C18:2, C20:2, C20:3 & C20:4

Table 9: The abundance of each fatty acid identified with mass spectrometry in oil extracted from the chicken intestines. The table shows the name of the fatty acids, the corresponding lipid number, area of each peak in the chromatogram and the calculated percentage of total area. The sum of saturated fatty acids, PUFAs, omega-3 and omega-6 fatty acids are also given.

Common name	**Lipid number**	**Area of peak**	**% of total area**
Myristic acid	C14:0	901.04	0.09
Palmitic acid	C16:0	131415.17	13.35
Palmitoleic acid	C16:1	39915.68	4.06
Stearic acid	C18:0	79753.23	8.10
Oleic acid	C18:1	472429.47	48.00
Linoleic acid	C18:2	193833.34	19.69
α-linolenic acid	C18:3	20435.80	2.08
Paullinic acid	C20:1	7373.65	0.75
Eicosadienoic	C20:2	1892.25	0.19
Dihomo-γ-linolenic acid	C20:3	4150.53	0.42
Arachidonic acid	C20:4	18151.91	1.84
EPA[a]	C20:5	2496.94	0.25
Docosatetraenoic acid	C22:4	2273.41	0.23
DPA[b]	C22:5	3015.82	0.31
DHA[c]	C22:6	6284.96	0.64
Σ Saturated[d]			21.54
Σ PUFA[e]			25.42
Σ n-3[f]			3.28
Σ n-6[g]			22.14

[a] Eicosapentaenoic acid, [b] Docosapentaenoic acid, [c] Docosahexaenoic acid
[d] includes C14:0, C16:0 & C18:0
[e] includes C18:3, C20:5, C22:5, C22:6, C18:2, C20:2, C20:3 & C20:4
[f] includes C18:3, C20:5, C22:5 & C22:6
[g] includes C18:2, C20:2, C20:3 & C20:4

It can be observed from Figure 16 that the salmon intestines had a more diverse fatty acid composition compared to the chicken intestines, and will contribute to the wide melting point of the fat obtained from the intestines. Small traces of other fatty acids, for instance pentadecanoic acid (C15:0), was also found in both materials. However, the amount of this compound was below the limit of quantification (LOQ) and was therefore disregarded.

Some of the peaks obtained from the chromatograms represent the same fatty acid, but were shifted resulting in different retention time. This was especially seen for DHA (C22:6) in the salmon intestines, which had acquired three peaks in this chromatogram. This was acknowledged as positional isomers of the fatty acid. The type of isomers were not determined, and these peaks were not taken into account in the calculations of the abundance given in Table 8 and 9. The fatty acid which was most abundant in both materials was oleic acid. Around 42 % and 48 % of the oleic acid was detected in salmon and chicken intestines, respectively. Oleic acid is a monounsaturated fatty acid and around 37-56 % of chicken fat constitutes of oleic acid [69]. This fatty acid made

up almost half of the fatty acid content in both materials, resulting in the fats of each material having very similar properties as oleic acid. The melting point of oleic acid was estimated to be around 13°C [70]. The second most abundant fatty acid was linoleic acid with approximately 16 % and 20 % in salmon and chicken intestines, respectively. Linoleic acid is one of the most consumed omega-6 PUFAs in the human diet, and is an essential nutrient [71]. According to Whelan & Fritsche (2013) [71] linoleic acid contributes to around 70-85 % of all PUFAs in meat such as chicken. This corresponds to the results obtained from the MS determination, as linoleic acid made up approximately 84 % of the total amount of PUFAs in the chicken intestines. Unlike the salmon intestines with 34 % of the PUFAs was linoleic acid, which was half the amount found in the chicken. The amount of fatty acids found in the chicken intestines was closely in agreement with the fatty acid content of raw hen eggs reported by Özcan et al. (2019) [72]. For instance, the stearic acid and the oleic acid content in raw hen eggs was around 6 % and 51 %, respectively, compared to 8 % and 48 % in the intestines. However, greater amounts of PUFAs were found in the intestines compared with the hen eggs. The fatty acid profiles of chicken by-products was conducted by Seong et al. (2015) [64]. The report describes PUFAs, omega-3 and omega-6 content in small intestines to be about 28 %, 1 % and 27 %, respectively. Oleic acid (C18:1) made up the largest and EPA (C20:5) made up one of the smallest part of the fatty acid profile of the small intestine [64]. These values are in agreement with the values given in Table 9.

Aursand (1994) [62] reported around 25 % PUFAs in the visceral fat from Atlantic salmon, which was lower than the amount found for the salmon intestines in this study and was closer to the amount found in the chicken intestines. The salmon intestines contained more of the PUFAs such as C18:2, C18:3, C20:3, C20:5, C22:5 and C22:6 compared to the chicken intestines. The majority of the PUFAs found in the salmon intestines were omega-3 PUFAs (25.15 %). This was larger than the amount found in the chicken intestines (3.28 %). The PUFAs have low melting temperatures, hence influencing the melting property of the salmon oil extracted. Some of these PUFAs were also found in the chicken intestines, however the abundance was much lower compared to the salmon intestines. This was also seen during experiments where the lipid from the chicken was more viscous compared to the oil from the salmon. The amount of long chain PUFAs, which are more susceptible to oxidation, can determine the rate of deterioration of the materials. As seen from the fatty acid composition, the salmon intestines contained more of these PUFAs and was therefore more vulnerable to deterioration compared to the chicken intestines. The lipid oxidation of dried salmon and chicken intestines is discussed further in Section 3.5, and can be correlated with the findings from the MS analysis.

The fatty acid composition can be used to study the properties of the fat separated during the thermal treatment. As mentioned, majority of the fatty acids found in the salmon intestines have low melting points. This resulted in the fat to be liquid in ambient temperature, unlike the fat from the chicken intestines which was more viscous. Thus, to obtain separation of fat in the chicken intestines require higher temperatures in comparison with the fat in the salmon intestines.

3.4 Drying of the materials

The thermal treatment and drying of the materials were distributed in several batches, as the amount of material varied for every batch and the amount obtained after drying also varied. The variation was dependent on the duration of the drying processes. The vacuum freeze drying lasted for 24 hours and the oven drying at 50°C lasted for 23 and 15 hours for the salmon and chicken intestines, respectively. The materials drying in the IR dryer lasted for 45 minuted and the oven drying at 100°C lasted for 3 hours. The oven drying was performed without humidity for both materials. The feasibility and costs of the different drying methods was not studied in this thesis.

The salmon was dried with three different methods: vacuum freeze drying, infrared drying and in an oven at two different temperatures. The results after the drying processes of the salmon intestines are pictured in Figure 17.

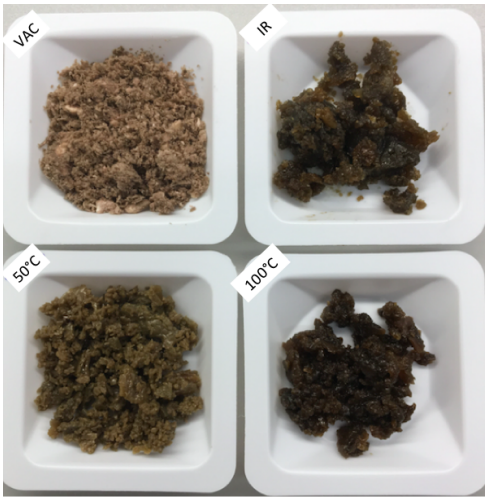


Figure 17: Salmon intestines dried with vacuum freeze dryer (VAC), infrared dryer (IR) and in oven at 50°C (50°C) and at 100°C (100°C).

The vacuum freeze dried salmon was porous and therefore could be pulverised to obtain a homogeneous powder. This was not possible to implement on the other samples as they were sticky and could not be easily pulverised. The vacuum freeze dried salmon was the lightest of all the samples, and had the lowest moisture content. The intestines dried in the oven at 100°C and in the IR dryer were sticky compared to the salmon dried in the vacuum freeze dryer and the oven dried at 50°C. These samples were also darker in colour. The intense colour of these samples could be result of a substantial quality change compared to the samples dried in the vacuum freezer dryer and in the oven at 50°C. The vacuum freeze dried salmon could be used as a reference for the dried samples as vacuum freeze drying is based on drying during sublimation of a frozen product. This yields a high quality of the product as deterioration processes are slowed down due to absence of liquid water, absence of oxygen under vacuum and the use of low temperature. However, vacuum freeze drying is approximately eight times more expensive than air drying and has a high operational cost which limits the use of this method to high-value products such as coffee and encapsulated aroma [28].

The chicken intestines was also dried with the same drying methods with the exception of IR drying due to issues with odour. Figure 18 shows the results obtained after drying the chicken intestines with the different drying methods.

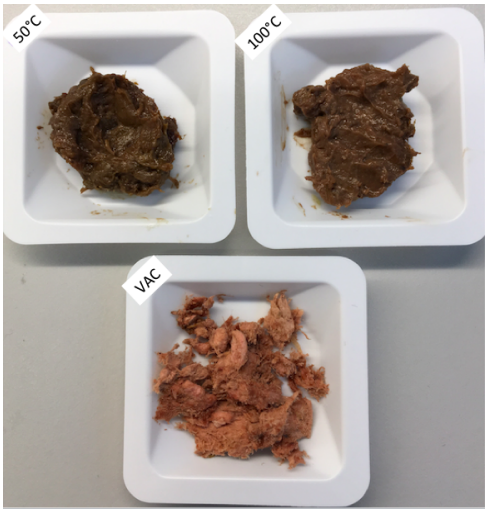


Figure 18: Chicken intestines dried with vacuum freeze dryer (VAC) and in oven at 50°C (50°C) and at 100°C (100°C).

A large difference in colour can be seen for the vacuum freeze dried sample compared to the other samples. As seen for the salmon intestines, the vacuum freeze dried sample was bright in colour and had a porous texture. The samples dried in the oven at 50°C did not completely dry, but was not able to be dried for more than 15 hours because the material was burnt. The sample dried at 100°C had a drying time of 3 hours, however the dried sample did not show the same properties as the salmon dried at the same temperature. The salmon intestines had crumbled into dried spheres which was not the case for the chicken intestines. The chicken intestines obtained paste-like properties. It can be seen that the chicken intestines either required a longer drying time or a higher temperature to achieve the same end result as the salmon, however the drying time could not be extended without burning the material which would reduce the quality drastically.

3.4.1 Drying processes

The drying processes were studied by weighing the material during each drying method. This was done to obtain an overview of the water removed over time. Drying at 50°C was conducted over 23 hours for the salmon intestines and for 15 hours for the chicken intestines. The time was reduced for the chicken intestines as the material was burnt after 15 hours. The progress of water removal in salmon intestines and chicken intestines is presented in Figure 19. The raw data are given in Appendix H.1.

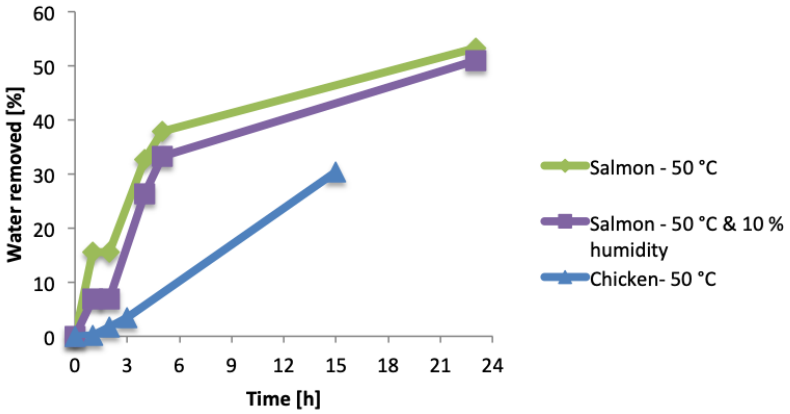


Figure 19: Water removed [%] from salmon and chicken intestines throughout drying at 50°C in oven. The salmon and chicken intestines were dried for 23 and 15 hours, respectively. The salmon intestines were dried in oven with zero and with 10 % humidity.

A rapid increase in water removed from the salmon intestines can be seen from the figure around 2 to 4 hours. The same pattern of water removed from the salmon intestines can also be seen for the two different ovens. It was seen that 10 % humidity did not have a large impact on the drying effect on the salmon intestines. The water removal from the chicken intestines was not as effective as for the salmon. The water content in the salmon and chicken intestines were around 65 % and 69 %, respectively. However, thermal treatment was conducted prior to drying resulting in almost 30 % and 13 % stickwater removed from salmon and chicken intestines, respectively. Thus, indicating that there are more water available in the chicken intestines after thermal treatment.

Both the salmon and the chicken intestines were dried for 3 hours at 100°C in the oven. Drying of salmon was in addition dried with both a oven with and without 10 % humidity. The water removal at 100°C is presented in Figure 20. The raw data are given in Appendix H.2.

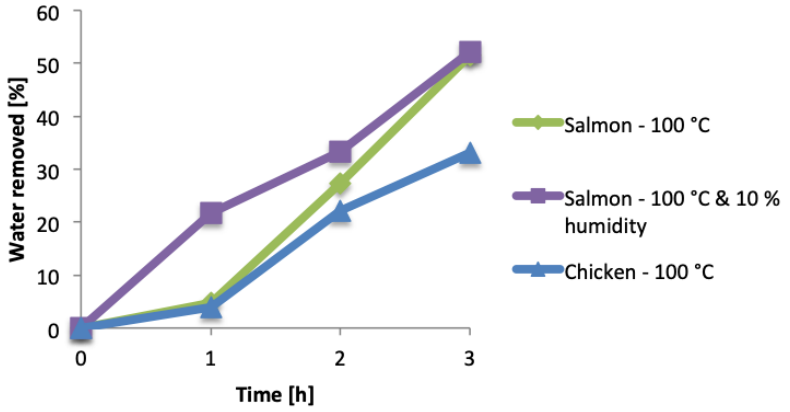


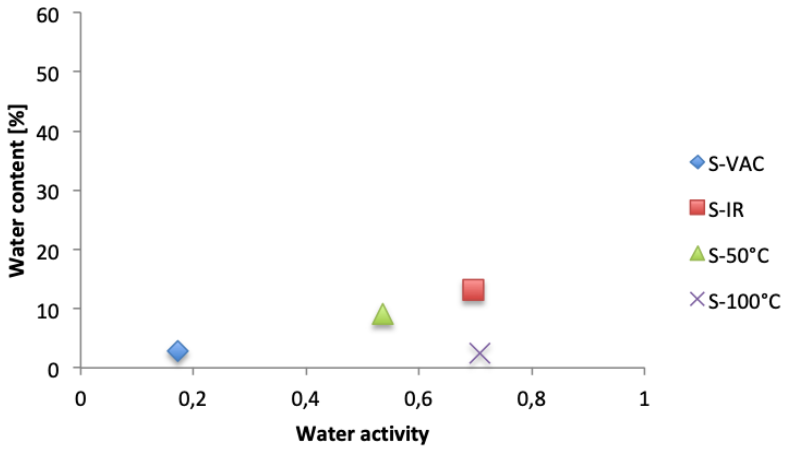
Figure 20: Water removed [%] during drying of salmon and chicken intestines at 100°C in oven. The salmon intestines were dried in oven with zero and with 10 % humidity.

The salmon intestines dried with the two different ovens showed a small difference in the progression of water removal. The sample of salmon intestines dried with 10 % humidity had a steeper increase in the percentage of water removed from the material compared to the oven without humidity. However, the amount of water removed after 3 hours was almost the same for both samples. It can therefore be concluded that the oven with humidity could decrease the drying time for drying at 100°C, but will not have a large impact for drying at 50°C. The oven with the humidity settings was not available for the drying of the chicken intestines as it was required to place the oven in a fume hood with good ventilation. The chicken intestines dried at 100°C without humidity had less water removed from the material compared to the salmon intestines.

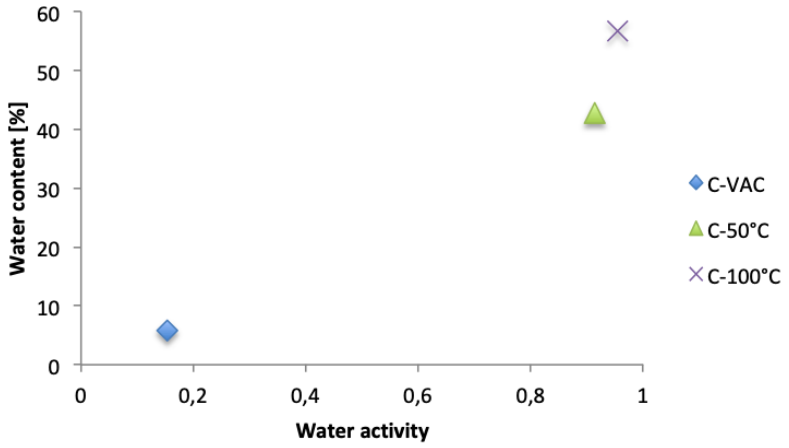
An effective removal of water can be seen for the drying at 100°C, yet a decrease in quality can be expected. This was analysed by studying the oxidation of lipids and proteins in the materials. Nonetheless, drying at 100°C decreases the drying time for both materials. The progress of water removal for the vacuum freeze drying was not recorded as this drying method required a closed environment during drying. The progression of water removal was also not recorded for the IR drying as this method only required 45 minutes of drying. The overall percentage of water removed during IR drying was around 47 %, which is less compared to the drying in oven. An overview with raw data for the IR drying are given in Appendix H.3.

3.4.2 Water activity and water content

Water plays a significant role in physical and biochemical changes occurring during drying of salmon and chicken intestines. The water activity and the water content of the dried materials were determined and the relationship between these parameters are plotted in Figure 20 **(a)** and **(b)** for salmon intestines and chicken intestines, respectively. The raw data for the water activity and water content of the dried materials are given in Appendix I and J, respectively.



(a) Salmon intestines



(b) Chicken intestines

Figure 20: Water activity plotted against water content in dried salmon intestines **(a)** and chicken intestines **(b)**. Samples are labeled with the drying methods (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

These plots can be compared to Figure 3, which shows the general rate of deterioration for foods as a function of a_w, and Figure 4, which shows the general water sorption isotherm of a typical food. Note that the water content in the dried salmon samples were all below 15 %, which indicate that all the methods acquired adequate drying of the salmon intestines. It also implies that these samples were more susceptible to lipid oxidation. The water activity had a larger variation where S-VAC had the lowest a_w, and S-IR and S-100°C had the largest a_w. The samples, except S-IR, were according to Figure 4, in region C of the diagram. This indicates that the water left in the samples was bounded to the material by strong chemical bonds. This makes the water difficult to remove. However, S-IR was on the boundary between region C and B which can be assumed to indicate that the sample contained both strongly bonded water and some water less firmly bound to the material, implying that the infrared drying was not as effective in removing the water from the salmon intestines. Thus, this sample was less vulnerable to lipid oxidation, but more subjected to enzymatic reactions. S-100°C and S-IR had high water activity making these samples more susceptible to growth of yeast and moulds compared to the other salmon samples.

The relation between a_w and water content in the dried chicken intestines varied with the different drying methods. C-50°C and C-100°C had a water content at 43 % and 57 %, respectively. C-VAC had a water content at 5 %, meaning that this samples can be found in region C of the water sorption isotherm. It can therefore be verified that the vacuum freeze drying of the chicken intestines was efficient. This was not the case for the oven dried samples, as both these samples were in region A of the isotherm. These samples contained free water which was only bound to the material by weak interactions. The free water can be easily removed, but was not removed during these drying methods. This shows that oven drying at these temperatures was not sufficient for proper drying of the chicken intestines.

C-VAC and S-VAC were low in both water activity and water content, which made these samples less susceptible to deterioration. The vacuum freeze drying resulted in highly porous products with small pores compared to microwave-dried products with larger number of small pores resulting in higher sorption of water according to Tsami (1998) [73]. C-50°C and C-100°C on the other hand were according to the deterioration diagram susceptible to growth of moulds and yeast. This was indeed seen during storage of C-50°C, and is discussed further in Section 3.5.

3.5 Storage experiment

3.5.1 Dried products

Salmon intestines

The dried salmon intestines after 30 days of storage is pictured in Figure 21. The cups are labeled with storage method on the right and the drying method on the left. The labelling of the samples is explained in detail in Appendix A.

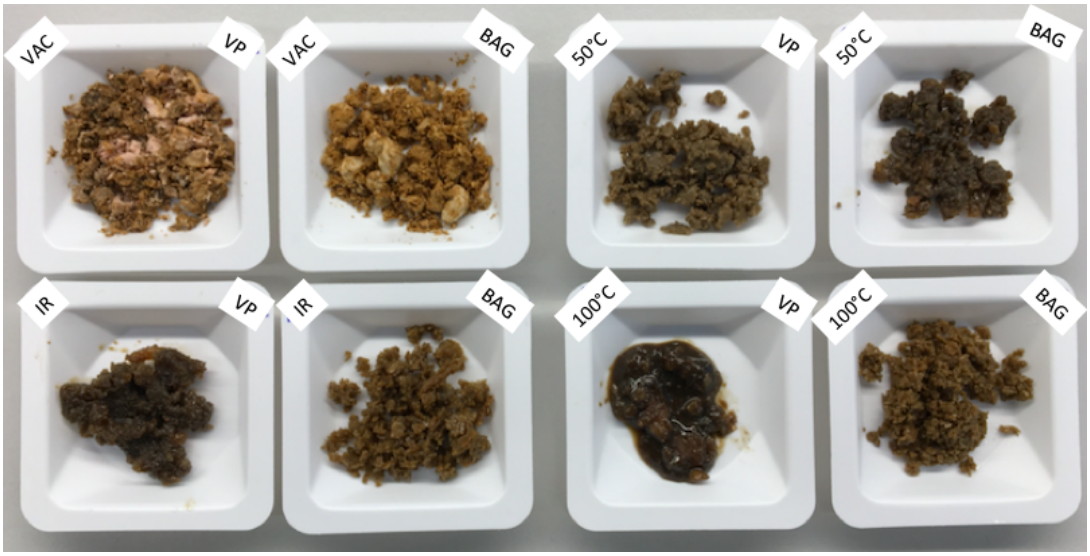


Figure 21: Dried salmon intestines after storage experiment over 30 days. The cups are labeled with storage method (VP = vacuum packed, BAG = plastic bag) on the right and the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C) on the left.

S-VAC, S-IR and S-100°C were light in colour after storage in the plastic bag. This was not the case for the S-50°C. This sample was darker in the plastic bag compared to the vacuum packed sample. The colour of all samples were measured and are presented and discussed further in Section 3.5.2. S-VP-100°C had a liquid consistency unlike the initial sample before storage (S-0-100°C) and the sample stored in the plastic bag (S-BAG-100°C). This could have been a result from the vacuum packing process as the this sample began to boil during reduction of pressure. This may have affected the quality of the sample, and is taken into account for the analysis of the other quality parameters.

Chicken intestines

The dried chicken intestines stored in vacuum packaging and in plastic bags are pictured in Figure 22. The cups are labeled with storage method on the right and the drying method on the left.

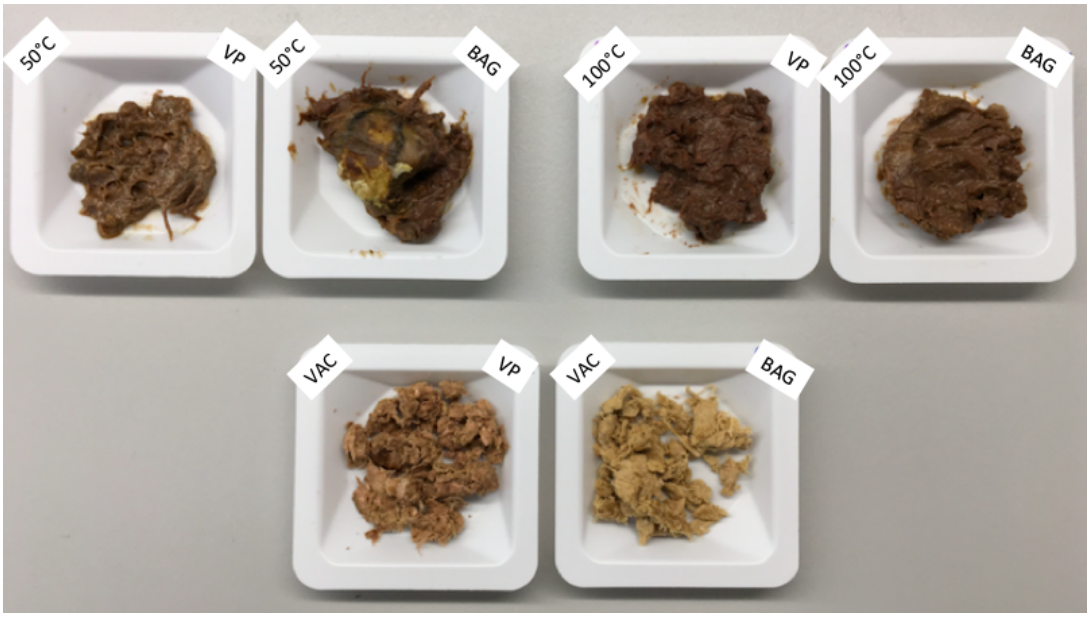


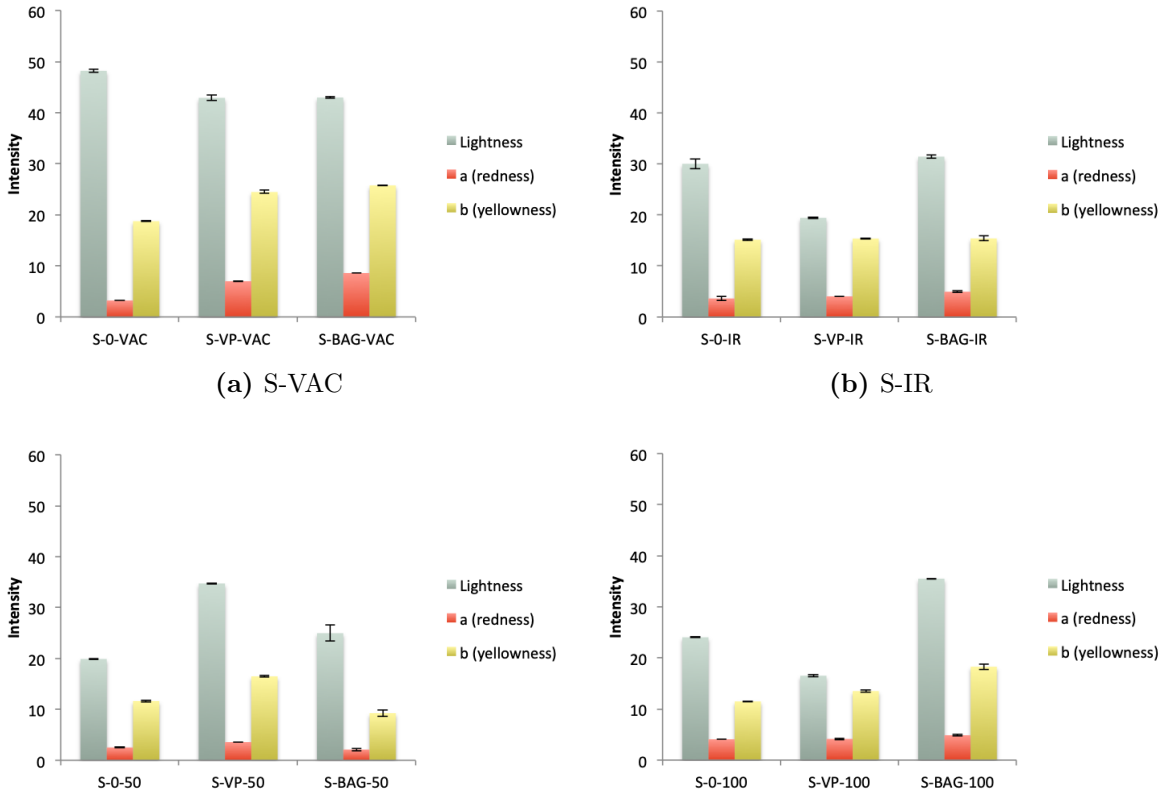
Figure 22: Dried chicken intestines after storage experiment over 30 days. The cups are labeled with storage method (VP = vacuum packed, BAG = plastic bag) on the right and the drying method (VAC = vacuum freeze dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C) on the left.

The same difference in colour in vacuum freeze dried salmon intestines was observed for the vacuum freeze dried chicken intestines as well. The material stored in a plastic bag was much lighter in colour compared to the vacuum packed intestines. The chicken intestines oven dried at 50°C and stored in plastic bag (C-BAG-50°C) had visible growth of mould and can be seen in Figure 22. Water activity for general mould growth is 0.8 [74] and the water activity for C-BAG-50°C was approximately 0.90.

3.5.2 Colour changes

Salmon intestines

The colour of the different dried salmon samples were measured using the Hunter L, a, b colour scale system. The lightness (L), redness (a) and yellowness (b) are given for each sample before storage and after storage in Figure 23. The raw data from the colour measurement are given in Appendix K. The labelling of the samples is explained in Appendix A.



(a) S-VAC **(b)** S-IR

(c) S-50°C **(d)** S-100°C

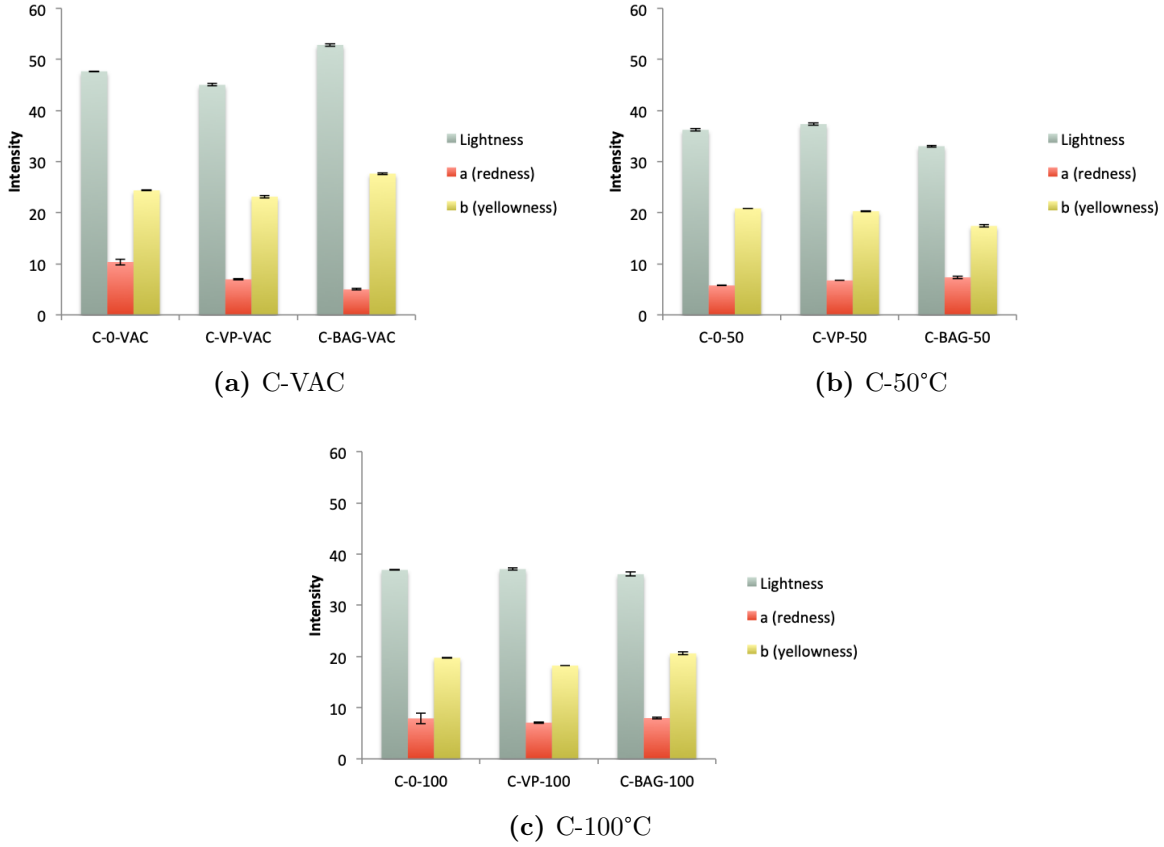
Figure 23: Colour measured for dried salmon intestines based on the Hunter L, a, b system, where L indicates lightness, a describes the redness and b indicates the yellowness of the product. The measurements are given as mean ± SD with n = 3. The samples are labeled with the storage method (VP = vacuum packed, BAG = plastic bag) and the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

S-VAC was the lightest of all the samples, and the lightness was only marginally reduced after the storage period. The redness and yellowness of the sample, however, increased during the storage period. S-50°C was darkest initially and had the least intensity of redness. The intensity of the parameters increased after storage, mostly for the vacuum packed samples compared to the sample stored in a plastic bag. This change could have been induced by the oxygen in the vacuum bag being trapped inside the bag causing

oxidation of proteins and lipids. Despite this, the opposite was observed for S-100°C and S-IR. The intensity of the parameters increased more for the stored sample in the plastic bag compared to the vacuum packed samples. This could have been a result of air being available in the plastic bag compared to the vacuum bag. It was also mentioned earlier that the vacuum packaging of S-100°C resulted in boiling of the material which could have influenced the colour change seen in Figure 23.

Chicken intestines

The lightness, redness and yellowness of different dried chicken intestines was measured based on the Hunter L, a, b colour scale and is presented in Figure 24. The raw data from the colour measurement are given in Appendix K. The labelling of the samples is explained in Appendix A.



(a) C-VAC

(b) C-50°C

(c) C-100°C

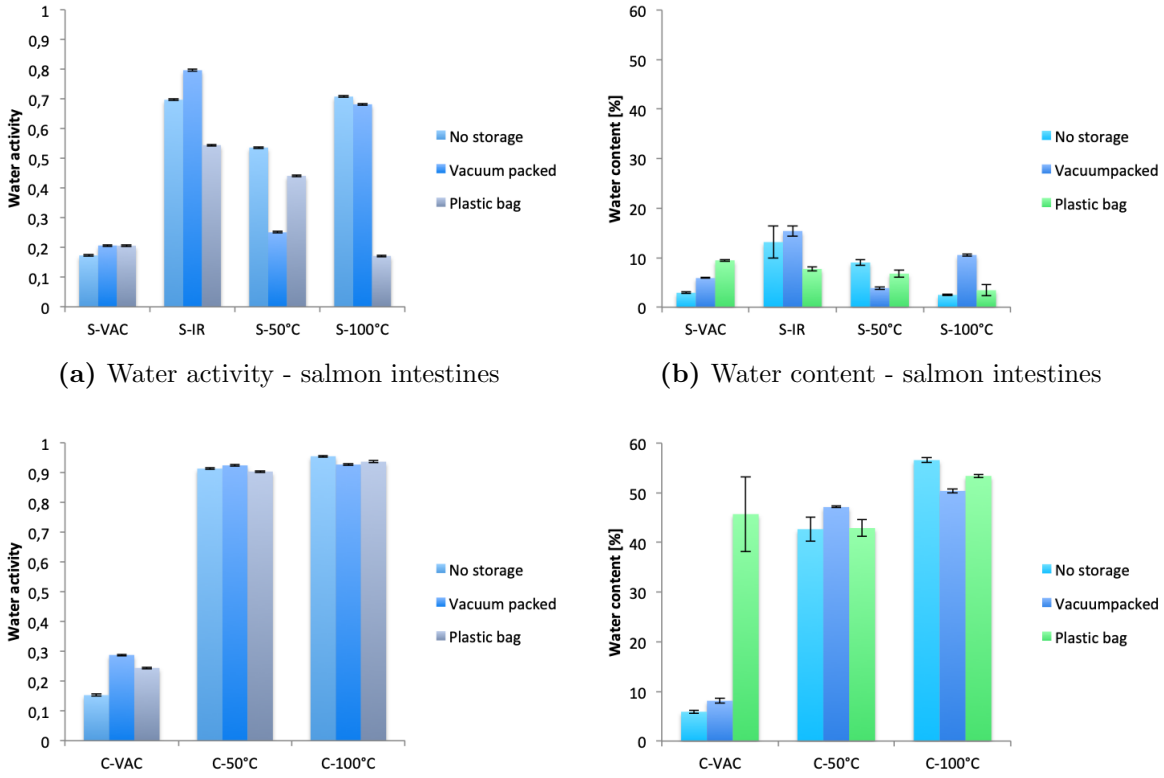
Figure 24: Colour measured for dried chicken intestines based on the Hunter L, a, b system, where **L** indicates lightness, **a** describes the redness and **b** indicates the yellowness of the product. The measurements are given as mean ± SD with n = 3. The samples are labeled with the storage method (VP = vacuum packed, BAG = plastic bag) and the drying method (VAC = vacuum freeze dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

The colour of C-VAC had a visible change and from Figure 22 it can be observed that the sample stored in a plastic bag became more yellow compared to the vacuum packed sample. This was not as clearly noted from the measured parameters.

From these colour parameters it can be assumed that the salmon intestines require better storage conditions than the chicken intestines, since a greater change in colour was observed in the salmon intestines. Relevant literature on colour changes in salmon and chicken intestines was not found. Modified atmosphere and use of inert gases, such as N_2 gas, could be a possible solution to reduce colour change and oxidation reactions occurring in the materials.

3.5.3 Water activity and water content

The water activity and the water content of different dried salmon intestines and chicken intestines before and after storage are presented in Figure 25. The raw data from the measurements of water activity are given in Appendix I and water content in Appendix J.



(a) Water activity - salmon intestines

(b) Water content - salmon intestines

(c) Water activity - chicken intestines

(d) Water content - chicken intestines

Figure 25: The water activity and water content of dried salmon intestines and chicken intestines for the different drying methods. The measurements are given as mean ± SD with n = 3. The samples are labeled with the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

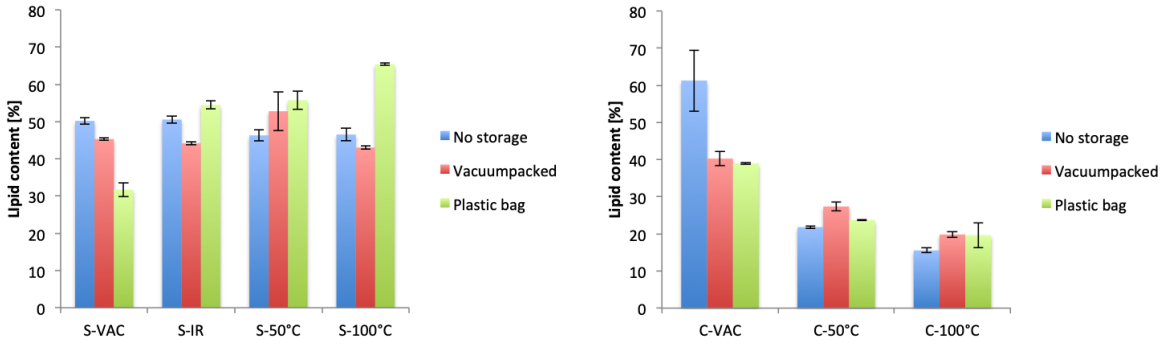
The water activity and water content increased after storage for S-VAC and S-IR. This could be the result of the porous structure formed in this samples after drying, causing the samples to re-hydrate easily compared to the oven dried samples [75]. The water content and a_w increased more for vacuum packed S-IR compared to plastic bag packed,

which was not expected. Variations in the water content and water activity was a result of samples being divided into several batches during the different drying methods.

The water content was measured at more than 40-50 % for the oven dried chicken intestines. This resulted in a_w around 0.9 which is sufficient for microbial growth. The results show that the oven drying of the chicken intestines were not suitable to obtain products with low water content (below 10 %) and low water activity (below 0.2). Neither the water content nor the activity increased or decreased significantly compared to the initial samples. This could be because these samples already had high a_w and water content, hence an increase or decrease would not have the same impact on the samples as the samples with low water content and low a_w. C-VAC, however, had the lowest water content and a_w but the water content increased for the sample stored in the plastic bag. Since vacuum freeze dried samples are known to obtain porous structure after drying, this increase in water content could be a result of easy re-hydration from the water available in the air inside the bag.

3.5.4 Lipid content

The lipid content was measured for the dried materials before and after storage, and is shown in Figure 26. The raw data are given in Appendix E.5.



(a) Salmon intestines **(b)** Chicken intestines

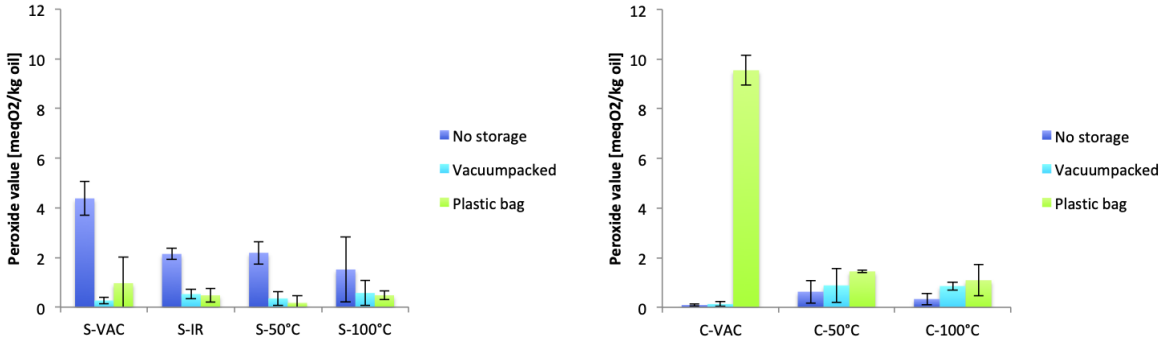
Figure 26: Percentage of lipid content in the dried salmon intestines (**a**) and in the dried chicken intestines (**b**) before and after storage. The lipid contents are given as mean ± SD with n = 3. The samples are labeled with the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

It was expected to observe a fairly similar lipid content in the samples treated with the same drying method. This was observed for the chicken intestines, however, C-VAC with no storage showed greater lipid content compared to the stored samples. The decrease could perhaps be a result of lipid degradation, although this must be further analysed. The values obtained from measuring lipid oxidation could give an indication of this hypothesis. The lipid content in the salmon intestines varied and could be attributed by the drying processes being separated into batches causing inconsistency in the water, lipid and protein content.

3.5.5 Lipid oxidation

3.5.5.1 Peroxide value

The peroxide value (PV) was determined for the samples and is presented in Figure 27 for both salmon intestines and chicken intestines. PV was measured in milliequivalent oxygen per kg oil. The raw data from this analysis are given in Appendix L.



(a) Salmon intestines **(b)** Chicken intestines

Figure 27: Peroxide value determined for dried salmon and chicken intestines. The peroxide values are given as mean ± SD with n = 3. The samples are labeled with the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

According to Huss (1988) [76] the acceptable limit for PV of crude fish oil is 7-8 meq O_2/kg oil. Refsgaard et al. (1998) [77] reported PV for fresh salmon oil to be around 0-2 meq O_2/kg oil. The initial PV for the salmon samples were higher than for fresh salmon oil, because the oil was being transferred to the surface during the drying processes resulting in higher level of oxidation. However, the value was still within acceptable limit for PV of crude fish oil. It was observed from the figure that the vacuum freeze drying of the salmon intestines had the highest amount of lipid hydroperoxides compared to the other drying methods of this specific material. These samples had low water content, making them prone to lipid oxidation. The high porosity in the material and the large surface area of matrix dried by the vacuum freezer could initiate diffusion of oxygen from the air and into the inner parts of the matrix. This will then accelerate the oxidation of lipids in the material [78]. The vacuum freeze drying is also conducted at a lower temperature compared to the other methods, which might retain lipases and other lipolytic enzymes in the material.

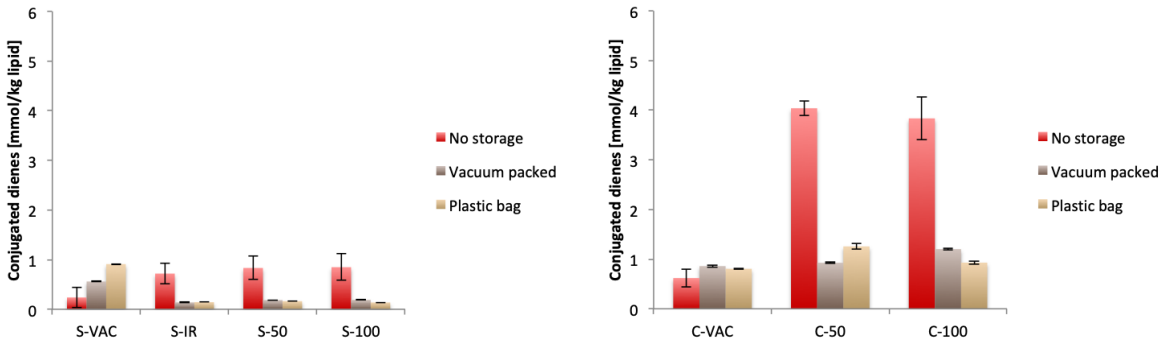
On the other side, C-VAC was not the product with the highest peroxide value for the chicken intestines. The peroxide value was rather low for this material and from the statistical analysis it was found that PV was not significantly different for the different drying methods of the chicken intestines. This implies that the type of drying methods conducted in this thesis did not have a significant impact on the peroxide value of the chicken intestines. However, a significant difference ($p < 0.05$) was found in the peroxide values between the different drying methods of the salmon intestines. This was expected

as the salmon contains long chain PUFAs and the oxidation of these are therefore more dependent of the drying methods.

The peroxide values decreased for the salmon intestines after storage in both vacuum and in plastic bag. The decrease could be a result of the lipid oxidation entering the later stages where breakdown of the primary oxidation products is greater than the formation. The same was observed for the chicken intestines, however, C-VAC stored in plastic bag had a much bigger PV compared to the other samples. The large amount was not expected, but could have been induced by the porous structure and the air in the plastic bag. The peroxide value increased for the stored chicken intestines unlike the salmon intestines. This could be an indication of low oxidation rate compared to the rate in the salmon intestines.

3.5.5.2 Conjugated dienes

The conjugated dienes are useful to monitor the early stages of the oxidation of the lipids. The conjugated dienes was measured and is given in Figure 28 for the dried salmon and chicken intestines. The raw data from the CD measurement are given in Appendix M.



(a) Salmon intestines **(b)** Chicken intestines

Figure 28: Conjugated dienes measured in dried salmon and chicken intestines. The measurements are given as mean ± SD with n = 3. The samples are labeled with the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

It was expected that CD would be more stable than the PV. The amount of conjugated dienes decreased after storage for all the salmon samples and chicken samples, except S-VAC and C-VAC. This is caused by breakage in the chains containing the conjugated dienes. This could indicate that the materials reached the later stages of the oxidation after 30 days, thus presumably indicating higher amounts of secondary lipid oxidation products. This was not seen in S-VAC and C-VAC as the CD increased after storage. This was unexpected as a porous structure and low temperature drying should have promoted the lipid oxidation, hence it was expected to observe late stages of oxidation. It was found from statistical analysis that there was a statistical significant difference (p

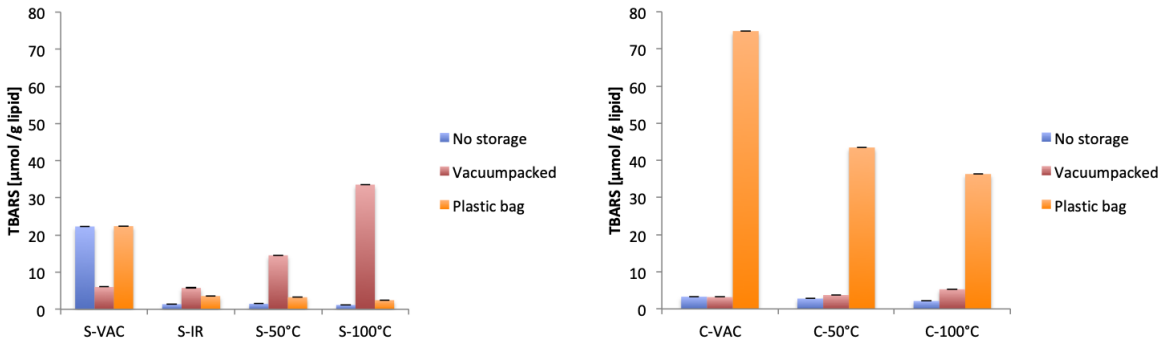
< 0.05) in the drying methods and the formation of conjugated dienes only for the salmon intestines and not for the chicken intestines. This is, as mentioned for the peroxide value, an indication that the long chain PUFAs are more prone to oxidation thus the drying method will have a significant effect on the level of oxidation.

It was observed larger amounts of conjugated dienes in the chicken intestines in total compared to the salmon intestines. It was assumed that the oxidation of lipids in the salmon intestines was rapid compared to in the chicken intestines. This would indicate that the chicken intestines were in the early stages of the oxidation whereas the lipids in the salmon intestines had reached a stage where breakdown of primary oxidation product was larger than formation of these products.

As both conjugated dienes and peroxide value determine the formation of primary lipid oxidation products, a correlation between the two parameters was calculated for all the samples. It was expected to see a correlation for all samples as reported by Wanasundara et al. (1995) [79] where PV and CD showed almost perfect correlation. The correlation coefficients are given in Appendix N. The plots were made for each material and all samples were included regardless of drying and storage method. The correlation coefficient was calculated to be 0.33 and -0.24 for the salmon and chicken intestines, respectively. Hence, only weak correlations were found and was not in agreement with Wanasundara et al. (1995) [79]. The reason for this could be the difference in each sample based on drying and storage, hence more tests should be evaluated to investigate the correlation.

3.5.5.3 TBARS

TBA values of dried salmon intestines and chicken intestines were measured using the TBARS assay. The results are presented in Figure 29. Standard curve and the raw data from this assay are given in Appendix O.



(a) Salmon intestines **(b)** Chicken intestines

Figure 29: TBA value of dried salmon and chicken intestines given in µmol TBARS/g lipid. The TBA values are given as mean ± SD with n = 3. The samples are labeled with the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

Amount of malondialdehyde (MDA), generated from decomposition of primary lipid ox-

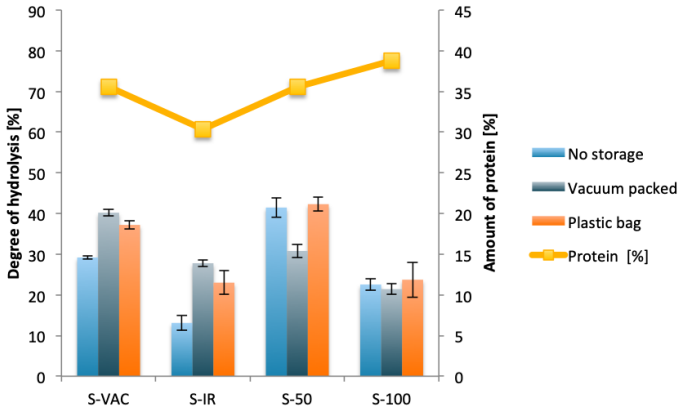
idation, is measured from the TBA assay [80]. Leung et al. (2018) [81] recorded a MDA concentration of about 10 µg/g lipid of raw untreated salmon oil, which corresponds to about 0.14 µmol/g lipid. This is relatively low compared to the results obtained from the experiment conducted in this thesis. The salmon intestines which were not stored, with the exception of vacuum freeze dried sample, had the lowest concentrations. The large TBA value of the vacuum freeze dried salmon could be attributed to the porous structure which is relatively more prone to take up oxygen, which initiates a more rapid oxidation compared to the other dried samples. It was also observed larger TBA values for the vacuum packed salmon samples compared to the samples stored in plastic bags. The plastic bags were sealed, however, air was left in the bag unlike the vacuum packed samples. The available air will therefore contribute to the rapid lipid oxidation. The results showed the opposite of what was expected, however it was reported by Cyprian et al.(2015) [82] that the oxidation products such as MDA, was higher at drying onset but reduced as the drying progressed. This study linked the reduction in TBA value in fish muscle to oxidative interactions with peptides and acid amines which results in formation of tertiary oxidative products [83]. Hence, this could explain the low concentrations in the plastic bags.

High concentrations of chicken intestines stored in plastic bags for all the different drying methods were observed. The air in the plastic bag could be the accelerating factor increasing the concentration. However, the large difference between TBA value of the chicken intestines and salmon intestines could be influenced by the moisture content in the samples. The salmon intestines contained water content between 2-10 % moisture which make these samples more prone to lipid oxidation compared to the chicken intestines with 40-50 % moisture content. C-VAC had the largest fat content (about 60 %) compared to the rest of the chicken samples (below 20 %). This could be the reason for C-VAC having the largest TBA value.

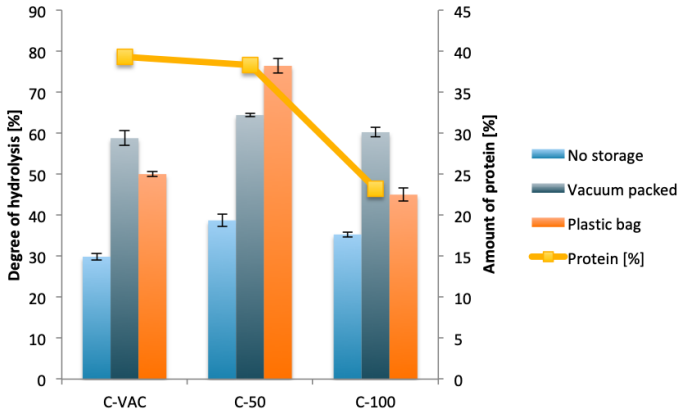
A statistical analysis between the TBA value and the drying methods showed statistical significant difference in the salmon intestines ($p < 0.05$), however a significant difference was not found for the chicken intestines. The reason for this difference was perhaps caused by the difference in the fatty acid composition, where the salmon intestines had an abundant amount of long chain PUFAs compared to the chicken intestines. PUFAs are more prone to oxidation, hence the different drying methods resulted in statistical difference in the level of oxidation for the salmon intestines.

3.5.6 Protein content and degree of hydrolysis

The total protein content was determined for the dried samples which were not stored. The protein content could therefore vary for the samples with storage compared to the initial samples, however this was not studied. The degree of hydrolysis of the proteins were also measured and are presented together with the protein content in Figure 30. The raw data from the total protein content in the dried samples are presented in Appendix C.2, and raw data from degree of hydrolysis assay are given in Appendix P.



(a) Salmon intestines



(b) Chicken intestines

Figure 30: Degree of hydrolysis (DH) of dried **(a)** salmon intestines and **(b)** chicken intestines both initial samples and stored samples. Protein content was measured for samples with no storage time. Protein content and DH are given as mean ± SD with n = 3. The samples are labeled with the drying method (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C, 100°C = oven dried at 100°C).

The protein content increased in the dried materials from about 13 % in the raw salmon intestines to about 30-40 % in the dried salmon intestines. The same increase was seen in the chicken intestines (about 12 % in the raw material and about 25-40 % in the dried material). This is due to removal of the moisture, resulting in more concentrated samples with proteins. The amount, however, varied for the different drying methods in both material. As seen from Figure 30, S-IR had the lowest amount and S-100°C had

the largest amount of total proteins. This was reflected in the water content determined, where S-100 had one of the lowest water content and S-IR had the largest water content of the salmon samples. Less water indicates more concentrated samples, thus greater protein content. The same was observed for the chicken intestines where C-VAC had a water content of about 5.9 % and C-100 contained almost 57 % water. The water content could also influence the total protein content since the water could contain small amounts of soluble proteins.

The degree of hydrolysis was measured to determine the degradation of proteins in the samples after drying. This information could be useful for hydrolysis processes, where the material is converted to hydrolysates with high protein content [84]. Statistical significant difference ($p < 0.05$) was found for DH dried with different drying method for both materials, indicating that the drying methods had a significantly different effect on the degree of hydrolysis. DH was determined to be lower for the salmon intestines when compared with the chicken intestines. This could be caused by the difference in water content, as C-50°C and C-100°C had a water content around 50 %. This could induce the degradation of the proteins resulting in higher DH. S-IR without storage had the lowest DH. This could be because of the low protein content in S-IR.

It can be observed that the DH increased after the storage period. However, it can be seen that the increase was dependent of the drying method as S-VAC, S-IR and S-50°C had a greater increase compared to S-100°C. The same was noted for chicken intestines where C-VAC and C-50°C had a greater increase compared to C-100°C. The drying at 100°C can inactive the enzymes in the materials which will reduce the degradation of proteins. Drying at 50°C will not inactive the enzymes, but induce the enzyme activity which is seen as DH had the greatest increase for the drying in oven at 50°C. The results after storage of the materials in either vacuum packed bags or plastic bags varied for the samples and did not show a specific trend to explain the results obtained.

There were some complications with the Kjeldahl method and the method had to be carried out three times. Thus, the total amino acid composition of one sample (C-VAC) was determined to verify the results obtained from the Kjeldahl method. The mean percentage of total amino acids in vacuum freeze dried chicken intestines, including the hydroxyproline content, was **20.0 ± 7.6 %**. The results from the total amino acid composition of one of the triplicates is given in Appendix Q and the results from the hydroxproline assay is given in Appendix R. The total amino acid content (TAA) should equal the total protein content, however, TAA was 19 % lower than the the protein content determined by the Kjeldahl method. This could be because TAA does not account for the following amino acids: tryptophan, proline and cysteine. Small amounts of urea may also be found in the chicken intestines which could add up to the total nitrogen content obtained from the Kjeldahl method. However, even with the amino acids not determined in the HPLC and the urea in the material, the difference in total protein content was too large, thus it can be concluded that the determination of total amino acid content was not adequate for this particular material.

3.6 Thermal properties of dried rest raw material

The approximate glass transition temperatures, melting energies and melting peaks for dried salmon intestines and chicken intestines were determined using DSC. The DSC curve for the dried salmon intestines (Figure 31) was used for determining the nature of glass transition. The protein-water complexes can show glass transition in a wide temperature range (−186°C to 150°C) depending on the moisture content. Triglycerides, which contain omega-3 PUFAs, show glass transition at ultra low temperatures. Pure triglycerides showed glass transition at −110°C [85], but the interactions with proteins and other components of salmon and chicken intestines were not investigated in this thesis. Thus, the use of DSC curve of the raw material will help separate these two types of glass transitions (lipids and proteins).

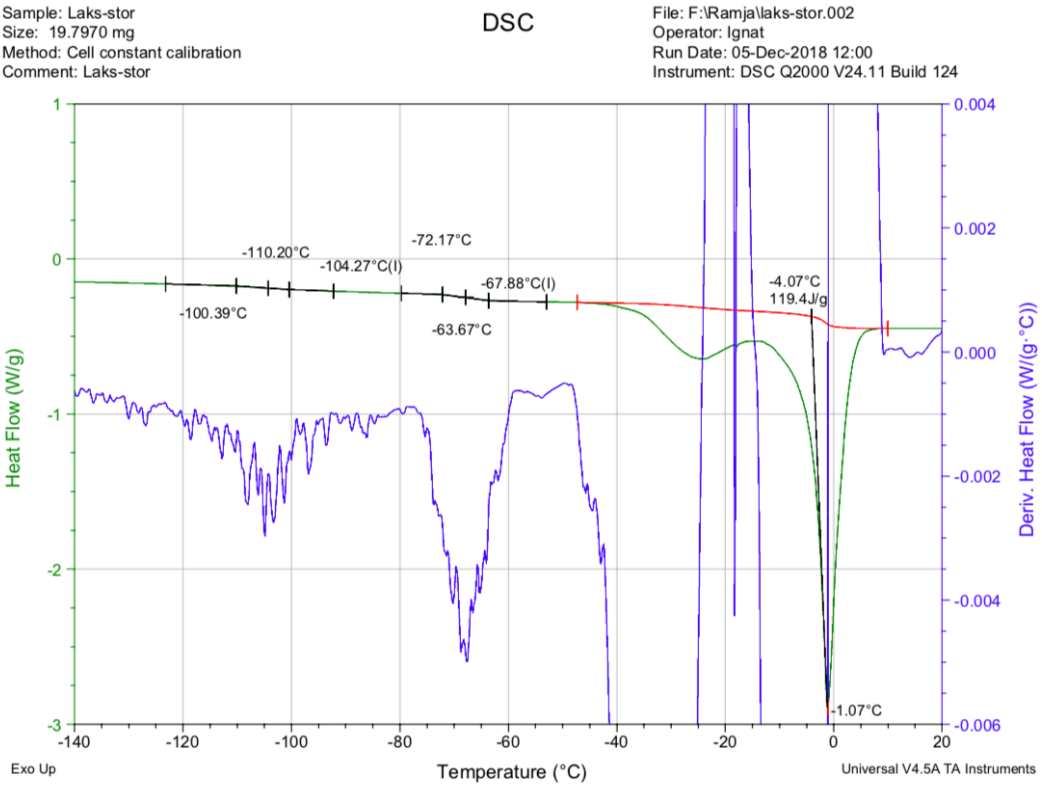


Figure 31: DSC curve of the salmon intestines including glass transition temperature (T_g) melting energy and melting peak.

The discussion on the glass transition uses the inflection temperature as T_g, as mentioned in the method chapter (Section 2.7.12). The raw salmon intestines showed two glass transition events in the curve. The first T_g was determined to be at approximately −104.27°C and the second at approximately −67.88°C. According to Tolstorebrov (2014) [85] the T_g of the triacylglycerides containing EPA and DHA was −111.78°C and −110.39°C, respectively. Thus, the high temperature glass transition (−67.88°C) can be attributed to protein-water interaction. The glass transition for salmon was found to be at −76.5°C in previous research conducted on salmon muscles by Tolstorebrov et al. (2013) [86]. Thus,

the low temperature glass transition (−104.27°C) found, was related to omega-3 fatty acids. The melting energy determined was approximately 119.4 J/g for the raw salmon intestines. The water content in the salmon intestines was determined to be around 65 %, which could be the reason for the large melting energy required. A summary of the values determined from the DSC analysis of the dried materials and S-RAW is given in Table 10. DSC curves of the dried salmon and chicken intestines are presented in Appendix S. The DSC curve of dried samples showed melting peak of lipids which crystallises in the temperature range from +5°C and −45°C. The glass transition of lipids were also detected for most of the samples. This indicates that the dried salmon intestines contained freezable fractions of lipids (which crystallises) and unfreezable fractions (which showed glass transition). This assumption was in agreement with the data presented by Tolstorebrov (2014) [85].

Table 10: Glass transition temperature, type of glass transition, melting energy and melting peaks determined from the DSC curves of the salmon intestines and the dried salmon (S) and chicken (C) intestines. S-RAW indicates the salmon raw material. The rest of the samples are labeled with the drying methods (VAC = vacuum freeze drying, IR = infrared drying, 50°C = oven drying at 50°C, 100°C = oven drying at 100°C).

Sample	**Type of T_g**	**Glass transition temperature [°C]**			**Melting energy [J/g]**	**Melting peak [°C]**
		Onset	**Inflection**	**End**		
S-RAW	lipid	−110.20	−104.27	−100.39	119.4	−1.07
	protein	−72.17	−67.88	−63.67		
S-VAC	lipid	−94.94	−86.07	−74.04	17.07	−20.11
S-IR	lipid	−102.80	−95.84	−91.04	22.21	−25.58
	protein	−56.90	−50.18	−49.00		
S-50°C	lipid	−107.36	−100.54	−93.72	16.78	−22.42
	protein	−66.01	−62.56	−57.44		
S-100°C	lipid	−107.77	−100.07	−93.13	19.79	−23.84
C-VAC	lipid	−99.90	−89.90	−77.92	19.73	−5.57
C-50°C	lipid	−74.10	−70.46	−65.45	120.8	−7.29
C-100°C	lipid	−64.13	−59.60	−52.41	72.68	−9.25

S-IR and S-50°C showed two T_g in their respective curves. The low temperature T_g on both curves were in the same range as for the low temperature T_g for S-RAW. However, the inflection points were slightly higher, indicating that the lipids were probably bound to proteins or other components in the material. The same pattern was observed for the high temperature T_g, which represent the structural change in the proteins. High temperature is necessary for separation of lipids from proteins, due to this S-IR and S-50°C showed visible glass transition related to both protein and lipids. The glass transition related to protein was detected at higher temperatures which can be explained by the low water

content (approx. 13 % for S-IR and approx. 9 % for S-50°C) and protein denaturation. S-100°C had one T_g at −100.07°C. This sample had very low moisture content (approx. 2.5 %) and the glass transition related to proteins could possibly be out of range or integrated in the melting peaks of lipids. S-VAC samples had very low moisture content (approx. 2.9 %) and high lipid fraction (approx. 50 %), and at the same time the low temperature glass transition was observed at −86.07°C which is high for the omega-3 fatty acids and low for proteins considering the low moisture content. For instance, Atlantic salmon muscle with moisture content at 5.7 % showed glass transition at +44.6°C according to Tolstorebrov et al. (2013) [86]. This can be explained by the vacuum freeze drying providing connections between lipids and proteins. Such structural changes influences the thermal properties. The structure is porous and perhaps lipids are absorbed on the surface of the material. The melting energy of lipids was low when compared with S-100°C. The lipid content in S-VAC was higher when compared with S-100°C, while the water content was almost the same. The low melting peak could therefore be a result of absorption of lipids on the surface because of the porous structure of S-VAC.

The vacuum freeze dried chicken intestines had a T_g at approximately −89°C, which is lower when compared to S-RAW. This could be interpreted as other lipid types, or less omega-3 lipids, found in the chicken intestines causing lower T_g. C-50°C and C-100°C had very high water content (almost 50 %), thus DSC curve showed quite big melting peak of water. These samples showed only one T_g at −70.54°C and −59.6°C, respectively. This glass transition could be mostly attributed by glass transitions of proteins, when the food contained high amounts of moisture. The relatively big difference between the results could be explained by protein denaturation at 100°C drying.

As a conclusion the high temperature drying resulted in good fat separation for salmon intestines. At the same time vacuum freeze drying methods, which is considered to be the most gentle drying method, resulted in low level separation of fat from proteins and possible absorption of lipids on surface of the material due to high porosity and low moisture content.

4 Conclusion

The main objective of this work has been to analyse the quality and stability of dried Atlantic salmon intestines and Norwegian chicken intestines, and evaluate if drying was a suitable preservation method for these types of materials. The study also included characterisation of the materials and analysis of effects of thermal treatment on the separation of fractions from the materials.

Seasonal variations in the chemical composition of the intestines were observed. The fat content decreased significantly from fall to spring for both animals. This has to be taken into account when determining optimal temperature of the thermal treatment. Thermal treatment above 65°C showed increasing fat separation for both materials, however the lipid content of the fat-reduced sediment increased as well due to separation of stickwater. 50°C was chosen and used as extraction temperature based on the relationship between lipid content of the fat-reduced sediment and the amount of fat separated.

The fatty acid composition of the oil extracted from the raw materials showed that the salmon intestines contained larger amounts of PUFAs (approx. 44 %) compared to the chicken intestines (approx. 25 %). Around 57 % and 13 % of the PUFAs were omega-3 fatty acids in the salmon and chicken intestines, respectively.

Vacuum freeze drying, infrared drying and oven drying at two different temperatures were conducted, and powder-like properties of the salmon intestines were obtained. The infrared drying of the salmon intestines was less effective compared to the other methods based on the water removal. Infrared drying was not conducted on the chicken intestines. The oven drying of the chicken intestines showed significant difference as these samples had water content above 40-50 %. This could presumably be result of shorter drying time due to the material being burnt. These samples obtained paste-like properties and based on the water sorption isotherm were highly susceptible to deterioration. Deterioration was observed in the level of lipid oxidation and degree of hydrolysis of the stored chicken samples, which were larger than the stored salmon intestines. Visible growth of mould was also observed for the oven dried chicken intestines stored in plastic bag. Significant differences in the oxidation level between the packaging methods was not found, except for the level of TBARS where the plastic bag stored chicken had more than ten times the TBA value compared to the vacuum packed samples.

The Kjeldahl method showed correlation between the protein content and the water content of the dried materials. Total amino acid composition assay was found not to be a useful method for measuring the protein content of the vacuum freeze dried chicken intestines.

DSC analysis on the glass transition temperature showed that high temperature drying separated the fat better than the vacuum freeze drying method. Absorption of lipids on the surface was observed for the vacuum freeze dried materials, making these samples more perishable.

Thermal treatments before drying have shown a large impact on the stability of the dried products. The optimal temperature of the thermal treatment was dependent on

the enzymes, microbes, fatty acid composition and the quality of the fractions separated. Oven drying at 50°C and 100°C was not suitable for the chicken intestines and other methods should be evaluated for this particular material. Significant differences in the oxidation rate for the two different packaging methods was observed for the chicken intestines and could be due to the high water content in these samples.

5 Further work

The work in this thesis have shown that there are possibilities to maintain good quality of dried rest raw material by using drying as a preservation method. However, drying limits the materials to only be used as pet food and are not suitable for human consumption. Hence, the applicability of the dried raw materials for human consumption should be further researched.

It is possible to implement enzymatic hydrolysis instead of thermal treatment for separation of the different fractions. A study utilising enzymatic hydrolysis with commercial enzymes should be investigated where the quality and yield of oil and protein fractions can be compared to the results in this thesis. Although earlier studies on herring have shown better results with thermal treatment, further analysis on salmon and chicken intestines is required. A better separation technique of the oil should be looked into as this may have affected the results from the thermal treatment. The quality of the oil separated should be investigated and the crude oil must be converted to refined oil to produce omega-3 rich oil acceptable for human consumption.

The feasibility and costs of the different drying methods should be studied. Hot air drying of the chicken intestines was shown not to be promising based on the results obtained from this thesis. The thermal treatment of the chicken intestines should therefore be tested out at higher temperatures to separate more fat as layers of fat was observed after oven drying of the material. Better drying methods should also be investigated for this material. Results obtained for the two different storage methods varied for the chicken intestines and could be due to large water content. The salmon samples did not show great variations for the different storage methods, hence further investigation in this area is not recommended for this particular material.

References

[1] Food and Agriculture Organisation. (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals.* (FAO report). Retrieved from: http://www.fao.org/3/i9540en/I9540EN.pdf
(retrieval date: January 22, 2019)

[2] PwC Seafood. (2018). *Økt foredling av sjømat og restråstoff i Norge - en analyse av muligheter, barrierer og lønnsomhet* [Increased processing of seafood and residual raw materials in Norway - An analysis of opportunities, barriers and profitability] (PwC report 01/2018). Retrieved from: https://www.innovasjonnorge.no/contentassets/817ae6b6cafd411d9d4bc15233977612/sluttrapport---okt-foredling-av-sjomat-og-restrastoff-i-norge.pdf
(retrieval date: January 22, 2019) *(in Norwegian)*

[3] Rustad T. (2002). Utilization of marine by-product. *Electronic Journal of Environmental, Agricultural and Food Chemistry, ISSN: 1579-4377*, pp. 458-463.

[4] Arvanitoyannis I. S. & Kassaveti A. (2008). Fish industry waste: treatments, environmental impacts, current and potential uses. *International Journal of Food Science and Technology, Vol.43*(4), pp. 726-745.

[5] Kystmiljø (2018). Om oss [About us]. Retrieved from: https://www.kmiljo.no/om-oss (retrieval date: January 22, 2019) *(in Norwegian)*

[6] Lindberg, D., Aaby, K., Borge, G. I. A., Haugen, J., Nilsson, A., Rødbotten, R. & Sahlstrøm, S. (2016). *Kartlegging av restråstoff fra jordbruket* [Mapping of rest raw materials from agriculture] (Report 67/2016). Retrieved from: https://brage.bibsys.no/xmlui/bitstream/handle/11250/2428846/Rapport%2B67-2016.pdf?sequence=1 (retrieval date: January 22, 2019) *(in Norwegian)*

[7] Chalamaiah, M., Dinesh kumar, B., Hemalatha, R., Jyothirmayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chemistry 135*(4), pp. 3020–3038.

[8] Norwegian Food Safety Authority. (2016, February 15). Mattilsynet har kontrollert håndtering av animalske biprodukter i villfiskmottak: Håndteringen må bedres [The Norwegian Food Safety Authority has controlled the handling of animal by-products in wild fish reception: Handling must be improved]. Retrieved from: https://www.mattilsynet.no/fisk_og_akvakultur/animalske_biprodukter/mattilsynet_har_kontrollert_haandtering_av_animalske_biprodukter_i_

villfiskmottak_haandteringen_maa_bedres.21742 (retrieval date: January 25, 2019) *(in Norwegian)*

[9] Rustad, T., Storrø, I., & Slizyte, R. (2011). Possibilities for the utilisation of marine by-products. *International Journal of Food Science & Technology, 46*(10), pp. 2001-2014.

[10] Hordur G. Kristinsson & Barbara A. Rasco (2000) Fish Protein Hydrolysates: Production, Biochemical, and Functional Properties. *Critical Reviews in Food Science and Nutrition, 40*(1), pp. 43-81.

[11] Coultate, T. (2016). *Food. The Chemistry of its Components* (6th ed., pp. 107-171). London: The Royal Society of Chemistry.

[12] Kenar, J. A., Moser, B. R., & List, G. R. (2017). Naturally Occurring Fatty Acids: Source, Chemistry, and Uses. In *Fatty Acids*, pp. 23-82. AOCS Press.

[13] Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S., & Linder, M. (2012). Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. *Trends in Food Science & Technology, 25*(1), pp. 24-33.

[14] Wall, R., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2010). Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutrition reviews, 68*(5), pp. 280-289.

[15] Wu, T. H., & Bechtel, P. J. (2008). Salmon by-product storage and oil extraction. *Food Chemistry, 111*(4), pp. 868-871.

[16] Ladikos, D., & Lougovois, V. (1990). Lipid oxidation in muscle foods: A review. *Food chemistry, 35*(4), pp. 295-314.

[17] Robards, K., Kerr, A. F., & Patsalides, E. (1988). Rancidity and its measurement in edible oils and snack foods. A review. *Analyst, 113*(2), pp. 213-224.

[18] Shahidi, F. & Wanasundara, U. N. (2002). Methods for measuring oxidative rancidity in fats and oils. *Food lipids: Chemistry, nutrition and biotechnology*, pp. 387-403.

[19] Papastergiadis, A., Mubiru, E., Van Langenhove, H., & De Meulenaer, B. (2012). Malondialdehyde measurement in oxidized foods: evaluation of the spectrophotometric thiobarbituric acid reactive substances (TBARS) test in various foods. *Journal of agricultural and food chemistry, 60*(38), pp. 9589-9594.

[20] Nofima. (2019). Rest raw material. Retrieved from https://nofima.no/en/forskningsomrade/marine-biotechnology/rest-raw-material/ (retrieval date: February 13, 2019)

[21] Food and Agriculture Organization. (1986). The production of fish meal and oil. FAO Fisheries Technical Paper No. 142. FAO: Rome, Italy. Retrieved from http://www.fao.org/docrep/003/x6899e/x6899e04.htm#3.3.2%20Packaged%20fishmeal%20plants (retrieval date: February 13, 2019)

[22] Bekkevold, S., & Olafsen, T. (2007). Råvarer med muligheter [Raw material with possibilities]. *Trondheim: RUBIN AS*, pp. 68-69.

[23] Baker, C. G. (Ed.). (1997). *Industrial drying of foods*. Springer Science & Business Media.

[24] Brennan, J. (2012). *Food Processing Handbook*. Weinheim, Germany: Wiley-VCH Verlag GmbH & KGaA, pp. 91-126.

[25] Lynum, L. (2011). *Konserveringsmetoder - og kjemiske reaksjoner ved tilberedning og lagring av mat* [Preservation methods - and chemical reactions in preparation and storage of food]. Trondheim: Tapir akademisk forlag.

[26] Rao M. A., Rizvi, S. S. H. & Datta, A. K. (1994). Thermal Properties of Food, In: Sweat, V. E. (Ed.) *Engineering Properties of Food*. (2nd edition, pp. 99-135). New York: Marcel Dekker, Inc.

[27] Bhandari, B. R., & Howes, T. (1999). Implication of glass transition for the drying and stability of dried foods. *Journal of Food Engineering, 40*(1-2), pp. 71-79.

[28] Bhandari, B. (2013). Handbook of food powders : Processes and properties (Vol. Number 255, Woodhead Publishing in food science, technology, and nutrition ;). Philadelphia, PA.

[29] Balasubramanian, S., Devi, A., Singh, K. K., Bosco, S. D., & Mohite, A. M. (2016). Application of glass transition in food processing. *Critical reviews in food science and nutrition, 56(*6), pp. 919-936.

[30] Troller J. & Chrisitan J. H. B. (1978). *Water activity and food*. New York: Acedemic Press, pp. 1-47.

[31] Labuza, T. P. (1980). *The effect of water activity on reaction kinetics of food deterioration. Food Technol, 34*(4), pp. 36-41.

[32] Rockland, L. B., & Stewart, G. F. (Eds.). (2013). *Water activity: influences on food quality: a treatise on the influence of bound and free water on the quality and stability of foods and other natural products*. Academic Press, p. 4.

[33] Roos, Y. H. (1993). Water activity and physical state effects on amorphous food stability. *Journal of Food processing and Preservation, 16*(6), pp. 433-447.

[34] Mujumdar, A. (2000). Equilibrium moisture relations for foods and bio-materials. In W. Kaminski & T. Kudra (Ed.), *Drying technology in agriculture and food sciences* (pp. 1-34). Enfield, NH: Science.

[35] Pathare, P. B., Opara, U. L., & Al-Said, F. A. J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and bioprocess technology, 6*(1), 36-60.

[36] HunterLab. (1996). Hunter Lab Color Scale. *Insight on Color, 8*(9), pp. 1-4. Retrieved from `https://support.hunterlab.com/hc/en-us/articles/203997095-Hunter-Lab-Color-Scale-an08-96a2` (retrieval date: February 15, 2019)

[37] Pomeranz Y. & Meloan C.E. (1994). Ash and Minerals. In *Food Analysis: Theory and Practice*, (pp. 602-624) Boston, MA: Springer US.

[38] Waterborg J. H. (2009). The Lowry Method for Protein Quantitation. In Walker J.M. (Ed.) *The Protein Protocols Handbook* (pp. 7-10). Totowa, NJ: Humana Press.

[39] Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). A thorough study of reactivity of various compound classes towards the Folin-Ciocalteu reagent. *Journal of Agricultural and Food Chemistry, 58*(14), pp. 8139–8144.

[40] PanReac AppliChem ITW Reagents. (2018). Nitrogen Determination by Kjeldahl Method. Retrieved from: `https://www.itwreagents.com/uploads/20180114/A173_EN.pdf` (retrieval date: March 4, 2019).

[41] Blackburn, S. (1978). *Amino acid determination : Methods and techniques* (2nd ed., rev. and expanded. ed.). New York: Marcel Dekker, pp. 7-37.

[42] Leach, A. A. (1960). Notes on a modification of the Neuman & Logan method for the determination of the hydroxyproline. *Biochemical Journal, 74*(1), pp. 70.

[43] Taylor, W. H. (1957). Formol titration: An evaluation of its various modifications. *The Analyst: the analytical journal of the Royal Society of Chemistry, 82*(976) pp. 488-498. Retrieved from: `https://pubs.rsc.org/en/content/articlepdf/1957/an/an9578200488` (retrieval date: September 1, 2018)

[44] Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology, 37*(8), pp. 911-917.

[45] Ke, P. J., Cervantes, E., & Robles-Martinez, C. (1984). Determination of thiobarbituric acid reactive substances (TBARS) in fish tissue by an improved distillation–spectrophotometric method. *Journal of the Science of Food and Agriculture, 35*(11), pp. 1248-1254.

[46] Crompton T.R. (2013). Differential Scanning Calorimetry. In *Thermal Methods of Polymer Analysis*, (pp. 87 -113). Smithers Rapra Technology.

[47] Christie, W. W. & Han, X. (1996). Lipid analysis. *Trends in Food Science & Technology, 11*(7), p. 145.

[48] Salimon, J., Abdullah, B. M., & Salih, N. (2011). Hydrolysis optimization and characterization study of preparing fatty acids from Jatropha curcas seed oil. *Chemistry Central journal*, 5, pp. 67.

[49] Skjellegrind, R. B. (2013). Enzymatisk hydrolyse av biprodukter fra laks: Produksjon av hydrolysater med helsebringende effekt [Enzymatic hydrolysis of byproducts from salmon: Production of hydrolysates with health beneficial effect] (Master's Thesis). Norwegian University of Science and Technology, Trondheim. *(in Norwegian)*

[50] Helrich, K. (Ed.) (1990). *Official Methods of Analysis of Association of Official Analytical Chemists*. (15th ed., pp. 69-70) United States of America: Association of Official Analytical Chemists, Inc.

[51] Lowry O. H., Rosebrough, N. J., Farr, A. L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, pp. 265-275.

[52] Buchi. (2013). Application Note No. 114/2013 Nitrogen & protein determination in meat products. Retrieved from: `https://static1.buchi.com/`

sites/default/files/FB91_AN_114_2013_Nitrogen_in_Meat_Products.pdf (retrieval date: March 9, 2019)

[53] American Oil Chemists' Society. (1990). Official methods and recommended practices of the American Oil Chemists' Society. AOCS-Pr.

[54] Aubourg, S.P. (1998). Lipid changes during long-term storage of canned tuna (Thunnus alalunga). *Journal of Food Examination and Research A, 206*(1), pp. 33-37.

[55] Mozuraityte, R., Kristinova V., Standal I.B., Evensen H. & Rustad T. (2017). Applicability of traditional and advanced methods for oxidative quality and stability on marine phospholipids. *European Journal of Lipid Science and Technology, 119*(3), 1600103.

[56] Ke, P. J., & Woyewoda, A. D. (1979). Microdetermination of thiobarbituric acid values in marine lipids by a direct spectrophotometric method with a monophasic reaction system. *Analytica Chimica Acta, 106*(2), pp. 279-284.

[57] Bartosova, Z. (personal communication, April 5, 2019).

[58] Tolstorebrov, I., Eikevik, T. M., & Bantle, M. (2014). Thermal phase transitions and mechanical characterization of Atlantic cod muscles at low and ultra-low temperatures. *Journal of Food Engineering, 128*, pp. 111-118.

[59] Tolstorebrov I. (personal communication, December 6, 2018).

[60] METER Group. (2019). AQUALAB 4TE. Retreived from https://www.metergroup.com/food/products/aqualab-4te-water-activity-meter/ (retrieval date: June 5, 2019)

[61] Jeyakumaran, R. (2018). *Cooling of poultry and marine rest raw material - Chemical and Thermal Characterisation and Effect of Storage Conditions on Quality* (Specialisation project). Norwegian University of Science and Technology, Trondheim.

[62] Aursand, M., Bleivik, B., Rainuzzo, J. R., Leif, J., & Mohr, V. (1994). Lipid distribution and composition of commercially farmed atlantic salmon (salmosalar). *Journal of the Science of Food and Agriculture, 64*(2), pp. 239-248.

[63] Mørkøre, T., & Rørvik, K. A. (2001). Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (Salmo salar) transferred to seawater as 0+ smolts or 1+ smolts. *Aquaculture, 199*(1-2), pp. 145-

157. Retrieved from: https://www.sciencedirect.com/science/article/pii/S0044848601005245?via%3Dihub (retrieval date: November 30, 2018)

[64] Seong, P. N., Cho, S. H., Park, K. M., Kang, G. H., Park, B. Y., Moon, S. S., & Van Ba, H. (2015). Characterization of chicken by-products by mean of proximate and nutritional compositions. *Korean journal for food science of animal resources, 35*(2), pp. 179-188.

[65] Xu, X. H-Kittikun, A. & Zhang, H.(2007). Enzymatic processing of omega-3 specialty oils. *Long-Chain Omega-3 Specialty Oils*, pp. 141-164.

[66] Carvajal, A., Slizyte, R., Storrø, I., & Aursand, M. (2015). Production of high quality fish oil by thermal treatment and enzymatic protein hydrolysis from fresh Norwegian spring spawning herring by-products. *Journal of Aquatic Food Product Technology, 24*(8), pp. 807-823.

[67] Luten, J. B. (2006). Production and characterization of a sockeye salmon (*Oncorhynchus nerka*) liver meal and dried powders from stickwaters. In S. Plante, A. C. M. Oliveira, S. Smiley & P. J. Bechtel (Ed.), *Seafood Research from Fish to Dish: Quality, Safety and Processing of Wild and Farmed Fish.* Wageningen Academic Pub, pp. 413-418.

[68] Ytterås R. (2019) *Effects of Storage Time and Temperature on Microbial and Enzymatic Activity in Chicken and Salmon Rest Raw Material.* (Master's thesis) NTNU, Trondheim.

[69] Nutter, M. K., Lockhart, E. E., & Harris, R. S. (1943). The chemical composition of depot fats in chickens and turkeys. *Oil and Soap, 20*(11), pp. 231-234.

[70] International Chemical Safety Cards. (2002). *Oleic acid.* Retrieved from: http://www.ilo.org/dyn/icsc/showcard.display?p_version=2&p_card_id=1005 (retrieval date: April 29, 2019)

[71] Whelan, J., & Fritsche, K. (2013). Linoleic acid. *Advances in Nutrition, 4*(3), pp. 311-312.

[72] Özcan, M. M., Al Juhaimi, F., Uslu, N., Ghafoor, K., Babiker, E. E., Mohamed Ahmed, I. A., & Alsawmahi, O. N. (2019). Effect of boiling on fatty acid composition and tocopherol content of hen, duck, and quail egg oils. *Journal of Food Processing and Preservation*, e13986.

[73] Tsami, E., Krokida, M. K., & Drouzas, A. E. (1998). Effect of drying method on the sorption characteristics of model fruit powders. Journal of food engineering, 38(4), 381-392.

[74] Coultate, T. (2016). *Food. The Chemistry of its Components* (6th ed., pp. 556). London: The Royal Society of Chemistry.

[75] Oikonomopoulou, V. P., Krokida, M. K., & Karathanos, V. T. (2011). The influence of freeze drying conditions on microstructural changes of food products. *Procedia food science, 1*, pp. 647-654.

[76] Huss, H. H. *Fresh Fish Quality and Quality Changes*. 1988. Rome, Italy: FAO.

[77] Refsgaard, H. H., Brockhoff, P. B., & Jensen, B. (1998). Sensory and Chemical Changes in Farmed Atlantic Salmon (Salmo s alar) during Frozen Storage. *Journal of Agricultural and Food Chemistry, 46*(9), pp. 3473-3479.

[78] Zhang, Z., Liu, Y., & Che, L. (2018). Effects of different drying methods on the extraction rate and qualities of oils from demucilaged flaxseed. *Drying technology, 36*(13), pp. 1642-1652.

[79] Wanasundara, U. N., Shahidi, F., & Jablonski, C. R. (1995). Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils. *Food Chemistry, 52*(3), pp. 249-253.

[80] Trevisan, M., Browne, R., Ram, M., Muti, P., Freudenheim, J., Carosella, A. M., & Armstrong, D. (2001). Correlates of markers of oxidative status in the general population. *American journal of epidemiology, 154*(4), pp. 348-356.

[81] Leung, K., Galano, J. M., Durand, T., & Lee, J. (2018). Profiling of omega-polyunsaturated fatty acids and their oxidized products in salmon after different cooking methods. *Antioxidants, 7*(8), pp. 96.

[82] Cyprian, O., Nguyen, V. M., Sveinsdottir, K., Jonsson, A., Tomasson, T., Thorkelsson, G., & Arason, S. (2015). Influence of smoking and packaging methods on lipid stability and microbial quality of C apelin (M allotus villosus) and Sardine (S ardinella gibossa). *Food science & nutrition, 3*(5), pp. 404-414.

[83] Odoli, C. O., Oduor-Odote, P., & Arason, S. (2019). The influence of lipid content and pretreatment methods on protein conformation in fish (capelin, Mallotus vil-

losus) during smoking and drying. *Food Science & Nutrition* 7(4), pp. 1446-1454.

[84] Gbogouri, G. A., Linder, M., Fanni, J., & Parmentier, M. (2004). Influence of hydrolysis degree on the functional properties of salmon byproducts hydrolysates. *Journal of food science, 69*(8), pp. C615-C622.

[85] Tolstorebrov I., Eikevik T. M. & Bantle M. (2014). A DSC determination of phase transitions and liquid fraction in fish oils and mixtures of triacylglycerides. *Food research international, 58*, pp. 132-140.

[86] Tolstorebrov, I., Eikevik, T. M., & Bantle, M. (2014). A DSC study of phase transition in muscle and oil of the main commercial fish species from the North-Atlantic. *Food research international, 55*, pp. 303-310.

Appendices

A Labelling of samples

The labelling of the samples are consistent and they are labeled with the type of material, type of storage and the drying method. Table A.1 shows the different labelling used throughout this thesis.

Table A.1: Labelling of samples used in this thesis.

Salmon intestines	**No storage**	**Vacuum packed for 30 days**	**Stored in plastic bags for 30 days**
Vacuum freeze dried	S-VAC/ S-0-VAC	S-VP-VAC	S-BAG-VAC
Infrared dried	S-IR/ S-0-IR	S-VP-IR	S-BAG-IR
Oven dried at 50°C	S-50°C/ S-0-50°C	S-VP-50°C	S-BAG-50°C
Oven dried at 100°C	S-100°C/ S-0-100°C	S-VP-100°C	S-BAG-100°C
Chicken intestines	**No storage**	**Vacuum packed for 30 days**	**Stored in plastic bags for 30 days**
Vacuum freeze dried	C-VAC/ C-0-VAC	C-VP-VAC	C-BAG-VAC
Oven dried at 50°C	C-50°C/ C-0-50°C	C-VP-50°C	C-BAG-50°C
Oven dried at 100°C	C-100°C/ C-0-100°C	C-VP-100°C	C-BAG-100°C

B Dry matter and ash content

The dry matter and ash content of chicken intestines and salmon intestines was determined to analyse the compositions of both rest raw materials. The raw data are given in Table B.1.

Table B.1: Raw data from dry matter and ash content analysis performed on chicken and salmon intestines.

Material	Salmon intestines			Chicken intestines		
	i	ii	iii	i	ii	iii
Weight of wet sample [g]	5.2657	5.6661	6.6443	3.5906	2.3074	1.9572
Weight of dry matter [g]	1.6883	1.9563	2.5035	1.0890	0.7216	0.5926
Dry matter percentage [%]	32.1	34.5	37.8	30.3	31.3	30.3
Mean dry matter percentage [%]		34.8			30.6	
SD		2.8			0.561	
Ash [g]	0.0560	0.0582	0.0616	0.0249	0.0172	0.0190
Ash percentage [g]	1.06	1.03	0.927	0.693	0.745	0.971
Mean ash percentage [%]		1.01			0.803	
SD		0.071			0.147	

C Total protein content

C.1 Rest raw material characterisation

The total protein content of rest raw material was determined using the Kjeldahl method. The raw data are presented in Table C.1. The concentration of the titrant, H_2SO_4, was 0.25 M for the samples presented in the table below.

Table C.1: Raw data of the protein content determined from Kjeldahl method of salmon and chicken intestines.

Material	**Weight of sample [g]**	**Titration volume [mL]**	**Nitrogen content [%]**	**Protein content [%]**	**Mean protein content [%]**	**SD**
Salmon intestines	1.7905	5.092	1.942	12.140	12.6	0.482
	1.5333	4.530	2.012	12.572		
	1.9807	6.055	2.096	13.102		
Chicken intestines	1.9221	5.510	1.962	12.261	11.9	0.289
	1.1587	3.253	1.890	11.813		
	1.5673	4.323	1.875	11.721		

C.2 Dried rest raw material

The total protein content of the dried rest raw material was determined using the Kjeldahl method. The raw data for salmon intestines and chicken intestines are given in Table C.2 and Table C.3. The concentration of the titrant, H_2SO_4, was 0.25 M. However, two samples of the dried chicken intestines were titrated with 0.1 M H_2SO_4. These samples are marked in the table below.

Table C.2: Raw data of the protein content determined from Kjeldahl method for dried salmon intestines. The samples were titrated with 0.25 M H_2SO_4.

Sample	**Weight of sample [g]**	**Titration volume [mL]**	**Nitrogen content [%]**	**Protein content [%]**	**Mean protein content [%]**	**SD**
Vacuum freeze dried	0.5635	4.866	5.887	36.796	35.6	1.028
	0.5169	4.266	5.605	35.032		
	0.5149	4.246	5.600	34.998		
IR dried	0.7345	5.389	5.012	31.322	30.4	0.857
	0.5911	4.140	4.749	29.679		
	0.6141	4.349	4.813	30.079		
50°C in oven	1.3456	11.172	5.747	35.922	35.6	0.423
	1.0252	8.351	5.619	35.117		
	1.0690	8.856	5.719	35.746		
100°C in oven	0.5987	5.654	6.466	40.415	38.8	1.411
	0.5296	4.700	6.046	37.788		
	0.5770	5.163	6.114	38.211		

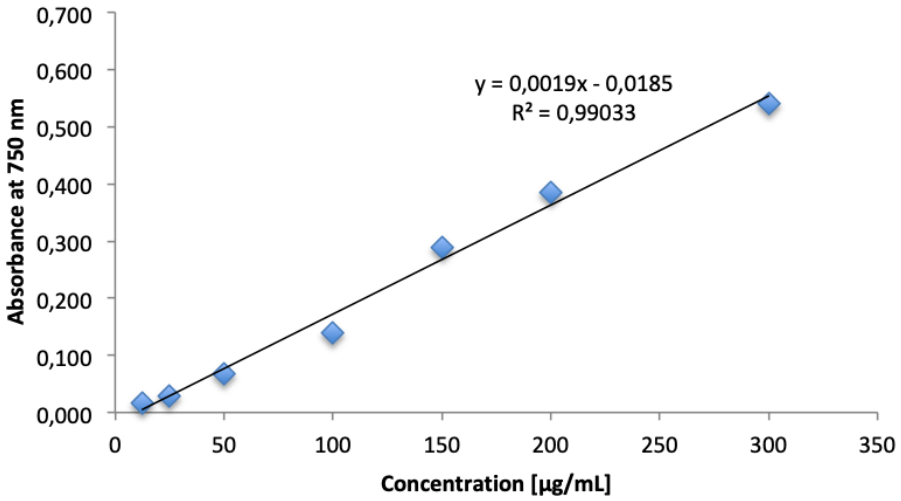
Table C.3: Raw data of the protein content determined from Kjeldahl method for dried chicken intestines. The samples were titrated with 0.25 M H_2SO_4.

Sample	**Weight of sample [g]**	**Titration volume [mL]**	**Nitrogen content [%]**	**Protein content [%]**	**Mean protein content [%]**	**SD**
Vacuum freeze dried	0.4053	3.919	6.554	40.963	39.3	2.554
	0.5209	4.455	5.820	36.376		
	0.5078	4.838	6.499	40.616		
50°C in oven	1.3038	6.802	3.586	22.413	38.3	13.786
	1.7265[a]	46.678	7.407	46.2936		
	1.3423[a]	36.514	7.406	46.287		
100°C in oven	1.3601	7.554	3.360	21.002	23.2	1.900
	1.0949	6.252	3.919	24.494		
	1.0458	5,870	3.847	24.045		

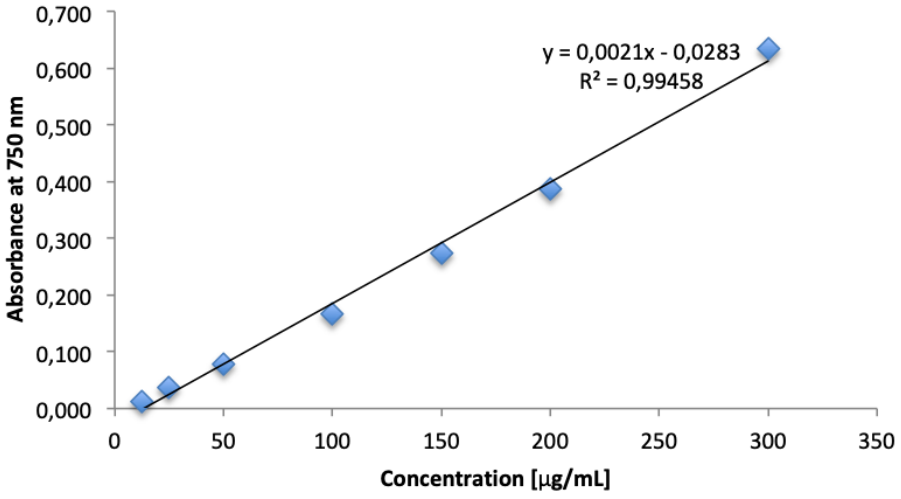
[a] These samples were titrated with 0.1 M H_2SO_4.

D Lowry method

Determination of soluble protein content was done using the Lowry method which was based on a standard curves composed during conduction of the method. The standard curves used for determination of protein content are given in Figure D.1 for both salmon intestines and chicken intestines.



(a) Salmon intestines



(b) Chicken intestines

Figure D.1: Standard curves for determination of soluble protein content based on the Lowry method. Concentration is plotted against absorbance measured at 750 nm for **(a)** for salmon intestines and **(b)** for chicken intestines.

The raw data from the Lowry analysis performed on the raw materials and the stickwater from these materials are given in Table D.1. The mean concentrations for each material are determined from the standard curves given above based on the absorbance measured.

Table D.1: Experimental data from the Lowry method for the raw materials and the stickwater separated from these materials with calculated mean concentrations, percentage of soluble proteins and the standard deviation (SD).

Sample	**Weight** [g]	**Dilution**	**Absorbance (750 nm)**	**Mean concentration** [µg/mL]	**Soluble proteins** [%]	**SD**
Salmon intestines	1.0600	1:100	0.145 0.143 0.138	84.47	8.0	0.004
Stickwater (salmon intestines)	1.1800	1:100	0.183 0.180 0.189	106.58	9.0	0.005
Chicken intestines	1.3005	1:100	0.175 0.170 0.183	97.29	7.5	0.01
Stickwater (chicken intestines)	1.2051	1:100	0.357 0.367 0.353	184.4	15.3	0.01

E Total lipid content

E.1 Characterisation of rest raw material

The total lipid content in the rest raw material was determined to analyse the composition of the materials. The raw data are given in Table E.1 for the salmon intestines and Table E.2 for the chicken intestines.

Table E.1: Raw data from the analysis of total lipid content in the salmon intestines.

Material	Salmon intestines					
	1.i	1.ii	1.iii	2.i	2.ii	2.iii
Weight of sample [g]		10.3			10.5	
Added chloroform [mL]	40	40	40	40	40	40
Chloroform transferred to tubes [mL]	2	2	2	2	2	2
Lipid after evaporation [g]	0.1277	0.1384	0.1383	0.1283	0.1355	0.1364
Lipid content [%]	24.7	26.8	26.8	24.5	25.9	26.1
Mean lipid content [%]	25.8					
SD	0.978					

Table E.2: Raw data from the analysis of total lipid content in the chicken intestines.

Material	Chicken intestines		
	1.i	1.ii	1.iii
Weight of sample [g]		11.9	
Added chloroform [mL]	40	40	40
Chloroform transferred to tubes [mL]	2	2	2
Lipid after evaporation [g]	0.1006	0.1025	0.1036
Lipid content [%]	16.9	17.2	17.4
Mean lipid content [%]	17.1		
SD	0.255		

E.2 Thermally treated rest raw material at 40°C

The total lipid content in the thermally treated rest raw material at 40°C were determined, and the raw data for salmon intestines are presented in Table E.3 and for chicken intestines in Table E.4.

Table E.3: Raw data from lipid content analysis of thermally treated salmon intestines. The material was both thermally treated at 40°C.

Material	Salmon intestines					
	1.i	1.ii	1.iii	2.i	2.ii	2.iii
Weight of sample [g]	11.1450			10.2992		
Added chloroform [mL]	40	40	40	40	40	40
Chloroform transferred to tubes [mL]	2	2	2	2	2	2
Lipid after evaporation [g]	0.1392	0.1333	0.1289	0.1119	0.1218	0.1221
Lipid content [%]	25.0	23.9	23.1	21.7	23.7	23.7
Mean lipid content [%]	23.5					
SD	1.07					

Table E.4: Raw data from lipid content analysis of thermally treated chicken intestines. The material was both thermally treated at 40°C.

Material	Chicken intestines		
	1.i	1.ii	1.iii
Weight of sample [g]	14.4544		
Added chloroform [mL]	40	40	40
Chloroform transferred to tubes [mL]	2	2	2
Lipid after evaporation [g]	0.1281	0.1314	0.1344
Lipid content [%]	17.7	18.2	18.6
Mean lipid content [%]	18.2		
SD	0.436		

E.3 Thermally treated rest raw material at 50°C and 65°C

The total lipid content in the thermally treated rest raw material at 50°C and 65°C were determined, and the raw data are presented in Table E.5 and E.6, respectively.

Table E.5: Raw data from lipid content analysis of thermally treated salmon intestines and chicken intestines. The materials were both thermally treated at 50°C.

Material	Salmon intestines			Chicken intestines		
	1.i	1.ii	1.iii	1.i	1.ii	1.iii
Weight of sample [g]		8.0059			9.8696	
Added chloroform [mL]	40	40	40	40	40	40
Chloroform transferred to tubes [mL]	2	2	2	2	2	2
Lipid after evaporation [g]	0.0407	0.0408	0.0413	0.0366	0.0362	0.0365
Lipid content [%]	10.2	10.2	10.3	6.14	6.07	6.12
Mean lipid content [%]		10.2			7.38	
SD		0.0803			0.0422	

Table E.6: Raw data from lipid content analysis of thermally treated salmon intestines and chicken intestines. The materials were both thermally treated at 65°C.

Material	Salmon intestines			Chicken intestines		
	1.i	1.ii	1.iii	1.i	1.ii	1.iii
Weight of sample [g]		6.5000			10.1942	
Added chloroform [mL]	40	40	40	40	40	40
Chloroform transferred to tubes [mL]	2	2	2	2	2	2
Lipid after evaporation [g]	0.0729	0.0692	0.0698	0.0449	0.0460	0.0466
Lipid content [%]	23.1	22.0	22.2	7.53	7.71	7.82
Mean lipid content [%]		22.4			8.99	
SD		0.630			0.169	

E.4 Thermally treated rest raw material at 80°C and 90°C

The total lipid content in the thermally treated rest raw material at 80°C and 90°C were determined, and the raw data are presented in Table E.7 and E.8, respectively.

Table E.7: Raw data from lipid content analysis of thermally treated salmon intestines and chicken intestines. The materials were both thermally treated at 80°C.

Material	Salmon intestines			Chicken intestines		
	1.i	1.ii	1.iii	1.i	1.ii	1.iii
Weight of sample [g]		9.6375			10.7433	
Added chloroform [mL]	40	40	40	40	40	40
Chloroform transferred to tubes [mL]	2	2	2	2	2	2
Lipid after evaporation [g]	0.1442	0.1493	0.1503	0.0905	0.0911	0.0917
Lipid content [%]	29.9	31.0	31.2	16.85	16.96	17.07
Mean lipid content [%]		30.7			17.0	
SD		0.679			0.115	

Table E.8: Raw data from lipid content analysis of thermally treated salmon intestines and chicken intestines. The materials were both thermally treated at 90°C.

Material	Salmon intestines			Chicken intestines		
	1.i	1.ii	1.iii	1.i	1.ii	1.iii
Weight of sample [g]		7.9575			7.6953	
Added chloroform [mL]	40	40	40	40	40	40
Chloroform transferred to tubes [mL]	2	2	2	2	2	2
Lipid after evaporation [g]	0.1064	0.1207	0.1158	0.0595	0.0578	0.0586
Lipid content [%]	26.7	30.3	29.1	15.46	15.02	15.23
Mean lipid content [%]		28.7			15.2	
SD		1.83			0.221	

E.5 Dried rest raw material

The total lipid content in the dried rest raw material is given in Table E.9 and E.10 for salmon intestines and chicken intestines, respectively.

Table E.9: Raw data from lipid content analysis for dried salmon intestines.

Material	Weight of sample [g]	Lipid [g]	Lipid content [%]	Mean lipid content [%]	SD
Vacuum freeze dried	5.1335	0.1261	50.3	50.2	0.926
		0.1300	51.8		
		0.1304	52.0		
IR dried	5.1974	0.1286	48.7	50.6	0.955
		0.1321	50.1		
		0.1334	50.6		
50°C in oven	4.9954	0.1117	30.8	49.3	1.46
		0.1163	32.1		
		0.1189	32.8		
100°C in oven	5.0253	0.1191	43.6	46.5	1.66
		0.1121	41.0		
		0.1195	43.8		

Table E.10: Raw data from lipid content analysis for dried chicken intestines.

Material	Weight of sample [g]	Lipid [g]	Lipid content [%]	Mean lipid content [%]	SD
Vacuum freeze dried	5.3528	0.1393	52.0	61.3	8.25
		0.1709	63.9		
		0.1818	67.9		
50°C in oven	5.0796	0.5580	22.0	21.8	0.357
		0.0544	21.4		
		0.0561	22.1		
100°C in oven	5.0138	0.0383	15.3	15.5	0.636
		0.0435	16.3		
		0.0403	15.1		

F Mass balance calculations

The fractions of samples after extraction from the thermal treatment were measured to determine the yield of each fraction. The sample can be fractionated into fat-reduced sediment, stickwater and fat. The fractions were weighed after separation to determine the total amount separated. The yield was calculated for fat, stickwater and for the fat-reduced sediment as given in Equation F.1. The calculation of mass balance is presented in Table F.11 and F.12.

$$\text{Yield } \% = \frac{\text{Weight of fraction [g]}}{\text{Weight of raw material [g]}} \tag{F.1}$$

Table F.11: Mass balance of salmon intestines.

		40°C	**50°C**	**65°C**	**80°C**	**90°C**
Fat	Total [g]	0.250	14.52	14.92	1.20	4.57
	Yield [%]	0.245	14.45	14.87	1.18	4.37
Stickwater	Total [g]	25.62	30.63	32.72	26.93	35.81
	Yield [%]	25.14	30.48	32.62	26.56	34.28
Fat-reduced sediment	Total [g]	62.33	47.45	46.10	56.35	55.72
	Yield [%]	61.17	47.21	45.96	55.58	53.34
Total	Sum total [g]	88.20	92.60	93.74	84.48	96.10
	Original weight [g]	101.90	100.50	100.31	101.39	104.46
	Difference in original weight and sum after separation [g]	13.70	7.90	6.57	16.91	8.36

Table F.12: Mass balance of chicken intestines.

		40°C	**50°C**	**65°C**	**80°C**	**90°C**
Fat	Total [g]	4.02	6.08	7.67	6.13	8.67
	Yield [%]	3.95	6.01	7.61	5.73	8.62
Stickwater	Total [g]	7.61	13.22	28.68	21.26	17.24
	Yield [%]	7.47	13.06	28.46	19.88	17.14
Fat-reduced sediment	Total [g]	84.62	76.10	61.98	78.66	70.21
	Yield [%]	83.06	75.20	61.51	73.54	69.78
Total	Sum total [g]	96.25	95.40	98.33	106.05	96.12
	Original weight [g]	101.88	101.20	100.76	106.96	100.61
	Difference in original weight and sum after separation [g]	5.63	5.80	2.43	0.91	4.49

´

G Fatty acid composition

The fatty acids identified from mass spectrometry of salmon intestines and chicken intestines are given in Table G.1. The table includes lipid number, molar masses of each fatty acid and the retention time.

Table G.1: Fatty acids detected from mass spectrometry of salmon intestines and chicken intestines presented with lipid number, molar mass [g/mol] and retention time [min]. The molar masses given do not take the negative ionisation mode into account, hence is presented as the original value.

Common name	**Lipid number**	**Molar mass [g/mol]**	**Retention time [min]**
Tridecylic acid (Internal standard)	C13:0	214.18	3.16
Myristic acid	C14:0	228.20	3.45
Pentadecanoic acid	C15:0	242.22	3.71
Palmitic acid	C16:0	256.23	3.97
Palmitoleic acid	C16:1	254.22	4.04
Stearic acid	C18:0	284.26	4.39
Oleic acid	C18:1	282.25	4.44
Linoleic acid	C18:2	280.24	4.55
α-linolenic acid	C18:3	278.22	4.68
Paullinic acid	C20:1	310.28	4.77
Eicosadienoic	C20:2	308.27	4.87
Dihomo-γ-linolenic acid	C20:3	306.25	4.97
Arachidonic acid	C20:4	304.24	4.92
EPA[a]	C20:5	302.22	5.01
Docosatetraenoic acid	C22:4	332.26	5.17
DPA[b]	C22:5	330.25	5.26
DHA[c]	C22:6	328.23	5.31

[a] Eicosapentaenoic acid, [b] Docosapentaenoic acid, [c] Docosahexaenoic acid

H Drying processes

H.1 Drying in oven at 50°C

Table H.1 shows the sample weight of every sample dried at 50°C. Some of the measuring intervals were different for each sample and is therefore marked with " - " if sample weight was not measured at the given time.

Table H.1: Raw data from drying salmon intestines and chicken intestines at 50°C in oven. A humidity of 10 % was used for the oven with the humidity setting.

Hours	**Salmon (oven)** [g]	**Salmon (oven & humidity)** [g]	**Chicken (oven)** [g]
0	58.23	58.85	161.2
1	49.19	54.80	160.9
2	49.19	54.80	158.3
3	-	-	155.6
4	39.19	43.30	-
5	36.19	39.30	-
15	-	-	112.1
23	27.19	28.80	-

H.2 Drying in oven at 100°C

The raw data from the drying process conducted at 100°C for both salmon intestines and chicken intestines are given in Table H.2.

Table H.2: Raw data from drying salmon intestines and chicken intestines at 100°C in oven. A humidity of 10 % was used for the oven with the humidity setting.

Hours	**Salmon (oven)** [g]	**Salmon (oven & humidity)** [g]	**Chicken 100°C in oven** [g]
0	62.0	69.0	164.9
1	59.0	54.0	158.5
2	45.0	46.0	128.5
3	30.0	33.0	110.2

H.3 Raw data from infrared drying

The percentage of water removed from eight samples of salmon intestines is presented in Figure H.1. The mean amount of water removed during IR drying was approximately 47 %. The raw data are presented in Table H.3.

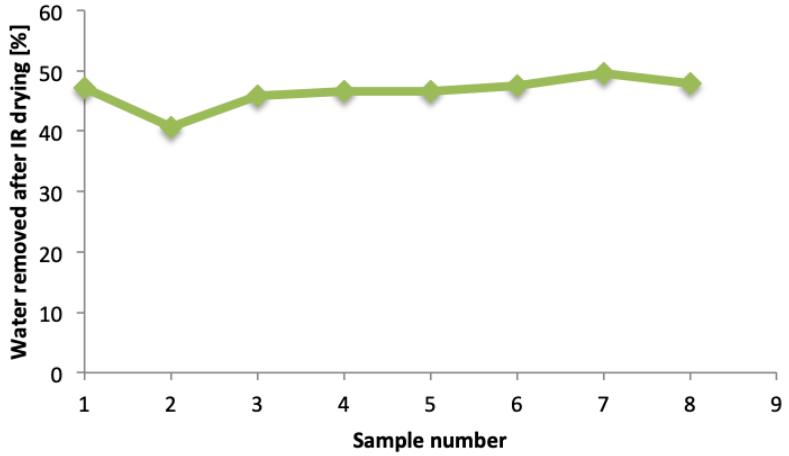


Figure H.1: Percentage of water removed during infrared drying of eight samples of salmon intestines.

Table H.3: Raw data of water removed from samples of salmon intestines during infrared drying.

Sample #	Sample weight [g]	Sample after drying [g]	Water removed [%]
1	41.09	21.70	47.2
2	50.61	30.01	40.7
3	48.02	26.03	45.8
4	44.77	23.90	46.6
5	38.49	20.53	46.7
6	37.05	19.42	47.6
7	30.6	15.44	49.5
8	33.27	17.30	48.0

I Water activity in dried rest raw material

The raw data from measuring water activity of dried salmon intestines and dried chicken intestines are given in Table I.1.

Table I.1: Water activity of dried salmon intestines (S) and dried chicken intestines (C). The samples are labeled with the drying method (VAC = vacuum freezed dried, IR = infrared dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Water activity**		
	No storage	**Vacuum packed**	**Plastic bag**
S-VAC	0.1731	0.2065	0.2057
S-IR	0.6971	0.7965	0.5444
S-50°C	0.5362	0.2508	0.4409
S-100°C	0.7078	0.6818	0.1715
C-VAC	0.1531	0.2879	0.2444
C-50°C	0.9135	0.9243	0.9036
C-100°C	0.9550	0.9271	0.9367

J Water content of dried materials

The water content of the dried materials were calculated based on the sample weight before and after the drying process. Equation J.1 was used to calculate the water content.

$$\left(1 - \frac{\text{Sample weight after drying}}{\text{Initial sample weight}}\right) \cdot 100 = \text{Water content [\%]} \tag{J.1}$$

The raw data from the measurement of the water content of the dried salmon intestines and chicken intestines are given in Table J.1 and Table J.2, respectively.

Table J.1: Raw data from measuring the water content in dried salmon intestines (S). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Initial sample weight [g]**	**Sample weight after drying [g]**	**Water content [%]**	**Mean water content [%]**	**SD**
S-0-VAC	2.184	2.117	3.068	2.91	0.181
	2.063	2.007	2.714		
	2.400	2.329	2.958		
S-0-IR	2.013	1.801	10.532	13.15	3.293
	2.226	1.851	16.846		
	1.359	1.195	12.068		
S-0-50°C	1.698	1.540	9.305	9.03	0.575
	2.367	2.169	8.365		
	2.891	2.619	9.409		
S-0-100°C	2.251	2.196	2.443	2.48	0.123
	1.220	1.188	2.623		
	2.221	2.168	2.386		
S-VP-VAC	1.290	1.213	5.969	5.89	0.096
	1.168	1.099	5.908		
	1.280	1.206	5.781		
S-VP-IR	2.106	1.775	15.717	15.38	1.037
	1.990	1.707	14.221		
	2.276	1.907	16.213		
S-VP-50°C	1.094	1.050	4.022	3.87	0.283
	1.362	1.307	4.038		
	1.243	1.199	3.540		
S-VP-100°C	1.585	1.414	10.789	10.54	0.218
	1.246	1.116	10.433		

	1.376	1.233	10.392		
S-BAG-VAC	0.962	0.873	9.252		
	1.306	1.182	9.495	9.47	0.204
	1.108	1.001	9.657		
S-BAG-IR	1.356	1.248	7.965		
	1.238	1.149	7.189	7.73	0.472
	1.206	1.109	8.043		
S-BAG-50°C	1.040	0.961	7.596		
	0.494	0.463	6.275	6.78	0.715
	0.805	0.753	6.460		
S-BAG-100°C	0.481	0.459	4.574		
	1.009	0.975	3.370	3.43	1.112
	1.275	1.245	2.353		

Table J.2: Raw data from measuring the water content in dried chicken intestines (C). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Initial sample weight [g]**	**Sample weight after drying [g]**	**Water content [%]**	**Mean water content [%]**	**SD**
C-0-VAC	1.121	1.051	6.244		
	1.014	0.957	5.621	5.90	0.316
	1.027	0.967	5.842		
C-0-50°C	2.321	1.281	44.808		
	1.624	0.923	43.165	42.68	2.411
	2.646	1.586	40.060		
C-0-100°C	1.867	0.817	56.240		
	1.811	0.776	57.151	56.59	0.491
	2.297	1.002	56.378		
C-VP-VAC	1.179	1.079	8.482		
	1.170	1.073	8.291	8.12	0.479
	1.360	1.257	7.574		
C-VP-50°C	1.996	1.058	46.994		
	1.544	0.817	47.085	47.13	0.166
	2.198	1.158	47.316		
C-VP-100°C	1.493	0.738	50.569		
	2.065	1.034	49.927	50.38	0.398
	1.143	0.564	50.656		
C-BAG-VAC	1.184	0.540	54.392		
	1.718	0.998	41.909	45.71	7.539
	2.055	1.216	40.827		

C-BAG-50°C	0.950	0.527	44.526	42.90	1.656
	1.718	0.980	42.957		
	2.055	1.208	41.217		
C-BAG-100°C	1.211	0.562	53.592	53.40	0.323
	1.047	0.486	53.582		
	1.156	0.543	53.028		

K Colour measurement of dried material

Data from measuring different colour parameters of the dried materials are given in Table K.1 and K.2 for salmon intestines and chicken intestines, respectively. The lightness (L), redness (a) and yellowness (b) were measured for the samples.

Table K.1: Raw data from measuring the colour of dried salmon intestines (S). The colour parameters measured were L (lightness), a (redness) and b (yellowness). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, IR = infrared dried 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Colour**			**Mean**			**SD**		
	L	a	b	L	a	b	L	a	b
S-0-VAC	48.01	3.26	18.70	48.19	3.22	18.75	0.284	0.036	0.050
	48.52	3.19	18.76						
	48.05	3.21	18.8						
S-0-IR	29.46	3.96	15.25	30.05	3.64	15.17	0.993	0.394	0.139
	31.20	3.20	15.01						
	29.5	3.76	15.25						
S-0-50°C	19.88	2.46	11.71	19.90	2.51	11.64	0.101	0.110	0.181
	20.01	2.64	11.77						
	19.81	2.44	11.43						
S-0-100°C	24.03	4.07	11.46	24.04	4.08	11.49	0.051	0.006	0.076
	24.00	4.08	11.58						
	24.10	4.08	11.44						
S-VP-VAC	42.40	7.04	24.81	42.90	6.99	24.46	0.505	0.050	0.311
	42.89	6.94	24.21						
	43.41	6.99	24.37						
S-VP-IR	19.58	4.09	15.47	19.45	4.06	15.39	0.121	0.042	0.100
	19.44	4.07	15.43						
	19.34	4.01	15.28						
S-VP-50°C	34.58	3.61	16.41	34.73	3.57	16.48	0.130	0.038	0.127
	34.81	3.55	16.41						
	34.8	3.54	16.63						
S-VP-100°C	16.67	4.33	13.67	16.52	4.15	13.52	0.204	0.159	0.216
	16.29	4.06	13.61						
	16.61	4.05	13.27						
S-BAG-VAC	43.02	8.55	25.8	42.97	8.60	25.76	0.132	0.042	0.045
	43.07	8.63	25.71						
	42.82	8.61	25.76						
S-BAG-IR	31.75	5.12	16.00	31.46	4.97	15.42	0.344	0.160	0.508
	31.08	4.8	15.07						
	31.55	4.98	15.18						
S-BAG-50°C	24.29	2.09	9.11	24.96	2.10	9.25	1.591	0.245	0.647

	23.82	1.86	8.69						
	26.78	2.35	9.96						
	35.59	5.03	18.91						
S-BAG-100°C	35.52	4.82	18.01	35.52	4.87	18.28	0.075	0.138	0.544
	35.44	4.77	17.93						

Table K.2: Raw data from measuring the colour of dried chicken intestines (C). The colour parameters measured were L (lightness), a (redness) and b (yellowness). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Colour**			**Mean**			**SD**		
	L	a	b	L	a	b	L	a	b
	47.74	10.04	24.39						
C-0-VAC	47.70	10.00	24.40	47.68	10.35	24.37	0.078	0.578	0.044
	47.59	11.02	24.32						
	36.27	5.74	20.84						
C-0-50°C	36.43	5.77	20.82	36.23	5.76	20.84	0.228	0.021	0.015
	35.98	5.78	20.85						
	36.93	7.3	19.71						
C-0-100°C	36.91	7.31	19.82	36.90	7.90	19.74	0.031	1.036	0.067
	36.87	9.10	19.70						
	44.89	6.92	23.23						
C-VP-VAC	44.90	7.07	23.39	45.03	6.96	23.14	0.234	0.100	0.300
	45.30	6.88	22.81						
	37.57	6.76	20.27						
C-VP-50°C	37.30	6.73	20.38	37.34	6.74	20.29	0.208	0.015	0.086
	37.16	6.74	20.21						
	36.85	7.22	18.3						
C-VP-100°C	37.14	7.03	18.19	37.06	7.08	18.23	0.187	0.127	0.064
	37.20	6.98	18.19						
	52.74	5.08	27.85						
C-BAG-VAC	52.64	5.05	27.66	52.85	4.99	27.66	0.276	0.131	0.195
	53.16	4.84	27.46						
	32.98	7.5	17.54						
C-BAG-50°C	32.85	7.51	17.53	33.02	7.35	17.41	0.199	0.269	0.217
	33.24	7.04	17.16						
	36.31	8.15	20.75						
C-BAG-100°C	36.26	8.03	20.76	36.06	7.97	20.61	0.391	0.216	0.257
	35.61	7.73	20.31						

L Peroxide value

The raw data from the PV determination are given in Table L.1 and L.2 for salmon and chicken intestines, respectively. B in the table indicates the amount of titrant which was titrated into a blank sample. The weight of the oil samples are based on the lipid percentages in the chloroform phases. The weight of oil in each sample is calculated using Equation L.1.

$$\frac{X}{2} \cdot 6 = \text{Weight of oil in sample [g]} \tag{L.1}$$

X is the % of lipid in 2 mL chloroform phase determined from the Bligh & Dyer method. This was divided by 2 and multiplied by 6 to determine the amount of oil in 6 mL of chloroform phase, which was used in the PV analysis.

Table L.1: Raw data from PV analysis conducted on dried salmon intestines (S). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, IR = infrared dried 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Volume titrated [mL]**	**Blank titrated [mL]**	**Weight of oil [g]**	**PV [meqO2/ kg oil]**	**Mean PV [meqO2/ kg oil]**	**SD**
S-0-VAC	5.883 7.334 *not found*	0.012	1.506	3.898 4.862 -	4.380	0.681
S-0-IR	3.322 3.562 2.910	0.012	1.518	2.181 2.339 1.909	2.143	0.217
S-0-50°C	2.468 3.725 2.964	0.012	1.389	1.768 2.673 2.125	2.189	0.456
S-0-100°C	2.758 0.075 3.548	0.012	1.395	1.968 0.045 2.535	1.516	1.305
S-VP-VAC	0.555 0.325 0.686	0.077	1.359	0.352 0.182 0.448	0.267	0.134
S-VP-IR	0.505 0.828 0.995	0.077	1.326	0.323 0.566 0.692	0.527	0.188
S-VP-50°C	0.973 0,802 0.124	0.077	1.584	0.566 0.458 0.030	0.351	0.283
S-VP-100°C	1.305	0.077	1.290	0.952	0.571	0.504

	0.076			-0.001		
	1.061			0.763		
	0.052			-0.026		
S-BAG-VAC	1.937	0.077	0.951	1.956	0.965	1.067
	1.647			1.651		
	1.008			0.569		
S-BAG-IR	0.355	0.077	1.635	0.170	0.476	0.272
	1.205			0.690		
	0.055			-0.013		
S-BAG-50°C	0.115	0.077	1.671	0.023	0.173	0.291
	0.926			0.508		
	0.749			0.343		
S-BAG-100°C	0.900	0.077	1.962	0.419	0.479	0.173
	1.399			0.674		

Table L.2: Raw data from PV analysis conducted on dried chicken intestines (C). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Volume titrated [mL]**	**Blank titrated [mL]**	**Weight of oil [g]**	**PV [meqO2/ kg oil]**	**Mean PV [meqO2/ kg oil]**	**SD**
	0.100			0.048		
C-0-VAC	0.237	0.012	1.839	0.122	0.094	0.041
	0.220			0.113		
	0.501			0.748		
C-0-50°C	0.092	0.012	0.654	0.122	0.629	0.460
	0.678			1.018		
	0.118			0.228		
C-0-100°C	0.292	0.012	0.465	0.602	0.335	0.232
	0.094			0.176		
	0.083			0.047		
C-VP-VAC	0.152	0.012	0.403	0.218	0.133	0.088
	0.102			0.094		
	0.381			1.157		
C-VP-50°C	0.447	0.064	0.274	1.398	0.884	0.691
	0.091			0.099		
	0.226			0.818		
C-VP-100°C	0.269	0.064	0.198	1.035	0.857	0.163
	0.206			0.717		
	3.996			10.082		
C-BAG-VAC	3.580	0.064	0.390	9.015	9.549	0.594
	3.965			10.003		

C-BAG-50°C	0.416 0.411 0.394	0.064	0.237	1.485 1.464 1.392	1.447	0.049
C-BAG-100°C	0.365 0.334 0.138	0.064	0.197	1.528 1.371 0.376	1.091	0.625

M Conjugated dienes

Conjugated dienes were measured using chloroform as solvent. The raw data used to compute the C-values of the dried salmon intestines and dried chicken intestines are presented in Table M.1 and M.2, respectively.

Table M.1: Concentration of each sample of dried salmon intestines (S), dilution, absorbance measured at 233 nm are presented with the calculated C-Values, Mean C-Values and the corresponding standard deviations. The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Conc. [g/mL]**	**Dilution**	**Absorbance (233 nm)**	**C- Value [mmol/ kg lipid]**	**Mean C-Value [mmol/ kg lipid]**	**SD**
S-0-VAC	0.064	0	0.769 0.147 0.244	0.473 0.090 0.150	0.238	0.206
S-0-IR	0.066	0	0.798 1.388 1.391	0.481 0.837 0.838	0.719	0.206
S-0-50°C	0.058	0	1.418 1.408 0.818	0.972 0.965 0.560	0.832	0.235
S-0-100°C	0.058	0	0.794 1.494 1.468	0.538 1.012 0.995	0.848	0.269
S-VP-VAC	0.061	0	0.859 0.865 0.868	0.557 0.561 0.563	0.560	0.003
S-VP-IR	0.096	0	0.341 0.323 0.344	0.141 0.133 0.142	0.390	0.005
S-VP-50°C	0.069	0	0.325 0.322 0.318	0.187 0.186 0.183	0.185	0.002
S-VP-100°C	0.054	0	0.257 0.261 0.259	0.188 0.191 0.190	0.190	0.001
S-BAG-VAC	0.051	0	1.188 1.186 1.167	0.916 0.915 0.900	0.910	0.009
S-BAG-IR	0.088	0	0.325	0.146	0.144	0.003

			0.326	0.147		
			0.313	0.141		
			0.314	0.165		
S-BAG-50°C	0.075	0	0.319	0.168	0.164	0.005
			0.301	0.158		
			0.283	0.141		
S-BAG-100°C	0.080	0	0.274	0.136	0.137	0.003
			0.272	0.135		

Table M.2: Concentration of each sample of dried chicken intestines (C), dilution, absorbance measured at 233 nm are presented with the calculated C-Values, Mean C-Values and the corresponding standard deviations. The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Conc. [g/mL]**	**Dilution**	**Absorbance (233 nm)**	**C- Value [mmol/ kg lipid]**	**Mean C-Value [mmol/ kg lipid]**	**SD**
			1.489	0.719		
C-0-VAC	0.082	0	0.847	0.409	0.619	0.182
			1.508	0.728		
			2.833	4.050		
C-0-50°C	0.028	0	2.720	3.889	4.039	0.145
			2.922	4.178		
			1.809	3.521		
C-0-100°C	0.020	0	1.878	3.655	3.835	0.433
			2.224	4.328		
			1.211	0.878		
C-VP-VAC	0.055	0	1.197	0.867	0.860	0.022
			1.152	0.835		
			1.052	0.932		
C-VP-50°C	0.045	0	1.054	0.934	0.927	0.011
			1.032	0.914		
			0.914	1.193		
C-VP-100°C	0.030	0	0.938	1.224	1.202	0.019
			0.912	1.190		
			1.090	0.802		
C-BAG-VAC	0.054	0	1.086	0.799	0.806	0.008
			1.107	0.815		
			1.007	1.205		
C-BAG-50°C	0.033	0	1.107	1.325	1.257	0.061
			1.038	1.242		

C-BAG-100°C	0.041	0	0.999 0.949 0.940	0.965 0.917 0.908	0.930	0.031

N Correlation between PV and CD

Correlation between PV and CD was plotted and are given in Figure N.1 and N.2 for salmon and chicken intestines, respectively. The plots contain all values obtained for the materials regardless of the storage method and drying method. The correlations were calculated using Microsoft Excel. Correlation is given as a value between 1 and -1, where 1 signifies perfect positive correlation, -1 signifies perfect negative correlation and 0 indicates no correlation. The calculated correlation coefficient was **0.33** and $\mathbf{-0.24}$ for the salmon and chicken intestines, respectively.

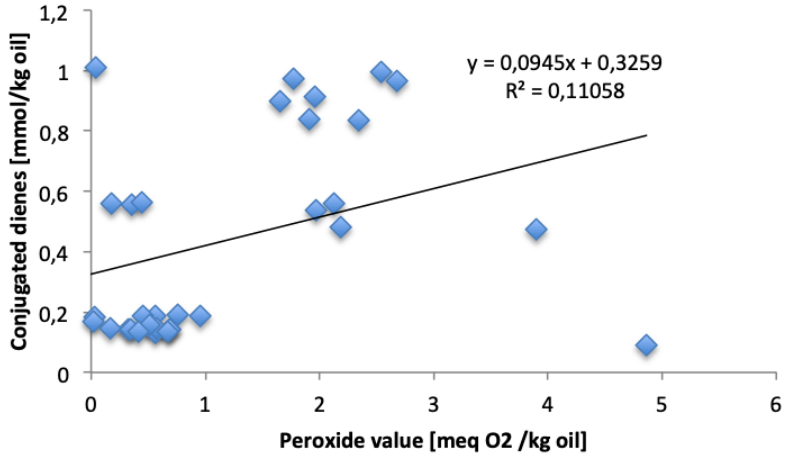


Figure N.1: Peroxide value plotted against conjugated dienes for the salmon intestines to determine correlation. Values of all samples are included regardless of drying and storage method.

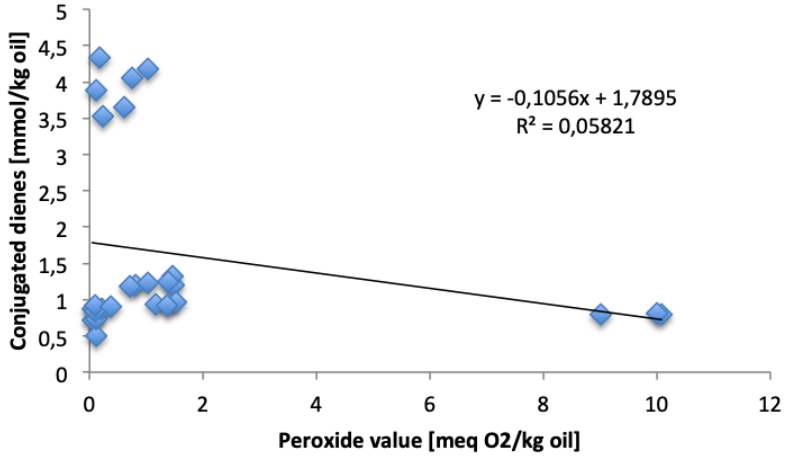
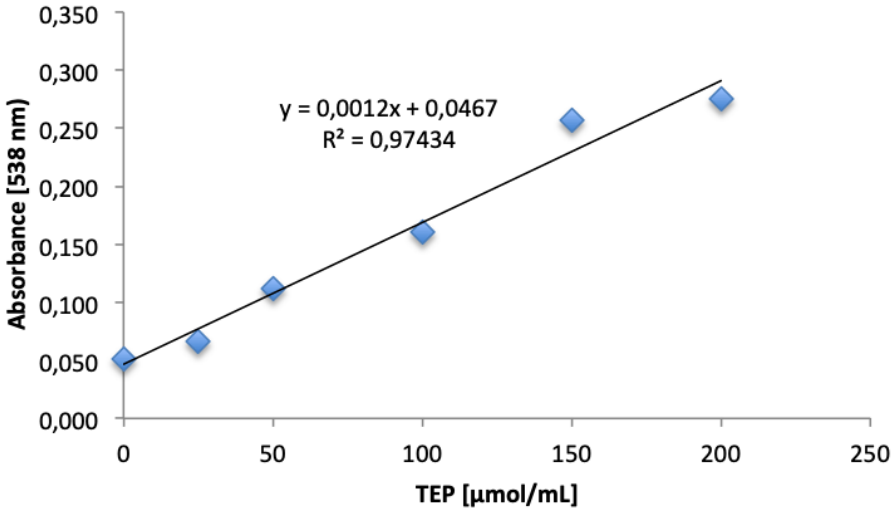


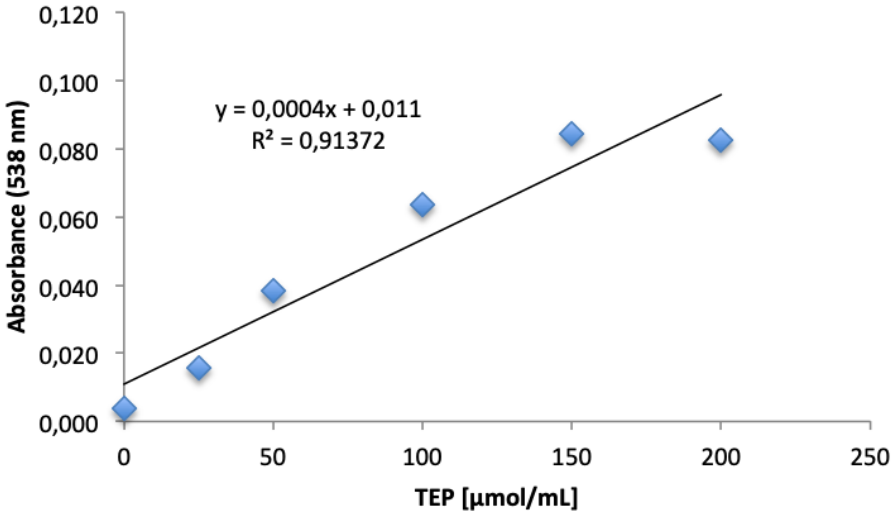
Figure N.2: Peroxide value plotted against conjugated dienes for the chicken intestines to determine correlation. Values of all samples are included regardless of drying and storage method.

O TBARS assay

Standard curves were made to measure the secondary lipid oxidation products using the TBARS method. The standard curves are given in Figure O.1.



(a) Initial samples



(b) Stored samples

Figure O.1: Standard curves for determination of secondary lipid oxidation products based on the TBARS method. Concentration was plotted against absorbance measured at 538 nm. **(a)** was the curve used for the initial samples which were not stored and **(b)** was used for the stored samples.

The raw data from the calculations of TBA value in the salmon and chicken intestines are given in Table O.1 and O.2.

Table O.1: Mean absorbance measured at 538 nm, lipid content in 200 µL sample, calculated µmol TBARS/ g lipid and standard deviation (SD) are given for the salmon intestines (S). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Mean absorbance (538 nm)**	**Lipid content in sample**	**µmol TBARS /g lipid**	**SD**
S-0-VAC	0.181	0.050	22.29	0.005
S-0-IR	0.131	0.051	1.38	0.006
S-0-50°C	0.129	0.046	1.48	0.006
S-0-100°C	0.112	0.047	1.17	0.002
S-VP-VAC	0.120	0.045	6.00	0.006
S-VP-IR	0.113	0.044	5.75	0.032
S-VP-50°C	0.088	0.053	14.52	0.018
S-VP-100°C	0.069	0.043	33.56	0.010
S-BAG-VAC	0.082	0.032	22.38	0.004
S-BAG-IR	0.089	0.055	3.57	0.008
S-BAG-50°C	0.083	0.056	3.24	0.010
S-BAG-100°C	0.075	0.065	2.45	0.004

Table O.2: Mean absorbance measured at 538 nm, lipid content in 200 µL sample, calculated µmol TBARS/ g lipid and standard deviation (SD) are given for the chicken intestines (C). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Mean absorbance (538 nm)**	**Lipid content in sample**	**µmol TBARS /g lipid**	**SD**
C-0-VAC	0.071	0.061	3.30	0.009
C-0-50°C	0.054	0.022	2.79	0.003
C-0-100°C	0.051	0.016	2.13	0.006
C-VP-VAC	0.062	0.040	3.19	0.005
C-VP-50°C	0.052	0.027	3.75	0.001
C-VP-100°C	0.053	0.020	5.29	0.003
C-BAG-VAC	0.069	0.008	74.82	0.006
C-BAG-50°C	0.093	0.005	43.47	0.053
C-BAG-100°C	0.068	0.004	36.32	0.020

P Degree of hydrolysis

The degree of hydrolysis was measured of the dried materials. Triplicates of each sample was weighed and the amount of titrated 0.001 M NaOH was measured. The raw data and the calculated DH together with the standard deviation are given in Table P.1 for the salmon intestines and in Table P.2 for the chicken intestines.

Table P.1: Weight of samples with dried salmon intestines (S) and the amount of NaOH titrated are presented. The computed degree of hydrolysis (DH) are also given with the corresponding standard deviation (SD). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, IR = infrared dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Weight [g]**	**NaOH titrated [mL]**	**DH [%]**	**Mean DH [%]**	**SD**
S-0-VAC	1.4686	17.174	28.76	29.20	0.39
	1.4792	17.646	29.34		
	1.5007	18.001	29.50		
S-0-IR	1.3795	6.534	13.64	13.10	1.87
	1.4002	7.117	14.64		
	1.4805	5.661	11.01		
S-0-50°C	1.3846	24.411	43.35	41.47	2.38
	1.3674	23.498	42.26		
	1.4587	23.011	38.79		
S-0-100°C	1.4682	15.259	23.45	22.54	1.47
	1.7032	15.737	20.85		
	1.5005	15.506	23.32		
S-VP-VAC	1.344	22.107	40.45	40.20	0.84
	1.3317	22.141	40.89		
	1.5091	24.099	39.27		
S-VP-IR	1.4217	13.299	26.94	27.78	0.84
	1.4292	13.782	27.77		
	1.6078	15.978	28.62		
S-VP-50°C	1.1925	14.733	30.38	30.79	1.67
	1.131	15.009	32.63		
	1.1548	13.790	29.37		
S-VP-100°C	1.5378	13.569	19.91	21.41	1.31
	1.239	12.258	22.32		
	1.2711	12.397	22.01		
S-BAG-VAC	1.0755	15.832	36.20	37.20	1.06
	1.2095	18.248	37.10		
	1.5223	23.711	38.30		
S-BAG-IR	1.2105	9.862	23.46	23.00	2.87
	1.317	11.714	25.61		

	1.3183	9.124	19.93		
S-BAG-50°C	1.0017	16.562	40.66		
	1.1771	21.081	44.04	42.30	1.69
	1.6328	28.018	42.20		
S-BAG-100°C	1.0737	13.649	28.68		
	1.0352	9.936	21.66	23.73	4.31
	1.2087	11.163	20.84		

Table P.2: Weight of samples with dried chicken intestines (C) and the amount of NaOH titrated are presented. The computed degree of hydrolysis (DH) are also given with the corresponding standard deviation (SD). The samples are identified with two labels. The first label is denoted to the storage experiment (0 = initial samples with no storage time, VP = vacuum packed for 30 days and BAG = stored in plastic bag for 30 days). The second label is denoted to the drying method used (VAC = vacuum freeze dried, 50°C = oven dried at 50°C and 100°C = oven dried at 100°C).

Sample	**Weight [g]**	**NaOH titrated [mL]**	**DH [%]**	**Mean DH [%]**	**SD**
C-0-VAC	1.3099	17.801	30.27		
	1.5127	19.597	28.86	29.87	0.879
	1.5242	20.848	30.47		
C-0-50°C	1.2367	21.196	39.18		
	1.8460	29.948	37.08	38.73	1.479
	1.2011	20.987	39.94		
C-0-100°C	1.6798	16.001	35.94		
	1.6112	15.028	35.20	35.29	0.611
	1.6066	14.788	34.73		
C-VP-VAC	1.6123	24.913	58.31		
	1.6212	24.609	57.28	58.78	1.79
	1.7971	28.940	60.77		
C-VP-50°C	1.3295	22.810	64.74		
	1.3503	22.910	64.02	64.42	0.36
	1.5982	27.311	64.48		
C-VP-100°C	1.1948	19.432	61.37		
	1.3905	22.185	60.20	60.24	1.12
	1.5590	24.434	59.14		
C-BAG-VAC	1.1322	14.819	49.39		
	1.1868	15.751	50.08	50.02	0.60
	1.2383	16.603	50.59		
C-BAG-50°C	1.5161	31.512	78.43		
	1.1280	22.469	75.16	76.43	1.75
	1.5093	30.282	75.71		
C-BAG-100°C	1.6225	19.665	45.73		
	1.6134	18.482	43.23	45.01	1.55
	1.3641	16.650	46.06		

Q Total amino acid composition

The total amino acid composition of vacuum freeze dried chicken intestines was performed to verify the total protein content obtained from the Kjeldahl method. The results from HPLC for **one of the triplicates** is shown in Table Q.1. The amount of each amino acid per gram sample was calculated based on Equation Q.1. C is the concentration of the amino acid in [µg/mL], V is the volume of the extract made of the sample in [mL], D is the dilution, m is the weight of the sample in [g] and 1000 is the conversion factor.

$$\frac{C \cdot V \cdot D}{1000 \cdot m} = \text{Total amino acid } [\text{mg/g}_{sample}] \tag{Q.1}$$

Amount of hydroxyproline in the sample was determined based on the hydroxyproline assay and is added to the results from the total amino acid content.

Table Q.1: The amino acid composition of the second parallel (out of three) of vacuum dried chicken intestines. The table shows the different amino acids recorded in the sample and the concentration of each. The sample weight was 0.0555 g, the extract volume was 10 mL and the sample had a dilution of 1:500.

Amino acids*	**Molar weight as bound to protein**				
	Mw_{aa} [g/mol]	**[µmol/ml]**	**[µg/ml]**	**[mg/g_{sample}]**	**% distribution**
Asp	115	1.6099	0.185	16.68	10.64
Glu	129	1.8699	0.241	21.73	13.87
Asn	114	0.0152	0.002	0.16	0.10
His	137	0.3769	0.052	4.65	2.97
Ser	87	1.0226	0.089	8.02	5.12
Gln	128	0.0335	0.004	0.39	0.25
Gly/Arg	98	1.4553	0.143	12.85	8.20
Thr	101	0.9081	0.092	8.26	5.27
Tyr	163	0.3423	0.056	5.03	3.21
Ala	71	1.6012	0.114	10.24	6.54
Aba	85	0.1030	0.009	0.79	0.50
Met	131	0.3139	0.041	3.70	2.36
Val	99	1.0515	0.104	9.38	5.98
Phe	147	0.6164	0.091	8.16	5.21
Ile	113	0.8225	0.093	8.37	5.34
Leu	113	1.5600	0.176	15.88	10.14
Lys	128	1.2301	0.157	14.19	9.05
Hyp[a]	131	69.6397	9.1228	8.22	5.25
Total				156.70	100

*Asp = aspartic acid, Glu =glutamic acid, Asn = asparagine, His = histidine, Ser = serine, Gln = glutamine, Gly = glycine, Arg = arginine, Thr = threonine, Tyr = tyrosine, Ala = alanine, Aba = amino butyric acid, Met = methionine, Val = valine, Phe = phenylalanine, Ile = isoleucine, Leu = leucine, Lys = lysine, Hyp = hydroxyproline.

[a]Determined from hydroxyproline assay with dilution rate 1:5.

R Hydroxyproline assay

The amount of hydroxyproline was determined for the vacuum freeze dried chicken intestines which was added to the total amino acid composition. This was done to verify the total protein content in this dried material which was obtained from the Kjeldahl method.

The standard curve obtained from the hydroxyproline assay is given in Figure R.1. The raw data and the calculated data from this assay are presented in Table R.1.

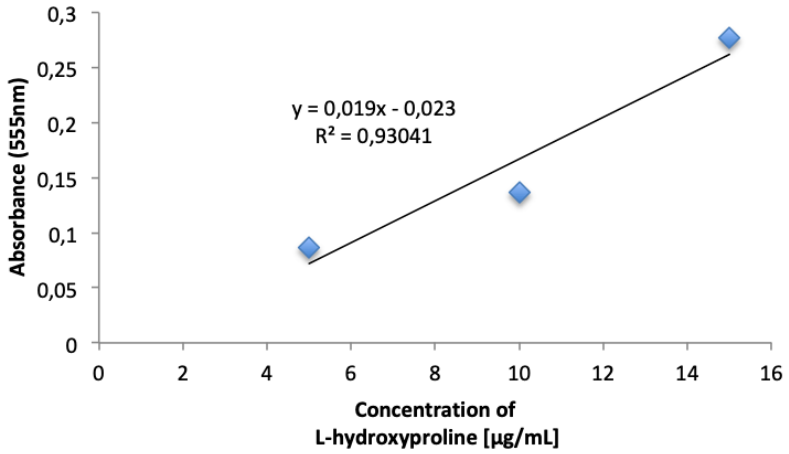


Figure R.1: Standard curve for determination of concentration of L-hydroxyproline [µg/mL]. The concentration is plotted against absorbance measured at 555 nm.

Table R.1: Raw data and calculated values from the hydroxyproline determination of vacuum freeze dried chicken intestines. The samples were diluted 1:5 and 10 mL of extract was made in this analysis.

Parallel	Mean OD (555 nm)	Mean conc. [µg/mL]	Sample weight [g]	Conc. [mg/g$_{sample}$]	Mean conc. [mg/g$_{sample}$]	SD
1	0.176	10.491	0.0510	10.286		
2	0.150	9.123	0.0555	8.219	8.6	1.6
3	0.134	8.263	0.0573	7.210		

S DSC curves for dried materials

The DSC curves obtained from the DSC analysis on the different types of dried salmon are given in Figures S.1, S.2, S.3 and S.4, and for the dried chicken intestines in Figures S.5, S.6 and S.7. The curves contain the glass transition temperatures (onset, inflection and end temperatures), melting peaks and melting energies determined for each material.

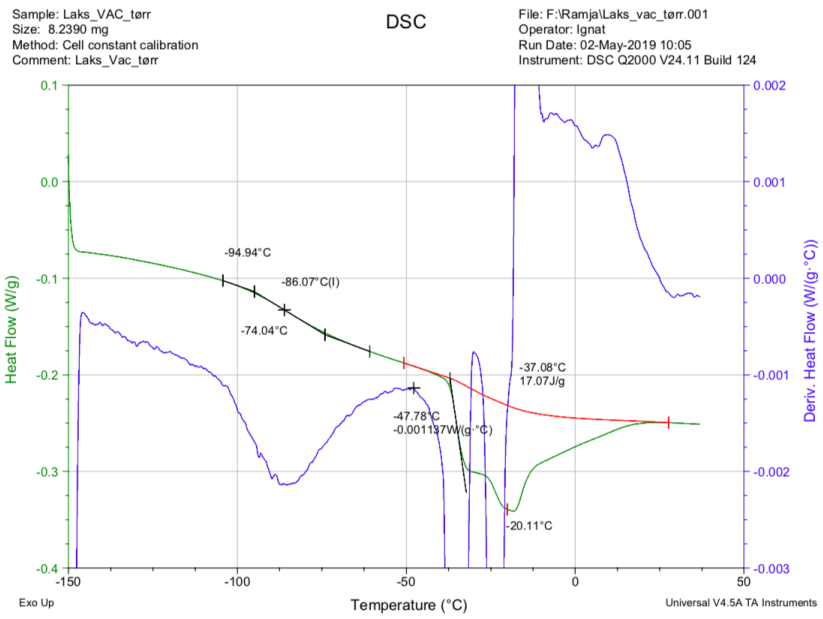


Figure S.1: DSC melting curves for vacuum freeze dried salmon intestines (S-VAC). Glass transition temperatures (onset, inflection and end temperatures), melting peak and melting energies are included in the curve.

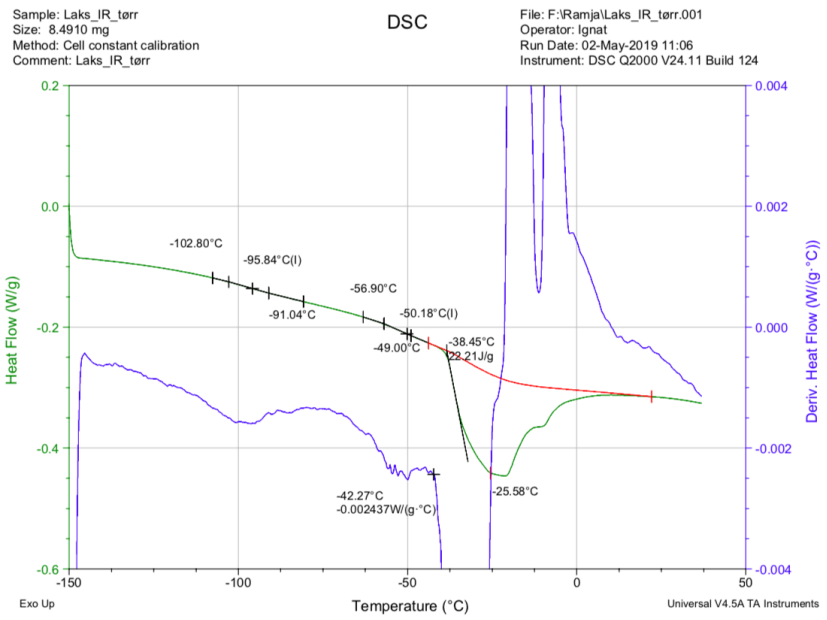


Figure S.2: DSC melting curve for infrared dried salmon intestines (S-IR). Glass transition temperatures (onset, inflection and end temperatures), melting peak and melting energies are included in the curve.

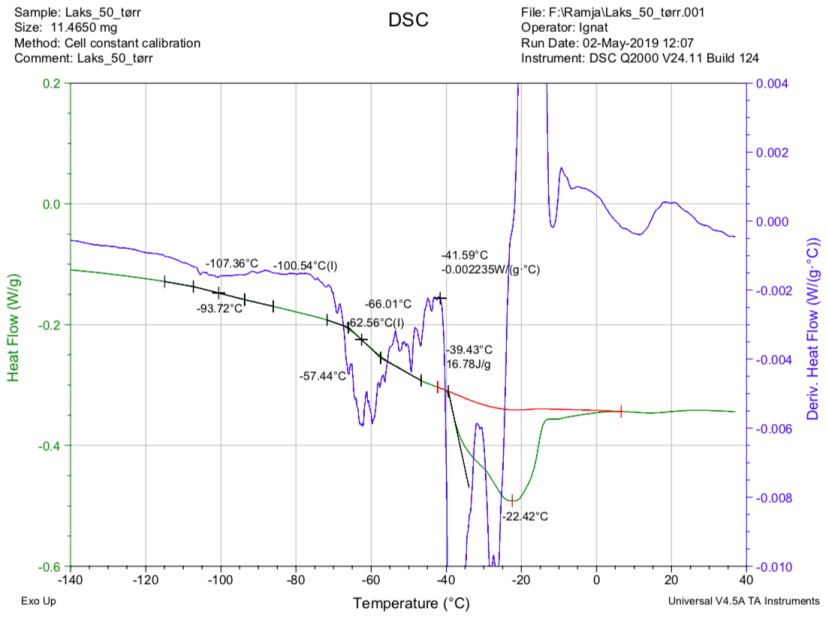


Figure S.3: DSC melting curve for oven dried salmon intestines at 50°C (S-50°C). Glass transition temperatures (onset, inflection and end temperatures), melting peak and melting energies are included in the curve.

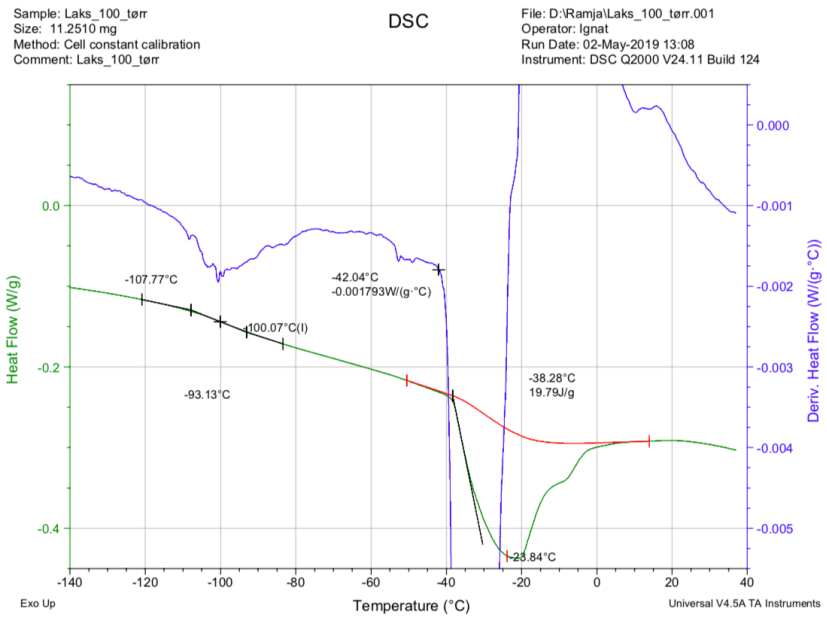


Figure S.4: DSC melting curve for oven dried salmon intestines at 100°C (S-100°C). Glass transition temperatures (onset, inflection and end temperatures), melting peak and melting energies are included in the curve.

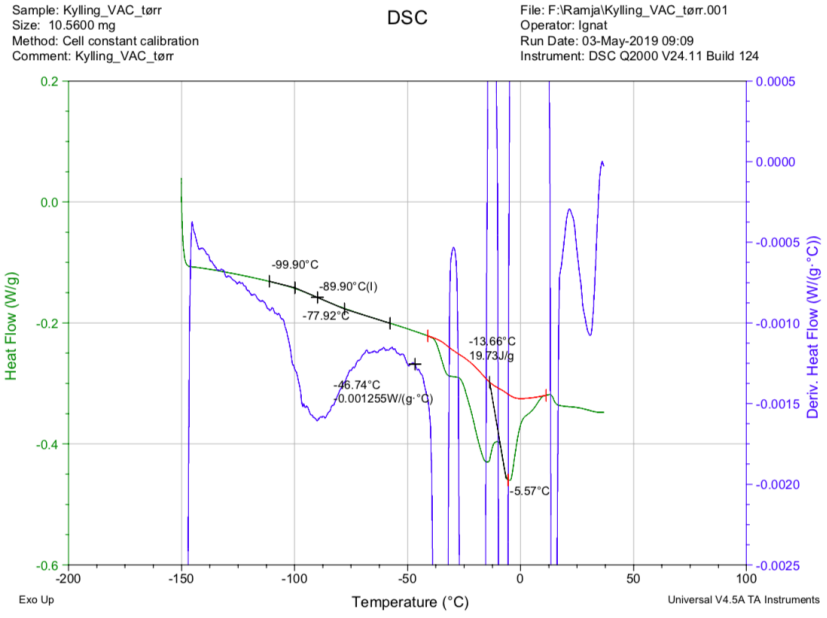


Figure S.5: DSC melting curve for vacuum freeze dried chicken intestines (C-VAC). Glass transition temperatures (onset, inflection and end temperatures), melting peak and melting energies are included in the curve.

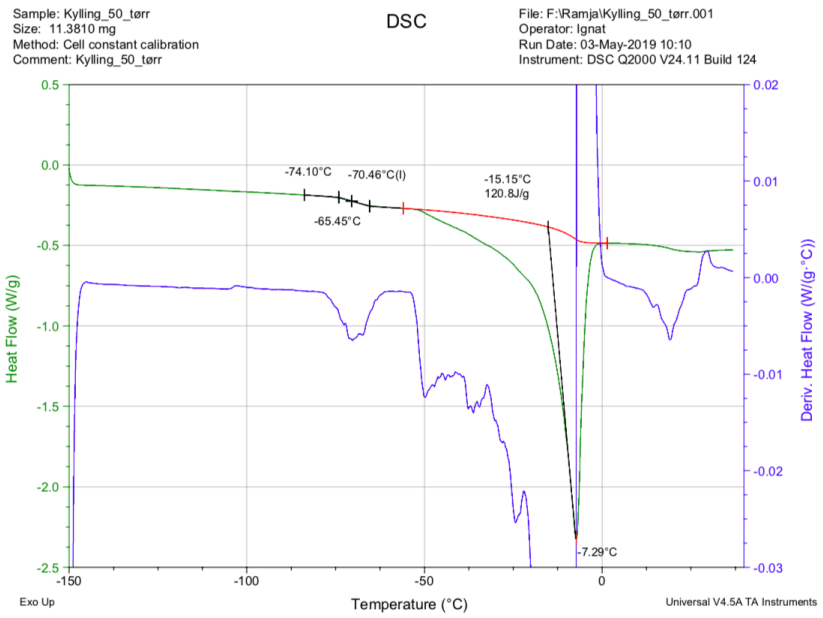


Figure S.6: DSC melting curve for oven dried chicken intestines at 50°C (C-50°C). Glass transition temperatures (onset, inflection and end temperatures), melting peak and melting energies are included in the curve.

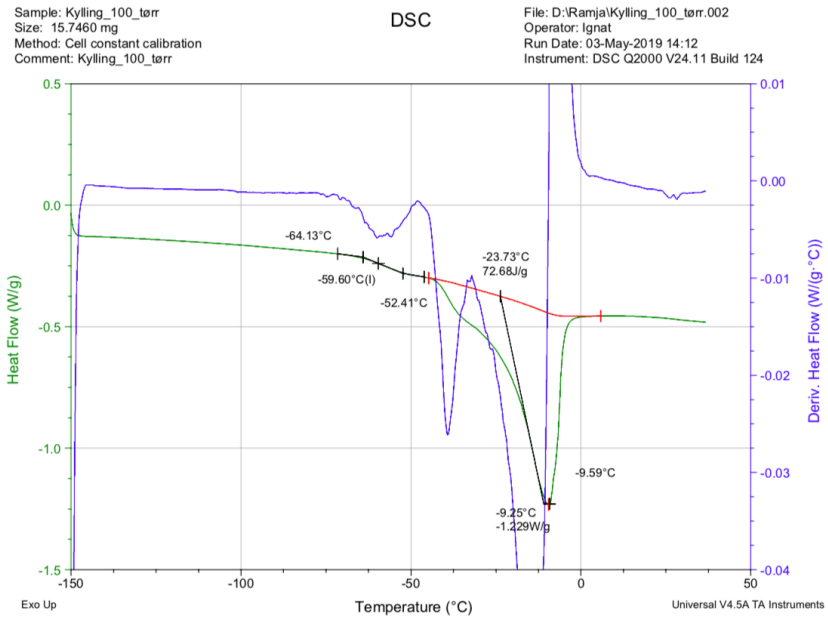


Figure S.7: DSC melting curve for oven dried chicken intestines at 100°C (C-100°C). Glass transition temperatures (onset, inflection and end temperatures), melting peak and melting energies are included in the curve.

Ramja Jeyakumaran

NTNU
Norwegian University of
Science and Technology